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论文汇编

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OR-001

Activation of MT receptors regulates differentiation of B cells in systemic lupus erythematosus

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Background Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by multiple organ involvement, including skin, kidney, blood, joint, mucosal and nerve involvement. The abnormal activation and differentiation of B cells play an important role in the development of SLE. Plasma cell secretion of a large number of autoantibodies is the main cause of systemic lupus erythematosus. The aim of this study was to investigate the role of melatonin receptors in B cell activation and differentiation and its influence on the development of SLE.
Methods To detect the expression of melatonin receptor (MT) in SLE, we detected the protein expression levels of MT1 and MT2 in peripheral blood mononuclear cells of SLE and healthy control by flow cytometry. We detected the melatonin levels in serum of SLE patients and healthy control by ELISA. In order to detect the role of MT receptor activation in B cell differentiation, we isolated human PBMCs and treated them with MT receptor agonists melatonin and Agomelatine after in vitro stimulation. At the same time, we isolated LPR mouse spleen B cells and induced differentiation in vitro. At the same time, MT receptor agonist melatonin and Agomelatine were used to treat B cells. Flow cytometry was used to detect the B cell differentiation.
Results The results of flow cytometry showed that the expression of MT1 and MT2 receptors was down-regulated in circulating B cells of SLE patients. ELISA results showed that the serum concentration of melatonin in SLE patients was lower than healthy control. The treatments of melatonin and Agomelatine could inhibit the differentiation of B cells and significantly reduce the proportion of plasma cells. Splenic B cells of LPR mice were cultured in vitro. Flow cytometry results showed that melatonin and Agomelatine treatment could inhibit the differentiation of B cells and significantly reduce the proportion of plasma cells.
Conclusions Activation of MT receptor inhibits B cell differentiation. Thus, MT agonists are potential therapeutic targets in SLE.

OR-002

Evaluation of the value of colloidal gold immunochromatography technique for BP180-NC16A specific antibodies in the diagnosis and monitoring of bullous pemphigoid

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Background Bullous pemphigoid (BP) involves elderly patients frequently, who have difficulty getting to hospital. Anti-BP180-NC16A antibodies could be used for BP diagnosis and the titer correlates to the severity of BP.
Objective Global validation of immune colloidal gold technique (ICGT) to detect anti-BP180-NC16A antibodies in the blood samples.
Methods: We performed a retrospective cohort study of 414 patients and 15 healthy donors to investigated the diagnostic and predictive value of ICGT.
Results ICGT showed 100% sensitivity in 26 BP patients with neurological complications. Perfect concordance was observed between plasma and serum (κ=0.980) samples with stability. The ICGT achieved sensitivity of 93.9% and specificity of 97.6% with strong agreement between the ICGT and ELISA (κ=0.902). The sensitivity was markedly higher in older patients, and in patients with

more classic lesions (blisters and erosions) ($P<0.05$). In follow-up, we also found ICGT-negative BP patients get consecutive positive strips 1 to 3 months prior to mild new activity or flare.
Limitations This is a retrospective, single-center, descriptive cohort study.
Conclusion ICGT shows high potential as a rapid and stable assay for immunological diagnosis of BP patients. Further investigations would be needed to reevaluate this technique in a larger-scale prospective study and multi-center design.

OR-003

The dual role of MED1 in cutaneous melanoma and its molecular mechanism

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Objectives The incidence of melanoma of the skin has grown globally, with approximately 324,635 new cases diagnosed in 2020. Despite accounting for a small part of skin tumors, cutaneous melanoma is the leading cause of mortality and causes nearly 57,043 deaths every year, which is mainly attributed to the metastasis of melanoma. At present, many biomarkers closely related to cutaneous melanoma have been found one after another. However, the treatment of cutaneous metastatic melanoma is limited to a certain extent and directly affects the prognosis of patients due to the mutation, heterogeneity, and drug resistance. Therefore, exploring the molecular mechanism of cutaneous metastatic melanoma and discovering new targets are of great clinical value to develop novel therapies and improve patients' prognosis of cutaneous melanoma.
Methods Firstly, we used multiple databases to compare the transcription level of MED1 in various types of cancer with the transcription level of MED1 in normal tissues and tumor tissues. Clinicopathological features, including the status of lymph node metastasis, were analyzed in metastatic melanoma patients from TCGA database. In order to study the role of MED1 in cutaneous melanoma progression, we constructed MED1 stable knockdown cells and corresponding control cells in mouse melanoma cell lines and transfected siMED1 to human melanoma cell lines. Then, the colony formation assay, transwell and cell wound healing assays were analysed in vitro. Further, in order to test whether MED1 could influence the metastasis of cutaneous melanoma in vivo. B16-luci cells stably transfecting shMED1 and shCtrl cells were injected to the C57BL/6 mice and detected by luciferase assay. To explore the mechanism, we analysed the activity of TGF-β signaling pathway and the expression of SMAD2. Next, co-IP assays and immunofluorescence were performed to confirm the interaction and identify the binding chains between MED1 and SMAD2 by PDBePISA. Lastly, we conducted the pulldown assay and CHX chase assays of SMAD2 to determine whether MED1 regulated the ubiquitination of SMAD2 in B16 cells.
Results In the present study, we found Mediator 1 (MED1) was highly expressed in patients with skin malignant melanoma from bioinformatics databases. Here, we demonstrated that MED1 knockdown could induce cellular EMT and promote the migration, invasion, and metastasis of malignant melanoma of the skin both in vivo and vitro. The increased EMT phenotype and migration in MED1 knockdown cells were mediated by the TGF-β signaling pathway via interaction between MED1 and SMAD2, a key transcription factor of the TGF-β signaling pathway. To further explore the mechanism, we found MED1 knockdown could protect SMAD2 from degradation via inhibiting SMAD2 ubiquitination, resulting in the transcriptional activation of TGF-β targeted genes. Thus, we speculated that MED1 inhibited TGF-β signaling to decrease cellular EMT and migration by SMAD2 ubiquitination in the metastasis of cutaneous melanoma.
Conclusions In summary, our findings elucidated the role of MED1 in melanoma metastasis and provided a target for the therapeutic strategies of cutaneous melanoma.

OR-004

Protective Effect of Isorhamnetin Against H2O2-Induced Oxidative Damage in HaCaT Cells and Comprehensive Analysis of Key Genes

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Isorhamnetin (ISO) is a methylated flavonol present in the leaves, flowers, and fruits of many plants, and has antitumour, anti-inflammatory, antioxidant, and anti-apoptotic properties, and it maybe the main active substance of Vernonia anthelmintica (L.) to treat vitiligo. However, the mechanisms underlying these effects remain unclear. Thus, we treated human keratinocytes (HaCaT) with hydrogen peroxide (H2O2) to generate an oxidative damage model, and pretreated it with ISO to evaluate the antioxidative effects of ISO. We found that pretreatment with ISO increased HaCaT cell viability, reduced malondialdehyde content, and enhanced superoxide dismutase activity. This reduced the loss of mitochondrial membrane potential, improved cell morphological damage, and inhibited apoptosis in H2O2-treated HaCaT cells. Furthermore, we focused on differentially expressed genes (DEGs) evaluated following the RNA sequencing of HaCaT cells treated with ISO. Enrichment analysis using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes helped us identify 51 significantly dysregulated DEGs associated with ISO. Furthermore, the protective effect of ISO could be related to the Wnt signalling pathway, and we predicted new transcripts and genes to complement and improve the original genome annotation information. Our study provides novel insights into key gene regulation in the progression of oxidative damage and the mechanisms of action of ISO.

OR-005

Risk SNP-mediated enhancer-promoter interaction drives keloid through lncRNA DEIK

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Keloid represents one extreme of aberrant dermal wound healing and is characterized by fibroblast hyperproliferation and excessive deposition of extracellular matrix. Genetics is a major factor for keloid predisposition and the genome-wide association study (GWAS) has identified a single nucleotide polymorphism (SNP) rs873549 at 1q41 as a susceptibility locus. However, the functional significance of this locus in keloid pathogenesis remains elusive. Here, we found that rs1348270, an enhancer located SNP in strong linkage disequilibrium with rs873549, mediated the interaction between the enhancer and the promoter of a lncRNA DEIK (Down Expressed In Keloids, formerly RP11-400N13.1). The risk variant was associated with decreased enhancer-promoter interaction and DEIK down-expression in keloid. Mechanistically, down-regulation of DEIK increased the expression of collagens and chondrocyte and osteocyte associated genes such as POSTN and COMP through up-regulating BMP2. Furthermore, correlation analysis revealed that DEIK expression was inversely correlated with BMP2, POSTN and COMP expression in keloid and normal fibroblasts. These findings uncover new mechanisms underlying genetic factor-mediated keloid predisposition and identify potential targets for keloid therapies.

OR-006

Computer Image Analysis reveals C-Myc as a potential biomarker for discriminating between Keratoacanthoma and Cutaneous Squamous Cell Carcinoma

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The distinction between Keratoacanthoma (KA) and Cutaneous Squamous Cell Carcinoma (cSCC) is critical, yet usually challenging to discriminate clinically and histopathologically. One approach to differentiate KA from cSCC is through assessing the immunohistochemical staining patterns of the threeindicators, β-catenin, C-Myc, and CyclinD1, which are critical molecules that play important roles in the Wnt/β-catenin signaling pathway. Ki-67, as a proliferation biomarker for human tumor cells, was also assessed as an additional potential marker for differentiating KA from cSCC. In this report, these four indicators were analyzed in 42 KA and 30 cSCC cases with use of the computer automated image analysis system. Computer automated image analysis is a time-based and cost-effective method of determining IHC staining in KA and cSCC samples. We found that C-Myc staining was predominantly localized in the nuclei of basal cells within KA patients, whereas cSCC staining was predominantly localized in the nuclei of diffuse cells. This C-Myc staining pattern has a sensitivity of 78.6 % and a specificity of 66.7 % for identifying KA. Moreover, positive rates of distinct expression patterns of C-Myc and Ki-67 may also serve as a means to clinically distinguish KA from cSCC. Taken together, our results suggest that these markers, in particular C-Myc, may be useful in differentiating KA from cSCC.

OR-007

RN7SL1 overexpression promotes cell proliferation in cutaneous T cell lymphoma by regulating hsa-miR-34a-5p

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Objective RN7SL1 enhanced the tumorigenesis and cell proliferation of cancers. The upstream mechanisms of RN7SL1 in regulating tumorigenesis and cell proliferation of cutaneous T cell lymphoma (CTCL) remained unclear.
Methods Two human CTCL cell lines HH and Hut78 were used in this study. RN7SL1 was knocked down by shRNA which were constructed by ligating 3 independently designed oligonucleotides encoding shRNA. Hsa-miR-34a-5p was inhibited by hsa-miR-34a-5p inhibitor. Levels of RN7SL1 and hsa-miR-34a-5p were detected by RT-qPCR. Protein levels of E-cadherin, N-cadherin, and Vimentin were detected by western blotting. The interaction between RN7SL1 and hsa-miR-34a-5p were detected by luciferase assay. HH and Hut78 cell proliferation and apoptosis were detected by MTT and Annexin V/PI assays, respectively. Migration was detected by transwell migration assay. Cytotoxicity of T cells was detected by LDH cytotoxicity kit. Proliferation and apoptosis of T cell co-cultured with HH and Hut78 cells were analyzed by CFSE and Annexin V/PI staining.
Results The numbers of viable RN7SL1-silenced cells were significantly lower than those of the parental cells and the scrambled vector cells indicating that RN7SL1 silencing induced proliferation arrest in cells. RN7SL1 knockdown induced cell cycle arrest but have no obvious effect on promoting spontaneous apoptosis. Long-term culture of the transduced cells didn't result in an increase in the annexin V/PI assays in RN7SL1-silenced cells, which indicates no obvious effect on spontaneous apoptosis. We will repeat each experiment for further verification. Hsa-miR-34a-5p was negatively correlated with RN7SL1. RN7SL1 could sponge hsa-miR-34a-5p to regulate the expression of RN7SL1. shRN7SL1 treatment increased hsa-miR-34a-5p level. It also inhibited cell proliferation, migration and immune escape ability while increased apoptosis ratio of HH and Hut7

cells. shRN7SL1 treatment in HH and Hut7 cells also promoted proliferation and inhibited apoptosis of T cells. These effects were all rescued by hsa-miR-34a-5p inhibitor.
Conclusion Long non-coding RNA RN7SL1 sponged hsa-miR-34a-5p to regulate proliferation, apoptosis and migration and immune escape abilities of CTCL.

OR-008

Low SOCS3 expression in CD4+T cells of Pemphigus vulgaris patients enhanced Th1 and Th17 cells differentiation and aggravated acantholysis by STAT activation

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Objective Pemphigus vulgaris (PV) is a chronic inflammatory autoimmune blistering disease. Aberrant SOCS3/STAT pathway activation is associated with many autoimmune diseases. This study explored the relationship between activation of the SOCS3/STAT pathway and abnormally increased proportions of Th1 and Th17 cells in the peripheral blood of PV patients as well as the effect of $CD4^+$ T cells with abnormal SOCS3/STAT pathway activation on acantholysis.
Methods In PV patients, the proportions of Th1 and Th17 cells in peripheral blood, the levels of IFN-γ and IL-17 in serum and the mRNA levels of SOCS3 and STAT1/3 in $CD4^+$ T cells were detected. Then, SOCS3-knockdown primary $CD4^+$ T cells were prepared, and cocultured with HaCaT cells. Finally, after SOCS3 knockdown and coculture, $CD4^+$ T cells were collected, and the proportions of Th1 and Th17 cells, the protein levels of STAT1/3 and p-STAT1/3, and the levels of IFN-γ and IL-17 were measured. After 2 days of coculture, HaCaT cells were collected, inflammatory factors mRNA expression and acantholysis were assessed.
Results In PV patients, the proportions of Th1 (P=0.016) and Th17 (P=0.045) cells and the levels of IFN-γ (P=0.010) were significantly increased. SOCS3 mRNA in $CD4^+$ T cells was significantly decreased (P=0.008), whereas STAT1 (P=0.043) and STAT3 (P=0.004) mRNA were significantly increased. After SOCS3 knockdown, the proportions of Th1 (P<0.001) and Th17 (P=0.006) cells, the levels of IFN-γ (P<0.001) and IL-17 (P=0.001), and the protein levels of p-STAT1 (P=0.001) and p-STAT3 (P=0.003) were significantly increased in the $CD4^+$ T-shSOCS3-1 group. In the coculture system, the proportions of Th1 (P<0.001) and Th17 (P<0.001) cells, the levels of IFN-γ (P<0.001) and IL-17 (P<0.001), and the number of cell fragments were significantly increased in the $CD4^+$ T-shSOCS3-1+HaCaT-PV-IgG group, whereas the protein level of desmoglein3 (Dsg3) was significantly decreased. In addition, PV-IgG significantly increased IFN-γ and IL-6 mRNA in HaCaT cells.
Conclusion Low SOCS3 expression in $CD4^+$ T cells from PV patients leads to overactivation of STAT, which causes $CD4^+$T cells to overdifferentiate into Th1 and Th17 cells. Additionally, PV-IgG-induced local inflammation in skin lesions, which is mediated by IFN-γ and IL-6, can aggravate this phenomenon. Furthermore, low SOCS3 expression in
$CD4^+$ T cells further exacerbates PV-IgG-induced acantholysis. Therefore, upregulating the expression of SOCS3 in $CD4^+$ T cells of PV patients and maintaining the balance of the IFN-γ/STAT1/SOCS3 and IL-6/STAT3/SOCS3 pathways can alleviate acantholysis in patients with PV.

OR-009

CXCR4high neutrophils predominate in psoriasis and trigger proinflammatory responses via glycolysis signaling

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Objective Epidermal infiltration of neutrophils is a hallmark of psoriasis, where their activation leads to release of neutrophil extracellular traps (NETs). However, the regulatory mechanisms for NETs formation remain enigmatic and how to target neutrophil therapy in psoriasis remains a difficult challenge. Emerging evidence indicate that neutrophils show plasticity and heterogeneity under certain physiological and pathological conditions, constituting different subpopulations.
Methods We investigated the proinflammatory function of CXCR4^{high} neutrophils by flow cytometry, immunofluorescence, and IMQ mouse model, and analyzed the functional and metabolic changes of CXCR4^{high} neutrophils by RNA sequencing. Co-culture model was used to study the interaction between CXCR4^{high} neutrophils and endothelial cells.
Results In this study, we present a new insight on the identity and functions of neutrophils in psoriasis by demonstrating that CXCR4^{high} neutrophils, with enhanced pro-inflammation functions and NETs formation, accumulate in circulation and psoriatic lesions and are reduced in the circulation upon biological therapy. Our findings also reveal that CXCR4^{high} neutrophils possessed increased glycolytic metabolism and high levels of lactic acid produced by psoriatic CXCR4^{high} neutrophils caused vascular remodeling including cutaneous vasodilation and enhanced vascular permeability in vivo and in vitro. Correspondingly, CXCR4 inhibitor and anti-CXCL12 antibody significantly reduced cutaneous vasodilation, vascular permeability, and psoriatic symptoms in an imiquimod-induced psoriasiform mouse model. Mechanistically, CXCR4 is regulated by transcription factors CREB, and targeting the transcription factor effectively modulates the pro-inflammatory effects of CXCR4^{high} neutrophils.
Conclusion Overall, these results thus identify an unprecedented role of the neutrophil subset in psoriasis.

OR-010

Tissue RNA Sequencing reveals novel biomarkers associated with postoperative keloid recurrence

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Keloids can be resected through surgery, but may still recur. The goal of this study was to explore the biomarkers to predict postoperative recurrence of keloids. Patients who underwent surgical treatment and postoperative superficial X-ray radiation from January 2019 to December 2020 were recruited, their clinical data collected and Keloid samples subjected to RNA sequencing analysis. Follow-up lasted for at least 1 year after surgery. By screening differentially expressed genes (DEGs) between postoperative recurrent and non-recurrent sample groups and constructing a co-expression network through the weighted gene co-expression network analysis (WGCNA), an immunity-related module identified was chosen for subsequent analysis. We constructed the DEG co-expression network in the key module, and used Molecular Complex Detection (MCODE) plug-ins in Cytoscape software to identify hub genes. The predictive accuracy of hub genes for postoperative keloid recurrence was evaluated using Receiver Operating Characteristic (ROC) curves. CIBERSORTx algorithm was used to quantify the infiltration of immune cells in keloid tissue. Gene set enrichment analysis (GSEA) was performed to investigate the biological functions of hub genes. Using MCODE algorithm, five hub genes (FERMT3, VEGFA, LCP1, CD86 and APBB1IP)

were identified in the key module. ROC curve analysis showed the AUC for the predictive value of five combined hub genes was 0.827. Immune infiltration analysis showed that T cells, mast cells, neutrophils, and macrophages were major components in the keloid immune microenvironment. GSEA showed that hub genes (LCP1, CD86 and ABB1IP) were involved in neutrophil-related biological processes. In our study, WGCNA and DEG analyses were combined to screen biomarkers in recurrent keloid tissue samples for the first time. Our findings provided potential prognostic markers for postoperative recurrence of keloids.

OR-011

Study on the difference of epidermal autophagy between psoriasis vulgaris with and without metabolic syndrome

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Objective To explore the relationship between autophagy, metabolic syndrome (MS) and psoriasis vulgaris, we detected the expression of epidermal autophagy related proteins (Beclin-1, LC3B, p62) in patients with and without MS.
Methods The clinical and pathological data of 80 patients with psoriasis vulgaris from December 2016 to September 2020 in the dermatology department of the first medical center of the PLA General Hospital were retrospectively analyzed (40 patients with MS and 40 patients without MS). Immunohistochemical staining was performed on the skin lesions of 80 patients to detect the expression of autophagy related proteins such as Beclin-1, LC3B, p62 at the epidermal level, and quantitative analysis was performed using ImageJ software. All data were statistically analyzed by SPSS 25.0 software. The baseline data and autophagy related protein expression levels were compared between the two groups. Moreover, the correlation between ImageJ score and PASI score, waist circumference, BMI, systolic blood pressure, diastolic blood pressure, high-density lipoprotein, triglyceride, fasting blood glucose, CRP and other indicators of 80 patients was analyzed.
Results A total of 80 patients with psoriasis vulgaris were included in this study, including 54 males and 26 females. The baseline data showed that there was no significant difference between the two groups in terms of age, gender, height, PASI score, CRP ($P > 0.05$); However, the weight, BMI, waist circumference, triglyceride, blood glucose and insulin levels of patients with MS were significantly higher than those without MS, and HDL-C was significantly lower than those without MS ($P < 0.05$). The results of immunohistochemistry showed that the ImageJ scores of Beclin-1, LC3B and p62 in the epidermis of patients with MS psoriasis were 16.31 ± 4.42, 4.49 ± 4.86 and 23.64 ± 8.45 respectively, while those without MS were 21.81 ± 5.99, 7.41 ± 7.59 and 17.38 ± 4.97 respectively, with significant differences ($P < 0.05$). Spearman correlation analysis showed that the immunohistochemical ImageJ score of Beclin-1 was significantly negatively correlated with waist circumference ($r = -0.368$, $P = 0.001$), triglyceride ($r = -0.327$, $P = 0.003$), blood glucose ($r = -0.226$, $P = 0.044$), and positively correlated with CRP ($r = 0.282$, $P = 0.011$); The immunohistochemical ImageJ score of LC3B was significantly negatively correlated with BMI ($r = -0.228$, $P = 0.042$) and blood glucose ($r = -0.292$, $P = 0.008$); The immunohistochemical ImageJ score of p62 was significantly positively correlated with waist circumference ($r = 0.283$, $P = 0.011$), BMI ($r = 0.312$, $P = 0.005$), triglyceride ($r = 0.225$, $P = 0.045$), while it was opposite to HDL-C ($r = -0.313$, $P = 0.005$).
Conclusion Compared with psoriasis patients without MS, the autophagy level of psoriasis patients with MS was significantly down regulated. This suggests that psoriasis patients with MS may affect the progress and treatment of psoriasis by inhibiting epidermal autophagy through metabolic syndrome related pathways. In addition, we should pay attention to the diet and weight changes of patients with psoriasis, and assist patients to change their lifestyle in the early stage to improve the condition of psoriasis.

OR-012

Immune and inflammatory dysregulation in pathogenesis of Hidradenitis Suppurativa

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Abstract: Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease with an estimated global prevalence of 0.3% to 4%. The clinical manifestations of the disease include painful nodules,abscesses, ulceration, sinus tracts or tunnels, and scar formation, which long-term impacts the quality of life in patients. To date, the complete pathological mechanism has not been elucidated yet. However, many studies have revealed specific immune factors involved in the pathogenesis of HS, which have emerged as key players in the pathological process. These key immune factors that have been investigated include inflammatory cytokines such as interleukin(IL)-17, IL-1β, tumor necrosis factor-alpha,chemokines,complements and some newly discovered molecules remaining to be explored. The curative effect of targeted therapies aimed at these immune factors has been confirmed by a certain quantity of clinical trials, which also provided factual evidence. This article will focus on the immune factors probably closely involved in the pathogenesis of HS.

OR-013

Effect of ultrasound combined with 4-hydroxyphenyl-retinamide lipid microbubble on COL1A1 expression in keloid fibroblasts LI

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Objective To investigate the effect of ultrasound combined with 4-hydroxyphenyl-retinamide (4-HPR) lipid microbubble on COL1A1 expression in keloid fibroblasts (KFs).
Methods KFs were cultured in vitro and divided into control group, 4-HPR lipid microbubble group and ultrasound combined with 4-HPR lipid microbubble group. The expression of COL1A1 mRNA and protein in KFs of each group were determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western Blot.
Results The expression of COL1A1 mRNA and protein in the 4-HPR lipid microbubble group and ultrasound combined with 4-HPR lipid microbubble group were lower than that in the control group. The difference was statistically significant ($P<0.05$). In particular, the expression of COL1A1 in the ultrasound combined with 4-HPR lipid microbubble group was decreased significantly.
Conclusion Ultrasound combined with 4-HPR lipid microbubble significantly inhibited the expression of COL1A1 mRNA and protein levels in KFs.

OR-014

Differential expression of hsa-miR-487b-3p in serum exosomes, a prospective biomarker for progressive vitiligo diagnosis

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Background Vitiligo is an acquired skin depigmentation disease. It is easy to be misdiagnosed at early stage and tend to relapse. Serum markers are important to monitor progression of

vitiligo. Exosomal miRNAs could act as the communication mediator between melanocytes and immune cells. Our study aims to provide a reference for diagnosis and evaluation of vitiligo progression via serum exosomal miRNAs.
Methods miRNAs were extracted from blood exosomes of 10 progressive vitiligo patients, 10 stable vitiligo patients (the progressive patients after treatment) and 10 healthy individuals. We profiled miRNAs expression by RNA sequencing and screened out potential miRNAs and plotted their ROC curves to explore sensitivity and specificity as biomarkers in differential diagnosis of progressive and stable vitiligo. We explore the correlation between expression of miRNAs and body surface area (BSA). Then, databases were used to predict gene targets of miRNAs. GO and KEGG were performed to analyze target genes.
Results Our results showed that 141 miRNAs were differentially expressed in serum exosomes of progressive vitiligo, 365 miRNAs differentially expressed in stable vitiligo compared with healthy individuals. Expression of hsa-miR-487b-3p in progressive vitiligo was significantly lower compared with healthy individuals, while there was no difference in stable vitiligo. Hsa-miR-487b-3p could serve as a biomarker for differential diagnosis of progressive and stable vitiligo based on ROC curve (AUC=0.840). The expression of hsa-miR-487b-3p was positively correlated with BSA.There were 41 genes of hsa-miR-487b-3p predicted via databases. KEGG were mainly enriched in phenylalanine metabolism, glycan degradation, protein export, etc.
Conclusion Serum exosomal hsa-miR-487b-3p could be a biomarker to distinguish progressive and stable vitiligo. The predicted target genes of hsa-miR-487b-3p were enriched in catabolism. Thus, down-regulation of hsa-miR-487b-3p in progressive vitiligo may accelerate catabolism in melanocyte and cause impairment of it.

OR-015

Discovery and functional characterization of long noncoding RNAs associated with familial acne inversa with NCSTN mutation Running title: Discovery and functional characterization of lncRNAs associated with FAI

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Background Long noncoding RNAs (lncRNAs) are associated with many dermatologic diseases. However, little is known about the regulatory function of lncRNAs in familial acne inversa (AI) patients with nicastrin (NCSTN) mutation.
Objectives To explore the regulatory function of lncRNAs in familial AI patients with NCSTN mutation.
Methods The expression profiles of lncRNAs and mRNAs in skin tissues from familial AI patients with NCSTN mutation and healthy individuals were analysed in this study via RNA sequencing (RNA-seq).
Results In total, 359 lncRNAs and 1863 mRNAs were differentially expressed between the two groups. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses revealed that the dysregulated mRNAs targeted by lncRNAs were mainly associated with the immune regulation, Staphylococcus aureus infection and B cell receptor signalling pathways. The lncRNA-miRNA-mRNA coexpression network contained 265 network pairs comprising 47 dysregulated lncRNAs, 8 miRNAs and 74 mRNAs. Conservation analysis of the differentially expressed lncRNAs between familial AI patients with NCSTN mutation and Ncstn keratinocyte-specific knockout (NcstnΔKC) mice identified 6 lncRNAs with sequence conservation; these lncRNAs may participate in apoptosis, proliferation and skin barrier function.
Conclusions These findings provide a direction for exploring the regulatory mechanisms underlying the progression of familial AI patients with NCSTN mutation.

OR-016

Memory B cells defect in patients with Netherton syndrome

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Background Comel-Netherton syndrome (NS) is a rare autosomal recessive disease caused by loss-of-function mutations in the SPINK5 gene, typically characterized by congenital ichthyosis, bamboo hair, atopic diathesis with elevated serum IgE levels. All evidence pointed to memory B cells defects in NS with patients. However, whether or how SPINK5 effects B differentiation in Germinal center and antigen specific memory B cells response in NS is still unknown.
Methods We used samples from patients with NS and samples from the Spink5 B-cell-specific knockout mice (Mb1Cre Spink5flox/flox) mice (BKO) to investigate the mechanism of SPINK5 in B-cell deficiency. we examined the numbers of peripheral B subsets, its abilities of proliferation and apoptosis, and MBC recall response after antigen specific stimulated BKO mice, as well as BCR signaling after soluble antigen stimulation.
Results We found that T cell-dependent antigen specific antibody production, maintenance and immune recall responses are defective in patients with NS. Furthermore, the differentiation of MBCs in germinal center in BKO mice mimicked the phenotype of patients with SPINK5 LOF mutation, having deceased levels of germinal center B cells and MBCs. B cells had reduced BCR clustering and defective activation of positive signaling but decreased negative signaling in NS patients.
Conclusions Overall, our study has provided a novel underlying molecular mechanism of how SPINK5 deficiency regulates the peripheral differentiation and maintenance of MBCs.

OR-017

Exosomes from conditioned medium of human keloid-derived mesenchymal stem cells promote the fibrosis of keloid via the TGF-β1/Smad pathway under tension

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Objective Keloids are considered to be dermal benign fibroproliferative tumors that characterised by invasive growth of keloid fibroblasts and aberrant deposition of extracellular matrix. The pathogenesis of the disease is still not clear and there is no effective treatment. Recently, mesenchymal stem cells (MSCs) have been suggested as a therapeutic potential cell source to treat fibrotic diseases. The conditioned medium and the exosome from mesenchymal stem cells (MSC-CM) is well known as a rich source of autologous macromolecules and is universally used for tissue regeneration in current clinical medicine. The exosome from MSC-CM can transport a diverse suite of macromolecules and play an important role of a cell-to-cell communication. However, the role of exosomes in keloid pathogenesis remains unclear. Here, we investigate the effect of the conditioned medium and the exosome from keloid-derived mesenchymal stem cells (K MSCs)on keloid fibrosis under mechanical tension.
Methods Exosomes were harvested from conditioned medium of K-MSCs stimulated with tension by a sequential centrifugation process. Treat keloid fibroblasts (K-Fbs) with conditioned medium and exosomes respectively. Cell proliferation, collagen synthesis and cell migration were assessed by Cell Counting Kit-8 (CCK-8) assay, hydroxyproline content analysis, and the scratch test respectively.Real-time quantitative PCR and Western blot were performed to measure the mRNA and protein expressions of Collagen I, Fibronectin, Alpha-Smooth Muscle Actin (α-SMA), and TGF-β1/Smad pathway proteins respectively.
Results CCK-8 analysis results demonstrated a significant difference ($P<0.05$) between the tension group and the control group from 1 to 4 days. The scratch test results showed that the cell migration rate of the tension group was significantly higher than the control group at 1 day and 2

days, and the difference was statistically significant (P<0.01). Real-time quantitative PCR showed that the mRNA expression level of collagen I, Fibronectin, and α-SMA were all significantly increased in the tension group compared with the control group, and the difference was statistically significant (P<0.01). Western blot revealed that the changes in protein expression levels of the three fibrosis markers were consistent with their mRNA changes (P<0.05). In addition, the expression of key signal molecules of the TGF-β/Smads signaling pathway, including TGF-β1, Smad2 and Smad3 proteins increased.
Conclusions The exosome from MSC-CM may enhance the biological activity of keloid fibroblasts under tension through regulating TGF-β/Smads signal transduction pathways, and may be involved in the fibrosis process of keloids.

OR-018

TWEAK depletes desmogleins in pemphigus vulgaris via JAK/STAT1-induced apoptosis

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Pemphigus vulgaris (PV) is a life-threatening autoimmune skin disease associated with a loss of epidermal keratinocyte adhesion because of autoantibodies targeting desmoglein (Dsg) 1 and 3. Recently, we have shown that tumor necrosis factor-like weak inducer of apoptosis (TWEAK) participates in cell adhesion via binding to fibroblast growth factor-inducible 14 (Fn14). Here, we further investigated the role of TWEAK-regulated Dsg1 and Dsg3 in keratinocyte adhesion. In lesional and perilesional biopsies of PV patients, TWEAK activation and Dsg1/3 reduction were detectable compared with healthy controls. This led us to speculate that TWEAK regulates Dsg1/3 to involve in PV pathogenesis. TWEAK-administrated human primary keratinocytes showed decreased Dsg1 and Dsg3, while Fn14 RNA interference preserved Dsg1/3 and protected cells from loss of adhesion, validating our speculation. TWEAK-stimulated keratinocytes also exhibited JAK/STAT1 activation, which is reported to inhibit Dsg3. In addition, Dsg1/3 reduction was efficiently blocked by Fludarabine, indicating that STAT1 mediates the TWEAK effects observed upon loss of Dsg1 and Dsg3. PV lesions and TWEAK-administrated keratinocytes also showed increased apoptosis characterized as increment of caspase-3, caspase-9, and caspase-14, which can induce apoptosis and cleave Dsg1/3. Furthermore, TWEAK-administrated human keratinocytes expressed increased CCL2, CCL5, and CXCL8, in accordance with increased infiltration of neutrophils, mast cells, CD68+ macrophages, as well as CD3+ T cells. These data indicate that TWEAK may exert an anti-adhesion role through reducing Dsg1/3 expression and inducing apoptosis via JAK/STAT1 pathways as well as triggering inflammation. Thus, TWEAK may serve as a biomarker or therapeutic target for PV in the future.

OR-019

Novel compound heterozygous CDH3 mutations in a Chinese case with hypotrichosis and juvenile macular dystrophy and a review of 116 cases from the literature

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Hypotrichosis with juvenile macular dystrophy (HJMD) is a rare autosomal-recessive genetic disorder characterized by short, curly, and sparse hair, progressive macular degeneration, decreased visual acuity, and even blindness during early life.To date, 30 HJMD pedigrees and 20

sporadic cases involving 36 mutations in the CDH3 gene have been reported. In this study, we present a sporadic Chinese HJMD case with novel compound heterozygous nonsense mutations and conduct a literature review of genotype-phenotype correlation in 116 HJMD individuals.
The proband was a ten-year-old girl, the first child of a non-consanguineous family. There was no family history of abnormal hair growth or visual impairment. She presented scalp hypotrichosis with sparse, soft and curly hair, especially of the peripheral hair. The hair pull test and scalp skin were normal. Her eyebrows and eyelashes were normal and she already had armpit hair. She had small papules on both cheeks and on the outer side of the upper arm. There were no associated abnormalities of her nails, teeth, or limbs. Dermoscopy revealed the hair diameter to be diverse and the percentage of pilosebaceous units with a single hair to be increased. Scanning electron microscopy showed the hair shaft had local deformation, peeling, and loss of cuticle but without complete 180° twists. Impairment of the proband's vision began at the age of 9 years. Her best-corrected visual acuity was 0.8 in left and right eyes. Fundus examination revealed a yellow lesion with a size of ~1 papillary diameter (PD) on the nasal side of the foveal center in the right macula . The left macula was normal. Optical coherence tomography showed partial continuity interruption in the outer retina.
The WES analysis discovered compound heterozygous mutations, c.2102_2103delAT (NM_001793.5)and c.2158C>T (NM_001793.6), in the CDH3 gene, which were confirmed by Sanger sequencing.The frameshift deletion (c.2102-2103_delAT) in exon 14 results in the replacement of tyrosine (Y) by tryptophan (W) at position 701 of the amino acid sequence and thus a stop codon occurred at position 713 caused by frameshift (p.Y701Wfs*13). The nonsense mutation (c.2158C>T) in exon 15 results in an arginine codon (CAG) being substituted by a stop codon(TAG) at position 720 of the amino acid sequence (p.R720*). It showed that the proband inherited the deletion mutation from her mother and the nonsense mutation from her father. Neither mutation was previously reported for HJMD and both are absent in the ClinVar, HGMD, gnomAD, and dbSNP databases.
We have reviewed 95 familial HJMD cases from 30 pedigrees and 21 sporadic HJMD cases. The patients with HJMD have been reported throughout the world but are mainly distributed in the Middle East. Only one case with hypotrichosis but no visual symptoms has been reported in China. Of 113 patients whose detailed clinical data were available, the male/female ratio was 48:65, the average onset of scalp hypotrichosis was approximately 1 month old, while decreased visual acuity developed from approximately 9 years old. Thirty-eight distinct CDH3 mutations, including 8 nonsense, 8 splicing, 8 missense, 11 frameshift, and 3 long fragment deletion mutations have been reported in HJMD patients. We also found it difficult to draw conclusions for phenotype-genotype correlation regarding CDH3 mutations on the basis of phenotypic heterogeneity and a limited sample size.
Our findings not only further expand the understanding of genetic and clinical spectrums of CDH3 mutations in HJMD, but also provide more insight into the genotype-phenotype correlation in HJMD.

OR-020

Study on the effect of antigen presenting element gene methylation on psoriasis

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Purpose To explore the relationship between the protein expression of antigen presentation related genes (TAP1, TAP2, LMP2, LMP7) and psoriasis and their expression differences.
Method Collect skin tissue samples from patients with psoriasis and normal people, the expression of antigen presentation related genes (TAP1, TAP2, LMP2, LMP7) was detected by immunohistochemistry SP method and Western blotting, respectively. The mRNA expression of antigen presentation related genes (TAP1, TAP2, LMP2, LMP7) was detected by real-time fluorescence quantitative PCR (RT-PCR). The methylation levels of TAP1, TAP2, LMP2 and LMP7

genes in the skin lesions of patients with psoriasis vulgaris and healthy controls were detected by methylation BSP method.
Result RT-PCR showed that there was no significant difference in the expression of TAP1, TAP2 and LMP2 between psoriasis patients and healthy controls, and the expression of LMP7 mRNA decreased significantly. Western blot showed that compared with healthy controls, the expression of TAP1 and TAP2 in psoriatic lesions was significantly increased, the expression of LMP2 had no significant change, and the expression of LMP7 was significantly reduced. Immunohistochemistry detection showed that the number of TAP1 and TAP2 positive cells in psoriatic patients' skin lesions was significantly higher than that in the healthy control group, the expression of LMP2 had no significant change, and the number of LMP7 positive cells was significantly lower than that in the healthy control group. Methylation detection results showed that the methylation level in psoriatic patients' skin lesions was significantly lower than that in healthy controls at the 125 locus of TAP2 gene ($p<0.05$).
Conclusion The increase of TAP1 and TAP2 protein expression levels may be related to the occurrence of psoriasis, and the decrease of TAP2 methylation level in patients with psoriasis may be related to the inhibition of antigen-presenting tissue proliferation and the abnormal proliferation of Kc cells to further tumor like cells. Methylation in the promoter region of TAP2 gene of APM member can inhibit gene protein expression, affect the assembly and presentation of HLA-I molecules, cause the down-regulation or deletion of HLA-I on the surface of keratinocytes, make cells escape the immune surveillance and killing of the body, and lead to the occurrence of psoriasis, which may be the epigenetic mechanism of psoriasis.

OR-021

44℃ hyperthermia activates AMPK/JNK signaling pathway to promote HSPA6 expression in HaCaT cells

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Objectives My team's previous research found that local hyperthermia can be clinically used for the treatment of HPV-related viral warts, such as common warts, flat warts, condyloma acuminatum, giant condyloma acuminatum, etc., and achieved good results. And for multiple HPV-related virus warts, when one wart is treated, the other untreated warts also appear to fall off, suggesting that local hyperthermia can clear multiple warts by activating the systemic immune response against HPV virus, and the mechanism is still to be further explored. AMPK is a highly conserved serine/threonine kinase, which is an important energy sensor. When the body is in an unfavorable stress state, AMPK is activated. After AMPK activation, it can simultaneously close the ATP-consuming anabolism and open the ATP catabolism pathway. , through this mechanism to maintain intracellular metabolic energy homeostasis. Mitogen-activated protein kinases (MAPKs) are protein serine/threonine kinases that transmit extracellular signals and cause many intracellular responses. The three most common MAPK pathways include extracellular regulated protein kinase (ERK) 1/2, p38, and c-Jun N-terminal kinase (JNK) 1/2. Many studies have found that MAPK plays an important role in controlling the response of cells to the environment and is widely involved in various physiological processes such as cell growth, cell differentiation,proliferation and apoptosis. Activating transcription factor 2 (ATF2)—also known as cyclic AMP (cAMP) response element (CRE)-binding protein 2 (CREB2) and CRE-BP1—is a member of the activating protein 1 (AP1) family of transcription factors that can be regulated by interaction with other AP1 family members,such as CREB, Fos, Maf, or Jun family transcription factors, homodimerize or heterodimerize to express many genes. HSPA6 is a member of the HSP70 family whose genes are present in the human genome and in marmosets, goats and camels,but not in mice and rats. Due to the lack of animal models to study HSPA6, the function of HSPA6 is known The expression of HSPA6 in the HSP70 family in epithelial cells was significantly stimulated by heat. The purpose of this study is to start with AMPK, JNK, ATF2 and HSPA6, to

study their expression and interaction in HaCaT cells after hyperthermia, and to provide ideas for explaining the mechanism of the efficacy of hyperthermia.

Methods 1. Research objects: The wild-type HaCaT cell line and the HSPA6 gene-knockout HaCaT cell line were cultured in a constant temperature CO2 incubator at 37°C as the control group,and heated in a 44°C water bath as the experimental group. 2. Treatment with inhibitors: The hyperthermia treatment group and the experimental group were divided into groups with and without inhibitors. A sterile pipette tip was used to aspirate the complete medium in the cell culture dish, and 200uM containing 200uM was added to the inhibitor group. AMPK inhibitor (dissolved in DMSO) or complete medium containing 200uM JNK inhibitor (dissolved in DMSO), and complete medium containing the same amount of DMSO was added to the no inhibitor group. The above operations were completed 2 h before the cells were warmed. 3. hyperthermia treatment: hyperthermia treatment: cells are plated,labeled as the control group and the experimental group, and the hyperthermia treatment is performed when the cell confluence reaches 70%-75%. Take it out from the 37°C constant temperature CO2 incubator and place it on the water surface of the 44°C water bath. After heating for 30 minutes, take it out and put it back into the 37°C constant temperature CO2 incubator to continue the cultivation. Extract nucleic acid or total protein from cells at different time points according to experimental needs for PCR or Western blot experiments. 4. Real-time quantitative PCR: Detect the mRNA expression of ATF2, and compare the expression difference of ATF2 mRNA in wild-type HaCaT cell line and HSPA6 gene knockout HaCaT cell line before and after hyperthermia. 5. Western blot (Western Blot, WB): detect the expression of AMPK, p-AMPK, JNK, p-JNK, ATF2,p-ATF2 and HSPA6, compare the above proteins before and after adding AMPK inhibitor, JNK inhibitor and hyperthermia Expression differences in wild-type HaCaT cells.

Results 1. Real-time quantitative PCR: (1) 30 min after hyperthermia at 44 °C, the RNA expression level of ATF2 in HaCaT cells and HSPA6-knockout HaCaT cells was significantly increased at the transcriptional level. 2. Western blot (Western Blot, WB): (1) In HaCaT cells, thermal stimulation at 44°C had no effect on the overall expression level of AMPK, but could induce an up-regulation of AMPK phosphorylation. (2) In HaCaT cells, Dorsomorphin had no effect on the overall expression level of AMPK, but could inhibit the up-regulation of p-AMPK expression induced by 44°C hyperthermia. (3) In HaCaT cells, the expression of HSPA6 was increased by 44℃ hyperthermia stimulation. (4) In HaCaT cells,Dorsomorphin inhibited the increase of HSPA6 expression induced by 44°C hyperthermia. (5) In HaCaT cells, thermal stimulation at 44°C increased the overall expression of JNK and induced the up-regulation of JNK phosphorylation.(6) In HaCaT cells, SP600125 could inhibit the up-regulation of JNK and p-JNK expression induced by 44°C hyperthermia. (7) In HaCaT cells, the expression of HSPA6 was induced to increase by 44°C hyperthermia stimulation. (8) In HaCaT cells, SP600125 inhibited the increase of HSPA6 expression induced by 44℃ hyperthermia. (9) In HaCaT cells, thermal stimulation at 44°C had no effect on the overall expression level of AMPK, but could induce an up-regulation of AMPK phosphorylation. (10) In HaCaT cells, Dorsomorphin had no effect on the overall expression level of AMPK, but could inhibit the heat-induced up-regulation of p-AMPK expression. (11) In HaCaT cells, thermal stimulation at 44°C increased the overall expression of JNK and induced the up-regulation of JNK phosphorylation. (12) In HaCaT cells, Dorsomorphin can inhibit the heat-induced up-regulation of JNK and p-JNK expression. (13) In HaCaT cells and HSPA6-knockout HaCaT cells, 44°C hyperthermia stimulation had no effect on the overall expression level of ATF2, but could induce the phosphorylation level of ATF2 to be up-regulated at site 112 94. (14) In HaCaT cells, thermal stimulation at 44°C had no effect on the overall expression level of AMPK, but could induce an up-regulation of AMPK phosphorylation. (15) In HaCaT cells,Dorsomorphin had no effect on the overall expression level of AMPK, but could inhibit the up-regulation of p-AMPK expression induced by 44°C hyperthermia.(16) In HaCaT cells, thermal stimulation at 44°C had no effect on the overall expression level of ATF2, but could induce the up-regulation of phosphorylation levels of ATF2 at 112 94 and T71 sites. (17) In HaCaT cells, Dorsomorphin had no effect on the overall expression level of ATF2, but could inhibit the up-regulation of p-ATF2 (112 94) and p-ATF2 (T71) induced by 44°C hyperthermia.

Conclusions 1.44℃ hyperthermia activates the AMPK/JNK signaling pathway to regulate the expression of HSPA6 in HaCaT cells. 2.44℃ hyperthermia activates the AMPK signaling pathway to regulate the phosphorylation of ATF2 in HaCaT cells.

OR-022

Hyperthermia inhibited the expression of EDN1/EDN2 affectting cell apoptosis and migration by reducing the binding of YAP1 and TEAD1

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Hyperthermia is an effective treatment for cancer and human papillomavirus (HPV) infections. Previous studies have found that keratinocytes in skin tissue undergo apoptosis after warm intervention, and the apoptosis rate in HPV-positive tissue is significantly higher than that in normal skin tissue.
Objective To investigate the effect of hyperthermia on HPV infected cervical cancer cells and condyloma acuminatum tissue.
Methods Caski cells were stimulated in a water bath at 44 ° C (as compared with 37 ° C) for 30 min. The CA tissue is split in two and subjected to the same treatment. Co-ip was used to detect protein binding. Rt-qpcr and Western-blotting were used to detect mRNA and protein expression. Cell cycle distribution, apoptosis and senescence were detected by flow cytometry. Transwell was used to detect cell migration.
Results High temperature activated YAP1 in Caski cells, which reduced its phosphorylation level and increased its nuclear entry. The total protein expression level of YAP1 in Caski cells and CA tissues did not change significantly under warm intervention, while the protein expression level of TEAD1 decreased, and the binding of the two decreased in Caski cells. High temperature promoted the transcription of YAP1 and TEAD1 target genes Cyr61, CTGF, BIRC5 in Caski cells, but inhibited the transcription of EDN1 and EDN2. High temperature and interference with EDN1/2 reduced the metastasis, proliferation and viability of Caski cells.
Conclusion Warming reduces the binding of YAP1 and TEAD1, and reduces the expression of their target gene EDN1/2, which affects the proliferation of Caski cells. These results suggest that YAP1 and TEAD1 may be a promising therapeutic target for the treatment of HPV infection.

OR-023

Microarray and bioinformatics investigation of circRNA - miRNA-mRNA expression profiles in severe acne lesions

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Objectives The purpose of this study was to look into the differences in circRNA expression in severe acne and its role in the ceRNA regulatory network.
Methods RNA microarray technology was used to determine differences in the expression of circRNAs, miRNAs, and mRNAs in six severe acne lesions vs six normal controls.
Biopsy specimens were obtained from the lesions of six patients with severe acne and six healthy volunteers who had plastic surgery.

The enriched circular RNAs were amplified and translated into fluorescent cRNA using a random priming technique (Arraystar Super RNA Labeling Kit; Arraystar). The tagged cRNAs were purified using the RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/g cRNA) were measured using the NanoDrop ND-1000. 1 μg of each labeled cRNA was fragmented by adding 5μl 10 × Blocking Agent and 1μl of 25 Fragmentation Buffer, then heating the mixture at 60 °C for 30 minutes, and then diluting the labeled cRNA with 25 μl 2 × Hybridization buffer. Before being affixed to the circRNA expression microarray slide, the gasket slide was filled with 50 μl of hybridization solution. The slides were incubated at 65°C for 17 hours in an Agilent Hybridization Oven. The hybridization arrays were washed, fixed, and scanned using an Agilent Scanner G2505C.

Researchers used Gene Ontology (GO) analysis to describe and classify differentially expressed circRNA-hosting genes. The functional role of DE mRNA is investigated using GO analysis, which considers biological processes (BPs), cell composition (CCs), and molecular function (MFs). Pathway analysis was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.kegg.jp/kegg) with the purpose of discovering relevant pathways connected to circRNA differential expression. When the $p < 0.05$ criterion was satisfied, enrichment analysis was proven to be significant.

miR-146a was chosen as the ceRNA hub for the prediction of circRNA-miRNA-mRNA network interactions. Circular RNAs with differential expression and a potential function for sponge miR-146a were predicted. The miRNA target interactions of circRNAs were estimated using the TargetScan and miRanda algorithms, with miRanda's max free energy values of< -10 and TargetScan's score percentiles of ≥50. Finally, three circRNAs, circ_102678, circ_102680, and circ_105040, were found to match the aforesaid screening criteria.

The regulatory relationship between miRNA and mRNA was estimated using miRanda and miRTarBase. The circRNA-miRNA-mRNA interaction network was created and diagrammed using Cytoscape software based on possible functional linkages between differential expressions of circRNAs, miRNAs, and mRNAs (version 3.4.0). Finally, to further validate the ceRNA network, we chose circ_102678 with the largest differential ploidy for in vitro cell function tests.

Primary human keratinocytes were cultured in the same way as previously described[23]. We transfected keratinocytes with circRNA_102678-specific siRNAs or NC using the jetPRIME transfection reagent (Polyplus) (designed and manufactured by GenePharma). For additional investigation, cells were collected 48 hours after transfection.

Total RNA was prepared and isolated using TRIzol (Invitrogen, Thermo Fisher Scientific, USA). The quality and quantity of RNA were determined using a nanodrop spectrophotometer (ND-1000, Nanodrop Technologies). The synthesis of cDNA was achieved via reverse transcription of 1 μg RNA. In the real-time PCR technique, denaturation at 95°C for 10 minutes was followed by 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds. All experiments were independently replicated at least three times for statistical analysis. The gene's expression was matched to the GAPDH reference gene. The samples were calculated using the $2^{-\Delta\Delta C}$ method.

Results CircRNA sequencing was used to identify the differentially expressed circRNAs between the acne cystic fluid tissue group and the control skin tissue group. Table S1 shows the general circRNA information in detail. The circRNA expression profile was statistically assessed using hierarchical clustering. We used scatter plots and volcano plots to show 1594 circRNAs with significantly altered expressions (fold change >2.0, P value 0.05) when comparing acne cystic fluid tissue with control skin tissues. 605 circRNAs were up-regulated and 989 circRNAs were down-regulated in acne cystic fluid tissue. Among the differentially expressed circRNAs, 240 distinct circRNAs were discovered that were not included in the circBase database. 156 of the 240 newly found circRNAs were upregulated in acne cystic fluid tissue, while 86 were downregulated.

miRNA sponges have been hypothesized as the most prevalent circRNA function to date, with the potential to override miRNA-mediated mRNA inhibition[25].We employed unsupervised hierarchical clustering to evaluate miRNA differential expression profiles in acne cystic fluid tissue and controls to define the role of the circRNA-miRNA network in AV. These differential miRNA profiles are exhibited for the acne cystic fluid tissue group and the control skin tissues group. A total of 582 miRNAs were significantly differentially expressed (fold-change >2.0, P-value 0.05) as

shown by the Volcano figure. When comparing acne cystic fluid tissue from patients with severe acne to the control group, 384 miRNAs were elevated and 198 miRNAs were downregulated.
High-throughput RNA sequencing was used to explore the differences in mRNA expression between cystic fluid skin tissues and control skin tissues. To find the differentially mRNAs, the filtering criteria fold change >2.0 and P-value 0.05 were utilized. The top 67 different mRNA expression profiles were shown in the heat maps analysis between the cystic fluid skin tissues group and the control normal skin tissues group. Volcano plots were used to demonstrate the differences in mRNA expression between the acne cystic fluid tissue group and the control skin tissues. A total of 18316 mRNAs were found, with 13075 showing substantial differences in expression. In the acne cystic fluid tissues of patients with severe acne, there were 4955 upregulated and 8120 downregulated mRNAs.
We used gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses to find host genes with variable circRNA expression. The function of differentially expressed genes (DEGs) in the acne cystic fluid tissues of patients with severe acne is then investigated further. DE mRNAs were significantly enriched in the top 10 GO terms, including regulation of cellular process (GO:0050794), regulation of biological process (GO:0050789), regulation of cellular metabolic process (GO:0031323), biological regulation (GO:0065007), regulation of primary metabolic process (GO:0080090), cellular macromolecule metabolic process (GO:0044260), regulation of nitrogen compound metabolic process (GO:0051171), and nucleobase-con. The top 10 enriched terms with statistical significance ($p < 0.05$) were identified among these differentially expressed circRNAs-derived genes, including Herpes simplex virus 1 infection, Fc gamma R-mediated phagocytosis, Endocytosis, Apoptosis, Pyrimidine metabolism, Notch signaling pathway, Fc epsilon RI signaling pathway, Sphingolipid signaling pathway, AGE-RAGE signaling pathway in diabetic complications.
CircRNA has been shown to have sponge adsorption sites for miRNA, which can impede miRNA regulation on target genes and hence indirectly influence gene expression[15]. Based on our high-throughput circRNA microarray assay results, we estimated the potential circRNAs-miRNAs-mRNAs network. The investigation of three highly down-regulated circRNAs in the acne cystic fluid tissues of individuals with severe acne revealed that 34 mRNAs shared 22 miRNAs in co-expression networks. Using the Cytoscape software, the anticipated target miRNAs and their host genes were displayed.
qRT-PCR results showed that the expression levels of circRNA_102678 in the si-circRNA_102678 group were markedly decreased compared with the si-NC group (P<0.001,). The following qRT-PCR demonstrated that miR-146a expression levels were significantly increased in the si-circRNA_102678 group compared with the si-NC group (p<0.01,). TRAF6 expression was reduced by circRNA_102678 knockdown (p<0.01). These results indicated that the expression of circRNA _102678 was negatively correlated with miR-146a expression in keratinocytes cells.
We used si-circRNA_102678 or si-NC to transfect keratinocytes. The expression levels of circRNA_102678 in the si-circRNA_102678 group were significantly lower than that in the si-NC group (P < 0.001,). The following data demonstrated that the si-circRNA_102678 group had significantly higher miR-146a expression levels than that of si-NC group (p < 0.01). circRNA_102678 knockdown reduced TRAF6 expression. These findings showed that circRNA_102678 expression in keratinocytes was inversely linked with miR-146a expression.
Discussion we discovered that 1594 circRNAs, 582 miRNAs, and 13075 mRNAs were differently expressed in acne cystic fluid skin tissues of six acne patients. Some functional circRNAs may have potential control mechanisms on AV, according to a high-throughput circRNA microarray test and bioinformatics analysis. Our findings shed light on the importance of circRNAs in the development of severe AV.

OR-024

The small-molecule ADP blocks regulatory factor X1 induced DNA demethylation and macrophage M1 polarization in autoimmune inflammation

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Object This study mainly explores the regulation and mechanism of regulation factor X1 (RFX1) on macrophage polarization in autoimmune diseases including inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE), and elucidates the mechanism of various diseases related to abnormal macrophage polarization.

Methods Western blot (WB) were used to detect the protein expression of RFX1 in CD14+ monocyte-derived macrophage (hMDMs) and mouse peritoneal macrophages (PMAs).

The RNA-seq was used to detect the differently expressed genes in RFX1-overexpressed or knockout PMAs. In addition, RT-qPCR and flow cytometry were used to detect the relative mRNA and protein expression of macrophage polarization related genes in hMDMs or PMAs infected with RFX1-overexpressed or control lentivirus. Besides, the expression of macrophage markers and proinflammatory cytokines in PMAs from Rfx1f/fLyz2-Cre (Cre) mice and Rfx1f/f (WT) mice was detected by RT-qPCR, Flow cytometry or ELISA.

The Cre mice and WT mice with colitis or SLE-like symptom was induced by 3% dextran sulfate sodium (DSS) or IMQ respectively. The damage of intestinal or kidney tissue was examined by H&E staining. The infiltrations of CD45+, neutrophils, macrophages, CD4+ and CD8+ cells in colon or kidney were detected by flow cytometry, as well as markers of M1 and M2 subtypes. The expression of macrophage polarization markers in colon or kidney were measured by immunohistochemistry.

Combination of RNA-seq and ChIP-seq was used to screen the target genes-APOBEC3A regulated by RFX1. Dual luciferase reporter and ChIP-qPCR were used to explore the direct control of RFX1 on APOBEC3A. Besides, we also detected the methylation of IL6, TNF and IL1B in hMDMs with APOBEC3A overexpression or monocytes/macrophages from healthy control or SLE patients.

Molecular docking technology was used to explore the potential small molecule inhibitors for DNA binding region of RFX1. The effect of selected small molecule inhibitor ADP on RFX1 activity was determined. The regulation of ADP on macrophage polarization in vitro or colitis were also detected.

Results The protein expression of RFX1 was increased significantly after M1 macrophage polarization.

The overexpression of RFX1 promoted the expression of M1-related marker genes in hMDMs or PMAs. Compared with control group, the expressions of M2-related marker genes were higher significantly in PMAs from Rfx1-deficient mice, while M1-related genes were significantly reduced.

In addition, deficiency of Rfx1 inhibited weight loss and atrophy and damage of colon, and protect the integrity of the intestinal structure in DSS-induced colitis. The infiltration of total immune cells (CD45+), neutrophils (CD11b+Gr1+), macrophages (CD11b+F4/80+), CD4+ and CD8+ cells in kidney were obviously reduced in CKO mice with colitis. The expression of M1-related markers genes was decreased, but the expression of M2-related genes was increased in intestinal macrophages from Cre mice. In Rfx1-deficient SLE-like mice, the immune cell infiltered in glomerulus and renal pathology scores were alleviated. Besides, the concentrations of anti-dsDNA and IgG in serum and the deposition of C3 and IgG in kidney tissue were markedly decrease in Cre mice. The infiltration of total immune cells (CD45+), neutrophils (CD11b+Gr1+), CD4+ and CD8+ cells were obviously reduced in CKO SLE-like mice. And the expression of CD86 in macrophage was reduced. The concentrations of cytokines IL-6 and TNF- and IL1 were also lessened significantly in serum of CKO mice induced with IMQ.

The analysis of RNA-seq combined with ChIP-seq showed that APOBEC3A may be the potential target genes for M1 macrophages polarization regulated by RFX1. Dual luciferase reporter and ChIP-qPCR assay indicated that APOBEC3A transcription was regulated by RFX1 directly. And

the demethylations of IL6 and TNF were increased by APOBEC3A or RFX1. Besides, the demethylations of IL6 and TNF were also increased in monocyte/macrophage from SLE patients.

ADP may be the inhibitor of RFX1 and inhibited M1-related gene expression. In addition, ADP administration alleviated intestinal injury in DSS-induced colitis.

Conclusion Both in vitro and in vivo experiments show that RFX1 promotes M1 polarization by APOBEC3A-regulated demethylation. And RFX1 may promoted the autoimmune inflammation, which was the potential target of unsolved inflammation in autoimmune diseases such as SLE or IBD. And ADP could serve as the potential inhibiter of RFX1, may be promising drug candidates for autoimmune inflammation.

OR-025

Isorhamnetin Protects Human Keratinocytes against H2O2 ultrastructural and functional alterations of mitochondria Damage

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Objective To explore the pre-protective effect of isorhamnetin (ISO) on mitochondrial structure and function (mitochondrial membrane potential, mitochondrial ATP content, mitochondrial DNA expression) in HaCat cells under oxidative stress.

Methods HaCat cells were taken as the research object and divided into control group, ISO group, H2O2 group and ISO+H2O2 group. Cells were pretreated with 60umol/L ISO and 600umol/LH2O2 were used to establish an oxidative stress model, and the cellular ROS was detected by flow cytometry. Mitochondrial membrane potential was observed by Confocal Fluorescence Microscopy; mitochondrial ultrastructure was observed by transmission electron microscope; mitochondrial ATP content was detected by ATP detection kit; mitochondrial DNA copy number was detected by RT-PCR.

Results Compared with the H2O2 group, the level of ROS in the cells pretreated with ISO decreased ($F=138.6$, $P<0.0001$), the mitochondrial membrane potential was restored ($F=9.728$, $P<0.0048$), and the ability of ATP generation was improved ($F=13.01$ $P<0.0019$), increased mitochondrial DNA copy number ($F=17.66$ $P<0.001$), improved the ultrastructure of damaged mitochondria, and observed isorhamnetin autophagosome phagocytosis of damaged mitochondria under electron microscope.

Conclusion ISO has a protective effect on the mitochondrial structure and function of normal human epidermal keratinocytes induced by H2O2. The results provide a certain experimental basis for the application of antioxidant ISO in the treatment of vitiligo.

OR-026

Exosomes containing miRNAs from keratinocytes under oxidative stress contributes to melanocyte loss in vitiligo

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Objective Vitiligo is a skin disease characterized by the destruction of epidermal melanocytes. Oxidative stress is closely related to the development of vitiligo. As the main constituent cells in the epidermis,keratinocytes regulates the viability and the function of melanocytes, but the underlying mechanism is still not clear. It has been proved that keratinocytes secrete quite a number of functional exosomes, therefore, we hypothesized that exosomes derived from keratinocytes under oxidative stress induce the destruction of melanocytes.

Methods We treated human keratinocytes (HaCaT) with H_2O_2 for 24h and exosomes from culture media were extracted by ultracentrifugation. Transmission electron microscope (TEM), scanning electron microscope (SEM), western blotting and Nanosight were used to identify the extracted exosomes. Then we incubated melanocytes (PIG1) with the above exosomes for 96h and the uptake of exosomes by melanocytes was accessed with Dil staining. The viability and apoptosis of melanocytes was tested by Cell Counting Kit and flow cytometry. Subsequently, small RNAs-seq was performed to screen the miRNAs enriched in exosomes derived from H_2O_2 treated- HaCaT (Exo.T) and the most significantly up- regulated miRNAs in Exo.T were selected for validation with qPCR. Then, we explored the effect of these miRANs on the viability and apoptosis of melanocytes and the underlying mechanism was explored. Finally, the role of Exo.T in the progression of vitiligo of mouse model was investigated.
Results Exosomes derived from HaCaT are small vesicles with double membranes, typically 50-100 nm in diameter. NTA showed a size distribution of these exosomes between 0 ~ 200 nm in diameter. Additionally, these harvested particles showed positive for exosomal marker proteins Alix, Hsp70, TSG101, CD9 and CD63. The above properties analysis identified these collected particles as exosomes.Interestingly, exosomal production was elevated alone with increased concentration of H_2O_2, suggesting that H_2O_2 promotes the secretion of exosomes from keratinocyte in a dose-dependent manner.Subsequently, we found that exosomes derived from keratinocytes were successfully intake by melanocytes after 24h incubation. Importantly, exosomes derived from H_2O_2 treated- HaCaT (Exo.T) significantly suppressed the viability and promoted the apoptosis of melanocytes after 96h incubation.
Then, the hierarchical clustering analysis of small RNAs-seq data showed the differential expression profile of miRNAs between exosomes derived from H_2O_2 untreated- HaCaT (Exo.C) or exosomes derived from H_2O_2 treated- HaCaT (Exo.T). The qRT-PCR results proved that the expressions of miRNAs miR-1246, miR-31-3p and so on were significantly increased in Exo.T as compared with those in Exo.C.Further, we found that miR-31-3p was the dominating miRNA responsible for the Exo.T induced-inhibition of viability and promotion of apoptosis in melanocytes by suppressing MITF-Bcl-2/CDK2 axis in both normal condition and oxidative stress.
Finally, Exo.T significantly enhanced the progression of vitiligo of mouse model via destruction of melanocytes.
Conclusion We propose that exosomes containing miRNAs from keratinocytes under oxidative stress contributes to melanocyte loss in vitiligo.

OR-027

Targeting Candida albicans Zinc Homeostasis with Hexyl-Aminolevulinate Ethosomes Impairs Biofilm Formation and Drug Resistance

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Background Candida albicans is prone to form highly drug-resistant biofilms that render conventional treatments ineffective, thereby resulting in chronic and recurrent infections. Our previous study showed that hexyl-aminolevulinate ethosomes (HAL-ES) act against C. albicans biofilm and weaken its drug resistance and pathogenicity; however, the mechanism involved remains unclear.
Purpose The purpose of this study is to systematically study the effects and mechanism of HAL-ES on *C. albicans* biofilm formation and drug resistance, so as to further provide experimental and theoretical basis for the clinical application of HAL-ES in the treatment of *C. albicans* biofilm-related infection.
Methods Crystal violet staining and germ tube formation assays were used to explore changes in early adhesion, mid-development and late maturation of *Candida albicans* biofilm formation. The sensitivity of the *C. albicans* cells to fluconazole was evaluated by a disk diffusion assay. A mouse

model of skin candidiasis to evaluate the therapeutic effect *in vivo*. RNA-seq and qPCR were used to analyze changes in gene expression in *C. albicans* biofilms. GO and KEGG enrichment analysis, as well as HUB gene screening by PPI network to analyze the mechanism and potential targets of HAL-ES against *C. albicans* biofilms.
Results We found that HAL-ES inhibited the early, developmental, and mature stages of biofilm formation compared with fluconazole, HAL, or ES. Notably, adhesion and hyphal formation were significant inhibited by post-drug effects even after brief exposure (2 h) to HAL-ES. Furthermore, HAL-ES mediated antifungal photodynamic therapy (aPDT) and its therapeutic effect *in vivo* also has been demonstrated in cutaneous candidiasis. RNA-sequencing and quantitative PCR showed that HAL-ES inhibited ribosome biogenesis by disrupting zinc homeostasis in *C. albicans*, thereby reducing the translation process in protein synthesis. Furthermore, HAL-ES downregulated the expression of multidrug-resistance genes and increased fluconazole susceptibility in *C. albicans*.
Conclusions This study reveals the multi-dimensional synergistic antibiofilm mechanism of HAL-ES and demonstrate its potential application in the treatment of candidiasis, especially drug-resistant and refractory infections caused by biofilms. We also provide a new target for the treatment of biofilm-related infections by zinc restriction in future studies.
Innovation Traditional antifungal drugs, such as azoles, polyenes, and echinocandins, mainly target cell wall and membrane metabolism. In bacteria, ~50% of antibiotics act on translation processes. Although protein synthesis pathways are not often targeted in antifungal drug studies, we demonstrated that HAL-ES effectively inhibited fungal protein synthesis by targeting zinc homeostasis, thus showing superior antibiofilm capacity and low potential for the development of resistance.

OR-028

Treatment of acne from lung and intestinal flora

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Acne is a multi-factor and multi-system disease. The pathogenesis of acne is complicated. Modern medicine believes that the pathogenesis of acne is mainly related to microbial infection, abnormal keratosis of hair follicles, sebaceous ducts and endocrine factors. In view of the pathogenesis of acne, anti-microbial infection, inhibition of lipid secretion, improvement of keratosis, regulation of sex hormones and other conventional treatment methods are commonly used for acne. However, most of them have side effects and high recurrence, so it is particularly important to seek other treatments for acne. Recent studies suggest that the gut microbiome may also be involved in the pathogenesis of acne, but the specific mechanism is still unknown. Huangdi neijing say "lung fur", "lung and large intestine list", by "the lung and fur", "lung and large intestine list" of the relationship between theory and acne, based on the theory of "gut - brain - skin axis" analysis of intestinal flora may be involved in the pathogenesis and treatment of acne, broaden the clinical diagnosis and treatment of acne, have very important theoretical and clinical value. Based on the fact that intestinal flora is involved in the pathophysiology of acne, probiotics can be given to patients with acne to improve their intestinal flora, so as to shorten the course of treatment and improve the efficacy. On the treatment of acne with Traditional Chinese medicine, the treatment and improvement of acne from the regulation of lung and intestinal flora, so as to broaden the dialectical thinking of conventional therapy. Modern pharmacological experiments and clinical studies on TCM and intestinal microecology are increasing day by day. TCM can also be a golden key to opening the door of intestinal microecology. This paper mainly expounds the relationship between intestinal microbiota and acne based on the theories of "lung and fur", "lung and large intestine surface and interior", "intestinal-brain-skin axis", as well as the related research on the treatment of acne by Traditional Chinese and western medicine.

OR-029

Comprehensive succinylome analyses reveal hyperthermia upregulates lysine succinylation of LACTB by downregulating Sirtuin 7 in human keratinocytes

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44℃ Local Hyperthermia could clear multiple HPV-infected skin lesions(warts) by targeting a single lesion which was considered a success of inducing antiviral immunity in human body. However, about 30% of the patients remain no response to the intervention. To find out novel molecular targets, in combination with local hyperthermia in anti-HPV immunity induction and improve the cure rates, we conducted the lysine succinylome in HaCaT cells (subjected to 44℃ and 37℃ water bath for 30min). A total of 119 proteins with 197 succinylated sites
were upregulated in the 44℃ treated HaCaT cells. GO annotation demonstrated that these proteins were mostly related to metabolic process. KEGG analysis showed that proteins associated with citrate cycle and fatty acid metabolism pathway were more likely to be succinylated. One succinylation site (K238) was upregulated in serine beta-lactamase- like protein
(LACTB), a regulator of mitochondrial lipid metabolism, which inhibited the cell survival. Sirtuin7 (SIRT7) acted as a desuccinylase interacting with LACTB. Carnitine palmitoyltransferase (CPT1A) and SIRT7 regulated the succinylated level of LACTB from opposite directions. In conclusion, inhibition of LACTB succinylation may serve as a novel target combined with local hyperthermia to improve the cure rates of HPV infected cutaneous and genital warts.

OR-030

New perspective of vitiligo pathogenesis: TRPML1 channel dysfunction

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Objectives To elucidate the role of TRPML1 in melanocyte autophagy under oxidative stress and its abnormity in the pathogenesis of vitiligo.
Methods The expression and ion channel function of TRPML1 was investigated by immunofluorescence and calcium imaging in both primary normal melanocytes (MC) and human vitiligo melanocyte cell line (PIG3V). After activating TRPML1 with MLSA1 (TRPML1 agonist), autophagy-related molecules were compared between PIG3V and MC by western blot. After interference with TRPML1 expression in PIG3V and MC, the autophagic vacuoles and mitochondrial structures were observed by electron microscopy with hydrogen peroxide (H2O2) treatment. After pretreatment with MLSA1, apoptosis-, autophagy-related molecules were compared between PIG3V and MC with H2O2 treatment by western blot and flow cytometry.
Results TRPML1 channel was expressed and functionally active in MC, and its activation promotes elevated expression of LC3-II and reduced apoptosis under oxidative stress. Interfering with its expression, there were fewer autophagic vacuoles and more disrupted mitochondrial structures in MC under oxidative stress. However, P62 expression was increased, and it was difficult to upregulate autophagy after TRPML1 activation in PIG3V.
Conclusion TRPML1 mediated lysosomal autophagy in melanocytes under oxidative stress, reducing cell apoptosis level. However, this mechanism is deficient in PIG3V, explaining the susceptibility of melanocytes to oxidative stress in vitiligo.

OR-031

Low Concentrations of Curcumin in Combination with Blue Light Irradiation Inhibits Cutibacterium Acnes Biofilm-simulated Inflammatory Response by Suppressing MAPK and NF-κB in Keratinocytes

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Background The skin disease acne vulgaris is characterized by chronic inflammation affecting up to 85–100% in adolescence, 10% of which is severe and 30% is moderate. Without proper control, this condition may lead to permanent disfigurement and even severe social and psychological problems. Cutibacterium acnes causes the acne inflammatory response, which is crucial to the pathological alterations of acne, by changing the composition of sebum and inducing a number of immunological responses. The inflammatory cascade of C. acnes may cause skin irritation and acne scar formation. Inflammation is a sophisticated defense mechanism against a wide range of hazardous stimuli, such as infections and irritants, but an overly aggressive inflammatory response causes damage to one's own tissues. Numerous studies have investigated the control of inflammatory signaling pathways in different disorders, focusing on potential molecular targets for anti-inflammatory therapy to counteract the harmful consequences of inflammation. The generation of pro-inflammatory cytokines is the most important biochemical mechanism by which the inflammatory response is mediated. There are several inflammatory factors associated with acne lesions, including tumor necrosis factor alpha (TNF-α), interleukin 8 (IL-8), and interleukin 6 (IL-6). Phosphorylation of molecules involved in the MAPK and NF-κB signaling cascade can promote the production of inflammatory factors. It is essential to target the NF-κB and MAPK pathways to inhibit the expression of inflammatory mediators in anti-inflammatory medications. The active ingredient in turmeric, curcumin, is a polyphenolic curcuminoid that has been proven to have antioxidant, anti-inflammatory, anti-apoptotic, and antibacterial activities. According to research on anti-inflammatory diseases, curcumin can reduce inflammation in a variety of conditions, including neuritis, fatty liver inflammation, arthritis, and nephritis, by inhibiting the NF-κB pathway and brain hypoxic injury, brain trauma, epilepsy, and acute vasculitis by inhibiting the MAPK pathway. Only our preliminary work on the ability of curcumin to prevent an inflammatory response by THP-1 cells to C. acnes stimulation via the NF-κB pathway exists in curcumin research in acne. We believe that curcumin is a good candidate for preventing C. acnes-induced inflammatory response that leads to acne. Treatment based on photosensitizers, light sources, and molecular oxygen has been used to treat a wide range of disorders, including cancer and non-cancerous diseases, with few side effects. Photodynamic treatment techniques are becoming widely accepted in the field of acne treatment. Several studies have shown that curcumin combined with phototherapy can reduce the activity of several pathogenic bacteria, including C. acnes, Streptococcus pyogenes, and Aeromonas gingivalis. Another study discovered that the combination of curcumin and blue light reduced inflammatory response by reducing Staphylococcus aureus infection in the epidermis of diabetic mice. However, there has been no research on the effectiveness of curcumin photodynamics in reducing acne inflammation.
Objective In this investigation, we sought to determine how curcumin, either alone or in conjunction with blue light, inhibits the production of inflammatory factors by C. acnes biofilm in human keratinocytes. Curcumin alone or curcumin-PDT inhibition via NF-κB and MAPKs may provide an attractive new approach to limit the generation of inflammatory molecules for treatment of acne.
Methods Following C. acnes biofilm stimulation, keratinocytes were treated with appropriate quantities of curcumin solution, and some of them were then treated with combined blue light irradiation. The amount of secreted protein was measured using an ELISA kit. The expression

levels of Toll-like receptor 2 (TLR2) and its downstream proteins were determined using western blotting.

Results C. acnes biofilms are prevalent in acne lesions; hence, we utilized C. acnes biofilms as a stimulus to cause inflammation. Following the building requirements, we created a successful in vitro model of C. acnes biofilm (shown in Fig.1). We chose the eighth day biofilm as the stimulus for the next investigation because the assay studies showed that C. acnes built the best biofilms on this day. According to the RT-qPCR and ELISA data, pro-inflammatory factors (TNF-α, IL-8, and IL-6) had considerably higher mRNA and protein levels than the control group. Additionally, TLR2 expression was upregulated in C. acnes co-culture, and this upregulation was more pronounced in the co-culture system using C. acnes biofilm. This showed that activation of the TLR2 receptor was directly related to the increase in inflammatory factors. We examined the expression of inflammatory factors after adding the selective antagonists of MyD88(ST2825), TLR2(C29), p38MAPK(SB203580), NF-κB(BAY11), and ERK1/2(U0126) to the aforementioned culture system to further investigate the activation of NF-κB and MAPK pathways after TLR2 activation. These results demonstrated that the aforementioned inhibitors had a considerable ability to reduce the expression profile of inflammatory markers(shown in Fig.2). These results imply that C.acnes biofilms can promote the expression of pro-inflammatory factors by enhancing TLR2 receptor expression, thus activating MAPK and NF-κB signaling pathways. In co-culture systems of keratinocytes and curcumin, curcumin concentrations < 20 μM did not affect the survival rate of keratinocytes. The survival rate of keratinocytes in the co-culture system of keratinocytes and curcumin PDT was not affected by curcumin concentrations lower than 10 μM. After co-culturing C. acnes biofilms with keratinocytes, we further investigated the effect of curcumin (5 μM) PDT on the survival rate of keratinocytes (shown in Fig.3). We discovered that below 5 μM, curcumin-PDT had no influence on the survival rate of keratinocytes. To examine how curcumin affects inflammatory mediators produced by C. acnes from biofilms, it was pre-incubated at a concentration of 20 μM in cell culture medium for 1 h. Curcumin (20 μM) significantly reduced IL-6, IL-8, and TNF-α mRNA and protein levels (shown in Fig.4). This demonstrates that curcumin dramatically reduced the inflammatory response caused by C. acnes biofilms. We investigated alterations in the TLR2/MAPK/NF-κB pathway following curcumin treatment of keratinocytes embedded in a C. acnes biofilm. The enhanced phosphorylation of p38 and ERK1/2 was reversed by curcumin, which also dramatically suppressed TLR2 expression. Additionally, we found that curcumin inhibited IκB phosphorylation and p65 nuclear translocation (shown in Fig.4). These findings implied that curcumin reduced the inflammatory response of C. acnes biofilm-induced keratinocytes via the MAPK and NF-κB pathways. Despite having little effect alone, curcumin (5μM) combined with blue light significantly reduced pro-inflammatory mRNA and protein levels in C.acnes biofilm-stimulated keratinocytes (shown in Fig.5). We investigated the effect of curcumin (5 μM)PDT on the keratinocyte TLR2/MAPK/NF-κB pathway generated by C. acnes biofilms. The elevated expression of TLR2 induced by C. acnes could not be considerably reduced by curcumin (5 μM) alone; when paired with blue light, the expression of TLR2 was restored to normal. Additionally, under curcumin (5 μM)PDT, the levels of p38 and ERK1/2 phosphorylation were restored to normal, as well as both p65 nuclear translocation of NF-κB and phosphorylation of IκB (shown in Fig.5).

Conclusion The appropriate concentration of curcumin or low-dose curcumin-PDT inhibits the inflammatory response induced by C. acnes biofilms in keratinocytes by blocking the TLR2, p38, ERK, and NF-κB p65 pathways. Based on these results, there is a strong theoretical basis for reducing acne inflammation by curcumin, either on its own or in conjunction with blue light.

OR-032

Dermal adipose tissue undergoes reversible dedifferentiation and differentiation during psoriasis pathogenesis

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Background/Objective Psoriasis, the most common chronic inflammatory skin disease in adults, is characterized by hyperplastic epidermis, elongated and increased vascularity in the dermis, and immune cell infiltration. Dermal white adipose layer (dWAT) has been recently recognized as a critical deep skin layer of defense, but whether and how dWAT plays a role in shaping the dermal immune response during psoriasis pathogenesis is unclear.
Methods The imiquimod-induced psoriasis-like mouse model was used to study how adipocyte lineage cells were regulated during the development of psoriatic inflammation. Histological, immunofluorescent, lipidomic analyses, adipocyte lineage tracing experiments and in vitro adipocyte differentiation assays were performed to trace and determine cellular and molecular changes to dWAT cells. Finally, human psoriatic skin samples were analyzed to validate our observations from mice.
Results Here by the imiquimod (IMQ)-induced psoriasis-like mouse model, we found that IMQ triggered a transient loss of dermal mature adipocytes during the acute inflammatory phase followed by a robust dermal adipogenesis response, including proliferation of adipocyte progenitor (AP) and new adipocyte formation, during the regression phase of skin inflammation. Using adipocyte lineage tracing mouse model, we showed that IMQ application promoted dedifferentiation of mature adipocytes into a population of highly pro-inflammatory and proliferative AP cells, which can re-differentiate into new adipocytes. During differentiation, mature adipocytes are subjected to lipolysis, a process by which adipocytes breakdown stored lipid. Lipidomics study revealed increased levels of the products of lipolysis in IMQ-treated mouse serum and immunohistological showed increased expression levels of lipolysis-associated lipase. Finally, immunohistological analyses of human skin samples validated our observations from mice in human psoriatic skin.
Conclusion Together our results have suggested that innate immune responses of dermal adipocytes and adipocyte progenitors may play an important role in activating dermal immune system during the development of psoriatic inflammation. Results from our study indicate that targeting lipolysis and/or adipogenesis maybe therapeutic for psoriasis.

OR-033

TRPA1 promotes melanosome phagocytosis in keratinocytes via PAR-2/ CYLD axis

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Background （1）Keratinocyte phagocytose melanosomes synthesized by melanocyte and transport them to the perinuclear region to form supranuclear caps (called ‘melanin caps’), which is an important physiological process that determines the amount of skin pigmentation and protects nuclear DNA from ultraviolet (UV) damage. Abnormal melanosome transfer can lead to skin pigmentary disorders. Hence, a thorough study of the cellular and molecular mechanisms involved in melanosome transfer is necessary to understand and treat pigmented skin diseases.

（2）TRPA1 is a calcium-permeant, non-selective, cation channel that has been implicated in the cellular sensing of physical and chemical stimuli such as UV radiation (UVR), heat shock, and oxidative stress. It has previously been shown to play a physiological role in sensory neurons, but recent studies suggest that the channel is also widely expressed in non-neuronal cells, the basal layer of the epidermis, dermis, and epithelium of hair follicles in cutaneous tissue. Previous studies have reported that TRPA1 was activated by physiological doses of UVR, resulting in early melanin synthesis. However, whether it participates in melanosome transfer remains unclear. Several mechanisms of melanosome transfer from melanocyte dendrites to keratinocytes have been suggested based on light and electron microscopy studies. These include phagocytosis, endocytosis, direct inoculation, and keratinocyte-melanocyte membrane fusion. However, no clear molecular mechanism has been established for melanosome transport.

（3）Protease-activated receptor-2 (PAR-2), a seven-pass transmembrane G-protein-coupled receptor, expressed by keratinocytes but not by melanocytes, regulates melanosome transfer via phagocytosis. PAR-2 activation increases the ability of keratinocytes to phagocytose melanosomes. After transfer to keratinocytes, melanosomes are moved to a position over the top of the nucleus, which is carried out with the assistance of microtubules. Microtubules have important functions, such as maintenance of cell morphology, subcellular transport, cellular signaling, cell migration, and formation of cell polarity. Microtubules are subject to various covalent modifications, one such modification is tubulin acetylation, which is associated with stable microtubules. In mammals, acetylation levels are mainly governed by the opposing actions of α-tubulin acetyltransferase 1 (ATAT1) and histone deacetylase 6 (HDAC6). Cylindromatosis (CYLD) is a tumor-suppressor gene that is mutated in a benign skin tumor syndrome called cylindromatosis. CYLD induces a delay in the G1/S transition of the cell cycle in both melanoma cells and keratinocytes by inhibiting the expression of cyclin-D1. Several lines of evidence suggest that CYLD regulates the levels of acetylated α-tubulin by acting as an endogenous inhibitor of HDAC6. The CYLD gene product is a deubiquitinating enzyme; TRPA1 is one of the substrates for the deubiquitinating activity of CYLD, and this deubiquitination has a net effect of increasing the cellular pool of TRPA1 proteins. Given the relevance of TRPA1 and CYLD and the critical role of PAR-2 in keratinocyte phagocytosis, we investigated the effect of TRPA1 on melanosome transport in keratinocytes by exploring the interaction between TRPA1 and PAR-2 or CYLD. This study aimed to investigate the roles and mechanism(s) of action of TRPA1 in keratinocytes.

Methods The correlation between TRPA1 expression levels and the ability of keratinocytes to phagocytize melanosomes was examined using melanin silver staining. TRPA1 depleted human epidermal keratinocytes and keratinocyte cell lines HaCaT were established using adenovirus-expressing shRNAs against TRPA1. The effects of TRPA1 on keratinocytes and HaCaT cells were determined using cell-based analyses, including light stimulation, calcium imaging, melanin phagocytosis, immunoblotting, and co-immunoprecipitation assays. The degree of epidermal pigmentation was determined in a guinea pig model.

Results TRPA1 mediated the phagocytic activity of keratinocytes. TRPA1 knockdown markedly suppressed melanosome transport to keratinocytes. Mechanistically, TRPA1 was required for PAR-2-induced melanosome phagocytosis in keratinocytes. Furthermore, TRPA1 activation indirectly stabilized microtubules by promoting the competitive binding of CYLD and acetylated α-tubulin. In addition, bortezomib (PS-341), a proteasome inhibitor, increased TRPA1 and CYLD expression and promoted phagocytic activity both in vitro and in vivo.

Conclusions Our findings firstly suggest that TRPA1 promotes melanosome transport in keratinocytes and reveal that TRPA1 is a regulator of PAR-2 activation and microtubule stability via the PAR-2/CYLD axis.

OR-034

Sporothrix globosa melanin regulates autophagy via the TLR2 signaling pathway in THP-1 macrophages

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Objective Melanin, an important virulence factor of pathogenic fungi, has been shown to suppress host immune responses in multiple ways. Our previous studies have demonstrated that the melanin derived from Sporothrix globosa not only resists phagocytosis and killing by macrophages, but also inhibits antigen presentation by downregulating the expression of major histocompatibility complex II in macrophages. Recently, elaborate mechanisms underlying the melanin-mediated inhibition of phagocytosis have been revealed. It was reported Aspergillus melanin selectively blocked nicotinamide adenine dinucleotide phosphate oxidase-dependent activation of LC3-associated phagocytosis (LAP), a noncanonical form of autophagy, suggesting that more research is needed to explore the relationship between melanin and autophagy. Autophagy is a vital cellular mechanism underlying the host's innate immunity against microbial infections. Despite previous studies showing that Candida albicans, C. neoformans and Aspergillus fumigatus can induce autophagy (or LAP), these studies indicated that the role of autophagy in manipulating immune responses varies greatly across different species of fungi. Therefore, we investigated the effect of melanin on autophagy in macrophages, which play a key role in controlling Sporothrix spp. infection, as well as the mechanism of melanin interaction with Toll-like receptor (TLR)-induced pathways.
Methods The wild-type strain of S. globosa (Mel+, FHJU12082703) and a melanin-deficient mutant (Mel-), which were identified based on phenotypic characteristics and the nucleotide sequence of the calmodulin gene, were co-cultured with THP-1 macrophages, in the presence or absence the autophagy modulators (chloroquine, wortmannin or rapamycin). Western blotting was performed to elevate the expression of the autophagy-related proteins (LC3A/B, Beclin1, and SQSTM1/p62). Transfecting with an adenovirus construct carrying LC3 tagged with double fluorescent protein and Laser scanning confocal microscopy were introduced to detect the the autophagic flux. The production of reactive oxygen species and multiple proinflammatory cytokines (interleukin-6, tumor necrosis factor-α, interleukin-1β, and interferon-γ) induced by Mel+ and Mel- in THP-1 macrophages were also assayed through commercial kit. To demonstrate the effect of Toll-like receptors on the functionality of autophagy in macrophagy challenged with S. globosa, the small interfering RNA targeted to TLR2 and TLR4 were used.
Results Obvious enhancements in the expression of Beclin-1 and LC3-II proteins, and a decrease in the levels of protein p62, occurred in THP-1 macrophages infected with S. globosa conidia in a time-dependent and concentration-dependent manner. The expression level of LC3 reached its peak at an MOI of 100, while those of Beclin1 and p62 peaked at an MOI of 10. The 24 h post-infection time point was when the highest expression levels of LC3-II and Beclin-1, and the lowest expression level of p62, were detected both in Mel+ and Mel- infected THP-1 cells. During the observation of the monitor autophagic flux, we found RFP-GFP-LC3 was successfully introduced into THP-1 macrophages challenged with both the Mel+ and Mel-. In addition to the accumulation of LC3, more red puncta were present in THP-1 macrophages infected with Mel+ conidia than in those infected with Mel- conidia or uninfected cells. These results suggested both Mel+ and Mel- S. globosa could induce autophagy in THP-1 macrophages. However, the expression of LC3-II and Beclin1 in THP-1 macrophages was enhanced in the absence of melanin, with the Mel- conidia group showing remarkably higher expression levels than the Mel+ conidia group. When THP-1 cells pretreated with chloroquine or wortmannin, both of them showed increasing expression of p62 protein and reducing expression of Beclin1 protein. Our results also showed that LC3-II accumulated under treatment with chloroquine or rapamycin, whereas LC3-II decreased under wortmannin treatment. Rapamycin treatment increased the protein level of Beclin1 and decreased the protein level of p62. S. globosa conidia (Mel+ or Mel-) were able to trigger a large increase in ROS production compared with that in the uninfected control group. In cells infected by both Mel+ and Mel- S. globosa conidia, pretreatment with chloroquine inhibited the enhancement of ROS

production, whereas pretreatment with rapamycin led to a trend toward even higher levels of ROS. Additionally, ROS levels were enhanced in the absence of melanin, with the Mel- conidia group showing remarkably higher expression levels compared with the Mel+ conidia group.We also evaluated the levels of four proinflammatory factors (TNF-α, IL-6, IL-1β and IFN-γ) in THP-1 macrophages preincubated with chloroquine or rapamycin, followed by infection with Mel+ or Mel- S. globosa conidia to activate autophagy. At 12 h post-infection, THP-1 macrophages secreted higher levels of TNF-α, IL-6, IL-1β and IFN-γ compared with untreated/uninfected control, with the Mel- conidia group showing significantly higher levels of all four factors compared with the Mel+ conidia group. By contrast, cells that were pretreated with chloroquine before infection exhibited trends towards decreasing levels of proinflammatory TNF-α, IL-6 and IFN-γ, and increasing levels of IL-1β, compared with untreated cells infected with Mel+ or Mel- S. globosa conidia. The results for these proinflammatory factors in cells pretreated with rapamycin before infection were completely opposite. The protein levels of both TLR2 and TLR4 in THP-1 macrophages increased after co-culture with Mel+ or Mel- S. globosa conidia. Furthermore, S. globosa-stimulated expression of LC3-II and Beclin1 was markedly attenuated, and p62 protein expression was elevated, in TLR2-siRNA cells subsequently infected with Mel+ conidia. By contrast, there were no significant differences in protein expression of autophagy markers between TLR4-siRNA and Ctrl-siRNA cells infected with Mel+ conidia. To further verify the above findings, confocal microscopy was performed to assess the numbers of autophagosomes to autolysosomes in S. globosa-infected THP-1 macrophages. Unsurprisingly, no significant differences in the numbers of autophagosomes to autolysosomes were observed in cells with TLR4-siRNA knockdown that were infected with Mel+ conidia, whereas there were significantly fewer red and yellow puncta in Mel+ S. globosa-infected cells with TLR2-siRNA knockdown compared with those with Ctrl-siRNA knockdown. These results demonstrated that TLR2, and not TLR4, mediated the activation of autophagy in S. globosa-infected THP-1 macrophages. Moreover, the transfection of THP-1 macrophages with TLR2-siRNA significantly attenuated the levels of ROS, TNF-α, IL-6, IFN-γ and IL-1β. Similarly, pretreatment of TLR2-siRNA knockdown cells with chloroquine or rapamycin led to downward and upward trends in expression levels, respectively, of these proinflammatory cytokines and ROS.
Conclusion This study is the first to elucidate the importance of autophagy in the immune response of macrophages against S. globosa, as well as the role of melanin as an S. globosa virulence factor involved in resisting the autophagy induced by S. globosa infection in vitro. This study also revealed that TLR2, as a cell surface receptor, may mediate S. globosa-induced autophagy in macrophages.
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OR-035

Study on the regulation and mechanism of Tianma Gouteng Yin on hair follicle cycle

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OBJECTIVE Hair plays an important role in maintaining temperature and protecting the scalp, excessive hair loss will seriously affect the mental health and quality of life of patients. At present, more and more researchers are devoted to screening the effective ingredients that can promote hair follicle growth from natural medicines and exploring their mechanism. Tianma gouteng yin (TGY) is a traditional Chinese preparation with the functions of calming the liver and quenching wind, clearing heat and activating blood circulation, while it has been found to promote hair growth in clinical. In this study, we further illustrate the molecular mechanism of hair follicle development induced by TGY.

METHOD In this experiment, a dual-dimensional network pharmacology technique was combined with in vitro and in vivo experiments to explore the potential mechanism of TGY promote hair growth. The active compounds of TGY were determined by synergistic analysis of database and UHPLC-MS/MS; Predicted the targets of TGY promoted hair growth based on Network pharmacology and Molecular docking; Constructed a model of hair follicle regeneration in female C57BL/6 mice, then observed the stimulatory effect of TGY on hair follicle regeneration and conducted histological analysis on it; The expression of Wnt/β-catenin signaling pathway related proteins in Dermal papilla cells (DPCs) and Vibrissa follicle were observed by immunofluorescence; The expression levels of Wnt/β-catenin signaling pathway-related proteins were detected by Western Blot.
RESULT Dual-dimensional Network pharmacology screened out 39 main active components of TGY, and there were 309 intersections between their corresponding 573 targets and 3938 targets related to hair growth. Gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis revealed that the treatment of hair loss using TGY was related to the Wnt/β-catenin signaling pathways, while Molecular docking results showed that quercetin, luteolin, fisetin, wogonin and tetrahydroalstonine which main active ingredients of TGY had strong binding ability to β-catenin and GSK-3β. Animal experiments shown that TGY induced hair follicle regeneration in mice in a dose-dependent manner and reduced the transition time from telogen to anagen of hair follicles at the same time. Immunofluorescence staining showed that TGY promoted the expression of β-catenin, p-GSK-3β and Lef2, inhibited the expression of GSK-3β in DPCs and rat vibrissa follicle. Finally, Western Blot results showed that TGY up-regulated the expressions of β-catenin and p-GSK-3β, down-regulated GSK-3β and p-β-catenin.
CONCLUSION TGY induced hair follicle regeneration by activating the Wnt/β-catenin signaling pathway.

OR-036

The implication of Macrophage Migration Inhibitory Factor (MIF) in vitiligo pathogenesis

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Background Vitiligo is a clinically common disfiguring skin disease, which has serious impact on the physical and mental health of patients. Vitiligo is mainly characterized by milky non-scaly depigmentation due to epidermal melanocytes destruction. In recent years, a large number of domestic and foreign studies have shown that despite various factors such as gene predisposition, oxidative stress, chemical agents exposure and autoimmune are involved in the pathogenesis of vitiligo, autoimmunity is the main culprit of the massive destruction of epidermal melanocytes in patients, of which the aberrant activation of $CD8^+$ T cells are the final and fatal step. However, the molecular mechanism of the aberrant activation of $CD8^+$ T cells is still unclear.
Macrophage migration inhibitory factor (MIF) has long been appreciated as a cytokine-, endocrine-, enzyme-, and chaperon-like pleiotropic molecule. Recent studies have shown that MIF widely regulates innate and adaptive immunity, and is intertwined in the activation of NLRP3 inflammasome in macrophages and dendritic cells as well as the activation and proliferation of T cells. Additionally, MIF has been validated to participate in the pathogenesis of various autoimmune diseases, such as psoriasis, systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis. Elevated MIF expression levels can also be detected in mouse models for autoimmune diseases like rheumatoid arthritis, type I diabetes, and Guillain-Barré syndrome. However, whether MIF is implicated in vitiligo pathogenesis warrants further clarification.
Based on the results, we propose the following hypothesis: MIF is excessively expressed in vitiligo patients and vitiligo mice. MIF-specific inhibitor intervention might protect epidermal melanocytes from destruction by suppressing the activation and proliferation of $CD8^+$T cells, thereby ameliorating the disease progression in vitiligo mice.

Objectives 1. Using clinical samples of vitiligo patients and vitiligo mouse model to detect the expression of MIF and analyze the relationship between MIF levels and disease progression and severity, and reveal the source of the redundant MIF expression;
2. MIF-specific inhibitor was used to treat vitiligo mice to clarify the role of MIF in vitiligo progression;
3. Using samples from vitiligo mice and patients, we combined the in vitro and in vivo experiments to clarify the regulatory effect of MIF on the phenotype and function of vitiligo $CD8^+$T cells and elucidate the underlying mechanism.
Methods 1. We determined the expression of MIF in the peripheral blood of vitiligo patients using ELISA, and analyzed the correlation between MIF levels in the peripheral blood of vitiligo patients and VASI scores. The expression of MIF in the lesional skin of vitiligo patients was also detected by immunofluorescence assay and Western blotting. The expression of MIF in PBMC of vitiligo patients was analyzed by flow cytometry to identify the source of the circulating excessive MIF.
2. Based on a mouse model for human vitiligo, MIF expression levels in peripheral blood of mice at indicated time points were detected by ELISA, and their correlation with depigmented area in vitiligo mouse tails was analyzed.
3.We treated vitiligo mice with ISO-1, a MIF-specific inhibitor, to observe the effect of ISO-1 intervention on disease progression in vitiligo mice at a gross level.
4. To clarify the effect of ISO-1 intervention on the disease progression of vitiligo mice, ear tissue immunofluorescence, whole-mount immunofluorescence staining and laser confocal microscopy scanning, flow cytometry analysis of skin and other methods were used to detect the epidermal melanocyte retention and skin $CD8^+$T cell infiltration in each group of mice. The expression levels of IFN-γ, MIF and IL-1β in the peripheral blood of mice in each group were detected by ELISA.
5. To clarify the effect of ISO-1 intervention on $CD8^+$T cells in vitiligo mice, transcriptional changes of $CD8^+$T cells in the inguinal lymph nodes of mice in each group were determined by RNA-sequencing.
6. Flow cytometry was used to detect the effect of MIF inhibition by ISO-1 on the frequency of $NKG2D^+CD8^+$T cells and $CD49a^+CD8^+$T cells in inguinal lymph node cells and peripheral blood of vitiligo mice, as well as the activation and proliferation capability of $CD8^+$T cells in each group of mice under in vitro stimulation conditions.
7. Flow cytometry was used to detect the effects of MIF inhibition with different concentrations of ISO-1 on the activation and proliferation of $CD8^+$ T cells from vitiligo mice and vitiligo patients. Besides, dopachrome tautomerase assays were conducted to examine the MIF activity in ISO-1-treated $CD8^+$T cells.
Results 1. MIF expression levels in peripheral blood and lesional skin of vitiligo patients were significantly higher than those of normal controls, and MIF levels in peripheral blood were positively correlated to vitiligo severity with significance. Monocytes and B cells might be the main source of the excessive MIF in peripheral blood of vitiligo patients.
2. In comparison with healthy controls, the expression levels of MIF in the peripheral blood of vitiligo mice was significantly elevated, and there was a significant positive correlation between MIF levels and vitiligo severity.
3. The disease progression of vitiligo mice treated with MIF inhibitor, ISO-1, was significantly slower than that of vitiligo mice treated with vector, which is mainly characterized by less white coat and tail skin depigmentation after the 7th week, most significantly at the 12th week.
4. Compared with normal controls, the epidermal melanocytes in the ears and tails of mice in the vitiligo group were significantly reduced, and the infiltration of $CD8^+$T cells in the tail epidermis and dermis was markedly increased. Compared with mice in the vitiligo group, the epidermal melanocyte retention in the ears and tails of mice in the ISO-1 group was significantly increased, the infiltration of $CD8^+$T cells in the tail epidermis and dermis was prominently decreased, and the IFN-γ and MIF expression levels in the peripheral blood were notably downregulated with those of IL-1β remaining largely unchanged.
5. A significantly up-regulated expression of *Ifng* (encoding IFN-γ), *Gzmb* (encoding Granzyme B), *Itga1* (encoding CD49a) and other genes in $CD8^+$T cells of inguinal lymph nodes from vitiligo mice was observed. And the expression of *Ifng*, *Gzmb*, *Itga1*, *Klrk1* (encoding NKG2D) and other genes in mouse $CD8^+$T cells of inguinal lymph nodes was significantly down-regulated in ISO-1 group compared with that in the vitiligo group.

6. Compared with normal controls, the expression of IFN-γ and Granzyme B and proliferation of mice $CD8^+$T cells of inguinal lymph nodes from the vitiligo group was considerably enhanced. The frequencies of $NKG2D^+CD8^+$T cells and $CD49a^+CD8^+$T cells in inguinal lymph nodes and peripheral blood were also increased with significance. In comparison with mice in the vitiligo group, the expression of IFN-γ and Granzyme B and proliferation of mice $CD8^+$T cells of inguinal lymph nodes from the ISO-1 group was markedly suppressed. $CD49a^+CD8^+$T cells and $NKG2D^+CD8^+$T cells frequencies in inguinal lymph nodes and peripheral blood were prominently downregulated.
7. MIF inhibitor ISO-1 could suppress the generation of IFN-γ and Gzmb in cell activation and proliferation of $CD8^+$T cells from vitiligo mice and vitiligo patients in a concentration-dependent manner. Among them, the 1600 μM concentration had the most significant effect on both cell activation and proliferation. And the activity of MIF in $CD8^+$T cells manifested significant diminution after ISO-1 treatment.
Conclusion The present study clarified that the expression levels of MIF in both lesional skin and peripheral blood of vitiligo patients were markedly increased. For the first time, we proposed that the excessively high levels of circulating MIF of vitiligo patients might be derived from monocytes and B cells. Furthermore, it was clarified for the first time that MIF is implicated in the development of vitiligo in mice, and MIF-specific inhibitor ISO-1 could not only reduce $CD8^+$T cells infiltration and promote the epidermal melanocyte retention in the skin of mice, but also downregulate the levels of IFN-γ and MIF in the peripheral blood, thereby delaying the disease progression in vitiligo mice. Additionally, ISO-1 treatment was also found to inhibit $CD8^+$T cell activation and proliferation capabilities and downregulate the frequencies of $NKG2D^+CD8^+$T cells and $CD49a^+CD8^+$T cells in inguinal lymph nodes and peripheral blood of vitiligo mice. Finally, we found that ISO-1 could directly act on $CD8^+$T cells from vitiligo mice and vitiligo patients to hamper their activation and proliferation. In this study, we systematically elucidated the underlying pathomechanism of MIF in the development of vitiligo, which enriched and improved the biological understanding of MIF in the pathogenesis of autoimmune diseases. MIF is promising as an important molecule in regulating the crux of vitiligo pathogenesis, which might play its role in the prevention and treatment of vitiligo in the future.

OR-037

Effective of a novel technique for sensitive skin treatment with optimal pulse technology: A clinical study

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Background and Aims Photomodulation is a non-photothermal effect that mobilizes energy and regulates cell activity at the mitochondrial level, and has been used to treat sensitive skin (SS) in recent years. Based on the photomodulated effect of optimal pulse technology (OPT), we developed a novel treatment mode (advanced OPT with low energy, three
pulses, long pulse width, AOPT-LTL) for the treatment of facial SS and evaluated its effectiveness.
Methods A total of thirty Chinese women with SS were included in this study. Patients were received different times of AOPT-LTL treatment with an interval of 2 to 4 weeks depending on the severity. Clinical improvement was evaluated by comparing baseline and post-treatment photographs. In addition, the skin objective signs and subjective symptoms, as well as adverse events and patient satisfaction were also analyzed and tabulated.
Results All included patients completed the treatment and follow-up period. After one course of treatment, 76.7 % of patients had a Symptom Score Reducing Index (SSRI) >20 %, suggesting treatment effectiveness(that the treatment is effective). Within two courses of treatment, all patients had SSRI >20 %, demonstrating significant improvement in skin sensitivity. The analysis of clinical photographs showed that facial dryness, desquamation, flushing, and skin color significantly improved, capillary density decreased, the dilated capillaries were retracted, and some blood

vessels were broken. During the treatment period, no obvious adverse reactions occurred in any patients, and the patients' satisfaction was high.
Conclusions This novel technique of AOPT-LTL might be an effective and safe modality for the treatment of SS.

OR-038

AhR promotes melanoma progression by regulating MerTK-mediated efferocytosis of macrophages

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Objective Melanoma is a highly malignant skin tumor, easy to early metastasize, and the 5-year survival rate of advanced patients is less than 5%. In recent years, the advent of immune checkpoint inhibitors (ICBs) has brought hope to melanoma patients, but ICBs still have problems of low treatment response rate and high incidence of primary and secondary drug resistance. Studies have shown that tumor microenvironment (TME) plays an important role in tumor immune escape and the occurrence of immunotherapy resistance. Macrophages are the largest number of immune cells in TME, accounting for about 50%. Macrophages in tumor microenvironment engulf apoptotic tumor cells through efferocytosis, thus promoting the release of anti-inflammatory cytokines and the polarization towards M2 phenotype. Efferocytosis is mediated by specific apoptotic recognition receptor MerTK and its phosphorylation, through which macrophages phagocytic tumor cells and then become M2 type polarization with immune tolerance and tumor-promoting effect. Tumor-associated macrophages (TAM) are mainly M2 type, which is closely related to the progression and poor prognosis of various tumors. By inhibiting the polarization of tumor infiltrating macrophages to M2-type TAM, or by reprogramming M2-type TAM to repolarize them into M1-type macrophages with anti-tumor activity, the inhibitory tumor microenvironment can be improved and the efficacy of anti-tumor immunotherapy can be improved. Our previous study showed that aromatic hydrocarbon receptor (AhR) and phagocytic receptor MerTK were highly expressed in macrophages in various immune cell subsets of tumor infiltration in melanoma patients, and activation of AhR could promote the polarization of tumor infiltrating macrophages to M2-type TAM. However, the mechanism of how AhR affects TAM polarization and tumor progression by regulating MerTK receptor expression in melanoma tumor microenvironment is still unclear.
Methods 1) scRNA-seq and immunofluorescence were used to detect and analyze the expression of AhR-MerTK in local macrophages of melanoma patients with PD-1 monoclonal antibody therapy resistance and non-resistance.
2) RNAseq and Chip-seq explored the regulatory mechanism of AhR on MerTK. Flow cytometry, WesternBlot, confocal and RT-PCR were used to detect the effects of AhR on macrophage phagocytic function, p-MerTK expression, M1 and M2 polarization.
3) Blocking the effects of AhR-MerTK and combined PD-L1 monoclonal antibody on tumor growth in melanoma mice;
4) The nano-drug delivery system loaded AhR inhibitor CH223191 targeting tumor-associated macrophages to explore its effect on the growth of melanoma mice.
Results 1) AhR and MerTK were highly expressed in tumor infiltrating macrophages of PD-1 monoclonal antibody therapy resistant patients compared with those of PD-1 non-resistant patients;
2) RNAseq suggested that AhR was closely related to macrophage MerTK and its phosphorylation mediated phagocytosis. Chip-seq and Chip-PCR et al. further verified that AhR promoted MerTK and its phosphorylation expression by promoting downstream protein kinase transcription, and macrophages enhanced efferocytosis and polarization toward M2.
3) AhRi combined with MerTKi can slow down the tumor growth of melanoma mice and improve the effect of PD-L1 monoclonal antibody therapy;

4) The system loaded with AhR inhibitor CH223191 can specifically target TAM, promote the polarization of M2-type TAM to M1-type, and then inhibit the tumor growth of melanoma bearing mice.

Conclusion Our study found that AhR in tumor macrophages could promote the downstream protein kinase ligand gene transcription, thereby promoting protein tyrosine receptor MerTK phosphorylation and activation, resulting in enhanced efferocytosis and polarization towards M2-TAM. Our study reveals the regulatory mechanism of AhR on MerTK-mediated efferocytosis for the first time, which enriches the theory of macrophage polarization and provides new ideas for immunotherapy of melanoma.

OR-039

Defining the Cell Type-specific Immune Response in Allergic Contact Dermatitis by Single-cell Transcriptomics

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Background/Objective Allergic contact dermatitis (ACD), one of the most prevalent occupational skin diseases in humans, is characterized by allergic delayed-type hypersensitivity to environmental, occupational and nutritional allergens. It has been extensively studied how contact allergens promote inflammation by activating the innate immune system and how innate immune cells cooperate with T cells and keratinocytes to initiate and drive an inflammatory response, but how ACD inflammation affecting immune cells and skin cells in the dermis, the primary site of immune cell residence in the skin, remains less explored . Here we aimed to explore the cell type specific immune responses in ACD and to determine how dermal fibroblasts are involved in shaping these immune responses.

Methods To model ACD skin in mouse, 8-week-old male ICR mice were sensitized with topical application of 1% dinitrofluorobenzene (DNFB) on the back skin, and 0.2% DNFB was topically applied to ear skin to elicitate ACD on the 6th day after sensitization. Ear skin samples were harvested 24 or 60 hours after excitation for analyses. Single cell RNA-seq (ScRNAseq) was performed by the droplet-based chromium 10x Genomics platform, and sequencing was performed by Illumina Novaseq6000 sequencer. The gene expression matrices of cells were obtained by Seurat 3.0 program of software R4.1.1, and two-dimensional t-SNE plots were used for visual display. The gene expression matrix and cell clustering information were introduced into Cellchat 1.1.3 program of software R4.1.1 to analyze the intercellular communication. Finally, qRT-PCR, flow cytometry, ELISA, histological and immunofluorescent analyses were performed to validate key observations from scRNA-seq.

Results ScRNAseq analysis of mouse ear skin tissues from control and ACD model grouped a total of 27,549 cells into 30 clusters by SNE plots. These cell clusters included immune cells such as neutrophils, macrophages, mast cells, basophils, and T cells, which were further reclustered into CD4+, CD8+, Treg, delta gamma T and natural immune lymphocytes (NK/ILC1 and ILC2). A variety of host non-immune cells were also identified, including keratinocytes, dermal fibroblasts, pericytes, vascular smooth muscle cells, muscle cells and endothelial cells, etc. Dermal fibroblasts (dFBs) were further reclustered into papillary dFB, reticular dFB, follicle-associated dFB, adipocyte progenitors and preadipocytes. Among all cell types, we found that the percentage of CD4+ or CD8+ T cells, basophils, mast cells and macrophages increased in ACD compared to control skin samples. By cell chat analysis, we found that dermal fibroblasts resided at a central location within the cellular interaction network that shaped immune cell activation in ACD. We further validated the increment, activation, inflammatory factor secretion levels, and tissue localization of most subpopulations in ACD skin by flow cytometry, qRT-PCR and immunofluorescence staining.

Conclusions Together, we have provided single cell insights into the immuno-cellular network that shapes the cell type specific inflammatory responses in ACD. Our results have suggested that

dermal fibroblasts are important mediators of the induction and amplification of the immune response in ACD, and this raises the possibility to alleviate ACD by targeting dFBs.

OR-040

MiR-210 negatively regulates dectin-1-induced inflammatory responses

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Objectives Our previous study identify three differentially expressed miRNAs in THP-1 cells after dectin-1 triggered by insoluble β-glucan from the cell wall of C. albicans (CaIG), including miR-210，miR-146a and miR-30. The roles of miR-210 in the innate immune of C. albicans infection remain unclear. We aim to study the regulation mechanism of miR-210 in dectin-1-ligand (CaIG)-induced inflammation in THP-1 cells.
Methods We transfect THP-1 cells with miR-210 mimic or inhibitor to study the regulation mechanism of miR-210 in dectin-1-ligand (CaIG)-induced inflammatory response.
Results We found CaIG-induced the expression of miR-210 in THP-1 cells in a time-dependent manner. MiR-210 was strongly upregulated and the response peaked at 24 hours. MiR-210 markedly downregulate CaIG-induced expression and production of TNF-α and IL-6 in THP-1 cells. MiR-210 inhibit CaIG-induced activity of Syk, IκBα and translocation of NF-κB p65 in THP-1 cells.
Conclusions Dectin-1-ligand (CaIG)-induced miR-210 acts as a negative feedback regulator of inflammation through down-regulation of the Syk-NF-κB pathway in THP-1 cells following dectin-1 stimulation.

OR-041

Effects of hyperthermia and hydrogen peroxide on NLRP3 inflammasome of keratinocytes

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Objective At present, hyperthermia is widely used and has achieved success in the treatment of many types of tumors. In addition, hyperthermia is also used in dermatology to treat deep fungal diseases, bacterial and viral skin infections. For example, hyperthermia has made important progress in the treatment of human papillomavirus (HPV) infection. HPV can cause various forms of skin and mucosal damage in various parts of the body. Its harm involves affecting beauty, lasting severe pain, affecting activities and causing benign and malignant tumor. Therefore, the clinical treatment of HPV infection is particularly important. Hyperthermia of viral warts has many advantages, including non-invasive, low recurrence rate, easy tolerance and so on. However, it also has disadvantages such as long treatment cycle and ineffective to some patients. Therefore, the research on the mechanism of hyperthermia in the treatment of viral warts is of great significance to further find improved treatment methods and improve the treatment effect. Studies have found that hyperthermia can induce local inflammation and promote keratinocytes to release a variety of inflammatory factors, including IL-1β Et al. The level of IL-1β may be related to the reactivity of hyperthermia. Studies have now shown that hyperthermia can activate NLRP3 inflammasomes in tumor cells, leading to death and release IL-1β. However, whether hyperthermia has the same effect in keratinocytes has not been reported. As a chemical, hydrogen peroxide is widely used in medical treatment. It is also used as an adjuvant treatment, which has made an important contribution to the treatment of a variety of diseases. It has been found that hydrogen

peroxide can activate temperature sensitive ion channel TRPM2, induce NLRP3 inflammasome activation and promote IL-1β release and cell death. We speculate that the stimulation of keratinocytes with hydrogen peroxide combined with hyperthermia may promote the further activation of inflammasomes and may improve the effect of hyperthermia. Therefore, this study intends to explore the effects of hyperthermia, hydrogen peroxide and inflammasomes on keratinocytes, the therapeutic mechanism of hyperthermia on warts, which provides a new idea for improving the therapeutic scheme of hyperthermia for HPV infection and improving the therapeutic effect.

Methods In this study, immortalized human keratinocytes (HaCaT) were selected as the experimental object. Firstly, CCK8 method was used to detect the cell activity of different concentrations of hydrogen peroxide on normal keratinocytes and keratinocytes treat with 44℃ hyperthermia. The appropriate concentration was selected to construct the injury model. The cytokine IL-1βand IL-18 secreted by normal keratinocytes, keratinocytes treat by 44℃hyperthermia, keratinocytes treat with low concentration hydrogen peroxide and keratinocytes co-treated with low concentration hydrogen peroxide and 44℃hyperthermia were measured by ELISA. And the proportions of necrosis, pyroptosis and apoptosis were measured by flow cytometry. In order to study whether hyperthermia and hydrogen peroxide can induce the activation of NLRP3 inflammasome and pyroptosis. Firstly, the mRNA related to pyroptosis and NLRP3 activation were detected by PCR. Then, Western blot, immunofluorescence and caspase-1 activity detection methods were used to determine the expression of pyroptosis related proteins and pyroptosis protein activity at the protein level. The changes of reactive oxygen species and intracellular calcium were further measured by flow cytometry.

Results 1. CCK8 results showed that the cell viability of HaCaT cells decreased after exposure to 44 ℃ for 30 minutes, and the effect of hyperthermia on HaCaT cell viability could be strengthened after H2O2 treatment. The cell viability decreased more significantly with the increase of H2O2 concentration, and the promoting effect hyperthermia was the most obvious on the viability of HaCaT cells when combined with 75μM H2O2 concentration. ELISA showed that 24 hours after hyperthermia and/or hydrogen peroxide treatment of keratinocytes, hyperthermia could induce cells to secrete pyroptosis protein IL-18, and hydrogen peroxide treatment promoted its expression. And the expression of IL-1βwas increased after cells were treated with hyperthermia or low concentration of hydrogen peroxide for 12h and 24h. Hydrogen peroxide also promoted IL-1β expression induced by hyperthermia; The results of flow cytometry showed that 44 ℃ hyperthermia could increase the apoptosis and pyroptosis rate of HaCaT cells, and hydrogen peroxide could promote that. The proportion of HaCaT cells treated with hydrogen peroxide alone did not show the same. 2. PCR results showed that the mRNA levels of ASC, IL-1β, TRPM2 and NLRP3 in hyperthermia group keratinocytes, while the mRNA level of Caspase-1 did not change significantly. And there was no significant change trend in the above mRNA in 75μM hydrogen peroxide group, but the above mRNA levels in hydrogen peroxide combined with hyperthermia group showed an increasing trend, which was more significant than that in hyperthermia group. Western-blot showed that the expression of NLRP3 protein increased significantly in the hyperthermia group, while the expression of cleaved caspase-1 P20 had no significant change. And 75μM hydrogen peroxide group also showed the same trend, but the expression of protein in hydrogen peroxide combined with hyperthermia treatment group showed a significant increase trend, which was more obvious than that in the groups treat with them separately. Immunofluorescence results showed that weak fluorescence could be observed in the cells of the control group. The 44℃ hyperthermia group and low concentration hydrogen peroxide group showed extensive NLRP3 fluorescence, and the red fluorescence was evenly expressed in the cytoplasm, and the hyperthermia was more obvious than the low concentration hydrogen peroxide group. In the 44 ℃ hyperthermia and low concentration hydrogen peroxide combined treatment group, NLRP3 fluorescence was observed in the cells, which was more remarkable than the groups treat with them separately. The detection results of Caspase-1 activity showed that both hydrogen peroxide group and hyperthermia could increase the activity of caspase-1 in cells, which was more obvious in hyperthermia group, and the activity of caspase-1 in hyperthermia and low concentration hydrogen peroxide combined treatment group was more significant. The results of flow cytometry showed that there was no significant change of

intracellular reactive oxygen species in hydrogen peroxide group, and the concentration of calcium ion increased slightly, but there was no statistical significance. The concentration of reactive oxygen species and calcium ion in 44 ℃ hyperthermia group also had no statistical significance. The content of reactive oxygen species and the concentration of calcium ions in hyperthermia and low concentration hydrogen peroxide combined treatment group increased significantly.
Conclusion 1. hyperthermia can make keratinocytes secrete IL-1βAnd IL-18 and lead to pyroptosis, while low concentration of hydrogen peroxide can promote this effect. 2. Hyperthermia can induce keratinocyte pyroptosis by activating NLRP3 inflammasome, and low concentration of hydrogen peroxide can significantly enhance the activation of NLRP3 and the hyperthermia effect in this process. The activation of NLRP3 inflammasome causing by hyperthermia may related to the increase of intracellular reactive oxygen species and calcium ion caused by TRPM2 activation.

OR-042

Study of innate immune response in generalized pustular psoriasis by single-cell RNA sequencing

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Objective
1. To characterize the PBMCs alterations of 5 patients with acute GPP at the single-cell transcriptional level.
2. To detect the mutation of IL36RN gene in GPP patients and explore the effect of IL36RN mutation on innate immune cell population.
3. To investigate the biological functions of various innate immune cell populations in GPP.
4. To explore the possible communication between innate immune cell populations.
Methods
Peripheral blood and clinical data of 5 Chinese patients with acute GPP were collected. DNA was extracted for mutation test of IL36RN gene and PBMCs were isolated for scRNA-seq. Data of 5 healthy individuals were obtained from Gene Expression Omnibus database for comparison. After clustering, cell-type annotation and mapping single-cell atlas of GPP patients, we compared the cellular composition between GPP and healthy controls (HC) as well as IL36RNmut group and IL36RNwt group. The mechanisms of innate immune involving in GPP were investigated by following bioinformatics methods including enrichment analysis, pseudo-time analysis and cellular communication network analysis.
Results
1. The homozygous mutation c.115+6T>C in IL36RN was identified in 3 out of 5 GPP patients. Meanwhile, GPP01 carried another heterozygous variation of C.140A>G. None of variations in IL36RN was detected in the other 2 patients.
2. After combined analysis of scRNA-seq data of PBMCs from 5 GPP patients and HC, we constructed an atlas of 29 cell groups, including T cells, NK cells, B cells, plasma cells, CD14+ monocytes, CD16+ monocytes, megakaryocytes, dendritic cells, and 6 groups of low-density granulocytes (LDGs) were identified based on marker gene expression. LDGs could be divided into CD10+LDGs and CD10-LDGs based on expression profile.
3. Compared with HC, the proportions of CD14+monocytes, LDGs, megakaryocytes and other innate immune cells in GPP group were significantly increased. There was no significant difference in the proportions of multiple innate immune cells between IL36RNmut group and IL36RNwt group.
4. Pseudo-time analysis showed that LDGs distributed along a unique cell trajectory. CD10+LDG cells are downstream of CD10-LDG cells. The comparison between GPP and HC groups showed that most LDGs in HC group were located at the downstream of cell trajectory, while LDGs in GPP group was mostly situated at the upstream.
5. Enrichment analysis showed that genes of functions on oxidation, phagocytosis and Fcγreceptor signal transduction were markedly up-regulated in CD14+ monocytes in GPP.

However, down-regulated genes of CD14+ monocytes were significantly enriched in ribosomal biosynthesis pathway. Up-regulated differentially expressed genes (DEGs) of LDG cells in GPP were significantly enriched in terms related to leukocyte transmembrane migration, chemokine signaling pathway, actin cytoskeleton regulation and MAPK signaling pathway. Moreover, neutrophil chemotaxis and migration pathways were activated in megakaryocytes in GPP patients.
6. Cell communication analysis revealed unique interactions between megakaryocytes and LDGs in GPP, such as PPBP_CXCR1, PPBP_CXCR2.

Conclusion

1. The single cell atlas of PBMCs from patients with acute GPP showed distinctive innate immune disorders charictrized by amplification of innate immune cells such as monocytes, LDG and megakaryocytes were observed.
2. Some patients with acute GPP carried the homozygous mutation c.115+6T>C in IL36RN，which may not affect the compositions of innate immune cell populations when comared with those without IL36RN mutations.
3. There existed CD10+LDG and CD10-LDG in patients with acute GPP. Both of them showed strong capacity of phagocytosis, chemotaxis and passing through vascular endothelial.
4. In patients with acute GPP, CD14+ monocytes were significantly activated，but their ribosome synthesis function was impaired, which suggests that CD14+ monocytes may play a vital role in the pathogenesis of GPP.
5. Megakaryocytes in patients with GPP might promote the activation and migration of CD10+LDGs through interaction with PPBP_CXCR1 and PPBP_CXCR2.
6. Further studies on innate immune system，especially neutrophils，would help clarify the key pathogenesis of GPP and find novel therapeutic targets.

OR-043

Homeostasis and immunofunction of invariant natural killer T cell in mice with imiquimod-induced psoriasis

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Background Psoriasis is a chronic, recurrent, inflammatory and systemic disease mediated by genetics and environment. Recent studies have shown that psoriasis is an autoimmune disease mediated by T cells. Natural killer T cells (NKT cells), helper T cells (Th cells) and regulatory T cells (Tregs) are all involved in the pathogenesis of psoriasis. Invariant natural killer T cells (iNKT cells) are type I NKT cells. iNKT cells are a unique lymphocyte subpopulation that share properties and express surface markers of both NK cells and T cells. The function of iNKT cells differ a lot from traditional T cells. α-GalCer, CD1d, and TCR form a complex to activate iNKT cells. The number of activated iNKT cells increase in a short time and rapidly produce large numbers of Th1 type cytokine Interferon-γ (IFN-γ), Th2 type cytokine Interleukin-4 (IL-4) and Th17 type cytokine interleukin-17 (IL-17), so as to quickly take effect. Previous studies have suggested that iNKT cells regulates the development of various inflammatory diseases. However, the expression and function of iNKT cells in psoriasis has not yet been clarified. The previous research of our team have confirmed that the proportion of iNKT cells are reduced in the peripheral blood of psoriasis patients. Here, we want to further explore the expression of iNKT cells in mice.
Objectives To explore the immunophenotype and function of iNKT cells in the skin draining lymphnodes and spleen of IMQ-induced psoriasis-like dermatitis mice and wide-type control mice.
Methods We used imiquimod cream to establish a psoriasis mouse model. Flow cytometry was used to detect whether the homeostasis and function of the skin draining lymph nodes and spleen iNKT cells of the imiquimod-induced psoriasis mouse model are compatible with the application of petrolatum cream in the wide-type control mice.
Results The proportion of iNKT cells and activated iNKT cells in the skin draining lymph nodes of imiquimod-induced psoriasis mice was higher than that of control mice, and the secretion IL-4 was

higher than that of control mice. The proportion of iNKT cells and activated iNKT cells in the spleen of imiquimod-induced psoriasis mice was higher than that of control mice, and the secretion of IL-17 was lower than that of control mice.
Conclusions iNKT cells are dysregulated in the skin draining lymphnodes and spleen of imiquimod-induced psoriasis mice. iNKT cells may play an important role in the pathogenesis of psoriasis.

OR-044

Staphylococcal enterotoxin B activates primary malignant T cells in cutaneous T cell lymphoma and promotes a Th2 tumor microenvironment

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Object Staphylococcus aureus (S. aureus) colonization has long been known to have a strong association with CTCL, and many patients die from complicating bacterial infections. Mycosis fungoides (MF), the most common subtype of CTCL, always exhibited a chronic clinical course with broken skin barrier and immune dysfunction, leading to profound immunosuppression and the risk of opportunistic infections. It has been believed that in the initial of MF, skin T cells received persistent antigen stimulation in a chronic inflammatory microenvironment, triggered by chronic bacterial/viral infection or environmental exposure, resulting in the depletion of T cell receptor (TCR) repertoires and malignant transformation. Although previous studies have demonstrated that staphylococcal enterotoxins (SEs) could function as superantigens and have long been suspected to be involved in CTCL, the complicated interplay between S. aureus and CTCL remains poorly understood. It has been revealed that SEA could stimulate CTCL cells growth by promoting the production of interleukin (IL)17 or IL10 in malignant T cells. However, most of CTCL cases, especially at advanced stages, exhibited a Th2-bias phenotype characterized by high expression of IL4, IL5 and IL13. It has been documented that microbiome could be involved in Th2 microenvironment in many diseases. It remains to be determined that whether SEs-producing S. aureus contributed to the Th2 phenotype of CTCL. We aimed toclarify the role of S. aureus in the skin microbiota of CTCL patients and investigate the immune response induced by SEs in malignant CTCL cells.
Methods Lesional skin biopsies were obtained from a cohort of 67 patients with CTCL recruited from the Skin Lymphoma Clinic of Peking University First Hospital and Qilu Hospital of Shandong University. Using traditional culture method and 16S rRNA sequencing, we characterized the clinicopathological features and disordered microbial community. To characterize the pathologic function of SEs in CTCL, PBMCs were collected from three Leukemic-CTCL patients and two healthy controls and were treated with SEs. Cell growth was measured with an MTS-based cell viability assay. Total RNA was extracted from PBMCs of two healthy donors and two L-CTCL patients after SEB treatment for 48 hours and used for gene expression analysis. Deconvolution analysis of PBMC transcriptomic data was performed to explore the key immune response in malignant T cells of CTCL.
Results 1) S. aureus colonization increased with the progression of the disease stage in MF/SS patients. The phenomenon of large cell transformation (LCT) and high lactate dehydrogenase level (LDH) were significantly more frequent in S. aureus positive group. SATB1, has been found to be associated with innate immune activation for S. aureus in CTCL and also involved in regulating IL13 expression. We found that skin lesions from S. aureus-positive patients exhibited an increased expression of SATB1, suggesting that S. aureus colonization may stimulate an immune response mediated by SATB1 and have the potential to induce Th2 cytokines. 2) We analyzed the 16S rRNA sequencing data from the 38 skin swab samples to explore the skin microbiota of CTCL skin. The results showed that Staphylococcusdominated the skin microbiota on MF lesional skin. Further

analysis revealed the high abundance of S. aureus on lesional skin was strongly related with decreased bacterial diversity, disturbing the equilibrium of skin microbiome. 3) Combined with PCR and enzyme immunoassay methods, we found that 34.8% S. aureus strains produced SEs. Interestingly, S. aureus strains isolated from the lesional and non-lesional skin of the same patient produced the same enterotoxins, indicating that they were from the same strain. SEB was the most frequently detected enterotoxins in this cohort and was presented in 17.4% S. aureus isolates. 4）We observed that SEB had direct effects on the growth of CTCL lines (MyLa, Hut78, MJ and Jurkat). Next, PBMCs were collected from three L-CTCL patients with high blood tumor burden (CD4/CD8 ratios >10) and two healthy controls and were treated with SEB. SEB conferred a proliferation signal in PBMCs from both L-CTCL patients and healthy controls, but induced distinct cellular responses between the two groups. 5) Deconvolution analysis of bulk RNA-sequencing on PBMCs showed that SEB exposure mainly upregulated the proportion of CD4+ memory T cells in the CTCL group. Since malignant T cells were derived from CD4+ memory T cells, this result strongly implied that SEB stimulated a proliferation of malignant T cells in CTCL. Then, we compared the transcriptional profiles between CTCL and healthy control groups after SEB exposure. We found SEB activated an IL4 and IL13 signaling in the CTCL group with enhanced IL-4, IL-5 and IL-13 expression, as well as an impaired anti-bacterial immune activity.

Conclusion Our study confirms the high prevalence of S. aureus colonization in CTCL patients and suggests that SEB fosters a Th2 phenotype accompanied by an impaired antibacterial activity, providing a microenvironment for tumor progression. These results provide new insights into the association between S. aureus and CTCL and may pave the way for future anti-S. aureus treatment or anti-enterotoxin treatment in CTCL.

OR-045

Interactions between antifungals and Everolimus against Cryptococcus neoformans

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Cryptococcus is the causal agent of cryptococcosis, a disease with high mortality mainly related to HIV immunosuppression and usually manifests with pneumonia and/or meningoencephalitis.Standard therapy is a typically an aggressive intravenous injection of an antifungal drug, followed by suppressive treatment taken orally for a period that varies depending on the patient's condition . Amphotericin B (AmB) (typically lipid formulations) plus 5-fluorocytosine (5FC) for induction treatment for 2 weeks, followed by fluconazole as suppressive therapy , is the recommended treatment. However, the high dosages needed for these infections, the severe toxicities of AmB have been a limiting factor in its use. Furthermore, in regions with higher disease load and death rates, the availability of 5-FC is limited. Cryptococcosis remains a challenging management issue. Combination therapy with drug repositioning has been seen as a potential option due to the scarcity of novel antifungal medicines.Everolimus (EVL), an analog of the naturally occurring macrolide rapamycin, is an orally bioactive inhibitor of the mammalian target of rapamycin (mTOR) serine/threonine kinase signal transduction pathway, which controls proliferation, cell growth, and survival. Its ability to directly inhibit tumor proliferation and cell growth and indirectly impede angiogenesis has garnered much interest as an anticancer drug. Herein, We examined the interaction of everolimus (EVL) with amphotericin B (AmB) and azoles [fluconazole (FLU), posaconazole (POS), voriconazole (VOR), itraconazole (ITR)] against Cryptococcus. Eighteen Cryptococcus neoforman clinical isolates were analyzed. Following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M27-A4, we conducted a broth microdilution experiment to determine the minimum inhibitory concentrations (MICs) of azoles, EVL, and AmB for assessing antifungal susceptibility. A fractional inhibitory concentration index (FICI) of less than and equal to 0.5 indicated synergy, with a range of 0.5 to 4.0 indicated indifference and a value more than 4.0 indicated antagonism. These experiments revealed that EVL had antifungal activity against C.

neoforman. Moreover, EVL, POS, AmB, FLU, ITR, and VOR exhibited MIC values ranging from 0.5-2 μg/mL, 0.03125-2 μg/mL, 0.25-4 μg/mL, 0.5-32μg/mL, 0.0625-4μg/mL and 0.03125-2μg/mL, respectively. The combination of EVL with AmB and azoles (POS, FLU, ITR, and VOR) exhibited synergistic antifungal effects against 16 (88.9%), 9 (50%), 11 (61.1%), 10 (55.6%) or 6 (33.3%) of analyzed Cryptococcus strains. In the presence of EVL, the MIC values of AmB and azoles were significantly lowered. No antagonism was observed. Subsequently, to analyse the interaction between antifungals and Everolimus against Cryptococcus neoformans., G. mellonella larvae were used and split into 14 different experimental groups: untreated (noninfected larvae hat received no treatments), saline (10 μL saline injected noninfected larvae), conidial (C. neoforman-infected larvae), POS (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with POS), ITR (200 μg/mL)-treated (treatment of C. neoforman- infected larvae with ITR), VOR (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with VOR), FLU (200 μg/mL)-treated (C. neoforman-infected larvae treated with FLU), EVL (200 μg/mL)-treated (C. neoforman-infected larvae treated with EVL), AmB (200 μg/mL)-treated (C. neoforman-infected larvae treated with AmB), and POS (200 μg/mL) + EVL (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with POS and EVL) groups, ITR (200 μg/mL) + EVL (200 μg/mL)-treated (treatment of C. neoforman -infected larvae with ITR and EVL) groups, VOR (200 μg/mL) + EVL (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with VOR and EVL) groups, FLU (200 μg/mL) + EVL (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with FLU and EVL) groups, AmB(200 μg/mL) + EVL (200 μg/mL)-treated (treatment of C. neoforman-infected larvae with AmB and EVL) groups. Each experimental group had 20 larvae (weighing between 0.3 and 0.4 g) included and the tests were conducted three times. All in vivo studies used a single C. neoformans isolate (G7). C. neoformans G7 conidia were counted using a hemocytometer at 106 CFU/ml after a 2-day culture on PDA at 28°C, after which the agar was rinsed with PBS. After incubating G. mellonella larvae at 37°C for 2 hours, all groups except the untreated and saline control groups received an injection of 10 μL of a conidial solution and were treated with appropriate antifungal medicines (5 μl). The larvae were placed in a 37°C incubator and inspected once a day for six days to determine their survival rate. In vivo analyses conducted using the G. mellonella model further confirmed that combination EVL+ POS, EVL+ FLU, and EVL+ITR treatment were associated with significantly improved larval survival following Cryptococcus spp. infection. However, EVL-AmB and EVL-VOR combination did not show a synergistic effect when G. mellonella infected with C.neoformans G7 isolate were treated, which was not consistent with in vitro experiments. It was speculated that the specific mechanism of drug interaction is different in vivo and in vitro. In conclusion, theses findings provide the first published evidence suggesting that a combination of EVL and AmB or azoles exhibit a synergistic effect and may be an effective antifungal disease treatment strategy for infections caused by Cryptococcus spp.. The current investigation expands prior results in the combination interactions between conventional antifungals and TOR inhibitors. Against Cryptococcus spp., EVL may augment the antifungal activity of AmB, POS, FLU, ITR, and VOR in vitro. In addition, for some individuals with clinical cancer, combining EVL with AmB or azoles may be a safe alternative for treating Cryptococcus infections. However, further research is required to clarify the underlying process and identify viable, safe therapeutic applications.

OR-046

Study on the application of HETI for injection in patients with mild to moderate depressed lacrimal sulcus

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Objective To analyze the application effect of Heiti for injection in patients with mild to moderate depressed lacrimal groove.
Methods From August 2022 to January 2023, 60 patients with mild to moderate sunken lacrimal groove admitted by Chengdu Qingyang Guanghua Jingfu Medical Beauty Clinic Co., Ltd. were

selected as the study subjects, and were divided into the observation group (30 cases, treated by injection of Heiti) and the control group (30 cases, treated by conjunctival approach to lower eyelid pouch repair) according to the difference of treatment methods of the patients. Compare the improvement effect of lacrimal groove with mild and moderate depression in the two groups. **Results** The improvement effect of lacrimal groove depression in the observation group was significantly better than that in the control group (P.
Conclusion Heiti for injection has a good effect in patients with mild to moderate depressed lacrimal groove.

如今由于生活节奏的加快、生活压力的不断增大，凹陷泪沟发生率也不断升高，这对于很多爱美的人群尤其是女性群体是无法忍受的，往往会要求临床给予纠正[1-2]。结膜入路下睑袋修复术是以往时期常用方式，这种方式会产生较大损伤，因此治疗过程中不良反应发生率较高[3-4]。近年临床逐步引入了注射用嗨体方式，不仅操作简单，效果也非常显著，是一种安全高效的治疗方式，患者的接受度、满意度更高[5-7]。为此，本文选取 2022 年 8 月至 2023 年 1 月期间成都青羊光华晶肤医疗美容诊所有限公司等三所医疗美容机构收治的 60 例轻中度凹陷泪沟患者，对注射用嗨体在轻中度凹陷泪沟患者中的应用效果进行了研究分析，现报告如下。

随着大众生活水平的不断提高，人们对面部年轻化的需求越来越高，尤其是女性群体，越来越多的人选择医美技术改善眼周问题。泪沟是沿着内眦往外下斜行，位于脸颊部交界处长约两厘米的凹陷，如果这种凹陷过深便会造成泪沟畸形，同时也属于一类眶周老化的早期改变，因此越来越受到人们重视[12-16]。以往时期对于泪沟凹陷的纠正多采用胶原蛋白、玻尿酸填充或手术纠正，但以上方式容易由于免疫性排斥或者手术刺激引发并发症等造成改善效果下降、患者满意度低的问题。临床通过深入研究发现，对泪沟凹陷的治疗，最关键的是选择安全高效的填充材料[17-19]。

本研究随访观察结果提示，观察组泪沟凹陷改善效果方面显著优于对照组（P 值均＜0.05），TTRS 评分改善显著优于对照组（P 值均＜0.05），且观察组患者对本次轻中度凹陷泪沟治疗满意度显著高于对照组（P 值均＜0.05），表明了注射用嗨体在轻中度凹陷泪沟患者中具有良好的应用效果。分析其原因，注射用嗨体延展性较好，用在泪沟填充上能有效促进泪沟局部色素改善，嗨体的组织成分包含了甘氨酸、脯氨酸、丙氨酸，这些成分都是合成胶原蛋白的重要原料，可以高效促进泪沟局部皮肤的胶原蛋白再生，并促进肌肤的弹性恢复，实现良好的治疗效果目标，这一结论也与陈元良[20]所报道的结论相符。

综上所述，注射用嗨体在轻中度凹陷泪沟患者中具有良好的应用效果。

OR-047

Dermal elastic fiber abnormality: an indicator of SLURP1-associated palmoplantar keratoderma?

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1 Background

Palmoplantar keratodermas (PPKs) refer to a heterogeneous group of keratinization disorders characterized by epidermal hyperkeratosis on the palms and soles. PPK of the Gamborg-Nielsen type (PPK-GN; OMIM #244850) is a rare autosomal recessive skin disorder characterized by hyperkeratosis and knuckle pads on the hands and feet caused by *SLURP1* mutations exclusively in patients from Sweden. The coexistence of punctate PPK and decreased and fragmented dermal elastic fibers was first reported in an Arabic female patient caused by a recessive mutation in *SLURP1*. However, to the best of our knowledge, abnormal dermal elastic fibers in PPK-GN has not been reported previously. The molecular basis for this condition is unknown.

2 Objective

To detect the pathogenic gene of individuals with PPK-GN accompanied with dermal elastic fiber abnormality and investigate the mechanism.

3 Methods

Whole exome sequencing (WES) followed by Sanger sequencing was performed to identify the gene responsible for the patients with PPK-GN from a Chinese Yi family. Quantitive RT-PCR, immunofluorescence, CCK-8 assay and ELISA were conducted to further demonstrate the role of SLURP1 for this unusual phenotype.

4 Results

4.1 Clinical data

A four-generational Yi family from China was investigated in this study. The affected individuals were two siblings and their uncle. Two sisters (patient 1 and 2) were referred to our outpatient clinic due to the skin lesions on the hands and feet. They were both 12 years old. Patient 1 suffered from acral lesions since the age of 4, and patient 2 developed similar symptoms since infancy. They denied any itching or pain on the lesions. The parents of patient 2 and patient 3 were consanguineous. On physical examination, the two siblings both exhibited a relatively well-defined translucent plaques surrounded by a papular border on the palms and soles, as well as multiple circumscribed, translucent and hyperkeratotic papules on the dorsal aspect of the hands and feet. Knuckle pads were observed on the dorsal aspects of the finger and toe joints.

4.2 Histopathology

Hematoxylin-eosin staining of a papule on the hand showed orthohyperkeratosis, a prominent granular layer with acanthosis in the epidermis. Verhoeff-Van Gieson staining revealed elastic fibers disappeared in the dermal papillary layer, and markedly fragmented elastic fibers in the reticular layer. Transmission electron microscopy was used to observe the elastin and microfibrillar component, which showed decreased elastin, and fragmentation and agglutination of the microfibrillar component in the skin lesion.

4.3 Identification of a novel *SLURP1* mutation in patients with PPK and abnormal dermal elastic fibers

In order to identify the mutated gene inherited by the patient's family, blood samples were collected from the three patients and their family members. We performed WES using the genomic DNA extracted from patient 2 and her parents. As patient 2 and patient 3 were born from healthy consanguineous parents, we focused on the homozygous variants that were present in patient 2. As a result, we obtained 25 homozygous variants. Using Sanger sequencing, we verified that only the nonsense mutation c.302C>A (p.S101X) in *SLURP1* was homozygous among all three patients and heterozygous / wildtype in their healthy family members. This variant was not present in 100 control individuals of Chinese Yi ethnicity. The Ser101 residue is highly conserved amongst species.

4.4 SLURP1 promoted the cell viability of dermal fibroblasts

To determine whether SLURP1 would affect the cell viability of dermal fibroblasts by silencing and overexpressing *SLURP1* in HDFs and assaying the cell viability using CCK-8 reagent. After the 48h transfection, the cell viability of HDFs in siRNA group was decreased comparing with normal control (NC) group, with a tendency to exacerbate with increase in transfection time. On the contrary, the cell viability of HDFs in pCMV6-SLURP1 group was increased after the 48h transfection when compared with the pCMV6-entry group, with a positive correlation with increase in transfection time. Those results indicated SLURP1 could promote the cell viability of HDFs.

4.5 SLURP1 increased the secretion of elastin

ELISA assay was used to detect the concentration of elastin in supernatants of HDFs transfected with *SLURP1*-siRNA or pCMV6-*SLURP1*. The concentration of elastin was significantly lower in the siRNA group when compared with the NC group, while it was significantly higher in the pCMV6-SLURP1 group than that in the pCMV6-entry group. The results showed SLURP1 could increase the secretion of elastin from HDFs.

5 Conclusion

We identified a novel homozygous nonsense mutation, c.302C>A, in *SLURP1* in a Chinese family of Yi ethnicity, which resulted in PPK-GN accompanied with abnormal elastic fibers. In addition, we first confirmed SLURP1 plays a role in the maintenance of dermal elastic fibers and may have a potential therapeutic effect on elastic fiber diseases by enhancing the proliferation and elastin expression of dermal fibroblasts. Dermal elastic fiber abnormality could be an indicator of *SLURP1*-associated PPK. More case observations and further studies are necessary to determine our findings and clarify the exact mechanism of SLURP1 on dermal fibroblasts.

OR-048

Gender disparity in clinical feature, lifestyle behavior and non-communicable diseases comorbidity among psoriasis patients in Shanghai

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Background Psoriasis vulgar has serious physical and mental impact on patients, and is an important public health issue. Researches indicate that gender is associated with the differences in clinical feature, disease severity, and quality of life for noncommunicable diseases (NCD), but studies on gender disparity in pathogenesis, treatment preference and NCD comorbidity in psoriasis patients is still limited. In this study, we aimed to explore the gender differences in clinical feature, lifestyle behavior and metabolic disorder comorbidity among psoriasis patients.

Methods A cross-sectional study was conducted from January to December 2021 in Shanghai. Psoriasis was confirmed according to guideline of the Chinese clinical dermatology(13). 1) pathogenic site: with skin damage manifested as localized or systemic; 2) clinical symptoms: mainly red inflammatory papules, maculopapular rash, patches of varying sizes, covered with multiple layers of silvery- white scales. After scraping the scale, there is membranous appearance and punctate bleeding. In this study, both of male and female psoriasis patients aged >18 years were included and psoriasis patients with neurological or psychiatric abnormalities were excluded. Finally, 2101 patients with psoriasis were recruited with cluster survey method, and questionnaire interviews were used to collect information covering the demographic feature, lifestyle habits, clinical feature and metabolic disorders. SAS9.4 were used for data analysis and P value less than 0.05 was considered as statistically significant.

Results The 2102 psoriasis patients included 1332 males (63.4%), 70% of them were over 35 years old and over half of them were overweight or obesity. 82.2% of them were married, and 50% of them had an education of college and above. The proportion of mental worker and manual workers were 39.3% and 34.0%, respectively. 62.5% of psoriasis patients were local residents, more than 59% of them had individual income over 5000 RMB per month, and over 50% of patients were overweight and obesity.

The prevalence of tobacco smoking, alcohol drinking was 31.2% and 12.6%, and male patients had higher prevalence of tobacco smoking (odds ratio (OR)=13.26, 95% confidence interval (CI): 9.54-18.44) and alcohol drinking (OR=14.44, 95%CI: 7.90-26.40) than female patients. Moreover, male patients had lower smoking initiation age, longer smoking duration and more daily cigarettes smoke consumption, in comparison with female psoriasis patients with tobacco smoking. In the study, psoriasis vulgaris was the predominant subtype of psoriasis (95.5%). The median value for psoriasis initiation age and disease duration was 33 years old (IQR: 22.1-46.0) and 9 years (IQR:4-18), respectively, and male patients had older age of diseases initiation and longer disease duration than female patients. The recurrence of psoriasis was mainly in winter (73.4%) and autumn (34.2%). The familial aggregation of psoriasis was 8.9% in fathers, 6.0% in mothers, 9.4% in siblings, and 5.1% in offspring.

The prevalence of diabetes, hypertension, hyperlipidemia, and metabolic syndrome were 13.2%, 28.5%, 23.4% and 21.5%, respectively. The prevalence of diabetes among male patients (14.9%) was higher than female patients (10.3%), the OR was 1.53 (95% CI:1.16-2.02). Logistic regression indicated that male patients had 1.87 times higher prevalence of hypertension than female patients (95% CI:1.52-2.30), even with the adjustment of potential confounders (OR=1.30, 95%CI: 1.00-1.68). Meanwhile, the prevalence of hyperlipidemia was 28.5% in male patients and 14.6% in female patients (OR=2.34, 95%CI:1.85-2.95), male patients had higher hyperlipidemia prevalence than female patients with control of potential confounding factors (OR=1.63, 95%CI:1.24-2.14). Male patients had 2.06 times higher prevalence of metabolic syndrome (95% CI:1.63-2.62), even with the adjustment of confounders (OR=1.60, 95%CI:1.22-2.10).

In comparison with the psoriasis onset time, we classified psoriasis patients into Group A for those whose NCD diagnosis time was behind the onset of psoriasis and Group B for those whose NCD

diagnosis time was ahead of the onset of psoriasis. The proportion of patients with NCD diagnosed after psoriasis onset (Group A) was 58.84%, 60.54% and 66.60% for diabetes, hypertension and hyperlipidemia, respectively. The proportion of patients in Group A for diabetes, hypertension, and hyperlipidemia were all higher than that in Group B, both for male and female psoriasis patients. Moreover, male patients had a higher proportion of NCD diagnosed after psoriasis onset (Group A) than female patients for diabetes, hypertension and hyperlipidemia, the differences were all statistically significant.

Conclusions Gender disparity in psoriasis patients indicates that male patients had more body weight issue, with fewer sleep time and higher prevalence of tobacco smoking, alcohol drinking and metabolic diseases. We recommend that more attention should be paid to male patients and provide diagnosis and treatment for metabolic diseases which is helpful for diseases recovery.

OR-049

Evaluation of cerebrospinal fluid ubiquitin C-terminal hydrolase-L1, glial fibrillary acidic protein, and neurofilament light protein as novel markers for the diagnosis of neurosyphilis among HIV-negative patients

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Objectives Neuronal damage is an important pathological feature of NS; thus, the markers associated with neuronal damage may be helpful in the diagnosis of NS. Neuronal damage markers have been suggested for the diagnosis of multiple sclerosis and Wilson disease. Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a compact, small, nearly spherical ubiquitin protein, highly specifically expressed by neurons, which is essential for removing abnormal proteins and preventing the accumulation of potentially toxic proteins in neuronal cells. It is associated with the occurrence and development of neurode- generative diseases, such as Alzheimer's disease, Parkinson's dis- ease, and amyotrophic lateral disease. Glial fibrillary acidic protein (GFAP) is a marker of astrocyte prolif- eration, which is involved in cytoskeleton formation and the main- tenance of tensile strength, and its expression is increased when neurons are damaged. Neurofilament light chain protein (NF-L) is an important part of the neuronal cytoskeleton and plays a key role in axonal radial growth and stability, thus ensuring proper nerve signal transmission. It is abnormally elevated in neurodegenerative, inflammatory, vascular, and traumatic diseases. UCH-L1, GFAP, and NF- L have been shown to be indicators of neuronal damage in many central nervous diseases, but the diagnostic utility of UCH-L1, GFAP, and NF-L in NS remains unclear. This study aimed to evaluate the possibility of using cerebrospinal fluid (CSF) ubiquitin C-terminal hydrolase L1 (UCH-L1), glial fibrillary acidic protein (GFAP), and neurofilament light protein (NF-L) for the diagnosis of neurosyphilis (NS).

Methods A cross-sectional study of 576 subjects was conducted from January 2021 to August 2022 to evaluate the diagnostic accuracy of CSF UCH-L1, GFAP, and NF-L for NS and analyze their correlations with CSF rapid plasma reagin (RPR), white blood cells (WBCs), and protein. Continuous variables are presented as medians (interquartile spacing), and categorical variables are expressed as frequencies (percentages). The Mann-Whitney U test was used for continuous variables with skewed distribution, the chi-square test was used for categorical variables, and the odds ratio (OR) was calculated. Spearman rank correlation was used to analyze the correlation among all parameters (CSF UCH-L1, GFAP, NF-L, CSF WBC, and CSF protein). The correlation of the results according to their r values was categorized as extreme (0.91-1.0), strong (0.71-0.9), moderate (0.41-0.7), weak, or poor (0-0.40). Receiver operating characteris- tic (ROC) analysis was performed to determine the performance of CSF UCH-L1, GFAP, and NF-L in the diagnosis of NS, and the opti- mal cut-off point was determined to correspond to the maximal Youden's index

(sensitivity + specificity - 100%). The sensitivity, specificity, and predictive value of the optimal critical values were calculated. The previously mentioned statistical analyzes were performed using SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as a two-sided P-value <0.05.

Results The NS, syphilis/non-NS, and non- syphilis control groups exhibited no significant differences in age (P-value = 0.065) or sex (P-value = 0.339). The serum RPR, serum TP-CIA, and CSF TP-CIA titers in the NS group were significantly higher than those in the syphilis/non-NS group (P <0.001). Compared with the syphilis/non-NS and nonsyphilis groups, patients with NS had higher levels of CSF WBC (P <0.001) and increased CSF protein (P <0.001). The level of CSF UCH-L1 was 951.92 (715.10-1263.51) pg/ml in the NS group, which was significantly higher than that in the syphilis/non-NS group (370.30 [231.23-543.10] pg/ml]) (P <0.001) and the nonsyphilis group (155.47 [56.29-679.33] pg/ml) (P <0.001). CSF UCH-L1 levels were higher in the syphilis/non-NS group than in the nonsyphilis group (P-value = 0.022). Similarly, CSF GFAP (692.19 [549.53-950.61] pg/ml) and CSF NF-L levels (87.15 [57.21-127.60] pg/ml) in the NS group were significantly higher than levels in the syphilis/non-NS group (409.62 [381.13-503.06] pg/ml, P <0.001; 30.41 [20.11-40.43] pg/ml, P <0.001) and the nonsyphilis group (407.46 [405.75-453.98] pg/ml, P <0.001; 20.99 [15.61-46.27] pg/ml, P <0.001). However, there was no statistical difference between the syphilis/non-NS and the nonsyphilis groups (P-value = 0.7696, P-value = 0.0777). To evaluate the use of CSF UCH-L1 levels as a screening test for NS, its screening accuracy was determined using ROC curve analysis. The area under the curve (AUC) for CSF UCH-L1 was 0.9177 (95% CI 0.8691-0.9663). Comparably, the AUC of CSF GFAP and NF-L were 0.8512 and 0.8848, respectively (95% CI 0.7782-0.9242; 95% CI 0.8222-0.9473). According to the maximal Youden's index, the optimal cut-offs for CSF UCH- L1, GFAP, and NF-L to distinguish NS from syphilis/non-NS were 652.25 pg/ml, 548.89 pg/ml, and 48.38 pg/ml, respectively. In addition, the chi-square test was used to analyze the correlation of CSF UCH-L1, GFAP, and NF-L with NS, and the results showed that patients with syphilis with CSF UCH-L1 ≥652.25 pg/ml were 67.76 times more likely to have NS than those with CSF UCH-L1<652.25pg/ml (OR, 67.76; 95% CI 22.25-206.31) (P <0.001). Likewise, pa- tients with syphilis with CSF GFAP ≥548.89 pg/ml and CSF NF- L≥48.38 pg/ml were 19.39 times and 49.97 times more likely to have NS than those with low levels, respectively (OR, 19.39; 95% CI, 7.92-47.45; and OR, 49.97; 95% CI, 17.46-143.01) (P <0.001). Based on the aforementioned ROC curve and optimal cut-off values established above, CSF UCH-L1 demonstrated a sensitivity of 85.11%, a specificity of 92.22%, and a negative predictive value (NPV) of 92.22% for NS diagnosis by using diagnostic criteria of NS as the reference standard. Similarly, the sensitivity and specificity of CSF GFAP for NS diagnosis were 76.60% and 85.56%, respectively. Moreover, the sensitivity and specificity of CSF NF-L for NS diagnosis were 82.98% and 91.11%, respectively. To further improve the sensitivity of NS diagnosis, CSF UCH- L1, GFAP, and NF-L were combined in a parallel testing format , which referred to one of two or three positive indicators. Using this algorithm, sensitivity increased more than that observed with a single indicator, reaching 83.67-93.62%, while specificity decreased, although it was still above 77.78%. The combination of CSF UCH-L1 and CSF NF-L was most optimal, yielding a high sensitiv- ity of 93.62% and specificity of 91.11% in identifying NS. Moreover, the NPV and κ values increased to 96.47% and 0.83, respectively. To further improve the specificity of NS diagnosis, a series com- bination testing format was used, which referred to two or three indicators that were all positive. By this algorithm, the specificity increased as high as 92.22-98.89%, but the sensitivity decreased significantly to 65.96-74.47%. The combination of CSF GFAP≥548.89 pg/ml and CSF NF-L≥48.38 pg/ml both had a specificity and posi- tive predictive value of 98.89% and 94.12% for the diagnosis of NS, respectively.

Conclusion NS is one of the most feared syndromes of syphilis owing to its serious, irreversible sequelae. To date, the di- agnosis of NS has relied on a combination of neurological manifestations in patients with reactive serological anti-T. pallidum test and CSF abnormalities. Usually, NS can be diagnosed with reactive CSF VDRL in conjunction with neurological signs or symptoms, but the test is insensitive. For patients with suspected NS but negative CSF VDRL, there is a lack of specific indicators to identify NS. Thus, the diagnosis of NS remains challenging and new indicators are urgently needed. In this study, we focused on markers associated with neu- ronal damage and evaluated the possibility of CSF UCH-L1, GFAP, and NF-L as novel markers for the diagnosis of

NS. We found that the CSF UCH-L1, GFAP, and NF-L levels in patients with NS were significantly higher than those in syphilis/non-NS patients and nonsyphilis participants. Interestingly, patients with syphilis with CSF UCH-L1 ≥652.25 pg/ml, CSF GFAP ≥548.89 pg/ml, and CSF NF- L ≥48.38 pg/ml were 67.76, 19.39, and 49.97 times more likely to have NS than those with low levels, respectively. Additionally, CSF UCH-L1, GFAP, and NF-L showed a sensitivity of 85.11%, 76.60%, and 82.98%, with a specificity of 92.22%, 85.56%, and 91.11%, respectively, for the diagnosis of NS using diagnostic criteria of NS as the reference standard.

OR-050

Prevalence of tobacco smoking and its association with disease severity among psoriasis patients in China: a cross-sectional study

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Objectives In recent years, a growing number of studies have indicated that environmental factors are vital triggers for the initiation of psoriasis, and tobacco smoking is one of the key environmental pathogenic factors. Due to tobacco induced heavy diseases burden, and its association with psoriasis, it is critical to understand the prevalence of tobacco smoking among patients with psoriasis for future tobacco control in this targeted population. But evidence on the association with psoriasis severity is still limited in China. In this study, we aim to examine the prevalence of tobacco smoking and explore the association between tobacco smoking and diseases severity among psoriasis patients.

Methods Data in this study was originated from the National Clinical Research Center for Skin and Immune Diseases (NCRCSID) in China. The NCRCSID was established in January 2020 and was aimed to build a database in China to collect extensive real-world data on psoriasis from 200 hospitals selected in all seven regions of China by a multi-stage sampling manner. In this study, 4529 participants aged 18-92 years were recruited, all participants had a clinical diagnosis of psoriasis and received a standardized personal interview with informed consent. Detailed information covering demographic feature, tobacco smoking and psoriasis history were collected through an electronic questionnaire, and clinical data was extracted from the HIS (Health Information System). SAS 9.4 was performed for data analysis, and a p-value of less than 0.05 was considered as statistically significant.

Results In this study, 4529 psoriasis patients were recruited and classified into non-smokers (3134, 69.2%), former smokers (281, 6.2%) and current smokers (1114, 24.6%). The average age of the 4529 patients was 38.6 years (SD:15.5), 64.1% of them were males and 70.4% of them were married. 33.0% of the 4529 psoriasis patients had college and above education, and nearly half of them were overweight or obesity, and approximately 97% of them had medical insurance.

The prevalence of tobacco smoking was 30.8%, with 24.6% for current smoking and 6.2% for former tobacco smoking. The median value of tobacco smoking years was 13.0 for total smokers (IQR: 8.0-12.0), and approximately one quartile of them had smoking duration over 20 years. The median number of daily consumed cigarettes was 10 with the IQR ranging from 8 to 20, and nearly 70% of them had smoking intensity less than 20 cigarettes per day.

The average PASI (Psoriasis Area and Severity Index) score for psoriasis patients was 9.4, with male patients had higher PASI score than females. For psoriasis lesion severity, the median score was 3.0 (IQR:0-6) for head and neck, 4.0 (IQR:2-6) for trunk, 4.0 (IQR:2-6) for upper limb and 5.0 (IQR:3-6) for lower limb. 16.1% of patients reported psoriasis family history, the proportion was higher in female patients but without statistically significance.

In this study, 4529 psoriasis patients were divided into three groups by their tobacco smoking status, and then recombined into condition 1 (non-smoker and former smoker) and condition 2 (non-smoker and current smoker) to explore the association between tobacco smoking and psoriasis severity. In comparison with non-smokers (8.3±7.5), former smokers (13.4±10.3) and current

smokers (11.5±9.4) had a higher PASI score. The proportion of PASI score over 7 was 44.2% in non-smokers, which was obviously lower than that in former smokers (66.9%), and in current smokers as well (58.1%).
Logistic regression analysis indicated that former smokers had higher PASI scores than non-smokers, the OR was 1.5 (95% CI: 1.0-2.3) for PASI score (3.0-7.0) compared with PASI score<3.0, 2.2 (95% CI: 1.5-3.3) for PASI score (7.1-13.0) compared with PASI score<3.0, and 4.2 (95% CI: 2.9-6.1) for PASI score>13 compared with PASI score<3.0, even with the adjustment of potential confounders (ORs were 1.2, 1.7 and 2.8, respectively). Likewise, current smokers also had higher PASI scores than nonsmokers, logistic regression showed that the OR was 1.8 (95% CI: 1.5-2.2) for PASI score (3.0-7.0) compared with PASI score<3.0, 1.9 (95% CI: 1.5-2.3) for PASI score (7.1-13.0) compared with PASI score<3.0, and 3.1 (95% CI: 2.5-3.8) for PASI score>13 compared with PASI score<3.0, even with the adjustment of potential confounders (ORs were 1.6, 1.5 and 2.3, respectively).
In the 1395 psoriasis patients who were tobacco smokers, scatter plot indicated that the PASI score was positively correlated with tobacco smoking years among smokers, and the findings were consistent both in current smokers and former smokers ($p<0.05$). Meanwhile, the scatter plot also demonstrated that the PASI score was positively correlated with the number of daily consumed cigarettes both in current smokers and former smokers ($p<0.01$).
Conclusions The prevalence of tobacco smoking was high, especially among male psoriasis patients and those with senior high education. Tobacco smoking was positively associated with psoriasis severity, moreover, both of smoking intensity and smoking duration were positively correlated with the severity of psoriasis in a dose-dependent fashion.

OR-051

Translation-Dependent Skin Hyperplasia Is Promoted by Type 1/17 Inflammation in Psoriasis

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Background Psoriasis vulgaris (PV) is a common, chronic, recurrent systemic inflammatory disease that affects 0.5-11.4% of adults worldwide. The typical histopathologic features of PV include epidermal hyperplasia, proliferation of dermal blood vessels, and an inflammatory infiltrate of leukocytes. It is well established that the interplay of aberrant immune responses and keratinocyte dysregulation plays a critical role in the pathogenesis of PV. Cytokines like tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-17A mainly produced by immune cells, are capable to directly activate keratinocytes, leading to excessive keratinocytes abnormal differentiation, proliferation, and the consequent production of more inflammatory mediators. Notably, the approaches like IL-17A or TNF-α monoclonal antibodies that cut off this feedback loop have exhibited tremendous effects in disease control. Despite these advances, the molecular basis underlying the positive feedback loop remains largely unidentified.
Due to the thriving forefront for omics-driven methods, the understanding of the underlying hub factors of psoriasis could be more efficient, including transcriptome, epigenomics, proteome, metabolomics and glycomics. Additionally, recent efforts are recapitulating multi-omics network analysis by recombining the isolated omics-modules to find more candidate biomarkers. In 2015, Swindell etc. applied RNA-seq and LC-MS/MS to interrogate the transcriptome and proteome of psoriasis lesions and found that global imbalance between the abundance of mRNA and protein, which was consistent with heightened protein translation process. Furthermore, other researchers also remarked the discordant and elevated translation level in psoriasis lesions, which

suggests that hyper-translation rate might be a very central key to illustrate the chaos between these effects. Based on their findings, there could be more therapeutic efficient by targeting specific stages of translation initiation, elongation or termination.

The correct process of mRNA translation is critical for cellular growth, proliferation, differentiation, and apoptosis. In eukaryotes, the complex of eukaryotic initiation factor (eIF) 4F is required for mRNA protein translation. The eIF4F complex is composed of eIF4E, eIF4A, and eIF4G. eIF4E binds to the mRNA 5' cap, and together with eIF4G (scaffolding protein) and eIF4A (mRNA helicase), form the eIF4F complex and recruit ribosomal subunits to the initiation codon. During this process, eIF4E is considered as the rate-determining protein, and its mutations and phosphorylation are associated with various malignant tumors such as melanoma and adenocarcinoma. However, the role of eIF4E in keratinocyte differentiation related to skin disorders is still poorly understood. Despite a recent study showing a substantial increase of eIF4E in psoriatic lesions from individuals with PV compared with that in non-lesional skin, the precise mechanisms of this phenomenon are not well investigated.

Objective To determine the role of eIF4E in keratinocytes abnormal differentiation in the context of psoriasis.

Methods

The expression of eIF4E in psoriatic skin lesions and normal skin from human subjects was examined by western blot and immunohistochemistry. In a murine model of psoriasis-like dermatitis that is induced by topical imiquimod, 4EGI-1 was used to inhibit eIF4E activities. To measure murine skin eIF4E and keratinocytes differentiation, immunofluorescence and western blot assays were conducted. Normal human epidermal keratinocytes (NHEK) were isolated, cultured, and stimulated with cytokines including TNF-α, IFN-γ, and IL-17A, respectively. Immunofluorescence and western blot were performed to test eIF4E and effect of 4EGI-1 in a co-culture system.

Results

1 eIF4E exhibits an epidermal pattern that is positively correlated with epidermal thickness in patients with PV

2 Epidermis hyperplasia is dependent on eIF4E in the context of psoriasis-like disease

3 Type 1/17 inflammation is sufficient to induce eIF4E expression and keratinocytes abnormal differentiation

Compared with healthy controls, skin lesions from patients with PV exhibited a higher expression of eIF4E, which was positively correlated with the epidermal thickness. This expression pattern of eIF4E was replicated by the imiquimod-induced murine model. Skin hyperplasia and eIF4E activities in the murine model were attenuated by the administration of 4EGI-1. Both IFN-γ and IL-17A, rather than TNF-α, are sufficient to induce NHEK abnormal differentiation. This effect can be disrupted by 4EGI-1.

Conclusion

In this study, we confirmed that eIF4E expression is higher in skin lesions of patients with PV and exhibits a positive correlation with epidermal thickness. After replicating this pattern of eIF4E in a murine model of psoriasis-like disease that is driven by imiquimod (IMQ), we further found that eIF4E is critically required to promote skin hyperplasia. Interestingly, both IFN-γ and IL-17A, rather than TNF-α, are sufficient to activate eIF4E in keratinocytes and lead to keratinocytes aberrant differentiation. Taken together, our study demonstrates that eIF4E is associated with abnormal differentiation of keratinocytes in the context of type 1/17 inflammation and the regulation of translation processes might be an alternative treatment approach for psoriasis.

Recently, there were several studies uncover “dark recesses” of psoriasis protein synthesis biology that is the discordant at the transcriptome and proteome levels. There was 7-fold elevated translation rate in psoriasis lesions and such hyper-translation might be a very central rate control for the treatment. There were many researches of eIF4E related to abnormal proliferation in tumor cells, but scarce report demonstrated its function in skin inflammatory disease related to abnormal proliferation and differentiation of keratinocyte cell (KC).

In this study, we found that eIF4E was predominantly over-expressed and activated in the epidermis of psoriatic lesions. This phenomenon was firstly found by Kjellerup, R. B that increased expression of eIF4E and 4E-BP1 in psoriasis. Besides, we found an elevated level of nuclear eIF4E

pattern occurred in psoriatic epidermis, which might be induced by certain inflammatory cytokines. As accumulating proofs have clarified the functional roles of eIF4E beyond the cytoplasm translational function, it could be a nuclear mRNA exporter for a set of mRNA related cell-cycle or differentiation. We also observed that expression of eIF4E increased progressively from normal differentiation to abnormal differentiation layer (ascertained by the expression correlation between K10 and eIF4E). This observation was consistent with the oncogenic function of eIF4E in squamous cell carcinoma (cSCC). But the nuclear expression pattern wasn't reported in cSCC, there would be further study research to dig deep into the nuclear effect of eIF4E on physiological and pathological process.

In vitro studies, we separated it into two main parts, including IMQ-induced mice model and cytokine-induced NHEK inflammatory cells. Under the IMQ stimuli, the eIF4E expression pattern was similar to human psoriatic lesion. IMQ-induced changes of cytoplasm/nuclear eIF4E expression correlated with the elevation of the abnormal differentiated KC marker K17 and proliferation marker of PCNA. The elevated eIF4E or nucleus translocation might activate the proliferation and differentiation of KC, which might be an important underlying pathogenesis of psoriasis.

Although type 1/17 inflammation is a major feature of PV, the precise mechanisms of inflammation-mediated skin hyperplasia is not well understood. A number of molecular pathways have been reported to contribute to inflammation-induced keratinocytes abnormal differentiation. Coherence with our results, IFN-γ and IL-17A could up-regulate K17 transcription through signal transducer and activator of transcription (STAT) 1 and STAT3. However, regarding to K10, although we showed in the psoriatic skin lesions that K10 has a weak and sporadic distribution, a study from Hattori et al. found that the expression of K10 in the ex vivo skin incubated with IFN-γ is not significantly altered. Thus, it would be interesting to investigate whether other mechanisms are involved in the impact of cytokines on keratinocytes normal differentiation. Further research is still required to understand the transition from normal to abnormal differentiation of keratinocytes in psoriasis. Besides, IL-22 was found to promote K17 expression through STAT3 and extracellular regulated protein kinases 1/2. STAT is a unique protein family that binds to DNA and promote DNA transcription. Unlike STAT family, eIF4E acts during the translation processes. Therefore, how different molecules act synergistically to control gene expression in the context of PV needs to be clarified in the future. Notwithstanding this, given that STAT3 can be co-regulated with eIF4E in malignance, they may exert a cooperation role in keratinocytes dysregulation in PV.

Notably, a number of studies have shown that type 1/17 cytokines are capable to alter eIF4E in other inflammatory conditions. For instance, TNF-α directly induces eIF4E phosphorylation in airway smooth muscle cells, leading to more chemokine secretion. IFN-γ mediates down-regulation of eIF4E in primary human macrophages during inflammatory responses. IL-17A can activate eIF4E signaling pathways in synovial fibroblasts in the context of rheumatoid arthritis, resulting in overexpression of L type amino acid transporter 1. It appears that eIF4E is not a unique molecule that merely regulate keratinocytes but may have broad effects on other cells and tissues. Future investigations using murine models to specifically delete eIF4E in keratinocytes would be necessary to verify its role in skin hyperplasia. Due to this, eIF4E inhibition might be more effective and safer in the form of topical treatment, rather than a systemic medication for PV.

In conclusion, we identified that eIF4E promotes keratinocytes abnormal differentiation that is responsive to type 1/17 inflammation. Pharmacologic strategies targeting eIF4E may have specific potentials to treat PV. What we found pave the way for more research into the precise mechanisms underlying gene translation in the context of PV.

OR-052

TAP2 drives HLA-B*13:01-linked dapsone hypersensitivity syndrome tolerance and reactivity

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Abstract Dapsone hypersensitivity syndrome (DHS) is restricted to human leukocyte antigen HLA-B*13:01. However, the positive predictive value for HLA-B*13:01 is only 7.8%. To explore potential coexisting factors involved in the occurrence of DHS, we carried out a genome-wide association study and a genome-wide DNA methylation profile analysis comparing DHS patients with dapsone-tolerant control subjects (all carrying HLA-B*13:01). No non-HLA SNPs associated with DHS were identified at the genome-wide level. However, the pathway of antigen processing and presentation was enriched in DHS patients and the gene transporter associated with antigen presentation (TAP2) was identified. Expression of TAP2 and its molecular chaperone, TAP1 were validated by qPCR and in vitro functional experiments were performed. The results showed DHS patients have higher mRNA levels of TAP1 and TAP2, and an enhanced capacity for antigen-presenting cell (APC) activating daspone specific T cells compared to dapsone-tolerant controls. Activation of dapsone-specific T cells was inhibited when TAP function of APCs was impaired. This study demonstrates that epigenetic regulation of TAP1 and TAP2 affects the function of APCs and is a critical factor that mediates the development of DHS.

Keywords: Dapsone hypersensitivity syndrome, antigen presentation, drug hypersensitivity, T cells, methylation

Introduction

Dapsone, also known as diaminodiphenyl sulfone, is an aniline derivative that is used for the treatment of infections and chronic inflammatory diseases. Dapsone hypersensitivity syndrome (DHS), caused by the intake of dapsone, is a life-threatening allergic syndrome characterized by fever, eruptions, lymphopathy, and hepatic function abnormalities (Richardus and Smith, 1989). DHS occurs in approximately 0.5–3.6% of patients treated with dapsone and has a high mortality of 9.9% (Zhang et al., 2013). Previous studies have identified a human leukocyte antigen, HLA-B*13:01, as a major risk factor for DHS, with a high negative predictive value of 99.8% and a low positive predictive value (PPV) of 7.8% (Zhang et al., 2013). Further analysis revealed five amino acid variants (positions 133, 142, -17, 11, and 13) in high-linkage disequilibrium with HLA-DRB1 that conferred susceptibility to this condition. When HLA-B*13:01 and DRB1 were combined as risk predictors, the PPV increased to 9.2% compared to HLA-B*13:01 alone (Yue et al., 2018). However, it remains unclear why a larger fraction of individuals carrying HLA-B*13:01 do not develop DHS after administration of dapsone.

It is well established that host genetic predisposition plays a critical role in the development of drug-induced severe cutaneous adverse reactions (SCARs). A series of HLA molecules have been associated with SCARs, including HLA-B*57:01 for abacavir hypersensitivity (Mallal et al., 2008), HLA-B*58:01 for allopurinol-SCARs (Hung et al., 2005), and HLA-B*15:02 for carbamazepine-Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (Chung et al., 2004). In addition, non-HLA molecules, CYP2B6 and CYP2C9, have been shown to be associated with nevirapine hypersensitivity (Carr et al., 2014; Ciccacci et al., 2013) and phenytoin-induced SCARs (Chung et al., 2014), respectively. Whether other non-HLA molecules are involved in the pathogenesis of DHS requires further investigation. In addition to genetic factors, epigenetic modifications, such as DNA methylation, regulate gene function, with an imbalance of this form of regulation contributing to disease etiology and progression (He et al., 2019). Methylated DNA modifications of the Psoriasis Susceptibility 1 Candidate 1 (PSORS1C1) gene and autophagy-related genes are involved in the pathogenesis of allopurinol-induced SCARs (Cheng et al., 2022) (Sun et al., 2017). A role for epigenetic modifications in the pathogenesis of DHS is unknown. Thus, to comprehensively investigate factors that mediate the development of DHS, a genome-wide association study (GWAS) and genome-wide DNA methylation profiling were performed in DHS patients and dapsone-tolerant control subjects. All individuals were positive for the HLA-

B*13:01 allele and were receiving dapsone for treatment. In vitro functional experiments were performed to elucidate the role of TAP1 and TAP2 in the pathogenesis of DHS. TAP1 and TAP2 were identified, by this study, as factors involved in DHS.

Results

Genome-wide association analysis

Genotyping data for 51 DHS patients and 218 dapsone-tolerant control subjects were initially analyzed. After QC filtering, 258,961 overlapping SNPs were analyzed by genome-wide association analysis. After imputation and quality control (QC), 3,574,594 SNPs outside the MHC region were evaluated by log-additive test. SNPs outside the MHC locus (n = 45) had a suggestive association with DHS ($P < 1.0 \times 10-4$). Among these 45 SNPs, 11 SNPs were previously identified (Zhang et al., 2013). 34 newly identified SNPs were subjected to validation.

A total of 62 DHS patients and 319 dapsone-tolerant control subjects were recruited for subsequent validation analysis. A total of 16 candidate SNPs with $P < 1.0 \times 10-4$ were successfully genotyped by the validation study. None of these non-HLA SNPs showed a significant association with DHS by replication analysis ($P < 0.05$) or by combined analysis at the whole-genome level. Summary statistics for the discovery and replication phases, as well as the combined analysis, are shown in Table S1.

Global genome-wide DNA methylation profile of DHS

Genome-wide DNA methylation profiles were compared between 31 DHS patients and 31 dapsone-tolerant control subjects (all carrying HLA-B*13:01) from the discovery set. A total of 759,996 CpG sites were identified by the Infinium Methylation 850k BeadChip, in which 11,181 CpG sites reached epigenome-wide significance (false discovery rate, FDR < 0.05). The distribution of these CpG sites within chromosomes is shown in Figure 1A. Among 11,181 CpG sites, there were 5,566 hypermethylated CpG sites (49.8%) and 5,615 hypomethylated CpG sites (50.2%). Based on the location of methylated regions, these 11,181 CpG sites were classified into six classes: untranslated regions (1,857, 16.6%), transcription start site (2,678, 24.0%), promoter (1,819, 16.3%), enhancer (1,626, 14.5%), gene body (5,493, 49.1%), and unclassified (2,133, 19.1%). The methylation status of the six classes is shown in Figure 1B.

Because promoter-associated methylation likely affects gene expression, the top 500 significant hypomethylation CpG sites which were annotated as “Promoter_Associated” by the Methylation Consortium were selected from 11,181 CpG sites. The heatmap for the 500 selected CpGs is shown in Figure 1C. To identify immune pathways and key immune-related genes involved in the pathogenesis of DHS, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO enrichment analysis found 575 biological processes were significantly associated with DHS ($P< 0.05$), including “GO:0002479:antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent”, which is a key step for activating T cells. KEGG Pathway enrichment analysis found 24 pathways were significantly associated with DHS ($P< 0.05$), including “hsa05340:primary immunodeficiency pathway”, which shared the key gene TAP2 (Figure 1D and 1E). Two CpG sites (site 1: cg23560159, Chr6:32805748 and site 2: cg13563634, Chr6:32805684) associated with TAP2 were selected for validation by pyrosequencing. The validation results showed that only Site 1 (cg23560159, Chr6:32805748) of TAP2 was consistent with the discovery set (Figure 2A and 2B).

mRNA expression analysis

To determine whether dysregulated methylation affects gene expression, qPCR was performed to compare gene expression between DHS patients and dapsone-tolerant control subjects. As expected, TAP2 mRNA levels were up-regulated in DHS patients compared to dapsone-tolerant control subjects (Figure 2C). TAP2 is essential for the MHC-I antigen presentation pathway (McCluskey et al., 2004), which in complex with TAP1 mediates unidirectional translocation of endogenous protein antigens into the endoplasmic reticulum. Expression of TAP1 was assessed. Compared to the dapsone-tolerant control subjects, mRNA levels of TAP1 were also up-regulated in DHS patients (Figure 2D). Correlation analysis demonstrated expression of TAP1, in peripheral blood mononuclear cells (PBMCs) of 43 donors, was positively related to TAP2 (Figure 2E).

Functional differences in antigen-presenting cells (APCs) between DHS patients and dapsone-tolerant control subjects

To determine whether dapsone specific T cells exist in the PBMCs of DHS patients, PBMCs from DHS patients and daponse tolerant control subjects (all carrying HLA-B*13:01) were stimulated with dapsone (100 μM) for six days and then analyzed by enzyme-linked immunospot (ELISPOT). After stimulation with dapsone, responding T cells were specifically activated to produce IFN-γ by PBMCs of DHS patients but not by PBMCs of daponse tolerant control subjects (Figure 3A). To determine whether CD4+T cells or CD8+T cells responded to dapsone, flow cytometric analysis was performed. After dapsone stimulation, CD8+T cells produced IFN-γ, with only slight CD4+T cell activation (Figure 3B). These results suggest that CD8+T cells and to a lesser extent CD4+T cells act as effector cell populations involved in the pathogenesis of DHS.
To explore functional APC differences between DHS patients and dapsone-tolerant control subjects, EBV-B cell lines were generated to serve as APCs and dapsone-specific T cells were generated to serve as effectors. HLA-B*1301-positive EBV-B cell lines from DHS patients or dapsone-tolerant control subjects were co-cultured with dapsone-specific T cells. Compared to dapsone-tolerant control subjects, dapsone treated EBV-B cell lines from DHS patients activated a greater dapsone-specific T cell response as judged by IFN-γ ELISPOT assay (Figure 3C). TAP1 and TAP2 mRNA levels of the EBV-B cell lines were assessed by qPCR. The results showed a consistent up-regulated expression of TAP1 and TAP2 by EBV-B cells from DHS patients compared to dapsone-tolerant control subjects (Figure 3D). These results suggest that functional differences in APCs are critical to the development of DHS, likely due to regulated expression of TAP2 and TAP1.
The dapsone-specific T cell response is TAP-dependent
TAP is the member of the superfamily of ATP-binding cassette transporters, that is composed of TAP1 and TAP2 (Dean and Annilo, 2005). The MHC-I antigen presentation pathway is TAP-dependent, with functional loss of TAP resulting in defective peptide loading and reduced surface expression of MHC-I (Chessman et al., 2008; McCluskey et al., 2004). To explore the immune significance of TAP in the activation of dapsone-specific T cells, TAP function was impaired by ICP47 treatment of C1R-HLA-B*13:01 APCs. In this manner, ICP47 was shown to inhibit surface expression of HLA-I molecules on the APCs (Figure 4A). Correlation analysis showed expression of TAP1 and TAP2 to be positively correlated with HLA-B (Figure 4B and 4C). When incubated with TAP impaired C1R-HLA-B*13:01 APCs, dapsone-specific T cells showed a reduced reactivity compared to incubation with C1R-HLA-B*13:01 APCs infected with a negative control lentiviral vector (Figure 4D).

OR-053

PRELP negatively regulate IL-17A mediated proliferation and inflammatory in psoriasis

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Objective Psoriasis is a common immune-mediated skin diease that it's global incidence is 1.9%. Psoriasis not only affects physical health, but also disturbs work ability, psycology and healthcare costs of patients. Psoriasis is a common immune-mediated skin disease. For histological, excessive proliferation and aberrant differentiation of keratinocytes is the main feature, cellular immune response involved T cells, dendritic cells(DCs) and releasing of various cytokine and chemokines also play an important role in inflammatory response of psoriasis. IL-17A is an important cytokine involved in pathogenesis of many inflammatory diseases including psoriasis. Blockade of IL-17A has much improved the clinical outcome for psoriasis. PRELP belongs to the class II subfamily of the small leucine-rich proteoglycan (SLRP) family, which binds type I collagen to basement membranes and type II collagen to cartilage. It involved in several paghogenesis process including osteoclastogenesis, infection of Moraxella catarrhalis in respiratory tract, hepatocellular caicinoma and so on. But it's role in skin as well as psoriasis had never been explored. We aimed to identify the role of PRELP in psoriasis.

Methods Bioinformatics analysis was used to identify differentially expressed genes in psoriasis lesions and non lesions as well as treatment by anti-IL17A biologics, RT-PCR was used to detect expression of PRELP and other inflammatory factor in cells and skin of mouse, western blot was used to detect expression of PRELP and key signal molecules in signal pathways, immunochemistry was used to identify expression of PRELP in slice of psoriasis patients, immunofluroscence was used to detect expression of PRELP in keratinocyte, transfection of plasmid, chromatin immunoprecipitation was used to explore combination fo stat3 with the promoter of PRELP, CCK8 and apoptosis assay were used to detect effect of PRELP on physiology of keratinocyte and flow cytometry was used to detect differentiation of CD4 T cells to TH17 cells both in vivo and in vitro.

Results Three datasets in regard to biologics targeting IL-17A were sellected, differentially expressed genes(DEGs) were identified between baseline and after-treatment, then most of those genes were identified differentially expressed between lesional(LS) and non-lesional(NL) tissue in psoriasis patients. Those differentially expressed genes then undergone functional enrichment analysis and protein interaction analysis, hub genes were sellected. Molecular functions of genes high expressed in lesion samples and down-regulated after treatments were mainly involved with"cell division", "cell proliferation" and "mitotic nuclear division". But the number of differentially expressed genes showing low expressions in lesion samples and up-regulated after treatments were too few to enable any clear detection of their functions.

The highest ranking hub genes included FBLN1, IGFBP5, PRELP, OGN, OMD, MFAP5, MGP and ISLR. Among them, PRELP(proline/arginine-rich end leucine-rich repeat protein) was up-regulated after treatments by the three biologics and low expressed in psoriasis lesions compared to non-lesions. We confirmed that PRELP was lowly expressed in imiquimod induced psoriasis model in mouse, IL-17A treated HaCat cells as well as lesions from psoriasis patients. Also, PRELP was low expressed in epidermis of psoriasis mouse but not dermis compared to un-treated mouse. Over expression of PRELP inhibited proliferation and promoted apoptosis of HaCat cells. PRELP could inhibit IL-17A mediated NF-κB and MAPK signalling molecules, as well as the release of cytokines contributing to psoriasis development, including TNF-α and IL-1β. Conversely, inhibition of PRELP in mouse psoriatic skin exacerbated psoriasis symptoms. Mouse treated with PRELP si-RNA in vivo increased epidermis thichness and inflammation in psoriasis model. Datasets analysis also found when psoriasis patients treated with brodalumab and methotrexate, high level of PRELP at baseline was associated with better therapeutic response, and that the expression level of PRELP was not associated with the therapeutic response with treatment of etanercept and ustekinumab. Which indicated the specific of PRELP in IL-17A pathway. K14-AAV-PRELP also be used to increase expression of PRELP in keratinocyte in mouse, results show that, overpression of PRELP in keratinocyte alleviated symptom of psoriasis in mouse with the decreased thickness of epidermis. Expression of IL-17A, IL23 also decreased in AAV group, which indicated PRELP will return to inhibit differentiation of CD4 T cells to TH17 cells. Mechanically, we found that IL-17A decreased expression of PRELP through induction of STAT3, a transcription factor that negatively regulates the transcription of PRELP.

Conclusion In this study, through bioinformatics analysis of datasets about biologics targeting IL-17A in psoriasis, prelp was identified a candidate gene. Experiments in vivo and in vitro verified that prelp could reverse IL-17A mediated pathogenesis of psoriasis. We postulate than PRELP is a negative regulator in IL-17A mediated signal in psoriasis. PRELP is a potential target for the treatment of psoriasis. In the further, drug or equipment that could increase PRELP will be effective to treat psoriasis.

OR-054

Study on the Role and Preliminary Mechanism of METTL3-mediated m6A Modification in Atopic Dermatitis

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the Third Xiangya Hospital

Background Atopic dermatitis (AD) is a common chronic inflammatory skin disease that is characterized by eczema-like lesions accompanied by intense pruritus. Prolonged recurrent pruritus has a significant adverse psychological impact on the patient. Sweating, mechanical irritation, and emotional stress can all aggravate itching in AD. Genetic, environmental, and immune factors are considered as important factors in its pathogenesis. In recent years, N6-methyladenosine (m6A) methylation modification has been identified as a widespread RNA modification in eukaryotes. It can affect cell growth, differentiation, transcriptional regulation, and disease occurrence and development by regulating the processes of RNA translation, degradation, stability, etc. Studies have shown that m6A methyltransferase METTL3 plays an important role in promoting tumor proliferation, normal hematopoiesis, leukemia cell myeloid differentiation, and cancer cell translation. Furthermore, METTL3 has been found to be downregulated in psoriatic skin lesions, and knocking down METTL3 can promote the development of psoriasis by inhibiting m6A methylation. This suggests that METTL3 may play an important role in inflammatory diseases. However, the role and mechanism of METTL3 in AD pathogenesis have not been elucidated. This study aims to explore the role and mechanism of METTL3 in AD, providing new ideas and strategies for its diagnosis and treatment.
Objective: To compare the differences of m6A level and METTL3 expression level in the skin lesion tissues between normal individuals and Atopic dermatitis (AD) patients. To establish an AD-like mouse model induced by a combination of carpotriol liniment and ovalbumin (OVA), and to detect the impact of METTL3-mediated m6A methylation on the occurrence and development of AD by inhibiting the expression level of METTL3.
Methods (1) Skin tissues from healthy control group and skin lesion tissues from AD patients were collected. The m6A methylation level in total RNA of skin lesions was evaluated using the m6A RNA methylation quantification kit. The expression difference of METTL3 in the skin lesion tissues of healthy control group and AD patients was detected using quantitative reverse transcription PCR (RT-qPCR), Western blot (WB), and immunofluorescence (IF) experiments.
(2) AD-like mouse model induced by OVA combined with carpotriol liniment was constructed, and their weight behavior was evaluated using an electronic balance and itches in ten minutes was assessed. Skin lesions such as erythema, papules, epidermal exfoliation, and scales at the site of skin lesions on the back of the mice were scored. Mice were euthanized on the 8th day of the experiment. The skin lesion tissue of the mice was collected for HE staining and RT-qPCR to detect the expression levels of relevant inflammatory factors to evaluate the success of the modeling. The RNA and protein levels of METTL3 in the mouse skin lesions were detected by RT-qPCR, WB, and Immunohistochemical analyses (IHC) in the successfully modeled mice.
(3) Repeat the same method to constructed a AD-like mouse model again, randomly dividing mice into four groups: ① Blank group (C), ② AD model group (AD), ③ Solvent control group (DMSO), and ④ METTL3 inhibitor group (SAH). The C and AD groups were subjected to the same modeling method as in the second part. The DMSO and SAH groups were respectively subcutaneously injected with diluted DMSO and SAH every day after modeling. The skin lesions, body weight, and scratching frequency of the mice were evaluated. On the day of euthanasia, Reflectance Confocal Microscopy (RCM) was used to evaluate the thickness of the epidermis and the degree of inflammation in the back of the mice. The skin lesion tissues of the mice were collected for HE staining, RT-qPCR, WB, IHC, and other experiments to detect the RNA and protein levels of METTL3 and evaluate the inhibitory effect of the inhibitor, further exploring the role of METTL3 in the pathogenesis of AD.
Results (1) The overall level of m6A methylation and the expression level of METTL3 in the skin lesions of AD patients were higher than those in the healthy control group.

(2) A mouse model of AD was successfully established by using a combination of carpotriol liniment and OVA applied to the back. Further detection found that the expression level of METTL3 in the skin lesions of the AD model mice was significantly higher than that in the healthy control group mice.
(3) By subcutaneously injecting the inhibitor SAH to interfere with the expression of METTL3 in the AD model mice, it was found that inhibiting the expression of METTL3 could slow down the development of AD and alleviate its severity. Specifically, the mouse skin lesion phenotype improved, the epidermis became thinner, the degree of inflammatory cell infiltration was reduced, and the level of inflammatory factors was lowered.
Conclusion (1) The overall m6A methylation level and the expression of METTL3 are significantly upregulated in the skin lesions of AD patients. (2) Animal experiments have shown that intervention in the expression of METTL3 can slow down the occurrence and development of AD by downregulating the m6A methylation level.

OR-055

Pam3CSK4 and anti-IgE alone or in combination regulate glycolysis / gluconeogenesis and inflammatory effector function of monocyte-derived inflammatory dendritic epidermal cells

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Objective Cell metabolism regulate activation and function of dendritic cells (DCs). In particular, pattern recognition receptors such as TLRs stimulation triggers mature, metabolic reprogramming and function of DCs. Inflammatory dendritic epidermal cells (IDECs) of patients with atopic dermatitis express toll-like receptor 2 (TLR2) and high-affinity IgE receptor (FceRI), but the metabolic changes of IDECs treated with TLR2 and FceRI alone or in combination have not been fully understood.
Method Human peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation by laying blood samples on the Lymphoprep^{TM}. $CD14^+$ monocytes were isolated using magnetic beads according to instructions of the manufacturers and confirmed by flow cytometry (the purity of $CD14^+$ cells was greater than 90 %). We seeded 10^6 $CD14^+$ monocytes/mL IMDM complete medium in 24-well plates. Cells were cultured with human recombinant IL-4 (75 ng / mL), Granulocyte-macrophage colony-stimulating factor (GM-CSF) (50 ng / mL), IgE (1 ug / mL) and β-mercaptoethanol (β-ME) (5 mM). On day 7, we exposed monocyte-derived IDECs (mo-IDECs) to Pam3CSK4 (TLR2 ligand) (1 ug / mL), anti-IgE (FceRI ligand) (5 ug / mL), Pam3CSK4 and anti-IgE for 24 h. After 24 hours of mo-IDECs treatment by Pam3CSK4 and anti-IgE alone or in combination, we applied flow cytometry to examine the expression of cell surface activation markers (CD80, CD83 and CD86), multiplex assay to quantify inflammatory factors (TNF-α, IL-6, IL-10, IL-1 β, IL-12p70, IL-23 and IL-18) in cell supernatant, RNA sequencing to detect the expression of metabolic pathway related genes and targeted energy metabolism to analyze the abundance of metabolites. In addition, we also analyzed the level of extracellular lactate and expression of phosphofructokinase 1 (*PFKL*, *PFKM* and *PFKP*) and fructose-1,6-bisphosphonates 1 (*FBP1*) by qPCR.
Result Compared with unstimulated group, Pam3CSK4 or / and anti-IgE significantly increased the expression of CD80, CD83 and CD86 ($p < 0.05$), while no difference in treatment anti-IgE ($p > 0.05$). To further assess the impact of Pam3CSK4 and anti-IgE alone or in combination on mo-IDECs effector function, we analyzed the release of cytokines, which included TNF-α, IL-6, IL-10, IL-1β, IL-12p70, IL-23 and IL-18. mo-IDECs displayed markedly enhanced production of inflammatory cytokines in response to stimulation with Pam3CSK4 or / and anti-IgE ($p < 0.05$). However, anti-IgE significantly stimulated the release of IL-1β and IL-18 ($p < 0.05$), while not other factors but

showed an weak increased trend. To determine the specific gene expression changes of mo-IDECs induced by Pam3CSK4 and anti-IgE alone or in combination, 4 samples from each group were selected for RNA sequencing. Genes with an | log2FoldChange | > 0 & p-value < 0.05 were categorized as differentially expressed. The results revealed that, in comparison with unstimulated group, treatment with Pam3CSK4 and anti-IgE alone or in combination significantly altered 3908 genes (2216 upregulated, 1692 downregulated), 1119 genes (766 upregulated, 353 downregulated) and 3697 genes (2056 upregulated, 1641 downregulated), respectively. Venn diagram illustrated that 255 common differentially expressed genes (144 upregulated, 110 downregulated) were identified either in Pam3CSK4 and anti-IgE alone or in combination. To further recognize the potential metabolic pathways involved in these differentially expressed genes in detail, we performed KEGG enrichment analysis, results showed that carbon metabolism was affected in different treatment groups. In addition to further investigate the metabolomic change, the pre-treated vs post-treated comparisons were performed. 5 samples from each group were selected for targeted energy metabolites using mo-IDECs induced by Pam3CSK4, anti-IgE and Pam3CSK4 in combination with anti-IgE, as well as unstimulated mo-IDECs. Differential metabolites between groups were identified with Variable Importance in Projection ≥ 1 and fold change ≥ 2 or fold change ≤ 0.5. We found that Pam3CSK4 and anti-IgE alone and in combination significantly altered 13 metabolites (7 upregulated, 6 downregulated), 13 metabolites (0 upregulated, 13 downregulated) and 5 metabolites (0 upregulated, 5 downregulated) through targeted energy metabolism analysis, and these differential metabolites were mainly enriched in glycolysis / gluconeogenesis in carbon metabolism through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and classified the results according to pathway types. Besides, to further explore the role of glycolysis / gluconeogenesis in different stimulated mo-IDECs, we found that pyruvic-acid was a common differential metabolite and downregulated through enrichment analysis using venn plots. Pyruvic-acid was converted to acetyl-CoA, amino acid and lactate, but we did not find an increase in these metabolites in different stimulated mo-IDECs. In order to further clarify the reason of pyruvic-acid reduction, we detected the changes of extracellular lactate and found that there were no significant difference ($p > 0.05$). The expression of *PFKL* and *PFKM* were upregulated in Pam3CSK4 and anti-IgE alone or in combination, while *FBP1* downregulated.

Conclusion Our data indicated Pam3CSK4 and anti-IgE, alone or in combination, mediate glycolysis / gluconeogenesis and inflammatory effector function of mo-IDECs to varying degrees.

OR-056

A lipid metabolism-related gene model reveals prognosis and immune microenvironment for cutaneous melanoma

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Background and Objective Skin cutaneous melanoma (SKCM), a devastating malignant tumor, causes 90% of skin cancer mortality. More than 232100 people developed SKCM and 55,500 patients died of the disease each year across the world. Recently, molecular-targeted drugs and immune checkpoint inhibitors were effective in treating unresectable or metastatic melanoma and improved the 5-year-survial rate. However, due to the limits on the applicable population, drug resistance and side effects, only a small number of patients benefited from them. Thus, it is necessary to explore a risk stratification method and to recognize prognostic genes for personalized targeted therapy of melanoma patients.

Lipids are essential structural basis of biological membranes, and also function as signaling molecules and energy source. Recently, lipid metabolism reprogramming has been regarded as a hallmark of tumors. Ever-increasing evidence indicated that lipid metabolism disorder was significantly correlated with tumorigenesis, tumor progression and treatment. In melanoma, researches have shown that lipid metabolic dysregulations could promote cell growth and metastasis, and therapeutic approaches targeting lipid metabolism could be applicable for

melanoma treatment. Furthermore, based on public datasets, the lipid metabolism-related genes (LMGs) prognostic signatures were developed in lung adenocarcinoma, breast cancer and osteosarcoma. While the correlations of LMGs with the prognosis of cutaneous melanoma patients is still elusive. Tumor microenvironment (TME) plays an crucial role in cancer progression, metastasis and treatment resistance. In addition, diverse components of TME have distinct metabolism programs, and lipid metabolism plays a critical role in the TME and its dysfunction affects the immune response. Therefore, targeting lipid metabolism could be considered as a new strategy for malignancy.
Herein, we first accumulated the relative expression of LMGs in cutaneous melanoma samples. Besides, we created a prognostic model with specific LMGs. Additionally, the characteristics of enriched pathways, immune infiltration in TME and immunotherapy response was also investigated.
Methods The data of SKCM patients was downloaded from TCGA and was taken as the training set, and GSE65904 as the validation data set. LASSO algorithm and multivariate Cox regression analysis were used to construct a prognostic risk model. Functional enrichment and immune infiltration analysis were used to reveal the possible mechanisms and the response to immunotherapy.
Results A predictive model consisting of four lipid metabolism-related genes was constructed, which could predict the survival of patients with cutaneous melanoma. Functional enrichment revealed enriched immune-associated pathways. Immune infiltrating analysis exhibited the distinct immune microenvironment. Real-Time PCR Analysis and Western blot were adopted to verify the results.
Conclusion A novel prognostic model of lipid metabolism-related genes, including the genes ADH4, ALDH7A1, HADH and HADHA,
was investigated in cutaneous melanoma. This model could be served as a promising biomarker to predict the prognosis and treatment response of these patients.

Abstract
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OR-057

Skin fibrosis pathogenesis dynamic analysis reveals a suppressive role for TREM2-dependent macrophages

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Objective. Systemic sclerosis (SSc) is a rare autoimmune disease with high morbidity and mortality rates, characterized by vasculopathy, immune dysfunction, and fibrosis of the skin and multiple visceral organs. Progressive skin fibrosis is the distinguishing hallmark of SSc, and the degree of skin thickness score correlates with internal organ involvement. Immunological abnormalities contribute to the onset and progression of SSc fibrosis, which triggered by vascular alternation because of the apoptosis and the release of damage-associated molecular patterns (DAMPs) of endothelial cells. Previous studies showed that multiple immune cells have been implicated in SSc. Macrophages were identified as one of the key drivers of the inflammatory and fibrotic manifestations in SSc, in which macrophages establish a complex communication system with ECM-producing myofibroblasts and endothelial cells via producing pro-fibrotic mediators and various cytokines/chemokines, suggesting their pathogenic role in tissue fibrotic progress. Interestingly, macrophages also showed the property of inflammatory resolution via efferocytosis to cleave cellular debris and apoptotic bodies. Moreover, in vitro-trained macrophages were anti-fibrotic in mice and pre-clinical models of SSc. These results suggested macrophages are at the crossroads of the SSc pathogenic processes, and might play a biodirectional role in SSc. Macrophages are plastic populations and can adopt various activation states depending on their dynamic microenvironment, implying that functionally important cell states are limited in time and space. However, macrophage heterogeneity, including new cell subpopulations or cell states, on the process of SSc fibrosis remains unclear. We aimed to investigate the comprehensive and dynamic pathogenesis of skin fibrosis using Drop-based single-cell transcriptome analysis and investigate the role of massively induced TREM2$^+$ macrophage subpopulation in the course of the disease, providing a basis for establishing new biomarkers and therapeutic approaches targeting SSc fibrosis.
Methods. Single-cell RNA-sequencing (scRNA-seq) helps dissect the dynamic process of disease pathology and investigate the transcriptional states of a heterogeneous cell population or cell states at a high resolution. We analyzed the transcriptomes of 95,362 skin cells, focusing on 956 macrophages from four-time points after bleomycin (BLM) treatment (d 3, 7, 14, and 28) and untreated control (d 0) mouse skin by single-cell RNA sequencing (10X Genomics). Confocal immunofluorescence microscopy of TREM2$^+$ macrophages validated transcriptional results, and the role of TREM2-dependent macrophages in skin fibrosis was investigated using knockout mice and intraperitoneal transferring TREM2$^+$ macrophages.

Results. We revealed the basal pathology's dynamism of skin fibrosis, which exhibited the major hallmarks and precise pathological processes associated with the signaling pathway. Initialization-phase cells (d3) were characterized by DNA and RNA polymerase activity, which might be associated with DNA damage of cells; whereas early-stage (d7) had a characteristic of inflammatory response, neurogenesis, response to hypoxia and mechanical stimulus, and signaling pathway such as JAK-STAT, suggesting tissue stress responses to injury. The features of vasoconstriction, myoblast differentiation, complement activation, and Notch signaling pathway were enhanced at the middle stage (d14), implying extensive and persistent tissue damage. In the last stage (d28), myoblast proliferation, collagen metabolism process, and VEGF signaling pathway were augmented, indicating the occurrence of irreversible fibrosis. Macrophages expressing TREM2 were massively induced at all-time of skin fibrosis. Grouping the L-R pairs by functional annotations revealed that receptors of vasculature, immune cells, and fibroblast recognized the upregulated ligands in TREM2$^+$ MΦs, which were implicated in angiogenesis, autophagy, and lipid metabolism via COX2 (Ptgs2)-CAV1, TGM2-SDC4/ITGB1, PTLP-ABCA1, SLPI-PLSCR1/4 L-R interactions, respectively. However, most chemokines expect CXCL16 in TREM2$^+$ MΦs were downregulated from d3 after BLM treatment, suggesting a decreased role in the recruitment of immune cells. We also detected increased receptors in TREM2+ MΦs receiving signals from other cells were involved in cell survival, phagocytosis, and calcium homeostasis via TNFR2 (Tnfrsf11b)-GRN, MET-SEMA4D/SEMA5A, CD48-CD2/CD244, FCGR1-BGP (Ceacam1), CD180-LY86, CXCR4-CXCL12, IL4RA-IL13 R-L interactions; and decreased receptors resulting in the dysregulation of coagulation and complement activation corresponding to fibrosis and hypercoagulative state in SSc (e.g., CD36-THBS1/COL1A1/SAA1, IL17RA-IL17A/IL17F, C5AR1-RPS19/GNAIL2). Thus, TREM2+ MΦs intensively interacted with the immune and stromal compartments, suggesting that TREM2$^+$ MΦs might be involved in the pathogenesis of skin fibrosis. Genetic ablation of *Trem2* in mice globally accelerated and aggravated skin fibrosis, whereas transferring TREM2hi macrophages improved and alleviated skin fibrosis. In addition, TREM2$^+$ macrophages of skin fibrosis undergoing fetal-like reprogramming in mice were also found in human SSc.

Conclusion. We attempted to dissect the dynamic features of basal pathogenesis and seek new cell states or cell populations to identify their function in skin fibrosis of SSc. Our data supported three conclusions: first, immune cells exhibited heterogeneous and kinetics as the disease progresses; second, massively induced TREM2-dependent macrophages suppressed skin fibrosis in a mouse model of BLM treatment; third, the features of TREM2$^+$ MΦs in skin fibrosis have a fetal-like reprogramming in mice and humans. These findings decipher the dynamic pathogenesis of skin fibrosis and identify TREM2 signaling as a major pathway by which macrophage subpopulation plays an anti-fibrosis function, highlighting TREM2 as a key target to develop advanced macrophage therapies for refractory fibrosis-like diseases.

OR-058

Metabolomic Profiling Differences among Vitiligo patients and Healthy Subjects in Plateau and Plain: A LC-MS-based Metabolomic Analysis

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Background Vitiligo is the most common disorder of depigmentation, which is caused by multiple factors like metabolic abnormality, oxidative stress and the disorders of immune. In recent years, several studies have used untargeted metabolomics to analyze differential metabolites in patients with vitiligo, however, the subjects in these studies were all in plain area.

Objective Metabolites altered in the plasma of vitiligo patients in plateau and plain areas, indicating that the pathogenesis of vitiligo patients from plateau and plain areas is distinct.

Methods We aimed to compare the serum metabolomic profiles among healthy subjects in plateau, healthy subjects in plain, vitiligo patients in plateau and vitiligo patients in plain and to explore the potential metabolic biomarkers and pathways. The serum metabolomic profiles of 30 subjects in plateau with vitiligo, 31 subjects in plain with vitiligo, 30 healthy subjects in plateau, and 32 healthy subjects in plain were determined by an untargeted metabolomic analysis utilizing liquid chromatography-mass spectrometry. A series of multivariate statistical analyses was subsequently used.

Results

Subject Characteristics

The vitiligo patients in plain were older than healthy subjects in plain ($P<0.001$). The ages between vitiligo patients in plateau and healthy subjects in plateau were comparable ($P=0.749$). The ages of healthy subjects in plain and plateau were also comparable ($P=0.384$). And there was no significant difference in sex proportions among the four groups ($P=0.079$).

Data Pre-processing

The superposition map of QC total ion current in positive and negative ion modes almost completely overlapped, indicating that the instrument and method remained stable and the data were reliable in this sample detection. PCA showed that the samples of vitiligo patients and healthy subjects have an obvious separation trend. In the PCA of samples of the plateau, the patients and healthy people were basically separated in the positive ion mode, and in the PCA of samples of the plain, the samples of vitiligo patients and healthy subjects showed a clear separation trend, indicating that the metabolome of vitiligo patients was significantly shifted compared with healthy subjects in both plateau and plain.

Analysis of the differential metabolites

Multivariate analysis indicated a distinct separation between the healthy subjects from plateau and plain areas in electrospray positive and negative ions modes, respectively.

A total of 48 differential metabolites were identified (Table 1). In addition, amino acids and carnitine metabolites were generally higher in the plateau population. In healthy subjects from the plateau region, the metabolite that was most significantly decreased was Allodeoxycholic acid, while the metabolite that was most significantly increased was sphingosine 1-phosphate (S1P).

Similarly, a distinct separation between vitiligo patients and healthy controls from plateau and plain areas was detected in the two ions modes. A total of 21 differential metabolites were identified in plateau (Table 2), and 40 in plain (Table 3). Increased metabolites were mainly amino acids and their metabolites, and the decreased metabolites were mainly phospholipids, glycerol monoesters and diethanolamine compounds in both areas. And in vitiligo patients from the plateau and plain regions, the most significantly increased metabolites were citric acid and S1P respectively.

Among the identified metabolites, the serum levels of sphingosine 1-phosphate (S1P) were markedly higher in vitiligo patients compare to healthy subjects in plain and markedly higher in healthy subjects in plateau compare to those in plain.

Metabolic Pathway Analysis

We confirmed the possible metabolites involved in the enrichment analysis and metabolic pathway analysis between healthy subjects in plateau and plain. According to the types of compounds involved in the metabolic pathways, it was summarized that differential metabolites mainly include amino acid metabolism, fatty acid metabolism, phospholipid metabolism, etc. And we confirmed the possible metabolites involved in the enrichment analysis and metabolic pathway analysis in plateau and plain, it was summarized that differential metabolites mainly include amino acid metabolism, fatty acid metabolism, phospholipid metabolism, tricarboxylic acid cycle, etc.

Analysis of ROC

The differential metabolites identified by screening were analyzed by ROC analysis to analyze its diagnostic significance for vitiligo. The closer the AUC value is to 1, the better the predictive ability is. Generally, AUC> 0.7 is considered to have good predictive ability. When all variables are used for prediction, the AUC is 0.987 and the 95% confidence interval (95 CI) is 0.924 in plateau, and the AUC is 0.992 and the 95 Cl is 0.949 in plain, so the model has a high diagnostic accuracy in the prediction ability of vitiligo disease.

Conlusion There are significant differences in serum metabolome between vitiligo patients and healthy subjects in both plateau and plain areas, as well as in healthy subjects from plateau and plain areas.S1P metabolism alteration may be involved in the pathogenesis of vitiligo. To sum up, our study shows that there are significant differences in serum metabolome between vitiligo patients and healthy subjects in both plateau and plain areas, as well as in healthy subjects from plateau and plain areas. We compared the metabolome of healthy subjects in plateau and plain areas, and on this basis found a significant difference in serum levels of S1P. Besides, S1P was significantly increased in patients with vitiligo in plain area. S1P may be involved in the pathogenesis of vitiligo and S1P receptor could be a promising therapeutic target for vitiligo.

OR-059

Oculocutaneous albinism type IV: Novel Compound Heterozygous Mutations in the SLC45A2 Gene in a Chinese case

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Background Oculocutaneous albinism is a rare autosomal recessive disorder characterized by a reduction of pigmentation in skin, hair and eyes, and oculocutaneous albinism type IV usually results from pathogenic pure or compound heterozygous mutations in the Solute carrier family 45, member 2 (SLC45A2) gene. The clinical presentations of oculocutaneous albinism type 4 (OCA4) patients often present with white or yellow hair and reduced skin pigmentation, which is characterized by a high degree of heterogeneity. Some individuals lose more pigment as they age, while others remain pigment deficient for life.
Objective To report a Chinese patient suspected of oculocutaneous albinism and identify the causing mutation.
Case report The proband is a 6-year-old Chinese Han boy. He was born with paler-than-normal skin all over his body with lighter hair and eyebrow. He has had mild ocular photophobia since the age of 3 years. His skin color has not changed significantly since birth. The hair color was slightly darker than before, while the eyebrows were still light in color with age. Strabismus, hyperopia, astigmatism and the absence of iris pigment were found in both eyes. He has been advised to wear glasses since June 2022. The cryptorchid repositioning has been performed in October 2018. His parents and brother were phenotypically normal.
Methods Peripheral blood samples were taken from the proband, his parents and elder brother after informed written consent was obtained. WES was performed in all samples. Genomic DNA were extracted from the test samples. Libraries were prepared to capture and enrich the exons and adjacent introns of the target genes. The enriched target fragments are sequenced using the Next-generation sequencing platform. Sanger sequencing was then used to verified the mutations. The sequenced DNA sequences are compared with the human genome reference sequence and evaluated for coverage of target regions and sequencing quality. Mutations were also analyzed bioinformatically and for pathogenicity, and the target regions included the coding sequence of the target gene and its adjacent ±10bp intron region.
Results In this text, compound heterozygous mutations of c.1304C>A (p.S435Y) and c.301C>G (p.R101G) in SLC45A2 gene were detected, which were inherited from the father and mother, respectively. The above variations were not detected in the elder brother and 100 other normal individuals. The missense variant in this gene is a substitution of base C for A at position 1304, resulting in a codon change from encoding Serine to Tyrosine at position 435. Also, the substitution of base C for G at position 301 changes codon 101 from encoding Arginine to Glycine. Based on the ACMG guidelines, we can interpret the c.1304C>A (p.S435Y) variant as a suspected pathogenic variant and the c.301C>G (p.R101G) variant as a clinically significant unspecified variant. The diagnosis of oculocutaneous albinism type 4 is confirmed.

We found about 90 mutations reported in SLC45A2 gene, including 46 homozygous mutations and 48 compound heterozygous mutations, which included missense, nonsense, insertions, deletions, and frameshift mutations. Of the cases, The largest number of mutations occurred at the c.469G locus, all at c.469G>A (p.D157N), followed by 7 indel mutations occurring at the c.986 locus, all at c.986del (p.T329Kfs*69). Irrespective of the nature of the mutation, all OCA4 patients showed the typical features of a reduction of pigmentation in skin, hair and eyes. Of these, 44.19% showed white hair, 39.53% showed blond hair, and 6.98% showed brown hair. 93.02% of the patients had iris changes. Of these, 44.19% showed blue iris, 16.28% showed blue-gray iris, and 11.63% showed brown iris. 74.42% of the patients had positive nystagmus and 20.93% had negative nystagmus. No particular genotype-phenotype correlation was found in the literature. Through phenotypic comparisons, we found that clinical studies alone could not distinguish between patients with a homozygous mutation and with a compound heterozygous mutation.

Treatment and prevention The current treatment is focused on correcting visual acuity. To improve this situation, symptomatic measures such as attention to light avoidance and wearing tinted glasses were performed. Patients should stay out of the sun from an early age, as prolonged exposure to the sun is a major risk factor for skin cancer. The annual eye exams and re-evaluation of vision to accurately correct refractive errors. And patients are advised to have a cancer screening assessment of the skin every six month.

Discussion: OCA is a group of genetic disorders that affect skin, hair and eye pigmentation due to abnormal production of melanin. The clinical phenotype of OCA patients includes generalized reduction of pigmentation (skin, hair, eyes, etc.), visual impairment, nystagmus, photophobia, etc. Tomita et al. published the first clinical case report of OCA in 1989, in which the patient had a complete lack of melanin in the eye and skin. The reason for this phenotype is the occurrence of nonsense mutations in tyrosinase. OCA is genetically heterogeneous, which can be classified into four main types based on the presence of mutations in four genes (TYR, OCA2, TYRP1 and SLC45A2). Of these, OCA types 1 (OCA1) and type 2 (OCA2) account for about 40% and 50% of OCA cases, respectively. In most populations, the prevalence of OCA4 is 1 in 100,000. In Japan, OCA4 is the second most common albinism after OCA1, with a 24 – 27 % prevalence. Among the pathogenic mutations of the SLC45A2 gene, p.D157N (c.469G>A) is the most common mutant allele in Japanese OCA4 patients. A comparison of findings in patients with the common D157N mutation and other mutations did not show major differences, and does not point to a particular genotype-phenotype correlation.

Conclusion The compound heterozygous mutation found in this study, c.1304C>A (p.S435Y) and c.301C>G (p.R101G), is first reported in literature. Treatment is focused on correcting visual acuity. Our findings further enrich the reservoir of SLC45A2 mutations in OCA4. Meanwhile, WES and Sanger sequencing were very effective in detecting the pathogenic gene in clinic.

OR-060

Predictive factors of atopic-like dermatitis induced by IL-17A inhibitors in patients with psoriasis: A 2-year follow-up study

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Objective Psoriasis is a chronic inflammatory disease caused by hereditary and environmental factors. Interleukin-17A (IL-17A) inhibitors, including secukinumab and ixekizumab, have shown high efficacy with a favourable safety profile and have gradually become a milestone in the treatment of psoriasis. However, several cutting-edge studies have reported the development of atopic-like dermatitis (ALD) in patients with psoriasis treated with IL-17A inhibitors, and the number of cases is increasing. The incidence rate of this CAE induced by biologics ranges from 1.0% to

12.1%. Although the pathogenesis of ALD has not yet been fully elucidated, it is assumed that the immune response is shifted from Th1/Th17-dominated to Th2-dominated through the inhibition of IL-17A (a Th1/Th17 cytokine), resulting in the onset of atopic dermatitis (AD) phenotype. In addition to immunological factors, recent evidence suggests that environmental factors, such as smoking habits and pet feeding history, play a significant role in the development of ALD. However, studies reporting the risk factors of ALD induced by IL-17A inhibitors are scarce. To date, evidence regarding the management of drug-induced ALD remains limited. Symptoms of ALD are similar to those of psoriasis. ALD can develop consequent to IL-17A inhibitor use, which can often lead to misdiagnosis and poor management. Thus, this study aimed to estimate the frequency of IL-17A inhibitor-induced ALD and explore its clinical correlates, risk factors, and treatments in a psoriasis cohort.

Materials and Methods

Study Design

This retrospective study included psoriasis patients (with or without psoriatic arthritis) who developed ALD during treatment with IL-17A inhibitors at our centre between July 2020 and July 2022. Data including age, sex, body mass index (BMI), type of IL-17A inhibitor, duration of IL-17A inhibitor use (weeks), presence of ALD, severity of AD (scoring atopic dermatitis index [SCORAD], eczema area and severity index [EASI] and numerical rating scale [NRS]), severity of psoriasis at the time of ALD onset (psoriasis area and severity index [PASI], body surface area [BSA], and dermatology life quality index [DLQI]), and personal and family history of atopic diseases were collected. Laboratory indices, including eosinophil levels and serum immunoglobulin E (IgE) levels, were also collected. Environmental factors, such as smoking habits (current smokers, past smokers and non-smokers), pet feeding history (owning or feeding pets in the present or having owned or fed pets in the past), and drug allergy history, were recorded. ALD lesions were defined as the classical form, with erythema, papule, lichenified and excoriated plaques, mainly at the flexure areas of the head and neck. All patients with ALD were evaluated by three dermatologists for agreement, and dermoscopy was used if necessary. A Naranjo score8 ≥4 indicated that ALD was related to IL-17A inhibitors. The extent and distribution of ALD and dermatological treatments were also reviewed, if available. In addition, treatment and outcomes of ALD were recorded. Patients lost to follow-up during the 2 years were excluded from the analysis.

Statistical Analysis

All analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Continuous variables were evaluated as means and compared using the t-test, if the data were normally distributed. Dichotomous variables were calculated as proportions and compared using the c2 test or Fisher's exact test. The Mann–Whitney U test was used for non-parametric data. All variables were considered significant at 0.1 level and any variables considered to be potentially clinically important occurrence-associated risk factors were evaluated in a stepwise fashion using a binary logistic regression model. Odds ratios (ORs) and 95% confidence intervals (CIs) were also determined. $P<0.05$ was considered statistically significant.

Results

Patients Characteristics

Herein, 226 patients with mild-to-severe psoriasis (with or without psoriatic arthritis) treated with IL-17A inhibitors between July 2020 and July 2022 were included. Participants' clinical features and demographics are shown in Table 1. The average age of patients was 45.45 years (standard deviation [SD] = 15.49), and 70 (31.0%) of the patients were women. The median duration of treatment with IL-17A inhibitors was 41 weeks. All patients had a plaque phenotype (with or without psoriatic arthritis). A total of 187 patients were treated with secukinumab and 39 with ixekizumab. A family history of atopic disease was identified in 8 (3.5%) patients, and a personal history of atopic disease was present in 24 (10.6%) patients. The average BMI was 24.88 kg/m2. Of the 226 patients, high serum IgE was recorded in 30 (13.3%) and elevated blood hypereosinophilia was reported in 9 (4.0%) patients. Regarding environmental factors, 12 (5.3%) patients had a history of drug allergy, and 31 (13.7%) had a pet feeding history. In addition, 147 (65.8%) patients were current or past smokers. As for their previous systematic therapies of psoriasis, 70 (31.0%) patients had been treated with acitretin, 33 (14.6%) with methotrexate, 7 (3.1%) with ciclosporin, and 24 (10.6%) with biologics.

Main Outcome

ALD was observed in 14 patients; the estimated incidence of ALD in our psoriasis cohort was 6.19%. Of these, 4 (28.6%) patients were women, and the average BMI was 23.93 kg/m2. Twelve patients were treated with secukinumab and 2 with ixekizumab. Of the 14 patients with ALD, 12 (85.7%) had a personal history of atopic disease, including asthma and atopic rhinitis, and 1 patient had both asthma and atopic rhinitis. Two (14.3%) patients had a family history of atopic disease. Pet feeding history was recorded in 5 (35.7%) patients, and only 1 patient had a history of drug allergy to penicillin. Of the 14 patients, 9 had elevated IgE levels, and only 1 patient showed blood hypereosinophilia. The median time between the first IL-17A inhibitor injection and the appearance of ALD was 25.96 weeks (range, 3.0–69.7 weeks). Seven (50.0%) patients were treated with acitretin and none were treated with methotrexate or ciclosporin before IL-17A inhibitor. Only 1 (7.1%) patient was bio-experienced. The distribution of ALD was single (5 patients, 35.7%) or multiple (9 patients, 64.3%; Table 2). All patients had affected flexural regions (Figs. 1a, 1b).

ALD were found on the trunk region in eight patients (57.1%, Fig. 1b), on the limbs in four patients (28.6 %) (Figs. 1a, 1c), and on the palmoplantar region in four patients (28.6%, Fig. 1d).

In addition, two patients (14.3%) had ALD on fold regions and one (7.1%) on the face and lips. Among patients with no history of atopic disease (n = 2, 14.3%), the distribution sites were single, and all lesions appeared on the trunk. Data regarding environmental factors showed that 6 of the 14 patients (42.8%) were current or past smokers. Five of the 14 (35.7%) patients have fed or currently feed a pet, and only 1 patient had a history of drug allergy. The average SCORAD score was 20.1, with the highest score being 55. The mean EASI score was 3.1, and the highest EASI score among patients with ALD was 12.2. The mean NRS score, which represented the degree of itching, among all patients that developed ALD was 2.7, with the highest score being 7.

Analysis of Predictors of ALD Lesions

To evaluate variables predictive of the occurrence of ALD due to the use of IL-17A inhibitors, we compared the positive and negative groups and found that most patients had a history of personal atopic diseases (85.7% vs 5.7%, P = 0.000). Logistic regression analysis confirmed that a personal history of atopic disease (OR 0.013, 95% CI 0.002–0.090; P = 0.000) and an elevated IgE level (OR 0.097, 95% CI 0.015–0.644; P = 0.016) before anti-IL-17A therapy were independent risk factors for ALD development. Other variables such as age, sex, BMI, the type of IL-17A inhibitor, pet feeding history, family history of atopic disease, PASI, BSA, DLQI, previous systematic therapy, smoking habit, drug allergy history, and elevated blood eosinophilia were not statistically significant predictors of ALD (Tables 1 and 3).

Management

Thirteen patients (92.9%) continued with the IL-17A inhibitor, of whom six were managed with topical steroids alone. Two patients received antihistamines alone. Five patients were administered topical steroids and antihistamines. A partial resolution was achieved in seven patients, while seven patients achieved complete resolution of ALD. Only one patient discontinued the IL-17A inhibitor treatment because of an explosion of ALD and switched to tofacitinib. In this patient, the ALD and psoriatic lesions improved without recurrence.

Conclusion In conclusion, ALD is a side effect of IL-17A inhibitors. The findings of our study suggest that a personal history of atopic disease and an elevated IgE level are risk factors for IL-17A inhibitor-induced ALD. After ALD develops, it is crucial to balance the risks and benefits of continuing the original drug. Personalised strategies are recommended according to the severity of ALD and psoriasis, and a prudent evaluation of the effect of skin lesions on the quality of life is important.

OR-061

Epidemiology and antifungal susceptibilities profiles of clinically isolated Aspergillus species in South China (2017-2021)

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Background Aspergillosis is a rising concern worldwide, while its prevalence is not well documented in China. In this retrospective study, the epidemiology and antifungal susceptibilities of Aspergillus were at Meizhou People Hospital, South China.

Methods The current retrospective study was performed at a tertiary care hospital named "Meizhou People's Hospital" in Meizhou, south China. The study included all aspergillosis cases reported from January 2017 to December 2022. The ethical review committee of the hospital approved the study following the standards of the Helsinki Declaration (Reference number: 2021-C-106).

The aspergillosis cases reported in the current study were classified as pulmonary aspergillosis, otoaspergillosis, invasive aspergillosis, and cutaneous aspergillosis. Briefly, pulmonary aspergillosis is a fungal infection caused by the Aspergillus species, primarily affecting the respiratory system. Otoaspergillosis is an Aspergillus infection that affects the ear, frequently resulting in otitis externa or otomycosis. Cutaneous aspergillosis is a type of Aspergillus infection that affects the skin, resulting in skin lesions or ulcers. Invasive aspergillosis is a severe and invasive form of Aspergillus infection that can spread from the respiratory system to other organs in the body [13].

The demographic and clinical data of all aspergillosis patients were collected from the hospital's electronic medical record using an Excel sheet (2021). The data included patients' characteristics such as gender, age, seasonal infection period, patient's type, reported department, sample source, infection type, and underlying patient status. Moreover, the laboratory record of Aspergillus species type and their antifungal-susceptibility tested were also collected.

In routine laboratory protocol, Aspergillus species are identified by combining colony morphology and matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis [14]. Furthermore, the antifungal susceptibilities testing (AST) was performed against five drugs: amphotericin B, caspofungin, itraconazole, voriconazole, and posaconazole (Sigma Aldrich, St. Louis, MO, U.S.A.). The AST was performed following the standard protocol of broth microdilution methods. The Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used as quality control strains. The AST results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [15-17].

Two researchers independently cross-checked all the information collected via Excel sheet to minimize possible errors. The quantitative findings were expressed as medians and interquartile ranges, while qualitative information was presented as absolute numbers and relative percentages. The characteristics of aspergillosis caused by various Aspergillus species (A. fumigatus, A. niger, A. flavus, and A. terreus) were analyzed using the chi-square test for categorical variables and ANOVA for continuous variables. For AST data, the ranges of minimum inhibitory concentrations (MICs), geometric mean (GM), and absolute numbers and percentages of MIC50, MIC90, wild type (WT), and non-wild type (NWT) isolates were quantified for each of the Aspergillus species types. Furthermore, the number of WT isolates against different antifungal agents recovered from inpatients and outpatients was statistically analyzed using the chi-square test. The p-values less than 0.05 were considered statistically significant. GraphPad Prism v.8.0.2 was used for statistical analysis and visualization of the data.

Results

In the current study, a total of 474 cases of aspergillosis were reported, in which high number of cases were caused by A. fumigatus (n= 357, 75.32%), followed by A. niger (n = 47, 9.92%), A. flavus (n = 42, 8.86%), and A. terreus (n = 28, 5.91%). The high number of cases were reported in the year 2018 (n = 140, 29.54%), followed by 2019 (n = 118, 24.89%), while only 18 (3.80%) cases

were reported in 2017. The fluctuation in number of cases each year was reported; however, a 5.94-fold increase in aspergillosis was observed from 2017 to 2021.

Among various departments of the hospital, a high number of cases were reported from the Intensive care unit (ICU) (n = 250, 52.74%), in which 210 (84%) cases were of A. fumigatus, 24 (9.60%) were A. flavus, and 8 (3.20%) each were A terreus and A. niger. Similarly, from the surgical department, a total of 13 (2.74%) cases were reported, of which 11 (84.62%) were A. fumigatus, and 2 (15.38%) were A flavus. A high number of cases from each department were caused by A. fumigatus except otolaryngology, in which 50.75% (n = 34) cases were A. niger among the 67 reported cases.

Among the sample types, sputum had the highest frequency of Aspergillus isolates (n = 294, 62.03%), and A. fumigatus (n = 256, 87.07%) was the most commonly identified species in sputum. A total of 101 (21.31%) cases of bronchoalveolar lavage (BAL) samples were reported, with A. fumigatus being the most frequently identified species in 88 (87.13%) samples. Ear secretion and pus samples had lower frequencies, with 41 (8.65%) and 29 (6.12%) samples, respectively. The A. niger was the most frequently isolated species in both sample types, with 19 (46.34%) samples from ear secretion and 16 (55.17%) samples from pus.

The demographic and clinical characteristics of aspergillosis caused by various Aspergillus species are presented. Based on gender, the high number of cases were reported in the male population (n = 315, 66.46%), while only 159 (33.36%) cases were reported from females. The same trend occurred for all species except A. niger, in which most cases (n = 29, 61.7%) were reported from the female population. Most cases were reported in senior adults with median age of 65 years and an interquartile range (52-76). Comparatively, the A. niger cases were reported in younger ages with a median age of 44 years (IQR; 35-56) followed by A. terreus (57-year 1QR; 37-76).

The distribution of Aspergillus species among various age groups revealed statistically significant differences ($p<0.05$). A. niger was more common in younger age groups (under 50 years), whereas A. fumigatus was more common in older age groups (over 50 years). Only 5 cases were reported in the age group 2-17 years, and all were A. niger. The distribution of A. terreus was relatively consistent across age groups, with the highest number of cases (n = 13, 30.96%) in the age group 51-60 years. A. flavus was highly prevented in age groups older than 50, and 13 (30.96%) cases were reported in age groups 51 to 60. These findings suggest that age may influence the prevalence of different Aspergillus species, emphasizing the importance of considering age as a factor in understanding the epidemiology of aspergillosis.

Seasonally, the distribution of Aspergillus species varies significantly. In the winter, A. fumigatus was the most prevalent species, whereas there was no significant difference in species distribution in the spring and summer. However, A. niger was the most common species in autumn, with a statistically significant difference from other species ($p = 0.0038$). This suggests that seasonal environmental factors may influence the prevalence of Aspergillus species.

Most cases were reported from hospitalized patients (n = 380, 80.17%), while only 94 (19.84%) cases were reported from outpatients. The distribution of Aspergillus species based on patient type (inpatients vs. outpatients) was statistically significantly correlated ($p<0.0001$). A. fumigatus (88.8%) was more frequently detected in hospitalized patients, whereas A. niger (57.45%) was more prevalent in outpatients.

Among the infection types, the high number of cases were reported having pulmonary aspergillosis (n = 406, 85.66%) followed by oto-aspergillosis (n = 56, 11.82%), while only six (1.27%) cases each were reported for invasive and cutaneous aspergillosis. The species distribution for pulmonary and oto-aspergillosis was statistically significant ($p <0.0001$), as the A. fumigatus (n = 344, 96.36%) was frequently reported in pulmonary aspergillosis patients followed by A. flavus (n = 31, 73.81%) and A. terreus (n = 15, 53.57%). On the other hand, A. niger (n = 29, 61.71%) was most frequently reported in oto-aspergillosis patients.

Gastrointestinal disorders (5.7%), hematologic malignancy (4.22%), cardiovascular disease (4.22%), and diabetes (2.54%) were some of the most prevalent underlying medical conditions. The association between underlying health status and different Aspergillus species was not statistically significant, except for hypertension ($p<0.0001$), for which 3 of 5 reported cases were infected with A. terreus and 2 with A. fumigatus. Out of the total cases, only 38 (8.02%) used

immunosuppressant drugs; however, no statistically significant relationship was found between immunosuppressive drug use and Aspergillus species distribution (p-value = 0.5731).
The antifungal susceptibilities profiles of Aspergillus species are summarized in table 2. Amphotericin B had relatively high MIC values against all species ranging from 0.125 to 1 for MIC50 and 0.25 to 4 for MIC90. A. niger had the highest percentage of amphotericin B NWT isolates (4/47, 8.52%), followed by A. terreus (2/28, 7.15%), A. flavus (2/42, 4.77%), and A. fumigatus (3/357, 0.85%). Caspofungin had lower MIC values and a 100% WT percentage against A. fumigatus and A. terreus, indicating that these species are more susceptible to caspofungin. In contrast, the percentages of NWT isolates in A. niger and A. flavus were 2/47 (4.26%) and 4/42 (9.53%), respectively. Among the tested azoles, posaconazole and voriconazole were more susceptible than itraconazole. A. niger, A. flavus, and A. terreus all showed 100% susceptibility to voriconazole. Similarly, A. flavus and A. terreus were 100% susceptible to posaconazole. For itraconazole, a high number of NWT isolates were reported in the case of A. terreus (2/28, 7.15%), followed by A. niger (2/47, 4.26%), A. fumigatus (10/357, 2.81%) and A. flavus (1/42, 2.39%).
Furthermore, we compared the WT isolates reported from inpatients and outpatients to determine the relative difference in susceptibilities between the two groups. Interestingly, we noted that the percentages of WT isolates in the case of amphotericin B and caspofungin were comparatively lower for inpatients than outpatients. On the other hand, for azole drugs, the percentages of WT isolates were comparatively higher for inpatients than outpatients. However, the difference was only statistically significant ($p<0.05$) in the case of itraconazole and posaconazole against A. fumigatus, while all others were statistically insignificant.
Conclusion The present study reported 475 aspergillosis cases, with a high proportion of cases caused by A. fumigatus. Among the non-fumigatus cases, A. niger was reported in high proportion, followed by A. flavus. A high number of cases were reported from ICU, indicating the immunocompromised status of the patients. Pulmonary aspergillosis was reported in high proportion showing that Aspergillus species are mainly involved in respiratory tract pathogenicity. Amphotericin B was reported as a better treatment option for A. fumigatus, while for non-fumigatus isolates, the triazole's susceptibility was greater than amphotericin B, which warrants further large-scale research confirmation. The Aspergillus species reported from outpatients were more susceptible to amphotericin B and caspofungin than triazoles, indicating that the agricultural use of triazole led to this resistance's emergence. Further extensive molecular-based surveillance studies under the umbrella of One-Health approaches are required for the continuous monitoring of Aspergillus species while will provide help in the management of aspergillosis

OR-062

Endogenous hydrogen sulfide deficiency and exogenous hydrogen sulfide supplement regulates skin fibroblasts proliferation via necroptosis.

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1. Objective
An excessive proliferation of skin fibroblasts usually results in different skin fibrotic diseases. Hydrogen sulfide (H2S) is regarded as an important autogenous gasotransmitter with various functions. The study aimed to investigate the roles and mechanisms of H2S on primary mice skin fibroblasts proliferation.
2. Methods
Cell proliferation and collagen synthesis was assessed with the expression of α-smooth muscle actin (α-SMA), proliferating cell nuclear antigen (PCNA), Collagen I and Collagen III. The degree of oxidative stress was evaluated by dihydroethidium (DHE) and MitoSOX staining. Mitochondrial membrane potential (ΔΨm) was detected by JC-1 staining. Necroptosis was evaluated with TDT

mediated dUTP nick end labeling (TUNEL), and expression of receptor interacting protein kinase 1 (RIPK1), RIPK3 and mixed lineage kinase domain like protein (MLKL).

3. Results

3.1. Promoted Proliferation of Skin Fibroblasts from cystathionine γ-lyase (CSE) knockout (KO) Mice was Reversed by Exogenous H2S Supplement. First, whether impaired endogenous Hydrogen sulfide (H2S) production influences skin fibroblast proliferation was investigated in CSE KO mice. It has been confirmed that more α-SMA respects stronger ability of fibroblast proliferation and PCNA participates in DNA replication and cell proliferation. Our study demonstrated that there was more α-SMA and PCNA in skin fibroblasts with CSE deficiency compared of WT mice, which was restored by NaHS (Figure 1A-F), suggesting that the promoted proliferation of skin fibroblasts from CSE deficient mice could be reversed by exogenous H2S supplement.

3.2. Excessive Synthesis of Collagen in Skin Fibroblasts from CSE KO Mice was Reversed by Exogenous H2S Supplement. There were more Collagen I and III in skin fibroblasts with CSE deficiency compared of WT mice, which was restored by sodium hydrosulfide (NaHS) (Figure 1G-J), suggesting that excessive collagen synthesis in skin fibroblasts from CSE deficient mice could be reversed by exogenous H2S supplement.

3.3. Promoted Oxidative Stress and Mitochondrial Membrane Potential Impairment in Skin Fibroblasts from CSE KO Mice was Attenuated by Exogenous H2S Supplement. Compared of WT mice, both DHE and MitoSOX red fluorescence were significantly increased in the skin fibroblasts from CSE KO mice, indicating an increased intracellular superoxide anion and mitochondrial superoxide in the skin fibroblasts of CSE KO mice. Moreover, above enhanced oxidative stress was significantly restored by NaHS (Figure 2A-B), suggesting that promoted oxidative stress production could be reversed by exogenous H2S supplement. In addition, compared of WT mice, red fluorescence for JC-1 aggregates was diminished, while green for monomers was significantly enhanced in skin fibroblasts from CSE KO mice, indicating a reduction in ΔΨm in CSE KO skin fibroblasts. After NaHS pretreatment, red fluorescence was significantly strengthened but green was suppressed (Figure 2C), verifying that impaired ΔΨm in skin fibroblasts from CSE deficient mice could be improved by exogenous H2S supplement.

3.4. Promoted Necroptosis in Skin Fibroblasts from CSE KO Mouse was Alleviated by Exogenous H2S. Compared of WT mice, TUNEL positive cells were significantly increased if CSE was dificient. Additionally, RIPK1 and RIPK3 expression were increased, and MLKL phosphorylation was also enhnaced, indicating a promotion in necroptosis. After NaHS pretreatment, TUNEL positive ratio, RIPK1 and RIPK3 level, and MLKL phosphorylation were all significantly inhibited (Figure 2D-H). It suggested that promoted necroptosis in skin fibroblasts from CSE KO mouse could be reversed by exogenous H2S supplement.

3.5. NaHS Suppressed Proliferation of Mouse Skin Fibroblasts with transforming growth factor-β(TGF-β) Stimulation. Above studies confirmed that endogenous H2S production disorder promoted proliferation of mouse skin fibroblasts, and exogenous H2S administration inhibited skin fibroblasts proliferation without any pathological factors. However, whether H2S has similar effects on the proliferation of skin fibroblasts with pathological stimulation remained unknown. Our further results demonstrated that compared of control group, TGF-β significantly enhanced α-SMA and PCNA expression, which was attenuated by NaHS (Figure 3A-F). It suggested that exogenous H2S supplement inhibited mouse skin fibroblasts proliferation with TGF-β stimulation.

3.6. NaHS Inhibited Collagen Synthesis in Mouse Skin Fibroblasts with TGF-β Stimulation. Compared of control group, TGF-β enhanced Collagen I and III expressions, which were attenuated by NaHS (Figure 3G-J). It suggested that exogenous H2S supplement inhibited collagen synthesis in mouse skin fibroblasts with TGF-β stimulation.

3.7. NaHS Attenuated Oxidative Stress and Improved Mitochondrial Membrane Potential in Mouse Skin Fibroblasts with TGF-β Stimulation. Compared of control group, both DHE and MitoSOX red fluorescence in skin fibroblasts with TGF-β stimulation were significantly strengthened, which were attenuated by NaHS pretreatment. Moreover, above enhanced intracellular superoxide anion and mitochondrial superoxide was significantly weaken by NaHS (Figure 4A-B), suggesting that exogenous H2S supplement attenuated oxidative stress in mouse skin fibroblasts with TGF-β stimulation. In addition, compared of control group, red fluorescence for JC-1 aggregates was diminished, while green for JC-1 monomers was significantly enhanced in skin fibroblasts with TGF-

β stimulation, which were restored by NaHS (Figure 4C). It suggested that exogenous H2S supplement improved ΔΨm in mouse skin fibroblasts with TGF-β stimulation.
3.8. NaHS Suppressed Necroptosis in Mouse Skin Fibroblasts with TGF-β Stimulation. Compared of control group, TUNEL positive cells were significantly increased in skin fibroblasts with TGF-β-stimulation. Additionally, the expressions of RIPK1 and RIPK3 were increased, and MLKL phosphorylation was also enhanced, which were all reversed by NaHS (Figure 4D-H). It suggested that exogenous H2S supplement suppressed necroptosis in mouse skin fibroblasts with TGF-β stimulation.

4.Conclusion

Endogenous H2S production impairment in CSE deficiency mice accelerated skin fibroblasts proliferation via promoted necroptosis, which was attenuated by exogenous H2S. H2S supplement also alleviated proliferation of skin fibroblasts with transforming growth factor-β1 (TGF-β1) stimulation. This study provides evidence for H2S as a candidate agent to prevent and treat skin fibrotic diseases.

OR-063

Clinical factors associated with remission of obese acanthosis nigricans after bariatric surgery

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Aims Acanthosis nigricans (AN) is a skin disease characterized by hyperkeratosis, pigmentation, papilloma-like hyperplasia, and a velvet-like rash. It occurs in folds of the skin on the neck, armpits, external genitalia, and face. The obesity-associated AN, the dominant type of AN described by Schwartz, is usually related to many metabolic syndromes such as obesity (OB), glucose and lipid metabolic disorders, sex hormone disorders, and hyperinsulinemia. AN is considered as a specific epidermal marker of metabolic disorders such as insulin resistance and early diabetes. It seriously affects the physical and mental health as well as the living quality of patients. Weight loss is a treatment option to improve the clinical outcomes in obese patients with AN. In terms of both short-term and long-term weight loss, bariatric surgery offers greater benefits than nonsurgical approaches. There are, however, the factors contributing to the remission of obese patients with AN after bariatric surgery are not very well known, which needs further research.Reflectance confocal microscopy (RCM) is an emerging non-invasive technology for diagnosis of many common skin diseases. In addition to capturing and analyzing skin thickness and pigmentation degree, RCM can also analyze various other parameters such as dynamics of microcirculation. Therefore, RCM may provide auxiliary prognostic values for AN after surgery.
In this study, we attempted to use RCM to assess dermatological changes in the pathological features of AN before and after bariatric surgery. In addition, we sought to determine which metabolic indices were most relevant to AN remission after surgery.
Methods The study included 319 obese patients who underwent bariatric surgery at our hospital. The subjects were divided into obesity (OB, n = 178) only and obesity with acanthosis nigricans (AN, n = 141) groups. The basic clinical and metabolic indices and the dermatological features via reflectance confocal microscopy (RCM) and histology were collected from patients prior to and after bariatric surgery.
Results 1) Based on statistical analysis, we found that there were no differences in gender (% male), rate of type 2 diabetes mellitus (T2DM) (%), birth weight, WC, HC, TC, TG, LDL-c, SBP, and DBP between the patients in OB group and AN group ($p < 0.05$). AN patients had significantly higher levels of duration of obesity, family history of obesity and diabetes, body mass index (BMI), FPG, FINS, and HOMA-IR, but lower levels of average age and HDL-c, as compared to patients in the OB group. For sex hormones, male patients had significantly higher but female patients had lower levels of testosterone (Testo) in the AN group than those in the OB group; other sex hormones did not show significant differences ($p < 0.05$).For the comorbidities, the AN group has

significantly more cases of gonadal disease than the OB group. The AN group also has more cases of fungal skin infection or bacterial folliculitis; but this increase was insignificant.
2) Dermatological features of all AN patients prior to surgery were evaluated by RCM. The severe and moderate AN groups had significantly higher levels of ET and SPS as compared with the mild AN group ($p < 0.01$). Dermal papillary rings and inflammatory cells containing medium and high reactive granular substances were observed (Fig.2B). The papillary rings in neck lesions contained more refractive particles than those in axillary lesions ($p<0.05$). When comparing the RCM images with pathological slides, epidermis thickness, and skin pigmentation were the same.
3) The AN score, BMI, FPG, FINS, and HOMA-IR were significantly lower at 3 months after surgery as compared with their levels before surgery . Testo levels were significantly higher in male but lower in female patients after surgery than before surgery ($p < 0.01$). RCM revealed there were significant reductions in ET, DBCF, and ECS, but increases in DDC, after surgery as compared with before surgery ($p < 0.01$). RCM and histology showed that there were significantly thinner epidermis, less pigment content, and fewer lymphocyte infiltrations in the acanthus and basal cell layers, and reduced blood flow in dermal small blood vessels at 3 months after surgery as compared with before surgery.The remissive rate of AN after surgery was about 86.5% (122 out of 141 AN patients). A univariate regression analysis was performed to identify the associated factors that contribute to AN remission following surgery. Changes in BMI, FPG, FINS, HOMA-IR, Testo (female), DBCF, ET, ECS and SPS after surgery were significantly positively correlated with changes in AN scores (ΔAN). Changes in Testo (male) and DDC were negatively correlated with ΔAN ($p < 0.01$). Next, we conducted a multivariate regression analysis to reveal the most crucial contributing factors to the remission of AN after surgery. We found that Testo (male) and BMI were the most critical factors in male patients. Testo (female) was the most critical factor in female patients. ET and SPS were the most critical dermatological factors for remission in AN after surgery.
4) The moderate and severe AN groups showed significantly higher rates of treatment ineffectiveness and abnormal Testo levels than the mild AN group . In addition, moderate and severe AN patients with treatment ineffectiveness showed significantly higher levels of HOMA-IR and Testo in female subjects, but lower level of Testo in male subjects, as compared with those mild AN patients with treatment ineffectiveness.
Conclusion In conclusion, our study demonstrates that bariatric surgery is an effective modality to manage the obesity-associated AN patients. Our research has found that Testo correlates closely with the remission of AN skin lesions after surgery. Our data also demonstrate that RCM is capable of reliably diagnosing and predicting the efficacy of AN, and can be used as a valuable non-invasive method for conducting clinical studies on patients with AN.

OR-064

Efficacy of adalimumab in pediatric generalized pustular psoriasis: case series and literature review

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Purpose In this article, we evaluate the efficacy of adalimumab in seven children with GPP. Furthermore, the pediatric pustular psoriasis treated with adalimumab in the literature was also reviewed.
Methods:
Patients
Data were collected on patients aged from 2 to 13 years old with GPP who are treated with adalimumab from 2019 to 2021 in Zhongda Hospital Southeast University, Nanjing, China. The diagnosis of GPP was confirmed by at least two dermatologists based on a combination of medical history, characteristic skin lesions, pathological biopsy, and related laboratory examination.
Exclusion criteria: patients with tuberculosis, hepatitis B, or abnormal liver function; HIV infection, severe bacterial infection, malignant tumors, and other severe immune system disorders.The

characteristic information including age, gender, age of onset, family/personal history, severity of psoriasis lesions, risk factors, concomitant diseases, treatment modalities, and the course of the disease was obtained from each patient. Written informed consents were presented by all eligible patients with the aid of their parents or legal guardians.

Treatment

Before treatment, a collection of examinations was performed on each patient, including a T-cell spot detection of tuberculosis infection, a chest CT scan, a routine blood test, a urine test, liver, and kidney function tests, an erythrocyte sedimentation rate (ESR) test, a C-reactive protein (CRP) test as well as hepatitis virus antigen and antibody tests. Patients with tuberculosis, serious viral, or bacterial infections were excluded. All patients were free of malignancy or severe internal organ disease.

All patients were subcutaneously administered with adalimu- mab according to the weight, with an initial dose of 40mg(weight >30kg) or 20mg (for weight <30kg) once in week 0, 40mg/20mg in week 1, and 40mg/20mg every other week thereafter.

Efficacy evaluation

The systemic/laboratory score, Physician Global Assessment (PGA) were assessed at clinic visits; baseline (week 0), week 1, and month 1.

The systemic/laboratory score ranged from 0 to 6 with four components included: fever, white blood cell (WBC) count, and CRP (Table 1).

The PGA was scored by the investigator based on the degree of three skin symptoms including erythema, edema, and pustulation, on a 6-point scale ranging from 0 (no evidence) to 5 (most severe). The scores for each symptom were then averaged and assigned to a PGA scale of 0 (clear), 1 (minimal), 2 (mild), 3 (moderate), 4 (severe), or 5 (very severe).

Results

Seven patients with GPP were seen at the Department of Dermatology of Zhongda Hospital Southeast University and received adalimumab. Four children were girls and three children were boys ranged from 2 to 13 years, with a mean age of 8.71±3.59 years. Average duration of disease was 2.69±2.63 years. All patients had a history of psoriasis vulgaris with a duration of 2.69 ± 2.63 years. The duration of onset of GPP is 6.71±2.14 days. Three patients had a history of cold prior to the onset of GPP. The other four patients had no identified triggers.

All of them had received prior treatments, and four of them only took topical treatment, none of them had ever received other biologic therapies before. Systemic treatments included compound glycyrrhizin (150 mg/d) (2 case) and acitretin (10 mg/ d3d) (1 case).

Three patients had family history of psoriasis, in which case, patient 2 is patient 6's cousin and patient 5's aunt had psoriasis vulgaris. No patients experienced concomitant diseases. The mean PGA and systemic/laboratory score at the initiation of adalimumab therapy were 4.57 ± 1.13 and 4 ± 2, respectively. All patients except patient 6 developed a fever over 38C.

All seven cases with GPP were treated with adalimumab mono- therapy. The initial treatment dose was 40 mg (weight >30 kg, 20 mg for weight <30 kg) at week 0, and then 40 mg/20 mg in week 1, and 40 mg/20 mg every other week thereafter. Two of them (patient 3 and patient 7) were treated with the dose of 20 mg, the other five patients received the dose of 40 mg.

After treatment initiation, the patients' skin conditions were obviously improved within the first week of treatment, fromPGA=5 in six cases and PGA=2 in one caseto PGA=2 in three cases, PGA=1 in three cases, and PGA=0 in one case. After two injections, complete clearance of the pustular lesions revealed in five patients with the PGA decreased to 0, two patients (patient 1 and patient 2) showed almost 90% clearance of the pustular and erythema with the PGA decreased to 1. The systemic/laboratory test score decreased from baseline to 0 at week 1 in all patients.

However, one patient (patient 3) experienced clinical side effects which presented with flu-like symptoms including a low-grade fever of 37.8 C, fatigue, cough, and sputum production. All these symptoms only lasted for four days. No side effects such as abnormalities in complete blood counts, urinalysis, liver, and kidney function were detected during adalimumab treatment.

Conclusion

In conclusion, subcutaneous injection of adalimumab every other week in the treatment of children with GPP has significant clinical efficacy with rapid clearance of skin lesions, providing a novel alternative for children with pustular psoriasis who responded poorly to traditional treatment or not

suitable for traditional treatment. Further studies and clinical trials on the details are needed to evaluate the efficacy and safety of adalimumab in pediatric patients with GPP including standardization of dosage, interval between injections, and total duration of treatment.

OR-065

Two cases of successful treatment of moderate to severe acne with an innovative acupuncture-debridement microsurgical technique and review of the literature

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Acne is a pilosebaceous chronic inflammatory disease, characterized by comedones, papules, pustules, nodules, cysts and scars [1]. Acne often occurs on the face of teenagers, showing serious impact on their physical and mental health, especially severe acne with nodules, erythema, atrophic and hypertrophic scars that greatly affect the appearance [2]. Microsurgical therapeutic technique of acupuncture and debridement is one of the most direct and effective treatment for acne. It is a minimally invasive operation to relieve the symptoms by eliminating sebum, purulent secretions, congestion and necrotic tissue through the hair follicle. Up to now, it has been widely used in medical cosmetic organizations, but not been valued and adopted in most hospitals probably due to the lack of standardized and detailed operational process.In order to standardize and generalize this immediate and effective treatment, we improved the traditional therapeutic technique of acupuncture and debridement by innovatively proposing a series of technical concepts including minimally invasive debridement, operational methods and scheme of combined medication, which have received good effects in clinical practice. Here we introduced two cases of successful treatment of moderate and severe acne with this innovative acupuncture-debridement microsurgical technique, and summarized the characteristics, mechanism and standardized operation of acupuncture and debridement according to relevant literature.

1. Case

1.1 Case 1

A 16-year-old male complained of continuous aggravation of facial acne and came to our clinic on September 11th, 2021. The patient had developed inflammatory papules of the whole face, starting from both cheeks since May, 2020. The patient had been treated with traditional Chinese medicine, fusidic acid cream and adapalene for more than 2 months without obvious effect. He was diagnosed as moderate acne, with Pillsbury's classification of grade III. There were multiple inflamed pustules with infection on both cheeks, comedones and papules on the forehead and pigmentation on the face. The patient signed informed consent for innovative acupuncture-debridement microsurgical treatment and clinical observation.

The patient was treated with the innovative acupuncture-debridement microsurgical technique and postoperative repaired system. The patient was treated twice with an interval of 14 days and was observed for 28 days after treatment. Photographs were taken before treatment (Fig.1). On the first follow-up 14 days after the first treatment, we could see that most pustules on the face had completely subsided with only small areas of pigmentation remained. Most comedones and papules on the forehead had completely relieved without pigmentation. The needling area recovered well without dilated pores or scar. Only 3-5 comedones and 1-2 papules recurred during the recovery period.

Then the patient received the second treatment and the follow-up 14 days later showed only local punctate pigmentation, his hair follicles healed completely. The patient was highly satisfied. Subsequent follow-ups showed continuous recovery without residual pigmentation, atrophic scar and recurrence.

1.2 Case 2

A 16-year-old female complained of a sudden outbreak of acne on both cheeks and forehead for one year. The patient had been treated with traditional Chinese medicine, topical fusidic acid

cream and oral isotretinoin for half a year with limited effect. And the lesions recurred rapidly after withdrawal of isotretinoin. According to the Pillsbury's classification, the patient was diagnosed as severe acne with grade IV. At the first visit, photographs were taken, showing multiple inflamed pustules and nodules on both cheeks, severe pigmentation and multiple comedones on the forehead. The patient signed the informed consent for the innovative acupuncture-debridement microsurgical treatment and clinical observation.

The patient received the same treatment as case 1. Fourteen days after the first treatment, most pustules and nodules on both cheeks had relieved significantly and most comedones on the forehead had disappeared with only small areas of pigmentation remained. The needling area recovered well without pore expansion or scars.

Then the patient received the second treatment. On the follow-up visit 3 months later, as we can see in Fig.4, most of her skin lesions had completely improved with only minor local pigmentation. The hair follicles of the affected area healed without dilated pores. On the follow-up visit one month thereafter, her facial pigmentation had further faded with only trivial atrophic scar remained. No recurrence was observed during the 6-months-follow-up till now.

2. Discussion

2.1 The mechanism of acupuncture and debridement

① Relieve the symptoms of acne directly and effectively

② Block the development of inflammation in acne

③ Acupuncture makes the subcutaneous mast cells redistributed

④ Promote the absorption of drugs

⑤ Reduce the formation of nodules and scars

⑥ Reduce the recurred incidence of acne

2.2 Indication and contraindication of acupuncture and debridement

Indication: comedones, papules, mature pustules and nodules, some mature cysts with purulent exudation, and pigmentation after acne.

Contraindication: polymerized cyst with severe infection and mutual adhesion.

Precautions: the treatment of acupuncture and debridement should avoid the dangerous triangle area of the face. At the same time, acupuncture and debridement might stimulate the immature acne which is not organized by purulent secretion in the early stage, leading to further development of inflammation [12].

2.3 Common types of acne debridement

At present, debridement of acne mainly includes fire-needle therapy in medical institutions and acne removal treatment in beauty institutions.

Fire-needle therapy is to place the tip of the fire needle in the outer flame of the alcohol lamp until it turns red and white, then stab it into the skin lesions vertically, and then quickly remove the needle. The fat plug, purulent secretion and bleeding are simply cleaned up with the needle or cotton swab. In clinical practice, it is mainly used as an auxiliary technique in combination with other physical therapy or facial mask, and the debridement is incomplete.

Acne removal treatment is to use an acne extruder to extrude the contents of acne. However, the diameters of acne needles and triangular needles used in beauty institutions are often too large. In addition, the technique and manipulation of the debridement haven't been standardized yet. The frequent treatment makes the pores expanded repeatedly and leaves the skin around the pores torn, resulting in various scars.

2.4 Innovation and standardized practice of acupuncture and debridement

In order to overcome the disadvantages of the above acupuncture and debridement therapy, we creatively put forward a series of treatment principle, operation standard and postoperative repairment schemes of combined medication for minimally invasive acupuncture and debridement. The innovative technique focuses on minimizing invasiveness. The improved needle with smaller diameter is adopted for operation. The needle is inserted into the main pores to open the passage of hair follicles without damaging their structure. We replace the sheared force of local pressing with the pushing force generated by pressing the muscle, thus reduce damage to the lesion and fulfill complete clearance of secretion, pus, tissue and congestion from the hair follicles.

2.4.1 Selection of needles

Generally, disposable needles of acupuncture with a length of 13mm (half inch) and a diameter of 0.25-0.4mm are used. Such needles can go through the pores without expansive damage to the pores and the surrounding skin tissues, thus reduce the pain of acupuncture.

2.4.2 Preoperative preparation

Acne around the eyebrows, cheeks and mandible are often extremely sensitive to the pain due to acupuncture and debridement. When treating acne of these special regions, we should pay attention not to crush the lesion during debridement. Additionally, 5% compound lidocaine cream is recommended for local anesthesia.

2.4.3 Timing and frequency of treatment

The timing of acupuncture and debridement depends on the maturity of pustules, nodules and cysts. It takes 3-5 days for pustules, nodules and cysts to mature. At the initial stage, redness, swelling, heat and pain are obvious. The skin lesions on the face are firm to the touch because there is no pus in them. When being pressed, no fluctuation could be felt. At the same time, there is no pus and white purulent protruding from the skin lesions. The skin lesions are bright red in color. When they mature, the patient feels no longer intense pain. The skin lesions become soft, and a large amount of pus is formed due to internal organization. When being pressed, fluctuation can be felt. At the same time, there are pus and white purulent protruding from the skin lesions, and the color of the skin lesions become dark red.

Patients are generally treated once every 2-3 weeks, depending on the recovery of the skin. Patients with severe acne can be treated once every 3-4 weeks.

2.4.4 Position and depth of needling insertion

The needle must be inserted through the main pores for acupuncture and debridement. There is no resistance with correct insertion, and the patient almost feels no pain. Generally, the pores of hair follicles in the center of the skin lesions are selected for insertion, especially those with white purulent protruding. The needling depth varies according to the type of skin lesions. Generally, the needling depth for comedones, papules and mild pustules is 3-5mm, and the needling depth for severe pustules, cysts and nodules is 5-8mm.

2.4.5 Technical points

The first point is the selection of pressing sites. Usually, the facial muscles 2-4cm away from the center of the acne are pressed. Most of secretions, pus and ecchymosis in the sebaceous gland are thoroughly removed through the pore of hair follicle expanded by the needle. Second, avoid pressing and pinching the skin lesions because they are weak and likely to form scars. After all, it is impossible to remove the purulent secretions in the sebaceous glands completely.

2.4.6 Post-operative repairment and evaluation of the effect of treatment

After treatment, the recombinant bovine basic fibroblast growth factor gel is used for recovery. At the same time, it is also very important to use the traditional Chinese medical facial mask to reduce swelling. The ingredients of the facial mask include 20g safflower, 20g Salvia miltiorrhiza, 15g Centella, 15g honeysuckle, 15g dandelion, 15g Forsythia suspensa, 15g Atractylodes macrocephala, 30g cuttlebone and 10g Sophora flavescens. These ingredients can quickly repair the damage of hair follicle, reduce swelling and promote healing quickly [13].

As the most direct and effective surgical debridement, acupuncture and debridement can remove the secretion, pus and congestion in the sebaceous glands of hair follicles, and alleviate the symptoms of acne effectively and quickly. This technique blocks further development of inflammation to prevent the acne from aggravation. In addition, it promotes the healing and repair of hair follicle expansion due to inflammation, thereby reducing the formation of atrophic scars and nodules [14]. The therapy of acupuncture and debridement can reduce the recurred incidence of acne and accelerate the absorption of topical drugs by dredging the duct of hair follicles. Finally, it has the function of local selection in acupoint and produce a good effect on detumescence and analgesia by stimulating the facial acupoints.

3. Summary

The innovative acupuncture-debridement technique and postoperative scheme of repair have achieved good therapeutic effects in patients with moderate and severe acne. In the process of clinical practice, the professional, standardized and innovative technique can significantly shorten the cycle of treatment and reduce the recurred incidence of acne. In addition, the skin lesions of patients recovered well, without sunken or hypertrophic scars. In a nutshell, the innovative

acupuncture-debridement technique for acne focuses on the concept of minimal invasiveness, thoroughness and optimum repairment. This technique might be referenced in standardization of surgical treatment for acne in clinical practice.

OR-066

BET inhibitors potentiate melanoma ferroptosis and immunotherapy through AKR1C2 inhibition

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Objective

Ferroptosis is a form of regulatory cell death driven by excess iron-dependent accumulation of lipid peroxidation, representing a vulnerability in cancers. Therapy-refractory melanoma cells with mesenchymal or dedifferentiated states are unexpectedly vulnerable to ferroptosis triggered by GPX4 inhibition, indicating that targeting ferroptosis holds great potential for melanoma treatment. However, there are several defensive mechanisms identified to control ferroptosis in melanoma, highlighting the necessity of exploring drugs that sensitize melanoma to ferroptosis. Our team has reported that BET inhibitors can suppress melanoma progression. In addition, we found that BET inhibitors sensitize melanoma cells to other anti-tumor drugs and they synergistically inhibit melanoma growth. However, it remains unclear whether BET inhibitors sensitize melanoma to ferroptosis and synergize with ferroptosis inducers in melanoma. Herein, the aim of this study is to explore the effect and regulatory mechanism of BET inhibitors on ferroptosis in melanoma, and to provide a new strategy for melanoma treatment.

Methods

To systematically identify anti-cancer drugs with the potential to augment ferroptosis, the spearman correlation analysis between ferroptosis level and drug sensitivity was performed to explore the potential candidates for sensitizing ferroptosis. Next, we conducted drug combination experiments to evaluate the combination effect of BET inhibitors and ferroptosis inducer RSL3 in melanoma cells. Besides, cell death inhibitor rescue assay, lipid peroxidation detection by flow cytometry and immunofluorescence, and electron microscopy assay were used to explore the type of cell death triggered by the combination of BET inhibitor and RSL3. In vivo, the subcutaneous A375 tumor-bearing model was constructed to explore the synergistic effect of BET inhibitor and GPX4 knockout in melanoma. In addition, the subcutaneous B16F10 tumor-bearing model was constructed to explore whether BET inhibitor potentiates the efficacy of ferroptosis-associated immunotherapy.

To clarify the mechanism by which BET inhibitors sensitize ferroptosis, we constructed stable BRD2/3/4 knockdown, BRD4 knockout and BRD4 overexpression melanoma cell lines to explore their sensitivity changes to RSL3 compared with control melanoma cells. To explore the differentially changed ferroptosis-related target proteins regulated by BET inhibitors, we performed RNA sequencing in melanoma cells treated with BET inhibitors or BRD4 silencing. We subsequently used western blotting analysis, qRT-PCR, immunofluorescence, and ChIP sequencing to verify the regulatory effect of BET inhibitor or BRD4 on the downstream target protein AKR1C2. Moreover, we constructed AKR1C2 knockdown, knockout and overexpression melanoma cell lines to investigate the regulatory effect of AKR1C2 on ferroptosis in melanoma cells. We also performed rescue experiments to explore whether the sensitization effect of BET inhibitors on ferroptosis was dependent on AKR1C2. Finally, to consolidate our findings, we explored the association between ferroptosis levels and BRD4 expression at a single-cell resolution, as well as the association of BRD4, AKR1C2 and ferroptosis levels with the prognosis and immunotherapy efficacy of melanoma patients.

Results

Bioinformatics analysis showed that BET inhibitors were potential candidates for sensitizing ferroptosis. In melanoma cells, four BET inhibitors (JQ1, NHWD-870, OTX015 and I-BET151)

showed strong synergistic effects when combined with ferroptosis inducer RSL3, respectively, with CI values less than 1. The cell death induced by BET inhibitors and RSL3 could be abrogated by ferroptosis inhibitor ferrostatin-1 (Fer-1), and the iron chelator deferoxamine (DFO), but not by inhibitors of apoptosis (Z-VAD-FMK), necroptosis (Nec-1s), or autophagy (CQ) in melanoma cells. Compared with BET inhibitors or RSL3 alone, the combination treatment triggered more accumulation of lipid peroxidation. Electron microscopy results showed that the combination of JQ1 and RSL3 caused striking ferroptosis-associated morphologic changes in melanoma cells, characterized by shrunken mitochondria with increased membrane density and reduced numbers of mitochondrial cristae. In vivo, BET inhibitors in combination with GPX4 knockout significantly inhibited melanoma growth and increased the levels of 4-hydroxynonenal (4-HNE), a major product of lipid peroxidation. Compared with monotherapy, BET inhibitors combined with anti-PD-1 antibodies also significantly suppressed melanoma growth and elevated the levels of activated cytotoxic CD8+ T cells including IFNγ+ and GZMB+ CD8+ T cells.

In addition, silencing BRD4 with shRNA or sgRNA sensitized melanoma cells to RSL3-triggered ferroptosis, and more importantly, overexpression of BRD4 protected melanoma cells from ferroptosis induced by the combination of BET inhibitors and RSL3. The results of RNA-seq suggested that AKR1C2 was most downregulated after BET inhibitor treatment or BRD4 silencing among candidate ferroptosis suppressors. Western blotting, qRT-PCR, immunofluorescence and ChIP-seq results showed that BET inhibitors impede the interaction between BRD4 and AKR1C2 promoter, thus downregulating AKR1C2 expression at mRNA and protein levels. Previously, we reported that BET inhibitors suppress STAT3 signaling via the BRD4/IL6 axis, and then we further verified whether STAT3 regulates AKR1C2 expression in the transcriptional level. Western blotting and ChIP-seq results indicated that BET inhibitors also inhibit AKR1C2 expression through BRD4/IL6/STAT3 axis. AKR1C2, a member of human hydroxysteroid dehydrogenases, plays a pivotal role in lipid peroxide demolition, thereby inhibiting ferroptosis. As expected, silencing AKR1C2 with shRNA or sgRNA, or treating with AKR1C2 inhibitors, sensitized melanoma cells to RSL3-induced ferroptosis. Rescue experiments further demonstrated that BET inhibitors potentiate ferroptosis by inhibiting AKR1C2 expression in melanoma cells. Moreover, data from single-cell RNA sequencing and spatial transcriptome clarified that BRD4 or AKR1C2 is negatively correlated with ferroptosis in melanoma. Kaplan-Meier survival analysis showed that melanoma patients with low BRD4/AKR1C2 expression and high ferroptosis score have longer progression-free survival or overall survival. Additionally, this group melanoma patients have better response to immunotherapy.

Conclusions

In summary, we demonstrated that BET inhibitors potentiate melanoma ferroptosis and immunotherapy through AKR1C2 inhibition. Mechanistically, BET inhibitors sensitize melanoma to ferroptosis through inhibiting AKR1C2 expression directly by BRD4 or indirectly by BRD4/IL6/STAT3 axis. Our study provides rationale for further preclinical and clinical investigation of BET inhibitors in combination with GPX4 inhibition or immunotherapy for melanoma treatments.

OR-067

Single-cell analyses reveal novel molecular signatures and pathogenesis in cutaneous T cell lymphoma

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Introduction Sézary syndrome (SS) is a rare and aggressive CD4+ leukemic variant of cutaneous T cell lymphoma (CTCL), characterized by diffuse pruritic erythroderma and atypical clonal T cells accumulated in the peripheral blood and skin, with high mortality and poor prognosis. Diagnosis of SS is difficult, due to the lack of specific markers for malignant lymphocytes. The limited treatment options for patients also reflected our poor understanding of disease pathogenesis. Single-cell RNA sequencing (ScRNA-seq) could reveal tumoral heterogeneity and identify tumor-specific molecular

signatures, with important implications for diagnosis and personalized disease treatment. Tumoral heterogeneity in SS has been described by used skin or PBMC samples in recent studies. However, intra-tumoral heterogeneity across disease compartments (e.g., skin, blood) is little known. In addition, ScRNA-seq combined with single-cell assay for transposase-accessible chromatin using sequencing (scATAC-seq) could elucidate the disease-associated transcriptional regulation mechanisms, providing new avenue to explore the pathogenesis of CTCL.

Objectives To facilitate the precise diagnosis of mycosis fungoides (MF)/SS, and provide targets for designing new effective medications in CTCL.

Materials and Method: We performed a comprehensive analysis by integrating single-cell transcriptomic data of 40,333 peripheral blood mononuclear cells (PBMCs) and 41,580 skin cells from SS patients and healthy controls (HCs). Moreover, single-cell transposase-accessible chromatin data of 11,058 PBMCs from SS patients and HCs were integrated with single-cell transcriptomic data to elucidate the pathogenesis of CTCL. Validation and functional investigation were done in an independent cohort including SS, MF, psoriatic erythroderma patients and HCs, as well as multiple cell lines.

Results

1. Identification and transcriptional characteristics of malignant CD4+ T cells in PBMCs and skin tissues

We acquired single-cell transcriptomes in 40,333 CD45+ immune cells from PBMCs of one SS patient and three HCs, and re-clustered of CD4+ T cells. By the results of clonotype and CNV analysis, we identified maligant CD4+ T cells with central memory T cell phenotype (SELL+CCR7+CD27+TCF7+S100A4+). A total of 41,580 qualified skin cells from an SS skin lesion and three normal skin biopsies were analyzed using scRNA-seq, and re-clustered of T-like cells. We identified skin-derived malignant CD4+ T cells, showing showing characteristics of the TCM and TRM phenotype (SELL+CCR7+CD27+TCF7+CCR4+CD69+NR4A1+).

2. Transcriptional heterogeneity between blood- and skin-derived SCs

Transcriptional comparisons and pseudotime trajectory analysis following the pooling and clustering of malignant CD4+ T cells from peripheral blood and skin were further performed. The results showed that TCM differentiation-associated genes KLF2 and S1PR1 were highly expressed in blood-derived SCs, and skin-derived SCs were accompanied by upregulation of TRM differentiation-associated genes NR4A1 and LGALS3. Blood- and skin-derived SCs presented the same dominant clone, indicating that phenotypic heterogeneity of SCs was caused by their adaptive plasticity to different tissue microenvironment.

3. Identification of specific marker genes correlated with MF/SS disease progression

By differential gene expression analysis and IHC experiment, we identified five potential biomarker genes (TOX, DNM3, KLHL42, PGM2L1, and SESN3) that were specifically overexpressed in both blood- and skin-derived SCs and could help to distinguish between SS and psoriatic erythroderma patients. And the expression of these genes was positively associated with disease progression of MF/SS. These genes could act as potential specific marker genes of malignant CD4+ T cells, facilitating the diagnosis of MF/SS, as well as differentiation between SS and EID.

4. ScATAC-seq revealed KLHL42-associated transcriptional regulation mechanism in malignant CD4+ T cells

To further investigate the mechanisms that regulating the expression of the aforementioned genes, we performed scATAC-seq for 11,058 CD45+ immune cells from PBMCs of one SS patient and three HCs. We found that KLHL42 overexpression in malignant CD4+ T cells corresponded to high accessibility of the KLHL42 region. Further TF motif analysis showed that the GATA3 motif was differentially enriched in malignant CD4+ T cells. GATA3 mRNA expression was significantly increased in malignant CD4+ T cells, suggesting that GATA3 positively regulated the expression of KLHL42 in malignant CD4+ T cells. Luciferase reporter gene assay showed that GATA3 expression significantly enhanced the transcription activity of KLHL42 promoter, while GATA3 knockdown significantly suppressed the mRNA and protein expression of KLHL42, confirming the activation of KLHL42 transcription by GATA3. Transcriptional activation of KLHL42 by GATA3 further upregulated KLHL42 in malignant CD4+ T cells when chromatin KLHL42 accessibility was high.

5. KLHL42 knockdown inhibited CTCL cell proliferation and promoted its apoptosis in vitro.

To further confirm the function of KLHL42 in CTCL, KLHL42 expression was silenced in Hut78 and HH cells via lentivirus-mediated transduction. CCK-8 assays demonstrated decreased cell growth rates in KLHL42-silenced Hut78 and HH cells compared to the control cells. Moreover, the expression of anti-apoptosis proteins, such as Bcl-2 and survivin, was suppressed by KLHL42 silencing. Annexin V-PI staining showed increased spontaneous apoptosis in KLHL42-silenced cells compared to the control cells. These results indicated that KLHL42 knockdown inhibited Hut78 and HH cell proliferation and induced their apoptosis.

Conclusions Our study revealed the transcriptional heterogeneity within SCs across different tissues and described the phenotypic plasticity of blood- and skin-derived SCs. A series of specific marker genes of SCs were identified in order to facilitate the diagnosis and prognosis of MF/SS, further illustrating the KLHL42-associated pathogenesis of CTCL and providing new insight for precision targeted CTCL therapy.

OR-068

The mechanism of RIPK3 O-GlcNAc glycosylation mediated by O-GlcNAc transferase to promote keratinocyte necroptosis in the pathogenesis of severe drug eruption

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The mechanism of RIPK3 O-GlcNAc glycosylation mediated by O-GlcNAc transferase to promote keratinocyte necroptosis in the pathogenesis of severe drug eruption

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Abstract

Purpose

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe, life-threatening mucocutaneous reactions characterized by widespread keratinocyte death, blisters, and mucosal sloughing. Drugs and infection, such as human immunodeficiency virus (HIV) and Mycoplasma, are the main causes of these
diseases. SJS/TEN can lead to complications in the liver, kidneys, and respiratory tract. Histologically, the skin of SJS/TEN patients separates at the dermo-epidermal junction accompanied by apoptosis and necrosis of epidermal keratinocytes. Classical apoptosis and necroptosis are the relevant cell death pathways critical for the extensive keratinocyte death observed in SJS/TEN. Necroptosis is a mode of cell death induced by extracellular factors such as TNF that plays an important role in inducing an inflammatory cascade. RIPK3 is a member of the receptor-interacting protein (RIP) family of serine/threonine kinases and contains an N-terminal kinase domain and a C-terminal RIP homotypic interaction motif (RHIM). Through RHIM-mediated protein interaction, RIPK3 forms a necrosome complex with RIPK1, and this complex is required for the induction of necroptosis, an inflammatory form of cell death. Both RHIM and kinase activity of RIPK3 are essential for activation of downstream effector protein MLKL and execution of necroptosis. In addition to having a central role in necroptosis, elevated RIPK3 activation has been shown to promote inflammatory responses in both cell-death-dependent and -independent manners. Glucose serves as a major nutrient to fuel cellular metabolic activities. Three major glucose metabolic pathways, namely glycolysis, the pentose phosphate pathway (PPP), and the hexosamine biosynthesis pathway (HBP), collaboratively determine
how glucose is processed. HBP is a unique glucose metabolism pathway leading to the generation of its end product uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is further utilized by the O-GlcNAc transferase (OGT) for protein modification, namely O-linked β-N-acetylglucosamine (O-GlcNAc). Many proteins involved in various fundamental biological processes, including transcription factors, kinases, and enzymes, have been identified as O-

GlcNAcylation targets. O-GlcNAc glycosylation is a classical post-translational modification of proteins, which plays a variety of biological functions such as regulating apoptosis and necrosis. Deletion of O-GlcNAc transferase (OGT), a key enzyme for protein O-GlcNAcylation, led to enhanced innate immune activation and exacerbated septic inflammation. Previous studies have shown that specific knockout of OGT can lead to programmed necrosis of cells. However, the role and mechanism of OGT-mediated glycosylation of O-GlcNAc in the pathogenesis of SJS/TEN remain unclear.

Method

FFPE paraffin section proteomic screening of healthy control, MPE and SJS/TEN significantly different proteins in skin tissues, RNA seq and O-GlcNAc glycosylation modification omics analysis of the expression levels of RIPK3 and p-MLKL key molecules of necroptosis after knockout OGT in SJS/TEN in vitro model. Phenotypic and mechanistic analysis was performed using Immunohistochemical (IHC) staining,
ELISA, western blotting, RT-PCR, non-targeted metabolomics, immunofluorescence, immunocoprecipitation, LDH reaease, cck-8 assay, ATP levels, and flow cytometry.

Results

Firstly, RIPK3 and p-MLKL, the key molecules of necroptosis, were highly expressed in the skin lesions of SJS-TEN in acute stage. RIPK3 levels were highly increased in the serum of SJS/TEN patients compared with maculopapular exanthems (MPE) patients, and positively correlated with C-reactive protein (CRP), Body surface area (BSA), the SCORe of Toxic Epidermal Necrosis (SCORTEN), and body temperature. Secondly, FFPE proteomics, western blotting, and IHC results showed that OGT expression was significantly lower in SJS/TEN patients than in healthy controls and MPE patients, and was significantly positively correlated with SCORTEN and BSA. In addition, LL37+AC2-26 stimulated primary keratinocytes to simulate SJS/TEN in vitro cell model, and the results showed that the levels of HBP (hexosamine biosynthesis pathway) and UDP-GlcNAc were significantly decreased, and the expression levels of OGT and O-GlcNAc total glycosylated protein were also significantly decreased. Transfection of OGT interference fragment and OSMI-1 inhibitor significantly promoted the expression of necroptosis markers about p-RIPK1, p-RIPK3, and p-MLKL in LL37+AC2-26 treated primary keratinocytes. Moreover, the necrosis rate, LDH release, ATP levels were also significantly increased in LL37+AC2-26 treated primary keratinocytes. In mechanism, knockout of OGT-mediated glycosylation at T467 site of RIPK3 promoted the apoptotic signal of primary KC programmed necrosis and induced cell necrosis. O-GlcNAcylation of RIPK3 on T467 inhibits RIPK3-RIPK1 and RIPK3-RIPK3 interaction. Finally, overexpression of OGT can significantly reverse the death effect of SJS/TEN animal models.

Conclusions

Our study shows for the first time that O-GlcNAc glycocation is involved in the occurrence and development of severe drug eruption. The low expression of OGT in SJS/TEN skin lesions significantly promotes the occurrence of keratinocyte necroptosis and is involved in the pathogenesis of severe drug eruption. It can be used as a reliable marker for the diagnosis of SJS/TEN, providing new ideas and new targets for the treatment of severe drug eruption. We also identified OGT-mediated RIPK3 O-GlcNAcylation at T467 as a key mechanism to block RHIM-mediated RIPK3-RIPK1 and RIPK3-RIPK3 interaction. Removal of OGT or RIPK3 O-GlcNAcylation promoted keratinocytes necroptosis, both of which are dependent on RIPK3 RHIM domain and kinase activity.

Key words: Necroptosis, O-glycosylation modification, OGT, severe drug eruption, keratinocytes

OR-069

Effect of biologic therapy on the risk of osteoporosis in patients with psoriasis

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Objective Psoriasis is a chronic and recurrent inflammatory disease that is mediated by both immunity and genetic susceptibility. Current evidence suggests a close association between psoriasis and bone diseases, such as osteoporosis and fractures. Osteoporosis is characterized by systemic bone loss, damage to the microstructure of bones, increased fragility, and susceptibility to fractures. Fracture can not only lead to disability, hypostatic pneumonia, deep vein thrombosis and other complications, which seriously threaten the health of patients, but also cause a huge economic burden to individuals, families, society and the country. Inflammatory factors, such as TNF-α and IL-17A, which play a crucial role in the pathogenesis of psoriasis, may serve as an essential molecular basis for the development of osteoporosis in patients with psoriasis. This suggests that biological agents could be effective in reducing the risk of osteoporosis while treating psoriasis. Currently, TNF-α inhibitors, IL-17A inhibitors and other biological agents are widely employed in clinical practice for the treatment of psoriasis; however, there is a lack of clinical research on the impact of various diagnostic and therapeutic approaches for psoriasis on bone metabolism andosteoporosis risk in patients with plaque psoriasis. This study aimed to assess the effects of adalimumab and secukinumab therapy on bone metabolism and osteoporosis risk in patients with psoriasis.

Methods A total of 26 psoriatic patients who received adalimumab or secukinumab in the First Medical Centre of Chinese PLA General Hospital from August 2022 to April 2023 were included in this study. None of the patients had received previous systemic therapy for psoriasis and no history of osteoporosis. The control group was given adalimumab, and the observation group was treated with secukinumab. The treatment was based on the recommended dose of biological agents and the recommended course of treatment. The starting dose of adalimumab is 80mg , with a second dose of 40mg given every other week and then 40mg every two weeks. The starting dose of secukinumab was 300mg once a week for 5 weeks, and then 300mg once a month. Before and after treatment, bone metabolism-related indexes included Osteocalcin, N-terminal lengthening peptide of type I collagen(P1NP), and β-carboxy-terminal peptide(β-CTX)were determined. The effects of different biological agents on the changes of bone metabolism related indicators in patients with psoriasis were analyzed.

Results A total of 26 patients were received during the observation period, of whom 10 were treated with adalimumab and 16 with secukinumab. During treatment with biologics, the patient did not receive any other medications that might affect bone metabolism. There was no significant difference in general data between the two groups (P>0.05). (1) After adalimumab treatment, osteocalcin levels were significantly increased in 10 patients with psoriasis (20.68±2.99 vs 21.80±5.16, P=0.001<0.05), while N-terminal lengthening peptide of type I collagen(PINP) was significantly increased (-5.83±4.66, P=0.003<0.05), while β-carboxy-terminal peptide (β-CTX) significantly decreased (0.08±0.06, P=0.003<0.05)， and the differences were statistically significant ; (2) After secukinumab treatment, osteocalcin levels were significantly increased in 16 patients with psoriasis (-2.87±2.4, P=0.000, P<0.05), while N-terminal lengthening peptide of type I collagen(PINP) was significantly increased (-20.48±24.14, P=0.004<0.05), while β-carboxy-terminal peptide (β-CTX) significantly decreased (0.16±0.08, P=0.000<0.05)，and the differences were statistically significant ; (3) Comparing with that in the control group, patients in the secukinumab group had significantly higher osteocalcin levels (-0.11±0.11, P=0.009<0.05), and the total N-terminal propeptide of type 1 collagen (P1NP) increased significantly (-0.18±0.18, P=0.003<0.05) and β-collagen degradation product assay (β-CTX) significantly decreased (0.11±0.06，P=0.000<0.05), and the differences were statistically significant.

Conclusion Our results suggest that biological agents can reduce the risk of osteoporosis in patients with psoriasis. Adalimumab and secukinumab can improve bone metabolism-related indexes in patients with psoriasis, and secukinumab has a more significant effect. The aim of this study is to compare the effects of different biological agents on the risk of psoriasis patients with osteoporosis, and to explore the optimal treatment for psoriasis patients with osteoporosis, so as to form a personalized treatment for patients with psoriasis. There is no clinical study on the effects of various psoriasis diagnosis and treatment methods on bone metabolism and osteoporosis in patients with plaque psoriasis at home and abroad. The specific mechanism of the effect of biological agents on bone metabolism related indicators in patients with psoriasis has not been clarified, but the present study confirmed the correlation at the clinical level in our study. The limitation of this study is the small sample size, which needs to be expanded for further exploration.

OR-070

Effectiveness and Recurrence of Trigger Points Acupuncture in the Treatment of Multiple Dermatopathy: A Retrospective Study

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Objective In clinical practice, we often treat body pain with the acupuncture of myofascial trigger points (MTrPs). Surprisedly, various concurrent focal skin diseases are also healed after their pains are relieved. This clinical phenomenon suggests that MTrPs may have some relationships with various focal dermatoses. Anatomical studies based on muscles, myofascias, and fascias have shown that the fascia usually has supporting and extending bands connecting with muscle fibres. It implies active muscle contraction and pathological contracture of MTrPs can all cause high fascia tension. The former (MTrPs) is a short-term physiological requirement, while the latter (high fascia tension) is a long-term pathological condition. If long-term chronic local fascial high tension can hinder the branch running of blood vessels and nerves passing through this location, then it will lead to the abnormality of the function and sense of its controlled effective site. Once the fascia disease occurs in a certain location, it may often be related to the occurrence of focal skin disease. Therefore, treating MTrPs, leading to the relaxation of the high fascial tension, will restore the neurovascular supply of local skin to normal. Furthermore, it will start the body's self-repair mechanism of focal skin diseases so they can be cured. Thereby, it is necessary to clinically observe the effects of treating skin diseases with this technology and whether there is a certain correlation between the MTrPs and dermatoses.
Methods A retrospective study was performed during a two-and-a-half-year period from April 2017 to October 2019. Our study comprised 120 patients with skin diseases of different parts and types. The clinical observation was divided into two stages. The patients in the early stage accounted for 50% (60 cases) who complained of pain that required treatment. Meanwhile, they had local skin problems. The other patients in the later stage were introduced by earlier patients to directly require treatment for their skin diseases, accounting for 50% (60 cases). The patients in the later stage also included 18 cases of systemic skin diseases. The common symptoms of patients' skin problems were itching or different changes (thickening, erythema, papule, moss, chaps, dark colour, and multiple chips, amongst others) or both of them in the local skin of limbs, neck or trunk, except the cases of vitiligo which only had skin whiten and annoying. Most patients had applied drugs externally long term to control the skin itching and the expansion of skin lesions. However, it could not be eradicated. It always recurred after stopping the medicine. Some skin diseases had bacterial and fungal infections. The diagnosis of skin diseases was based on the outpatient records of the dermatology department. In our study, the acupuncture technique based on the MTrPs principle was used for treatment. Except for a small amount of targeted medicine with bacterial and fungal

infections, traditional Chinese herbs for Qi and blood tonifying medicines had been given to some patients with poor physiques. However, no other medicine related to skin had been used. One hundred and twenty patients with dermatoses were treated with MTrPs acupuncture. Since the common clinical features of dermatoses are itching or local discomfort, the location of skin lesions, and frequency of itching, a modified comprehensive visual analogue scale (VAS) itching score was applied to evaluate:1) 1—10 points for itching or discomfort as its different degree, 0—1 position for none, 2) 1 point for the sites of skin lesion less than 2—3, 2—3 points for 3—5 sites with lesion area less than 30%, and 4 points for above five positions with body surface area more than 30% as systemic and multifocal dermatoses; 3) Pruritus frequency: 0—1 points for none, 1 point for frequent pruritus in 1—30 minutes, 2 points for frequent pruritus in 30 minutes to 2 hours, and 3 points for persistent pruritus more than 2 hours. The above comprehensive VAS scores were evaluated before and after the treatment course. Furthermore, all data were collected from the records of clinical outpatients. The data were divided into two parts (focal and systemic) to make statistical analyses separately. All patients were followed up for three years (mostly by telephone). The effective rate was determined according to the VAS scores, respectively. 0—1 is excellent or cured, 1—2 is good or improved, and 2—3 is medium or effective; the scores over 3 are poor or ineffective. The photos of skin changes before and after treatment were taken for visual comparison. Statistical processing of the obtained results and the reliability of differences were assessed by a paired t-test of the two groups. All statistical analyses were completed using Microcal origin 7.0 (OriginLab Software, Inc., Northampton, MA, USA). The level of significance of all the tests was set at $p<0.01$. The results were presented as mean values and standard deviation.

Results After treatment with MTrPs acupuncture, 102 cases of focal dermatoses were cured, except for two patients with focal vitiligo that only showed effectiveness (the affected skin darkened a little with no VAS changes before and after) and were classified as ineffective. Their average VAS scores before and after the treatment course were statistically decreased from 8.25±1.65 to 0.59±0.36. The t-test showed that the t-value is 47.13, $p<0.01$, with a highly significant difference. The cure rate was 98%, and the average frequency of acupuncture was 5.2±2.3. There was no recurrence in the 3-year follow-up.

Conclusions The occurrence of skin diseases cannot be regarded as an independent event. Myofascial trigger points, such as high tension of local fascia, high or low sensitivity of nerves, and dysfunction of neurovascular operation, amongst others, may indirectly cause a series of local skin lesions in the body. Therefore, the MTrPs may be the pre-pathological factors that lead to local skin diseases. If the pre-pathology of skin diseases can be removed, the skin lesions as the post-pathology can repair themselves, similar to the traditional Chinese medicine for preventing disease. Thereby, the acupuncture treatment of MTrPs is effective for focal dermatoses. However, some systemic and multifocal dermatoses may partly be cured relative to MTrPs. Other undetected factors may exist, which dermatologists need to explore further.

OR-071

Mechanical stimuli-induced CCL2 restores adult mouse cells to regenerate hair follicles

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Aged cells have a reduced ability to regenerate tissues. Thus, it is intriguing to reactivate the adult cells to regenerate.

Aims Here, we used hair follicles as a model to study how mechanical stimuli induce hair regeneration using hair plucking and organoid culture approaches in adult mice.

Methods We plucked the hairs from the dorsal back skin of 6-month-old CD1 mice, in which hair follicles are at the telogen phase and harvested these samples at PPD0 to PPD4 daily, when hair follicles gradually transition from telogen to anagen. We also harvested samples at PPD10, when

the hair follicles enter full anagen. The skin samples were separated into epidermis and dermis, which were then dissociated into single cells using different enzymatic strategies. The cells were remixed and transplanted into the wound bed created on the dorsal back skin of the nude mice using the planar hair-forming assay. Then we examined the morphology and structure of the hair follicles regenerated from the PPD3 skin cells by phase-contrast microscopy. H&E, immunostaining and AP staining were also used to show the morphological and structural characterization of the regenerated hair follicles. To identify molecular changes involved in plucking-induced hair regeneration, we performed RNA-sequencing (RNA-seq) analysis of the PPD0 and PPD3 samples, which have the least or most hair regenerative ability among all the tested time points in the reconstitution assay. We also examined the extra-follicular inhibitory and excitatory signals that maintain HFSCs quiescence. To test CCL2 and CCR4 function in plucking-induced hair regeneration, we performed an in vivo assay by injecting a CCL2 inhibitor or CCR4 neutralizing antibody into the plucked skin. To examine if the cells from the plucked skin have a distinct cellular behavior during skin organoid culture, we harvested the skin from different post-plucking days. To investigate if chemokine signaling is involved in human skin organoid formation, we analyzed the scRNA-seq data from D29 and D48 samples when skin cells are primed to generate hair follicles. We further tested if this principle can be applied to restore adult cells to self-organize during skin organoid culture.

Results Using phase-contrast microscopy, we observed adult cells can also regenerate all four types of hairs, although the hairs look a little thinner in diameter than the physiologically-developed ones. In addition, we observed that most of the hairs regenerated from adult cells are Zigzag type. The scanning electron microscopy shows that the hair fiber in the reconstituted group has normal cuticle structures similar to that of the normal scaly hairs. H&E staining shows complete morphology of the skin with epidermal and dermal layers, as well as hair follicles along with the newly formed sebaceous gland in the reconstituted skin. K14 immunostaining shows that the skin has essential epithelial layers including the basal epidermis and outer root sheath of the hair follicle. The serial frozen section reveals that these epithelial cells are mostly derived from the grafted donors, as indicated by the fluorescent cells grafted from K14H2BGFP transgenic mice, in which the basal epidermal cells where express K14 are inserted with a nucleotide sequence that encodes green fluorescence protein. AE13 and AE15 immunostaining shows that the newly formed hair follicles have normal inner root sheath. Using immunostaining for hair follicle stem cell markers including CD34 and Sox9, we observed that hair follicle stem cells are correctly located in the bulge area in telogen and anagen hair follicles. Alkaline phosphatase (AP) staining and NCAM immunostaining reveal that the reconstituted hair follicles have normal dermal papilla and dermal sheath structures. KEGG enrichment analysis reveals that the Chemokine signaling pathway ranks first among the differential gene ontology terms. Volcano plots and heatmap show that among the up-regulated genes at PPD3 vs PPD0, many Chemokine signaling pathway genes including chemokine (C-C motif) ligands (CCL) and chemokine (C-X-C motif) ligands (CXCL) were significantly up-regulated. The Bmps that maintain HFSCs quiescence were significantly decreased in PPD3 vs PPD0 skin cells. Whereas Shh, Nog, Fgfs, Fst, and Fstl1 that can activate HFSCs to transition from telogen to anagen were significantly increased in PPD3 vs PPD0 skin cells. Among the CCL immune chemokines, we observed that Ccls and Cxcl are more significantly increased in PPD3 vs PPD0 skin. CCl2 expression is up-regulated in the hair follicle keratinocytes soon after plucking at PPD1 and peaked at PPD3. Using immunostaining for CD34, we verified that the plucked hair follicles only have one layer of stem cells in the outer bulge region. Upon injury caused by plucking, the epithelial cells including the bulge stem cells, and some of the cells located in the interfollicular region are quickly positive P16. Epithelial cell proliferation is more obvious in the bulge and secondary hair germ region at PPD2 and PPD3. P-cadherin which marks the secondary hair germ, is also gradually increased in the secondary hair germ and lower bulge regions. In the skin organoid, K14 immunostaining and quantification show that the epidermal aggregate size is increased in the PPD2, PPD3, and PPD4 groups, compared to that of the PPD0 and PPD1 groups in which tiny aggregates are formed. Immunostaining and quantification show that P-cadherin+ and P63+ cells are significantly increased in PPD2, PPD3, and PPD4 groups, compared to that of the PPD0 and PPD1 groups. TSNE plots show 12 cell types with representative gene expression, including basal keratinocytes (CXCL14+), intermediate keratinocytes (KRT1+), Peridermal keratinocytes (KRT4+),

Fibroblasts (PRRX1+), cycling cells (MKI67+), Neuron cells (PLP1+), etc. And the exemplary chemokine signaling pathway genes including CCL2 and CXCL2 are expressed in the keratinocytes and fibroblasts, but their receptors such as CCR4, and CCR10 are weekly expressed. By RNA-seq analysis of adult and newborn mouse cells, we observed that Chemokine signaling pathway genes including CCL and CXCL genes are significantly decreased in adult cells, compared to the newborn mouse cells. We added CCL2 recombinant protein from D0 to D3, and added Wnt10a recombinant protein from D1 to D4. We then added MMPs from D3 to D7 to trigger the coalescence of aggregate. In addition, we added PKC inhibitors throughout the cultivation period to decrease epidermal cell differentiation. Immunostaining and quantification for K14 show that the addition of CCL2 leads to an enlarged aggregate in size, compared to that of the control with small aggregate formation. And the addition of MMPs led to the coalescence of the aggregate at D4 followed by the formation of a planar skin at D7, in which the epidermal layer is localized at the culture insert side and the dermal layer faces the air side. The restored adult organoids were then grafted onto the dorsum of nude mice, where significantly more hairs were regenerated compared to that of the control group.

Conclusion we elucidate the mechanism by which mechanical stimulus induces hair regeneration at the microenvironmental regulation level using the hair plucking and organoid culture models. Our study reinforces the concept of immune regulation in hair regeneration under mechanical stimuli. The immune modulation in the skin organoid culture system also provides therapeutic potential for future clinical application.

OR-072

Shikonin induces ferroptosis in HaCaT cells by inhibiting the Nrf2/HO-1/GPX4 signaling axis

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Purpose : Psoriasis is an immune-mediated disease characterized by papules and scaling skin. The global patient population is estimated to be as high as 125 million. The excessive proliferation of keratinocyte are the clinical characters of psoriasis. Currently, corticosteroids, vitamin D3 analogues, phototherapy, biologics and oral methotrexate are the primary drugs used for the clinical treatment of psoriasis. However, these drugs often carry the risk of relapse and adverse reactions, and in severe cases, may lead to mental disorders. Therefore, the development of novel drugs with high efficacy and low toxicity is necessary for the treatment of psoriasis. Shikonin(SHK) is a natural compound for the treatment of psoriasis, but its mechanism has not been elucidated. Recent literature has reported the role of SHK in ferroptosis and it has also been found in psoriasis that ferroptosis can exacerbate the progression of psoriasis and exacerbate inflammation. Ferroptosis as a newly discovered form of cell death has only just been discovered in the study of psoriasis. Meanwhile, studies have shown that Shikonin promotes lipid peroxidation by inhibiting Nrf2 signaling, thus leading to ferroptosis. The present study was aimed to investigate the capability and the mechanism of Shikonin(SHK) on HaCaT cells.

Methods We engaged a CCK-8 assay kit (Dojindo, Japan) and lactate dehydrogenase (LDH) assays kit (Beyotime, China) to measure cell viability. We also used the Annexin V- fluorescein isothiocyanate (FITC) Apoptosis Detection Kit and 7-AAD cell viability assay kit (Beyotime, China) to determine the cell viability and apoptosis. The cellular concentrations of MDA and GSH were measured using MDA Assay Kit, GSH and GSSG Assay Kit (Beyotime, China). What's more, Intracellular and mitochondrial ferrous iron content was assessed using the FerroOrange probe (Dojindo, Japan) and the Mito-FerroGreen probe (Dojindo, Japan). Cellular mitochondrial ROS was measured using Mito-Tracker Green (Beyotime, China) and MitoSox Red (Thermo Fisher Scientific, USA). For the cellular lipid ROS, we used the BODIPY-581/591 C11 (Thermo Fisher Scientific, USA). Finally, we used Western blot to detect protein expression including Nrf2, HO-1, GPX4, FTH1, NCOA4, and 4HNE in HaCaT cells treated with SHK. In short, We applied SHK to HaCaT

cells and used western blot assay, specific kits assay and confocal microscopy imaging to explore the mechanism of action of SHK in HaCaT cells.

Results To determine the effect of SHK on HaCaT cell death, the cells were treated with a series of different concentrations of SHK and then assessed for their viability using the CCK-8 assay. Concentrations of SHK below 2 μM slightly enhanced cell viability, while concentrations above 2 μM resulted in a time-dependent decrease in cell viability and an increase in the release of lactate dehydrogenase. The iron-chelating agents Deferoxamine B meylate (DFOM), 3-methyladenine (3-MA), and chloroquine (CQ) could inhibit iron-induced cell death and autophagy and also inhibited cell death induced by SHK. In order to assess the effect of SHK on HaCaT cell death, we used V-FITC/7-AAD staining to observe membrane-associated proteins. At high doses of SHK stimulation, a significant number of HaCaT cells underwent apoptosis, which could be inhibited by DFOM. Our data indicated a dose-dependent increase in MDA levels and a concomitant decrease in GSH levels, suggesting elevated lipid peroxidation in the cells. To further delineate the extent of lipid peroxidation within the cells, we employed the C11 BODIPY 581/591 probe to measure lipid reactive oxygen species (ROS) levels. Our results demonstrated that SHK treatment augmented lipid ROS levels in HaCaT cells, whereas the effect was attenuated by DFOM treatment. In parallel, we assessed mitochondrial ROS levels, which exhibited a similar trend to that of lipid ROS. Collectively, these findings substantiate that SHK effectively increases lipid peroxidation levels in HaCaT cells. Moreover, we evaluated intracellular ferrous ion levels, another crucial hallmark of ferroptosis. In line with the observed changes in lipid peroxidation levels, SHK treatment elicited an increase in ferrous ion content both within the cells and in the mitochondria. Taken together, these results provide evidence that SHK induces HaCaT cell death via the ferroptosis pathway. To investigate the potential mechanism of SHK induced ferroptosis in HaCaT cells, we treated cells with SHK, which resulted in an decrease in Nrf2 and HO-1 expression followed by a decrease in NCOA4 expression, a decrease in FTH1 expression, and an increase in the expression of lipid peroxidation end product 4-HNE. These findings suggest that SHK may induce ferroptosis in HaCaT cells by regulating Nrf2 regulated glutathione metabolism and promoting ferritinophagy. To further investigate whether Nrf2 mediates SHK induced ferroptosis in HaCaT cells, cells were treated with the Nrf2 activator NK-252, which effectively restored the expression of Nrf2 and HO-1, reduced the expression of NCOA4, and increased the expression of GPX4. These results provide additional evidence to support the view that SHK induces ferroptosis in HaCaT cells by regulating Nrf2 regulated glutathione metabolism and ferritinophagy. In a word, results of the current study found that SHK induced ferroptosis in HaCaT cells by enhancing intracellular and mitochondrial ferrous and lipid peroxidation levels, and that ferroptosis inhibitors reversed SHK-induced ferroptosis. Further studies have shown that SHK regulates GPX4 and ferritinophagy through Nrf2 /HO-1.

Conclusions Our results suggested that SHK induces ferroptosis in HaCaT cells by downregulating Nrf2/HO-1 mediated GPX4 and ferritinophagy, including regulating intracellular and mitochondrial levels of ferrous ions and lipid peroxidation. Therefore, SHK can be developed as a new clinical drug for the treatment of psoriasis, and may represent a new treatment method to improve the recovery of psoriasis by targeting ferroptosis.

OR-073

Proteomics in combination with secretomics approach to unravel multiple mechanisms of skin protection benefit of 12-hydroxystearic acid

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Background 12-hydroxystearic acid (12-HSA) is a cosmetic ingredient used in cosmetic formulation as emulsion stabilizer etc. and for skin appearance and skin barrier protection benefits. Published reports indicate that 12-HSA could attenuate UV and oxidative stress-induced hyperpigmentation, inflammation and compromised barrier function in living skin equivalent (LSE) model[1] and acts as a PPAR agonist to help with epidermal homeostasis and barrier function [2],

and enhance skin's natural immunity through elevating level of LL37 AMP in skin explant [3]. Mass spectrometry-based proteome/secretome approach is a powerful strategy to obtain comprehensive picture on protein expression changes within cells and the cross-talk of individual cells. Here, this advanced approached was leveraged to identify and quantify all proteins, including secreted by cells, to reveal underlying mechanism of action on 12-HSA from a holistic perspective.

Methods Bother proteomic and secretomic profiling of keratinocytes treated by 12-HSA at 10μM were investigated here. The primary human keratinocytes were cultured using serum-free medium in six-well plate. 12-HSA was added at 24 hours after adding calcium when keratinocytes were 80% confluency. Three biological replicates were carried out for each group. At 72 hours, cells were harvested and lysed for proteomics analysis. Meanwhile, the cell supernatant was collected for characterizing secreted proteins. Protein expression level was quantified using isobaric labeling Tandem Mass Tag (TMT) platform. Then, 12-HSA significantly regulated expressed and secreted proteins were identified based on statistics analysis. Function analysis of these proteins were conducted using IPA to investigate the underlying mechanisms and interaction between expressed and secreted protein to explore holistic skin benefits of 12-HSA.

Results Totally, 6375 proteins were identified in 12-HSA treated keratinocytes and 3180 proteins were identified in the supernatant by using TMT platform. There were 2485 proteins were identified in both the proteome and secretome. There were about 700 proteins being only identified in the secretome including collagens, MMPs, RNASE7, S100A7, etc.

Firstly, the differentially expressed proteins were identified and their involved pathways were investigated. 12-HSA significantly regulated 162 proteins' expression in keratinocytes and these altered proteins were significantly involved in cholesterol biosynthesis, epithelial adherens junction signaling, integrin pathway and DNA repair pathways liker NER pathway etc. through IPA analysis. In details, 12-HSA regulated skin barrier related proteins like CASP14, SDC1. Interestingly, 12-HSA significantly regulated the expression of DKK1 and RAB32, two key proteins in regulating skin pigmentation and thickness, and regulated CRABP2 which is key marker for anti-aging effect of retinol. 12-HSA also down regulated RHEB, which was reported to inhibit autophagy, as indication that 12-HSA can boost autophagy.

Secondly, secreted proteins changed by 12-HSA were investigated and there were 306 proteins' secretion significantly changed. The significantly changed secretion proteins mainly involved in cytokine signaling, cellular immune response. Furthermore, cell senescence was predicted to be inhibited by secreted proteins through IPA analysis. In details, 12-HSA upregulated ABCA12 acting on lipid synthesis, SERPINB3 and IVL to boost skin barrier function and IL1RN to perform anti-inflammation effect.

Comparison between altered expressed proteins and secreted proteins were conducted, while there were only 6 proteins being changed in both studies. However, there were strong interaction between expressed proteins and secreted proteins through network analysis. There are 81 links between altered expressed and secreted proteins through IPA analysis, like the interaction between secreted MEK1 and expressed DKK1, the expressed PLCG1 and secreted IVL protein.

Conclusion

The proteomics in combination with secretomics study showed that 12-HSA maintained skin homeostasis by interfering multiple biological progresses. These perturbated proteins indicated that 12-HSA delivered multiple skin benefits including 1) skin barrier formation through regulating lipid synthesis, cell differentiation and interaction of cells with matrix; 2) skin lightening through regulating DKK1 to inhibit melanogenesis and RAB32 to interfere melanosome transport; 3) anti-aging effect through CRABP2. Moreover, the network analysis showed the cross-talk between expressed and secreted proteins to deliver desired benefit.

In summary, the proteomics and secretomics study unraveled biological processes involved by 12-HSA through different perspectives. The combination of proteomics and secretomic study shed lights on the impact of 12-HSA on cell-cell communication and provided new indication on mechanisms understanding.

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OR-074

A clinical study on the efficacy of lip cream containing dipalmitoylhydroxyproline, octanoic acid/capric triglyceride, and citrus fruit extract for lip skin moisturizing, repairing and anti-wrinkle

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Objective The stratum corneum of the skin of lips is very thin, and the skin of lips does not have sebaceous glands and sweat glands, which can not secrete oil and sweat to moisten the lips like ordinary skin, so the barrier and moisturizing function of the skin of lips is much lower than that of ordinary skin, resulting in dry skin, dandruff, wrinkles and other problems [.] Therefore, lip skin is more sensitive and fragile than ordinary skin and needs more care. The purpose of this study was to observe the effectiveness of a lip repair cream containing dipalmitoyl hydroxyproline, caprylic acid/caprylic acid triglyceride and citrus fruit extract on moisturizing and repairing lip skin, anti-wrinkle and lightening lines.
Methods A total of 31 healthy subjects aged 18-60 years with dry, peeling lips and obvious lip lines were recruited.Using self-control method, the subjects applied the sample lip cream evenly on their lips after cleaning their lips every day for 28 consecutive days, the moisture content in the cuticle of the lip skin, the moisture loss through skin and the skin softness of the lip were measured at D 0, D 14 and D 28, respectively. At the same time, the doctor evaluated the dryness and texture uniformity of the lip skin before and after using the sample.
Results According to the inclusion criteria, 33 healthy subjects were selected to carry out the experiment, and the final number of effective cases was 31, including 7 males and 24 females, with an average age of 36.7±2.0 years. The ambient temperature is 20.2-21.4 ℃ and the humidity is 42.0%-52.0%RH.
After 14 and 28 days of using lip cream, the moisture content of the subjects' lip skin was significantly increased compared with that before using the product ($p < 0.001$), an increase of 24.72% and 43.47%, respectively. The results showed that after 14 and 28 days of using the sample lip cream, the water content of the lip skin stratum corneum increased significantly, indicating that the sample has a good short-term and long-term hydrating effect.
After 14 and 28 days of using the samples, the water loss in the skin decreased significantly (p & Lt; 0.01) by 6.58% and 8.41% , respectively, the results show that the sample has good short-term and long-term barrier repair function.
After 14 and 28 days, skin softness and skin L * value were significantly increased (P & LT; 0.05) , and skin a * value was significantly decreased (p & lt; 0.001) , the skin softness increased by 4.49% and 14.04% , the skin L * value increased by 3.58% and 3.79% , and the skin a * value decreased by 5.90% and 10.06% , respectively, and reduce the lip skin flush problem, but also make it more shiny, brighten the effect, image contrast also further shows the state of the lip skin changes, you can see that the lips after the use of samples more bright, the reddening problem has also eased.
In addition to the use of non-invasive instruments to determine whether lip products are effective, subjective evaluation of the lip skin can also intuitively reflect the effect of the product. After 14 days and 28 days of sample use, the expert scored the dryness degree and texture (texture) uniformity

of lip skin according to the description of the subjects. Compared with before use, the dryness degree of lip skin and texture uniformity of skin were significantly improved ($p < 0.001$). Both subjective assessment and objective instrumenting indicators showed that the skin condition of lips was significantly improved after the use of samples, and the results of both were consistent, which could confirm each other and make the evaluation results more perfect.
Compared with pre-use, the area and volume of labial lines decreased significantly at 14 and 28 days, and the area and volume of labial lines decreased by 29.89% and 37.34% , respectively (P & Lt; 0.001) , and the volume of labial lines decreased by 29.53% and 28.62% , respectively (P & Lt; 0.01) .Compared with before use, the lip lines were significantly reduced, indicating that the sample had good short - and long-term anti-wrinkle effect.
Conclusion After applying the lip cream containing dipalmitoylhydroxyproline, octanoate/capric triglyceride, and citrus fruit extract, the moisture content of the cuticle, skin softness, and skin L * value of the lip skin significantly increased, the moisture loss, a * value and the uniformity of dry and texture of lip skin were significantly improved, which indicated that the lip cream had good short-term and long-term moisturizing and repairing effects. The area and volume of lip lines were also significantly reduced, indicating that the cream has good anti-wrinkle effect. There were no adverse reactions in all subjects.

OR-075

Diverse immune environments in cutaneous granulomatosis by quantitative multiplexed immunofluorescence

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Introduction
Cutaneous infectious granulomatosis is a heterogeneous group disease, characterized by a skin inflammatory reaction of multiplex clinical features of nodules, ulcers, or verrucous plaques. Infectious granulomas of the skin are usually chronic and localized skin infections, the agent being mycobacteria(Non-tuberculous mycobacteria, NTM) or fungus (Sporothrix schenckii) possibly(1). M. marinum is the most common type of NTM that can cause skin lesions and sporotrichosis is a chronic subcutaneous infection caused by the rapidly growing dimorphic fungus S. schenckii(2,3). Their pathogenic mechanisms of them are discussed rarely. Pathogenesis and treatment must understand the immune microenvironment of the infectious granuloma structures. The structure of infectious granuloma caused by different pathogens may be heterogeneous(4).
Infectious granulomas are organized immune cell aggregates that originate in response to persistent stimuli of an infectious nature. At its most basic level, a granuloma is a compact, organized immune aggregate of macrophages surrounded by myeloid, T, and B cells, such as TB granulomas(4). Whether the immune microenvironment of cutaneous infectious granuloma caused by different pathogens is similar to lung TB granuloma has not been studied. In this study, we aim to investigate the immune microenvironment of cutaneous infectious granuloma of M. marinum and S. schenckii infection in this multiplexing technique.
Materials and Methods
13 M. marinum infections and 13 sporotrichosis patients were contained in this study. All patients were confirmed with clinical features, histopathology, polymerase chain reaction(PCR), and positive culture specimens. The myeloid panel include CD11b(rabbit monoclonal, 1:400, CST, product number 48274S), CD68(rabbit monoclonal, 1:1000, CST, product number 76437S) and CD66b(rabbit monoclonal, 1:200, Abcam, product number ab197678). Lymphoid cell panel include CD8(rabbit monoclonal, 1:400, CST, product number 85336S), CD4(rabbit monoclonal, 1:200, CST, product number 48274S), and B cell panel CD20(rabbit monoclonal, 1:400, CST, product number 48750S).

Multiplex immunofluorescence(mIHC) staining protocol according to the Akoya opal methods. Tyramide signal amplification (TSA) visualization with fluorophores Opal 520, Opal 540, Opal 570, Opal 620, Opal 650, and Opal 690(Akoya Biosciences). The Fluorescence images were acquired on a Vectra-Polaris Automated Quantitative Pathology Imaging System (Akoya Biosciences). Akoya's inForm advanced image analysis software was used to quantitatively imagery and analyze the extracted data.

Spearman correlation coefficient (r) was calculated for the correlation analyses between different cell marker distributions. Wilcoxon–Mann–Whitney tests were applied to identify immune markers that reached a significant difference in density levels among granulomas with positive and negative AFB staining for M. marinum infection or PAS staining for S. schenckii infection. All $p < 0.05$ values were considered statistically significant.

Results

1. Heterogeneous immune environment on M. marinum and S. schenckii infection granulomas with macrophages, neutrophils, T cells, B cells, and myeloid cells.

The demographics of the patients are shown in Supplemental Table 1. Histologically, granulomatous inflammatory infiltrate in the dermis and/or hypodermis is the common feature, the main infiltrate is composed of macrophages(5).

To characterize the immune cell infiltrate, we used seven-colour mIHC (CD20, CD4, CD8, CD68, CD11b, CD66b, and DAPI) to estimate different immune infiltrate subpopulations in the large area of M. marinum and S. schenckii infection granulomas from 13 patients, respectively. M. marinum and S. schenckii-caused infection granulomas were counted for ~178937 /~233631 cells by Akoya's inForm advanced image analysis software, respectively. Diverse cellular compositions of two group granulomas environments were observed. B and T lymphocytes and myeloid cells were distributed heterogeneously in different granulomas in two groups(Supplemetal Figure 1).

To explore the expression of immune cells with six markers expressing in the immune microenvironment of the granuloma structure in all samples. We analyzed and compared the expression frequency of all immune cells labeled by six markers in Fig. 1A-D. Huge differences in the expression of all marker-expressing immune cells were found. Macrophages, neutrophils, T cells, B cells, and myeloid cells were quantified by their antibodies markers and the extracting data showed heteroplasmy of the immune microenvironment. The results of immune cell frequency in CD4+ T cells($p=0.0070$) are still dominant expression compared with other immune cells of most samples, whether in the granuloma of M. marinum or S. schenckii infection(Fig.1E-F). The relative frequency of macrophages(CD68+) in M. marinum and S. schenckii infection granulomas peaked at the top in most samples.

2. Immune cell densities on M. marinum and S. schenckii infection granulomas.

Macrophages are the primary host cells that initiate an immune response to NTM, it is really important to define the infection within macrophages for understanding the pathogenesis of NTM disease, including M. marinum infection(6). The immune cell densities in the granulomas of two groups were quantified and analyzed using the mIHC panels of antibodies targeting the T cell marker CD4+ and CD8+, CD68+ macrophages, B cell marker CD20+, CD66b+ neutrophils, and CD11b+ myeloid cells. All granulomas observed heterogeneity in immune cell densities of five types of immune cells(Fig. 2A-B).

We use Spearman correlation analysis to determine the association between the immune cell densities of macrophages, neutrophils, T cells, B cells, and myeloid cell markers mutually. Macrophages marker CD68+ was only strongly correlated with myeloid cell marker CD11b+ ($r=-0.66$, $p=0.0017$) in M. marinum infection granuloma(Fig. 2C). Macrophages marker CD68+ was strongly correlated with T lymphoid cells(CD8+, $r=-0.76$, $p=0.004$)and B cells(CD20+, $r=-0.70$, $p=0.009$) in S. schenckii infection granuloma(Fig. 2D).

Compared with negative acid-fast bacilli (AFB) (n=8) on microscopic examination, no significant difference was found between the AFB-positive (n=5) group and the AFB-negative group in the six marker-labeled immune cells in M. marinum infection ($p > 0.05$) in Supplemental Table 2. A significant difference ($p=0.0255$) in CD68+ macrophages cell densities between the periodic acid-Schiff (PAS) stain-positive group (n=8) and negative group (n=5) in S. schenckii infection granulomas were found in Supplemental Table 3.

To compare whether the structure of granuloma caused by two pathogens(bacteria and fungus) is dissimilar, we try to analyze the cell densities between M. marinum infection granuloma and S. schenckii granuloma. The density of CD4+ and CD4+/CD8+ cells was statistically different between M. marinum and S. schenckii granuloma (p=0.0065; p=0.0070) in Fig. 2E. Significant difference was not found in other immune cells.

3. Spatial pattern in M. marinum and S. schenckii infection granulomas.

To understand the spatial pattern of immune cells is crucial to know the immune microenvironment of cutaneous infection granulomas. Though the whole granuloma analysis approach may not reflect the true size of the lesion, we still found some obvious distribution patterns of the immune environment. Our results show the easy approach to distinguishing granulomatous or non-granulomatous structures of various stages. The granuloma structures are primarily dense aggregations of immune cells. Macrophages accumulate or disperse within the granuloma structure in all samples. Immune cells other than macrophages surround the granuloma structure in most samples of M. marinum and S. schenckii infection(Fig. 3A-B).

In negative AFB/PAS samples, immune cells are distributed within the granuloma structure except for macrophages. The positive AFB/PAS group has a more dense immune density of granulomas than the negative AFB/PAS group. We find many stages of granuloma formation on the granuloma pattern map of M. marinum and S. schenckii infection samples, some granuloma structures are chaotic with various immune cells randomly distributed. No significant difference was found between all immune cells between AFB positive and negative groups. Except for the significant differences in macrophages(p=0.0255) between the PAS-positive and negative groups, no differences were found in other immune cells between the two groups (Fig. 3C-D).

Conclusion

To our knowledge, there is no study applying quantitative mIHC to explain cutaneous infection granuloma. The composition of the immune microenvironment of cutaneous infectious granuloma caused by two different pathogens(M. marinum and S. schenckii) is similar and different. The immune microenvironment of cutaneous infectious granuloma is heterogeneous. One limitation is the small number of cutaneous granuloma samples examined in our study. The cell density of CD4+ T cells and CD4+/CD8+ T cells may reflect the different pathogenesis of M. marinum and S. schenckii that invaded the host.

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Figures:

Figure 1：Immune environments on M. marinum and S. schenckii infection granulomas with all marker-expressing immune cells.

A, B. Seven markers of CD8, CD20, CD66b, CD4, CD11b, CD68, and DAPI are expressed in M. marinum and S. schenckii infection granulomas separately. C, D. Cell frequency of six marker-labeled macrophages, T cells, B cells, myeloid cells, and neutrophils in M. marinum and S. schenckii infection granulomas. E. Comparative results of the relative frequency of six marker-

labeled immune phenotypes between M. marinum granulomas and S. schenckii infection granulomas. A significant difference in the frequency of CD4+ T cells(p=0.0007) in the two groups (p value＜0.05 significant).

Figure 2：Immune cell densities on M. marinum and S. schenckii infection granulomas.

A, B. M. marinum and S. schenckii infection granulomas with higher expression of macrophages marker CD68+ and lower expression of B cell marker CD20+, neutrophils marker CD66b+ and myeloid cell marker CD11b+ of cell densities(cell/mm²). C, D. Spearman correlation shows the relationship between two types of immune cells mutually(p value ＜ 0.05 significant). E. Comparative results of the immune density of six marker-labeled immune phenotypes of M. marinum and S. schenckii infection granulomas. The density of CD4+ and CD4+/CD8+cells was statistically different between the two groups (p=0.0065; p=0.0070).

Figure 3 ： Spatial patterns of the immune microenvironment in M. marinum and S. schenckii infection granulomas.

A. Positive AFB and negative AFB of M. marinum infection granulomas and positive PAS and negative PAS of S. schenckii infection granulomas, respectively. B. Immune pattern images corresponding to figures A. C, D. Comparative results of immune density differences between different immune cells in the positive AFB and negative AFB groups of M. marinum infection granulomas. E. Comparative results of immune density differences between different immune cells in the positive PAS and negative PAS groups of S. schenckii infection granulomas. The density of CD68+ macrophages was statistically different between the positive PAS and negative PAS groups in S. schenckii infection granulomas(p=0.0255).

OR-076

Effect of Fibroblasts on the Structure of Human Hair Follicle Stem Cells-Derived Hair Follicle Organoids and its Mechanism

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Objective Androgenetic alopecia (AGA) is the most common clinical pathology of hair loss, with strong patient intention for treatment but limited efficacy. The construction of hair follicle organoids could facilitate further AGA-related drug discovery, gene therapy and tissue engineering techniques. However, this technology is still faced with long culture cycles and poor feedback on individual heterogeneity. Therefore, this study was based on the isolation, culture and identification of hair follicle stem cells (HFSC) and hair follicle fibroblasts (HFFB) from the occipital hair follicles of AGA patients to investigate the effect of fibroblasts on the structure of human hair follicle stem cell-derived hair follicle organoids and the underlying mechanisms.

Materials and Methods Spatial transcriptomics (ST) technology was used to explore the spatial location information of HFSC and HFFB to provide data support for the selection of "seed cells" for constructing hair follicle organoids. Secondly, Human primary HFSC and HFFB were isolated, cultured, subcultured, cryopreserved and revived, and human primary EKC and DFB were revived and cultured. The expression of HFSC and HFFB-related genes was detected by real-time fluorescent quantitative polymerase chain reaction (RT-qPCR), the expression and localization of HFSC and HFFB-related proteins were detected by immunofluorescence staining, and the expression and localization of HFSC and HFFB-related proteins were detected by flow cytometry. The expression of cell surface antigens or intracellular antigens related to HFSC and HFFB was detected by technique. HFSC was constructed and differentiated into HFSCO; by selecting the volume ratio of HFSC and HFFB, the method of group transfer and the differentiation medium, the suitable conditions for adding HFFB were found out, and the differentiated HFSCFBO was constructed, and the hair follicle organoid was observed and recorded under an inverted microscope. Viability of hair follicle organoids was detected by live and dead cell staining. The

morphological changes of hair follicle organoids were detected by hematoxylin-eosin staining (H&E staining), the expression of adipocyte markers in hair follicle organoids was detected by Oil Red O staining, and the characteristics of hair follicles in hair follicle organoids were detected by immunofluorescence staining Expression and localization of markers. Finally, based on transcriptome sequencing, we investigated the relevant enrichment pathways to explore the mechanism of action of HFFB affecting hair follicle-like organ formation. **Results** The spatial distribution of HFSC and HFFB gene expression in skin tissues was defined by spatial transcriptome sequencing, and spatial differential expression analysis showed that HFFB promoted epithelial lineage differentiation and tissue regeneration more than interfollicular fibroblasts. The spatial differential genes of HFFB and IFFB were obtained by spatial differential gene expression analysis. The up-regulated genes of HFFB include KRT17, CST6, KRT15, S100A2, SCGB2A2, MUCL1, KRT6B, KRT5, and SCGB1D2, etc., which are closely related to the growth and development of hair follicles; GO and Reactome enrichment analysis found that the differential genes were mainly enriched in the biological processes of epithelial development, tissue regeneration and homeostasis. The HFSC isolated and cultured from human occipital hair follicles can grow adherently, showing polygonal shape, and can be stably proliferated, passaged, cryopreserved and revived. RT-qPCR showed that the gene expression levels of stemness genes KRT15, LHX2, SOX9, LGR5 and ITGA6 in HFSC were higher than those in EKC. Immunofluorescence staining showed the high expression of HFSC-related protein markers KRT15, KRT19, CD200, ITGA6, CD71, WIF1, FST and p63 and their expression locations. Flow cytometry showed that the proportion of cells expressing KRT15+ /FST+, FZD1+ /WIF1+, CD29+/CD49f+ and CD71+ specific intracellular and cell surface antigens in the primary extracted HFSC was higher, that is, the purity of HFSC was higher. RT-qPCR showed that the gene expression levels of HFFB-related genes COL1A1, FN1, FBN1, SOX2, ALPL, NOG, PLCG2 and LEF/TCF were higher than those of DFB. Immunofluorescence staining showed the high expression of HFFB-related protein markers VIMENTIN, ASMA, S100A4 and COL1A1 and their expression locations. Flow cytometry verified that the proportion of cells expressing CD90 + /CD13 + and CD90 + /CD26 + related surface antigens in HFFB was higher. Furthermore, the vesicle-hair bulb polarized structure of HFSCO was observed under an inverted microscope, and 300.6 ± 507.7 hair follicle organoids could be induced by one follicular unit through statistical analysis of the number of primary HFSC cells. When the volume ratio of HFSC and HFFB reaches 1:3, the ideal hair follicle organoid structure can be induced in the microenvironment simulated by matrigel and adding factors by successively centrifuging HFSC and HFFB. After culturing for 5-7 days, the germination of HFSCFBO spheres was observed under an inverted microscope, which showed that rod-shaped structures protruded from the spheres, and the tails were hair-like structures. The proliferation level of HFSCFBO was better than that of HFSCO, and the survival period of HFSCFBO was as long as 60 days. H&E staining showed that the histomorphological structure of HFSCO and HFSCFBO was similar to that of normal hair follicles. Oil red O staining showed the expression of adipocyte-related markers in HFSCFBO.HFFB increased the induction and survival rates of hair follicle organoids and contributed to the structural maturation. Further mechanistic studies revealed that HFFB significantly promoted the production of several epidermal growth factors and was involved in the induction of HFSC into hair follicle organoids through the upregulation of IGF-1R, Wnt/β-catenin and MAPK/p38 pathways. **Conclusion** human HFSC and HFFB were successfully used as "seed cells" to differentiate and induce characteristically stable and structurally mature hair follicle organoids. The beneficial role of HFFB in influencing the maturation of HFSC towards organoid differentiation is confirmed and further relevant pathways are revealed. In summary, the findings of this study could provide some theoretical basis for hair regenerative medicine research, hair growth and development research and the identification of new drugs for AGA.

OR-077

A Case-control Study Exploring the Association Between Cosmetic Use and Acne Risk: Implications for Prevention and Clinical Practice

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Introduction & Objectives According to epidemiological surveys, cosmetics are considered to be one of the risk factors for acne, especially for young women. Some studies have referred to acne caused by cosmetics as "Acne Cosmetica" due to the presence of comedogenic ingredients in cosmetics. Additionally, research has shown a dose-exposure relationship between cosmetics and acne, indicating a negative association between overall cosmetic exposure and postpubertal acne that is dependent on the dosage. Certain categories of cosmetics have also been identified as risk factors for postpubertal acne. While previous studies have provided valuable insights into the link between cosmetics and acne, focusing more on the effect of the overall cumulative dose of cosmetics on the risk of acne, there is a lack of prevalence investigations on the cumulative dose of individual types of cosmetic products and comedogenicity ingredients cause the risk of acne. Therefore, we analyzed the relationship between cosmetics and acne occurrence in three aspects: the type of cosmetic exposure, the cumulative dose of cosmetics, and the use of cosmetics containing comedogenicity ingredients.

Materials & Methods In this case-control study, a totally 151 females (81 acne patients and 70 controls) who used cosmetics were randomly selected from the Department of Dermatology at Sun Yat-sen Memorial Hospital, Sun Yat-sen University from October 2022 to April 2023. The demographic information, type of cosmetics, dosage, and product name in the past 28 days were collected through a self-administered questionnaire, and the product's ingredient list was consulted by product name to assess the product's containing comedogenicity ingredients. Further, we compared the demographic information, family history, dietary habits, and related cosmetic data were compared between the two groups with or without acne by using the nonparametric test for continuous variables or X2 test for categorical variables. Univariate and multivariate logistic regression analyses were conducted to evaluate the relation between the types of cosmetics, the cumulative dose or the use of cosmetics containing comedogenicity ingredients and the risk of acne separately. Factors that were significant and considered meaningful in demographic information, family history, and dietary habits were used as corrective factors in the multivariate regression model.

Results In terms of the basic information, family history, and dietary habits of the interviewed population, a total of 66.9% of the participants were over the age of 25, the proportion of the patient group is 35.1%, while in the control group it is 31.8%, there was no significant difference between the two groups. The occupational distribution differed between the case and control groups, with a higher proportion of Students in the case group and a higher proportion of Professionals in the control group. The occupational distribution differed significantly between the two groups ($p < 0.05$). With a significant between-group difference ($P = 0.03$), the proportion of first-degree relatives with an acne history among the analyzed acne family history was 13.9%, 13.9%, and 31.8% for fathers, mothers, and siblings, respectively. However, this finding was only significant for parents. In terms of dietary habits, milk consumption was significantly different between the two groups ($P < 0.05$). We discovered that the use of facial cleanser (P=0.04), foundation (P=0.03), and powders (P=0.01) were risk factors for acne, while the effect of each cosmetic type on acne was facial cleanser (OR:3.59) > powders (OR:2.86) > foundation (OR:2.13) in that order. After correction of age (<25 vs≥25 years old), occupation, father acne history, mother acne history, and milk consumption, only the use of powders significantly increased the risk of acne [OR:3.47; 95%CI:1.58-7.59, P=0.02]. In addition, moisturizers were found to be the independent risk factors for acne after controlling the other risk factors, and the higher the dose of the product used led to a higher risk of acne occurrence[OR:1.03; 95%CI:1.01-1.05, P=0.03]. Moreover, the use of facial cleanser containing comedogenicity ingredients was found to be an independent risk factor for acne[OR:2.49;

95%CI:1.23-4.90, P=0.01]. Based on the collected data from the respondents regarding the ingredients of their facial cleansers, stearic acid, myristic acid, and glyceryl stearate SE were identified as the most prevalent comedogenic ingredients.

Conclusion In this study, we observed a significant association between the use of powders and an increased risk of acne in females, with a 3.47-fold higher risk compared to those who did not use powders. Additionally, we found that higher doses of moisturizers were associated with a greater risk of acne occurrence. Moreover, the use of facial cleansers containing comedogenicity ingredients was independently associated with a higher risk of acne, resulting in a 2.49-fold increased risk of acne occurrence. This study suggests that the use of certain types of cosmetics may increase the risk of acne, in relation to the dosage of cosmetics used and comedogenicity ingredients. Therefore, we suggest that patients should be careful to choose appropriate products, avoid cosmetics containing comedogenicity ingredients, and control the amount of cosmetics used. Further research is needed to better understand the mechanisms underlying the relationship between cosmetics and acne, to develop effective preventive strategies for acne.

OR-078

The roles and potential intervention therapies of KLF2 in CTCL progression

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Introduction Cutaneous T cell lymphomas (CTCLs) comprise a group of T cell non-Hodgkin lymphomas with clonal expansion of skin-homing T cells, which counts for the second highest incidence of extra-nodal non-Hodgkin lymphoma. Mycosis fungoides (MF), the most common type of CTCL, usually presents with patches and plaques and usually has an indolent clinical course in the early stage. The main treatments are skin-targeted therapies. Progression occurs in 25% of patients with early-stage diseases, presenting rapidly progressive skin tumors, aggressive extracutaneous spreading, resistance to conventional treatments, and poor prognosis. However, little is known about the progression mechanisms of CTCL and the management of advanced-stage patients is a huge clinical obstacle. At present, systematic therapies, including interferon-alpha 2b, methotrexate, and bexarotene, are used to treat early refractory MF and advanced cases. Due to the poor skin barrier function of MF patients, multi-drug combination therapies will increase the risk of secondary infection and the incidence of adverse drug reactions. Moreover, advanced-stage patients are often resistant to these drugs, resulting in the need for chemotherapy or bone marrow transplantation finally, which greatly reduced the quality of life. Therefore, new and safe treatment modalities for these types of CTCLs are in urgent need.

Krüppel-like factor 2 (KLF2) was negatively regulated by paternally expressed gene 10 (PEG10), which potentiated large-cell transformation of CTCL， leading to cell resistance to treatment and poor prognosis of patients. We found KLF2 was significantly downregulated in tumorous T cells compared with normal reactive T cells in a published single-cell RNA sequence data of CTCL patients. Previous reports suggested that KLF2 is expressed in naive T cells, downregulated in activated cells, and again expressed in mature memory T cells. Its level is negatively correlated with T-cell activation, which can inhibit cell proliferation, size, and activation. However, the roles of KLF2 in CTCL are unclear. Moreover, statin, a clinically lipid-lowering drug, has been reported to induce KLF2 expression in human and mouse T cells and reduce inflammatory and pathogenic responses. Statins can up-regulate KLF2 expression by inhibiting the mevalonate pathway, activating the Rac1-Rab7-autophagy axis, down-regulating the expression of m6A demethylase FTO and so on. As lipid-lowering drugs that have been used clinically for many years, the pharmacokinetics and safety data of statins have been verified by multiple parties. Common side effects of conventional bexarotene medications for CTCL include abnormal high triglycerides, which may require the use of lipid-lowering drugs such as statins.

Objectives We aimed to explore the roles of KLF2 in CTCL and potential intervention strategies. It is also hoped to further explore the novel roles of statins in CTCL therapy via inducing KLF2 expression, to find clinically safe and effective drugs to effectively control the progression of the disease, and ultimately achieve the goal of improving patients' prognosis and quality of life.
Materials and Method We performed KLF2 knockdown and overexpression tests in CTCL cell lines to validate cell viability and apoptosis. We evaluated cell phenotypes of viability and apoptosis in a series of drug-exposure tests under the treatment of statins and/or combined with traditional CTCL treatment drugs in CTCL cell lines and primary cells. Further transcriptome sequencing analysis and validation tests were performed to evaluate the downstream regulation mechanisms.
Results Based on published single-cell RNA sequencing data, we found KLF2 was significantly downregulated in tumorous T cells compared with normal CD4+ T cells in advanced-stage CTCL patients. Moreover, KLF2 was significantly downregulated in 7 well-recognized CTCL lines compared with normal CD4+ T cells from 3 healthy controls. Overexpressing KLF2 inhibited cell viability and induced apoptosis in two CTCL lines, Hut78 and Myla. Both simvastatin and atorvastatin could upregulate KLF2 mRNA and protein expression levels, depress cell viability and induce cell apoptosis in a concentration-dependent manner in multiple CTCL lines. Moreover, they could also confer increased sensitivity to bexarotene, which was used in advanced CTCLs with adverse effects of hyperlipidemia. Further transcriptome sequencing analysis of CTCL cells under simvastatin treatment discovered simvastatin could confer growth disadvantages in CTCL cells via multiple classical pro-apoptosis, proliferation-inhibition, and inflammatory response pathways, such as E2F, G2M, MYC, apoptosis, P53, as well as TNF-a signaling via NFKB pathways. Key downstream genes participate in cell proliferation and apoptosis, such as PLK1， CDC20， RHOB，and BMF, whose expression levels were significantly related to the CTCL patients' prognosis. Silencing KLF2 expression rescued partially simvastatin-induced viability inhibition in CTCL cells. Moreover, key downstream genes upon simvastatin treatment were confirmed in KLF2-overexpressed CTCL cells. Finally, we confirmed tumorous CD4+ T cells are more sensitive to simvastatin compared with normal CD4+ T cells in primary CTCL cells from PBMC with blood involvement.
Conclusions These results provide new insights into the pathogenic mechanisms of KLF2 underlying CTCL progression. Stains, clinically lipid-lowering drugs, may play a novel role in CTCL therapy via inducing KLF2 overexpression.

OR-079

Mutation Detection of the SMARCAD1 gene and Basan Syndrome with Cutaneous Basal Cell Carcinoma

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1. Background

Basan syndrome is a rare ectodermal dysplasia and autosomal-dominant monogenic disease characterized by high penetrance and variable expression. Malformations of the ectodermal tissue can result in various abnormalities affecting the hair, teeth, nails, and sweat glands, along with potential involvement of the thyroid and thymus glands. After birth, individuals with Basan syndrome typically exhibit distinct clinical features, including the absence of dermal ridges on the palms and soles, neonatal acral blisters, facial milia, adult traumatic blistering and fissuring, reduced or absent sweating on the palms and soles, and digital contractures.
The incidence of Basan syndrome remains unclear, with only 15 families reported worldwide to date. The disease is primarily caused by mutations in the SMARCAD1 gene located on chromosome 4q22.3, including c.378+3A>T, c.378+1G>T, c.378+5G>A, c.378+2T>G, and c.378+1G>A. Additionally, Elhaji et al. (2021) identified a 50.9 kb deletion, a non-tandem

duplication, and an inversely complex rearrangement in a Canadian family with Basan syndrome, as well as a 12 kb deletion at the exon-intron junction (c.374_3787del) in a Dutch family.

In this study, we present a new Chinese family affected by Basan syndrome, in which the proband exhibited both Basan syndrome and cutaneous basal cell carcinoma (cBCC). Xiong et al. (2022) discovered an association between Basan syndrome and cutaneous squamous cell carcinoma (cSCC). However, no previous research has established a link between Basan syndrome and cBCC. Our objective is to identify the pathogenic gene mutation responsible for Basan syndrome in this family and explore the possible connection between Basan syndrome and BCC. This investigation contributes to a deeper understanding of the pathogenesis and clinical characteristics of Basan syndrome, with potential implications for the diagnosis of related dermatological conditions.

2. Method

2.1 Proband and Pedigree investigation

The proband was a 38-year-old male who presented with dry skin on his hands and multiple eruptive milia on his face at birth. At the age of 23, he developed a callus-like rash and gradually experienced joint contracture in the fifth finger, without any other associated discomfort. He had previously been diagnosed with psoriasis (and was cured) at 19 years old and with cutaneous basal cell carcinoma (cBCC) due to a rash on his chest at 35 years old . Physical examination revealed flexion contracture of proximal interphalangeal joints of the fifth finger on both hands, atrophy and mild pigmentation on the dorsal skin of the hands, punctate-type palmoplantar keratosis merging into plaque, absence of fingerprints, and poor nail development with slight longitudinal striations on the surface of the nail plate of the right middle finger (Figure 1(a)).

Five individuals in this family were affected by Basan syndrome exhibiting similar clinical manifestations. There was no history of cancer in the family other than the proband. The pedigree of the family, illustrating the familial relationships among the individuals, is depicted in Figure 2. This pedigree serves to provide a visual representation of the inheritance pattern and familial clustering of Basan syndrome within the family. Before sample collection, we obtained written informed consent from the patient, ensuring their understanding and agreement regarding the purpose and procedures of the study. Additionally, explicit permission has been obtained to publish photographs of the patient, respecting their privacy and confidentiality.

2.2 Sanger sequencing and Haematoxylin and eosin staining

Peripheral blood genomic DNA was extracted from the nine family members. Whole exon sequencing was performed using the Illumina HiSeq Nova 6000 sequencer, and Sanger sequencing was used to confirm the mutation. In addition to the genetic analysis, the proband's skin biopsies with cBCC in 2017 were subjected to detailed histopathological examination

2. Result

2.1 Pedigree Investigation.

The proband had dry skin at birth and developed callus-like rashes on his hands at the age of 23. Gradual joint contracture of the fifth finger joints occurred without other discomforts(Figure 1(a)). As the proband aged, excessive keratinization of the palms and soles became increasingly apparent, and fingers gradually became thinner with vertical ridges appearing on the nails. Additionally, pigmented spots appeared on the hands and feet. The proband had previously been diagnosed with psoriasis at age 20, and there was no history of psoriasis in the family. At age 36, the proband was diagnosed with basal cell carcinoma (BCC) when seeking medical attention for a rash on their chest. The proband's mother and sister exhibited no fingerprints and had dry skin. The mother demonstrated progressive hyperkeratosis and joint contracture of the fingers. The proband's 8-year-old son had dry skin on hands and feet and did not sweat.

2.2 SMARCAD1 gene mutation.

To identify the underlying genetic defects, WES was performed in all nine members of family, including five who were affected (I-2, II-2, II-3, III-1, III-2) and four unaffected (I-1, II-1, II-4, III-3), confirming a heterozygous mutation in the SMARCAD1 gene NM_001254949.2: c.378+5G>A in I-2, II-2, II-3, III-1 and III-2. Then, the WES result was further confirmed by Sanger sequencing (Figure 3). This mutation site leading to Basan syndrome was first reported by Xiong et al. (2022).

2.3 Histopathology of the skin biopsies.

The histopathological examination of skin biopsies obtained from the proband (II-2) revealed distinctive features. Clusters of basaloid cells were observed, extending from the basal layer of the epidermis into the papillary layer of the dermis, and arranged in a characteristic palisade pattern. These basaloid cells exhibited enlarged and deformed nuclei, demonstrating pleomorphism, along with the presence of atypical nuclei and mitotic figures. Furthermore, the stroma surrounding the basaloid cells exhibited a mucinous matrix, and focal lipid vacuoles were also observed (Figure 4). This histopathological profile provides valuable insights into the cellular and structural abnormalities associated with Basan syndrome in the proband.

3. Discussion

In this study, we reported a novel Chinese family with Basan syndrome and the proband had a history of cutaneous basal cell carcinoma (cBCC). Through genetic analysis, we identified the pathogenic mutation site as c.378+5G>A in SMARCAD1 gene. These findings provided further insight into the genetic basis of Basan syndrome and its association with cBCC.

The genetic pattern of the family reported in this article is consistent with autosomal dominant inheritance. This rare mutation has been reported in public databases and was considered as moderate evidence of pathogenicity (PM2). The mutation was detected in multiple affected individuals of this family, which is considered as supporting evidence (PP1-Moderate). Xiong et al. (2022) found that it may cause splicing variation and regarded it as strong evidence of pathogenicity (PS3-Moderate). Meanwhile, the clinical manifestations are typical of Basan syndrome, which serves as additional supportive evidence (PP4). According to the guidelines published by the American College of Medical Genetics and Genomics (ACMG), this type of mutation should be classified as a potential pathogenic mutation of Basan syndrome.

The SMARCAD1 gene, located on chromosome 4q22.3, belongs to the SWI/SNF subfamily and encodes an ATPase of the SNF2 helicase subfamily. It is expressed in various tissues and organs and functions as part of a protein complex. SMARCAD1 gene has revealed three primary protein functions. Firstly, it is involved in gene silencing and the formation of heterochromatin. Secondly, it plays a role in DNA replication and repair processes. Lastly, it regulates a wide range of transcription factors and multiple subtypes of alternative splicing transcript variants. This regulatory activity allows it to control the expression of target genes involved in cell cycle regulation and developmental processes.

In 2011, Janna Nousbeck and Eli Sprecher conducted linkage analysis and haplotype analysis on a family, revealing the existence of two isoforms of the SMARCAD1 gene with distinct transcription start sites. The short isoform, referred to as the skin-specific isoform, is predominantly expressed in skin fibroblasts, with lower expression in keratinocytes and esophageal tissues. Skin-specific isoform may influence genes involved in dermatoglyph and sweat gland development. On the other hand, the long isoform is more widely expressed but at lower levels across various tissues. The long isoform consists of 24 exons and encodes a protein comprising 1028 amino acids which is expressed ubiquitously at low level. Mutations in the short isoform of SMARCAD1 have been associated with Basan syndrome; however, the precise underlying mechanisms remain unclear.

The ectodermal lineage, which encompasses various tissues including the skin, is regulated by highly conserved signaling pathways, such as the WNT (Wingless/Integrated), BMP (Bone Morphogenetic Protein), and FGF (Fibroblast Growth Factor) pathways. Therefore, the investigation of the signaling pathways associated with the ectodermal lineage can help understand the specific signaling pathway of the skin-specific isoform of SMARCAD1.

Basan syndrome, and Huriez syndrome (HRZ, OMIM181600) share significant clinical similarity and genetic mutations, prompting some scholars to suggest that they are phenotypic variations of the same disease. Mutations in HRZ are all located at upstream of intron 1 of the SMARCAD1 gene, which is the same mutated gene as Basan syndrome. Additionally, several studies have reported an increased incidence of cBCC in patients with HRZ. The co-occurrence of Basan syndrome and cBCC in our patient suggests a potential association between these two diseases. There are two plausible explanations for this observation. One is that the mutation in the SMARCAD1 gene that causes Basan syndrome may also contribute to the developmento of cBCC. The SMARCAD1 gene is involved in various cellular processes, including gene silencing, DNA replication and repair, and regulation of transcription factors. Dysregulation of these processes due to the mutation in SMARCAD1 may predispose individuals to the development of cBCC. The other

explanation is that the heightened susceptibility to skin tumors in patients with Basan syndrome makes them more likely to develop cBCCs. The underlying mechanisms for this increased susceptibility could involve impaired DNA repair mechanisms or altered regulation of cell growth and differentiation.
However, definitive conclusions regarding the relationship between cBCC and Basan syndrome cannot be drawn at this stage for the small number of cases. Further research involving a larger cohort of patients with Basan syndrome is warranted to better understand the exact mechanism underlying the association between these two conditions. This case highlights the importance of careful monitoring for the development of skin tumors in patients with Basan syndrome, given the potential increased risk. It is crucial to ensure prompt treatment of any skin tumors for optimal patient outcomes and to minimize the potential complications associated with cBCCs.

OR-080

Antitumor activity of chidamide combined with curcumin in CTCL cells

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Objective Cutaneous T-cell lymphoma (CTCL) is a rare group of non-Hodgkin lymphoma originating from skin, which is characterized by T-cell lymphoproliferative disorders. Chidamide, a Chinese original antineoplastic agent with independent intellectual property rights, and curcumin, is a constituent of the traditional medicine known as turmeric, both have exerted effect in the treatment of tumors individually by means of inhibiting the cell cycle, inducing apoptosis, inhibiting tumor neovascularization and regulating active oxygen. And our preliminary experiment results showed that chidamide and curcumin showed good suppressed effect on CTCL in vitro alone, but chidamide combined with matrine has not been tested in cutaneous T-cell lymphoma (CTCL). Thus our experiments is designed to evaluate the inhibitory effect of chidamide combined with curcumin on the proliferation of CTCL cell line HH and Hut78, as well as their promotive effect on its apoptosis, and to explore their therapeutic mechanisms in CTCL.

Methods

1.Cell lines and reagents
Two CTCL cell lines, HH (CRL-2105™) and Hut78 (CRMTIB-161™) were used in this research. They were got from the American Type Culture Collection (ATCC; Manassas, VA, USA). The HH cell line was derived from the peripheral blood of an aggressive CTCL patient. The Hut78 cell line originated from the peripheral blood of a SS patient. These two cell lines were cultivated in RPMI 1640 medium (Gibco, Eggenstein, Germany) plus with 10% fetal bovine serum (HyClone Laboratories, Logan, UT) and 1% penicillin-streptomycin solution.
2.Evaluation of cell viability and survival
Exponentially growing cells were seeded into 6-well plates (2×105 cells/ml per well), before the treatment with dimethyl sulfoxide (DMSO) vehicle or 10 μmol/L curcumin or 0.4 μmol/L chidamide or 10 μmol/L curcumin +0.4 μmol/L chidamide for 24, 48, or 72 h. At each set point, the 100 μl suspension was transferred to a 96-well plate and added with 20 μl of MTS reagent (Promega, Madison, WI, USA). After 2 h incubation in 37℃， the relative cell viability was measured at 490 nm using a 96-well plate spectrophotometer. The IC50 value was calculated using GraphPad Prism5 Software. Each experiment was performed in triplicate and repeated at least 3 times.
3.Flow cytometric cell apoptosis assay
Annexin V-FITC cell apoptosis kit (BD, USA) was used to detect cell apoptosis. Two cell lines were respectively treated with DMSO or 0.4 μmol/L chidamide or10 μmol/L curcumin alone, or in combination for 48h. the cells in suspension were collected and incubated with Annexin V-FITC for 5 min and then with PI for 15min in the dark. Cell apoptosis was measured on FACScan instrument
4.RT-PCR

Total RNA was isolated using the RNeasy Mini Kit according to the manufacturer's instruction. The first strand cDNA was synthesized from total RNA by reverse transcription according to the manufacturer's instructions. Gene-specific quantitative real-time PCR assays for caspase-3, Fas, bcl-2, NF-κB p65 and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) were performed according to the manufacturer's instructions. GAPDH was used as an internal standard of mRNA expression for normalization. The results were expressed as mean±SD in three independent experiments.

5.Protein extraction and Western blotting

Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto nitrocellulose (NC) membranes. The NC membranes were blocked in 5% skim milk in Tris buffered saline with Tween-20 (TBST) at 22°C to 25°C for 1 h, and then washed one time by TBST and incubated in primary antibody in refrigerator at 4°C for one night. TBST was used to wash the NC membranes 3 times, after the membranes incubated with secondary antibodies at 25°C for 1 h. and then the membranes were taken images by LI-COR Odyssey® Imaging System. The primary antibodies used in this study included anti-Caspase3 (9662, Cell Signaling, Technology, USA), anti-E-cadherin (ab1416, abcam, 1:5000), anti-Bcl-2 (4223S, Cell Signaling Technology USA), anti-BAD (ab32445, Abcam,), anti-P-Bad (ab129192, Abcam), and anti-GAPDH as an internal control (PPLYGEN 1:10000). All antibodies were used in 1:1000 dilutions, unless specified otherwise.

Results

1. Chidamide and curcumin significantly inhibited the growth of HH and Hut78 cells in a time-dependent manner. After 48-hour culture, the combination showed significantly stronger inhibitory effect on cell proliferation compared with 24-hour or 72-hour culture.
2. Chidamide, when administered together with curcumin, showed a strong synergistic effect, the IC50 values were less than the curcumin alone, and the CI values were less than 1.
3. As flow cytometry showed, the percentage of apoptotic cells was significantly higher in the combination treatment group than in the single drug treatment. Compared to the single drug, chidamide combined with curcumin showed more significant effect on proliferation inhibition and apoptosis induction of HH and Hut78 cells.
4. RT-PCR and WB results shows that the combination could increase the protein expression of cleaved caspase-3 and Fas, and decrease the expression NF-κB, and Bcl-2.
5. The effect and outcome of chidamide in combination with curcumin in NOD/SCID mice are still ongoing.

Conclusions Taken together, our data provided that chidamide combined with curcumin exhibits synergistically antitumor activity in both CTCL cells, which may be potential treatment option for CTCL.

OR-081

Human umbilical cord mesenchymal stem cell-derived exosomes alleviate vitiligo by reprogramming IFNγ-induced macrophages and prohibiting $CD8^+$ T lymphocytes migration via HDAC1-STAT1-CXCL9/10 axis

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Purposes Vitiligo is an autoimmune disease characterized by the impairment of epidermis melanocytes so that patches of depigmentation devastate the social life of patients. While the employment of Janus kinase (JAK) inhibitors has led vitiligo treatment to a new era, the undesirable side effect is still concerning. Human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-Exos) inherit potent immunosuppressive capacity and unique immune

compatibility from their parental cells, besides, the characteristic of readily accessibility and storage allow hUCMSC-Exos to become a sustainable candidate for treating autoimmune diseases. Nevertheless, whether hUCMSC-Exos could resolve vitiligo and its underlying mechanism is still obscure. Herein, we aim to gauge the therapeutic effect and translational potential of hUCMSC-Exos for vitiligo treatment in this study.

Methods hUCMSC-Exos were separated via high-speed differential ultracentrifugation and identified by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA) and immunoblotting. To study the therapeutic effect of hUCMSC-Exos upon vitiligo *in vivo*, flow cytometry analysis and whole-mount immunofluorescence staining were performed to quantify the melanocytes, $CD8^+$ T cells and macrophages in the tail skin of the melanoma/treg-induced vitiligo mouse model. Then, ELISA and transwell migration assays were utilized to reveal the capacity of IFNγ-induced macrophages to recruit $CD8^+$ T cells *in vitro*. Finally, the underlying mechanism of hUCMSC-Exos on impairing the CXCL9/10 secretion of macrophages and suppressing the migration of $CD8^+$ T cells was investigated via immunoblotting, immunoprecipitation (IP) and immunofluorescence staining analysis.

Results We initially isolated hUCMSCs and their exosomes, which possess high quality in accordance with standard guidelines. Then, we demonstrated that hUCMSC-Exos could alleviate vitiligo potently by suppressing the infiltration of $CD8^+$ T cells and impairing its cytotoxic function in multiple tissues of vitiligo mice. In particular, the infiltrated $CD8^+$ T cells in the tail skin of mice were positively correlated with the number of macrophages in the immune microenvironment. *In vitro*, we observed that IFNγ-induced macrophages could recruit $CD8^+$ T cells through chemokines CXCL9 and CXCL10, whose secretion could be hampered by hUCMSC-Exos. Moreover, we found that STAT1 was phosphorylated and translocated in the nucleus of macrophages in lesions of vitiligo. Finally, we proved that hUCMSC-Exos disrupt STAT1 phosphorylation and obstruct the transcription of CXCL9/10 of macrophages by reducing HDAC1-mediated post-translational modification on STAT1.

Conclusions Overall, hUCMSC-Exos could resolve vitiligo by suppressing the HDAC1-STAT1-CXCL9/10 axis of macrophages, thereby hampering the migration of cytotoxic $CD8^+$ T cells. These findings may provide a rationale for hUCMSC-Exos as a promising therapy for vitiligo.

OR-082

In situ sprayed NIR-responsive hydrogel as a transdermal MTX delivery platform for stratum corneum barrier penetration against psoriasis

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Background Psoriasis is a chronic inflammatory skin disease that involves keratinocyte over-proliferation and abnormal activation of immune cells. The classic morphology of psoriasis is characterized by sharply demarcated erythematous plaques covered with silvery scales, and the pathogenesis involves the hyperproliferation and abnormal differentiation of keratinocytes and feed-forward of inflammation. Nowadays, many medications have been developed for psoriasis, but conventional systemic drugs involve immunosuppressants (e.g., methotrexate (MTX) and ciclosporin) failed to meet the demands in practice due to their severe liver and renal toxicity. And monoclonal antibody such as anti-interleukin-17, is costly and outside the realm of affordability for most of the patients. Therefore, developing more effective treatment options with fewer adverse effects for psoriasis is highly desirable.

Topical pharmaceutical formulation, which directly acts on diseased skin and avoids systemic toxicity, is one of the attractive alternatives within the field of anti-psoriasis treatment. However, the thickened stratum corneum (SC) of psoriasis prevents drug permeation and absorption across the skin, resulting in poor clinical efficacy and low retention in the epidermis. Over the past decades,

the great progress in medical nanotechnology has provided possible opportunities and solutions to improve topical therapeutics. For example, liposomes and lipid nanoparticles, whose unique phospholipid bilayer structure favors skin permeation, have been used as a mainstream transdermal delivery system and can entrap various types of anti-psoriasis drugs such as curcumin and vitamin D analogs. However, the skin penetration and retention of these novel preparations are still restricted by their carriers which usually have low liquid viscosity and lead to an unsatisfactory therapeutic outcome. To expedite the transdermal drug delivery in psoriasis, microneedle patches that possesses sufficient rigidity to penetrate the cuticle for subcutaneous drug delivery have been proposed. Nevertheless, the thickened SC is rough, irregular and less organized which may hinder the effective adhesion and desired effects of microneedles in moderate-to-severe psoriasis. Therefore, the strategy to alleviate psoriasis with topical anti-inflammatory therapy remains challenging due to limited therapeutic effects and low skin penetration of drugs. Developing the formulation with both enhanced drug penetration performance and high viscosity on the skin surface can be an efficient method to meet the demands for timely symptom relief and extend drug retention in psoriasis therapy.

Methods Herein, we designed a near-infrared (NIR) light-triggerable thermosensitive hydrogel-based drug reservoir, GNRs+MTX@PLEL, which was fabricated by mixing methotrexate (MTX) and gold nanorods (GNRs) into PDLLA-PEG-PDLLA (PLEL) hydrogel against psoriasis. It is expected to increase both the penetration depth and permeation rate of MTX. The unique feature of thermosensitive PLEL hydrogel makes it achievable to simply spray the mixed formulations into skin lesion sites and finally form a stable gel in situ (above 37℃). Upon further near-infrared (NIR) laser irradiation, GNRs generated at high temperatures (above 45℃) could promote the further transformation of gel to sol and induce not only a higher rate of formulations permeation but also apoptosis of keratinocyte, which yield a combined efficacy to mitigate psoriatic lesions. To characterize PLEL@GNR+MTX hydrogel, 1H-NMR spectra, SEM, the temperature dependence of storage (G′) and loss modulus (G″), thermographic images, MTX release profiles, the in vitro thermosensitive properties were determined, respectively. Then cell viability, live-dead KCs, JC-1 staining, and flow cytometric analysis were utilized to confirm the apoptosis induced by the PLEL@GNR+MTX hybrids hydrogel attributed its synergistic effect of chemotherapy and photothermal therapy. Skin permeability, the in vivo photothermal effect, skin irritation, toxicological studies, the psoriasis area and severity index (PASI) score, immunohistochemistry images, and histological staining were employed to evaluate its therapeutic efficacy in the imiquimod (IMQ)-induced psoriatic mouse model.

Results In this composite hydrogel, NIR light irradiation could activate the photothermal GNRs, and the generated local hyperthermia could not only induce keratinocyte apoptosis but also boost MTX release for psoriasis treatment in a synergistic way. Additionally, PLEL@GNR+MTX hydrogel played a crucial role in releasing MTX in an on-demand way under NIR light irradiation. What's more, rapid phase transition enhanced hydrogel penetration through stratum corneum upon NIR light irradiation on the IMQ-induced psoriasis mice model attributed to its optimal permeability, long local retention, and drug release in response to heat stimuli. More importantly, in vivo tests further confirmed that PLEL@GNR+MTX hydrogel combined with NIR light irradiation had excellent phase-change properties and promoted drug penetration in the stratum corneum. Besides, due to the excellent photothermal effect of GNRs, the elevated temperature induced by NIR could also exfoliate the skin, producing a synergistic effect for MTX delivery. Finally, the combination treatment of PLEL@GNR+MTX hydrogel and NIR light irradiation could effectively relieve psoriasis inflammation. It is hoped that the present light-activatable hydrogel-based platform may hold promise for fighting against psoriasis.

Conclusion Overall, the light-activatable hydrogel-based platform could utilize NIR as a "trigger switch" to release MTX more precisely, and photothermal-enhanced anti-psoriasis treatment during the reversible phase change process. As expected, this "smart" hydrogel local delivery system could optimize the therapeutic effects against psoriasis in vivo and in vitro.

OR-083

Study on the influence of different parameters of pneumatic injector on the depth of intradermal injection

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Objective (s) Through adjusting the parameters of the injection flow (Flow, mL/min) and injection time(Time,s), to observe the effect to the depth of skin injection and the change of skin tissue structure when pneumatic injector under different parameters. To provide experimental basis for equipment improvement and precise clinical application of the pneumatic injector.

Method(s) ①Measure the skin thickness and skin elasticity of the shoulder, back, lateral abdomen, and abdominal skin of Bama miniature pigs through the non-invasive skin testing method. According to the measurements, select the injection site and injection region, to minimize the effect of the mechanical properties of the skin itself on the injection efficiency.

②Use the pneumatic injector to perform a needle-free injection of 0.1% methylene blue (0.1% MB) dye solution into the skin of Bama miniature pigs. Combine the following two parameters in pairs: injection flow(F, mL/min): 1, 3, 5, 7; injection time (T, s): 1, 2, 4, 8; then conduct the needle-free jet injection experiment according to the parameters respectively. Experimental group: According to different jet flow (F), the experimental group was divided into F1, F3, F5 and F7 groups, and according to different jet duration (T), the experimental group was divided into T1, T2, T4 and T8 groups. Pairwise combinations are divided into 16 subgroups: F1 group was divided into F1T1、F1T2、F1T4、F1T8 subgroup, F3 group was divided into F3T1、F3T2、F3T4、F3T8, subgroup, F5 group was divided into F5T1、F5T2、F5T4、F5T8 subgroup. Control group: local application of 0.1% MB staining solution.Parameters F and T of each injection cell were adjusted according to the experimental group, and 0.1% MB of dyeing solution was injected. During the injection process, ensure that the direction of the jet is perpendicular to the skin surface, and the distance between the nozzle and the skin surface is fixed at 1.0cm to avoid exerting pressure on the skin. At the same time, filter paper is used to absorb the staining liquid remaining on the skin surface. Linear sequence of injection in the injection cell to avoid duplication. After the injection, the injection area was cleaned 3 times with normal saline (pH 7.4, 0.9% sodium chloride) for 1 minute.

③Observe the penetration of 0.1% MB dye solution through the skin under different parameters through frozen sections;④Measure the injection depth, and statistically analyze the relation between the parameters (F, T) of the pneumatic injector to the injection depth.⑤Select The parameters of layered injection into the epidermis and dermis. According to H&E（Hematoxylin-eosin ） staining and PAS （ PeriodicAcid-Schiffstain ） staining, observe the effects of corresponding parameters of needle-free jet injection on the skin tissue structure. Use the electron microscopy to observe the effects of needle-free jet injection on the skin ultrastructure（Without interrupting the blood supply, a small piece of the target tissue is cut with a sharp scalpel and quickly immersed in 2.5% glutaraldehyde working solution at room temperature）.

Result(s) ①Based on the results of non-invasive skin measurement, the experimental area for needle-free jet injection was selected as the skin on the lateral abdomen attached to the bone structure of Bama miniature pigs which was the upper and middle skin of the lateral abdomen of Bama miniature pigs. ②With different injection parameters, 0.1% MB of the staining solution can be deposited on different skin layers such as the stratum corneum, epidermis, dermis, and hair follicles. Under the same flow, the injection depth increases with time, with a statistically significant difference ($P<0.05$); At the same injection time, the injection depth increased with the increase of flow rate, with a statistically significant difference ($P<0.05$); ③When the injection parameter is F3T1, it can break through the stratum corneum barrier and inject drugs into the epidermis. When the injection parameter is F3T1, it can break through the basement membrane barrier and inject most drugs into the dermis; ④ Needle-free jet injection can cause a certain degree of damage to the

structure of the cuticle, desmosomes between epidermal cells, and desmosomes in the DEJ （Dermal-epidermal juncation，DEJ） region when it is injected into the epidermis and dermis.
Conclusion(s) ①The injection depth of needle-free injection is affected by the parameters of F and T; within a certain range, the injection depth increases according to the increase of F and T separately.②Under different parameters of F and T, needle-free injection can cause different degrees of damage to the skin epidermis, the basement membrane and the superficial dermis. The degree of damage can be controlled through adjusting the parameter settings.③The drug delivery into the skin through needle-free injection is achieved by destructing the barrier of skin epidermis and barrier of epidermis and dermis.④Through adjusting the injection parameters of F and T, the precise layered injection into the epidermis and dermis under the condition of slight reversible damage can be achieved, which provides the experimental basis for equipment improvement.

OR-084

Trem2 promotes diabetic skin wound healing prognosis via macrophage-fibroblast regulating hierarchy in a Lif dependent way

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Background The immunoglobulin superfamily protein Trem2 (triggering receptor expressed on myeloid cells 2) is primarily expressed on myeloid cells where it functions to regulate macrophage-related immune response induction. While macrophages are essential mediators of diabetic wound healing, the specific regulatory role that Trem2 plays in this setting remains to be established.
Objective This study was developed to explore the potential importance of Trem2 signaling in the process of diabetic wound healing and to clarify the underlying mechanisms through which it functions.
Methods and Results Following wound induction, diabetic model mice exhibited pronounced upregulation of Trem2 expression, which was primarily evident in macrophages derived from the bone marrow. No cutaneous defects were evident in mice bearing a macrophage-specific knockout of Trem2 (T2-cKO), but they induced more pronounced inflammatory responses and failed to effectively repair cutaneous wounds, with lower levels of neovascularization, slower rates of wound closure, decreased collagen deposition, and higher M1 macrophage infiltration levels following wounding. Both collagen I and α-smooth muscle actin were expressed at lower levels in the skin of these T2-cKO animals following wounding, and transcriptional profiling revealed that these mice engaged more robust pro-inflammatory responses in the wounded skin as evidenced by the activation of multiple inflammation-related pathways. RNA-sequencing analyses further demonstrated that Trem2 plays a role in the programming of functional macrophage phenotypes in the context of diabetic wound healing through mechanisms at least partially associated with activator protein (AP)-1 inhibition. Trem2 was further found to disrupt AP-1 activation in these macrophages through the modulation of normal MAPK signaling. In an experimental system in which fibroblasts and macrophages were co-cultured, macrophages from T2-cKO mice were found to suppress the in vitro activation and proliferation of dermal fibroblast through the upregulation of leukemia inhibitory factor (Lif). Injecting soluble Trem2 in vivo was also sufficient to significantly curtail inflammatory responses and to promote foot ulcer wound healing in this murine model system.
Conclusions In summary, these analyses offer novel insight into the role of Trem2 signaling as a mediator of myeloid cell-fibroblast crosstalk that may represent a viable therapeutic target for efforts to enhance diabetic wound healing.

OR-085

Effects of fecal microbiota transplant on DNA methylation in patients with systemic lupus erythematosus

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Aim To explore the mechanism of fecal microbiota transplant (FMT) in the treatment of SLE.
Methods We included 850K methylation chip sequencing in14 SLE patients participating in clinical trials, including 8 in responders group (Rs) and 6 in non-responders group (NRs) to find differential methylation sites. Pyrosequencing and bisulfite sequencing were used to verify the methylation levels of screened CpG sites and IFN-related genes. Finally, we treated PBMC of SLE patients with selected methylation-related metabolites to verify its effect on DNA methylation.
Results We found that the serum of S-adenosylmethionine (SAM), methylation group donor, was upregulated after FMT, accompanied by an increase in genome-wide DNA methylation level in Rs. We further showed that the methylation levels in promoter regions of Interferon-γ (IFN-γ), induced Helicase C Domain Containing Protein 1 (IFIH1), endoplasmic reticulum membrane protein complex 8 (EMC8), and Tripartite motif-containing protein 58 (TRIM58) increased after FMT treatment. On the contrary, there was no significant change in the methylation of IFIH1 promoter region in the NRs after FMT, and the methylation level of IFIH1 in the Rs was significantly higher than that in the NRs at week 0. Finally, we found that hexanoic acid treatment can up-regulate the global methylation of peripheral blood mononuclear cells in SLE patients.
Conclusion Overall, our results delineate changes in methylation levels after FMT treatment of SLE and reveal possible mechanisms of FMT treatment in terms of the recovery of abnormal hypomethylation.

OR-086

Cbf-β is required for the development, differentiation, and function of murine mucosal-associated invariant T cells

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Aim Mucosal-associated invariant T (MAIT) cells are an evolutionarily conserved subset of T cells, which mediate functions that link innate and acquired immunity in a broad spectrum of diseases, including infectious, autoimmune and skin diseases. Although transcription factors such as PLZF, SATB1, miR-181a/b-1, miR-155, Drosha, and SAP have been shown to regulate MAIT cell development and differentiation, a significant gap in our knowledge of MAIT cell regulation remains. Method: Using a previously published scRNA-seq dataset, we observed a significant increase in Cbf-β expression in stage 3 MAIT cells, which led us to investigate the effect of Cbf-β on MAIT cells. Result: We found that Cbf-β deficiency in murine MAIT cells impaired MAIT cell development and interrupted MAIT1 and MAIT17 cell differentiation. Additionally, inducible deletion of Cbf-β inhibited the activation and cytotoxicity of peripheral MAIT cells.
Discussion Our work elucidates the mechanism of MAIT cell development and identifies a novel target, Cbf-β, for modulation of MAIT cells for applications in MAIT cell-based immunotherapies.

OR-087

Serum uric acid levels in patients with erythrodermic psoriasis

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Background Mounting evidence has shown elevated serum uric acid levels in patients with psoriasis when compared to healthy controls. However, it remains unclear whether the elevated uric acid concentration is unique to psoriasis or common to other skin diseases. Therefore, this study compared serum uric acid levels in patients with erythrodermic psoriasis, erythrodermic eczema, or idiopathic erythroderma. Moreover, the association between serum levels of uric acid and several inflammatory markers in patients with erythrodermic psoriasis was also explored.
Methods 270 patients diagnosed with erythrodermic psoriasis (n = 94), erythrodermic eczema (n = 59), or idiopathic erythroderma (n = 117) were included in this study. Serum levels of uric acid, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and neutrophil-to-lymphocyte ratio (NLR) were measured before treatment. The differences in serum uric acid levels among different groups were analyzed using linear regression analysis. The associations between serum levels of uric acid and inflammatory markers were tested by Pearson correlation analysis.
Results After adjustment for gender, serum uric acid concentrations were significantly higher in patients with erythrodermic psoriasis than patients with erythrodermic eczema or idiopathic erythroderma (Table 1). Pearson correlation analysis revealed a negative correlation between uric acid levels and NLR in erythrodermic psoriatic patients ($r = -0.219$, $p = 0.043$*) (Figure 1). However, no significant correlation was observed between serum uric acid levels and serum CRP or ESR levels in this group of participants.
Conclusions In conclusion, a higher serum uric acid level was identified in patients with erythrodermic psoriasis as compared to patients with erythrodermic eczema or idiopathic erythroderma. Besides, uric acid concentrations correlated negatively with NLR in erythrodermic psoriatic patients.

OR-088

YAP1/Piezo1 involve in the changes of lymphatic vessels in photoaging develop to squamous cell carcinoma induced by UVR

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Abstract
Background Cutaneous squamous cell carcinoma (cSCC) is the most common cancer. The alterations of lymphatic-centered immune microenvironment are essential in transforming from photoaging to cSCC. Studying the mechanism will be beneficial for finding new targets to early predict cSCC.
Aims To investigate the constant changes of the lymphatic-centered immune microenvironment in transforming from photoaging to cSCC induced by ultraviolet irradiation (UVR).
Methods TIMER2.0 was used to analyze whether YAP1/VEGFC signaling pathway is involved in lymphangiogenesis in head and neck squamous cell carcinoma (HNSCC). Meanwhile, lymphatic-centered immune microenvironments alterations and the related cumulative survival time were also analyzed. With the accumulated UVR, skin photoaging developed and gradually progressed into actinic keratosis and cSCC on SKH-1 hairless mice. The skin lymphatic-centered immune microenvironment was evaluated at the 0th, 8th, 12th, 16-18th, and 20-24th week of UVR. Skin phenotype was assessed using optical coherence tomography (OCT) and skin image. H&E and

Masson's trichrome staining evaluated epidermis and dermis. The structure of lymphatic vessels (LVs), blood vessels, and different types of T cells were evaluated by immunohistochemistry staining. The expression of Piezo1 whose deletion in adult lymphatics led to substantial valve degeneration, VE-cadherin that maintained the permeability of LVs, and YAP1 were evaluated by immunohistochemistry staining as well. Besides, the drainage function of LVs was assessed by Evans Blue assay in vivo.

Results TIMER2.0 analysis indicated that VEGFC genes high expressed in HNSCC. YAP1 gene expression was positive correlated with VEGFC in HNSCC. LV density increased in human cSCC. More LVs in HNSCC were beneficial to prolong the survival time. VEGFC gene overexpression was positive correlated to CD8+T cell infiltration. More CD8A+T cells and CD8B+T cell infiltration in HNSCC extended survival time. When YAP1 gene overexpression and high infiltration of endothelial cells took place simultaneously might prolong the survival time of HNSCC patients. And high infiltration of CD8+T cells prolonged the survival time as well. In animal studies, UVR-induced eight weeks and 16-18 weeks were two turning points. The density of LVs in UV-8w was the least. When photoaged skin developed into AK lesions (UV-16-18w), LV slightly exceeded healthy skin and proliferated sharply in cSCC (UV-20-24w). YAP1 expression was almost consistent with LV but rose after the photoaging stage. The drainage of cSCC mice induced by UVR was better than that of photoaged skin and worse than that of health skin. The dynamic alterations of LVs number, Piezo1 expression, and collagen might be reasons for it. The expression of Piezo1 was in the highest point after 8 weeks of UVR, then gradually descended to the platform. The total T cells increased slowly, but the infiltration of CD4+T cells increased, and CD8+T cells decreased after eight weeks of UVR. The CD8+T cells and CD4+T cells increased sharply in UV-16-18w and UV-20-24w groups.

Conclusion The lymphatic-centered immune microenvironment underwent adaptive changes under continuous UVR via regulating YAP1/VEGFC. During the formation of cSCC, there is two turning points, eight weeks (photoaging) and 16-18 weeks (precancerous). YAP1, Piezo1, LVs, and immune cells constantly changed with the skin state induced by UVR.

OR-089

Bioinspired Nanoparticles Enhances Protection Against UV-induced Skin Damage through Inhibiting Oxidative Stress and Inflammation

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Excessive and prolonged ultraviolet radiation (UVR) exposure causes photodamage, photoaging, and photocarcinogenesis in human skin. Taking precautions such as using broad-spectrum sunscreen is becoming a prevalent lifestyle among people. Nevertheless, artificial UV filers widely utilized in the current commercial sunscreen bear undesirable effects on the environment and human beings. Melanin distributed in the human epidermis serves as a natural shield to minimize UVR damage. Synthesized as a mimic of melanin, polydopamine nanoparticles (PDA NPs) possesses a similar structure with natural melanin and biocompatibility characterization. In this study, we reported a sunscreen based on melanin-inspired PDA NPs. We validated that PDA NPs sunscreen could make a difference in photoprotection and inhibit photoaging in UVR-damaged lesions of mice, which is reflected in epidermal thickness normality, healthy barrier function maintenance, ordered collagen arrangement, and less inflammation occurrence. Additionally, we found that PDA NPs are efficiently intake by keratinocytes, exhibiting robust ROS scavenging and DNA protection ability with minimal cytotoxicity. Collectively, the biocompatibility and full photoprotective properties of PDA NPs sunscreen displayed superior performance to those of commercial sunscreen. This work provides new insights into the development of a melanin-mimicking photoprotection material for sunscreens.

OR-090

Serum level of galectin-9 as a potential biomarker for high risk of malignancy in dermatomyositis

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Objectives Galectin-9, as immune checkpoint protein, plays a role in regulating autoimmunity and tumour immunity. Therefore, we explored the pathophysiological link between galectin-9 and malignancy in cancer-related DM (CRDM).
Methods Serum galectin-9 were quantified via enzyme-linked immunosorbent assay, and its association with serological indices was evaluated using Spearman analysis. Receiver operating characteristic (ROC) analysis was utilized to determine the cut-off value of galectin-9.
Results Serum levels of galectin-9 were significantly higher in DM patients [23.38 (13.85-32.57) ng/ml] than those in healthy controls (HCs) [6.81 (5.42-7.89) ng/ml, $P < 0.0001$], and were positively correlated with the cutaneous dermatomyositis disease area severity index activity (CDASI-A) scores ($r_s = 0.3065$, $P = 0.0172$). DM patients with new-onset and untreated cancer (new-CRDM) [31.58 (23.85-38.84) ng/ml] had higher levels of galectin-9 than those with stable and treated cancer (stable-CRDM) [17.49 (10.23-27.91) ng/ml, $P = 0.0288$], non-cancer-related DM (non-CRDM) [21.05 (11.97-28.02) ng/ml, $P = 0.0258$], and tumour patients without DM [7.46 (4.90-8.51) ng/ml, $P < 0.0001$]. Serum galectin-9 levels significantly decreased [27.79 (17.04-41.43) ng/ml vs. 13.88 (5.15-20.37) ng/ml, $P = 0.002$] after anti-cancer treatment in CRDM patients. The combination of serum galectin-9 and anti-transcriptional intermediary factor 1-γ (anti-TIF1-γ) antibody (AUC = 0.889, 95% CI 0.803-0.977) showed the highest predictive value for the presence of cancer in DM.
Conclusion Increased galectin-9 levels were related to tumor progression in CRDM, and galectin-9 was downregulated upon cancer treatment. Monitoring serum galectin-9 levels and anti-TIF1-γ antibodies might be an attractive strategy to achieve tumour diagnosis and predict CRDM outcome.

OR-091

Dermatoscopic and skin ultrasound evaluation in lupus miliaris disseminatum faciei

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We describe a case of lupus miliaris disseminatus faciei (LMDF) in a 21-year-old female with a 5-months history. Clinical evaluation revealed multiple, dome-shaped, reddish-yellow papules, distributed symmetrically on nearly the entire face, especially the central area. Dermoscopy showed linear and arborizing vessels on a yellow-reddish background, some with targetoid follicular plugs. The ultrasonographic evaluation of the papules on the central area as well as the lesions on the cheeks showed alterations in dermis tissue. The evaluation with a 50 MHz probe showed multiple well-defined peanut-like hypoechoic area. The diagnosis of LMDF was confirmed via histopathology. To our knowledge, this is the first reported case where ultrasound has been used for evaluation for LMDF.

OR-092

Vitamin A and E concentration at early gestation associated with reduced risk of early-onset atopic dermatitis

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Background Maternal nutrition has been associated with allergic diseases in offspring, studies about maternal antioxidative vitamins and infant atopic dermatitis (AD) are limited and inconclusive.
Objectives We aim to quantify the association of maternal vitamin A and E levels during early gestation with early-onset infant AD risk in a prospective cohort.
Methods: Pregnant women (n=456) from MKFOAD (NCT02889081), recruited at 12–14 weeks' gestation, had serum vitamin A (retinol) and vitamin E (alpha tocopherol) concentrations examined by liquid chromatography mass spectrometry. Infant AD that occurred within 12 months was diagnosed according to Williams' criteria. Infant transepidermal water loss (TEWL) was tested on foreheads and cheeks at birth and age 42 days. The association of maternal vitamin levels with infant AD was assessed with discrete time survival analysis.
Results In total, 121 (26.5%) infants developed AD before 12 months old. Per ug/mL increase in maternal vitamin E levels was associated with an 8% reduction in the risk of early-onset infant AD (aHR 0.92, 95% CI 0.87–0.98). Infants born to mothers with the highest tertile of vitamin E had a 46% reduced risk of AD (aHR 0.54, 95% CI 0.32–0.92) compared with the middle tertile. Maternal vitamin E levels showed a weak negative correlation with forehead TEWL at birth (r=–0.14, P=0.011). We found no association between maternal vitamin A levels with infant AD.
Conclusions We provide the first evidence of association of higher maternal vitamin E concentration during early gestation with reduced risk of subsequent early-onset infant AD. Our findings support a protective effect of sufficient maternal vitamin E levels during early pregnancy on early-onset infant AD.

OR-093

Skin barrier function in newborns within 96 hours after birth: A cross-sectional study

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Background The skin barrier function varied with age in children and adults, but the limited study was focused on the neonatal period. We aimed to elaborate on the age and related factors with the skin barrier of full-term newborns within 96 hours after birth.
Methods Based on the prospective cohort MKNFOAD (NCT02889081), we examined transepidermal water loss (TEWL), stratum corneum hydration (SCH), skin pH, and sebum content at three anatomical sites (cheek, forehead and volar forearm) in 383 full-term infants within 96 hours after birth. Multivariable linear regression analysis was used to analyze the association of skin barrier parameters with postnatal age adjusted by infant gender, parents' allergy history, delivery mode, amniotic fluid characteristics and birth weight.
Results This study included 383 infants, 197 (51.4%) boys and 186 (48.6%) girls. From birth to 96 hours, the TEWL values of the cheek, forehead or volar forearm were stable and were not associated with age (P>0.05). The pH values of the three anatomical sites showed a negative association with age （-0.184（-0.250～-0.118）；-0.309（-0.401～-0.217）；-0.371（-0.446～

-0.298）, P<0.001), which means pH value decline by 0.184 (cheek), 0.309 (forehead), 0.371 (volar forearm) for age increment. The SCH value of all three anatomical sites appeared a positive association with age (3.059(1.962~4.156); 2.705(1.598~3.813); 1.477(0.649~2.304), P≤0.001), which means SCH value increase by 3.059 (cheek), 2.705 (forehead), 1.477 (volar forearm) for age increment. And the sebum content of the forehead also appeared to have a positive association with age (7.231(1.792~12.670)), which means the sebum content value rises by 7.231 for age increment. The sebum content in the forehead of boys was significantly higher than that of girls (p<0.001). The above four parameters were not associated with delivery mode.
Conclusion During the first 96 hours after birth, TEWL is stable, pH has a relatively obvious decline, while SCH and sebum content are rising with age. These findings may provide a new theoretical basis for neonatal skin physiology and clinical strategies for guiding newborn skin care.

OR-094

FGFR3 upregulates interferon-stimulated genes via the JAK1-STAT1 signaling pathway in HPV E2 stable expression keratinocytes

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Objective Human papillomavirus (HPV) infections are common skin infectious diseases. The HPV E2 protein as an early express protein of HPV has been demonstrated to interact with fibroblast growth factor receptor 3 (FGFR3) to produce tyrosinase phosphorylation inhibiting HPV DNA replication in HPV infections. Nevertheless, the role and mechanism of FGFR3 in HPV infections remain unclear.
Methods We detected FGFR3 and interferon-stimulated genes (ISGs) expression using common wart samples and evaluated the role and mechanism of FGFR3 in ISGs via the overexpression of FGFR3 in HPV E2 stable expressing cell lines.
Results The expression of FGFR3 and ISGs in patients with common warts was higher than in normal skin adjacent warts via RNA-seq and immunohistochemistry detection. To understand the role and mechanism of FGFR3 in stable HPV E2 stable expressing keratinocytes, we evaluated changes in the level of ISGs and JAK1-STAT1 signal after the overregulation of FGFR3 in HPV E2 stable expressing keratinocytes, (HaCaTs) Human Epidermal Keratinocyte line and Normal Human Epidermal Keratinocytes (NHEKs). This study showed that FGFR3 phosphorylated by the HPV E2 protein and induced JAK1-STAT1 activation, thereby playing antiviral immunity via promoting the expression of ISGs. In addition, FGFR3 and HPV E2 had co-localization and interaction in keratinocytes.
Conclusion Our findings revealed the important role of FGFR3 in innate antiviral immunity in stable HPV E2 stable expressing keratinocytes and may serve as a potential target for HPV infection therapy.

OR-095

Preparation of clindamycin hydrochloride-loaded microneedle patch and experimental study on treatment for acne in a rat model

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Objective To study the preparation of clindamycin hydrochloride loaded sustained-release microneedle patch (GM-CLin-MN) by Gelatinmethylacryloyl (GelMA) photocrosslinking, to obtain the microneedle patch with stable physical and chemical properties, high drug loading, controlled release, no cytotoxicity and skin irritation, and to use it in the treatment of acne rat model, observe its effectiveness and explore the possible mechanism of action.
Methods 1. Preparation and evaluation of microneedle patch: GelMA was synthesized by grafting gelatin with methacrylic anhydride, photoinitiator was added, and GelMA microneedle patch loaded with clindamycin hydrochloride (GM-Clin-MN) was prepared by polydimethylsiloxane (PDMS) mold.The morphology and structure of the microneedles were observed by light microscopy, scanning electron microscopy, and fluorescence microscopy; the composition of the microneedles was detected by infrared spectroscopy; the drug loading of clindamycin hydrochloride and the in vitro release curve were determined by UV spectrophotometry; the mechanical properties of the microneedles were detected by a pressure tester to observe whether they achieved the mechanical strength of effectively puncturing the skin.The depth of insertion into the skin was measured by local rhodamine 6G staining andhistopathological sections after puncture of the abdominal skin of rats; the swelling performance and needle tip separation ability of the needle tip were assessed by observing the needle tip morphology at different times after microacupuncture into the skin. 2. Human skin fibroblasts (HSF) were co-cultured with microneedles to assess their cytotoxicity by CCK-8 assay; chicken embryo chorioallantoic membrane was contacted with microneedles to assess their skin irritation by Hen's egg test on the chorioallantoic membrane (HET-CAM); microneedles were co-cultured with P. acnes in vitro to assess the in vitro antibacterial performance by observing the growth of colonies by plate coating method. 3. The rat model of acne was established by applying 2% coal tar + subcutaneous injection of Propionibacterium acnes.After successful modeling, they were divided into negative control (PBS application) group, blank microneedle patch (GM-MN) group, clindamycin hydrochloride gel (Clin-Gel) group, and clindamycin loaded microneedle patch (GM-Clin-MN) group, with 10 animals in each group.The gross morphological changes, HE staining and immunohistochemical staining (IL-1β, MMP2, IL-10 protein expression) of acne lesions before and after treatment were compared between the groups to evaluate their efficacy on acne and explore the possible mechanism of action.
Results 1. ① GelMA with photocrosslinking characteristics was successfully prepared. ② Clindamycin-loaded microneedle patch (GM-Clin-MN) with the appearance of 2 cm*2 cm square patch and needle tip array 20 * 20 was successfully prepared.The needle tip showed a pyramid shapeof about 500 μm*500 μm *800 μm with a distance of 700 μm between the needle tips. ③ GM-MN can effectively load the hydrophilic antibiotic clindamycin hydrochloride with a needle tip loading of (0.49 ± 0.025) μg/Neddle.The drug release was rapid with a cumulative release rate of (54.8 ± 2.1)% on Day 1, followed by a slow release with a cumulative release rate of (72.1 ± 1.5)% on Day 10.④ The mechanical strength of GM-Clin-MN needle tip is greater than 0.50 N/Needle at 0.3 mm, which can effectively puncture the skin to reach the dermis. ⑤ The swelling rate after microacupuncture into the skin was (185.4 ± 12.1)%, and at 10 min and the needle tip could be separated from the basal layer, with separable characteristics. ⑥ GM-Clin-MN was co-cultured with human skin fibroblasts (HSF), and CCK-8 results showed that microneedle material did not affect cell proliferation, indicating that microneedles were not cytotoxic. ⑦ GM-Clin-MN was applied to the chorioallantoic membrane of chicken embryos, and HET-CAM assay verified that GM-Clin-MN had no skin irritation and had good application safety.⑧ GM-Clin-MN was co-cultured

with P. acnes, and the excellent antibacterial properties of GM-Clin-MN were verified by plate coating method. 2. ①Gross morphological changes: Compared with PBS group, GM-MN group and Clin-Gel group, the color of skin lesions in GM-Clin-MN group was significantly lighter than before after treatment, most of them returned to the original color of rat ears, the swelling was significantly reduced than before, most of the cysts and nodules dissipated, and the thickness of auricle was significantly reduced. ($P < 0.05$) ② Compared with PBS group, GM-MNgroup and Clin-Gel group, GM-Clin-MN group had clear demarcation of each layer of auricular tissue after treatment, smaller hair follicle volume, less contents, nearly normal sebaceous gland volume, more regular arrangement of subcutaneous collagen fibers, very few inflammatory cell infiltration, and no significant telangiectasia.③ Immunohistochemistry was used to detect the expression of IL-1β, MMP2 and IL-10. Compared with PBS group, GM-MN group and Clin-Gel group, GM-Clin-MN could significantly reduce the expression of pro-inflammatory cytokines IL-1β and MMP2 in acne lesions ($P < 0.05$), and its IOD values were 0.111 ± 0.004 and 0.114 ± 0.009, respectively.It significantly increased anti-inflammatory factor IL-10 expression ($P < 0.05$), and its IOD value was 0.248 ± 0.005.It has a significant inhibitory effect on P. acnes induced acne inflammatory response, and may reduce scarring in acne by decreasing the expression of MMP2.
Conclusion The clindamycin hydrochloride loaded microneedle patch prepared is a new transdermal drug delivery system with stable physical and chemical properties, high drug loading, controlled release and no cytotoxicity and skin irritation, and the microneedle patch has significant effect on acne rat model, providing theoretical basis for the clinical development of new topical preparations for acne treatment.

OR-096

Anionic surfactants Sodium Dodecyl Benzene Sulfonate may alleviate Atopic dermatitis inflammatory

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Objectives Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases. Surfactants in detergents are believed to exacerbate AD by disrupting the skin barrier. However, the mechanism of the effect of surfactants on the inflammatory response of AD remains unclear. We sought to investigate the role of doanionic surfactant sodium dodecyl benzene sulfonate (SDBS) in AD inflammatory response.
Methods We intervened by topically applying 0.1% and 1% SDBS on MC903-induced AD-like mice. The degree of skin lesions was evaluated by macroscopical and histological methods. Quantitative real-time polymerase chain reaction (QRT-PCR) was adopted to detect the mRNA expression of thymic stromal lymphopoietin (TSLP), interleukin (IL)-4, IL-13 in skin lesions. Serum levels of IgE and TSLP levels was tested by ELISA.
Results Topical application of 0.1% and 1% SDBS can alleviate the clinical symptoms of MC903-induced AD-like dermatitis in mice, which includes a significant reduction in skin lesion thickness and frequency of scratching behavior. Using SDBS in mouse AD-like skin lesions can reduce TSLP levels in both skin lesions and peripheral blood, decrease the expression of IL-4 and IL-13 in the skin, and total IgE levels in peripheral blood.
Conclusions Low-dose SDBS may not worsen the inflammatory response in AD patients and could potentially improve it.

OR-097

ZWZ-3 down-regulates BIRC5 inhibits proliferation and metastasis of melanoma through the β-Catenin/HIF-1α/VEGF/MMPs pathway

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Background Melanoma is one of the most malignant tumors with high metastatic potential. Although it represents only 4% of dermal tumor cases, melanoma is the most dangerous and deadly form of dermal tumor, accounting for 75% of skin cancer-related deaths. Early-stage melanoma can be treated with surgical intervention, with relatively high 5-year survival rate, yet metastatic melanoma signifificantly shorten survival rate. Due to its aggressiveness, melanoma patients are often diagnosed at advanced stages with local infifiltration or distant metastasis, which cannot be treated with surgery alone. In addition, melanoma is one of the most potent drug-resistant cancers. Thus, multifunctional theranostic agents with the potential of simultaneous imaging-guided, tumor targeting and treatment are urgently needed to improve the timely diagnosis and treatment of the disease.

Objective: We designed and synthesized a novel hemicyanine-based flfluorescent probe ZWZ-3, and investigated its application for melanoma imaging and treatment both in vitro and in vivo. At the same time, we investigated the specific mechanism of ZWZ-3 in melanoma proliferation and metastasis.

Methods In vitro experiments, the mitochondrial targeting of ZWZ-3 was determined by fluorescence microscopy, the anti-proliferation effect on tumor cells was detected by MTT and colony formation assay, and the anti-metastatic effect on tumor cells was investigated by wound healing assay and transwell assay. A zebrafish tumor model was used to assess the therapeutic effect of ZWZ-3 in vivo. Subcutaneous xenograft models, histopathology, and immunohistochemistry have been used to verify the anti-tumor, toxic, and side effect in vivo. Mechanistic insights into the inhibition of tumor metastasis by ZWZ-3 were obtained through analysis of tumor tissue sections in mice. RNA sequencing was performed to identify the effect of ZWZ-3 on gene expression. The expression level of BIRC5 in melanoma and its influence on prognosis were determined by biogenic analysis. siRNA was used to inhibit Birc5 gene expression in the B16F10 cell line.

Results ZWZ-3 preferentially accumulated in melanoma cells via a process that depended on the organic anion-transporting polypeptide (OATP), which targeted mitochondria on the hemicyanine cationic nitrogen. A subcellular localization assay, using colocalization with a mitochondrial tracker (MitoTracker Green) in B16, A375 and RAW264.7 cells, showed that ZWZ-3 preferentially accumulated in the mitochondria of tumor cells in a time-dependent manner. In addition, we investigated the effect and molecular mechanism of ZWZ-3 in melanoma. Given the tumor targeting and imaging property of ZWZ-3, further investigation of anti-tumor effects of ZWZ-3 was conducted. The effect of ZWZ-3 on cancer cells proliferation was evaluated by MTT assay. ZWZ-3 displayed relatively strong activity against B16 and A375 cell lines (IC50 0.2 and 0.43 μM, respectively) at 72 h. Additionally, ZWZ-3 suppressed melanoma cells viability in a concentration and time-dependent manner. Similarly, in a colony formation assay, ZWZ-3 suppressed proliferation of both cell lines and reduced colony numbers in a dose-dependent manner. To explore whether ZWZ-3 induce apoptosis in melanoma cells, we treated B16 cells or A375 cells with different ZWZ-3 concentrations, then analyzed them by flflow cytometry. Treatment with 0.31 μM ZWZ-3 for 24 h led to an apoptosis rate of 55.25%, and this effect was dose-dependent, since 0.63 μM led to a rate of 80.71%; 1.25 μM, 82.14%; and 2.5 μM, 84.71%. These data indicated that ZWZ-3 induced melanoma cell apoptosis in a dose-dependent manner. Western blot analysis displayed that ZWZ-3 increased the levels of Bax, cleaved caspase 9, cleaved caspase 3 and cleaved PARP. These effects of ZWZ-3 were reversed by NAC (a pharmacological inhibitor of ROS), suggesting that the flfluorescent probe ZWZ-3 induces mitochondria-mediated apoptosis through the ROS pathway. ZWZ-3 could inhibit the migration ability of melanoma cells. ZWZ-3 inhibited the ability of

A375 and B16F10 cells to metastasize in a time- and concentration-dependent manner, as assessed using the wound healing assay. Similarly, ZWZ-3 inhibited melanoma cell migration in a concentration-dependent manner in the transwell migration assay. To evaluate their tumor suppression ability in vivo, we selected the zebrafish model. Zebrafish have emerged as a valuable model system for studying melanoma, which can be rapidly used for in vivo assessment of anti-cancer drugs and drug targets. In zebrafish, the melanoma cell lines GFP-A375 and Dil-B16F10 were used to evaluate the anti-tumor effects of ZWZ-3. We inoculated zebrafish with either GFP-A375 or Dil-B16F10 cells and cultured them in a medium containing various concentrations of ZWZ-3, and any alterations in fluorescence were recorded. We observed a significantly lower fluorescence intensity of ZWZ-3-treated group than that of the control group, and the tumor cell volume was also reduced in a dose-dependent manner. At the same time, different concentrations of ZWZ-3 did not affect the morphology of zebrafish compared to the control group. It signifificantly suppressed tumor growth of A375 xenograft tumor in mice without notable side effects. Histological and immunohistochemical analyses revealed that ZWZ-3 induced apoptosis and inhibited tumor cell proliferation. We performed RNA sequencing in melanoma cells after the treatment with ZWZ-3 and found that Birc5, which is closely associated with tumor metastasis, was significantly downregulated. Bioinformatics analysis and the immunohistochemical results of tissue chips for melanoma further confirmed the high expression of BIRC5 in melanoma and its effect on disease progression. Moreover, Birc5 knockdown significantly inhibited melanoma cell proliferation and metastasis, which was correlated with the β-Catenin/HIF-1α/VEGF/MMPs pathway. Additionally, histological and immunohistochemical analyses revealed that ZWZ-3 inhibited tumor cell metastasis by downregulating HIF-1α, VEGF, and MMP9.

Conclusions ZWZ-3 represents a novel theranostic agent that can be used to effectively targeting, detecting, and treating melanoma. It could also help monitoring disease progression and response to treatment. Meanwhile, ZWZ-3 could downregulate BIRC5 and inhibit melanoma proliferation and metastasis through the β-Catenin/HIF-1α/VEGF/MMPs pathway. Therefore, BIRC5 represents a promising therapeutic target for the treatment of melanoma.

OR-98

A novel pathogenic gene KRT10 with compound heterozygous mutation inherited pattern causing skin fragility

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Objective In the previous study, the whole exome sequencing analysis was performed in a cohort of 46 pedigrees and 13 sporadic cases with epidermolysis bollusa (EB) in the Han Chinese population. However, two pedigrees were not carried a known pathogenic gene. After strict screening, we identified that two pedigrees carried the complex heterozygous mutations on the tail domain in the *KRT10* gene (NM_000421.5). Interestingly, we were concerned that none of their family members who carried homozygous or heterozygote mutations in *KRT10* suffered from a similar disease. In this study, we performed the knock-in mouse model and the keratin filaments assembly in vitro to explore the genetic pattern of two families.

Method The whole genome exon sequencing, sanger sequencing, immunofluorescence confocal microscopy, and transmission electron microscopy were performed in 2 families.

To construct of mice model, we performed the sequence alignments between human and mouse *KRT10* genes. Cas9 mRNA and gRNAs were designed to construct the mutant fragment. Cas9 mRNA, gRNA and Donor vector samples were microinjected into C57BL/6JGpt mouse zygotes to obtain F0 progeny mice. Positive F0 generation mice were mated with wild-type background mice to obtain F1 progeny mice. After crossing different homozygous mice, we

generated compound heterozygote mice. We studied the mouse model by observing the clinical phenotype, detecting the expression of mutant gene protein and transmission electron microscopy.

To investigate the sequences important for assembly of keratins into 10-rim filaments, we used a combined approach of `transfection of mutant keratin cDNAs into epithelial cells in vivo, and in vitro assembly of mutant and wild-type keratin 10. The high performance liquid chromatography system (Cytiva) were used to further purify keratins. The morphology of the nanofibrils was examined by a Philips CM-10 electron microscope.

Result

(1) Clinical information. Proband 1 is a 24 years old female who suffered from disease at 17 years old. Her skin involvement was relatively mild, with blisters, papules, and scars localized predominantly at trauma sites. No mucosal, nail, or other system was involved. Her family members weren't affected. Histopathological examination revealed non-inflammatory blisters between epidermal and dermal . And immunofluorescence results showed loosely connected in spinous layer and K10 expression decreases around the blistering.

Proband 2 is a 30-year-old male who was referred to our clinic for blisters, erosion, papule, nodule, and cysts on his face, trunk, and limbs. No other systems are accumulated. He developed the disease for 14 years, with no family history. We took a skin biopsy from his father as a volunteer, and all indicators were normal. Pathology inspection result of the lower leg skin showed subepidermal blisters . Around the blistering, immunofluorescence results showed K10 expression decrease . Transmission electron micrograph results revealed that separation occurred on the junction of dermal and epidermal. The structure of the basement membrane zone disappeared. Compared with normal control, keratin fibers aggregated to shorter clumps with disorder distribution.

(2) Sequencing analysis revealed biallelic mutations. Proband 1 and 2 carried compound heterozygous mutations in KRT10 on two alleles.

(3) Transgenic mouse model of KRT10 insertion mutation. The mouse model of KRT10 insertion mutation using the CRISPR/Cas9 system was successfully established. The compound heterozygote mice formatted blister after the mechanical stress. This phenotype occurred in 6-month mice, appeared inconspicuous at birth, and displayed no overt phenotype. Whereas no skin fragility appeared in mice that carried other genotypes. A histological examination showed blisters located between the dermis and the superficial epidermal layers. Immunofluorescence identified the blister in the basal layer but not in the spinous layer. The results are consistent with results of humans.

(4) Marked ultrastructural differences in compound heterozygote. We performed transmission electron microscopy in mouse back skin. Compared to the wild-type, heterozygous, and homozygous mice, the results showed total cytoskeleton collapsed in compound heterozygous mice. The spinous and basal keratinocytes lose their normal columnar structure and become flat and irregular. And the perinuclear cisterna swelling and the altered organelles distribution were also found. The keratinocyte junction was markedly widened, even occurring epidermal cleft.

Ultrastructural results showed compound heterozygous mice were markedly impaired in desmosome, hemidesmosome, and keratin filaments. The length of desmosomes appeared to be markedly reduced, and internal protein structure disappeared. Hemidesmosomes are irregular and rudimentary with rare keratin IFs. And keratin clumps and short keratin filaments aggregate in the keratinocytes. The extent of damage was distinctly smaller in homozygous mice than in compound heterozygous mice, which is sufficient to maintain cell function. Overall, the damage to cell function gradually increased in wild-type, heterozygote, homozygote, and compound heterozygote.

(5) Kinetics of intermediate filaments (IFs) assembly in vitro. In vitro, assembly of equimolar mixtures of wild-type keratin 1 (K1) and keratin 10 (K10) proteins under optimal buffer conditions resulted in the formation of regular IFs of normal diameter with a smooth surface and average of 1.96 mm long. To reflect the in vivo situation with different alleles, we evaluated in vitro filament formation in a molar mixture containing 50% wild-type K1, 25% one-type K10, and 25% other-type K10 according to the phenotypes. The average length of heterozygous IFs and homozygote IFs is 1.12 to 1.30 mm and 7.30 to 8.54, respectively. However, the compound heterozygous IFs are only 0.41 to 0.53 mm in length, and the surface of these filaments showed irregularities and protrusions. The result indicated that the structural features (length and regularity of width) of the IFs got worse gradually with the increased copy numbers of insertion mutations.

Conclusion Using a knock-in mouse model and the keratin filaments assembly in vitro, we identified that the compound heterozygous mutations in *KRT10* lead to the unstable filament and lower filament-forming efficiency than homozygous and heterozygote ones. These observations have a profound understanding of the genetic model of compound heterozygous mutations, and they give a new perspective on the function of the tail domain.

OR-99

The role of B-cell ferroptosis in systemic lupus erythematosus

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Objective Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by the dysregulation of B cell subpopulation and function. Recent studies have suggested a potential role of ferroptosis, an iron-dependent form of regulated cell death, in the pathogenesis of SLE. This study is to demonstrate that B-cell ferroptosis occurs both in lupus patients and MRL/*lpr* mice. In addition, we treat MRL/*lpr* mice with liproxstatin-1 (LPX-1), a potent ferroptosis inhibitor, to explore whether it can alleviate lupus symptoms in vivo and investigate the underlying mechanisms.
Methods (1) We first used a single-cell data set GSE135779, which contains peripheral blood mononuclear cells (PBMC) samples from 7 adult patients with SLE and 5 matched healthy donors (HD), to explore the proportion of B cell subsets and differential pathway enrichment between lupus patients and healthy people. Next, we collected and analyzed the clinical data from SLE patients and healthy donors. Non-targeted 1H nuclear magnetic resonance (NMR) spectroscopy was conducted on plasma samples from SLE patients and healthy donors, profiling a total of 30 metabolites, and the serum levels of malondialdehyde (MDA) were detected. The peripheral blood $CD19^+$ B cells were isolated from the lupus patients and healthy donors by magnetic bead sorting technology. We observed the mitochondrial morphology of B cells by transmission electron microscope and detected the expression of glutathione peroxidase 4 (GPX4) by western blot. Finally, human peripheral blood B cells were cultured with serum from lupus patients or healthy donors, and intervened with ferroptosis inhibitor, necroptosis inhibitor, and apoptosis inhibitor, respectively. Flow cytometry was used to detect the cell survival rate of each group. (2) MRL/*lpr* mice of 14-week-old and 18-week-old were used as lupus mouse models, while MRL/Mpj mice and BALB/c mice of the same age were used as normal controls, with 5 mice in each group. We observed the morphology of spleens and lymph nodes, detected the serum levels of anti-dsDNA antibodies by enzyme-linked immunosorbent assay (ELISA), and examined kidney pathology by hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS) staining. The proportion of $CD19^+$ B cells and plasma cells in the spleen was detected by flow cytometry. The spleen $CD19^+$ B cells were sorted by magnetic bead sorting technology. Then we observed the mitochondrial morphology by transmission electron microscopy, detected the ferrous ion content and lipid peroxidation level by fluorescence confocal microscopy and flow cytometry, and compared the expression of GPX4, SLC7A11, and SLC3A2 by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot. Through RNA sequencing (RNA-seq) analysis, we explored gene expression and pathway enrichment differences in splenic B cells from 14-week-old and 18-week-old MRL/*lpr* mice. (3) 8-week-old female MRL/*lpr* mice were randomly divided into LPX-1, vehicle, and control groups, with 5 mice in each group. The LPX-1 group received LPX-1 (10 mg/kg) by intraperitoneal injection, the vehicle group received equal volumes of 10% dimethyl sulfoxide (DMSO), and the control group received no treatment. Treatments were administered every other day for 8 weeks. All mice were sacrificed at 16 weeks of age. We observed the lupus symptoms and the characteristics of ferroptosis. Through RNA-seq analysis, the effects of the ferroptosis inhibitor on B cell-related pathways were elucidated.
Results (1) Single-cell data analysis demonstrated that the proportion of B-cell subset in SLE patients was significantly reduced. The pathways related to cell death, iron metabolism, and

reactive oxygen species (ROS) were significantly enriched in B cells from lupus patients. Clinical data analysis showed that the percentage of peripheral lymphocytes in SLE patients was negatively correlated with anti-dsDNA antibody levels (n = 34) and SLE disease activity index (SLEDAI) scores (n = 35). Analysis of lymphocyte subpopulations (n = 15) revealed a marked elevation in the percentage of T lymphocytes and a reduction in the percentage of B lymphocytes in SLE patients. Compared to healthy donors, the plasma of lupus patients exhibited significantly elevated levels of creatinine, glucose, triglyceride, and acetic acid, and notably decreased levels of acetone, lactic acid, pyruvic acid, and ethylenediaminetetraacetic acid (EDTA) (n = 13). In addition, the serum levels of MDA were elevated in lupus patients (n = 12). Then, peripheral blood $CD19^+$ B cells isolated from lupus patients showed ferroptotic characteristics in the mitochondria and expressed significantly lower levels of GPX4 than that of healthy donors. Finally, the serum from SLE patients significantly decreased the survival rate of peripheral blood B cells, which was greatly restored after LPX-1 intervention, showing no significant difference from that of the HD group. (2) Compared with normal control mice, 14-week-old MRL/*lpr* mice exhibited significant splenomegaly and lymphadenopathy, elevated serum levels of anti-dsDNA antibodies, substantial glomerular inflammatory cell infiltration, and renal tubular swelling, mesangial hypercellularity, and matrix expansion; the splenic B cells exhibited shrank and smaller mitochondria, with decreased or even disappeared mitochondrial cristae, iron deposition, abundant amounts of lipid ROS, downregulated expression of GPX4, SLC7A11, and SLC3A2. In addition, the proportion of $CD19^+$ B cells in the spleen of lupus mice was significantly decreased, with an elevated proportion of plasma cells. At 18 weeks of age, the condition of MRL/*lpr* mice worsened, with a more pronounced manifestation of ferroptosis in splenic B cells, and an overall upward trend in the proportion of plasma cells. Gene set enrichment analysis (GSEA) analysis revealed significant enrichment in the pathways associated with positive regulation of B cell activation, B cell-mediated immunity, B cell receptor (BCR) signaling, and positive regulation of B cell differentiation in splenic B cells from 18-week-old MRL/*lpr* mice when compared to the 14-week-old group. Of note, ferroptosis repressors (GPX4, SLC7A11, SLC3A2) expression was negatively correlated with the expression levels of B cell differentiation, plasma cell formation, and oxidative stress pathway gene sets. (3) Compared with the vehicle and control group, MRL/*lpr* mice treated with LPX-1 exhibited normal spleen and lymph node morphology and downregulated serum level of anti-dsDNA. The renal damages of MRL/*lpr* mice were dramatically tempered after LPX-1 treatment. Besides, splenic B cells after treatment exhibited normal mitochondrial morphology, significantly reduced iron deposition and lipid ROS, and upregulated expression of GPX4, SLC7A11, and SLC3A2. The proportion of $CD19^+$ B cells was increased after treatment, while plasma cells decreased significantly. GSEA analysis showed that LPX-1 treatment significantly inhibited B cell activation, B cell immunity, and BCR signaling pathways while activating the pathway to negatively regulate B cell differentiation.

Conclusion The findings of this study provide new insights into the pathogenesis of lupus and suggest that B-cell ferroptosis is involved in the development of this autoimmune disease, both in human and mouse models. Moreover, inhibiting ferroptosis could be a promising therapeutic strategy for alleviating lupus symptoms in vivo, by its regulatory function in B cell differentiation and plasma cell formation. These results highlight the importance of further research into the mechanisms underlying B-cell ferroptosis in lupus and its potential as a target for novel therapies.

OR-100

Clinical application of molecular subtypes associated with prognosis of mycosis fungoides based on immunohistochemical staining algorithm

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Abstract Our previous single-cell study defined two molecular subclassifications of mycosis fungoides, the T_{CM} group and T_{CyEM} group, which have distinct differences in tumor microenvironment and prognosis. In the current study, we generated an IHC panel to identify the 2 subtypes in paraffin tissue using antibodies to the gene signatures of each group (CD27 and TOX for T_{CM} group, GZMA and HOPX for T_{CyEM} group). A total of 126 cases of MF were included, and 69 of them with RNA-seq data were used to determine the cut-off value of each marker. Finial, the cut-off (CD27-0.5, TOX- 0.5, GZMA- 0.2, HOPX-0.1) were used to divided the 126 patients into three groups, the T_{CM} group (n=35), T_{CyEM} group (n=70) and undefined group (n=21). The T_{CM} group and T_{CyEM} group identified by the IHC panel matched the RNA-seq data with high sensitivity (85%) and presented a significant difference in progression free survival (P<0.001). Thus, we thought the IHC panel and cut-off value will aid in identifying the 2 molecular subtypes in clinical practice, but we need more MF patients with different ethnical backgrounds to validate.

Background According to our recent single-cell analysis on CTCL (Liu, et al. Nature Communications, 2022), we establish a subtyping scheme based on the molecular features of malignant T cells and their pro-tumorigenic microenvironments: the T_{CyEM} group, demonstrating a cytotoxic effector memory T cell phenotype, shows more M2 macrophages infiltration, while the T_{CM} group, featured by a central memory T cell phenotype and adverse patient outcome, is infiltrated by highly exhausted CD8+ reactive T cells, B cells and Tregs with suppressive activities. We have identified the 13-gene and 27-gene signatures in the T_{CyEM} and T_{CM} group, respectively. Considering the limited sample size of single-cell sequencing, we try to validate this subtyping scheme in a larger patient cohort with routine IHC, so it can be clinically applicable.

Methods

Patient information

We included 126 cases of MF with available FFPE samples and clinic-pathological data from our institutions. All cases were reviewed by at least 2 hematopathologists to confirm the diagnosis of MF according to the current WHO criteria. Sixty-nine patients among them had RNA-sequencing data and had been divided into two subtypes by the gene signatures we mentioned before.

Development of IHC panel

We considered commercial antibodies against molecules within the gene signatures set of each subtype and were expected to show reactivity on FFPE tissues. We selected GZMA, GZMH, HOPX, CLU for T_{CyEM} group, and CD27, TOX, CD40L, KIR3DL2, SMS, PON2, LGALS9 and FOXP3 for T_{CM} group. Each antibody was tested in at least 12 sections using different antigen retrieval methods and dilution concentration. Finally, four markers with optimal staining were selected for the IHC panel (CD27 and TOX for T_{CM} group, GZMA and HOPX for T_{CyEM} group).

Digital image acquisition and analysis

All 126 FFPE skin specimens were obtained and 4-μm-thick sections were used for the four markers staining. All slides were scanned with the NanoZoomer 2.0-RS C10730-13 (Hamamatsu Photonics) and analyzed using NDP.view 2.7.52 software. The number of positive cells / tumor cells was manually calculated in three representative regions of each slide at ×800 magnification. And the average positive rate of the three regions was calculated as the final positivity rate.

Statistical analysis

The mRNA expression data were treated as a continuous variable and IHC positivity was presented as percentages. Spearman correlation was used to assess the association between mRNA expression levels and IHC positivity. Scatterplots display the relationships with linear regression. Sensitivity, specificity and overall accuracy were determined by comparing the IHC-defined subtype

to the RNA-seq classification. Patient characteristics were compared between groups using the χ2 or Fisher exact test.

The Kaplan-Meier method was used to estimate the progression free survival (PFS) distributions. PFS was calculated from the date of initiation of diagnosis to the first date meeting the criteria for progressive disease (PD) or death of any cause. Disease progression was defined as progression to a more advanced TNMB classification (excluding a change from T1a or T2a to T1b or T2b, respectively) or death owing to disease. The log-rank test was used to compare survival distributions by IHC based diagnosis. Cox proportional hazards regression assessed clinical and pathologic characteristics with PFS. All statistical tests were 2-sided and P values <0.05 were considered to be statistically significant. The data analysis for this manuscript was conducted using SPSS (version 26).

Results

Patient characteristics

A total of 126 patients were included in the analysis (79 men [62.7%] and 47 women [37.3%], M:F ratio: 1.68). The median (range) age of diagnosis was 48 (15-77) years. More than half of the patients had early-stage MF (n=67, 53.2%), and 59 (46.8%) had advanced-stage MF. The median (range) follow-up duration was 21 (1-144) months.

Development of an antibody panel for IHC

Four antibodies were selected, including 2 with nuclear staining (TOX and HOPX), 1 with membranous staining (CD27) and 1 with cytoplasmic granules staining (GZMA) (*Figure 1*).The selected antibodies showed significant positive correlations (P<.05) between the percentage of positive cells and the corresponding mRNA expression levels except for TOX (*Figure 2*), which indicated that IHC staining results can be used to distinguish between two molecular subtypes.To determine the optimal positive thresholds for each marker staining, a receiver operating characteristic curve (ROC) was constructed using 69 training samples and their corresponding percentages of positive cells. In order to maximize the allocation of patients to their respective molecular subgroups, the optimal positive thresholds were selected at the point of maximum Youden index (*Figure 3*). According to the ROC curve results, the optimal positive thresholds for TOX/CD27/GZMA/HOPX were 0.515, 0.543, 0.225, and 0.142, respectively.The thresholds for IHC positivity were determined based on the percentage of staining that optimally divided the RNA-seq defined subtypes. This resulted in 50% for TOX and CD27 immunostains, 20% for GZMA and 10% for HOPX.In order to use the threshold for clinical IHC interpretation, the positive thresholds were rounded. The final selected thresholds for TOX/CD27/GZMA/HOPX were 50%, 50%, 20%, and 10% respectively. The discrimination results in the 69 training set patients are shown in *Figure 4*.

To further classify the two subtypes, we defined TOX ≥50%, CD27 ≥50%, GZMA <20% and HOPX <10% as T_{CM} IHC signatures, and TOX <50%, CD27 <50%, GZMA ≥20% and HOPX ≥10% as T_{CyEM} IHC signatures. For each specimen, if its T_{CM} IHC signatures more than T_{CyEM} IHC signatures，it would be classified into T_{CM} group, and vice versa. But in some cases, the T_{CM} IHC signatures would be equal to the T_{CyEM} IHC signatures, which were defined as undefined group. Using this IHC score method, we accurately classified 85% (59 of 69) of the RNA-seq defined case (*Figure 5*)

Clinicopathological characteristics and outcomes between the two subtypes

Using the IHC subclassification method, we analyzed the other 57 patients without RNA-seq data, together with the 69 patients mentioned before.Finally, 35 patients were classified into T_{CM} group, 70 patients into T_{CyEM} and 21 into undefined group. In consistent with our previous study, the T_{CM} group presented with adverse prognosis (T_{CM} VS. T_{CyEM}, P<.001). While there was no significant difference between undefined group with the other two groups as we expected (*Figure 6*)

We compared the clinicopathological characteristics of the T_{CM} group with the T_{CyEM} group. There was no significant difference of age at diagnosis and stage between two groups. while in TCM group, there were more female patients. Besides, the T_{CM} cases more often presented with folliculotropism and large cell transformation, which were all associated with poor prognosis (Table 1).

To eliminate the effects of these factors on prognosis, we performed univariate and multivariate Cox regression analyses. The results showed that the molecular subtypes of T_{CM} and T_{CyEM} can be

independent prognostic factors, regardless of age, stage, follicular subtype, and large cell transformation (Table 2）.

Conclusion

In the current study, we generated an immunohistochemistry (IHC) algorithm to identify the 2 molecular subtypes based on our previous single-cell study. The four markers panel classified the two groups with high sensitivity (85%) and showed a significant difference in progression free survival (PFS) (P<.001). We look forward to further validating this panel in more MF patients with different ethnical backgrounds.

OR-101

Potential role of N6-Methyladenosine of RNAs in the Pathophysiology of Atopic Dermatitis

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Objective To explore the role of m^6A modification in the pathophysiology of atopic dermatitis(AD).
Methods We first performed an Arraystar m^6A-mRNA epitranscriptomic microarray to screen differentially expressed genes (DEGs) and their m^6A levels, m^6A-related enzymes in patients with AD.
Results We identified 598 upregulated genes and 432 downregulated genes using mRNA-seq (fold changes ≥ 2 and $P < 0.05$) and discovered that 181 DEGs simultaneously occurred with hypo-m^6A and down-mRNA (hypo-down), and 532 DEGs simultaneously occurred with hyper-m^6A and up-mRNA (hyper-up). “Hypo-down” occurred in 54 biological processes (BPs) and two hsa pathways, while “hyper-up” occurred in 522 BPs and 27 hsa pathways. According to the sort of enrichment scores and the Fisher’s P value, we identified 16 discrepant pathways of interest, including 10 upregulated pathways and 6 downregulated pathways. The heatmap shows the sequence of the m^6A and mRNA levels of DEGs involved in these pathways. Moreover, we found that the difference in mRNA expression of “writer” WTAP was most apparent between NC and AD.
Conclusion Collectively, our findings revealed that m^6A modification may be closely related to the pathogenesis of AD, which can be further explored in the future.

OR-102

Genome-wide meta-analysis and fine mapping prioritize potential causal variants and genes for leprosy

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Objective Genome-wide association studies (GWAS) have discovered 35 susceptible leprosy loci, but the cumulative effects of these loci can only partially explain the overall risk of leprosy, and the causal variants and genes within these loci remain to be clarified. Here we performed a meta-GWAS analysis of leprosy in two newly generated (5,007 cases and 4,579 controls) and multiple previously published (2,277 cases and 3,159 controls) datasets to further characterize the genetic architecture of leprosy.
Methods The imputation of genotype datasets were based on a Chinese population-specific reference panel (China Metabolic Analytics Project, ChinaMAP). A comprehensive fine-mapping analysis was conducted including colocalization analysis, functional annotation and manual checking of epigenomic information from the Epimap database. Furthermore, we used gene-set, tissue and cell-type enrichment analyses to further reveal the pathogenetic mechanism of leprosy.

Results Four novel and 14 previously reported risk loci were identified from these datasets, increasing the known leprosy risk loci of explained genetic heritability from 23.0% to 38.5%. 20 causal variants (eight protein-damaging and 12 regulatory variants) and 16 causal genes were nominated. The biological annotation revealed the causal variants, especially in novel locus chr12:57,665,085 - 58,665,085 and CSK, located within immune-relevant or immune-specific regulatory elements. Gene-set analysis and tissue and cell specificity analysis revealed the important role of the synergistic antimicrobial responses between phagocytes and T cells through multiple immune-related pathways in leprosy.
Conclusion Our study highlighted the key roles of immune related tissues, cells and implicated PD-1 pathways in the pathogenesis of leprosy. Our study identifies candidate causal variants, and elucidates potential regulatory and coding mechanisms for genes underlying leprosy.

OR-103

Toxic epidermal necrolysis due to Lenalidomide Treatment of Multiple Myeloma:A Case Report

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Toxic epidermal necrolysis (TEN) is a rare yet severe delayed allergic reaction primarily affecting the skin. This dermatological emergency is triggered by the use of specific drugs and can be life-threatening. The implicated medications commonly associated with TEN encompass a range of pharmaceutical classes such as anticonvulsants, nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, antiretrovirals, anti-tuberculosis drugs, anti-anxiety drugs, and anti-gout agents. Notably, the occurrence of TEN is considered an uncommon phenomenon, but its severity and potential fatality warrant urgent attention and prompt management.
Lenalidomide, a highly effective immunomodulatory drug, is frequently employed in the treatment of patients with multiple myeloma (MM). However, in this particular case report, we present an unforeseen manifestation of TEN in a 67-year-old female patient diagnosed with MM who had undergone lenalidomide treatment. Notably, the patient displayed the development of a distinctive rash approximately 19 days following exposure to lenalidomide. This cutaneous eruption was accompanied by the presence of Nikolsky's sign, indicating the detachment of the epidermis upon slight pressure, and an extensive area of epidermal detachment exceeding 30% of the patient's total body surface area. The immediate cessation of lenalidomide was implemented to halt the progression of the adverse reaction. The therapeutic management strategy employed for this case consisted of a combination of intravenous dexamethasone, immunoglobulin (IVIG) administration, appropriate antibiotic therapy, and comprehensive supportive care. The treatment regimen was consistently administered for a duration of two months until a discernible improvement in the patient's overall health status was observed, coupled with a transition of the affected skin from a blistered state to a predominantly dry and crusted appearance. Furthermore, it is noteworthy to highlight that this reported case represents the first occurrence of TEN induced by lenalidomide within the Han Chinese population, further emphasizing the significance of documenting such rare adverse events in specific ethnic groups. Our findings underscore the importance of promptly initiating combined therapy with corticosteroids and IVIG to manage lenalidomide-induced TEN. They also serve as a reminder for clinicians to exercise heightened vigilance regarding this severe side effect.

OR-104

TRPV3 mediates Propionibacterium acnes-induced chemotaxis and inflammation in sebocytes

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Objective TRPV3 mediates itch and inflammation in keratinocytes and plays an important role in atopic dermatitis, however, whether and how TRPV3 is involved in the pathogenesis of acne, a common chronic inflammatory disease of the pilosebaceous unit, remains unclear. Thus, we explored the effects of TRPV3 on sebaceous glands (SGs) inflammation, its relation to Propionibacterium acnes (P.acnes), and the underlying mechanisms in vitro and in vivo.

Methods Formalin-fixed and paraffin-embedded human or mouse skin sections were used for in situ hybridization using the RNAScope system or stained with H&E, anti-Myeloperoxidase (MPO), TLR2, and p65 antibody for immunohistochemical (IHC) staining. Total RNAs were extracted from human SZ95 sebocytes and transcriptome sequencing was performed using Illumina HiSeq X Ten, differential expression data were ranked through log2 fold change, and performed Gene set enrichment analysis (GSEA) to figure out enrichment for KEGG pathways. Targeted quantification of fatty acids in the culture supernatants of human sebocytes was carried out by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Acne-like mouse model was established by intradermal injection of 20μl Living P. acnes (1.0 × 108 CFU suspended in PBS) or PBS into the ears of 8 weeks Trpv3-/- and C57BL/6J WT mice. TRPV3-overexpression and TRPV3-knockout cell lines were generated using TRPV3-plasmid transfection and CRISPR/Cas-9 technology, respectively. Real-time quantitative PCR and enzyme‐linked immunosorbent assay（ELISA）were conducted to detect the mRNA and protein levels of chemokines and cytokines in human SZ95 sebocytes and skin lesions of mice, respectively. Human primary neutrophils were isolated from the peripheral blood of healthy subjects and a transwell assay was performed to evaluate the chemotaxis of supernatant from human SZ95 sebocytes under different treatments to human neutrophils. Western blot was performed to determine the phosphorylation level of p65 as well as the protein expressions of TRPV3 and TLR2.

Results To investigate whether TRPV3 is associated with the occurrence of acne, we first detected the expression and localization of TRPV3 in facial lesions of healthy controls and acne patients. We observed that TRPV3 was significantly upregulated in the SGs of acne patients compared with healthy controls and mainly expressed in mature sebocytes. Since P.acnes, a Gram-positive anaerobic bacterium commonly colonized in human pilosebaceous follicles, stimulates inflammation and is associated with the development of acne, we wondered if the upregulation of TRPV3 was associated with P.acnes. Our results showed that P.acnes treatment promoted the protein level of TRPV3 both in human SZ95 sebocytes and acne-like mouse models. To further clarify how P.acnes affected the level of TRPV3, lipid profile changes in human sebocytes were evaluated. By LC-MS/MS, we found that the content of arachidonic acid (AA), a kind of unsaturated fatty acid involved in the pathogenesis of acne, increased in the supernatant of human sebocytes under P.acnes stimulation. We further found that topical treatment of AA promoted TRPV3 expression as well as inflammatory cell infiltration in the lesional skin of mice, hinting that P.acnes upregulated the level of TRPV3 through regulating lipid profiles in human sebocytes.

We next examined how TRPV3 is involved in the development of acne. Overexpression of TRPV3 lead to increased IL-1α, IL-1β, IL-6, IL-8, TNF-α, CXCL1, and CXCL2 release in human SZ95 sebocytes. Besides, the supernatant from TRPV3-overexpressed sebocytes exhibited enhanced chemotactic abilities on human primary neutrophils compared with Mock transfection control. Conversely, TRPV3 silencing attenuated P.acnes-induced acne-characteristic cytokines and chemokines release as well as the chemotaxis of neutrophils. Next, we sought to determine

whether the effects of TRPV3 in vitro also occurred in vivo, acne-like mouse model was constructed by intradermal injection of live P.acnes to the ears of wild-type (WT) and Trpv3-/- mice. We observed reduced weight and thickness of acne-like lesions in Trpv3-/- mice compared with WT mice. The levels of pro-inflammatory cytokines and chemokines both decreased in mice lesional tissue homogenate. Besides, IHC staining for the neutrophil-recruiting chemokines such as CXCL1 and MPO representing neutrophil infiltration were stronger in the lesional skin of acne patients compared with healthy controls, suggesting an important role of neutrophils in the development of acne. Further results showed that CXCL1 and MPO are both weaker in Trpv3-/- mice than in WT mice. Moreover, pharmacological inhibition of TRPV3 by its specific antagonist Trpvicin also improved acne-like inflammatory phenotype in WT mice. The above results demonstrated that TRPV3 may contribute to the inflammatory process of acne.

To further explore the underlying mechanisms of TRPV3 in acne-related inflammation, we performed RNA-sequencing in Mock transfected and TRPV3-overexpressed human sebocytes. GSEA results revealed that Toll like receptor (TLR) signal and Nuclear factor kappa-B (NF-κB) signal pathway were enriched in TRPV3-overexpressed human sebocytes. Western blot was carried out to validate the sequencing results, and results showed that TLR2 level and the phosphorylation form of p65 were upregulated after TRPV3 overexpression, while TRPV3 knockout abolished P.acnes-induced TLR2 and p-p65 expression in human SZ95 sebocytes, indicating that TRPV3 overexpression leads to the activation of TLR2-NF-κB signaling pathway. Besides, the expression of TLR2 and p-p65 induced by P.acnes was significantly lower in Trpv3 KO mice than in WT mice and pharmacological inhibition of TRPV3 by Trpvicin obtained similar results. These results suggested that TRPV3 may have an impact on acne-related inflammation through the regulation of the TLR2-NF-κB signaling pathway.

Conclusion Our study revealed increased TRPV3 expression in human acne SGs. P.acnes upregulated the content of AA by modulating sebum metabolism and subsequently promoted TRPV3 expression in human sebocytes as well as acne-like mouse model. Furthermore, we revealed that TRPV3 activates NF-κB signaling by regulating TLR2 expression and then promotes downstream inflammatory cytokines, chemokines releases, and neutrophil infiltration. Genetic deletion or pharmacological inhibition of TRPV3 both ameliorated acne-like inflammatory phenotype. Overall, these findings suggest a previously unreported association between TRPV3 and P.acnes, which is involved in the development of acne inflammation and may suggest TRPV3 as a novel therapeutic target for acne vulgaris and other disorders of the pilosebaceous unit.

OR-105

Loss of Fra1 in keratinocytes induces mitochondrial dysfunction and promotes the pathogenesis of psoriasis.

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Objective To elucidate the regulatory mechanism of Fra1 in psoriasis pathogenesis.

Methods The CRISPR technology was utilized to generate a Fra1-deficient cell line in the human keratinocyte cell line HaCaT. Psoriasis-like inflammatory models were induced using imiquimod (IMQ) in HacaT cells. Epidermal-specific Fra1-deficient mice were generated through the Cre-Flox system, employing Krt14Cre mice and Fra1flox/flox mice. Psoriasis-like dermatitis models were established in mice through the application of imiquimod. BrdU staining was employed to assess cell proliferation, Annexin V and PI staining were used for apoptosis detection, Elispot was utilized to measure the secretion of IFNγ and IL6, cell scratch assay assessed cell migration ability, JC1 determined mitochondrial membrane potential, cellular oxygen consumption, and glycolytic capacity were evaluated using Seahorse assay. Fra1 expression, mouse dermatitis phenotype, and inflammatory infiltration were analyzed using HE staining, immunohistochemistry, immunofluorescence, and flow cytometry. Epidermal tissues from Fra1-deficient mice were subjected to liquid chromatography-mass spectrometry (LC-MS/MS) based data-independent

acquisition (DIA) proteomics. Differential protein analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG), and Gene Set Enrichment Analysis (GSEA) was utilized for pathway analysis to identify relevant regulatory pathways. Western blotting and qPCR were conducted to verify protein expression. Mitochondrial phenotypes were observed using transmission electron microscopy. Data analysis and graphical representations were performed using FlowJO, GraphPad Prism, and R software.

Results Fra1 expression significantly increased in patients with psoriasis and wild-type mice induced by IMQ, particularly in keratinocytes. The loss of Fra1 amplified the proliferative ability of HaCaT cells, weakened their migratory capability, and heightened their susceptibility to apoptosis. Following IMQ treatment, the apoptosis of Fra1-deficient cells was significantly enhanced, while the migratory ability was further reduced, with no discrepancy observed in the proliferative ability. After IMQ treatment of HaCaT cells, there was a significant enhancement in the expression of IFNγ and IL6, while the secretion of IFNγ in Fra1-deficient cells was considerably reduced. The loss of Fra1 in mice resulted in more severe dermatitis, remarkable hyperkeratosis, and intensified infiltration of immune cells. After IMQ-induced psoriasis in mice, the expression of S10A8 and S10A9 was significantly elevated in Fra1-deficient mice, indicating a more pronounced inflammation. The analysis of differential protein quantification revealed that differential proteins were predominantly enriched in the oxidative phosphorylation and JAK-STAT signaling pathways. The expression of mitochondrial respiratory chain-related proteins ATP5MG, ATP5PB, and NDUFA9 in the epidermal tissues of Fra1-deficient mice demonstrated a substantial increase compared to control mice before IMQ treatment. After IMQ induction, these proteins' expression increased in both mice groups without any significant difference. The expression of ATP6V1C1 remained unchanged in response to IMQ-induced protein expression following Fra1 deficiency, underscoring the direct targeting of ATP6V1C1 by Fra1. Moreover, impaired mitochondrial function was observed in Fra1-deficient HaCaT cells, as evidenced by a noteworthy decrease in oxygen consumption. After IMQ treatment, the reduction in oxygen consumption was more prominent in Fra1-deficient cells. Additionally, Fra1-deficient cells displayed an increase in glycolysis capacity, which subsequently decreased in both groups of cells after IMQ treatment, albeit without any significant difference. Electron microscopy of the mouse epidermis revealed a substantial increase in mitochondria in keratinocytes of Fra1-deficient mice after IMQ induction, albeit with abnormal mitochondrial structure. Notably, Fra1 presented a considerable genetic predisposition in Han Chinese, Japanese, and European populations.

Conclusion The aforementioned findings demonstrate that the deficiency of Fra1 exacerbates the psoriasis-like dermatitis model induced by IMQ. Impairment of Fra1 in keratinocytes could compromise the functionality of cellular mitochondria, and perturb cellular oxidative phosphorylation and glycolysis levels, thereby triggering premature cell apoptosis and facilitating the onset and progression of the psoriasis phenotype. In summary, our discoveries shed light on the involvement of the metabolic microenvironment within keratinocytes in the pathogenesis of psoriasis, thus enhancing the comprehension of the mechanisms underlying disturbances in the epithelial microenvironment in psoriasis.

OR-106

Therapeutic observation of PDL combined with ultra pulse carbon dioxide lattice laser in treating hypertrophic scar in children

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Objective To observe the clinical efficacy of PDL combined with ultra pulse carbon dioxide lattice laser in the treatment of hypertrophic scar in children.
Methods Forty-five patients with hypertrophic scar were selected and treated with pulsed dye laser (PDL, 585 nm) combined with carbon dioxide lattice laser for 4 times with an interval of 8 weeks. Vancouver Scar Scale (VSS) was used to evaluate the hypertrophic scars before and after treatment. The vessels and thickness of scar were measured by dermoscopy and high frequency skin ultrasound. The efficacy was evaluated 8 weeks after treatment.
Results The results of curative effect evaluation showed that 27 cases had obvious effect, 13 cases were effective and 5 cases were ineffective, the effective rate was 88.9%. There were statistically significant differences in VSS score, skin thickness and dermatoscopic score before and after treatment ($P<0.05$). None of the patients had severe adverse reactions such as skin blister and ulceration after laser treatment.
Conclusion PDL combined with ultra pulse carbon dioxide lattice laser is safe and effective in the treatment of hypertrophic scar in children.

OR-107

BCAT2 promotes melanoma progression by activating lipogenesis via the epigenetic regulation of FASN and ACLY expressions

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Aims Melanoma is the most lethal skin cancer originating from the malignant transformation of epidermal melanocyte. The dysregulation of cellular metabolism is a hallmark of cancer, including in melanoma. Aberrant branched-chain amino acids (BCAA) metabolism and related enzymes has been greatly implicated in the progression of multiple types of cancer, whereas remains far from understood in melanoma.
Methods Immunoblotting and immunofluorescence staining analysis were used to verify the expression status of BCAT2 in melanoma. The role of BCAT2 in melanoma cell proliferation, invasion, migration, as well as tumor growth and metastasis, were investigated both *in vitro* and in *vivo*. A panel of biochemical assays and RNA-sequencing technology were used to investigate the underlying mechanism.
Results Firstly, we found that BCAT2 expression was prominently increased in melanoma, and highly associated with clinical stage. Then, it was proved that the deficiency of BCAT2 led to impaired tumor cell proliferation, invasion and migration *in vitro*, and tumor growth and metastasis *in vivo*. Further, RNA sequencing technology and a panel of biochemical assays demonstrated that BCAT2 regulated de novo lipogenesis via the regulation of the expressions of both FASN and ACLY. Mechanistically, the inhibition of BCAT2 suppressed the generation of intracellular acetyl-CoA, mitigating P300-dependent histone acetylation at the promoter of FASN and ACLY, and thereby their transcription. Ultimately, zinc finger E-box binding homeobox 1 (ZEB1) was identified as the upstream transcriptional factor responsible for BCAT2 up-regulation in melanoma.
Conclusion Our results demonstrate that BCAT2 promotes melanoma progression by epigenetically regulating FASN and ACLY expressions via P300-dependent histone acetylation.

Targeting BCAT2 could be exploited as a promising strategy to restrain tumor progression in melanoma.

OR-108

A novel XPC splicing mutation in a compound heterozygous pedigree with xeroderma pigmentosum

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Background

Xeroderma pigmentosum (XP) is a rare autosomal recessive genetic disease characterized by hypersensitivity to ultraviolet (UV) radiation, sunburn, skin pigmentation, and an increased risk of neurological degeneration, skin cancer, and ocular disease. The main pathogenesis of XP is UV-induced DNA damage repair system deficiency. XP is classified into eight different groups according to different pathogenic genes involved. These subtypes are XP-A (XPA), XP-B (ERCC3), XP-C (XPC), XP-D (ERCC2), XP-E (DDB2), XP-F (ERCC4), XP-G (ERCC5), and XP-V (POLH). XP-A to XP-G are associated with gene defects in nucleotide excision repair (NER) pathway, which is a major mechanism for repairing UV-induced DNA damage. A genotype-phenotype correlation between gene mutations and clinical outcomes is reported in XP patients. Identification of pathogen mutations and XP genotype will promote the quick development of early diagnosis and genetic counselling of patients.

Objectives

To identify the pathogenic mutation and genotype in a Chinese XP pedigree, in which both the two children exhibit typical symptoms while their parents were asymptomatic.

Methods

The clinical information and family history were collected to analyze the genetic pattern of the pedigree. Additionally, we obtained whole blood samples from members of four family lines. Hematoxylin-eosin staining of the biopsy was performed to validate the pathological features of the lesion. Whole-exome sequencing (WES) and Sanger sequencing were used to analyse variations and confirm the causative gene in the patients and their parents. DbscSNV_SCORE, Spidex, SIFT, Polyphen2_HDIV, Polyphen2_HVAR, MCAP, Mutationtaster and REVEL bioinformatics prediction software was used to predict the effect of candidate variants on proteins.

Results

The proband is an 8-year-old boy. Skin reddening and red macules presence on his face when he was eight months old. With increasing age, the dense brown freckle-like rash on the face、anterior and other exposed areas, and dry skin all over his body. Despite the patient reporting no history of sunburn, his symptoms aggravated after exposure to sunlight. The proband's 10-year-old sister exhibited similar clinical characteristics. A black 0.5 cm × 1 cm hyperpigmented nodule with central ulceration and scab formation was observed on the right side of her nose. Histopathological examination (HE) of the skin lesions was performed and revealed a basal cell carcinoma (BCC). This patient also exhibited dry skin and the presence of small dark brown macules on her face and limbs. Their parents were healthy and none of the similar clinical manifestations was observed. Both patients were clinically diagnosed with XP.

Whole exome sequencing was performed in this pedigree. The results revealed the patients carried a compound heterozygous variations of c.1735C>T and c.299+1G>A in XPC gene that were inherited from their asymptomatic parents. The mutation c.1735C>T was present in the father, and c.299+1G>A in the mother. None of the homologue mutation was found in the recessive model and no other possible pathogenic variations were found.

Based on the human gene mutation database (HGMD), c.1735C>T is a reported mutation that leads to a premature termination codon, resulting in a truncated protein. As such, c.1735C>T is graded as 'pathogenic'. Meanwhile, c.299+1G>A, which is located in the shear site region of the

second exon of XPC, is an unreported SNP with unknown function. Two XPC variants were confirmed by Sanger sequencing and co-isolated from the disease phenotype in the family.
XPC gene encodes a 940 amino acid protein that plays an essential role in the initiation of the NER. Hence, mutations in the XPC gene can result in failure to recognise and repair DNA damage. Protein function prediction was performed through multi-platform bioinformatics analysis, the novel splicing XPC variant was predicted to have a change in amino acid sequence, which may affect protein features, this maybe a pathogenic splicing variant in patients with XP-C.
Conclusion
A newly identified heterozygous mutation in the XPC gene (NM_004628: c.299+1G>A) may affect the expression level of the XPC gene by altering the splicing of the transcript, resulting in the absence of NER function. When complemented with the presence of c.1735C>T, this rare combination of mutations causes XP-C. Our study not only expands the pathogenic range of XP, but also promotes the development of early diagnosis and genetic counselling in Chinese Han population. Supported by Yunnan Fundamental Research Projects (grant NO. 202201AT070065)

OR-109

An outbreak of Mycobacterium marinum infections associated with handling seabass in China

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Background In December 2019, based on a report from a dermatologist describing seven patients with similar persistent nodules on their upper extremities after stabbed by seabass, an outbreak of Mycobacterium marinum infection in China was identified and investigated.
Methods We performed interviews and epidemiological investigations on patients and fish retailer. Pathological examinations, real-time quantitative PCR (qPCR), and bacterial cultures were performed on skin tissues. The fish tissues were evaluated by cultures and qPCR. Isolates from patients and fish were sequencing by whole-genome sequencing (WGS).
Results Totally, 217 patients (44 men and 173 women with a median age of 57 years) were recruited, including 157 laboratory-confirmed, 56 probable, and four suspected patients. The infection peak (35%) was observed in October 2019 due to increased consumption of fish for Chinese traditional Mid-Autumn Festival. Isolates from patients and infected fish were all confirmed to be M. marinum and two clusters with polyclonal strains were involved. Comparative analysis of strains from fish and humans confirmed transmission from seabass to human.
Conclusions This study describes the largest documented human outbreak of seabass-associated M. marinum infection. The public should be aware of the risk of this infection when handling fish.

OR-110

Current Status and Trend of Treatment Pattern and Patient Characteristics of Pediatric Psoriasis in China: A multicenter, retrospective observational study

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Current Status and Trend of Treatment Pattern and Patient Characteristics of Pediatric Psoriasis in China: A multicenter, retrospective observational study

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Objectives Approximately one-third of the patients developed psoriasis in childhood. The characteristics and treatment patterns of adult psoriasis have been investigated in many studies in China, while large-scale studies about pediatric psoriasis are scarce. In recent years more biologics have been approved for use in children, and the treatment paradigm for pediatric psoriasis has undergone a change in China. Our study aimed to analyze the current status and trend of real-world characteristics and treatment patterns of pediatric psoriasis in China.

Methods Patients first diagnosed with psoriasis before age 18 between 2016 and 2023 in 2 tertiary children's hospitals (Beijing Children's Hospital, Capital Medical University, and Tianjin Children's Hospital) in China were included. Data on demographic characteristics, clinical characteristics, and treatment patterns were retrospectively collected through de-identified electronic medical records (EMR) databases and hospital information systems (HIS).

Results A total of 5,768 patients (53.2% male) were included with a mean age of 7.7 ± 3.3 years old, and most were in the 7-10 years old (42.3%). 5,388 (93.4%) patients were in the outpatient group, and the remaining 380 (6.6%) were in the inpatient group. The most common type of psoriasis in children was psoriasis vulgaris (n = 5,431, 94.2%), followed by pustular psoriasis (n = 295, 5.1%), erythroderma psoriasis (n = 24, 0.4%) and psoriatic arthritis(n = 18, 0.3%). The proportion of patients with pustular psoriasis, arthritis psoriasis and erythrodermic psoriasis were significantly different between the inpatient group and outpatient group ($p < 0.001$). The lesions were mainly localized in the extremities (34.7%), trunk (33.7%), and scalp (25.0%). Regarding treatment regimes, topical treatment remains the most common choice for psoriasis in children. Systemic treatments included acitretin (4.4%), biologics (2.53%), methotrexate (2.18%), glucocorticoids (0.94%), and cyclosporine(0.31%). The use of methotrexate and acitretin among outpatients was 11.8% and 2.13% in 2016, respectively, while it decreased to 1.27% and 1.66% in 2022. Conversely, the proportion of biologics increased over time in the outpatient group, from 2.6% in 2016 to 11.62% in 2022. The proportion of patients in the inpatient group was higher than that in the outpatient group with the treatment of biologicals (14.2% vs. 1.7%, $P < 0.001$), and the use of them also increased over time in the inpatient group, from 3.9% in 2016 to 52.2% in 2022,

respectively. Among these biologics, secukinumab, adalimumab, and recombinant human type II tumor necrosis factor receptor-antibody fusion protein accounted for 54.8%, 31.5%, and 19.2% of patients, respectively.
Conclusion Psoriasis vulgaris is the predominant clinical subtype of pediatric psoriasis. Topical treatment remains the main option. The application of traditional systemic medications in pediatric psoriasis decreased over time, while the use of biologics significantly increased among inpatients. Still, the usage rate remains relatively low among outpatients.

OR-111

STING-induced GBP1 promotes cutaneous squamous cell carcinoma via the Jak/STAT signaling pathway

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Objective This study aimed to investigate the function of GBP1 in cutaneous squamous cell carcinoma.
Methods The expression of GBP1 in patient cutaneous squamous cell carcinoma tissues was determined by immunohistochemistry. By using combination approaches of GBP1 knockdown and overexpression, the effect of GBP1 on cell proliferation was detected by CCK8 assay in SCL-1 and A431 cell lines. The effects of GBP1 on cell cycle and cell apoptosis were detected by flow cytometry, and the expression of cell cycle and cell apoptosis-related proteins was detected by RT-qPCR and Western blot analysis. After GBP1 knockdown in A431 cells, RNA sequencing was performed, and KEGG/GO analysis was used to analyze the enrichment pathways of downstream differentially expressed genes. After inhibiting the STING pathway in cutaneous squamous cells by using STING inhibitor C-176, the cell proliferation was detected by CCK8 assay, and the GBP1 expression was detected by RT-qPCR and Western blot analysis.
Results Immunohistochemistry showed that GBP1 was highly expressed in patient cutaneous squamous cell carcinoma tissues. In vitro functional experiments showed that GBP1 promoted the proliferation of squamous cell cells, inhibited cell apoptosis, and promoted cell invasion and migration. After inhibiting the STING pathway, GBP1 expression was down-regulated and cell proliferation was inhibited. The KEGG analysis following RNA sequencing demonstrated that GBP1 knockdown mainly affected Jak/STAT signaling pathway.
Conclusion In summary, GBP1 can be activated by the STING pathway and is involved in the development of cutaneous squamous cell carcinoma through Jak/STAT signaling pathway. GBP1 may be used as a therapeutic intervention target for cutaneous squamous cell carcinoma.

OR-112

The Effect of a Standardized Nutraceutical to Improve Hair Coverage & Texture in Postpartum Women

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Introduction Most women experience changes in hair quality during their pregnancy including physical attributes of the hair like texture (i.e. softness and shine) as well as increased hair fall. Several factors contribute to the metabolic and hormonal alterations in the body during pregnancy such as malnutrition and other stress conditions which subsequently then impacts their hair. During pregnancy, more hair follicles remain in the anagen phase and then quickly switch to the telogen phase postpartum. As a result, hair may continue to shed anywhere from 3 to 6 months postpartum (entering a phase known as telogen effluvium). Cosmetically significant hair regrowth can take up to 12 to 18 months. In principle, the hair shedding experienced after childbirth should

improve on its own, yet reports of brittle hair, slower growth, and hair not getting “back to normal” are common amongst postpartum women. Increased hair shedding or fall can impact a women’s self-confidence especially during the postpartum period. The role of nutrition in hair health continues to be demonstrated. There is evidence that different botanicals, vitamins and minerals and other nutrients (i.e. collagen peptides) all play a critical role in overall hair health. It has previously been reported that the use of a standardized nutraceutical improved physical perception of hair (i.e. less shedding, breakage, etc) in a postpartum population consuming the product for eight months. This study further evaluated the impact on self-reported feelings of hair texture (i.e. softness, shimmer, etc), confidence and scalp coverage in postpartum women as these are also of concern to this population.

Methods

This was an 8-month multi-site, subjective single-blind prospective study evaluating the efficacy and safety of a standardized nutraceutical for hair health. This study was reviewed and approved by a local IRB. Secondary objectives included the assessment of the product to improve hair coverage, texture and self-confidence in postpartum women. Fifty-two non-breastfeeding women having given birth within the previous 12 months entered in the 8-month study. All Fitzpatrick skin types were included in the study. All hair types (straight, wavy, curly and kinky) were included in the study. All subjects had non-complicated pregnancies/births and were otherwise healthy at study entry. Subjects came into the clinic for repeated visits at baseline, and at days 60, 90, 120, 180 and 240, where standardized digital images were taken, and a self-perception questionnaire was administered. The questionnaire consisted of 24 questions and was a 4 choice Likert scale (Strongly Agree, Agree, Disagree or Strongly Disagree). Subjects consumed the standardized nutraceutical once daily as 4 capsules with a meal. Compliance to the program was determined via collection of bottles at each visit. The occurrence of adverse events was captured at each visit.

Results

39 subjects (mean age 32.1y) completed the 8 month study. Time since birth averaged 129 days (range: 7-241) or 18 weeks (range 1-34). Overall results from subject questionnaires support that the formulation improves perception of hair coverage and texture (including softness and shine). A significant proportion of patients reported more scalp coverage, part line improvements, baby hair growing out, and hair texture improvements as early as 60 days after starting the supplement. These results were either maintained or continued to improve for subjects throughout the duration of the study. Subjects also reported improvement of their hair with regards to softer and shinier hair. For softer hair, 91.8% of postpartum women agreed or strongly agreed that their hair was softer at Day 60. At Day 90 it increased to 95.6% of postpartum women reporting softer hair (agreed or strongly agreed). By Day 120, all postpartum women (100%) reported having softer hair (agreed or strongly agreed). Similar data patterns were observed for all of the additional variables in the study. Additionally, subjects reported feeling more confident (100% of subjects at Day 60) and would recommend this product to a friend (100% of subjects at Day 90). Subjects demonstrated high compliance with taking the product. As previously reported, the product was noted by patients as easy to add to their daily routine and there were 5 AEs during the trial (reported by 2 subjects).

Conclusion

There have been very limited offerings to support hair health during the postpartum period to date and in fact, providing any type of therapy can be difficult during this biologically-sensitive period. This study demonstrated that the consumption of a standardized nutraceutical by postpartum women resulted in improved subjective feelings about their hair texture, scalp coverage and overall confidence. The postpartum women in the trial would strongly recommend the product to their friends. By addressing the hormonal fluctuations, stress, and nutritional gaps that affect the hair growth cycle post-childbirth, the “normal” hair shedding and extended recovery can be mitigated. Although further research is needed, the results from this study suggest that providing a standardized nutraceutical to help replenish nutrients that support the body through the postpartum period can lead to healthier hair.

OR-113

eIF4E and p-eIF4E play the role of pathogenic genes in psoriasis and the inhibition of eIF4E phosphorylation ameliorates Imiquimod-induced psoriasis-like skin lesions in mice

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Objective
To investigate the role eIF4E and p-eIF4E played in psoriasis and to figure out whether eFT-508 (Tomivosertib, eIF4E phosphorylation inhibitor) can relieve the disease severity and become a promising candidate for the psoriasis treatment.
Methods
We first verified the expression of eIF4E and p-eIF4E in psoriasis patients' lesional skin. Then we constructed an in vitro psoriasis cell model by adding five cytokines (M5: IL-17A, IL-22, oncoprotein M, IL-1α, and TNF-α) to keratin-forming cells. 1αand TNF-α) to construct an in vitro psoriasis cell model. Then, we verified the expression of eIF4E and p-eIF4E with or without M5 in HaCaT and NHEKs keratinocyte cell lines. Then, we demonstrated the effect of eIF4E on the abnormal proliferation and inflammatory state of keratinocytes by using eIF4E-specific small interfering RNA (si-eIF4E). We selected si-eIF4E with the highest knockdown efficiency in HaCaT and NHEKs keratinocyte-forming cell lines, respectively, to directly verify the effect of eIF4E on keratinocyte proliferation using CCK-8 assay. The effect of eIF4E on the proliferation of keratinocytes was indirectly verified using the EdU experiment. We performed qRT-PCR experiments to verify the expression of IL1β, CXCL10, and IL23 mRNA levels in keratinocytes after the knockdown of eIF4E. We performed ELISA experiments to verify IL1β, CXCL10, and IL23 proteins level secreted by keratinocytes after the knockdown of eIF4E.
We then added eFT-508, a phosphorylation inhibitor of eIF4E, to the HaCaT cell culture medium to verify the effect of p-eIF4E on HaCaT cell proliferation and inflammation. We first determined the non-toxic concentration of eFT-508 for HaCaT cells. Secondly, we verified the effect of eFT-508 on the inhibition of eIF4E phosphorylation at different concentrations, and we took the highest dose at the non-toxic concentration as the experimental concentration for this experiment. The effect of p-eIF4E on the proliferation of keratinocytes was verified by CCK-8 and EdU assay. We performed qRT-PCR experiments to verify the expression of IL1β, CXCL10, and IL23 mRNA in keratinocytes after adding eFT-508. We performed ELISA experiments to verify the secretion of IL1β, CXCL10, and IL23 protein by keratinocytes after adding eFT-508. After that, we used whole transcriptome sequencing to verify which pathways play more critical roles after knocking down eIF4E. We also verified the expression of some essential pathway proteins to explore the possible mechanisms of eIF4E and p-eIF4E in keratinocytes. Next, to confirm the therapeutic potential of eFT-508 in psoriasis, we used C57 mice. After eliminating back hair, we applied imiquimod (IMQ) for seven days to create a mouse model of acute psoriasis inflammation. We divided mice into five groups, the entire control group (coated with petroleum jelly), the disease model group (IMQ), the low-dose treatment group (IMQ + 10 mM eFT-508), the medium-dose treatment group (IMQ + 15 mM eFT-508) and the high-dose treatment group (IMQ + 20 mM eFT-508). Mice in the treatment group were coated with eFT-508 on the back on days 1, 3, 5, and 7 of the IMQ application, and mice were executed and sampled on day 8. On days 2, 4, 6, and 8 of the modeling, we used the PASI score to quantify the skin damage in mice with psoriasis. We took H&E staining to visualize the thickness of dorsal skin in mice, and the spleen index was calculated to assess the systemic inflammatory status of mice.
Results
1.eIF4E and p-eIF4E highly express under psoriasis condition, eIF4E promotes M5-induced abnormal proliferation and inflammatory state of keratinocytes

To vividly show that eIF4E and p-eIF4E were highly expressed in psoriatic tissues, we did IHC staining in tissue sections from psoriatic patients (n=6) and healthy individuals (n=6). We could see that eIF4E and p-eIF4E were significantly highly expressed in the skin tissues of psoriatic patients, which matches to the previous study. Then, we validated the expression of eIF4E and p-eIF4E in M5-stimulated HaCaT and NHEKs cells. As expected, eIF4E and p-eIF4E were also significantly highly expressed in the psoriasis model groups. These results all suggested that eIF4E and p-eIF4E may be associated with the development of psoriasis.

2.eIF4E promotes M5-induced abnormal proliferation and the inflammatory state of keratinocytes

To validate the role of eIF4E played in keratinocytes under the psoriasis condition, we transfected si-eIF4E into HaCaT and NHEKs cells to reduce the expression of eIF4E. We chose the most effective one from three si-eIF4E in HaCaT and NHEKs cells, respectively. And, we decided to apply si-eIF4E 1 in the HaCaT cells and si-eIF4E 2 in the NHKEs cells. Next, we validated the effect of eIF4E on cell proliferation using CCK-8, EdU, and flow cytometry analysis of cell cycle distribution and found that inhibition of eIF4E expression inhibited the abnormal proliferation of keratinocytes in the psoriatic state. Cell cycle distribution showed that si-eIF4E tended to block cells into G0/G1 phase. For the inflammatory state, we found that si-eIF4E inhibited the expression of cytokines such as IL1β, CXCL10 and IL23 at mRNA and protein levels in keratinocytes.

3.eFT-508 inhibits M5-induced abnormal proliferation and inflammatory state of keratinocytes

To verify whether the function of p-eIF4E in the psoriasis model is consistent with that of eIF4E, we added the eIF4E phosphorylation inhibitor eFT-508 to the supernatant of HaCaT cells. We first confirmed the safe dose of eFT-508 in HaCaT cells and found that cell viability was significantly inhibited after 72 hours at concentrations greater than 8 μM. Secondly, we confirmed by WB that eIF4E phosphorylation was progressively inhibited as the concentration of eFT-508 increased. Therefore, in this study, we took 8 μM as the experimental dose of eFT-508. Next, we performed the CCK-8 and EdU experiments and found that eFT-508 inhibited the abnormal proliferation of keratinocytes in in vitro psoriasis model. Besides, the mRNA and protein levels of IL1β, CXCL10 and IL23 in keratinocytes were also reduced after the addition of eFT-508. These experiments all suggested that, as demonstrated in other studies, the effect of p-eIF4E was consistent with the effect of eIF4E and may play the role of effect-amplifier of eIF4E.

4.eIF4E interferes with the transcription of many cytokines in the IL17 pathway and with the AKT pathway by affecting the translating of NBS1

We set up si-NC+M5 (n=3) and si-eIF4E+M5 groups (n=3) in HaCaT cells, extracting total RNA after 48 hours, and sent the obtained samples for transcriptome sequencing. The results showed that the knockdown of eIF4E increased the transcription of 212 genes and repressed the transcription of 94 genes. The differentially expressed genes were mainly inflammatory factors that enriched in the cytokine interaction pathway and downstream of the IL17 pathway. At the protein translation level, the knockdown of eIF4E reduced expression of cyclin D1 in in vitro psoriasis models of HaCaT and NHEKs cells, which explained why si-eIF4E could block cells into G0/G1 phase. Besides, si-eIF4E affected the expression of IL1β, Wnt 5a, and NBS1. NBS1 is a known activator of AKT, and the decrease of eIF4E expression reduced the p-AKT protein level and interfered with the AKT pathway naturally. The same conclusion was obtained by adding eFT-508 in the HaCaT cells . These results further suggested that, at a mechanistic level, p-eIF4E's effect was consistent with that of eIF4E under the psoriasis condition.

5.eFT-508 ameliorated IMQ-induced psoriasis-like skin damage in mice

Mice were treated as described above to induce a psoriasis model and were divided into five groups randomly: control group (vaseline), IMQ group (IMQ+DMSO), low dose group (IMQ+eFT-508 10mM), medium dose group (IMQ+eFT-508 15mM) and high dose group (IMQ+eFT-508 20mM). It can be seen that the mice in the IMQ group had the most severe psoriasis with apparent erythema, scaling, and thickness and had the highest PASI score, compared to the mice with eFT-508 applied on the back having significantly reduced psoriasis status on the skin surface, the pictures of all mice could vividly show the result. H&E staining also showed that the epidermal thickness of the mice in the eFT-508 group was significantly reduced, and the PASI score also decreased compared to the IMQ group. Moreover, the splenic index of eFT-508-applied mice decreased with the increasing drug concentration. Besides, qPT-PCR showed reduced levels of IL1β, CXCL10, and IL23 mRNA in the skin tissues of the high dose group mice, and WB showed reduced protein levels

of p-eIF4E, cyclin D1, IL1β, Wnt 5a, VEGF-α, NBS1 and p-AKT in the skin tissues of eFT-508-applied mice. These results were consistent with those obtained from in vitro experiments and indicated that eFT-508 has promising therapeutic effects in psoriasis.

Conclusion

eIF4E and p-eIF4E are the causative genes of psoriasis, and eFT-508 may be a promising candidate for anti-psoriasis drugs.

OR-114

The role and mechanisms of PGLYRP1 on macrophage innate immune response against Cryptococcus neoformans through NF- κB signaling pathway

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目的 新生隐球菌是一种能够引起隐球菌性脑膜炎等严重致死性疾病的重要病原真菌。隐球菌性脑膜炎治疗棘手，病死率高。其临床结局取决于宿主的免疫状态，巨噬细胞作为天然免疫的第一道防线，与宿主的保护性免疫密切相关。暴露于新生隐球菌后巨噬细胞的状态在受到一些特定因素的刺激后，如细胞因子、细胞间接触物和代谢物等，可表现出高度可塑性。肽聚糖识别蛋白1(PGLYRP1)作为机体的一种多功能天然免疫蛋白，既对微生物有直接的抑制或杀伤作用；又在调控机体免疫反应中发挥着重要的角色，可在不同疾病中发挥着抗炎和促炎的效应。基于前期临床芯片数据的 mRNA 差异性表达谱，我们提出假设：具有免疫调节活性的蛋白 PGLYRP1，可能参与巨噬细胞抗新生隐球菌感染的免疫调节过程。

方法 1. 研究 PGLYRP1 蛋白在宿主新生隐球菌感染中的表达情况：前期对隐球菌性脑膜炎患者和健康人的外周血单个核细胞（PMBC）进行 mRNA 表达谱的芯片分析，随后通过实时定量荧光 PCR，Elisa 法对隐球菌性脑膜炎患者 PBMC 和血浆中的 PGLYRP1 mRNA 及蛋白水平进行验证；然后进一步构建新生隐球菌小鼠感染模型，应用 Elisa 法和免疫荧光技术检测 PGLYRP1 在新生隐球菌小鼠感染模型中的时空表达情况，以初步明确 PGLYRP1 是否参与了宿主新生隐球菌感染进程。

2. 研究 PGLYRP1 蛋白对新生隐球菌的直接作用：通过采取 PGLYRP1 蛋白与新生隐球菌直接共孵育实验，使用酶标仪测定新生隐球菌的增殖曲线，台盼蓝检测其活力，墨汁染色检测新生隐球菌荚膜大小以及单克隆形成实验检测菌落负荷，来判断 PGLYRP1 蛋白对新生隐球菌有无直接抑制或杀伤作用。

3. 研究 PGLYRP1 蛋白对体外新生隐球菌感染后巨噬细胞表型的影响：通过采取 PGLYRP1 蛋白、巨噬细胞以及新生隐球菌共孵育体系。采用单克隆形成实验检测巨噬细胞对新生隐球菌的胞内杀伤、吉姆萨染色检测巨噬细胞的吞噬功能、Elisa 法检测巨噬细胞上清细胞因子与趋化因子的释放、实时荧光定量 PCR 和 WB 检测胞内细胞因子 mRNA 和蛋白水平以及流式细胞术检测细胞上清活性氧的产生等，来明确 PGLYRP1 对巨噬细胞抗新生隐球菌感染时具体发挥的功能。

4. 研究 PGLYRP1 在体内对新生隐球菌病感染进程的影响：进一步构建 PGLYRP1-KO 小鼠。建立 PGLYRP1-KO 和 WT 新生隐球菌小鼠感染模型：观察小鼠的生存期、组织菌落负荷（肺、脑）、组织 HE 和 PAS 染色切片（肺、脑）以及采用 Elisa 法对组织上清细胞因子进行检测（肺、脑、脾），来探究 PGLYRP1 在体内对新生隐球菌病感染进程的影响。

5.研究 PGLYRP1 蛋白对新生隐球菌感染后巨噬细胞内潜在通路的影响：接着我们通过高通量测序方法，检测 PGLYRP1 对巨噬细胞在抗新生隐球菌感染中转录组水平的变化，同时对差异性基因进行 GO 和 KEGG 的富集分析，以鉴定显著变化的调控通路。

6. 研究 PGLYRP1 蛋白对新生隐球菌感染后巨噬细胞内关键通路的作用机制：最后我们采用实时荧光定量 PCR 验证了显著变化通路相关分子的 mRNA 水平；采用 WB 和免疫荧光检测通路关键分子的表达；并通过加入该通路抑制剂，检测巨噬细胞表型的变化，以明确 PGLYRP1 蛋白在抗新生隐球菌感染时巨噬细胞内关键通路的作用机制。

结果 1. 宿主新生隐球菌感染 PGLYRP1 表达结果显示：mRNA 表达谱芯片分析提示在隐球菌性脑膜炎患者的 PBMC 中 PGLYRP1 基因明显升高。实时定量荧光 PCR 和 Elisa 法验证发现患者 PBMC 和血浆中的 PGLYRP1 mRNA 和蛋白水平都明显升高。在小鼠感染模型中，相比于 PBS 对照组，新生隐球菌感染组小鼠 PGLYRP1 在不同组织内呈现不同时间点的增高。

2. 体外实验结果显示：PGLYRP1 蛋白抑制巨噬细胞内新生隐球菌的胞内复制；PGLYRP1 蛋白促进巨噬细胞在抗新生隐球菌感染时促炎细胞因子和趋化因子的产生；PGLYRP1 蛋白促进巨噬细胞在抗新生隐球菌感染时活性氧的产生。然而 PGLYRP1 蛋白对新生隐球菌并无直接的作用；PGLYRP1 对巨噬细胞在抗新生隐球菌感染时氮化物的产生、吞噬功能并无显著性的影响。

3. 体内动物实验结果显示：相较于 WT 小鼠，PGLYRP1-KO 小鼠感染新生隐球菌后生存期明显缩短；HE 染色和 PAS 染色显示更严重的肺部感染以及更多的炎症细胞浸润；肺和脑组织显示更重的菌落负荷；组织上清细胞因子谱检测结果显示 PGLYRP1-KO 小鼠更偏向于非保护性细胞免疫反应。

4. RNA-seq 测序以及 GO 和 KEGG 富集分析显示，加入 PGLYRP1 蛋白后，巨噬细胞高表达基因在 NF-κB 信号通路上显著富集。实时荧光定量 PCR 结果显示 NF-κB 通路关键基因如：MMP9、TNF、ICAM1 和 CD40 在 PGLYRP1 组明显升高。WB 结果显示 NF-κB 信号通路关键分子 P65 和磷酸化的 P65 在 PLGYRP1 组升高。免疫荧光结果显示在 PLGYRP1 组 P65 发生核转位。WB 结果显示 PGLYRP1-KO 小鼠肺组织中 NF-κB 表达与 WT 小鼠无差异。加入 NF-κB 抑制剂后，巨噬细胞抗新生隐球菌感染能力下降。

结论 PGLYRP1 在隐球菌性脑膜炎中显著高表达，高表达的 PGLYRP1 蛋白能促进巨噬细胞在抗新生隐球菌感染时促炎细胞因子、趋化因子和活性氧的产生；PGLYRP1 在小鼠体内感染进程中发挥着保护性的作用。在机制上，PGLYRP1 可能通过调控 NF-κB 信号通路增强巨噬细胞抗新生隐球菌的天然免疫反应。该研究揭示了 PGLYRP1 蛋白在巨噬细胞抗新生隐球菌过程中的作用机制，为隐球菌病的免疫治疗提供新的思路，具有潜在的临床转化价值。

OR-115

The prevalence of Mycoplasma genitalium infection among different anatomic distribution and macrolide and fluoroquinolone-associated mutations among men who have sex with men in Shenzhen, China

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Background The antimicrobial resistance of Mycoplasma genitalium (MG) has been rising alarmingly, but few studies have examined the macrolide and fluoroquinolone-associated mutations of MG among men who have sex with men (MSM) in China. Our study aimed to investigate the prevalence of MG infection at the pharynx, urethra and rectum, the prevalence of macrolide and fluoroquinolone resistance and potential risk factors for MG infection.

Methods 162 participants completed a questionnaire regarding demographic characteristics and sexual behaviours. MG infection was detected by nested polymerase chain reaction. Predictive markers associated with macrolides and fluoroquinolones resistance were detected using PCR primers targeting region V of the 23S rRNA gene, parC and gyrA sequence. Univariate logistic regression was used to evaluate risk factors associated with MG.

Results Based on the sexual behavior of the participants, 124 pharynx swabs, 132 urethral swabs and 89 rectal swabs were collected from 162 MSM. MG was detected in 13.0%(21/162) of

the participants. The prevalence of MG at the pharynx, urethra and rectum was 9.7%(12/124), 6.1% (8/132) and 7.9% (7/89), respectively. Of the 21 MG-positive participants, 1 participant was infected with all 3 sites and 4 participants were infected with 2 sites. 27 MG-positive specimens from 162 participants were collected and available to detect mutations associated with resistance to macrolides and fluoroquinolone. 33.3% (9/27), 11.1% (3/27) and 22.2% (6/27) of specimens produced sufficient amplicon for detecting resistance mutations in 23S rRNA, gyrA and parC genes, respectively. For which sequencing data were available, 33.3% (9/27), 3.7%(1/27) and 7.4%(2/27) specimens harboured mutations in 23S rRNA, parC and gyrA genes, respectively. 11.1%(3/27) specimens carried a strain with dual-class mutations. All of the MG-positive participants (21/21) received a 14-day doxycycline regimen. Due to the covid-19 pandemic, only 19.0%(4/21) of participants returned for the test of cure(TOC) 2 weeks after treatment and all 4 participants were cured. No significant risk factors(e.g. demographic or sexual behaviour) associated with MG infection were identified.
Conclusion The finding of the high prevalence of macrolide and fluoroquinolone-resistant MG in China suggested that strategies of practicing resistance-guided therapy and building a surveillant net are clinically essential. Doxycycline exhibits high efficacy in the treatment of resistant MG.

OR-116

Next-generation sequencing for diagnosis and prognosis in early-stage syphilis

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梅毒是一种慢性性传播疾病，由苍白密螺旋体 (T.苍白球).诊断通常依赖于血清抗体的检测，而不是T.苍白球，这限制了对预后的评估。宏基因组下一代测序(mNGS)允许对临床样本中的细菌、病毒、真菌和寄生虫进行基于序列的检测。我们目前的前瞻性研究招募了诊断为早期梅毒的患者，以评估NGS 技术的诊断和预后价值。目前的研究结果表明 mNGS 作为早期梅毒诊断和预后评估的补充工具的适用性。

OR-117

Polyphenol Nanoparticles Boosted Photoprotective Efficiency and Stability of Sanshool

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Objective Sanshool has demonstrated photoprotective efficiency in photodamaged skin, while it could be instable after ultraviolet radiation. Polyphenol based materials have excellent ultraviolet absorption capacity and antioxidant capacity. The Catechol structure and polymerization stacking mode in its structure endow it with rich physical and chemical properties, which can act as both structural and functional units, and also introduce their own functions to realize the preparation of multifunctional polyphenol-based materials. This study aimed to boost the photoprotective efficiency and stability of sanshool by restructuring with natural polyphenols.
Methods We characterized the structure of polyphenol-based sanshool functional materials through polymer physicochemical methods, including morphology, size, component ratio, stacking method, bonding method, responsiveness, etc. Furthermore, we explored the impact of the changes in the above structures on their final properties, including UV light absorption ability, antioxidant ability, and tissue repair ability. Then we studied the safety, stability, effectiveness, and related molecular mechanisms of materials through skin photodamage models.

Results Epigallocatechin gallate (EGCG), tea polyphenols, and oligo proanthocyanidin (OPC) were assembled with sanshool to form structurally stable functional materials (Figure 1). After ultraviolet radiation, the ultraviolet B (UVB) absorption ability of EGCG-Sanshool and Tea-Sanshool functional materials did not show a significant decrease, while OPC-Sanshool functional material slightly decreased. Among them, EGCG-Sanshool functional materials had the best ultraviolet absorption stability (Figure 2). The ABTS and DPPH free radical scavenging abilities of EGCG-Sanshool, Tea-Sanshool, and OPC-Sanshool functional materials were significantly improved before and after UVB exposure compared to sanshool. Among them, the EGCG-Sanshool functional materials had the best free radical scavenging abilities before and after UVB exposure (Figure 3). The animal experimental results showed that the EGCG-Sanshool functional material had the best improvement effect on photodamage in mice, significantly superior to sanshool, and had a sustained and stable effect (n=6) (Figure 4). The pathological HE staining results showed that the EGCG-Sanshool functional material significantly improved the tissue morphology of mice with photodamage, which was significantly superior to sanshool. The double skin thickness and light injury score of the mouse skin was in accordance with this (Figure 5). The production of reactive oxygen species (ROS) was significantly enhanced after UVB exposure, and was suppressed by EGCG-Sanshool, Tea-Sanshool and OPC-Sanshool functional materials (Figure 6a,b). The superoxide dismutase (SOD) content was significantly increased by EGCG-Sanshool (Figure 6c). The percentage of apoptosis was increased by UV radiation, and was suppressed by EGCG-Sanshool functional material (Figure 6d,e). The γH2AX in HaCaT and skin tissue was significantly enhanced after UV exposure, and EGCG-Sanshool and Tea-Sanshool functional material could decrease it (Figure 7).
Conclusion EGCG, Tea polyphenols and OPC could be assembled with sanshool to form structurally stable functional materials. These three functional materials have superior photoprotective efficiency than sanshool. EGCG-Sanshool functional materials showed the best free radical scavenging abilities, antioxidative abilities and photoprotective abilities before and after UVB exposure.

OR-118

Efficacy and safety of dupilumab in the treatment of 123 cases of atopic dermatitis

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Objective To evaluate the efficacy and safety of dupilumab in Chinese patients with atopic dermatitis (AD).
Methods An ambispective cohortcomparativestudy with 123 AD patients treated with dupilumab at the Department of Dermatology, the Second Xiangya Hospital of Central South University, from July 2020 to March 2022 were conducted to evaluate the efficacy and safety of dupilumab. Primary outcomes include mean scores of eczema area and severity index (EASI), patient-oriented eczema measurement (POEM), peak pruritus numerical rating scale (NRS) and dermatology quality of life index (DLQI) before and after 4-,8-,12- and 16- week treatment, and adverse reactions and events were recorded. Comparison of scores before and after treatment was performed using paired t test or repeated measures analysis of variance, Mann-Whitney U test was used for the comparison of efficiency among patients with different types of skin lesions or different IgE levels and multiple regression models based on robust standard errors was used to analyze the factors influencing the efficacy.
Results Among the 123 patients, 107 were enrolled into the efficacy analysis, and 85 (79.44%) completed at least 4 weeks of treatment, including 6(7.06%)achieving EASI75 and 23(27.6%) achieving EASI50, and the EASI, NRS, POEM, DLQI scores (10.41 ± 6.72、4.12 ± 1.74、8.60 ±

4.29、7.81 ± 4.38, respectively) significantly decreased compared with those before treatment (18.08 ± 10.69、7.21 ± 2.01、16.88 ± 5.74、12.95 ± 5.95, respectively; all P < 0.001) in the 85 patients. Among the 107 patients, 47 (43.93%) completed at least 16 weeks of treatment. Among the 47 patients, 23(82.14%) of 28 adult and 17 (89.47%) of 19 adolescents and children achieved 75% or greater improvement in EASI score; the EASI, NRS, POEM and DLQI scores before the treatment all significantly differed from those 4, 8, 12,16 weeks after treatment (all P < 0.001), and all the scores were significantly lower at weeks 4, 8, 12 and 16 than at the previous adjacent time points (all P < 0.001). At 4 weeks during the treatment, the EASI improvement rate was significantly lower in the AD patients with prurigo nodularis than in those without (U=151.00，P=0.006), while there was no significant difference in the EASI improvement rate between AD patients with and without xeroderma and those without (P > 0.05); at week 16 during the treatment, there was no significant difference in the EASI improvement rate between patients with prurigo nodularis or xeroderma and those without (both P > 0.05). Multiple regression models based on robust standard errors at week 16 showed that the improvement degree in the EASI score was not correlated with the type of skin lesion (β=3.2，P=0.075), but correlated with age (β=-0.22，P=0.030), whether patients were in adulthood (β=9.54，P=0.049), immediate family history (β=7.46，P=0.017); the improvement degree in the NRS score was correlated with the type of skin lesion (β=0.55，P=0.032), age (β=-0.04， P=0.033), weight (β=-0.05， P=0.020), whether patients were in adulthood (β=2.06， P=0.003), and whether patients received combined treatment with antihistamines (β=-1.91， P=0.001). Adverse reactions: among the 123 patients, 6 (4.88%) developed conjunctivitis, and 2 (1.63%) developed facial erythema. Adverse reactions: vitiligo-like changes occurred on the right forehead of 1 patient, and 3 patients discontinued the treatment with dupilumab due to Henoch-Schonlein purpura, distal axonal damage in peripheral nerves in both upper limbs, and epilepsy, respectively. The causal relationship between these adverse events and dupilumab was unclear.

Conclusions Dupilumab is effective in the treatment of AD with high overall safety, and can serve as a new treatment option for AD patients with an unsatisfactory response to traditional treatment.

OR-119

ZQT formula inhibits G-MDSCs glycolysis through down-regulating p21/HIF-1α/GLUT1 signal in psoriasis model mice

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Background Psoriasis is a chronic, immune-mediated inflammatory skin disease that affects the quality of life and mental health of approximately 150 million adults and children worldwide. The Ze-Qi-Tang (ZQT) formula is a classic compound recipe in China for lung disease, but its mechanism for psoriasis is not clear. This study aimed to investigate the therapeutic effect of the ZQT formula on psoriasis and explore the underlying molecular mechanism.

Methods PBMC from psoriasis patients and healthy donors were collected to detect the number of MDSCs by flow cytometry and the expression of p21, HIF1α, and GLUT1 in MDSCs by IF. Psoriasis was induced in mice by daily application of imiquimod, and the mice were randomly assigned to control, methotrexate, and ZQT formula groups on the day of model establishment. The severity of psoriasis symptoms was scored daily, and skin pathology was evaluated using HE staining. ELISA was used to measure the concentrations of IL17A and IL23 in mouse spleen and skin. Flow

cytometry was used to detect changes in immune cell populations, including CD4+CCR6+T cells, MDSC, et al. in the spleen and skin of mice. The immunosuppressive activity of MDSCs on T cells was assessed using CFSE. Gene and protein expression of MDSC immunosuppressive activity indicators Arg1 and iNOS were detected using RT-qPCR and WB. Anti-Gr1 was used to remove MDSCs from psoriasis mice, and flow cytometry was used to detect G-MDSCs and CD4+CCR6+T cells. G-MDSCs from control and ZQT groups were sorted, and mRNA sequencing was performed. Seahorse energy metabolism analysis was used to measure the glycolysis level of G-MDSCs after ZQT formula administration, and RT-qPCR was used to detect glycolysis-related gene expression in MDSCs. RT-qPCR and WB were used to detect p21 and GLUT1 expression in G-MDSCs, and immunofluorescence was used to detect p21, GLUT1, and HIF1α expression in G-MDSCs in mouse skin psoriasis lesions after ZQT formula intervention.

Results The number of MDSCs was significantly increased in psoriasis patients, with the increased expression of p21, HIF1α, and GLUT1 in MDSCs. ZQT formula significantly alleviated psoriasis-like skin lesions in mice, reducing erythema and scale scores, skin thickening, and the number of CD4+CCR6+T cells. Flow cytometry showed that the ZQT formula significantly inhibited the number of MDSCs in psoriasis mice, but had no significant effect on other immune cells. The immunosuppressive activity of G-MDSCs on T cells was enhanced after ZQT formula intervention, and gene and protein expression of Arg1 and iNOS, the indicators of MDSC immunosuppressive activity, increased. After anti-Gr1 treatment, the number of G-MDSCs in psoriasis mice decreased significantly, while CD4+CCR6+T cells were no longer suppressed. Differential genes of G-MDSCs after ZQT formula intervention were related to glycolysis, and the level of glycolysis of G-MDSCs in psoriasis mice was significantly reduced. GLUT1 and p21 expression in G-MDSCs decreased, and the expressions of GLUT1, p21, and HIF-1α in G-MDSCs were significantly reduced in mouse skin.

Conclusion In conclusion, the results of this study suggest that the ZQT formula has therapeutic effects on psoriasis through the inhibition of G-MDSCs and reduction of CD4+CCR6+ T cell infiltration, which are the main pathogenic immune cells of psoriasis. The mechanism of action may be attributed to the down-regulation of glycolysis in G-MDSCs mediated by the p21/GLUT1/LDHA signaling pathway.

Keywords: ZQT formula; psoriasis; G-MDSCs; glycolysis

OR-120

Targeting upregulation of the immunosuppressive activity of MDSCs with indirubin as a novel strategy to alleviate psoriasis

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Background Psoriasis is a chronic and incurable skin disorder that causes inflammation. There is an urgent clinical need for new treatments. We identified the natural compound indirubin as a potential potent agent for the treatment of psoriasis, but it's therapeutic effect and underlying mechanisms were not well understood.

Methods Peripheral blood and skin tissues from psoriasis patients and healthy individuals were collected. Bioinformatics analysis was performed to investigate SLC7A5 expression and associated signal pathways in psoriasis skin lesions. A mouse model of psoriasis was established. Indirubin was administered separately or in combination with MDSCs depletion or adoptively transferred MDSCs. JPH203, rapamycin, and siRNA were further used to investigate the potential mechanism by which indirubin regulates MDSCs.

Results Psoriasis patients had increased numbers of MDSCs in their blood and skin lesions, with high expression of LAT1. The upregulation of SLC7A5 expression and the arginine synthesis pathway was observed in psoriasis skin lesions. The number of MDSCs was increased, while their inhibitory effect on psoriatic T cells was decreased. Indirubin decreased LAT1 expression on the surface of MDSCs, inhibited mTOR pathway activation, upregulated Arg1 expression in MDSCs, and enhanced the immunosuppressive activity of MDSCs while inhibiting CD4+CCR6+ T cells.
Conclusion This study demonstrates indirubin's pharmacological and therapeutic effects, providing a basis for future clinical application in treating psoriasis.

OR-121

Differential molecular signatures of CD30-positive tranformed MF and cutaneous anaplastic large cell lymphoma and their identifying markers to improve diagnosis

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Objecive CD30-positive transformed mycosis fungoides (TMF) presents a challenge in terms of differential diagnosis with cutaneous anaplastic large cell lymphoma (cALCL), especially when co-existing with mycosis fungoides (MF). While several clinical indicators and molecular markers have been proposed for diagnosing cALCL and TMF, some clinical indicators may lose their discriminatory value when dealing with lesions occurring in the early stages of MF disease. Furthermore, the unconvinent practice precludes some molecular findings in a clinical setting. The rarity of cALCL and CD30-positive TMF has hindered our understanding of the pathogenesis and molecular features of both diseases, making it difficult to identify effective biomarkers for improving diagnosis.
The objective of this research is to compare the clinicopathological data and gene expression profiles of cALCL and CD30-positive TMF in order to achieve the following aims:
1) Identify distinct transcriptional programs between the two diseases.
2) Determine different hub regulators associated with distinct transcriptional programs.
3) Uncover molecular features that contribute to the observed clinicopathological differences.
4) Identify diagnostic biomarkers capable of differentiating CD30-positive TMF lesions from cALCL lesions.
Methods We assembled a cohort of 25 CD30-positive TMF samples (from 23 cases) and 25 cALCL samples (from 25 cases) that were meticulously annotated with clinicopathological information. The clinicopathological information was compared to identify the differential indicators. In our cohort, 16 CD30-positive TMF lesions and 15 cALCL lesions had bulk transcriptomic data, and 4 of them had single-cell sequencing data. Transcriptome data on isolated cutaneous anaplastic cells, normal T cells, and untransformed MF lesions were obtained from publicly available datasets. A comprehensive analysis of gene expression profiles of CD30-positive TMF and cALCL was performed, including differential gene expression analysis, weighted gene co-expression network analysis, functional enrichment analysis, regulon-target network analysis, T-cell phenotype identifying, and tumor microenvironment cell fraction analysis.
The differentially expressed genes (DEGs; log2 fold change ≥1 or ≤ -1 and adjusted p-value <0.05) that were significantly associated with the disease (Wilcoxon test, $p<0.01$) were carefully screened for candidate identifying biomarkers. To make it applicable for clinical diagnosis, we designed a cost-effective immunohistochemical staining method as an alternative to high-throughput sequencing. Commercially available antibodies for candidate molecules were selected based on their expected reactivity on formalin fixation and paraffin embedding (FFPE) tissues and clear staining of the lymphoid cells and minimal background. Three antibodies were chosen, including 2 with nuclear staining (BATF3, TCF7 transcription factors) and 1 with membranous staining (CD27).

The thresholds for positivity were determined based on the percentage of tumor cell staining that optimally divided the diseases with a minimal error rate, estimated by the area under the receiver operating characteristic (ROC) curve. To ensure convenience for clinical utility, we approximated the statistical thresholds.

Results Comparing the clinicopathologic data of the two groups, we observed distinct patterns associated with each diagnosis. TMF was characterized by a higher frequency of poor outcomes, body-disseminated lesions, progressive disease course, elevated LDH levels, pruritus, epidermotropism, and positive CD20 immunostaining. On the other hand, cALCL showed a higher occurrence of relapse, spontaneous regression, and neutrophil infiltration.

The percentage of Ki-67 positive tumor cells was similar in both diseases, indicating cell growth advantages supported by upregulated cell cycle and cell division-associated genes in both tumor cell types. Notably, the mechanism underlying cell enlargement differed between cALCL and CD30-positive TMF, as the signature for large cell transformation was significantly enriched in the CD30-positive TMF group. Comparative gene expression analysis identified 495 upregulated genes and 391 downregulated genes, enabling robust classification of CD30-positive TMF from cALCL samples through unsupervised hierarchical clustering of the top 200 differentially expressed genes (DEGs), highlighting characteristic transcriptional profiles in both entities.

CD30-positive TMF exhibited enrichment in TCR signaling pathways, Rho/Ras protein signal transduction, NFKB pathways, and interleukin-4 signaling, indicative of a Th2-skewing phenotype in MF tumor cells. Additionally, CD30-positive TMF demonstrated an enriched B cell activation signature, aligning with the higher presence of CD20-positive cells observed in the histological features of CD30-positive TMF lesions. cALCL demonstrated enhanced T cell cytotoxicity, staphylococcus aureus infection, and activated reactive oxygen species and ATP metabolic processes.

Notably, cutaneous anaplastic large cells in cALCL exhibited overexpression of HLA II type genes. In CD30-positive TMF lesions, an enrichment of TCF7+ exhausted T cell signatures was observed. Key transcription factors associated with distinct T cell phenotypes showed higher scores for exhausted T cells (TCF7), regulatory T cells (Tr1, iTreg) (IKZF2), and naive T cells (LEF1) in CD30-positive TMF, whereas the Th17 phenotype (BATF3, RORC) was significantly enriched in cALCL. C CD30-positive TMF exhibited an abundance of B cells, dendritic cells, and neurons, which correlated with the histological features and pruritus symptom.

To distinguish CD30-positive TMF lesion from cALCL, lesion we employed selected molecules (BATF3, TCF7, CD27) to generate an immunohistochemistry (IHC) algorithm. Notably, the selected antibodies exhibited significant positive correlations between the percentage of positive cells and the corresponding mRNA expression levels. Leveraging these identified markers, we re-diagnosed samples in our cohort and identified two CD30-positive TMF samples showing prominently positive BATF3 immunostaining and negative TCF7 and CD27 immunostaining. Upon reviewing the clinical histology, we reconsidered the diagnosis of these two patients as cALCL arising in the context of MF.

Conclusion In summary, we revealed the unique gene expression programs associated with CD30-positive TMF and cALCL, offering valuable insights into their underlying molecular mechanisms. Notably, we observed contrasting neoplastic T cell phenotypes driven by specific transcription factors, as well as correlations between tumor microenvironment (TME) cell components and clinicopathological data for both entities. Leveraging these distinct gene expression programs, we have identified specific identifying markers as potential diagnostic tool to enhance the effectiveness of discriminating CD30-positive TMF from cALCL. These findings contribute to a deeper understanding of the molecular landscape of CD30-positive TMF and cALCL and also have profound clinical implications, enabling more accurate diagnoses and tailored treatment strategies for improved patient outcomes.

OR-122

Individual and Disease-specific Genome, Transcriptome, and Metabolome Signatures Shape the Functional Diversification of Cutibacterium acnes in Atopic Dermatitis and Healthy Skin

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Individual and Disease-specific Genome, Transcriptome, and Metabolome Signatures Shape the Functional Diversification of Cutibacterium acnes in Atopic Dermatitis and Healthy Skin

Purpose

Atopic dermatitis (AD) is a chronic inflammatory skin disease, closely related to the disorder of skin microbiota. Cutibacterium acnes (C. acnes) is the most abundant commensal bacteria on healthy skin. While extensively studied for its pathogenic role in acne vulgaris, growing evidence suggests that C. acnes and its sebum metabolites play important roles in maintaining healthy skin homeostasis by regulating skin pH, resisting colonization by Staphylococcus aureus, and modulating immune responses.

AD patients have reduced sebaceous gland secretion capacity, the abundance of C. acnes is reduced in both lesions and non-lesions skin in AD patients. With children pubertal development, C. acnes abundance significantly increases in acne patients, accompanied by a distinct acne microbiome. Sebum content may affect the content and composition of C. acnes metabolites, and we propose that appropriate sebum concentrations may contribute to maintain the beneficial functions of C. acnes.

C. acnes has phylotype diversity in healthy skin, the diversity of C. acnes phylotype decreased in acne lesions. The intraspecific heterogeneity of C. acnes may be an important reason for maintaining its beneficial function. However, the intraspecific diversity in different skin sites of healthy skin has not been studied in detail, and the characteristics of C. acnes phylotypes in AD patients have not been reported.

Therefore, the purpose of this study is to reveal the component and functional signatures of C. acnes intraspecific heterogeneity in strain level in different skin sites of AD patients and healthy individuals, using combined analysis of whole-genome sequencing (WGS), transcriptome, and metabolome, and dig out specific C. acnes metabolites that can reduce AD inflammation.

Methods

12-40 years old healthy people, AD patients, and acne patients were recruited in this research. A 2^2 cm^2 skin area was scraped with swab. Each swab was then inoculated in Columbia Blood Agar plates in anaerobic environment to enrich C. acnes. Colonies with colony morphology similar to C. acnes were randomly selected on the plates, and were purified to single colony. Purified C. acnes was inoculated into Brucella anaerobic broth, cultured until logarithmic growth phase, then extracted total DNA. Sequencing libraries were made according to the Illumina standardized protocol. Sequencing adapters and low-quality reads were removed from the sequencing reads using Trimmomatic. Cleaned reads were then assembled using SPAdes software.

Gene coding sequences of the isolate genomes were predicted using Prokka. The pan-genome and core-genome analysis was then carried out using the Roary pipeline. The core-genome alignment generated form Roary was used to construct a SNP-based approximately-maximum-likelihood phylogeny tree by RAxML. Single-locus sequence typing (SLST) and multilocus sequence typing (MLST) of all strains was specified based on the C. acnes SLST and PubMLST database. C. acnes horizontal genes were analyzed using HGTector. Positive selection estimated by calculating the ratio of the nonsynonymous substitution rate to the synonymous substitution rate (dN/dS).

One C. acnes strain from each individual and each skin site was selected for matched WGS, transcriptome, and metabolome analysis, identified by MALDI-TOF MS. Total RNA was extracted from C. acnes cultures in the logarithmic growth phase for RNA-seq, and the culture supernatant was subjected to non-targeted LC-MS metabolome analysis. The RNA-seq reads were aligned to the reference genome C. acnes ATCC6919 using Bwa. LC-MS data were processed using Progenesis QI software.
Primary human keratinocytes were cultured and treated with C. acnes culture supernatant. Cell viability was assessed using the CCK-8 assay. The MC903-induced AD-like mice model was treated with 10mM L-carnosine daily for 9 days. The expression of inflammatory factors was measured by qRT-PCR.

Results

To compare the C. acnes genetic heterogeneity within and between individuals, we designed 4 representative sampling sites (face, forearm (AM), antecubital fossa (AF), and foot) for 11 healthy people. Face, AM, and AF surrounding the lesions from 10 moderate-to-severe AD patients were sampled. Surrounding face lesions and non-lesion of AM and AF from 11 moderate acne patients were sampled. We randomly selected, isolated and purified 5–20 colonies per skin sites with colony morphology consistent with C. acnes for WGS. All together, we obtained 1234 high-quality C. acnes genomes that passed purity and coverage filters.
We classified C. acnes colonies according to SLST and MLST scheme. We found C. acnes exhibits multiscale genetic heterogeneity and plentiful individual specificity. Specifically, the genome heterogeneity of strains in NC group was significantly higher than that in AD and acne, inter-site strain genetic diversity was significantly higher than intra-site, and healthy individuals had relatively higher skin site specific phylogenetic structuring. The multi-scale genetic diversity of C. acnes indicates that each individual has a unique phylotypes distribution pattern. The subtype diversity decreased in acne group, with the dominant SLST F4 group belonging to the MLST subtype IA1. Consistent with previously C. acnes WGS research, there was no intra-individual site-specific aggregation of SLST subtypes in our data.
By analyzing the pan-genome of C. acnes, we discovered that the accessory genome showed considerable subject-specific variations, indicating individual specificity. KEGG pathways enrichment analysis of accessory genes revealed glycolipid and amino acid metabolism pathways were enriched in the acne group. Interestingly, we observed the enrichment of quorum sensing (QS) related genes in both acne and NC group, including the LuxS gene involved in bacterial intercellular communication.
We measured the genetic diversity of individual strains using average SNP distance, divided into the top 50% and bottom 50%, and found that individuals with low genetic diversity in the AD and acne groups experienced significantly more HGT events in accessory genes. As for the inter-site variation, the NC group exhibited a lower number of HGT events in AM and AF, while it in AD group was significantly higher in face. The glucose metabolism and amino acid metabolism KEGG pathway were enriched in the acne group, indicated that HGT in the accessory genome plays a significant role in shaping the functional gene differences and individual specificity of C. acnes.
We examined positive selection in both the core and accessory genes of C. acnes, found most of them belonged to purification selection in the core genome. Notably, the acne group showed higher selection pressure in accessory genes associated with glycolipid and amino acid metabolism pathways. The accessory genome shared by different skin sites exhibited higher positive selection pressure on the face in the AD and acne group, suggesting the presence of a unique ecological niche.
Transcriptome analysis of C. acnes revealed distinct gene expression profiles among the three groups. In the AD group, the COG function classification involved in fatty acid synthesis, triglyceride hydrolysis, and fatty acid β-oxidation were found to be down-regulated, indicating a reduced ability to utilize lipids as carbon sources. The acne group showed down-regulation of anti-oxidative stress related genes, suggesting a reduced ability to withstand the high oxidative stress environment on the host skin. Non-targeted metabolome analysis showed increased levels of triglyceride metabolites in AD-derived C. acnes strains, suggesting a lower ability to utilize long-chain fatty acids as carbon sources.

To investigate whether the sebum concentration affects the gene expression of C. acnes, we treated keratinocyte with medium-to-high sebum concentration (0.1%-0.25%) C. acnes culture supernatant for 4 hours, found the relative cell viability significantly reduced. After adding C. acnes supernatant to keratinocyte for 4 hours, only the acne group with high sebum concentration (0.25%) significantly up-regulated the expression levels of IL-1β, IL-6, IL-8, and TNF-α, while the NC and AD group had no obvious pro-inflammatory effect at various sebum concentrations. Additionally, the culture supernatant of C. acnes significantly reduced the expression of TSLP in keratinocytes, regardless of the strain source and sebum concentration.

L-carnosine, an antioxidant and anti-inflammatory metabolite, exhibited the highest content in AD group and the lowest in acne group in C. acnes metabolome. MC903-induced AD-like mice treated with 10 mM L-carnosine, resulted in milder symptoms, H&E staining of ear sections further confirmed a decrease in epidermal thickness and inflammatory cell infiltration in the ears of AD mice treated with L-carnosine. RT-qPCR analysis showed significantly lower expression levels of inflammatory cytokines, including IL-4, IL-13, IL-6, IL-8, IL-33, and TSLP, in the L-carnosine-treated AD mice compared to the AD model control.

Conclusion

In conclusion, this study investigated the genetic, transcriptomic, and metabolic heterogeneity of C. acnes in healthy individuals, AD, and acne, insights into the role of C. acnes heterogeneity and its interactions with the host in maintaining skin health. Our findings revealed that C. acnes exhibits genetic diversity at multiple levels and demonstrates individual specificity, particularly in healthy skin. However, C. acnes strains derived from acne patients exhibited enhanced energy metabolism and fatty acid synthesis abilities. The culture supernatant of these strains induced toxicity and up-regulated inflammatory factors in keratinocytes, possibly due to increase of sebum levels. Interestingly, L-carnosine, a metabolite produced by C. acnes, was highly expressed in AD-derived strains supernatant and demonstrated anti-inflammatory effects in an MC903-induced AD-like mice model. Manipulating C. acnes and its metabolites may maintain skin microbiota functions and hold promise as a potential treatment strategy for AD and acne.

OR-123

Decreased HMGCS1 Inhibits Infammatory Response in Keratinocytes and Ameliorates Imiquimod-induced Psoriasis in Mice by the STAT3/IL-23 Axis

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Introduction

Psoriasis is an autoinflammatory disease characterized by the excessive proliferation of keratinocytes, requiring a large amount of cholesterol to form cell membranes. Patients with psoriasis are prone to coronary heart disease and metabolic syndrome. An essential mechanism of this systemic inflammatory is lipid metabolic disorders. However, the specific mechanism has not been elucidated.

3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) is a catalytic enzyme for the first step of cholesterol synthesis and the mevalonate pathway. The mevalonate pathway also has been found to modulate the immune response of various cancers and cardiovascular disease. Recent study has found HMGCS increased by 1.5-fold compared with control in epidermis In a mouse model of acute barrier disruption. Moreover, interaction of HMGCS1 with Gal-7 facilitated cholesterol deposition in keratinocytes.

Objectives

We aim to explore the role of lipid metabolism-related molecules, HMGCS1 in psoriasis. We aim to advance the understanding of the lipid metabolic-inflammatory interaction in psoriatic keratinocytes and provide a possible target for the treatment of psoriasis.

Materials and Method

We analyzed two Gene Expression Omnibus (GEO) data sets to Compare the HMGCS1 mRNA expression level between healthy persons and psoriatic patients, and between psoriatic patients before and 12 weeks after treatment with TNF-α antagonist or JAK inhibitor. We assessed HMGCS1 protein expression level in tissue samples from psoriatic patients and imiquimod-induced psoriatic mice with immunohistochemical (IHC) staining. We assessed the expresion level of immunoinflammatory factors associated with psoriasis in TNF-α stimulated psoriatic cell model using qRT-PCR. We performed Gene ontology (GO) enrichment analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis after knockdown of HMGCS1by siRNA transfection. Furthermore, we assessed the migration and proliferation of HMGCS1-knockdown HaCat cells using scratching assays and cell cycle analysis. We assessed the expression and activation of STAT3/IL-23 Axis by WB. In imiquimod (IMQ)-induced psoriatic mice, we used intradermal injection of HMGCS1 siRNA to locally lower the expression of HMGCS1. We compared the phenotypic changes between control group, IMQ group, IMQ+NC-siRNA group and IMQ+HMGCS1-siRNA group. We evaluated the Psoriasis area and severity index (PASI) scores, including cumulative, erythema, thickness and scaling scores. We measured the epidermal thickness and evaluated the Baker scores based on histopathological level. We further assessed the expression level of immune inflammatory factors including IL-23 in four mouse groups using IHC and qRT-PCR.

Results

Microarray data analysis revealed that the expression of HMGCS1 in psoriatic lesions was higher than that in normal skin tissues, and the expression level of HMGCS1 decreased after treatment with TNF-α antagonist or JAK inhibitor. Gene Set Enrichment Analysis (GSEA) analysis showed that higher expression of HMGCS1 positively correlated with TNF-α_NFκB signal pathway, inflammatory pathway, IL6_JAK_STAT3 pathway, oxidative phosphorylation pathway activation and G2M cell cycle transition. Further immunohistochemical staining confirmed HMGCS1 was aberrantly upregulated in the skin lesions of both psoriatic patients and imiquimod-induced psoriatic mice. TNF-α significantly increased the expression level of HMGCS1, as well as proinflammatory cytokines including IL-1β, IL-8, IL-23 and innate immune mediator S100A8 in HaCat cells. Gene ontology (GO) enrichment analyses of differentially expressed genes showed protein kinase regulator activity as one of the significantly enriched biological processes in HMGCS1 knockdown HaCat cells. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that the differential expressed genes of keratinocytes transfected with HMGCS1-siRNA significantly enriched in 4 signal pathways. 3 of these pathways were closely related to glucose and lipid metabolism, which were involved in regulating cell proliferation, differentiation, apoptosis and other biological processes. Decreased HMGCS1 downregulated migration and proliferation of HaCat cells. HMGCS1 knockdown remarkably triggered the delay of scratch gap closure and led to growth arrest in the G1/S transition. Decreased HMGCS1 also reduced the expression of IL-23 and the phosphorylation level of STAT3. In IMQ-induced psoriatic mice, we found that PASI score, epidermal thickening, pathological Baker score improved in the HMGCS1-knockdown group. And Expression level of inflammatory cytokines IL-23, IL1-β, chemokine CXCL1, innate immune mediator S100A7-9 were downregulated in the epidermis.

Conclusion

Decreased HMGCS1 can improve the TNF-α stimulated psoriatic keratinocytes model and imiquimot-induced psoriatic mice model. HMGCS1 may affect psoriasis through the STAT3/IL-23 axis, which provides a possible target for the treatment of psoriasis..

OR-124

Effects of exogenous electric field on migration and immunomodulatory functions of skin dendritic cells in mice

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Objective To explore the galvanotaxis effects of exogenous electric field (EF) on mouse skin dendritic cells and whether it can regulate immune responses.
Methods Through inducing cultivation Bone Marrow-Derived Dendritic Cells (BMDCs) of wild-type and P110 γ (flox/flox, CD11c-Cre) mouse, with EF treatment, the movement trajectory of BMDC cells was observed and plotted in real-time, and the directionality, trajectory velocity, and displacement velocity of cell movement were analyzed. WB was used to detect the changes of cell protein under the treatment of electric field, and Phalloidin staining showed the changes of cytoskeleton protein. Analysis of BMDC cell galvanotaxis trajectories and changes in skeletal proteins with PI3K/AKT signaling pathway inhibitor Wortmannin added and knockout of P110γ gene after EF treatment. Transwell assay was used to detect the chemical chemotactic effect of electric field on BMDC cells. We utilized the injection of BMDC with corresponding treatment in mouse paw pads to detect the effect of EF on competitive migration of BMDC in vivo. Flow cytometry was performed to detect the antigen phagocytosis ability (FITC-Extran) and maturation activation ability of BMDC cells under EF treatment. DC-T cell co-culture assay was conducted to detect the proliferation effect of EF-induced BMDC cells on CD4+and CD8+T cells, and BMDC proteomics analysis was performed. Animal experiments were carried out to detect EF effect of contact hypersensitivity (CHS) intensity on wild-type mice or P110γ gene knockout mice or mice with switching the direction of EF. Flow cytometry was used to detect the changes in antigen phagocytic capacity of BMDC 3 days after the EF was applied. Under the addition of CCL21 by WB, the effect of P110γ gene knockout on signal transduction pathway of G-protein-coupled receptor was detected.
Results The exogenous EF could promote the directional migration of BMDC cells and activate the downstream signal pathway of CCR7 with participation in regulating the formation of stress fiber polymerization of cytoskeleton proteins and synergistically promote the chemotaxis of BMDC and promote the migration of BMDC in vivo and in vitro. The immediate effect of EF treatment has no effect on the antigen phagocytosis ability of BMDC cells and did not affect cell maturity. Co-culture of DC-T cells could inhibit the proliferation of CD4+T cells and promote the proliferation of CD8+T cells. Proteomic analysis showed that inter group differential protein enrichment analysis was mainly focused on Lysosome, Phagosome, Regulation of actin cytoskeleton, and other aspects. EF stimulation 3 days before sensitization could enhance the intensity of CHS in mice. After 3 days of EF treatment, the antigen phagocytic ability of BMDC cells increased. P110γ gene knockout affected the activation of P38 and Cytoskeleton protein signaling pathways.
Conclusion The exogenous EF treatment could promote the directional migration of BMDC cells and affect the intensity of CHS in mice.

OR-125

Analysis of clinical characteristics in classic Kaposi's sarcoma complicated with metabolic syndrome and establishment of interferon therapy efficacy evaluation model

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(XJYS1707)

Objective To analyse the clinical features of Kaposi's sarcoma complicated with metabolic syndrome and establish a model for evaluating the efficacy of interferon therapy with classical Kaposi's sarcoma based on blood lipid and blood cell count, and assess the effectiveness of this model.
Methods One hundred sixty-seven patients treated with classic Kaposi's sarcoma in Xinjiang Uygur Autonomous Region People's Hospital were enrolled as research subjects from January 2012 to December 2021. According to whether they achieved pathological complete response (CR) or partial response (PR) within interferon treatment, they were divided into two groups: response group (n=151) and disease group (n=16). Logistic regression was used to analyse the risk factors of the efficacy of interferon therapy with classical Kaposi's sarcoma, and a nomogram regression model of the above risk factors was constructed. ROC curve, calibration curve, and decision curve analysis are used to evaluate the value of predictive models.
Results The expression of lipoprotein A in the response group was lower than that in the disease group [(188.98±175.53) mg/L vs (361.04±253.84) mg/L, P<0.01]. The expression of HDL cholesterol in the response group was higher than that in the disease group [(1.0272±0.31496) mmol/L vs (0.87562±0.13342) mmol/L, P <0.05), the remaining factors did not differ significantly between groups. Logistic regression analysis showed that high lipoprotein A was an independent risk factor for the efficacy of interferon in classic Kaposi's sarcoma (P<0.01). When the risk threshold probability was 1%~8%, 10%~37%, and 48%~49%, the net benefit of the model evaluating the efficacy of interferon therapy with classic Kaposi's sarcoma was positive.
Conclusion The efficacy evaluation model of classical Kaposi's sarcoma based on blood lipid and blood cell count has specific differentiation, calibration degree, and clinical application value and can assist clinical decision-making within a certain risk threshold.

OR-126

HOTAIRM1-mediated Regulation of miR-107 and Fatty Acid Synthase Signaling in Cutaneous T-cell Lymphoma

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Objective To investigate the comprehensive biological roles and clinical significance of the long noncoding RNA (lncRNA), HOX antisense intergenic RNA myeloid 1 (HOTAIRM1), in cutaneous T-cell lymphoma (CTCL).
Methods Quantitative reverse transcription PCR (qRT-PCR) was employed to analyze the expression of HOTAIRM1 in CTCL tissues. In addition, a comprehensive set of experimental techniques including Cell Counting Kit-8, plate colony-formation assay, flow cytometric apoptosis analysis, transwell assay, bioinformatics analysis, and dual-luciferase reporter gene experiments were conducted.
Results We elucidated the downregulation of HOTAIRM1 in CTCL tissues, correlating with prolonged treatment duration and poorer drug response.The overexpression of HOTAIRM1 in Hut-

78 and HH cell lines significantly inhibited cell proliferation and invasion, while inducing apoptosis in vitro. Through bioinformatics analysis, we identified miR-107 as a target of HOTAIRM1. Dual-luciferase reporter gene and RNA immunoprecipitation assays confirmed the competitive endogenous RNA role of HOTAIRM1 in relation to miR-107. Furthermore, enhanced expression of miR-107 exhibited the potential to reverse the effects of HOTAIRM1 overexpression in vitro. Inhibition of miR-107 suppressed CTCL cell proliferation and invasion, while inducing apoptosis in vitro. In mouse xenograft models, overexpression of HOTAIRM1 and inhibition of miR-107 impaired tumorigenesis. Further, we found that miR-107 post-transcriptionally regulates fatty acid synthase (FASN) by binding to its 3′ UTR and reduces its protein levels and the 3′UTR luciferase reporter activity, which are blunted by the miR-107 inhibitor and mutation in the miR-107 binding site in the 3′ UTR. Knock-down of endogenous miR-107 levels increased FASN levels in a dose-dependent manner. Overexpression of miR-107 led to significant accumulation of malonyl CoA, accompanied by enhanced cell proliferation. All these events were prevented in the presence of the miR-107 inhibitor. While overexpression of FASN could attenuate miR-107 mediated cell proliferation. This was followed by increased triglyceride formation and lipid accumulation in the presence of miR-107. These indicate that miR-107 inhibits FASN levels by binding to its 3′ UTR and this interaction promotes malonyl CoA and lipid accumulation, and cell proliferation in CTCL.
Conclusion In conclusion, our study validates HOTAIRM1 as a novel tumor-suppressive lncRNA in CTCL and proposes the HOTAIRM1/miR-107/FASN axis as a potential therapeutic target for this disease. These findings highlight the important role of miR-107 in promoting lipid accumulation and cell proliferation in CTCL, offering insights into the diverse etiologies observed in this disease.

OR-127

A unique and transitional progenitor cell type between nerve cells and skin cells

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Skin is a somatosensory organ, Merkel cells (MC) mediate the perception of light touch in the skin. Together with touch dome keratinocytes, MCs composed the distinguished sensory epithelium which is maintained by its specific tissue stem cell. Identifying the progenitor of MC and its underlying mechanism in early development is critical for the regeneration of touch sensation. We proposed its development, as well as regeneration, goes through three cellular stages. Single cell analysis reveals that there exists a two-stage development model, confirmed by immunofluorescence staining for stage-specific markers. In vitro culture system for MC is established for the first time and the underlying regulatory mechanism is investigated. Taking together, here we illustrate the development profile of Merkel cell.

OR-128

Evaluation of chromatin accessibility in memory T helper and T regulatory cells of patients with psoriasis

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Objective Genomic studies on psoriasis, which have rapidly progressed in the last decade, have revealed more than 90 risk loci. However, the identified regions are broad and usually do not indicate specific genes. Thus, the molecular mechanisms underlying the effect of these variants must be elucidated. However, the elucidation of these molecular mechanisms is limited by various factors, including unknown cell types, tissue types, unknown causal variants due to linkage disequilibrium (LD), and the regulatory function of unknown non-coding risk variants. According to a commonly assumed model to explain the molecular mechanisms of risk loci, non-coding risk alleles disrupt transcription factor (TF) binding within cell-type-specific regulatory elements (REs), Previously, we had reported that memory Th (mTh) and memory Treg (mTreg) cells undergo remodeling in patients with psoriasis. Thus, this study focused on the open chromatin regions in mTh and mTreg cells. The effects of these genetic variations were used to understand the impact of disrupting TF binding motifs on chromatin accessibility and gene expression and to elucidate the cell-type-specific regulatory mechanisms underlying genetic risk for psoriasis.

Method This study examined the assay for transposase-accessible chromatin with sequencing (ATAC-seq) and RNA sequencing(RNA-seq) profiles of mTh and mTreg cells in psoriasis in up to 178 samples.

Result We profiled the chromatin accessibility landscape for 89 psoriasis cases and 89 normal controls, yielded 356 genome-wide chromatin accessibility profiles. All ATAC-seq data included in this study passed a minimum threshold of enrichment of signal over background with most samples showing a characteristic fragment size distribution with clear nucleosomal periodicity. We normalized accessible peaks for GC content, peak length and sequencing depth. In total, 65,593 and 78,760 final peaks were identified in mTh and mTreg cells, respectively. Additionally, 31.68% and 28.63% of open chromatin regions were mapped near the promoter in mTh and mTreg cells, respectively, while 22% and 22.1% were mapped in the distal intergenic regions, respectively. This distribution was similar to that reported in previous studies using DNase sequencing.

Linkage disequilibrium score regression (LDSC) was performed to evaluate whether the SNP heritability of psoriasis is enriched in open chromatin regions or other pre-defined genomic features . In mTh cells, 2.87% of SNPs (n = 13,248) located in ATAC-seq peaks explained 12.52% of the SNP heritability of psoriasis (4.35-fold enrichment, P value = 0.44). Meanwhile, in mTreg cells, 3.46% of SNPs (n = 36,402) located in ATAC-seq peaks explained 44.1% of the SNP heritability of psoriasis (12.74-fold enrichment, P value = 0.77). The SNP heritability enrichment had the tendency of

decrease as the peak width increased (100 bp, 300 bp, 1 kb, 2 kb, 5 kb, and 10 kb) . To predict the association of ATAC-seq peaks with the genes that they are regulate, we implemented a strategy based on the correlation of ATAC-seq accessibility and gene expression across all samples (N=151 with matched RNA-seq and ATAC-seq). Using a false discovery rate (FDR) cutoff of 0.05, we identified 1173 unique links between distal ATAC-seq peaks and genes in mTh cells and 757 unique links in mTreg cells. To identify genetic variants that influence chromatin accessibility within cell types, chromatin accessibility quantitative treat loci (caQTL) analyses were performed separately for Th and Treg cells using 65,593 and 78,760 peaks, and 6,596,894 genetic variants. The population stratification was strictly controlled in association tests using a mixed linear model including a kinship matrix as a random effect and 10 genotype multidimensional scaling (MDS) components as fixed effects10,11. Additionally, principal components (PCs) were included across chromatin accessibility profiles of Th and Treg cell as fixed effect co-variants to reduce the impact of unmeasured technical variation. After the stringent multiple testing correction, significant caQTLs were identified for 8320 peaks (caPeaks) corresponding to 7744 unique lead caQTL variants in mTh cells and 5850 caPeaks corresponding to 5572 caQTL variants in memory Treg cells. To predict the regulatory function of caPeaks, the caPeaks of Th and Treg cells were compared with non-caPeaks. The caPeaks in Th and Treg cells were frequently located in enhancers (41.35% vs. 36.73% in Th; 26.53% vs. 22.4% in Treg) and promoters (30.39% vs. 28.5% in Th; 30.3% vs. 25.37% in Treg). These results suggest that most genetic variants affect chromatin accessibility by altering the sequence (and presumably TF binding sites) at the caPeaks, then have functional effect on diseases. Next, the peaks or genes influenced by genetic variations were compared. The proportions of peaks in memory Th and Treg cells were 12.68% and 7.43% respectively, whereas those of genes were 5.82% and 6.67%, respectively. Which indicated caQTL could explain more variance than expression quantitative trait loci (eQTLs).

This study identified caQTL for which the lead variant exhibited high LD ($r2 > 0.8$) with an eQTL lead variant for 18191 and 19552 autosomal genes of memory Th and memory Treg cells, respectively, based on RNA-seq. In memory Th cells, 381 caSNPs were in strong LD with at least 1 eQTL lead variant and 92 caSNPs were in strong LD with more than one eQTL lead variant. Meanwhile, in memory Treg cells, 426 caSNPs were in strong LD with at least 1 eQTL lead variant and 92 caSNPs were in strong LD with more than one eQTL lead variant. This study identified 393 and 450 caPeak-gene pairs in Th and Treg cells, respectively. Within these RE-Gene pairs, we found many genes differentially expressed in memory Th and Treg cells involved in pathogenesis of psoriasis such as Smad, GSTM, CTS, S100. totally, 43 DE caQTL and eQTL colocolization were found.

Conclusion This study provides a resource to understand the impact of genetic variation on gene regulation in human Th and Treg cells, as well as an additional layer of information to explain the function of common variants associated with the risk of psoriatic traits. While caQTLs can pinpoint functional regulatory variants, the modest sample size and analyses restricted to common variants limit fine-mapping potential and highlight the importance of considering LD proxies. Future studies focusing on more sample size and other cell-type specific caQTLs and eQTLs will give us more clues on understanding the pathogenesis of psoriasis.

Come see my poster

PO-001

Development and Evaluation of a New Nomogram to Accurately Predict the Risk of Severity in Patients with Psoriasis: a Large Multicenter Study

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Objective The aim of this study was to develop a risk prediction model for severe psoriasis in patients with psoriasis based on common clinical features
Methods Characteristics of patients with psoriasis were collected, and AIC was applied to initially screen the characteristics, and univariate and multivariate COX regression analyses were used to further select characteristics and develop a predictive model for severe psoriasis. The predictive model was calibrated, evaluated and clinical utility power was assessed by internal validation using bootstrap validation, using calibration plots, C-index, area under the curve (AUC) and decision curve analysis, and the model was externally validated using two independent cohorts.
Results The investigators constructed a novel prediction model based on indicators commonly found in actual clinical practice through univariate and multifactorial COX regression. The new model, named Psoriasis-22, consists of 22 statistically significant parameters that include Age at visit (HR: P<0.001), Sex (P<0.001), BSA (P<0.001), Delphi (P<0.001), DLQI (P<0.001), BMI (P = 0.1520), Height(P<0.001), Weight (P<0.001), Family history of psoriasis (Yes P<0.001), Level of education (Master degree or above P =0.0208), Career (Students P<0.001), Smoking history (Regular Smoking P<0.001), Drinking history (Occasional Drinking P =0.046), Employment status, Working status (Full-time, P =0.002, Students P<0.001), Marital status (Widowed P =0.031, Divorce P =0.553), Combined diseases (Yes P = 0.021), Self perceived symptoms (Pain P= 0.033), Seasonal relation (Light summer and heavy winter P =0.191), Fingernail and toenail involvement (P>0.05), Degree of fingernail and toenail involvement (P>0.05), Scalp involvement (Yes P<0.001), Facial involvement (Yes P<0.001), Palmoplantar involvement (Yes P<0.001), Involvement of joint areas(Yes P = 0.002), and External genital skin involvement (Yes P= 0.384). After COX multivariate regression results, indicators such as BMI, Height, History of alcohol consumption, History of smoking, Combined diseases, Seasonal relationships, Segree of nail involvement, Palmoplantar involvement, ang External genital skin involvement were excluded at P>0.05. The final prediction model incorporated 13 common indicators, called “Nomogram-13”. The predictive equation built for the nomogram was ln{h(t)/ h0(t)}= Surv(Course of disease, PASI) ~-0.027233*Age+0.348152*Sex[Male]+0.013300*BSA+0.007678*Weight-0.422263*Family.history+-0.549284*Education[Master degree or above]+0.855417*Working status+Nail involvement site[-0.130470*Toenail or Fingernail+-0.248031*Toenail and Fingernail]+0.380347*Scalp involvement+0.156960*Facial involvement+-0.275930*Involvement of joint areas. The cox prediction model was applied to predict the risk of severe psoriasis based on the current clinical characteristics. Each predictive feature in the nomogram was assigned segments of different lengths, with the length of the line segment representing the importance of that disease feature. If the clinical features listed in the model are present during the course of a visit to the physician with psoriasis, the higher the total score obtained, the higher the risk of the patient evolving into severe psoriasis.
The decision curves suggest that the nomogram is effective in guiding clinical practice. We also built a very convenient QR code version and web version for use in real-world clinical work. We evaluated the discriminative power of the constructed prediction model for different patients by comparing the gap between the predicted results and the actual situation. To validate the ability of our constructed model to predict patients with psoriasis, also known as discrimination, calibration was assessed by examining plots of the predicted versus actual probabilities of the model. The calibration curves of the nomogram used to predict the risk of developing severe disease in patients with psoriasis showed good agreement (Figure 3A). The predictive accuracy (discriminatory power) of the nomogram was measured by the consistency index (C-index). The C-index of the predicted nomogram was 0.71063(95% CI: 0.6910-0.7302), which was determined to be 0.7126(95% CI:

0.6900-0.7353) by bootstrap=1000 validation. At the time of testing, the C-index was determined to be 0.7804 (95% CI: 0.7023- 0.8585) and 0.7301 (95% CI: 0.6716- 0.7886) by external validation cohort 1 (HKU-Shenzhen Hospital data after June 1, 2022) and external validation cohort 2 (Xinjiang Uygur Autonomous Region People's Hospital after June 1, 2022), respectively, indicating that the prediction model has good discrimination. In addition, the PASI>10 probability 1-year, 3-year and 5-year AUC of the prediction model were 0.7067 (95% CI: 0.6569-0.7565), 0.6834 (95% CI: 0.6445-0.7123), and 0.7235 (95% CI: 0.7023-0.7447) respectively.
Conclusion This new nomogram allows easy and fast assessment of risk in patients with severe psoriasis with good efficiency. It is expected to be widely used in real-world clinical work.

PO-002

30% supramolecular salicylic acid in the treatment of sweat herpes: a case report

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Objective To analyze the effect of 30% supramolecular salicylic acid in the treatment of sweat herpes.
Methods One patient with sweat herpes was selected and treated with 30% supramolecular salicylic acid. The clinical effect was observed.
Results From the 2nd to the 5th day of treatment, the patient's skin lesions were flushed and desquamated in a large area, with mild itching and dryness; After 2 weeks, the skin was smooth, without depigmentation and pigmentation; The patients were followed up for 6 months, and there was no aggravation or recurrence of skin.
Conclusion Choosing 30% supramolecular salicylic acid to treat the patients with sweat herpes can achieve good curative effect, and the operation is simple, convenient, safe and reliable, which is worth popularizing.

临床中也将汗疱疹称之为出汗不良性湿疹，具体是指掌趾、指趾屈侧皮肤对称发生的复发性水疱性皮肤病，属于一种慢性炎症性疾病；青少年为该疾病的主要发病人群，而手足部则是主要的发病部位[9-10]。汗疱疹患者常常伴手足多汗、瘙痒，病程可能持续几周或者几个月，该疾病虽然能治愈，但是复发风险却较高[10-11]。现阶段临床中在对汗疱疹患者进行治疗时，可供选择的治疗手段较多，包括光化学疗法、放射治疗、中医中药治疗、局部注射肉毒毒素、激素药物等[11]。

相关临床研究结果显示，汗疱疹的发生、发展与红色毛癣菌等真菌感染存在显著相关性[12]。水杨酸的抑菌效果比较理想，微生物实验结果发现，水杨酸对白色假丝酵母菌、金黄色葡萄球菌等的抑制效果比较显著[13]。除此之外，水杨酸能对汗腺分泌进行有效抑制，所以能对汗疱疹患者的手足多汗症状进行有效改善。水杨酸是一种脂溶性化合物，无论是低浓度水杨酸或者是高浓度水杨酸，均能促成、松解、剥脱角质[14]。水杨酸能对胶质细胞相互间的桥粒连接进行有效溶解，对细胞间的黏附进行有效松解，让细胞代谢有效脱落，能对表皮增生进行有效抑制，所以临床中常常将水杨酸当成换肤剂[15]。

水杨酸还具有抗炎、抗湿疹的效果，水杨酸能对环氧合酶 COX2 进行有效抑制，让前列腺素 E 明显减少；除此之外，该药物还能对核因子 NF-KB 炎症通路进行有效抑制，同时对诱导型一氧化氮酶激活进行有效抑制，让一氧化氮过度释放明显减少[16]。临床研究发现，水杨酸能让炎症反应有效减轻，同时对皮肤新陈代谢进行有效改善[17-18]。相关实验结果显示，水杨酸抗炎能力相当于氢化可的松的 82%，相当于阿司匹林的 77%，相当于消炎痛或保泰松的 63-66%[19-20]。本研究中，治疗第 2 至第 5 天，患者皮损处基地潮红，大面积脱屑，同时伴轻度的瘙痒感和干燥；2 周后皮肤光滑，无色素脱失及色素沉着；对患者进行为期 6 个月的随访，皮肤无加重、无复发。但是本次研究并没有发现和提及超分子水杨酸治疗汗疱疹的具体作用机制，还需要进行深入的分析、探讨。

总之，选择 30%超分子水杨酸对汗疱疹患者进行治疗能取得较好疗效，而且操作简单方便、安全可靠，值得推广。本次研究也为临床治疗汗疱疹提供了新的思路。

PO-003

Barrier function and ultrastructure characteristics of epidermis in patients with primary cutaneous amyloidosis

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Background Previous studies on primary cutaneous amyloidosis (PCA) mainly have focused on exploring genetic mutation and components of amyloid in patients with PCA. However, studies on skin barrier function in PCA patients are scarce.
Objective We sought to detect the skin barrier function in patients with PCA and to characterize ultrastructural features of PCA lesions compared with healthy people.
Methods A total of 191 patients with clinically diagnosed PCA and 168 healthy individuals were enrolled in the study. Transepidermal water loss (TEWL), stratum corneum hydration (SCH), pH, and sebum were assessed noninvasively with the GPSkin Barrier (G-POWER, korea), Hanna Instruments HI 99181N Skin pH Meter (Asch, Japan), Sebumeter (HANNA, Italy) in accordance with standardized protocols. The ultrastructural features of PCA lesions were examined under transmission electron microscope (TEM). The length of basement membrane zone and the intercellular space of basement cells were measured using calibrated ImageJ software. The expression of proteins related to skin barrier function was examined by immunohistochemistry staining. Results were presented as means ± SEM. A threshold of $p < 0.05$ was accepted as statistically different and $p > 0.05$ considered non-significant by Student's t-test, Mann Whitney test, or Chi-square test.
Results The epidermal barrier function was measured in 285 skin lesions from 191 patients with clinically diagnosed PCA, including 113 back lesions, 116 shin lesions and 56 neck lesions. The number of each tested location, i.e., back, shin and neck, in the control group was 168, from 168 healthy individuals. The shins and back were the primary involved body sites (Figure.1A and 1B). The diagnosis of PCA was confirmed histologically with Congo red stain (Figure.1C) in all patients. The genenal information of PCA patients and healthy individuals were listed in table 1. There was no statistical difference in gender and in age between PCA group and control group. All investigated lesion skin areas displayed higher TEWL and pH values, and lower sebum levels and SC hydration levels in PCA group compared with the same site area in control group (Table.2). These results indicated that the skin barrier function was impaired in PCA patients.
3.2 The ultrastructure characteristics of PCA lesions under Transmission electron microscopy
It is well known that the function of the skin barrier is closely related to the physical structure of the skin. So, we want to know whether the skin structure of the lesions is altered in PCA patients. Under Transmission electron microscopy, the amyloid of the kerationcyte cytoplasm can be observed in stratum spinosum and stratum basale in the PCA lesion skin (Figure.2A-2D). In stratum basale there was some apoptotic kerationcytes (Figure.2E). The basement membrane zone was folded and bifurcated, and the hemidesmosomes were discontinuous (Figure.2F). The number of hemidesmosomes was significantly less in the PCA groups than that in the control subjects (Figure.3A-3C). We also found that the intercellular spaces of the stratum basale in the PCA group were wider than that of control group (Figure.3D-3F).
Hemidesmosomes are multimeric protein complexes which adhere epithelial cells to the underlying extracellular matrix. The integrins alpha6beta4 (α6β4) -heterodimer is a major component of hemidesmosomes. To validate the visualization of hemidesmosome distribution through transmission electron microscopy, indirect immunofluorescence analysis for α6β4 integrin was performed on skin tissues from PCA patients and control subjects. Previous studies12-14 has demonstrated apolipoprotein E (ApoE) deposit in PCA lesions, so in this study we used ApoE staining as a marker of amyloid deposition in the immunofluorescence analysis of PCA lesion skin. Figure 4A showed that integrin α6 continuously distributed on the basement membrane zone in the control group, and its expression

reduced significantly in PCA patients compared to the control group (Figure.4B). There was no significant difference in β4 expression between PCA patients and controls (Supplemental Figure.2). Under Transmission electron microscopy, we also found fibroblasts in superficial dermis were surrounded by a massive of islet-like amyloid substance and looked like phagocytosing the amyloid (Supplemental Figure.1A and 1B), which has been confirmed by immunofluorescence staining with anti-vinmentin and anti-apolipoprotein E antibodies (Supplemental Figure.1C).

3.3 The expression of skin barrier-related proteins in patients with PCA

Filaggrin (FLG) and loricrin (LOR) are important components of skin barrier-related proteins. The barrier dysfunction correlates with the downregulation of barrier-related molecules such as FLG and LOR. E-cadherin specifically in the developing epidermis is the main component of adherens junctions (AJs) which has recently been highlighted as an essential structure of the skin barrier function.15 Therefore we detected the expression of filaggrin, loricrin and E-Cadherin by immunofluorescence staining in PCA patients and control group. Our results showed that the expression level of E-Cadherin in PCA skin lesion tissues was significantly lower than that in control group, while no significant difference in expression of FLG and LOR was detected between two groups (Figure.5).

Conclusion Our study revealed that individuals with PCA displayed skin barrier dysfunction which may be related to alterations in epidermal ultrastructure and a decrease in the skin barrier-related protein E-Cadherin. However, the molecular mechanisms underlying skin barrier dysfunction in PCA remain to be elucidated.

PO-004

Retrospective Analysis of 397 Dermatoses Inpatients Associated with Blood Eosinophilia

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Objective To analyze and summarize the clinical characteristics of dermatoses associated with blood eosinophilia (DABE), and to explore the relationship between blood eosinophilia and various dermatoses.

Methods In this retrospective study, we analyzed the clinical data of 397 inpatients with DABE admitted to the Department of Dermatology, Southwest Hospital of Army Medical University, from January 2018 to January 2023. SPSS23.0 statistical software was used for the statistical analysis of data. Measurement data were compared by using t-test, analysis of variance or rank sum test, and enumeration data were compared by using the chi‑square test or Fisher's exact test. $P < 0.05$ means the difference is statistically significant.

Results A total of 397 patients (267 males, 67.3%; median 59 years, range 45-70 years) were included and grouped according to blood absolute eosinophil counts (AEC): mild eosinophilia, $0.5\times10^9/L \le AEC < 1.5\times10^9/L$ (n=292, 73.6%); moderate eosinophilia, $1.5\times10^9/L \le AEC < 3\times10^9/L$ (n=70, 17.6%); severe eosinophilia, $AEC \ge 3\times10^9/L$ (n=35, 8.8%).

There were statistically significant differences in the age distribution ($P < 0.05$) and the proportion of atopic history ($P < 0.001$) among the three groups. The severe eosinophilia group had a higher proportion (23/35, 71.5%) of old patients and a lower proportion (1/35, 2.9%) of atopy history. 66 patients (66/363, 18.2 %) developed leisions and elevated blood eosinophils due to drug explosure. The proportion of drug sensitization in the severe eosinophilia group (10/35, 32.3%) was higher than that in the other two groups, but the difference was not statistically significant ($P = 0.076$).

From the perspective of clinical manifestations, almost all DABE patients (383/397, 96.5%) exhibited pruritus symptoms, which were independent of AEC levels ($P = 0.549$). Localized skin lesions were more common in the mild eosinophilia group, while generalized skin lesions were observed in the moderate and severe eosinophilia groups. The morphological spectrum of skin lesions in DABE patients was wide, and the most common lesions were erythema (348/397, 87.7%) and papules (208/397, 52.4%). The incidence of skin vesicles was significantly higher in the

moderate eosinophilia group than in the mild and severe groups ($P = 0.03$). The incidence of other lesion types was independent of blood eosinophil levels.

We selected two blood parameters, serum total IgE and blood LDH, to analyze their association with blood eosinophilia. Serum total IgE was elevated in 68.4% (132/193) of DABE patients, and LDH levels were elevated in 27.7% (67/242) of DABE patients. In the mild eosinophilia group, the serum total IgE median was significantly lower than those in the other two groups ($P < 0.001$). In contrast to mild and moderate groups, elevated LDH was more common in the severe group, and their LDH levels were also higher ($P < 0.001$).

Then, we were interested in whether increased blood eosinophilia corresponded to eosinophilic infiltration in the skin and bone marrow. Histopathological examination of skin biopsies was performed on 155 patients, of whom 71.9% (105/155) had cutaneous eosinophilic infiltration. There was no significant difference in skin eosinophil infiltration between different groups ($P = 0.629$). The most common histopathologic characteristics are spongiosis and hyperplasia. Bone marrow biopsy histopathology showed that 93.2% (41/44) of DABE patients were accompanied by bone marrow eosinophil infiltration, and most of them were from the moderate or severe eosinophilia groups. Screening for the FIP1-like1-platelet-derived growth factor receptor (*F/P*) fusion gene, which has been associated with hypereosinophilic syndrome (HES), was negative in 2 patients. For the immunophenotype analysis of peripheral blood lymphocytes, no abnormal T and B lymphocytes were found in all 3 patients.

The most common diagnoses in DABE patients were eczema/dermatitis (207/397, 52.1%), followed by drug eruption (66/397, 16.6%), systemic disease (50/397, 12.6%) including HES or tumor, autoimmune bullous diseases (34/397, 8.6%), psoriasis (23/397, 5.8%), and other diseases (17/397, 4.3%). The diagnosis of eczema/dermatitis was dominant in the mild eosinophilia group ($P < 0.001$), while the diagnosis of systemic disease (HES or tumor) was more common in the severe eosinophilia group ($P < 0.001$).

In this study, we also evaluated the response to therapeutic drugs in different groups with blood eosinophila. Glucocorticoids (370/392, 93.2%) were the most commonly used drug to treat DABE, followed by antihistamines (322/397, 81.1%), immunosuppressants (129/392, 32.5%), antibiotics (14/392, 3.5%), retinoids (14/392, 3.5%), biologics (5/392, 1.3%), other drugs (7/392, 1.8%). In the mild eosinophilia group, the usage rate of antihistamines was significantly higher than that in the moderate and severe groups ($P = 0.032$), while the usage rate of antibiotics was opposite ($P < 0.001$). There was no statistically significant difference in the usage rate of other drugs among the three groups.

Conclusions Our study revealed three distinct patterns: (1) Mild eosinophilia associated with localized skin lesions, atopic history, mildly elevated total serum IgE level, diagnosed with eczema/dermatitis, and frequent antihistamines use. (2) Moderate eosinophilia has the characteristics of both mild group and severe group. (3) The severe eosinophilia group had a high proportion of elderly people without atopic history, but with acute onset, generalized skin lesions, and high level of LDH, and the majority of them were diagnosed with systemic diseases (HES or tumor).

PO-005

Efficacy and safety of once-daily bilastine 20 mg vs. levocetirizine 5 mg for the treatment of chronic idiopathic urticaria: a multicenter, double-blind, double-dummy, phase III, non-inferiority, randomized clinical trial

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Aim Chronic idiopathic urticaria (CIU) is the most common form of urticarial in China and has a long course, with episodes of remission and recurrences, multiple comorbidities, which lead to increasing financial burden. The standard first-line treatment of CIU is standard-dose second-generation H1 antihistamines and the available guidelines do not make clear recommendations for the priority of selecting second-generation H1 antihistamines. Bilastine is an enzimidazolepiperidinic derivative and a highly selective inverse agonist for H1 receptors, with favorable cardiac safety profile and lowest brain histamine H1 receptor occupancy rate compared with other antihistamines, which attributes to minimal impact on daily activity. Previous studies demonstrated a better control of dermatological symptoms with a faster onset of action compared to desloratadine and rupatadine, as well as a comparable management in CIU comparing to levocetirizine. Since most available evidence is from Western studies performed on Caucasians, this study aimed to examine the efficacy and safety of bilastine 20 mg once daily vs. levocetirizine 5 mg once daily in Chinese patients with CIU.

Methods This multicenter, double-blind, double-dummy, phase III, non-inferiority, randomized clinical trial (CTR20181967) enrolled adults with CIU for at least 6 weeks. Participants must have a clinical history of CIU for at least 6 weeks prior to screening, characterized by erythematous skin wheals accompanied by itching, occurring regularly, at least two times per week. Participants were 1:1 randomized to a 28-day treatment with bilastine 20 mg/day and bilastine-like dummy placebo or levocetirizine 5 mg/day and levocetirizine-like dummy placebo. The primary endpoint was the change from baseline in the Total Symptom Score (TSS3) of reflective symptoms. The secondary endpoints included the changes from baseline in the daytime and nighttime scores of TSS3 for each domain (itching intensity, wheals number, and maximum size of wheals) during treatment (reflective) and the changes from baseline in the Urticaria Composite Score (UCS2, reflective), instantaneous TSS3 and UCS2 during treatment (assessed by the participants and investigators, independently), the areas under the TSS3 and UCS2 (reflective) curves, the investigator’s overall Global Clinical Impression (GCI) scores, Dermatology Life Quality Index (DLQI), the overall assessment of discomfort caused by CIU by using visual analog scale (VAS) and the impact on sleep by using a 5-point scale. The non-inferior margin was 0.8.

Results This study randomized 288 participants (144 in each group). In the analysis of the primary endpoint among the PP analysis set, the baseline TSS3 (reflective) were 4.06±2.040 and 4.43±2.102 in the bilastine and levocetirizine groups, respectively. The mean TSS3 (reflective) on day 28 were 1.53±1.914 and 1.37±1.876 in the bilastine and levocetirizine groups, respectively, both decreased compared with baseline. The mean changes from baseline in TSS3 (reflective) on day 28 were -2.54±2.594 and -3.06±2.469 in the bilastine and levocetirizine groups, respectively

(Table 2). The LS means (95% CI) of difference between the two groups was 0.17 (-0.19, 0.54), indicating non-inferiority (Figure 2). The domain scores of itching intensity, wheals number, and maximum size of wheals in the daytime and nighttime were all decreased from baseline in both groups and the changes were similar between the two groups (all P>0.05). The areas under the TSS3 score curves were 44.085±43.3506 and 41.444±41.5494 in the bilastine and levocetirizine groups, respectively (P=0.604). The participant-assessed TSS3 (instantaneous) decreased from 3.19±2.696 at baseline to 1.34±1.868 on day 28 in the bilastine group and from 3.20±2.752 to 1.06±1.773 in the levocetirizine group, respectively. There were no significant differences in the changes from baseline to day 28 in the two groups (-2.13±2.935 vs. -2.04±2.682, P=0.829). The investigator-assessed TSS3 (instantaneous) were both decreased on day 28 compared with baseline, with a similar change of -2.12±2.761 and -2.29±2.934 in the two groups (P=0.861), respectively (Table 2). The UCS2 (reflective) were 2.59±1.284 and 2.90±1.374 at baseline, respectively. The UCS2 (reflective) decreased to 0.98±1.222 and 0.87±1.187 on day 28 in the bilastine and levocetirizine group (Table 2). There were no significant differences in the changes in UCS2 (reflective) (-1.62±1.633 vs. -2.05±1.593, P=0.171). The participant-assessed and investigator-assessed UCS2 (instantaneous) were decreased on day 28 in both groups (Table 2). There were no significant differences in the changes in participant-assessed (-1.3±1.88 vs. -1.4±1.68, P=0.127) and investigator-assessed UCS2 (instantaneous) (-1.3±1.80 vs. -1.5±1.82, P=0.522, Table 2). Regarding the investigator's GCI, the proportions of participants with marked improvements (51.1% vs. 590.7%, P=0.420), moderate marked improvements (29.5% vs. 34.3%, P=0.964), and minimal slight improvement (14.4% vs. 9.3%, P=0.805) were similar between the two groups. The DLQI scores assessed on day 28 were 4.1±4.63 and 3.7±4.28 in the bilastine and levocetirizine groups, respectively. The improvement of QoL was similar between the two groups (-6.1±5.1 vs. -7.2±5.75, P=0.209). After 28 days of treatment, the discomfort caused by CIU in the two treatment groups was significantly reduced. The mean VAS score at baseline and day 28 of the bilastine group decreased from 58.50±22.13 to 20.53±20.72, while it decreased from 61.68±23.21 to 16.55±20.55 in the levocetirizine group. There were no significant differences between the VAS scores of the two treatment groups on days 14 and 28 (P=0.193 and P=0.116, respectively). The proportions of patients whose sleep were not impacted by CIU at baseline were 10 (7.2%) and 11 (7.9%) in the two groups, respectively (P=0.536); after 28 days of treatment, they were 80 (57.6%) and 89 (63.6%), respectively (P=0.707, Table 2). A total of 30.2% (n=42) and 34.0% (n=48) of the participants in the bilastine and levocetirizine groups experienced treatment-emergent AEs (TEAEs), respectively. There were no dose adjustments or treatment interruptions due to TEAEs in this study, but one (0.7%, anxiety) participant in the bilastine group and two (1.4%, one with blurred vision and one with diarrhea and lethargy) participants reported treatment discontinuation due to TEAEs. The most common TEAEs reported by ≥3% of the participants in either group (bilastine vs. levocetirizine) were sinus arrhythmia (3.6% vs. 2.8%), elevated blood triglycerides (0.7% vs. 5.0%), ECG T wave abnormality (0.7% vs. 4.3%), hyperuricemia (1.4% vs. 4.3%), and somnolence (2.9% vs. 4.3%, Table 3). No SAEs were reported in this study, and all TEAEs were mild to moderate. Moderate TEAEs were reported in five (3.6%) participants in the bilastine group and three (2.1%) in the levocetirizine group. The moderate TEAEs in the bilastine group included upper respiratory tract infection (n=2), fatigue (n=1), anxiety (n=1), and contact dermatitis (n=1). The moderate TEAEs in the levocetirizine group included lethargy (n=1), upper respiratory tract infection (n=1), and drug-induced liver injury (n=1).

Conclusion Bilastine significantly improved CIU symptoms and could improve patients' quality of life and sleep qualities, similar to levocetirizine. Bilastine was safe and tolerable, and no new safety signals were found in the Chinese CIU population. This study supports bilastine as an effective and safe treatment option for Chinese CIU patients.

PO-006

Reactive oxygen species-responsive sprayable hydrogel dual blocking inflammatory loops for the treatment of psoriasis

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Purpose Psoriasis (Ps) is a multifaceted disease related to chronic dysimmunity and genetic disease, which manifests in skin symptoms of demarcated erythematous and scaly lesions, accompanied by other systemic inflammatory comorbidities, like psychological illness, metabolic disturbance, arthritis, and cardiovascular disorders. It has been affecting appropriately 125 million people worldwide, in which the age group of 60–69 years is recognized as a weighty psoriasis burden according to the Global Burden of Disease (GBD) 2019 study. According to the clinical features, psoriasis is classified into cutaneous psoriasis and systemic psoriasis. Among the variants in cutaneous psoriasis, plaque psoriasis, also known as psoriasis vulgaris, is the most common phenotype, affecting~85-90% of patients with psoriasis. The histopathological feature of psoriatic lesions is parakeratosis in the thickened stratum corneum, the remarkably thickened epidermis with elongations into the dermis, and an abundance of different immune cells from dermis infiltration into the epidermis. Numerous studies have currently revealed that the direct or indirect cross-talking among different cell types in epithelial immune niches, plays a vital role in the pathogenesis of psoriasis and predominately emphasized the trigger role of oxidative stress in these cell types dysfunctions. Multiple inflammatory circuits mediated by these pathogenetic cells can disequilibrate the redox system of KCs, leading to inflammation and hyperproliferation of KCs. Psoriatic skin inflammation remains a critical challenge due to the crosstalk between keratinocytes and immune cells.

Methotrexate (MTX) is considered to be an immunosuppressive regulator involved in mediating the suppression of these pathogenetic cells. It has been systemically used in the treatment of moderate-to-severe psoriasis. Currently, the introduction of hydrogel, liposome, polymers and microneedles nanocarriers, makes contributions to the transdermal delivery of MTX and other antipsoriatic drugs for the circumvention of overwhelming systemic adverse reactions in conventional therapy. However, desired therapeutic efficacy would be hindered by singleagent topical MTX therapy against multiple inflammatory loops in the pathogenesis of psoriasis. Thus, it could be further contributed to an increased risk of side effects and drug resistance caused by overused high-dose MTX. Recently, anti-inflammation of nanomaterials had been employed to inherent ability for the treatment of RA and psoriasis, such as cationic polymers, Au, FA-Ag and manganese ferrite/ceria codecorated nanoparticles. The hydrogel has been identified as the most competitive candidate for percutaneous treatment of skin diseases, which can be equipped with tunable functions via the incorporation of various nanoparticles due to its characteristics of good adhesiveness and skin retention. Therefore, it’s an alternative strategy to fabricate a self-therapeutic multifunctional hydrogel nanocarrier and precisely controlled release of MTX in a specific manner for simultaneously inhibiting propagation of inflammatory circuits and enhancing low-dose MTX therapeutic effects to repress the aggravation of psoriasis. It may be inadequate to eradicate complicated pathogenesis only via single-mode therapy. Therefore, targeting proinflammatory macrophages and "activated" KCs simultaneously could be emerged as an effective strategy for the treatment of psoriasis. To provide optimal combinatory therapeutics, a nanocomposite-based hydrogel was constructed by loading methotrexate (MTX) and CeOZnO nano-enzyme to realize combined multiple target therapy of psoriasis.

Method Herein, a sprayable multifunctional hydrogel was developed to simultaneously block the feedforward amplification of inflammatory loops. In our previous work, we reported a good biocompatible AA-[Zn(OH)4]2− (denoted as ZnO) hybrid mesoporous microspheres. On this basis, CeOZnO nanoparticles was synthesized by doping a certain amount of Ce in the synthesis reaction

which demonstrated excellent antioxidant activity. The reactive oxygen species (ROS)-responsive hydrogel was fabricated through a Schiff-based reaction between ε-polylysine (EPL)-coated CeOZnO nanoparticles and methotrexate (MTX)-loaded self-assembled aldehyde Pluronic F127 (FCHO) micelles.
Result We developed a sprayable, self-healing, and adhesive hydrogel for anti-psoriasis treatment. Specifically, the assembled CeOZnO nano-enzyme homogeneously dispersed in the hydrogel, not only enabling the hydrogel with expedite gelation behavior and stable rheological properties by interacting with the polymer matrix, but also inducing macrophages polarizing toward M2 phenotype by scavenging ROS. In vitro experiments showed that CeOZnO nano-enzyme inhibited the activation of p65 in proinflammatory macrophages and suppressed ROS-induced STAT3-cyclin D1 signaling in KCs, resulting in simultaneous blockade of the key nodes of innate and adaptive cytokine networks. Meanwhile, the redox-responsive hydrogel inhibited excessive proliferation of keratinocytes via a sustained and spatiotemporal controlled release of MTX. In addition, the Pluronic F127 nanomicelles was conducive to the enhancement of transdermal delivery of MTX through the thickened skin layers of psoriasis area. As a result, this hydrogel potentiated the immunomodulatory function of MTX in a psoriasis-like mouse model. In particular, the multifunctional hydrogel is comprised of MTX with extreme low concentration, which shows good biocompatibility. Therefore, this multifunctional hydrogel is believed to be a promising nanoplatform for the transdermal delivery of MTX, characterized by remarkable anti-psoriatic efficacy and the potential for clinical translation.
Conclusion In summary, our composite hydrogel demonstrated an enhanced anti-psoriasis effect and represented a combined strategy for relieving inflammation and inhibiting keratinocyte hyperplasia. Through a synergistic effect of MTX and CeOZnO nano-enzyme, the composite hydrogel exhibited extraordinary anti-inflammatory and anti-proliferation capacities against psoriasis. In vivo and in vitro experiments, including qRT-PCR, ELISA and immunofluorescence analyses, were demonstrated. As expected, this composite hydrogel could optimize the therapeutic effects against psoriasis via synergistic multitherapy.

PO-007

The safety of ixekizumab in Chinese adults with moderate-to-severe plaque psoriasis: results from a prospective, multicenter, observational study

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Background We reported an observational study of ixekizumab for the treatment of moderate-to-severe plaque psoriasis in routine clinical practice in China which showed ixekizumab is well-tolerated and effective in real-world clinical practice. Here, we present a further safety analysis of this study.
Methods In this prospective, observational, single-arm, multicenter, post-marketing safety study, adults (≥18 years) with moderate-to-severe plaque psoriasis receiving ixekizumab were recruited

at dermatology departments in hospitals across China and prospectively followed for 12 weeks or until their last dose of ixekizumab. Safety was assessed during follow-up visits 2 weeks (± 5 days) and 12 weeks (± 3 weeks) after the first dose of ixekizumab, and on the telephone 6 weeks (± 5 days) after the first dose of ixekizumab. The primary outcome of the study was the safety of ixekizumab over 12 weeks in routine clinical practice, assessed based on the incidence of adverse events (AEs) and serious AEs (SAEs). In this analysis, we evaluated AE of special interest (AESIs) including injection site reactions, hepatic events, cytopenias, allergic reactions/hypersensitivity, infections, major adverse cardiovascular events (MACE), malignancies, inflammatory bowel disease, and interstitial lung disease, identified using MedDRA search strategies. We also analyzed AEs in subgroups of patients by age (<65/≥65 years), sex, body weight (<60/60–<80/≥80 kg), renal impairment, hepatic impairment, history of tuberculosis, history of HBV infection, recent or active infection, history of allergic reaction/hypersensitivity, and number of ixekizumab 80 mg injections after baseline until day 105 (0–1/2–4/5–7).

Results The safety population comprised 663 of the 666 patients enrolled in the study across 26 hospitals in China. At least one AESI was reported in 224 (33.8%) patients and considered related to ixekizumab in 181 (27.3%) patients; the most common were injection site reactions (n=131, 19.8%), infections (n=80,12.1%) and allergic reactions/hypersensitivity events (n=59, 8.9%). Most of these 3 AESIs were mild and no severe events were reported. One patient had moderate hypersensitivity, which was classified as an SAE (considered medically significant); the patient had a history of allergy. No MACE or cases of interstitial lung disease were reported. One case of lung neoplasm (not related to ixekizumab) and one case of ulcerative colitis was reported. A total of 6 hepatic events were reported in 6 patients (0.9%), which were all mild in intensity. Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) illustrated that most patients had liver function test results within the normal range and no patient had results that met Hy's law.

In subgroup analysis, a higher proportion of female patients had at least one AE over 12 weeks versus males (99/186, 53.2% of female vs 184/477, 38.6% of male, p=0.0006) and the proportion of patients with ≥1 AE increased with an increasing number of ixekizumab injections after baseline (61/188 [32.4%] of those who received 0–1 injection; 151/338 [44.7%] for 2–4 injections; and 61/106 [57.5%] for 5–7 injections; p=0.0001). No significant differences in the incidence of AEs or AEs considered related to study drug (judged by investigators) were observed across the other subgroups, such as age and body weight.

Conclusions Ixekizumab was well tolerated in Chinese patients with moderate-to-severe plaque psoriasis in this real-world study with no difference in safety across most of the subgroup analyses.

PO-008

The role of exosome-derived FCN2/A in host resistance to Cryptococcus neoformans

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Objective Cryptococcosis is an infectious fungal disease caused by *Cryptococcus spp*., of them *Cryptococcus neoformans* is most common in cryptococcosis. Due to the peculiar neurotropism of *C.neoformans*, it often causes fatal cryptococcal meningitis，which is lethal to the patients. At present, in the treatment of cryptococcal meningitis, antifungal drugs such as amphotericin B are mainly used, which not only have serious drug-related side effects, but also are prone to problems such as fungal resistance. This article aims to investigate the effect of FCN2/A protein in exosomes on macrophages and mice against *C.neoformans*. to provide a new theoretical basis and scheme for the immune adjuvant therapy of patients with cryptococcosis.

Method

1. The *C.neoformans* H99 infection mouse model and the PBS control mouse model were constructed by the nasal inhalation method, and the mouse plasma exosomes were extracted (H99-EXO: plasma exosomes of the C.neoformans infected mouse group; PBS- EXO: plasma exosomes from PBS-treated mice), morphological identification of exosomes was carried out by nanoflow cytometry; the uptake of exosomes of different mouse plasma by macrophages was verified by confocal microscopy; H99-EXO and PBS-EXO were incubated with mouse macrophages (Raw264.7 macrophage cell line and BMDM primary macrophage cell) for 24 hours, the treated macrophages were co-incubated with the standard strain of *C.neoformans* H99. By observing the related phenotypes of the interaction between macrophages and *C.neoformans*: killing, phagocytosis, IPR (intracellular proliferation rate) and the release of inflammatory factors and nitric oxide in the cell supernatant of H99-EXO and PBS-EXO cocultured mouse macrophage were detected to explore the effect of H99-EXO on macrophages against *C.neoformans* infection; H99-EXO and PBS-EXO were injected into healthy mice by intraperitoneal injection, and 24 hours later they were infected with *C.neoformans* and observed: mouse survival time, tissue colony load (lung, brain), HE and PAS tissue stained (lung, brain), tissue inflammatory factor release (lung, brain), to explore the effect of H99-EXO on the anti- C.neoformans infection ability of mice.
2. 8 patients with cryptococcal meningitis and 8 healthy controls during the same period were included. Plasma exosomes from patients with cryptococcal meningitis and healthy
controls were extracted by using a plasma exosome extraction kit, and exosome proteomics was used to explore the differentially expressed protein. The differential protein expression between patients and healthy control was analyzed, and Western-Blot was used to verify the differential protein FCN2 in plasma exosomes of cryptococcal meningitis patients, healthy control, and FCN2 mouse homologous protein FCNA in plasma exosomes of *C.neoformans* infected, PBS treated group; H99-EXO and PBS-EXO were incubated with mouse macrophages for 24 hours, and then the mRNA expression levels of FCNA in each group were verified by real-time quantitative PCR; The expression level of FCNA protein were analyzed to explore whether FCNA in H99-EXO and PBS-EXO could be taken up by macrophages.
3. Using Lipofectamine RNA iMAX transfection reagent, FCNA-negative control and FCNA-small interfering RNA were transfected into mouse Raw264.7 macrophages, and BMDM of FCNA knockout mice and wild-type mice was extracted. The cells were co-incubated with *C.neoformans* H99 standard strain, and the phenotypes related to the interaction between macrophages and *C.neoformans* were observed: killing, phagocytosis, IPR (intracellular proliferation rate) and the release of inflammatory factors and nitric oxide in the cell supernatant of knockdown and knockout FCNA macrophages were detected. was to explore the effect of FCNA protein on the function of macrophages against *C.neoformans*. And mouse survival time ， HE、PAS tissue stained (lung, brain) were used to explore the effect of FCNA knockout mice on the ability of anti- *C.neoformans* infection.
4. Transcriptomic analysis was performed on mouse BMDM macrophages co-incubated with H99-EXO and PBS-EXO and FCNA knockout and wild-type mouse BMDM macrophages, and the same differential genes in the two groups of transcriptomics was selected. RT PCR was done to confirm the selected differentially expressed genes, to initially reveal the possible targets of FCNA protein in mouse plasma exosomes in regulating macrophage killing and phagocytosis of C. neoformans.

Results

1. Firstly, different infection time gradients were set up. It was found that compared with the PBS-treated group, the plasma exosomes extracted after 14 days of infection mouse with the *C.neoformans* H99 were different in diameter and concentration; Macrophages could directly take up the exosomes derived from different mouse plasma, and the uptake of exosomes by macrophages gradually increased with time. The phagocytosis and killing of *C.neoformans* were enhanced, the intracellular proliferation ability of *C.neoformans* in macrophages was weakened, the release of pro-inflammatory inflammatory factors was increased, and there was no significant difference in the release of nitric oxide; Healthy mice were reinfused with H99-EXO and PBS-EXO and then infected with *C.neoformans*. Mice reinfused with H99-EXO had prolonged survival, decreased lung and brain fungal load, increased protein expression of IL10 and MCP-1 in brain tissue, and alleviated lung damage.

2. After proteomic analysis of plasma exosomes from patients with cryptococcal meningitis and healthy controls, a total of 158 differential proteins were found, of which 144 were down-regulated and 14 were up-regulated. Five differential proteins were screened out and verified by Western-Blot experiments. Combined with the verification results and literature review, it was finally determined that FCN2/A protein was the main research object in this study. We found that FCN2 protein was lowly expressed in the plasma exosomes of patients with cryptococcosis, and the FCN2 mouse homologous protein FCNA was also lowly expressed in the plasma exosomes of C.neoformans infected mice; H99-EXO and PBS-EXO were co-incubated with murine macrophages, it was found that compared with the blank control group (without exosome co-incubation) there was no significant difference in the mRNA expression of FCNA, but the FCNA protein expression was higher than that in the blank control group, proving that FCNA in exosomes were taken up by macrophages.
3. After Comparison with the FCNA-NC group , the FCNA knockdown mouse Raw264.7 was found to have enhanced phagocytosis and killing of *C.neoformans* weakened intracellular proliferation ability of *C.neoformans* in macrophage, increased release of pro-inflammatory inflammatory factors. The ability of anti- *C.neoformans* of macrophage may be positively correlated with the efficiency of FCNA gene knockout. Compared with the wild-type group, BMDM of FCNA knockout mice has enhanced phagocytosis and killing of *C.neoformans*, weakened intracellular proliferation ability of *C.neoformans* in macrophage, and pro-inflammatory inflammatory factors release increases. There was no obvious nitrogen release in the supernatant of macrophage cells in the above two groups. The FCNA knockout and wild-type mice model of *C.neoformans* infection was constructed, and it was found that the survival time of mice of FCNA knockout group was prolonged and the lung damage was alleviated compared with the wild-type group.
4. Transcriptomic analysis of the BMDM of H99-EXO and PBS-EXO co-incubated mice and the BMDM of FCNA knockout and wild-type mice showed that there were 20 differentially expressed genes in common between the two group, of which 4 were identical. After verification by Real-time quantitative PCR, it was found that the expression of C1ql3,
Pcdhb3, and Shroom1 genes were up regulated in the macrophages of the two groups, which was consistent with the transcriptomic results of the two groups. Based on the previous results, it is suggested that the ability of FCNA protein in mouse plasma exosomes to regulate macrophages against Cryptococcal infection may be related to the changes in the expression of *C1ql3, Pcdhb3* and *Shroom1* genes in macrophage.
Conclusion The ability of FCNA protein in mouse plasma exosomes to regulate macrophage resistance to cryptococcal infection may be related to the changes in the expression of *C1ql3, Pcdhb3* and *Shroom1* genes in macrophages.

PO-009

Single-cell RNA and ATAC Sequencing Revealed Distinct Molecular Characteristics of B Cells in Systemic Lupus Erythematosus and Sjogren's Syndrome

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Objective The pathogenetic mechanisms of systemic lupus erythematosus (SLE) and primary Sjögren's syndrome (pSS), two forms of autoimmune diseases (ADs), are not well understood. Aberrant of B-cell differentiation are critical for the development of ADs. While single-cell transcriptome and chromatin accessibility of B cells have not been extensively revealed. It seems

the differentiation and function of B cells are likely determined by the synergistic action of a set of transcription factors (TFs), rather than one individual TF.
Methods CD45+CD19+ B-cells were sorted by Flow cytometry, then entered into single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq). ArchR algorithm was used to integrate scRNA-seq and scATAC-seq data to investigate the transcriptome and chromatin accessibility of B-cell subpopulations and differentiation trajectories.
Results We generated scRNA-seq data for 29,318 and scATAC-seq data for 25,827 B cells from healthy controls (HC), SLE, and pSS. Naive B cells (NB), Effector B cells (EB), Double negative B cells (DNB), Activated memory B cells (AB) and Classical memory B cells (CMB) were annotated. We analyzed different B cell populations and found that NB had the largest number of differentially expressed genes (DEGs).We found both common and specific features for NB in pSS and SLE. Differential expression pattern and functional enrichment of the cell-type specific genes were highlighted. Integration of the scRNA-seq and scATAC-seq revealed that the characteristics of NB expansion. To explore the fate determination TFs of B cells in ADs, we performed motif enrichment of high-resolution cell type-specific ATAC-seq peaks of four B cells clusters. We identified seven TFs (RELA, NF-κB1, NF-κB2, ZNF148, SPI1, SPIB, and SPIC) highly enriched in NB, eight TFs (RELA, NF-κB1, NF-κB2, POU3F4, POU2F2, POU5F1B, POU2F3, and POU2F1) enriched in DNB, 19 TFs enriched in AB, and 20 TFs enriched in CMB. Importantly, we observed that different B-cell subpopulations were enriched with varying quantities of TFs, which seemed to correlate with the stage of differentiation that the B cells were in. Furthermore, different B-cell subpopulations were enriched with both overlapping and non-overlapping TFs. B-cell differentiation trajectories showed a group of TFs profiles that contribute to cell fate determination. Selective gain and loss of these TFs may influence the differentiation and maturation of B-cell subpopulations.
Conclusion In this study, we revealed the composition of B cell subpopulations and transcriptional heterogeneity in SLE and pSS peripheral blood by combining scRNA-seq and scATAC-Seq at the individual cell level and describe their molecular characteristics and differentiation trajectories. Our analysis provided high-resolution insights into the diverse molecular events occurring at the single-cell level. We hypothesize that B cells may be able to mitigate the development of ADs by altering cell fate through the modulation of TFs. These selective pressures perhaps begin during the transformation of mature NB and persist through all stages of disease development, resulting in a clinically diverse and heterogeneous regulatory landscape for SLE and pSS.

PO-010

EPSTI1 induced by IFN-γ contributes to keratinocyte hyperproliferation and inflammation in psoriasis by activating NFκB/STAT3 pathway and Wnt5a pathway

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Objective This study aimed to determine the expression level of Epithelial Stromal Interaction 1 (EPSTI1) in psoriasis and cell disease models, to study the effect of EPSTI1 on keratinocyte proliferation and psoriasis-like inflammation, and to explore the mechanism of upstream and downstream genes.
Method Transcriptome sequencing, bioinformatics analysis, immunohistochemical and qRT-PCR were used to detect the expression of EPSTI1 in psoriasis lesions. CCK-8 and flow cytometry analysis were performed to measure cell proliferation. QRT-PCR was used to evaluate the expression of inflammatory cytokines. Western blot and qRT-PCR analysis were performed to detect expression of molecules related to cell cycle, inflammation and NFκB/STAT3 and Wnt5a signaling pathway. Bioinformatics websites JASPAR, PROMO and GeneCards were used to predict target upstream genes between IFN-γ and EPSTI1. STRING database was used to predict

related signal pathway of EPSTI1. QRT-PCR was performed to measure the expression of EPSTI1 after different treatments.
Result In this study, we found EPSTI1 was significantly upregulated in psoriasis tissues and cell models. The expression of EPSTI1 was higher in lesional skins than uninvolved skins. Through overexpression and knockdown of EPSTI1, we confirmed its pro-proliferative and pro-inflammatory effects. These phenotypes were associated with NFκB/STAT3 and Wnt5a signaling pathway. Through bioinformatics analysis, we confirmed that EPSTI1 was upregulated by IFN-γ via JAK-STAT1 pathway, which could be blocked by JAK1/JAK2 inhibitor Ruxolitinib.
Conclusion Here we first reported the function of EPSTI1 in psoriasis and these results suggest that EPSTI1 could promote keratinocyte proliferation and inflammation by NFκB/STAT3 and Wnt5a signaling pathway. Also, the expression of EPSTI1 was regulated by IFN-γ via JAK-STAT1 pathway. Our results suggest that EPSTI1 could play roles in development of psoriasis.

PO-011

FOXO3a overexpression inhibits cell growth and fibrosis through β-catenin regulation in keloid fibroblasts

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Background Abnormal expression of FOXO3a has been documented in keloids, a type of fibroproliferative disorder characterized by excessive fibroblast proliferation and an accumulation of extracellular matrix. However, the functional role of FOXO3a in keloids remains largely elusive duo to limited information available on this topic. Thus, our study aimed to investigate the effects of aberrant FOXO3a expression on keloid cell growth and fibrosis, as well as the underlying mechanism.
Methods Primary cultured keloid fibroblasts (KFs) was transfected with lentivirus, siRNA, or a corresponding negative control. Cell proliferation, cell cycle distribution, cell apoptosis and extracellular matrix related protein were then analyzed.In addition, the protein expression of β-catenin was mesured. After transfection with lentivirus-mediated FOXO3a-plasmids, β-catenin was overexpressed, and then the cell viability and extracellular matrix related protein were determined again.
Results The relative cell proliferation in KFs was significantly reduced by FOXO3a overexpression ($p < 0.05$). FOXO3a overexpression displayed a significant blockage at the G1/S transition and significantly increased the percentages of G0/G1 phase. Moreover, the results showed that FOXO3a overexpression significantly induced cell apoptosis and statistically downregulated the protein expression levels of β-catenin. However, the cells transfected with FOXO3a siRNA in NF showed contrary results. Additionally, the effects of siRNA FOXO3a on cell prolieration and apoptosis were reversed by downregulation of β-catenin.
In conclusion, our findings indicate that the overexpression of FOXO3a plays a crucial protective role in keloids by effectively inhibiting cell growth and fibrosis. These effects are likely mediated through the regulation of β-catenin expression in keloid.

PO-012

A Review of Immune Cells Involved in the Development of Acne

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Acne is a chronic inflammatory disease of hair follicle sebaceous gland unit, various types of immune cells participate in the inflammatory process of acne. The development of acne is closely related to environmental factors, and the colonization of *C.acnes* in hair follicle plays a prominent role. *C.acnes* shows activities that can promote the development of acne skin lesions. It can activate innate immunity and specific immunity, activate a variety of immune cells and promote their secretion of a large number of cytokines, causing inflammatory reactions, leading to hair follicle damage, promoting hyperkeratosis of hair follicles, promoting the proliferation and differentiation of sebaceous gland, leading to sebum production and abnormal secretion. In addition, inflammation caused by C.acnes can be mediated by androgens, which trigger an inflammatory response through immune cells. In summary, acne can be considered an immune-mediated chronic inflammatory skin disease, characterized by a pathological manifestation caused by a network of various immune cells and their secreted inflammatory factors.

Skin epidermal cells are the first stop of contact with antigens such as *C.acnes*. The exogenous proteases produced by *C.acnes* activates PAR-2 expressed on keratinocytes, causing the mRNA expression of IL-1α, IL-8, TNF-α, hBD-2, cathelicidin LL-37 and MMPs. A large amount of *C.acnes* on the skin can also stimulate the secretion of IL-1α and IL-1β by sebocytes, It can induce keratinocytes in the infundibulum of hair follicle to produce IL-6 and IL-8, cause hair follicle inflammation and rupture, and the production of MMPs, leading to scar formation. In vitro studies have shown that *C.acnes* can upregulate IL-8 secretion in human SZ95 cells through TLR2 dependent signaling. In addition, *C.acnes* IA and IB type isolates can activate PAR-2 on SZ95 sebocytes, thereby inducing pro-inflammatory cytokines and hBD-2 transcription in sebocytes; *C.acnes* can induce the activation of caspase-1 and the secretion of IL-1 in sebaceous gland by activating NLRP3 inflammasome; Sebaceous gland cells can also secrete IL-6, TGF-β and IL-1β induce the differentiation of Th17 cells, promoting the inflammatory response in acne. When skin traditional dendritic cells (cDCs) are stimulated by *C.acnes*, a subset of cDC1s $EpCAM^+CD59^+Ly\text{-}6D^+$cDC1s activates and secretes VEGF- α, control the recruitment, survival, and function of neutrophils in inflamed skin, and regulate the amplitude of the skin's innate immune response to bacteria. *C.acnes* initiate neutrophil aggregation at the site of acne by producing low molecular weight chemokines. After phagocytic bacteria, neutrophils release inflammatory factors such as lysosome enzymes and hydrogen peroxide to damage hair follicle epithelium. Skin fibroblasts stimulated by androgen can secrete FGF10 and other growth factors. FGF10 can inhibit the mRNA expression of cell keratin (CK) 1 and CK10 in human keratinocytes, reduce the ratio of CK10 /CK14, change the differentiation of keratinocytes in acne lesions, and lead to abnormal keratinization of skin. The involvement of mast cell in acne is related to the effect of substance P (SP) secreted by them on Sebaceous gland. SP can induce sebaceous gland cells to express nerve growth factor, and can also up regulate the lipid production of sebaceous gland cells, which may cause the reproduction of *C.acnes* and aggravate the inflammatory reaction caused by *C.acnes*.

The involvement of mononeuclear phagocyte system in acne was investigated in vitro. After stimulating peripheral blood monocyte with *C.acnes*, they can be induced to differentiate into two different innate immune cell subsets $CD209^+$ macrophages and $CD1b^+$ dendritic cells. $CD209^+$ cells can effectively engulf and inhibit the growth of *C.acnes*, playing a role in the host's defense against *C.acnes*. $CD1b^+$ cells are efficient antigen-presenting cell of T cells, which can indicate the response of adaptive T cells. In addition, excessive accumulation of *C.acnes* can cause monocyte to release IL-12 and IL-8. *C.acnes* can also induce and activate the expression of NLRP3 in human monocyte through various mechanisms, and then release IL-1 β Inflammatory mediators.

Except for the inflammatory response caused by *C.acnes*. Squalene in the sebum of acne patients can stimulate the proliferation of keratinocyte, the activity of Lipoxygenase and the activation of NF-

κB, thereby promoting the secretion of IL-6. Triglycerides in sebum are decomposed by bacteria to release monounsaturated fatty acid (MUFAs), inducing changes in the proliferation and differentiation of keratinocytes. The qualitative change of sebum can also induce the secretion of IL-1 by keratinocytes, promoting excessive keratinization of hair follicles.

In addition to the involvement of innate immune cells, adaptive immune response also plays an important role in the occurrence of acne. Research has found that Th1 cell transcription factor T-bet and effector factor IFN- γ and CXCR3 are elevated in acne lesions. Key genes *IL23A, IL6*, and *TGFB1* involved in the activation and differentiation of Th17 cells and Th17 cell products IL-17A, IL-22, IL-26, and TNF are also expressed at high levels in acne lesions. The number of IL-17A$^+$ lymphocytes in the dermis papillary and around the hair follicle sebaceous gland of acne lesions increased significantly. IL-6, TGF-β And IL-1β secrete by sebocytes can induce CD4$^+$CD45RA$^+$ immature T cells to differentiate into Th17 cells. *C.acnes* can not only induce Th17, but also induce Th17/Th1 response to participate in the inflammation of acne. Different *C.acnes* strains also have the ability to induce Th17 to secrete IFN- γ or the ability of IL-10, and exhibiting pathogenicity or protection, respectively. In addition, diet mediated abnormalities in sebum volume and composition lead to increased levels of free palmitate in hair follicles. Free palmitate can stimulate Th17 differentiation and IL-17 mediated keratinocyte proliferation. B cells only exist in severe acne and late stages of the acne skin lesion cycle. By producing cytokines, B cells regulate the collagen synthesis of fibroblasts, leading to dermal damage and deep changes in the structure of sebaceous gland, resulting in the formation of atrophic scars in acne.(supported by the National Natural Science Foundation of China (Project Number: 82160600))

Poster session

PO-013

Multiple xanthoma tuberosum in one case

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A case of multiple nodular xanthoma of the buttocks, elbows and right hand is reported. The patient, a 30-year-old male, had a mass on buttocks for more than 10 years, and papules and plaques on elbows and right hands for 2 years. Dermatological examination revealed a soft yellowish-red round mass with a diameter of about 4cm on the left buttock. On the metacarpophalangeal joints of the right index finger and bilateral elbows, brown-yellow papules and nodules of the size of soybeans to broad beans were observed. Some fused papules and nodules were in the shape of plaques and lumps with slightly hard quality, with a maximum of about 3cm×2cm×1.5cm. Histopathological examination showed that the epidermis was generally normal, with a large number of foam cells (xanthoma cells) infiltrating in the middle and deep dermis. The cytoplasm of the tumor cells was slightly stained and foamy, and the nuclei were small and vesicular-like, which was consistent with the histopathological changes of xanthoma.

PO-014

Therapeutic potential of targeting drug in pemphigus

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Pemphigus is a heterogeneous group of autoimmune skin disorders characterized by blistering of the skin and mucosal membranes, potentially affecting the quality of life if left unchecked. The current mainstay of treatment is systemic corticosteroids and immunosuppressive agents. Nevertheless, long-term use of these drugs can easily cause infections and other life-threatening adverse reactions. Therefore, many targeted therapies have been gradually introduced and used for the treatment of pemphigus. Among these therapies, rituximab has been approved as a first-line treatment for moderate to severe cases of pemphigus vulgaris (PV) in some countries. With an increasing number of targeted therapies are being tested in clinical trials, in this study, we review the research progress on the mechanism of targeted therapies for pemphigus.
Pemphigus diseases are a group of autoimmune blistering diseases affecting the skin and mucous membranes. The main clinical manifestations of pemphigus are intra-epidermal blistering and flaccid blisters, caused by the loss of cell-cell adhesion due to autoantibodies against cell adhesion proteins desmogleins 1 (Dsg1) and desmogleins 3 (Dsg3)[1]. Pemphigus diseases include pemphigus vulgaris (PV), pemphigus foliaceus (PF), pemphigus vegetans, pemphigus erythematosus, pemphigus herpetiformis, paraneoplastic pemphigus (PNP), IgA pemphigus, and drug-induced pemphigus. PV is the most common and severe form of pemphigus, mediated by anti-Dsg1 and anti-Dsg3, affecting oral mucosa and skin. Painful oral lesions are usually the first manifestation. Compared to PV, PF is mainly mediated by anti-Dsg1 and only affects the skin[1]. Due to the defects in the skin barrier, leakage of exudate onto the skin surface can easily attract bacteria to multiply on-site, with subsequent skin infections that may threaten the life of patients. The diagnosis of pemphigus requires clinical presentation and histopathology consistent with pemphigus and a positive direct immunofluorescence (DIF) microscopy or serologic detection of autoantibodies against epithelial cell surface antigens. The Pemphigus Disease Area Index (PDAI) and the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) are the usual scoring systems used to evaluate the extent and activity of pemphigus[2]. Since the advent of glucocorticoid (CS) therapy for the treatment of pemphigus in 1950, the prognosis of pemphigus has largely and rapidly improved. Afterward, using CS alone or combined with immunosuppressive agents is the main treatment for pemphigus[1-5]. Despite controlling the disease, long-term high-dose CS therapy inevitably causes some adverse reactions, such as headaches, insomnia, obesity, fluid retention,

osteoporosis, cardiovascular diseases, type 2 diabetes mellitus, and insulin resistance. During the past few decades, treatment methods have been tremendously updated based on a better understanding of the pathogenesis. In this study, we review the recent advances in the mechanism of targeted therapy for pemphigus. According to our current understanding of pemphigus pathogenesis, we classified the emerging targeted therapy into five categories: (1) elimination of autoreactive B cells[6]; (2) modulation of autoantibody (IgG) half‐life[6]; (3) inhibition of inflamatory markers; (4) immunological checkpoint receptors agonists; (5) inhibition of the blister‐inducing activity of autoantibodies[6].

PO-015

Up-regulation of FOXO3a inhibits the proliferation and extracellular matrix in keloid fibroblast cells

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Background and purpose Keloid is a fibroproliferative disorder resulting from abnormal wound healing processes, and is believed to be unique to human skin. Common risk factors are genetic predisposition, wound trauma, foreign body reactions, mechanical stretch, and immune dysfunction, but there are still mechanisms that are not clear and challenge in addressing the clinical problem of recurrence and drug resistance. Forkhead box protein O3a（FoxO3a）, a member of the forkead family of transcription factors characterized by a highly conserved forkhead DNA-binding domain, is involved in cell metabolism, proliferation, differentiation and apoptosis. Based on the detailed roles of FOXO3a in anti-fibrosis and anti-cancer, there might be especially relevant to keloid. This study aimed to intend to investigate the effect of FOXO3a on keloid at the cellular and tissue levels and further elucidate the related mechanisms.
Methods The expression pattern of FOXO3a in keloid was initially analyzed usage of GEO database. Immunohistochemical techniques were used to observe FOXO3a protein distribution and expression, and tissue proteins were extracted for semi-quantitative detection by Western blot. Cellular proteins were extracted for FOXO3a Western blot assay. To determine the expression level of FOXO3a in keloids, establish the establishment of lentivirus-mediated stable transplants for overexpression of FOXO3a KFs and knock down FOXO3a in normal fibroblasts using siRNA technology. Cell proliferation, cell cycle, apoptosis changes and the expression of extracellular matrix-associated proteins were detected by CKK8,flow cytometry and Western blot, respectively. The Western blot was used to detect the protein of β-catenin in tissues. The correlation between FOXO3a and β-catenin was analyzed by Pearson's correlation algorithm. Furthermore, co-immunoprecipitation was used to further reveal the specific relationship between the two.
Results A total of 250 differential genes were selected between keloid and normal skin gene chips, and 2720 differential genes were selected between keloid and normal scar gene chips. The common differential gene species were obtained from keloid comparison with normal scar and normal skin, respectively, contained FOXO3a. FOXO3a immunohistochemistry showed a significant decrease in FOXO3a positive expression in keloid dermis compared with normal tissues ($P<0.05$). A lentivirus-mediated FOXO3a overexpression KFs stable transfer strain was established, and the results detected by qRT-PCR and Western blot, respectively, showed that FOXO3a expression was significantly higher at both the nucleic acid and protein levels ($P<0.05$) while siRNA treatment was performed in normal fibroblasts, and Western blot results showed that the three sequences were effective sequences ($P< 0.05$) and the knockdown rate was about 60%.The proliferation ability of KFs cells with up-regulated FOXO3a was significantly decreased ($P<0.05$) and the apoptotic level was significantly higher ($P<0.05$), the percentage in the G0/G1 phase of the cell cycle was significantly higher ($P<0.05$), while the percentage in the S phase was significantly reduced ($P<0.05$), and correspondingly, the expression of fibrosis-phenotype-related proteins collagen I, collagen III, FN and α-SMA proteins in KFs was significantly reduced ($P<0.05$).The proliferation ability of NFs with FOXO3a knockdown was significantly higher ($P < 0.05$), the

percentage of cells in G2/M phase of the cell cycle was significantly increased, and the protein expressions of α-SMA protein and external matrix-associated proteins collagen I, collagen III, and FN of the corresponding NFs were significantly increased (P<0.05).Next, β-catenin, the main coregulator of FOXO3a, was found to be significantly higher in keloid tissues than in normal tissues in Western blot results (P<0.05) .A negative correlation was found between the two by Pearson calculation (P<0.05).In cell co-immunoprecipitation experiments, it was found that FOXO3a could bind to β-catenin, and the binding of FOXO3a to β-catenin interfered with the binding of β-catenin to TCF4.

Conclusion Taken together,these findings indicated that FOXO3a relieves cell proliferation, apoptosis and extracelular matrix expression by negatively regualting beta-catenin,which indicates the potential role of FOXO3a in the keloid formation.

PO-016

Clinical characteristics and treatment of 14 patients with SAPHO syndrome

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Objective Toanalyze and summarize the clinical features of 14 patients with SAPHO syndrome.

Methods Theclinical data, treatment and curative effect of 14 patients with SAPHO syndrome were analyzed retrospectively.

Results There were 8 males and 6 femaleswith an average age of onset of (28.43 ± 13.38) years.All 14 patients had skin lesions (8palmoplantar pustulosis, 2 acne vulgaris, 3 cystic acne, and 1 acne conglobata).The bone and joint involved in the lesions in imaging were theanterior chest wall (13/14), sacroiliac joint (11/14), limb bone and joint(6/14), spine (6/14), shoulder joint (3/14), and pelvis (2/14).Thirteen patients were treated with non-steroidal anti-inflammatory drugs,glucocorticoids, disease-modifying anti-rheumatic drugs and tumor necrosis factor-α(TNF-α) antagonists, of which 9 patients were successively treated with TNF-αantagonists.Thirteen patients were followed up. Nine patients had poor control of skin lesions or bone and joint swelling and pain symptoms after treatment with glucocorticoids, non-steroidal anti-inflammatory drugs and DMARDs, of which 5 patients had different degrees of improvement of skin and bone and joint symptoms after treatment with TNF-α inhibitors,2 patients were changed to Yisaipu after aggravation of rash after combined use of infliximab, and 1 patient refused treatment.

Conclusion In this group, SAPHO syndrome is predominantly male.The main bone and joint involved is the anterior chest wall.The main skin lesions are palmoplantar pustulosis.Palmoplantar pustulosis is more common in females and severe acne is more common in males.TNF-α antagonists are effective in refractory patients, especially in combination with DMARDs to rapidly control osteoarticular disease, but may aggravate skin lesions.For this disease, it is recommended to use a multidisciplinary collaborative diagnosis and treatment model to give patients the best diagnosis and treatment plan and comprehensive medical resources and improve the prognosis and quality of life.

PO-017

Research advances of biomarkers associated with malignant progression from actinic keratosis

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Actinic keratosis (AK) is a precancerous skin damage caused by long-term sun exposure, which may progress to cutaneous squamous cell carcinoma(cSCC) at a rate estimated between 0.025% and 16% for an individual lesion per year. Bowen's disease (BD) is an in-situ squamous cell carcinoma of epidermis. The clinical manifestations and histopathological types of AK, BD and cSCC are diverse. Their differential diagnosis and early diagnosis of cSCC have always been a research hotspot. In this review, p16, lumican, Hsp70, p53, Claudin-1, MMP-13, EZH2 and other biomarkers related to malignant progression from AK were reviewed in order to provide a reference for early clinical diagnosis and further research.

PO-018

Correlation between chronic spontaneous urticaria and serum anti-FcεRI antibody and C5a

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Objective 35% to 40% of patients with chronic urticaria have IgG autoantibodies to the alpha subunit of the FceRI receptor, and 5% to 10% of patients have IgG anti-IgE autoantibodies. These antibodies activate mediators such as histamine from basophils and mast cells, which require activation of the classical complement pathway. The relationship between serum anti-FceRI autoantibodies and C5a levels in patients with chronic spontaneous urticaria was preliminarily explored for the relationship and impact of two pathways that stimulate mast cell degranulation; , development and prognosis.
Methods From October 2021 to October 2022, the condition evaluation scale and skin lesion photos of patients diagnosed with chronic spontaneous urticaria (meeting the admission and exclusion criteria) admitted to the Dermatology Outpatient Department and the Inpatient Department of the First Affiliated Hospital of Shihezi University School of Medicine were collected. A total of 40 cases of blood samples and 40 cases of blood samples from healthy people who have undergone physical examination (meeting the inclusion and exclusion criteria) were obtained. The clinical data of the two groups were obtained through medical history inquiry and questionnaires, and the antibody levels between the two groups were analyzed and compared by SPSS 26.0 system. difference.
Results The levels of serum anti-FcεRI antibody and C5a in patients with chronic spontaneous urticaria were significantly higher than those in healthy people (P value < 0.01), and there was a positive correlation between the levels of serum anti-FcεRI antibody and C5a in patients with chronic spontaneous urticaria There was no positive correlation between serum anti-FcεRI antibody and C5a levels and disease severity in patients with chronic spontaneous urticaria (all P>0.05).
Conclusion Mast cell-related autoantibodies-anti-FcεRI and C5a are associated with chronic spontaneous urticaria; the increase of C5a level promotes the increase of anti-FcεRI level; serum anti-FcεRI antibody and C5a levels have no correlation with the severity of the disease.

PO-019

The mechanism of asiaticoside in promoting the healing of diabetic ulcers by activating the Wnt/β-catenin signaling pathway

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Objective Diabetic ulcers (DU) are the most common complication of diabetes. Although the level of medical care in China has been improving, the mortality and amputation rate caused by DU are still at a high level. Promoting cell proliferation and migration, collagen synthesis, angiogenesis and skin regeneration are very important for DU wound healing. There are many
physiological activities of Chinese herb Centella asiatica, and its monomer Asiaticoside (AC) plays a regulatory role in cell proliferation, angiogenesis and inflammatory response. This study is to explore the effects and mechanisms of AC on DU wound healing through cell and animal experiments.
Methods In the first part, we treated the HFF-1 cells with different concentrations of AC (0, 62.5, 125, 250, 500, 1000μM) for 24h. Based on the preliminary results of our studies, HFF-1 cells were divided into five groups: Control group, Model group, 125μM group, 250μM group and 500μM group. The effects of AC treatment for 24 hours on the viability, proliferation and
migration of HFF-1 cells were detected by CCK-8 assay, cell scratch assay, Transwell assay, flow cytometry and cell cycle analysis. In order to clarify the relationship between mechanism of the effect of AC on the viability, proliferation, migration of HFF-1 cells and Wnt/β-catenin. Western blot was used to detect Wnt1, Wnt3a, Wnt4, Wnt5a and Wnt7a in HFF-1 cell supernatant, GSK3β, phos-GSK3β(Ser9), nuclear β-catenin, total β-catenin in cell lysate and expression of downstream target genes Axin-1 and c-myc of Wnt/β-catenin
signaling pathway. In addition, HFF-1 cells were stimulated by both Wnt pathway inhibitor (DKK-1 20ng/mL) and the best concentration of AC (250μM). They were divided into Model group, 250μM-AC group and DKK-1 + 250μM-AC group. CCK-8 assay, cell scratch assay, Transwell assay, flow cytometry and cell cycle analysis were used to detect the effect of AC treatment on the activity, proliferation and migration of HFF-1 cells after 24 hours and its relationship with Wnt/β-catenin signaling pathway. The expression of GSK3β, phos-GSK3β(Ser9), nuclear β-catenin, total β-catenin in HFF-1 cell lysates and the downstream target genes Axin-1 and c-myc of Wnt/β-catenin signaling pathway were detected
again by Western blot. In the second part, we randomly divided 54 rats into blank control group (Control group), diabetic model group (Model group), 4%AC group, 8% AC group, 16%AC group, positive control group (recombinant bovine basic fibroblast growth factor, rb-bFGF) (n=9). All rats were intraperitoneally injected with 1% streptozotocin (STZ) to establish a diabetic rat model, except blank control group. Two full-thickness wounds of 12 mm in diameter were made on the dorsal surface of anaesthetized rats. The distance between both wounds was approximately 15 mm, that is the formation of DU model. The general state, blood glucose, body weight, water consumption, diet, wound healing area and
wound healing rate of rats in each group were observed. On the 3rd, 7th and 12th day after treatment, the skin tissue in and around the wound was cut. The structure of wound tissue, the number and distribution of inflammatory cells, fibroblasts, collagen and capillaries during wound healing were observed by H&E staining and Masson staining. In addition, we detected the expression of Wnt1 protein in the wound tissue of rats in each group on the 3rd, 7th and 12th day after treatment by immunohistochemistry. Western blot was used to detect the expression of Wnt/β-catenin signaling pathway-related proteins GSK3β, phos-GSK3β (Ser9), nuclear β-catenin, total β-catenin and downstream target genes Axin-1 and c-myc in the wound tissues of rats in each group on the 3rd, 7th, and 12th days after treatment.

Results 1. Relative to the normal group, when the concentration of AC was 1000μM, the viability of HFF-1 cells was remarkably reduced, showing certain cytotoxicity (P<0.01). AC at 125μM, 250μM and 500μM were chosen for follow-up experiments.
2. In a high glucose environment, HFF-1 cells were treated with AC at a concentration of 250 and 500μM for 24h. The results of CCK-8 assay, cell scratch assay, Transwell assay, flow cytometry, and cell cycle analysis showed that compared with the diabetes Model group (the Model group), AC could promote the viability, proliferation and migration of HFF-1 cells (P<0.05).
3. In a high glucose environment, HFF-1 cells were treated with AC at a concentration of 250 and 500μM for 24h. Western blot results showed that compared with the Model group, the expression of Wnt1 and Wnt4 in cell supernatant, phos-GSK3β(Ser9), total β-catenin and nuclear β-catenin in cell lysate were up-regulated (P<0.05). At the same time, the Wnt/β-catenin signaling pathway was activated to transcribe the downstream target genes c-myc and Axin-1, which increased the expression of c-myc and decreased the expression of Axin-1 (P<0.05). AC may promote the viability, proliferation and migration of HFF-1 cells by activating the Wnt/β-catenin signaling pathway under high glucose environment.
4. In a high glucose environment, AC at a concentration of 250μM acted on HFF-1 cells for 24 hours. The results of CCK-8 method, cell scratch test, Transwell assay, flow cytometry and cell cycle analysis showed that compared with the Model group, the results showed that, AC promoted the viability, proliferation and migration of HFF-1 cells (P<0.05). Adding Wnt
pathway inhibitor DKK-1 reversed the effect of AC (P<0.05).
5. In a high glucose environment, HFF-1 cells were treated with AC at a concentration of 250μM for 24h. Western blot results showed that compared with the Model group, the expression of phos-GSK3β(Ser9), total β-catenin and nuclear β-catenin in cell lysate were up-regulated (P<0.05). At the same time, the Wnt/β-catenin signaling pathway was activated to transcribe the downstream target genes c-myc and Axin-1, which increased the expression of c-myc and decreased the expression of Axin-1 (P<0.05). After adding Wnt pathway inhibitor DKK-1, the expression of the above proteins were reversed (P<0.05). It was further proved that AC may promote the viability, proliferation and migration of HFF-1 cells by activating the Wnt/β-catenin signaling pathway under high glucose environment.
6. After a single high-dose intraperitoneal injection of 1% STZ, rats appeared listlessness, thin body, reduced exercise, dry hair, heavy odor in the cage, and wet bedding. Compared
with the Control group, the diabetic rats had significantly higher blood sugar, decreased body weight, and increased water and diet (P<0.01). Compared with the Model group, the wound area of the 8% AC group was significantly reduced on the 7th and 12th day, and the healing rate was significantly increased (P<0.01). Compared with the Model group, the
8% AC group had fewer inflammatory cells, more fibroblasts, thicker granulation tissue, abundant new capillaries, and the most collagen fiber deposition. It is suggested that AC can promote the healing of DU wounds, reduce the area of wounds, and accelerate the healing rate of wounds, and the best effect is at 8% concentration.
7. The results of Western blot on the wounds of rats showed that compared with the Model group, the phos-GSK3β(Ser9), total β-catenin and uclear β-catenin expression in the wound tissue of the diabetic rats in the 8% AC group were significantly up-regulated on the 7th and 12th day. (P<0.01), the Wnt/β-catenin signaling pathway was activated to transcribe the downstream target genes c-myc and Axin-1, which significantly increased the expression of c-myc and decreased the expression of Axin-1 (P<0.01). The immunohistochemical results of rat wounds showed that in contrast with the Model group, Wnt1 content increased after AC administration. When the AC concentration was 8%, the Wnt1 content increased most remarkably, and there was significant statistical significance (P < 0.05). AC can up regulate the expression of Wnt1 in skin tissue of diabetic wound model. In conclusion, AC
may promote the healing of DU through Wnt/β-catenin signaling pathway.
Conclusion Based on the high glucose-induced HFF-1 cell impaired model and DU rat model, we found that AC can enhance the activity, proliferation and migration of HFF-1 cells, promote
the proliferation of fibroblasts, the increase of granulation tissue, the formation of capillaries and the deposition of collagen fibers in the wound microenvironment, which may promote the healing of DU. The mechanism may relate to Wnt/β-catenin signaling pathway.

PO-020

Therapeutic Effect and Molecular Mechanism of Oncostatin M-regulated Senescence-autophagy Crosstalk in Psoriasis: An Update

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Background Psoriasis is a common but difficult-to-treat chronic, inflammatory skin disorder. Although the etiology of psoriasis is not fully understood, dysregulations in cell programmed death in keratinocytes were reported, suggesting its role in the pathogenesis of psoriasis. In our previous studies, we found that Oncostatin M (OSM) was significantly overexpressed in psoriatic lesions, and it was related to the expressions of typical cellular senescence and autophagy markers. We therefore hypothesized that OSM-regulated senescence-autophagy interaction in keratinocytes is related to the maintenance of stability in epidermal cells and skin barrier, and thus playing an important role in the pathogenesis of psoriasis.
Objective To explore the molecular mechanism of psoriasis from the aspect of senescence-autophagy interaction in keratinocytes regulated by OSM, anticipating the provision of a novel biomaker for targeted therapy in psoriasis.
Methods We utilized whole transcriptome sequencing, proteomics, immunohistochemistry and immunostaining for assessment of the clinical samples; primary cell culture, CRISPR-Cas9 genome editing, non-viral transient and lentivirus-mediated stable transfections, real-time polymerase chain reaction (rtPCR), immunoprecipitation, Western blot for investigation of the therapeutic effects of OSM; protein-protein interaction (PPI) online databases incorporating with dynamic transcriptomic and proteomic inputs for exploration of the role of OSM in the PPI network.
Results A significant level of overexpression in OSM and its receptor OSMR limited to the murine psoriasiform skin was observed. We report that a novel fusion protein targeting OSM we since synthesized incorporating soluble OSMR, glycoprotein (gp) 130, and human IgG fragment crystallizable (fc) region, could competitively bind to OSM and effectively neutralize its inhibitory function in proliferation of A375 human melanoma cells. The role JAK-STAT pathway plays in the relationship between OSM and senescence-autophagy interaction in keratinocytes was indicated in bioinformatics, PPI and Western blot. The use of the aforementioned novel fusion protein was well received in the psoriasiform murine model, a reversal of the level of overexpression in OSM and normalization of autophagy level in the skin, as well as an inhibition of the downstream phosphorylation of STAT3 were also achieved.
Conclusion: Our findings demonstrated the role of OSM in the regulation of senescence-autophagy interaction in keratinocytes, identified its molecular mechanism and biological function in the pathogenesis of psoriasis, and introduced a novel fusion protein targeting OSM as a promising therapeutic measure in the treatment of psoriasis.

PO-021

Inhibition of YAP reduces Smad4 expression and sumoylation in keloid fibroblasts

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Objective YAP is a key effector molecule in the Hippo signaling pathway, which can affect the proliferation, migration, and apoptosis of keloid fibroblasts (HKFs) and plays an important role in keloid formation. TGF-β (transforming growth factor-β, TGF-β) signaling pathway is recognized to

be involved in regulating the whole process of trauma repair including keloid development, while Smad4 is a common component in Smad-mediated TGF-β signaling. The aim of this study was to explore the cross-linking between Hippo and TGF-β signaling pathways and the possible mechanisms in keloid fibroblast activation.
Methods Fibroblasts were isolated from keloid tissues and cultured in vitro. siRNA interference was used to reduce the expression of YAP or Smad4, and changes in the expression levels of relevant factors in HKFs were studied by quantitative RT-PCR and western blot. In addition, the interaction of YAP and Smad4 sumoylation was examined by Co-Immunoprecipitation (CoIP). Apoptosis and proliferation of HKFs were analyzed by flow cytometry and BrdU assay after YAP inhibition with addition of CA3, inhibition or enhancement of Smad4 sumoylation.
Results Inhibition of YAP reduces Smad4 expression and expression of SUMO1, 2 and 3 in keloid fibroblasts, but knockdown of Smad4 did not change the expression of YAP, SUMO1, 2 and 3. Enhancing sumoylation of Smad4 increased the HKFs proliferation and reduced HKFs apoptosis, while inhibition of Smad4 sumoylation has opposite effects. CoIP confirmed that the SUMO1 bind to Smad4 and downregulation of YAP reduced the binding of SUMO1 and Smad4.
Conclusion YAP promote keloid formation through cross-talking of Hippo and TGF-β signaling pathways mediated by YAP and Smad4 interaction.

PO-022

The IRF7/CTSS axis is a pivotal component for cutaneous scar formation and may serve as a potential therapeutic target

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Background and objective Scarring after skin injury is a challenging clinical problem. Fibroplasia in adult skin due to a variety of causes, including trauma, burns, infection, or surgery, often results in multiple types of scarring, among which the most difficult pathological types are hypertrophic scars and keloids. Scars can cause discomfort, such as itching or reduced mobility, and can also have adverse effects on mental health. This psychological pressure makes patients seek effective means to beautify or reduce scarring. However, the current scar treatment methods are still limited. Studies have shown that the skin in the early embryo will regenerate perfectly after injury, but this ability is lost in the late embryo, resulting in two completely different outcomes due to embryonic development. Early studies on embryonic trauma were mostly based on large mammalian models, while mice were gradually included in the scope of research due to their short gestation period, low cost, and high homology to humans. However, due to the high difficulty of surgery, the scope of current research is still limited. Unlike near-normal healing after age-injury in mice in early gestation (embryonic day of 16, E16), wound healing in late gestation (embryonic day of 18, E18) and adulthood is accompanied by fibrosis. And the mechanisms that lead to this transition are largely unknown. Therefore, we aimed to explore the causes of scarring from the perspective of fetal development, find the key genes that lead to scarring in the late embryo and combine them with adult scar treatment to provide new ideas for clinical treatment of pathological scars.
Methods 1. In the first part of this study, C57B1/6J mice were used as the research object, and the mouse fetal full-thickness skin wound model was stably constructed at different embryonic stages (E16 and E18), and the generalized repaired images of E16 and E18 were photographed. The photo shows the difference between embryonic E16 and E18 healing after injury. The tissue after injury was also obtained for H&E staining to analyze the difference in the tissue structure of the repaired area between the E16 and E18 groups. The collagen structure of the two groups was analyzed by Sirius red staining, and the difference in collagen structure was evaluated by FD (fractal dimension) and L (lacunarity). At the same time, immunohistochemical staining of TGF-β3 was performed on the skin sections of the E18 scarring group to evaluate the scars. 2. The second part of this study is based on the results found in the first part. 24 hours after injury, the skin tissue

of mice on day E18 and their self-controls were selected for transcriptomic sequencing (n=3). The results were subjected to bioinformatics analysis, including GO analysis and KEGG analysis, involving the biological process, molecular function and cellular components of differentially expressed genes as well as enrichment of related functional signaling pathways. qRT-PCR was used to verify the expression of the screened differential genes between the sample 24h after E18 injury and the normal E18 controls. 3. In the third part of this study, qRT-PCR, Western Blot and immunohistochemical staining experiments were used to examine the changes of CTSS mRNA and protein levels in different embryonic stages after injury. The mechanism of CTSS in vitro was explored in human skin fibroblasts. After synthesizing CTSS knockdown plasmids, cells were transfected and the knockdown effect was confirmed by qRT-PCR and Western Blot experiments. Protein expression levels of collagen type I, collagen type III and fibronectin were detected. Then, the expression of IRF7 in the damaged tissues of embryos at different stages was detected by qRT-PCR, Western Blot and immunohistochemical experiments. The possible binding sites of IRF7 and CTSS were predicted using bioinformatics analysis tools, and the IRF7 overexpression plasmid and blank control were synthesized. Combined with dual luciferase reporter experiments and chIP experiments, the binding of IRF7 to the CTSS promoter region was confirmed. Meanwhile, IRF7 was overexpressed in fibroblasts, and the changes of CTSS levels were detected by qRT-PCR and Western blot experiments. The mRNA and protein levels of scarring-related genes (collagen type I, collagen type III and fibronectin) were tested to verify the mechanism of the IRF7/CTSS regulatory axis in scarring, and the reverse experiment was used to further clarify the regulatory role. Finally, qRT-PCR, Western blot, and immunohistochemical experiments were used to detect the expression levels of IRF7 and CTSS in skin tissue of human keloid patients. The adult mouse hypertrophic scar model was used to study the in vivo mechanism of CTSS, and the CTSS inhibitor LY30003283 was administered to the injured skin to observe the curative effect. The scar area and the expression of myofibroblast marker α-SMA (alpha smooth muscle actin) were detected by H&E staining and immunohistochemical experiments.

Results 1. In the embryonic stage E16 and E18, the lesions healed within 48 hours, but the skin was completely regenerated as normal on E16 and the skin was scarred on E18. The results of H&E staining and Sirius red staining showed that the tissue structure on E16 and E18 days was significantly different, and the healed tissue after E18 day was similar to the scar structure. The expression of TGF-β3 in the scar area after injury at E18 was less than that in the control group, demonstrating the activation of the scar-producing effect in the injury area. 2. Through transcriptomic sequencing of the skin tissue of mice 24 hours after injury on day E18 and their own normal controls, 283 differentially expressed genes were found, including 258 up-regulated genes and 25 down-regulated genes. Biological process included dysregulated genes that were increased in responses to stimuli, metabolic processes, and intercellular communication. Molecular functions are more reflected in protein binding and nucleic acid binding. The top ten most relevant signaling pathways were analyzed by GO and KEGG enrichment, and the most significant common pathway in the two results was found—TLR signaling pathway. The differential genes of the TLR signaling pathway were verified to be consistent with the sequencing results. 3. The CTSS in the TLR pathway was selected according to the significance and difference fold of the differential genes, and it was verified that it showed differences in temporal expression after E16 and E18 injury. The E18 day group that produced scarring was significantly up-regulated in the injured tissue. In vitro experiments confirmed that inhibiting the expression of CTSS can significantly reduce the expression of collagen type I, collagen type III and fibronectin in human skin fibroblasts. At the same time, the only transcription factor IRF7 in the TLR pathway was found to exhibit consistent temporal expression differences in CTSS. Dual-luciferase reporter assays and chIP assays confirmed the binding of IRF7 to the CTSS promoter region; overexpression of IRF7 demonstrated the up-regulation of CTSS expression by IRF7. Up-regulation of the IRF7/CTSS regulatory axis can promote the expression of collagen type I, collagen type III, and fibronectin in fibroblasts, and the up-regulation of downstream proteins by overexpression of IRF7 can be reversed by knockdown of CTSS. IRF7 and CTSS were also significantly up-regulated in the skin tissue of patients with keloids; CTSS inhibitors in vivo could significantly reduce the hyperplasia of scars and inhibit the activation of myofibroblasts.

Conclusion 1. The skin of E16 and E18 days of embryonic development can be repaired after 48 hours. E16 wounds can be healed without scars, but scars appear after E18 wounds in the late embryonic stage. Day E18 is a critical developmental time point for the transition from scarless repair to scarring in embryonic wounds. 2. At 24h after trauma on day E18 in the late embryonic stage of mice, it is in the transitional stage from the inflammatory response period to the proliferative remodeling period after trauma, in which the activation of TLR signaling pathway plays an important role. 3. The differential expression of the IRF7/CTSS regulatory axis in late embryos promotes the formation of post-traumatic scars, and IRF7 acts as a transcription factor to activate the transcription of CTSS. 4. IRF7 and CTSS have different expressions after trauma in different embryonic stages. The differential expression can last from late embryonic stage to adulthood, which is closely related to the formation of pathological keloids in adulthood. 5. Targeted inhibition of CTSS expression can effectively inhibit scar formation and myofibroblast activation after trauma in adult mice.

PO-023

Case report：multiple body rash (head, face, scrotum, hand and foot syndrome) caused by sunitinib malate capsule

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A 34-year-old male patient presented to the clinic for one month with multiple skin rashes all over his body. The patient was diagnosed with renal cell carcinoma in 2020,and underwent "right nephrectomy" at West China Hospital. He received sunitinib malate capsules50mg qd orally from April 26, 2020. He developed a rash all over his body a week later. Scalp folliculitis, facial dermatitis, scrotal dermatitis, hand-foot syndrome. He got better when he stopped taking the medicine. Sunitinib malate is a small molecule that inhibits multiple receptor tyrosine kinases (RTK), some of which are involved in tumor growth pathologic angiogenesis and tumor metastasis. Common skin adverse reactions include rash, hand-foot syndrome, skin discoloration and dry skin.

PO-024

Study on the mechanism of microRNA-203b-3p targeting COL17A1 in aging skin affecting the stemness and proliferation of epidermal stem cells

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Objective To investigate the changes in the expression level of COL17A1 in the skin of young and old mice during skin aging and its effect on the stemness and proliferation of epidermal stem cells (ESCs), and to explore the effects of microRNA-203b-3p (miR-203b-3p) on the expression of COL17A1 of ESCs in the skin of mice of different ages.
Methods A total of 24 C57BL/6 mice aged 2 months (young) and 24 months (old) were taken, 12 in each group. Full-thickness skin samples were taken from the same position on the lower back of mice. After hematoxylin-eosin staining, the age-related changes of epidermal thickness were observed under microscope. The changes of basement membrane and hemidesmosomes in skin of different ages were observed under transmission electron microscope. The expression level of COL17A1 in the skin samples was detected by real-timefluorescent quantitative PCR, western blotting and immunofluorescence. Human ESCs were randomly divided into blank control group,

transfection reagent group, empty plasmid group (U6-MCS-Ubiquitin-Cherry-IRES-puromycin group), knockdown plasmid group (U6-COL17A1-Ubiquitin-Cherry-IRES-puromycin group) according to the random number table method. After transfection for 48 h, the changes of COL17A1 mRNA were detected by real-time fluorescent quantitative PCR. The expression levels of COL17A1 and CK14, a stemness marker of ESCs, were detected by western blotting. The changes in cell proliferation were compared by CCK-8 method. Bioinformatics analysis was used to predict miRNAs that may act on the 3' untranslated region (UTR) of COL17A1 mRNA. After transfecting the target miRNA mimic into human ESCs for 48 h, the expression of COL17A1 protein was detected by western blotting. The miRNAs that could cause the decrease expression of COL17A1 were screened and their expression level changes with age in the skin of young (18-25 years old) and elderly (over 70 years old) people were searched through the miRNA sequencing database GSE114006 on the GEO website. Screening of miRNAs with increasing expression in human aged skin, real-time fluorescent quantitative PCR was used to verify the changes of their expression levels in the skin of mice of different ages. With the patient's
informed consent, the fresh foreskin tissues of healthy adult male were collected after surgery, and human ESCs were extracted and divided into siCHECK-WT-COL17A1+miR-203b-3p negative control group, psiCHECK-WT-COL17A1+miR-203b-3p mimic group, psiCHECK-MUT-COL17A1+miR-203b-3p negative control group, psiCHECK-MUT-COL17A1+miR-203b-3p mimic group, respectively transfected with the corresponding sequence. After transfection for 48 h, the luciferase reporter gene detection kit was used to detect the firefly luciferase and renilla luciferase of each group. The gene expression level was reflected by their ratio. The number of samples in the cell experiment was 3. Two independent sample t test, one-way analysis of variance, Mann-Whitney or Kruskal-Wallis rank sum test were performed on the data, and the difference was statistically significant with $P<0.05$.

Results After hematoxylin-eosin staining, the average thickness of the epidermis was (13.24 ± 2.23) μm of young mice and (3.67± 0.71) μm of old ones, which was only 27.72% of that of young mice ($Z=-2.882$, $P=0.004$). Transmission electron microscopy showed that the basement membrane of young mice was intact, containing a large number of hemidesmosome structures (9.00 ± 2.16 pieces/10 μm) which were arranged in an orderly manner and evenly distributed in the epidermis-dermis junction zone. Meanwhile, the morphology of the basement membrane of old mice was intermittent, with fewer hemidesmosomes (3.67 ± 0.75 pieces/10 μm) which was unevenly distributed ($Z=-2.908$, $P=0.004$). The real-time fluorescent quantitative PCR method found that the expression level of COL17A1 mRNA in the skin of old mice was only 11.92% of that of young mice ($t=10.61$, $P<0.01$). Western blotting results showed that the expression level of COL17A1 protein in the skin of young mice was significantly higher than that of old ones, about 3.98 times ($t=6.846$, $P<0.01$). The results of immunofluorescence experiments showed that COL17A1 was mainly distributed in the basal layer of the epidermis and the hair follicle bulb in the skin of young mice; while the expression of COL17A1 in basal layer of epidermis and hair follicle bulb of the old mice was significantly reduced with about 31.34% the relative fluorescence intensity of young mice ($Z=-2.242$, $P=0.025$). For human ESCs, the real-time fluorescent quantitative PCR method found that compared with the blank control group, the mRNA and protein expression levels of COL17A1 in the transfection reagent group and the empty plasmid group did not change significantly ($P>0.05$). In the knockdown plasmid group, the mRNA and protein expression levels of COL17A1 were significantly reduced ($P=0.002$, 0.034). At the same time, compared with the blank control group, the ESCs stemness marker CK14 in the transfection reagent group and the empty plasmid group was
not changed significantly ($P=0.504$, 0.631), but reduced in the knockdown plasmid group ($P=0.015$). The CCK-8 method detected that compared with the blank control group, the absorbance of cells in the transfection reagent group and the empty plasmid group was not changed significantly at 450 nm ($P=0.985$, 0.984) but reduced in the knockdown plasmid group ($P=0.041$). According to bioinformatics analysis, hsa-miR-203a-3p, hsa-miR-203b-3p, hsa-miR-512-5p, has-miR-124-3p, hsa-miR-28-5p, hsa-miR-590-3p, and hsa-miR-329-5p may bind to the 3' UTR region of COL17A1 mRNA. Western blotting showed that the expression level of COL17A1 decreased slightly after the ESCs were incubated with the mimics of hsa-miR-329-5p ($P=0.031$). The expression level of COL17A1 decreased significantly after the ESCs were incubated with the mimics of hsa-miR-203b-

3p and hsa-miR-203a-3p (P=0.0004, 0.0007). Searching through the expression data of these three miRNAs in the GSE114006 database, we found that the expression levels of hsa-miR-329-5p, hsa-miR-203b-3p, and hsa-miR-203a in the skin of the elderly were respectively 1.05 times (t=0.792, P=0.431), 1.47 times (t=3.270, P=0.002) and 1.04 times (t=0.776, P=0.441) of that of the young. It was further verified in the skin of young and old mice that the expression level of miR-203b-3p in the skin of old mice was significantly higher than that of the young (Z=-2.882, P=0.004). After the transfection of the dual luciferase plasmid for 48 h, the luciferase/renilla luciferase ratio was 5901.15 ± 739.57 of psiCHECK-WT-COL17A1+miR-203b-3p mimic group and 10244.55 ± 645.84 of the psiCHECK-WT-COL17A1+miR-203b-3p negative control group (t=7.662, P=0.002). Meanwhile, the luciferase/renilla luciferase ratio was 5653.55 ± 60.76 of the psiCHECK-MUT-COL17A1+miR-203b-3p mimic group and 6184.73 ± 594.25 of the psiCHECK-MUT-COL17A1+miR-203b-3p negative control group (Z=-0.655, P=0.513).
Conclusions COL17A1, as a hemidesmosome constituent protein, was mainly distributed on the basement membrane in the epidermis. Its mRNA and protein expression levels decreased with age, which may cause epidermal stem cells to detach from the epidermal basement membrane. Decreased expression of COL17A1 could inhibit the expression of CK14 and the proliferation of ESCs, which may be the reason for the thinning of the epidermal layer and the slowing down of wound healing in aging skin. The increased expression of miRNA-203b-3p in aged skin could target and bind to the 3' UTR region of COL17A1 mRNA, hinder the post-transcriptional translation process, and reduce the expression of COL17A1 protein in aging skin.

PO-025

The study on the pharmacological effects of Shengqing anti-itch lotion against chronic eczema model caused by DNFB

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Objective To study the therapeutic effect of Shengqing anti-itch lotion against chronic eczema, providing reliable references to clinical treatment.

Methods repeatedly paint 2，4-DNFB （dinitrofluorobenzene）on the skin of mice to replicate chronic eczema model, meanwhile add Shengqing anti-itch lotion of different concentration for the purpose of inspect the drug effect.
Results After the model mice was stimulated, its back skin gradually thicken and became rough and dry, carrying with blood scab on its surface. And there was just a small amount of hair remaining growing on it. The mice appeared to be agitated and kept turning round frequently, and the skin of its back thicken, skin inflammatory cell infiltration obviously increased. Compared with the skin of mice of model group, the mice skin of dosage groups carrying Shengqing anti-itch lotion that was twice as much as the dosage for clinical application, that was the same as the dosage for clinical application, and that was half of the dosage for clinical application, appeared to thicken less seriously and showed no dryness. And almost all hair in the stimulated parts remained growing. As for the behaviors, the mice were relatively quiet, and would not turn round to lick as frequently as the model mice would. Shengqing anti-itch lotion can obviously inhibit DNFB from thickening the skin of mice and inflammatory cell infiltration. There are some differences between dosage groups and model groups statistically.
Conclusion Shengqing anti-itch lotion possesses certain preventive and therapeutic effect against chronic eczema; the mechanism of action remains to be further discussed.

PO-026

The study on the Dermal Hypersensitivity and Irritation of Shenqing Anti-itch Lotion

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Objective To study the dermal Hypersensitivity and irritation of shenqing anti-itch lotion.
Methods repeatedly smearing shenqing anti-itch lotion on guinea pigs' local skin to evaluate the drug's dermal Hypersensitivity to animal skins; smearing shenqing anti-itch lotion in concentration for clinical use on rabbits' damaged skin and healthy skin, once a day, for seven times in a row, having day-to-day observation and recording the symptoms occurring on local skin of the animals at each time point, thus to evaluate the dermal irritation of shenqing anti-itch lotion.
Results There is no local reaction or edema on the kin of guinea pigs after application of shenqing anti-itch lotion's hypersensitivity and excitation within 6 to 72 hours, and there is no systemic anaphylaxis such as asthma and astasia, with average response value of 0 and sensitization rate of 0. Shenqing anti-itch lotion has no clear irritation on healthy or damaged skin of rabbits.
Conclusion The test results show that shenqing anti-itch lotion has no hypersensitivity to guinea pigs' skin, and has no clear irritation to rabbits' healthy or damaged skin.

PO-027

Mechanism of acrolein increasing with age in epidermis on epidermal stem cells by regulating mitochondrial fission via Drp1

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Introduction Acrolein is a lipid peroxidation unsaturated aldehyde product, which can disrupt the redox balance. We aimed to explore the age-related changes of acrolein in skin and its effect on mitochondria of epidermal stem cells (ESCs) and explore the protective effect of hydralazine on wound healing in aged skin.
Methodology Western blotting and immunofluorescence were used to explore the age-related expression changes and distribution of acrolein in skin. ESCs were incubated with acrolein in vitro to explore its effects on mitochondrial, apoptosis and cell proliferation. Protein inhibitors were used to determine the signaling pathways affected by acrolein in ESCs. In vivo experiments were conducted to explore the effect of hydralazine on acrolein in aged skin.
Results The content of acrolein in skin increased with age, especially in epidermis. In vitro experiments revealed the damage of acrolein in the aspects of proliferation and apoptosis of ESCs. More specifically, acrolein caused the dysfunction of mitochondrial in ESCs, manifested as decreased membrane potential and increased membrane permeability of mitochondria. Further studies found that acrolein promoted the translocation of Drp1 to mitochondria through AMPK and ERK pathways, causing excessive mitochondrial division, and finally leading to mitochondrial fragmentation of ESCs. Drp1 inhibitor Mdivi-1 can alleviate the mitochondrial damage and cell apoptosis caused by acrolein. The acrolein scavenger hydralazine can significantly reduce the level of acrolein in skin of old mice with mitochondrial damage alleviated and wound healing promoted.
Conclusion Acrolein caused mitochondrial fragmentation through Drp1 in ESCs which may be the reason for delayed ounds healing in aged skin. Hydralazine could eliminate age-increasing acrolein in skin, and thus promote wound healing in aged skin via improved mitochondrial function. Applicability to Clinical Practice Oxidative stress product scavenging such as hydralazine may be used to accelerate the healing of burns or wounds in aged patients in clinical.

PO-028

Multiple cutaneous ulcers in an HIV male

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A 45-year-old male presented with multiple cutaneous ulcers distributed in the trunk, arms and legs. The patient was positive for HIV and a definite diagnosis of AIDS was made. Depend on the bacterial culture and pathological examination, he was diagnosed as infection of Pseudomonas aeruginosa. Pseudomonas aeruginosa is a common disease-causing bacteria for HIV-positive group, which often resulted in folliculitis and ulcers. But ulcers are usually small and solitary. It is supposed that if the immune function is severely suppressed, the ulcers could be multiple, irregular, extensive, rapid spreading and have arcuate margins. The case highlights the importance of screening for HIV when cutaneous ulcers are multiple or atypical.

PO-029

Acute toxicity and Skin Irritancy Test of Shenqing Anti-itch Lotion

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Objective To study the safety of Shenqing Anti-itch Lotion by testing the acute toxicity and skin irritancy test on the skin of mice.

Methods Single or multiple-dosage of Shenqing Anti-itch Lotion were spread on the normal skin and damaged skin without hair in the back of white guinea pigs and it was observed whether Shenqing Anti-itch Lotion could induce acute toxicity. Smearing shenqing anti-itch lotion in concentration for clinical use on rabbits' damaged skin and healthy skin, once a day, for seven times in a row, having day-to-day observation and recording the symptoms occurring on local skin of the animals at each time point, thus to evaluate the dermal irritation of shenqing anti-itch lotion.

Results and Conclusion It indicated that Shenqing Anti-itch Lotion did not induce acute toxicity, and shenqing anti-itch lotion has no clear irritation to rabbits' healthy or damaged skin.

PO-030

Perspiration keratosis along the Blaschko line: a case report and literature review

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Patient, female, 45 years old. Left forechest, axilla, and flexion of upper limbs had a linear brown rash for more than 1 year. Dermatological examination: brown patches and macules of millet to soybean size distributed along the Blaschko line along the left forechest, left underarm and left upper limb flexion, with clear boundaries. Some lesions showed light brown dike ridges at the edge and dark center. Histopathology of skin lesion: slight hyperkeratosis, hair follicle horn thrombus, incomplete keratosis column, granular layer below it disappeared. Diagnosis: Linear perspiration keratosis (along the Blaschko line). Compound clobetasol propionate ointment was given for external treatment, and the lesions were relieved after 2 weeks, and now the follow-up is ongoing.

PO-031

Study on the correlation between systemic immune inflammatory indexes and arthritic psoriasis

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Objective To explore the relationship between systemic immune inflammatory indicators such as NLR, MLR, PLR, SII and the severity of psoriatic arthritis (PSA).
Methods 50 patients with PSA and 50 patients with pigmented nevus were selected from the dermatology department of the first medical center of the PLA General Hospital from July 2019 to may 2022. The clinical data of age, sex and systemic immune inflammation were recorded. Mann Whitney U rank sum test was used to analyze the clinical data of the two groups, and the differences of inflammatory indicators in patients with PSA of different severity were compared. Spearman correlation test was used to analyze the correlation between inflammatory indicators and PASI score of psoriasis, draw the receiver operating characteristic curve (ROC) and calculate the area under the curve (AUC) to evaluate the efficacy of inflammatory indicators in predicting severe PSA.
Results A total of 50 patients with PSA and 50 patients with nevus were included. The age of the PSA group was 45.50 (34.00 ~ 56.25) years old, and the age of the nevus group was 40.00 (33.75 ~ 49.25) years old. The difference was not statistically significant ($p = 0.291$). NLR, MLR and SII in PSA group were higher than those in nevus group, and NLR and SII in severe PSA group (PASI ≥ 10 points) were higher than those in mild / moderate PSA group (PASI < 10 points), the difference was statistically significant ($P < 0.05$). PASI score of patients with PSA was positively correlated with NLR and SII, and the correlation coefficients were $r=0.501$ ($P < 0.001$) and $r=0.542$ ($P < 0.001$) respectively. Through ROC curve analysis, the AUC was NLR 0.746 (95% CI 0.610~0.882) and SII 0.761 (95% CI 0.627~0.896). The prediction efficiency of SII is higher than that of NLR. The optimal critical value is 460.5, the sensitivity is 84.6%, the specificity is 62.5%, and the Youden index is 0.485.
Conclusion NLR, MLR and SII and have the advantages of simplicity, accuracy and economy, which can provide a basis for the diagnosis and disease evaluation of PSA and have guiding significance for clinical practice. At the same time, it also proves that inflammation plays an important role in the pathogenesis of PSA.

PO-032

Single cell insights into the heterogeneity and involvement of dermal adipocyte lineage cell in the immuno-modulatory network during psoriasis pathogenesis

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Objective Psoriasis, a common inflammatory skin disease with a worldwide incidence of approximately 2%, is significantly associated with obesity. The increasing prevalence of systemic medical complications associated with obesity requires a better understanding of the mechanisms. Dermal white adipose tissue (dWAT), the major skin layer that expands during obesity, has been suggested to be immunologically active, but how dWAT is involved during psoriasis pathogenesis remains largely unexplored. In this study, we aimed to clarify the role of dWAT-related adipocyte lineage cells in regulating dermal immune system and psoriatic inflammation.
Methods We studied how adipocyte lineage cells interact with myeloid cells including macrophages and neutrophils by single-cell RNA-sequencing (ScRNAseq) and Cell-Chat R package analysis. Furthermore, we investigated the differentiation path and regulatory mechanism of adipocyte

lineage cells by pseudotime and RNA velocity analysis by Monocle and velocyto R packages and transcription factors (TF) analysis by SCENIC R package. Finally, scRNAseq data from human psoriatic skin samples was analyzed to validate results obtained from mouse skin samples.
Results By ScRNAseq analysis, we defined the highly heterogeneous dermal fibroblast subpopulations, including papillary and reticular fibroblasts, dermal papilla and several adipocyte lineage cell subtypes (adipocyte progenitors (AP), preadipocytes (pAd) and adipocytes (Ad)). Psoriatic inflammation promoted the accumulation of a highly proinflammatory AP subpopulation, which resided at a central location to interact with myeloid immune cells, including neutrophils and macrophages. Pseudotime and RNA velocity analyses defined the
differentiation trajectory for this AP population and TF analysis identified CEBPβ and NFKB as the key transcription factor driving AP activation. Finally, scRNAseq analysis of human psoriatic skin cells validated our observation from mice.
Conclusion Our results have suggested that adipocyte progenitor may play an important in shaping the immune system during psoriasis pathogenesis. Results from our study provide novel insights into why obesity is a risk factor for psoriasis and our findings suggest that targeting adipogenesis maybe a novel approach to treat psoriasis.

PO-033

Recent advance in the role of EMT in keloid

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Epithelial-to-mesenchymal transition (EMT) is a process associated with the loss of polarity of epithelial cells and their development into mesenchymal cells with invasive and migratory properties. EMT has been shown to be involved in wound healing, organ fibrosis and development of cancer. The research of keloids is focused on keloid fibroblasts. In recent years, scholars have begun to pay attention to the phenomenon of keloid keratinocytes invading the surrounding normal skin. There is evidence that EMT is associated with the aggressive, continuously growing tumor-like characteristics of keloids. The article focuses on the relationship between EMT and keloid, and provides a new direction for the treatment of keloid.

PO-034

Preliminary study of pathogenic gene and functional mechanism in one family with Vohwinkel syndrome

Hongyu Chen
Preliminary study of pathogenic gene and functional mechanism in one family with Vohwinkel syndrome

Background Mutilating palmoplantar keratoderma was first described and named by Vohwinkel in Germany in 1929. It is a rare autosomal dominant genetic disease,which is mostly familial, and there are also scattered cases reported. The disease is more common in white women than in infants, and its course is progressive. Typical clinical symptoms include: symmetrical diffuse palmoplantar keratosis, and the surface of palmoplantar presents honeycomb pits;typical starfish-shaped or strip-shaped hyperkeratotic plaques can be found on the back of the hand, foot and knee joint extension. The distal interphalangeal joint is annularly narrowed and gradually severed (pseudo Ahun's disease-like changes). At present, two pathogenic genes related to mutilated palmoplantar keratoderma have been identified by location cloning, namely GJB2 gene encoding gap linking protein Cx26 located on chromosome 13q11-12 and LOR gene encoding pocket nail protein located on chromosome 1q21.3. The mutilated palmoplantar keratoderma caused by GJB2 gene mutation is often accompanied by different degrees of neuropathic deafness, which is called Vohinkel mutilated palmoplantar keratoderma (OMIM 124500), also known as Vohinkel syndrome.

The mutilated palmoplantar keratosis caused by LOR gene mutation is often accompanied by ichthyosis,but there is no hearing loss. It is called onychomycosis (OMIM 650117), which is considered as a special type of Vohinkel mutilated palmoplantar keratoderma. Clinically, we collected a family of mutilated palmoplantar keratosis with sensorineural deafness, and studied the pathogenic genes of this family.

Objective To detect the mutation of GJB2 gene in a Chinese family with Vohwinkel syndrome and deafness.

Methods Peripheral blood samples from 3 patients and 3 asymptomatic individuals in this family, lesional tissue samples from the proband and skin tissue samples from 6 healthy individuals who were not from the family were collected, and the clinical data of the family members were analyzed. The genomic DNA of family members was extracted, and Sanger sequencing was used to sequence the exons and flanking sequences of GJB2, GJB3, GJB4,GJB6,GJA1,KRT1 and KRT10 genes in the proband. Immunohistochemistry was used to detect the skin lesions and normal tissues of the proband. Expression of skin Cx26 protein. Furthermore, the model of overexpressing GJB2 mutant (V37I,Y155C,V37I&Y155C) and wild-type HaCaT was constructed to study the function of mutant gene.

Result There were 22 patients in the Vohwinkel mutilating palmoplantar keratoderma family involving three consecutive generations. 3 patients presented with palmoplantar keratoderma, plaques, mild sensorineural neuropathy, deafness, and constricted rings of fingers and toes. Two heterozygous missense mutations in GJB2 gene, c.109G>T and c.464A>G, were detected in all patients, respectively causing the 37th valine in the first transmembrane domain (TM1) to be replaced by isoleucine. The substitution (p.V37I) and the tyrosine at position 155 in the third transmembrane domain (TM3) were replaced by cysteine (p.Y155C), and 1 patient was not involved in this study. One patient presented with mild sensorineural hearing loss, and a heterozygous missense mutation c.109G>T (p.V37I) was found in GJB2 gene. No mutation of GJB2 gene was found in 3 normal persons in this family. No GJB3, GJB4， GJB6,GJA1,KRT1 and KRT10 gene mutations were found in the probands. Immunohistochemical analysis showed that the expression of connexin Cx26 in the skin lesions of the proband was higher than that of the normal skin. Western blotting showed that the expression of Cx26 in HaCaT cells infected with GJB2 gene mutants (V37I, Y144C,V37I&Y155C) overexpressed by lentivirus was higher than that in the overexpression group and wild-type group. Compared with the single mutants (V37I， Y144C), the amount of extracellular regulated protein kinase (ERK),c-jun amino-terminal kinase (JNK) and phosphorylated c-jun amino-terminal kinase (P-JNK)， GJB2 wild-type overexpression lentivirus infected HaCaT and mutant (V37I， Y144C,V37I&Y155C) overexpressed lentivirus infected HaCaT were both significantly higher than HaCaT .

Conclusion The GJB2 gene is the causative gene responsible for the clinical phenotype of Vohwinkel mutilating palmoplantar keratoderma in this family. GJB2c.109G>T(p.V37I) and c.464A>G(p.Y155C) mutations lead to the typical clinical phenotype of Vohwinkel's palmoplantar keratoderma,which may be gain-of-function mutations. GJB2 gene mutation may regulate Cx26 protein expression abnormally through MAPK pathway to produce clinical phenotype.

PO-035

Detection of Sebaceous Gland Hyperplasia with Dermoscopy and Reflectance Confocal Microscopy

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Sebaceous gland hyperplasia (SGH) is a benign cutaneous proliferation of the sebaceous glands that occurs in approximately 1% of the healthy population, mainly males. Our aim was to describe dermoscopy and reflectance confocal microscopy features of sebaceous gland hyperplasia. Thirty-

one patients with sebaceous gland hyperplasia diagnosed according to the clinical and histopathological standards were examined using dermoscopy and reflectance confocal microscopy (RCM) from March 2018 to January 2022. Dermoscopically, lesions revealed yellowish-red background and faintyellow background in 25 cases (80.65%) and 6 cases (19.35%) respectively. White-yellowish lobulated structures in the center of the lesion were presented in 31 cases (100%) and umbilications in 19 patients (61.29%). Crown vessels in the peripheral of the lesions were observed in 11 cases (35.48%) while irregular linear vessels on the surface of the lesions in 18 cases (58.06%). Under the reflectance confocal microscopy, all lesions presented a honeycombed pattern in the epidermis and typical morulae-shaped
sebaceous lobules in dermis. Dilated follicular infundibulum was observed in 15cases (48.39%) and dilated vessels in 26 cases (83.87%). In conclusion, dermoscopy and RCM enable us to describe the imaging features of SGH. Combing the two useful tools together provides a non-invasive basis for the accuracy of clinical diagnosis.

PO-036

Correlation analysis of immune infiltrates and abnormal keratinization between SPRRs gene and psoriatic lesions based on single cell sequencing data

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Objective Correlation analysis of small proline rich proteins (SPRRs) and psoriatic lesions skin (LS) were studied at the level of single cells.
Methods Single cell sequencing data from psoriatic skin biopsies were subjected to quality control, dimensional-reduction clustering and cell annotation. Cell clusters were analyzed for differential gene expressions (DGEs), cell communication and pseudotime analysis to explore cell-to-cell heterogeneity.
Results Compared with the expression in the non‐lesional skin and healthy normal skin, keratinocytes, plasmacytoid dendritic cells and plasma cells exhibited a significant distribution in the LS. The activeness of interactions number, interaction strength and level of pathway enrichment of cell communication were higher in LS than in the other groups. Keratinocytes only interact with plasmacytoid dendritic cells in LS. DGEs analysis revealed overexpression of SPRRs (SPRR1A, SPRR2A, SPRR2B, SPRR2D, SPRR2E, SPRR2F, SPRR2G) was mainly observed in keratinocytes. GO enrichment analyses showed that they were mainly related to epidermis development, keratinization and keratinocyte differentiation. IL-17 signaling pathway was shown to be significantly enriched. Seurat gene set analysis revealed the infiltration score of SPRRs was the highest in keratinocytes. Pseudotime analysis showed that the expression of SPRRs in keratinocytes increased first, then decreased, and finally increased along with pseudotime.
Conclusion Keratinocytes and plasmacytoid dendritic cells are involved in the pathogenic processes of LS, and SPRRs is primarily overexpressed in keratinocytes, which suggests that they may play an important role in LS formation and may serve as a new target for psoriasis treatment.

PO-037

A Case of Large Eccrine Poroma and Literature Review

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History A 75-year-old man presented with a large fleshy pink mass on the right sole. The lesion started more than 10 years back as pea-sized nodule, which gradually increased in size. Over the

past year, the nodule grew quickly accompanied with occasional bleeding. There's also occasional pain caused by the lesion that interferes with walking.
Clinical findings On the lateral margin of the right plantar, there is a 2.5x4.0cm oval raised plaque with clear border, moist and reddish surface, surrounded by a hyperkeratotic collar, accompanied with swelling and exudation.(Figure 1)
Biopsy Histological examination of a small fragment shows a well-defined tumor mass connected to the epidermis. The tumor cells were mostly cuboidal, consistent in size and shape, closely arranged, and intercellular Bridges were visible. The nuclei were small and regular, and the cytoplasm was medium. Some of the cells were rich of glycogen and showed clear cell transformation. some of the tumor cells formed duct-like structures. The nuclei around the tumor do not show palisade arrangement.(Figure 2)
Treatment Eccrine poromas are benign neoplasms. Extended resection is not necessary. The defect after resection were repaired with transposition flap.(Figure 3)
Literature review: Eccrine poroma is benign neoplasm arising from the intraepidermal portion of the eccrine duct epithelium. It is a relatively rare tumor which accounts for about 10% of eccrine gland tumors, 0.005%~0.01% of cutaneous tumor. At present, its etiology and pathogenesis are not clear, which may be related to scar, sun damage, radiation, trauma and human papillomavirus infection. The lesions are usually located in the extremities, but can occur in the skin of any part of the body. They are most common in middle-aged and elderly people. No gender or racial difference is involved. Lack of obvious clinical features in eccrine poroma leads to rather high clinical misdiagnosis. Pedicled vegetations may be misdiagnosed as skin tags. Pigmented papules and nodules may be misdiagnosed as pigmented nevus, melanoma or seborrheic keratosis. Ulcerative nodules may be misdiagnosed as squamous cell carcinoma. Exophytic red nodules with bleeding are easily misdiagnosed as pyogenic granuloma. Nodules in the head and face are easily misdiagnosed as basal cell carcinoma. The diagnosis depends mainly on histopathological examination. It is reported that dermoscopy can be used to assist diagnosis. The characteristics of eccrine poroma under dermoscopy mainly include white interleaved area around blood vessels, yellow unstructured area, milky red globules, unclear blood vessels and blunt round branch vessels. Unlike other malignant tumors, asymptomatic hemorrhage of eccrine poroma can not be used as an indicator of benign and malignant tumor. More important factors should be considered, such as the presence of significant tumor cell atypia, abundant mitotic figures, and obvious invasive growth model. The malignant conversion rate from eccrine poroma to eccrine porocarcinoma is 18%. The average progression is about 8.5 years. The survival time of metastatic eccrine porocarcinoma is about 5 to 24 months. As benign tumor, the treattment of eccrine poroma is easy. Liquid nitrogen cryotherapy, CO2 laser, electric cautery and surgery can be applied to remove different lesions.

PO-038

Dermatoscopic and skin ultrasound evaluation in lupus miliaris disseminatum faciei

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We describe a case of lupus miliaris disseminatus faciei (LMDF) in a 21-year-old female with a 5-months history. Clinical evaluation revealed multiple, dome-shaped, reddish-yellow papules, distributed symmetrically on nearly the entire face, especially the central area. Dermoscopy showed linear and arborizing vessels on a yellow-reddish background, some with targetoid follicular plugs. The ultrasonographic evaluation of the papules on the central area as well as the lesions on the cheeks showed alterations in dermis tissue. The evaluation with a 50 MHz probe showed multiple well-defined peanut-like hypoechoic area. The diagnosis of LMDF was confirmed via histopathology. To our knowledge, this is the first reported case where ultrasound has been used for evaluation for LMDF.

PO-039

Chemotherapy-induced alopecia

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Damage to hair follicles after exposure to toxic chemotherapy drugs can lead to massive hair loss, commonly referred to as chemotherapeutic alopecia (CIA). The degree of alopecia is often related to the length of chemotherapy and the type, dosage and combination of drugs. CIA has a great negative impact on patients' body image, sex, self-esteem and other aspects, thus greatly reducing patients' quality of life. Current research results show that there is no way to prevent or cure CIA. This article reviews the current research progress on the related prevention and treatment measures at home and abroad, so as to provide theoretical support for further exploring the mechanism of CIA and finding more suitable prevention and treatment measures.

PO-040

Research progress on individualized treatment of atopic dermatitis

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Atopic dermatitis is a common chronic inflammatory skin disease, mainly caused by Th2 type immune response,characterized by dry skin, eczema-like dermatitis, severe pruritus and recurrent attacks.Atopic dermatitis is a common chronic inflammatory skin disease, mainly caused by Th2 type immune response, characterized by dry skin, eczema-like dermatitis, severe pruritus and recurrent attacks.

Dupilumab is a human monoclonal antibody that inhibits both IL-IL-4 and IL-13 signaling pathways by blocking the common IL-4 receptor a (IL-4Ra), and down-regulates TH2 inflammation in a variety of allergic diseases, including atopic dermatitis, asthma and other possible allergic diseases. Dupilumab can inhibit the mRNA expression of genes activated by T cells, dendritic cells or eosinophils.

IL-31 cytokines are another key Th2 cytokines involved in the pathogenesis of AD and are described as mediators that induce intense itching. IL-31 and its receptors IL-31R and OMSR are highly expressed in the dorsal root ganglion of the cutaneous sensory nerve, which mediates the pruritus response.Nemolizumab is a humanized anti-IL-31RA antibody. It can prevent IL-31 from acting on neurons, thus inhibiting itching.For patients with moderate and severe AD, when the local treatment effect is not good, the efficacy of Nemolizumab is better and the overall tolerance is good.

Fezakinumab is a human IgG1 λ anti-IL-22 monoclonal antibody, which can directly bind to IL-22, prevent the formation of IL-22 / IL-22 receptor complex (IL-22R-1), and strongly inhibit a variety of inflammatory mediators mainly produced by keratinocytes. The molecular drug effect of Fezakinumab existed only in the high baseline IL-22 drug group, but not in the low IL-22 drug or placebo group.It only has clinical effect in patients with severe AD, but it has stable clinical effect and good tolerance.

JAK-STAT is the main signal pathway of immune function regulation, which can block a series of signal pathways of cytokines, growth factors and hormone receptors. The imbalance of Jak-STAT signal pathway will lead to the disorder of cytokines and play a key role in the pathogenesis of AD. Abrocitinib is a selective JAK1 inhibitor that regulates the signal transduction of IL-4, IL-13 and other cytokines involved in AD and pruritus without inhibiting the inhibition of JAK2,minimizing the risk of neutropenia and anemia. Baricitinib regulates these intracellular signaling pathways by partially inhibiting JAK1 and JAK2, reducing the phosphorylation and activation of STATs.

Topical glucocorticoid (TCS) is a first-line anti-inflammatory treatment for skin inflammation. The appropriate TCS can be selected according to the age, location, drug effect and skin

lesions, and timely relief and induction therapy can be carried out to reduce inflammation and itching. TCS can be divided into four levels according to strength. Skin atrophy may occur after long-term use of very powerful TCS Therefore,the use of powerful corticosteroids should be limited in time in sensitive skin areas such as face and folds, and moderate and weak TCS can be recommended to avoid skin atrophy.

The mechanism of topical calcineurin inhibitors （TCI） is to inhibit the synthesis of pro-inflammatory cytokines, inhibit the signal transduction pathway of cell proliferation, and have strong anti-inflammatory effects, including tacrolimus and pimecrolimus.Compared with TCS, TCI does not cause skin atrophy, and it is beneficial to use in eyelid, mouth and armpit.

Atopic dermatitis is one of the most common chronic skin diseases in the world, but its individualized treatment is still at an early stage. Classification of atopic dermatitis based on pathophysiology can provide a new direction for individualized treatment.

PO-041

Recent Advances of mTOR Related Signaling Pathways in the Pathogenesis of Psoriasis

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Mammalian target of rapamycin (mTOR) is considered as an important signaling hub for coordinating various cell and tissue responses. Recent studies have found that mTOR related signaling pathways play important roles in the pathogenesis of psoriasis, including immune imbalance, keratosis, and angiogenesis. In this article, we overview recent advances for the role of mTOR related signaling pathways in the pathogenesis of psoriasis, and provide new molecular targets for the treatment of psoriasis.

PO-042

Skin and the cell “power plant”: mitochondrial dysfunction mediating skin aging

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Cellular aging begins in fetal life. When humans are still young, the skin is firm and can quickly return to normal when some damage occurs. When the aging factors in the cells accumulate to a certain level, much greater than the repair factors, the cells go into senescence and apoptosis. And when the metabolic rate of most cells in the whole tissue slows down and senescent cells accumulate, then the whole tissue will go to irreversible aging. Skin aging is the most visual manifestation of the aging process in the body, leading to cumulative changes in appearance, skin structure and skin function. Skin aging is influenced by both intrinsic aging processes and extrinsic factors that regulate it. Mitochondria, an organelle that provides energy for life activities, are known as the "power plant" of cells and are commonly found in plant and animal cells. Current research has identified mitochondrial dysfunction as a potential driver of aging that integrates the regulation of various endogenous and exogenous factors throughout the skin aging process. In this review, we will describe the characteristics of mitochondrial dysfunction and the mechanisms of how mitochondrial dysfunction mediates skin aging.

PO-043

The metagenomic next-generation sequencing in diagnosing tuberculoid leprosy: a case report

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A 64-year-old male with edematous erythema and papules on face with numbness for 2 months, and eyes swelling for 3 days . There was no thickened nerves. Histopathology showed dermal granuloma nodules, no foam-like tissue cell and caseous necrosis. Acid fast staining and PAS staining were both negative. Metagenomic next-generation sequencing (mNGS) detected 3 sequences of mycobacterium leprosy in skin tissue. This case highlights that mNGS maybe an effective supplementary diagnostic method when traditional method results is negative.

PO-044

A Case of Linear lichen planus pigmentosus

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A 34-year-old woman presented with Grey or brown-coloured maculopapules on the trunk and right arm presenting in a linear pattern for 3 months.On examination, she had a linear streak of grey and brown maculopapules on the trunk and right arm following of Blaschko's lines.Skin biopsy showed Keratinization of epidermis with extensive basal cell edema and liquefaction, a few lymphocytes infiltrate around small vessels in superficial dermis and more pigment cells distributed around the blood vessels.Dermoscopy showed grey-yellow pigmentation in epidermis.The patient treated with tacrolimus ointment,and still in follow-up.

PO-045

Mast cell hyperplasia in young children was combined with 1 case of congenital hypothyroidism

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Child, female, 1 year and 7 months. The reddish-brown spot on the right femur has been around for more than 1 year and has gradually enlarged in the past 3 months. Diagnosis 29 days after birth: congenital hypothyroidism. Dermatological examination: a broad bean-sized round dark red spotted papule can be seen on the extensor side of the right femur, clear boundaries, infiltration, toughness, no exudation, blisters, etc., positive Darier sign, negative Nicholas sign, superficial lymph nodes of the whole body are not touched and enlarged. Lesion histopathology: (right femoral) epidermis is mildly thickened, the base is intact, the dermis is densely packed with cytoplasm, the nucleus is centered, the mast cells and some eosinophils of fine particles in the cytoplasm, a small amount of mast cells in the subcutaneous fat layer, and eosinophils infiltrate. Immunohistochemical staining: CD117 most +, CD68 more +, CD45 more +, CD20 scattered in a small amount +, CD25 part weak +, CD2-, CD3 scattered in a small amount +, Lysozyme part +, S-100 scattered in +, MPO-, TDT-, Ki67+20-30%. Special dye Gimesa mostly +. Diagnosis: skin mast cell hyperplasia. Cetirizine drops orally and fluticasone propionate ointment for external use of the right femoral rash darkened in color, local flattening, the therapeutic effect is acceptable, is still being followed up.

PO-046

Effects of Itraconazole on Hypertrophic Scars in Rabbit Ears

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Objective To investigate the effect of itraconazole (ITZ) on hypertrophic scar fibrosis in rabbit ears.
Methods A rabbit ear hypertrophic scar model was established, and 25 New Zealand rabbits were randomly divided into blank group (group A), immediate intervention group after surgery (group B1, B2), and post-epithelialization intervention group (group C1, C2), there were 5 rabbits in each group, and the drugs were administered continuously for 28 days. During the treatment period, dermoscopy was used to examine the regression of scar blood vessels; scar specimens were prepared, the pathological changes were observed by hematoxylin-eosin staining（HE） and Masson staining, and the Hypertrophic scar Index (HI) and Collagen Volume Fraction (CVF) were determined; and the protein expressions of α-smooth muscle actin (α-SMA), fibronectin (FN), collagen type I (Col 1), collagen type III (Col 3), vascular endothelial growth factor (VEGF) were detected by Western Blot and immunohistochemistry.
Results The pathological structure of the grouped scar tissue after itraconazole intervention was significantly improved, HI and CVF were decreased, and the expressions of α-SMA, FN, Col 1, Col 3 and VEGF in the scar tissue were significantly decreased.
Conclusion Itraconazole can effectively inhibit the formation of hypertrophic scars in rabbit ears, and it is feasible to explore the feasibility of itraconazole as a prevention and treatment strategy for hypertrophic scars.

PO-047

The mechanism of Wen’s Ershen Dihuang Decoction in the treatment of psoriasis vulgaris in mice

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Objective We undertook this study to describe the mechanism of Wen’s Ershen Dihuang Decoction of psoriasis action may involve the EGFR/ERK/AQP3 pathway.
Methods 54 BALB/c mice were randomly divided into 9 groups, A group (blank group), B group (model group), C group (Wen’s Ershen Dihuang Decoction group), D group (NSC228155 group), E group (Wen's Ershen Dihuang Decoction + NSC228155 group), F group (Bortezomib group), G group (Wen's Ershen Dihuang Decoction + Bortezomib), H group (PD153035 group), I group (U0126 group). After shaving the back area of mice, all the groups were smeared with 62.5 mg of 5% imiquimod cream for 8 consecutive days, except for group A. Groups D and E were intraperitoneally injected with EGFR agonist NSC228155, groups F and G were injected intraperitoneally with the ERK agonist Bortezomib, 1 mg·kg-1·d-1, twice a week. Group H was injected intraperitoneally with EGFR inhibitor PD153035, 8 mg·kg-1·d-1. Group I was intraperitoneally injected with ERK inhibitor U0126, 3 mg·kg-1·d-1; Group C, E, G were intragastrically administered Wen's Ershen Dihuang Decoction, 38.64g·kg-1·d-1. Other mice were gavaged with UP water, 0.4 ml·d-1 for 8 consecutive days. On the last day, the mice were evaluated and scored using PASI, the water and oil content were measured by the intelligent skin tester. The weight of the kidney, lung, spleen and body were recorded to calculate the organ index. The pathologic changes in skin tissue were observed by hematoxylin-eosin (HE) stain and Baker score. The expression level of AQP3 mRNA in peripheral blood of mice in groups A, B, C were measured by real-time PCR. Immunohistochemical staining was used to detect the expression of AQP3 in

kidneys, lungs, and skin lesions in groups A, B, C. The expression level of AQP3, EGFR, p-EGFR, ERK and p-ERK in skin tissue were detected by Western Blot.

Results

(1) PASI score: Group A had no skin lesions, and the PASI score was 0. Compared with group A, group B ($P<0.05$), group D ($P<0.01$), group E ($P<0.05$), group F ($P<0.01$), group G ($P<0.05$), and group H ($P<0.05$) increased PASI score. Compared with group B ($P<0.05$), group D ($P<0.01$), and group F ($P<0.01$), group C effectively reduced the PASI score of psoriasis animal model. Compared with group D, group H had a lower PASI score ($P<0.01$); group G had a lower PASI score compared with group F ($P<0.01$).

(2) Histopathological features and Baker score: The epidermal structure of mice in group A was complete. Group B, group D, and group F showed obvious spines in the epidermis of layer hypertrophy, the number of layers increased, the epidermis elongated in a clubbing shape, hyperkeratosis, parakeratosis, telangiectasia, hyperemia and lymphocyte infiltration were seen in the dermis. Combined with the above PASI scores, the model was successfully replicated. The histopathological changes in group C were significantly relieved.

Baker score: The Baker score of group A was 0. Compared with group A, group B ($P<0.01$), group C ($P<0.01$), group D ($P<0.01$), group E ($P<0.01$), group F ($P<0.01$), group G ($P<0.05$), group H ($P<0.01$), and group I ($P<0.01$) elevated the Baker score. Compared with group B ($P<0.05$), group D ($P<0.01$), and group F ($P<0.01$), group C significantly reduced the Baker score of animal skin lesions. Compared with group D, group H ($P<0.05$) had a lower Baker score; compared with group F, group I ($P<0.01$) had a lower Baker score.

(3) Organ index: The results showed no difference in the lung organ index and kidney organ index among the groups. Compared with group A, group B ($P<0.01$), group D ($P<0.01$), group E ($P<0.05$), group F ($P<0.01$), group G ($P<0.01$), group H ($P<0.05$), group I ($P<0.01$) increased the spleen organ index. Group C could down-regulate the spleen organ index.

(4) AQP3 mRNA expression in peripheral blood: Compared with group A, group B ($P<0.05$) and group C decreased the expression level of AQP3 mRNA. Compared with group B, group C ($P<0.05$) had higher level of AQP3 mRNA in peripheral blood, which was consistent with the trend of clinical trial results.

(5) Water and oil content of skin lesions:

Compared with group A, group B ($P<0.01$), group D ($P<0.01$), group F ($P<0.01$), group G ($P<0.05$), group H ($P<0.05$), and group I ($P<0.05$) decreased the water content of skin lesions. Compared with group B ($P<0.05$), group D ($P<0.05$), group F ($P<0.05$), and group C increased water content of skin lesion.

Compared with group A, group B ($P<0.01$), group D ($P<0.01$), group F ($P<0.05$) and other groups had lower oil content in skin lesions; Compared with group B, group C increased the oil content of skin lesions($P>0.05$).

(6) AQP3 (IOD) expression in kidney, lung and skin:

Compared with group A, group B ($P<0.01$) and group C($P<0.05$) had a statistically significant decrease in the expression of AQP3 (IOD) expression in kidneys; Compared with group B, group C ($P<0.05$) increased AQP3 (IOD) expression.

Compared with group A, group B ($P<0.01$) and group C ($P<0.05$) decreased the expression of AQP3 (IOD) in the lungs; Group C ($P<0.05$) increased the expression of AQP3 (IOD).

Compared with group A, group B ($P<0.01$), group C ($P>0.05$) down-regulated the AQP3 (IOD) expression; compared with group B, group C ($P<0.05$) increased AQP3 (IOD) expression in the skin.

(7) EGFR, p-EGFR, ERK, p-ERK, AQP3 protein expression in skin tissue:

Compared with group A, group B, group D, and group F decreased the expression of AQP3; Compared with group B ($P<0.05$), group D ($P<0.01$) and group F ($P<0.05$), group C significantly increased the expression of AQP3.

Compared with group A, group B ($P<0.01$) and group D ($P<0.01$) increased the expression level of total EGFR in skin tissue. The level of total EGFR in group D ($P<0.05$) was higher than in group B; Compared with group B, group C ($P<0.01$) decreased the expression level of EGFR protein. Compared with group D, group E ($P<0.01$) and group H ($P<0.01$) decreased the expression level of EGFR protein.

The expression of p-EGFR in group B (P<0.01), and group D (P<0.01) were higher than in group A . Compared with group B, group C (P<0.05) decreased the level of p-EGFR. Compared with group D, the expression of p-EGFR was down-regulated in group E. The expression of p-EGFR in group D was higher than in group H (PD153035 group) (P<0.05).
The expression of ERK in groups B and F was enhanced than in group A; Compared with group B, group C down-regulated the expression of ERK in skin lesions; Group G down-regulated the level of ERK elevated by agonists. Compared with group A, the level of p-ERK increased in group B, group D, and group F. While group C, group E, group G, group H, and group I down-regulated the level of p-ERK.

Conclusion

(1) Wen's Ershen Dihuang Decoction could effectively improve the PASI score, histopathological features in model mice, reduce Baker score, do no obvious damage to lung, kidney and spleen.
(2) Wen's Ershen Dihuang Decoction increased the AQP3 mRNA expression in peripheral blood, water, and oil content of skin lesion, simultaneously increased the expression levels of AQP3 in the lungs and kidneys.
(3) Wen's Ershen Dihuang Decoction could down-regulate the levels of EGFR, ERK, p-EGFR, and p-ERK in psoriatic skin lesions, and up-regulate the expression of AQP3.
Above all, the therapeutic effect of Wen's Ershen Dihuang Decoction on psoriasis vulgaris may involve the EGFR/ERK/AQP3 pathway.

PO-048

Juvenile Pityriasis Rubra Pilaris

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Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disease that affects men and women of all ages, which presents with hyperkeratotic follicular papules, erythematous-desquamative plaques, palmoplantar keratoderma. Its classification into five subgroups is based on age at onset, clinical course, morphologic features, and prognosis. We present a type III pityriasis rubra pilaris. The differential diagnosis includes psoriasis vulgaris, perifollicular keratosis, lichen spinulosus. We reported a 4-year-old girl, without a prior history of eczema or psoriasis, presented with a 4-weeks history of extensive erythematous scaly eruption which started on her face and scalp, and spread rapidly to her torso and limbs. There were well-circumscribed, red-orange, waxy plaques on the patient's palms and soles (Figure1A-1F). Her growth and psychomotor development were normal. Histopathology showed basket-like hyperkeratosis, diffuse orthokeratosis and spotted parakeratosis, and a dermal superficial perivascular lymphohistiocytic inflammatory infiltrate (Figure 1G). The final diagnosis was Juvenile pityriasis rubra pilaris (type III).

PO-049

Study on the mechanism of Wen's Qingxin Kaixuan formula in the treatment of psoriasis-like mouse based on the theory of "heart commanding the exterior"

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Bjective This study aims to discuss the possible mechanism of Wen's Qingxin Kaixuan formula in the treatment of psoriasis through mechanism research, and then explain the scientific connotation of the theory of "heart commanding the exterior".

Methods Using imiquimod cream-induced psoriasis-like mice as the animal model, 30 BALB/c male mice were randomly divided into blank group (Control), model group (Model), Hippo pathway inhibitor group [XMU-MP-1(1mg/kg)], Qingxin Kaixuan formula group [QXKX(24.25g/kg)] and Hippo pathway inhibitor + Qingxin Kaixuan formula group [XMU-MP-1(1mg/kg) + QXKX(24.25g/kg)]. Each group has six mice, which were given with oral gavage once a day for 7 days. Mice in Control and Model groups were given equivalent volume of UP water. The body weight of mice was measured every day, and the back skin condition was also observed. The PASI score of the mice skin lesions was scored at the end of the experiment on the 7th day. Mice were sacrificed by anesthesia, the spleen tissues were taken and weighed, the spleen index of the mice in each group were calculated, and the proportion of Th17 and Treg cells in spleen were detected by flow cytometry; the back skin tissues were collected, and the skin pathological changes of the mice in each group were observed by HE staining. Meanwhile, the Baker scores were calculated. The mRNA expressions of RORγt and Foxp3 in the skin tissues were detected by qPCR method, and the protein expressions of Hippo pathway molecules such as MST1/2, YAP and TAZ in the skin tissues were detected by Western Blot.

Results

(1) Comparison of the skin lesions: compared with the Control group, the exposed skin on the back of mice in the Model group and XMU-MP-1 group showed obvious thickening of skin lesions with large areas of erythema and scales, which were similar to human psoriasis-like skin lesions. The total PASI score of skin lesions in the two groups were significantly increased ($P < 0.05$). Compared with the Model group and XMU-MP-1 group, the symptoms of skin lesions in the QXKX group were significantly improved, and the total PASI score was significantly reduced ($P < 0.05$); The skin lesions in the XMU-MP-1+ QXKX group had a certain degree of improvement, and the total PASI score decreased, but the difference was not statistically significant ($P > 0.05$). The total PASI score of mice in the QXKX group was lower than that in the XMU-MP-1+QXKX group, but the difference was not statistically significant ($P>0.05$).

(2) Comparison of the body weight and spleen index: compared with the Control group, the mice weight in the other groups was significantly reduced, the spleen was significantly enlarged, and the spleen index was significantly increased ($P < 0.05$). The mice weight in the Model group, XMU-MP-1 group, XMU-MP-1+ QXKX group showed the trend of XMU-MP-1+ QXKX > Model group > XMU-MP-1 group. The weight differences among these three groups were not statistically significant ($P > 0.05$). The spleen index of these three groups showed the trend of XMU-MP-1+QXKX group <Model group <XMU-MP-1 group, only the difference of spleen index between XMU-MP-1 group and XMU-MP-1+QXKX group was statistically significant ($P<0.05$). Compared with these three groups, the mice weight in the QXKX group was significantly decreased ($P<0.05$), while the spleen index was increased, and only the difference of the mice weight between QXKX group and XMU-MP-1+QXKX group was not statistically significant ($P>0.05$).

(3) Comparison of the pathological change of skin lesions and Baker score: compared with the Control group, the skin lesions of the Model group and XMU-MP-1 group showed obvious psoriasis-like pathological histomorphology, and the Baker scores were significantly increased ($P < 0.05$). Compared with the Model group and XMU-MP-1 group, the psoriasis-like pathological histomorphology of mice in the XMU-MP-1+QXKX group was improved, and the Baker score was significantly reduced ($P<0.05$). In the QXKX group, the improvement of psoriasis-like pathological histomorphology was the most obvious, and the Baker score was significantly decreased ($P < 0.05$). Compared with the QXKX group, the psoriasis-like pathological histomorphology of mice in the XMU-MP-1+QXKX group was more obvious, and the baker score was significantly higher ($P < 0.05$).

(4) Comparison of the outcomes of Th17/Treg balance: compared with the Control group, the proportion of Th17 cells in the spleen and the mRNA expression of RORγt in the skin lesions in the Model group, XMU-MP-1 group, and XMU-MP-1+QXKX group were significantly increased, while the proportion of Treg cells in the spleen and the mRNA expression of Foxp3 in the skin lesions were significantly decreased ($P<0.05$). The proportion of Th17 cells in the spleen and the mRNA expression of RORγt in the skin lesions of the three groups showed the trend of XMU-MP-1 group > Model group > XMU-MP-1 + QXKX group, while the proportion of spleen Treg cells and the mRNA expression of Foxp3 in skin lesions showed the trend of XMU -MP-1 group < Model group < XMU-

MP-1+QXKX group. There were no significant differences in the above outcomes among these three groups (P>0.05). Compared with these three groups, the proportion of Th17 cells in the spleen and the mRNA expression of RORγt in the skin lesions in the QXKX group were significantly decreased, while the proportion of Treg cells in the spleen and the mRNA expression of Foxp3 in the skin lesions were significantly increased (P<0.05).
(5) Comparison of the protein expression of Hippo pathway molecules: compared with the Control group, the protein expression of MST1/2 in the skin lesions of the Model group and XMU-MP-1 group was significantly decreased, while the protein expression of YAP and TAZ was significantly increased (P<0.05). The protein expression of MST1/2 in the Model group, XMU-MP-1 group, and XMU-MP-1+QXKX group showed the trend of XMU-MP-1+QXKX group≈Model group>XMU-MP-1 group, while the protein expression of YAP and TAZ showed the trend of XMU-MP-1+ QXKX group<Model group<XMU-MP-1 group. There was no significant difference in the above outcomes among these three groups (P>0.05). Compared with these three groups, the protein expression of MST1/2 in the QXKX group was significantly increased, the protein expression of YAP was significantly decreased (P<0.05). The protein expression of TAZ in the QXKX group was decreased, however, there was no significant difference between the QXKX group and XMU-MP-1+QXKX group (P>0.05).

Conclusion

1. Wen's Qingxin Kaixuan formula can significantly improve the appearance and pathological histomorphology of psoriasis-like mice, reduce the total PASI score, Baker score and spleen index, and restore the immune function of mice.
2. Wen's Qingxin Kaixuan formula may play a role in regulating the immune imbalance of Th17/Treg in psoriasis-like mice by activating Hippo pathway.
3. The inhibition of Hippo pathway activity may be related to the pathogenesis of psoriasis. Its role in regulating the immune balance of Th17/Treg in psoriasis has not been clear, which needs to be confirmed by further research.

PO-050

A case of special manifestations of lower extremity ulcer

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The patient, male, 53 years old, was admitted to the hospital due to "nodules, plaques and ulcers in lower extremities accompanied by pain for six months, aggravated for one month". Medical history: Half a year before admission,the patient presented red nodules in his left leg without obvious inductions,accompanied by pain, which was ignored. The nodules were enlarged, redness andswelling were obvious, and the central ulceration and pus were discharged. Hewas repeatedly treated in other hospitals, and his condition did not improve significantly after oral and external drug treatment. The rash affected both legs and feet,partially fused into plaques, and the surface burst into ulcers, causing obvious pain. After repeated treatment,the effect is not good. One month ago,the patient's legs and feet were red and swollen, and the pain was aggravated. Since the onset of the disease, the patient has good spirits, good appetite, no obvious abnormalities in urine and feces, poor sleep, and no significant changes in body weight.
Past history: Had hypertension for 5 years.
Physical examination: no obvious abnormality was found in heart, lung and abdomen. Specialist physical examination: symmetrical distribution of pigeon egg to palmsize plaques were seen on both legs and feet,with obvious redness and swelling on the base, and a large amount of exudation,purulent secretions, pus and blood, and brown crusts were seen on the surface,with a lot of pus flowing out. Part of the surface ulceration, the formation of ulceration, visible on the surface of a large number of purulent secretion.Redness and swelling of both legs and feet are obvious. Auxiliary examination: secretions tuberculosis bacillus smear test, fungal smear test, liver and kidney function, blood lipid,blood sugar, routine urine and feces test, infection markers: no special. Four items of immunoglobulin: white 1gA 4.10g/L. Four items of coagulation test :Fbg 5.4g

/L. Plasma procalcitonin detection :PCT 0.051ng/mL. CT plain chest scan: left superior septal emphysema: possible interstitial changes in both lungs. Several nodules in both lungs. A little chronic infection in the upper tongue segment of the left lung: a little subpleural effect in the lower lobe of both lungs. Ecg: normal. Abdominal color ultrasound: fatty liver. Vascular color doppler ultrasound of lower limbs: irregular intima thickening with plaque formation in the arteries of both lower limbs. First pus culture:Staphylococcus aureus, MRS positive.Second abscess culture: Klebsiella pneumoniae pneumonia subspecies and Staphylococcus aureus infection, MRS positive.Third pus culture: pseudomonas aeruginosa infection. Macrogene sequencing suggested staphylococcus.
Pathological examination: "Left leg": epidermal pseudoepitheliomatous hyperplasia, ulcer formation, inflammatory granulation tissue hyperplasia, swelling of some small vascular endothelial cells, occlusion of tube wall.
Treatment: amoxicillin potassium clavulanate,piperacillin tazobactam, ceftazidime, methylprednisolone sodium succinate were given successively. Local debridement,dressing change, external use of fusidic acid cream.
Diagnosis: 1. Pyoderma gangrena; 2. Skin infection of lower extremities (Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa); 3. Hypertension grade 3(moderate risk), fatty liver

PO-051

Investigation of the mechanism of Cdc37/Akt signaling pathway in the proliferation and apoptosis of keratinocytes and the effect of shikonin

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Objective Accelerated proliferation and reduced apoptosis of epidermal keratinocytes, shortened mitotic cycle and epidermal turnover time are characteristic manifestations of psoriasis. Studies have shown that keratinocytes are not only involved in the initiation of psoriasis, but also interact with immune cells in the further development of psoriasis and the relapse process. Therefore, exploring the mechanism and inhibition strategy of abnormal proliferation of keratinocytes and achieve clinical translation will be valuable for psoriasis treatment.
Cell division cycle 37 (Cdc37) has chaperone molecular activity and specifically mediates the binding of multiple protein kinases to heat shock protein (HSP) 90, forming a specific molecular chaperone complex that protects protein kinases from degradation and plays a significant role in cell cycle, signal transduction and gene expression. It has been shown that Cdc37 expression is elevated in a variety of malignant neoplasm tissues and in some benign diseases. However, the expression and mechanism of action of Cdc37 in psoriatic keratinocytes have not been reported. Therefore, this study will first verify the expression of Cdc37 in skin lesions of psoriasis patients by database search and immunohistochemical assay of clinical samples; Then we will further observe the effect of Cdc37 expression alteration on proliferation, cell cycle and apoptosis of keratinocytes; Finally, we will investigate the potential molecular mechanism of Cdc37 function.
Zicao is used in clinical as a Chinese herbal formula for the treatment of psoriasis. Shikonin is the main active ingredient of Zicao, which has various biological activities such as anti-inflammatory, anti-viral and anti-tumor. Several basic studies have confirmed the possible mechanism of its effect on psoriasis, but there is no clinical use of shikonin to treat psoriasis. More basic researches are still needed to provide the evidences for the effectiveness and safety of its clinical application. We have discovered in previous studies that shikonin inhibited keratinocytes proliferation and induced apoptosis. It has also been shown that shikonin regulated Cyclin A, Cyclin B, Cyclin D, Cyclin E, CDK1, CDK2 and other cycle-related proteins that interact with Cdc37 to inhibit theproliferation of tumor cells. However, there are no studies on the association of shikonin with cell cycle-related

factors in psoriatic keratinocytes. This study will explore whether shikonin affects the abnormal alteration of keratin-forming cells induced by Cdc37 both in vivo and in vitro, and explore the possible targets of action in an attempt to provide important scientific data for the elucidation of the pathogenesis of psoriasis and the clinical application of shikonin.

Methods

1. The GEO database was retrieved to obtain datasets of the expression of Cdc37mRNA between psoriasis patients and healthy controls. Datasets with organisms other than homo sapiens, cases with few samples or cases irrelevant to psoriasis were excluded. Paraffin blocks were collected after clinicopathological diagnosis, and immunohistochemical staining was used to observe the differences in the expression of Cdc37 protein levels in psoriatic lesions and controls, as well as the differences in its distribution in the various layers of the skin.
2. Cdc37 overexpression cell model LV-Cdc37 was constructed by lentiviral transfection, and the transfection efficiency was verified by RT-qPCR and Western blot; cellular models of Cdc37-HSP90 complex inhibition were constructed with Hsp90 inhibitor 17-AAG. Based on the successful construction of cell models, the cell proliferation rate was detected by CCK-8 and the proportion of cells in proliferative phase was detected by the EdU assay; The changes of cell cycle distribution and apoptosis between different groups were detected by flow cytometry. Combining the signaling pathway mapping in the KEGG database and related research advances in psoriasis, the expression differences of pathway molecules were verified by Western blot technique.
3. Clarification whether shikonin affects the role of Cdc37/Akt in keratinocytes: The effect of shikonin on the proliferation rate of LV-Cdc37 cells was detected by CCK-8; The proportion of LV-Cdc37 cells in proliferative stage was detected by EdU method; The changes of cell cycle and apoptosis of LV-Cdc37 cells after shikonin intervention were detected by flow cytometry; The expression changes of related protein molecules were verified by Western blot. Psoriatic mouse model induced by imiquimod was used to verify the effect of shikonin on psoriatic lesions; The expression changes of related protein molecules in animal models were verified by Western blot.

Results

1. Two eligible datasets were retrieved from the GEO database, and the statistical results both showed that the expression of Cdc37mRNA was significantly increased in psoriasis patients' skin lesions compared with that in non-lesioned and normal human skin tissues, and no significant difference was seen in the expression of Cdc37mRNA in psoriasis patients' non-lesioned and normal human skin tissues. Immunohistochemical assays of skin tissues from psoriasis patients and normal controls showed that Cdc37 was distributed to the basal cell layer of the epidermis in healthy skin, whereas in the lesions of psoriasis patients, Cdc37 was expressed not only in the basal layer but also in the spiny layer cells. The results quantified with Image Pro-Plus 6.0 software also suggested that the expression of Cdc37 was significantly higher in the skin lesions of psoriasis patients than in the control group.
2. The results of CCK-8 assay showed that LV-Cdc37 cells proliferated significantly faster from day 3 compared with HaCaT cells, while 17-AAG inhibited the proliferation rate of LV-Cdc37 cells. The results of EdU assay showed that the proportion of EdU-positive cells in LV-Cdc37 cells was significantly higher, representing a significant increase in the proportion of cells in proliferative phase, while 17-AAG reduced the proportion of cells in proliferative phase in LV-Cdc37 cells. After flow cytometric detection of the cell cycle, the results showed that the proportion of cells in the G1 phase was reduced and the proportion of cells in both the S and G2/M phases was increased in LV-Cdc37 cells compared with EV cells; the proportion of cells in the G1 phase was increased and the proportion of cells in both the S and G2/M phases was reduced in LV-Cdc37 cells after 72h of 17-AAG intervention at a concentration of 10nM. The results of apoptosis detection by flow cytometry showed that the proportion of apoptotic cells was significantly reduced in LV-Cdc37 cells compared with EV cells; 17-AAG caused a significant increase in the proportion of apoptotic cells in HaCaT cells. The results of Western Blot showed that compared with EV cells, p-Akt, Cyclin D1, Bcl-xL expression was elevated and BAD protein expression was reduced in LV-Cdc37 cells, while Akt, p-ERK1/2, CDK4/6, Cyclin D2, Cyclin D3, Bcl-2 and C-casp-9 proteins were not changes. In order to clarify the key regulatory point, MK2206, an inhibitor of Akt phosphorylation site, was used to co-culture with LV-Cdc37 cells, and then Western blot was used to detect changes in the

expression of related proteins again, which showed that the expression of p-Akt, Cyclin D1 and Bcl-xL was reduced and the expression of BAD was increased.
3. Effect of shikonin on Cdc37/Akt signaling pathway: Treatment of LV-Cdc37 cells with shikonin (0.5μM or 1μM) for 72h reduced the proliferative activity of LV-Cdc37 cells, increased the proportion of G1-phase cells and reduced the proportion of both S-phase and G2/M-phase cells in LV-Cdc37 cells, and significantly increased the proportion of apoptotic cells in LV-Cdc37. The results of Western Blot showed that shikonin did not affect the expression of Cdc37, but significantly decreased the protein expression of Akt and downstream p-Akt, CyclinD1, Bcl-xL in concentration-dependent manner, and significantly increased the expression of BAD protein. In vivo experiments showed that shikonin significantly improved imiquimod-induced skin lesion in mice, and Western Blot showed that shikonin played a role by reducing the expression of Akt protein and reducing its phosphorylation.

Conclusion

1. Cdc37 expression is elevated in skin lesions of psoriasis patients compared to normal controls.
2. With Akt as the key regulatory point, Cdc37 promoted the phosphorylation of Akt in keratinocytes, further increased the expression of Cyclin D1 and differentially regulated the expression of Bcl-2 family proteins (Bcl-xL and BAD), then accelerated the proliferation of keratinocytes, inhibited the apoptosis of keratinocytes, and promoted the transition of keratinocytes from G1 phase to S phase , and these effects were accomplished jointly by binding to HSP90.
3. Shikonin reduced the expression of Akt protein, decreased its phosphorylation, and then regulated the expression of downstream protein molecules CyclinD1, Bcl-xL and BAD, which inhibited the accelerated proliferation of keratinocytes induced by Cdc37, reduced the proportion of cells transitioning from G1 to S phase, and induced apoptosis, ultimately improving the severity of psoriatic lesions in mice.

PO-052

Sodium thiosulfate inhibits epithelial-mesenchymal transition in melanoma via targeting the Wnt/β-catenin signaling pathway

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Background Melanoma is the most common form of skin cancer. Given its high metastasis and high recurrence, its therapies are constantly updated.
Objective The study aims to prove the efficacy of sodium thiosulfate (STS), an antidote to cyanide or nitroprusside poisoning, in melanoma treatment.
Methods We tested the effect of STS by culturing melanoma cells (B16 and A375) in vitro and establishing melanoma mouse models in vivo. The proliferation and viability of melanoma cells were measured by the CCK-8 test, cell cycle assay, apoptosis analysis, wound healing assay, and transwell migration assay. The expression of epithelial-mesenchymal transition (EMT)-associated molecules and the Wnt/β-catenin signaling pathway-related molecules were determined by Western blotting and immunofluorescence.
Results The high metastasis of melanoma is considered to be linked to the EMT process. The scratch assay using B16 and A375 cells also showed that STS could inhibit the EMT process of melanoma. We demonstrated that STS inhibited the proliferation, viability, and EMT process of melanoma by releasing H2S. STS-mediated weakening of cell migration was related to the inhibition of the Wnt/β-catenin signaling pathway. Mechanistically, we defined that STS inhibited the EMT process via the Wnt/β-catenin signaling pathway.
Conclusions These results suggest that the negative effect of STS on melanoma development is mediated by the reduction of EMT via the regulation of the Wnt/β-catenin signaling pathway, which provides a new clue to treating melanoma.

PO-053

A case of comprehensive treatment of pustular psoriasis

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The patient, female, 26 years old, came to our hospital for "recurrent erythema, pustules, scales and itching all over the body for 19 years and recurrence for 10 days". 19 years before admission, the patient developed erythema and pustules all over the body for unknown reasons. The rash first appeared on the trunk, manifested as small patches of erythema and scattered miliary-sized papules, with needle tip to miliary-sized pustules visible on it, partly fused into a lake of pus, accompanied by itching. During the course of the disease, the patient had repeated chills and fever, with a maximum body temperature of 40°C, no chest tightness, tightness of breath, abdominal pain, diarrhea, and joint pain and other discomforts. After the patient uses 30mg of Acitretin, hormone ointment, Chinese medicine and other treatments, the rash can be controlled, but it often recurs. Ten days ago, the patient had no obvious cause and the rash worsened again. The rash spread all over the body, showing erythema, papules, and pustules, accompanied by mild itching and pain. During the course of the disease, the patient had relapsed chills and fever, and the highest body temperature could reach 40°C. , No chest tightness, tightness of breath, no abdominal pain, diarrhea, no joint pain and other discomforts. The patient had a history of thalassemia and was not treated. The family history is nothing special. Specialty situation: The rash spreads all over the body, with the trunk and limbs as the weight. It is manifested as a large number of red plaques ranging from nails to palms and scattered miliary to mung bean-sized pustules. Silvery white scales can be seen on them, and some of the pustules merge into pus lakes. Austenite sign is positive, isomorphic reaction is positive, no joint swelling, psoriasis nail is seen, no bundle of hair is seen. Auxiliary examination: hemoglobin 96g/L, albumin 27.2g/L. Treated with Acitretin, compound glycyrrhizin, hormone ointment, supplemented with fluid rehydration, wet compress, intravenous infusion of albumin and other comprehensive treatments.

PO-054

Tranexamic acid may promote melanocores clustering in keratinocytes through upregulation of Rab5b

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Background Tranexamic acid (TXA) is a promising therapeutic agent in melasma that can act on multiple pathophysiologic mechanisms of melasma. However, it is unclear whether TXA affects melanin in keratinocytes.
Objectives To explore the effect of TXA on melanocores in keratinocytes.
Methods The melanocore-incorporated keratinocytes were constructed by co-incubating normal human epidermal keratinocytes (NHEK) with melanocores. After being treated with TXA, autophagy- and melanin-related protein expressions were detected. Then transcriptome sequencing was used to compare the genetic changes in melanocore-incorporated keratinocytes before and after TXA treatment and further verified the differentially expressed genes. At the same time, the distribution of melanocores in human keratinocytes was observed by transmission electron microscopy.
Results We found that TXA does not promote melanin degradation in primary keratinocytes by inducing autophagy. Protein transport and intracellular protein transport-related genes were enriched after TXA treatment, and Rab5b was significantly upregulated. Transmission electron microscopy showed that the percentage of melanocores distributed in clusters increased after treatment with TXA, which was reduced after Rab5b silencing. In addition, results suggested that melanocores could colocalize with Rab5b and lysosome-associated membrane protein1 (LAMP1).

Conclusions Our study found that Rab5b may be involved in the melanocore distribution in keratinocytes. TXA may promote the clustering distribution of endocytic melanocores through upregulation of Rab5b, representing a potential mechanism of TXA treatment against melasma.

PO-055

The Effect of 5-Aminolevulinic Acid Photodynamic Therapy in Promoting Pyroptosis of HPV-Infected Cells

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5-aminolevulinic acid photodynamic therapy(ALA-PDT) is highly effective in the treatment of condyloma acuminata (CA). Previous research has indicated that ALA-PDT could induce cell death by different mechanisms, including apoptosis and autophagy, but the role of pyroptosis in ALA-PDT remains uncertain. Thus, this study aimed to explore whether pyroptosis is a potential mechanism of ALA-PDT killing human papillomavirus (HPV) infected cells. HPV-positive HeLa cells were exposed to ALA-PDT, then cell viability assay, lactate dehydrogenase release (LDH) assay, detection of reactive oxygen species (ROS), quantitative real-time PCR (qPCR), and western blot were used to evaluate pyroptosis induced by ALA-PDT. Results suggested that ALA-PDT enhanced the expression of NLRP3, caspase-1, GSDMD, and the production of inflammatory cytokines such as IL-1β and IL-18. In addition, ALA-PDT induced the production of ROS and led to the destruction of the cell membrane. The inhibition of pyroptosis reduced the killing of HeLa cells by ALA-PDT. This study demonstrates that ALA-PDT induces pyroptosis in HPV-positive cells, which provides some explanation for the mechanism of ALA-PDT to treat CA and HPV infection-related diseases.

PO-056

A case of photosensitive drug eruption caused by candesartan

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A male patient with the age of 56 year old visited the doctor for the recurrent facial erythema for 2 months. One month before the onset of erythema, the patient began to take candesartan axetil tablets orally for the treatment of hypertension. The instructions of the drug indicated the side effect of photosensitivity. After stopping the drug and taking prednisone, hydroxychloroquine and ebastine orally, the rash improved quickly. However, the rash still recurred from time to time, and worsened after exposure to the sunlight. The persistent photosensitive reaction was considered.

PO-057

SIRT7 orchestrates melanoma progression by simultaneously promoting tumor cell survival and immune evasion via the activation of unfolded protein response

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Melanoma is the most lethal skin cancer originating from the malignant transformation of epidermal melanocyte. While the progress of targeted therapy and immunotherapy has gained revolutionary advances in improving the treatment outcome, the prognosis of patients with melanoma remains unoptimistic. During tumor progression, melanoma frequently encounters stress from both endogenous and exogenous sources in tumor microenvironment. SIRT7 is a nicotinamide adenine dinucleotide (NAD+)-dependent nuclear-localized deacetylase with versatile biological functions in maintaining cell homeostasis. Nevertheless, whether SIRT7 regulates tumor cell biology and anti-tumor immunity in melanoma under stressful tumor microenvironment remains elusive. Herein, we reported that SIRT7 orchestrates melanoma progression by simultaneously promoting tumor cell survival and immune evasion via the activation of unfolded protein response. We first identified that SIRT7 expression was the most significantly increased one in sirtuins family in response to stress. Then, we proved that the deficiency of SIRT7 potentiated tumor cell death under stress in vitro and suppressed melanoma growth in vivo. Mechanistically, SIRT7 selectively activated IRE1α-XBP1 axis to potentiate pro-survival ERK signal pathway and the secretion of tumor-promoting cytokines. SIRT7 directly de-acetylated SMAD4 to antagonize TGF-β-SMAD4 signal, which relieved the transcriptional repression on IRE1α, so that led to the activation of IRE1α-XBP1 axis. What's more, SIRT7 up-regulation eradicated anti-tumor immunity by promoting PD-L1 expression via IRE1α-XBP1 axis. Additionally, the synergized therapeutic effect of SIRT7 suppression and anti-PD-1 immune checkpoint blockade was also investigated. Taken together, SIRT7 can be employed as a promising target to restrain tumor growth and increase the efficacy of immunotherapy in melanoma.

PO-058

ER stress facilitates anti-cancer immunosurveillance and improves immunotherapy efficacy in melanoma

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Tumor cells frequently suffer from endoplasmic reticulum (ER) stress resulted from both increased accumulation of misfolded proteins and stressful tumor microenvironment characterized by hypoxia, acidosis and nutrients deprivation. Previous studies have extensively elucidated the role of ER stress and unfolded protein response (UPR) in melanoma pathogenesis, especially in the regulation of tumor cells' malignant behaviors. However, the effect of tumorous ER stress on tumor-infiltrating lymphocytes and anti-cancer immunosurveillance, as well as the underlying mechanism, remains far from understood. Herein, we demonstrated that tumorous ER stress facilitates anti-cancer immunosurveillance and improves immunotherapy efficacy in melanoma. We firstly found that the activation of UPR was positively associated with the feature of tumor-infiltrating lymphocytes in TCGA SKCM database and melanoma tissues. Then, pharmacological induction of ER stress exerted better anti-tumor effect in immunocompetent mice and was highly dependent on CD8+T cells. In parallel, the profile of immune cells in tumor microenvironment was significantly re-shaped. Subsequent mechanistic studies revealed that tumorous ER stress facilitated the expression and secretion of multiple chemokines and cytokines via IRE1α-NF-κB pathway, which was related to increased infiltration and anti-tumor capacity of CD8+T cells. Furthermore, tumorous ER stress also promoted the expression of PD-L1 via IRE1α-NF-κB axis, which brought synergized

therapeutic effect on melanoma along with anti-PD-1 antibody. Taken together, our results demonstrate that tumorous ER stress facilitates anti-cancer immunosurveillance and improves immunotherapy efficacy via the regulation of chemokines, pro-inflammatory factors and PD-L1 expression. The combination of pharmacological ER stress inducer and anti-PD-1 antibody could be a potent synergic approach for melanoma immunotherapy.

PO-059

Photoprotective potential of Dendrobium nobile Lindl polysaccharides against UVB-induced oxidative stress and apoptosis in HaCaT cells

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Objective Dendrobium nobile Lindl polysaccharides (DNP) contain various pharmacological activities. Nevertheless, the effects of DNP on skin photodamage remain unclear. Herein, we investigated whether DNP ameliorate ultraviolet B (UVB)-irradiated skin photodamage in HaCaT cells and its potential mechanism of protective effects.
Methods HaCaT cells were pretreated with DNP for 24 hours and exposed to UVB irradiation. After 24 hours, the cell viability was measured by cell counting Kit-8 (CCK-8) assay. Reactive oxygen species (ROS) and antioxidant biomarkers, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and malondialdehyde (MDA) content were analyzed. Cellular apoptosis and cell cycle were detected respectively with Annexin V-FITC/PI staining and PI staining by flow cytometry. Additionally, fluorescence microscopy of apoptotic cells was performed by Honest 33258 staining. The expression levels of several apoptotic-associated and mitogen-activated protein kinase (MAPK) signaling proteins were determined by western blot analysis of UVB-irradiated HaCaT cells with or without DNP pretreatment.
Results DNP exhibited the inhibitory effect against UVB-induced cell death on HaCaT cells. While DNP significantly decreased ROS and MDA content and restored the activities of SOD, CAT and GSH-Px, thereby effectively alleviating UVB-induced oxidative damage. The flow cytometry analysis suggested that DNP regulated HaCaT proliferation by influencing the cell cycle transition from G0/G1 to S phase, culminating in cell cycle arrest at the S phase, with a statistically decrease of cells in G2 phase. Notably, DNP caused significant inhibition of UVB-induced apoptosis, as evidenced by immunofluorescence, flow cytometry and western blotting. Moreover, DNP prevented UVB-mediated increases in cleaved caspase-3, p53, and the ratio of Bax/Bcl. In addition, DNP inhibited apoptosis through down regulating phosphorylation of MAPKs signaling pathway activated by UVB irradiation.
Conclusion These findings suggested that DNP treatment alleviated UVB irradiation-induced oxidative stress and apoptosis through MAPK signaling pathway in HaCaT cells.

PO-060

Thalidomide attenuates skin inflammation by inhibiting cathelicidin LL37 mediated TLR4/MyD88/NF-κB signaling pathway in rosacea

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Background Rosacea is a chronic inflammatory cutaneous disease characterized by immune system anomalies and vascular hyperreactivity. Thalidomide is a synthetic derivative acid with anti-

inflammatory and anti-angiogenic properties. However, the potential molecular mechanism of its therapeutic effects is still unclear.
Objectives Based on bioinformatics methods,screening the signal pathways and key genes related to the pathogenesis of rosacea,and exploring the effect and potential molecular mechanisms of thalidomide in rosacea.
Methods Bioinformatics methods screened the signal pathways and key genes related to the occurrence of rosacea. Mice were intradermally injected with LL37 to induce rosacea-like features and intraperitoneally administered with thalidomide. The expression of key genes (tlr4 and camp) and inflammatory cytokines (TNF-α and IL-1β) was detected by RT-qPCR. The number of CD31 positive microvessels and inflammatory cytokines were measured by immunofluorescence. The effect of thalidomide on inhibiting TLR4/MyD88/NF-κB signaling was determined by immunofluorescence and western blot.
Results We found that toll signal played a key role in the pathogenesis of rosacea by bioinformatics analysis. Our animal research also showed that toll signal was highly expressed in the skin of rosacea-like mice. Moreover, rosacea-like symptoms including
skin erythema and histopathological alterations, as well as the elevated pro-inflammatory cytokines (IL-1β and TNF-α) were ameliorated by thalidomide treatment. Furthermore, thalidomide repressed the angiogenesis by reducing the number of CD31+ cell and downregulating the expression levels of VEGF in rosacea.We further demonstrated that thalidomide attenuated the expression of TLR4, MyD88 and P-NF-κB in the skin of rosacea mice.
Conclusion Our research suggested that thalidomide ameliorated skin inflammation by inhibiting the cathelicidin LL37 mediated TLR4/MyD88/NF-κB signaling pathway in rosacea.

PO-061

CircRNA _105040 aggravates Cutibacterium acnes biofilm-induced inflammation via the miR-146a/MAPK/NF-κB axis

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Objective We explored at the function and probable mechanism of circRNA 105040 in the induction of inflammatory reactions in keratinocytes by Cutibacterium acnes biofilms.
Methods We had previously performed the circRNA microarray research on six different pairings of severe acne lesion and normal skin tissue. Additionally, using data from circRNA microarray analysis, we ruled out three circRNAs (circ 105040, circ 102678, and circ 102680) that could control miR-146a expression. We initially chose a circ 0105040 for the follow-up investigation out of the aforementioned 3 circRNAs.
First, we ran the two tests below to demonstrate that circ 0105040 is circRNA. 1. circ 105040 was amplified in cDNA using divergent primers, but not in genomic DNA, as shown by qRT-PCR (gDNA). The GAPDH negative control. 2. Fluorescence in situ hybridization (FISH) was utilized to re-examine the expression level of circ 105040 after qRT-PCR was performed to demonstrate the expression levels of linear FLNA and d circ 105040 after being treated with or without RNase R. By conducting the next two tests, we further investigated the distribution of reformed cyclic RNA in keratinocytes. 1.Using qRT-PCR, the distribution of circ 105040 subcellular in human keratinocytes was determined. 2. To identify the subcellular localization of circ 105040 in human keratinocytes, RNA fluorescence in situ hybridization (FISH) was performed.
By using qRT-PCR and Elisa, the levels of IL6, IL8, and TNF-a were measured in keratinocytes treated with 2 ×108 CFU/ml heat-inactivated biofilm-derived C. acnes with or without circ 105040 knockdown or overexpression.Western blotting examination of p-IkBa, IkBa, p-p38, p38, and p-ERK1/2 in human keratinocytes treated with 2×108 CFU/ml heat-inactivated biofilm-derived C. acnes, circ 105040 siRNA, overexpression circ 105040, or control.The expression of NF-KB P65

was measured in human keratinocytes treated with 2 ×108 CFU /ml heat-inactivated biofilm-derived C. acnes through stable circ 105040 siRNA, transfection of circ 105040, or vector.
The sequence of miR-146a that may bind circ 105040 was predicted by a biological information source. After co-transfection of the circ 105040 WT or mutant plasmid with miR-146a or miR-NC, luciferase activity was found. Circ 105040 expression levels in human keratinocytes as measured by RNA immunoprecipitation (RIP) test. RIP dependability was examined by Western blot using the Ago2 protein. Circ 105040 enrichment upon transfection with biotin-miR-146a or miR-NC was confirmed using a miRNA pull-down experiment. In human keratinocytes transfected with the circ 105040 siRNA or control, qRT-PCR was utilized to demonstrate the relative expression of circ 105040 and miR-146a. The relative expression of circ 105040 transfected with miR-146a or miR-NC was demonstrated using qRT-PCR in combination with anti-miR-146a or anti-miR-NC.
mRNA and protein levels of si-circ 102678 or si-NC transfected traf6 and IRAK1 were measured. The relative protein levels of IRAK 1 and TRAF6 were measured in keratinocyte after transfection with si-NC, anti-miR-146a, sicirc 102678, or anti-miR-146a co-transfected with si-circ 102678. qRT-PCR and Elisa investigation evaluated the relative amounts of IL6, IL8, and TNF-a expression in keratinocyte transfected with si-NC, anti-miR146a, sicirc 102678, or anti-miR-146a combined with sicirc 102678.
In order to determine the relative expression of TRAF6 and IRAK1 in human keratinocytes transfected with si-circ 105040 or si-NC, qRT-PCR was used. TRAF6 and IRAK1 transfected with si-circ 105040 or si-NC in human keratinocytes were analyzed by Western blotting. TRAF6 and IRAK1 protein expression in human keratinocytes following transfection with si-NC, anti-miR-146a, si-circ 102678, or si-circ 102678 and anti-miR-146a co-transfection. Human keratinocytes transfected with si-NC, anti-miR-146a, si-circ 102678, or si-circ 102678 and anti-miR-146a were stimulated with 2× 108 CFU/ml heat-inactivated biofilm-derived C. acnes, and the expression levels of IL6, IL8, and TNF-a were measured by qRT-PCR and Elisa.
Results A total of 1594 circRNAs were differentially expressed between the severe acne cystic fluid and normal skin tissue. Among those, 605 circRNAs were upregulated and 989 circRNAs downregulated (fold change ≥ 2, $p < 0.05$). Based on our recent report, miR-146a has been proved to play a key role in acne lesions. Therefore, we screened out three circRNAs(circ_105040, circ_102678,circ_102680) that may regulate miR-146a expression through circRNA microarray analysis data. fluorescence in situ hybridization (FISH) was used to re-examined that the expression level of circ_105040 was significantly reduced in acne tissues compared with the adjacent normal controls.
The results shown that divergent primers of circ_105040 was only amplified from cDNA, but not from gDNA. The RNase R degradation assay was used to illustrate whether circPPP1R12A could be resistant to digestion with RNase R treatment. The qRT-PCR results confirmed that linear transcripts of FLNA was remarkably degradation, while circ_105040 was retained after RNase R treatment. Nuclear mass separation assay and RNA fluorescence in situ hybridization (FISH) assay were used to demonstrated that circ_105040 was mainly localized in the cytoplasm.
The mRNA expression of IL-6, IL-8 and TNF-α in keratinocytes co-cultured with biofilm-derived acne C was significantly reduced by qRT-PCR. Meanwhile, ELISA showed that increased circ_105040 expression remarkably promoted the secretion of IL-6, IL-8, and TNF-a. We found that overexpression circ_105040 in keratinocytes obviously increased p-IKBA, P-P38 and P-ERK1/2 under biofilm-derived C. acnes induction. In addition, immunofluorescence assays results demonstrated circ_105040 restricted biofilm-derived C. acnes induced NF-kB p65 translocation. Taken together, these results reveal that circ_105040 can remarkably promotes biofilm-derived C. acnes induced inflammatory response in primary human keratinocytes through NF-KB and MAPK pathways.
We further validation the effects of knockdown circ_105040 in primary human keratinocytes under biofilm-derived C. acnes. As opposed to overexpressing circ_105040, qRT-PCR and ELISA assay analysis showed that transfected circ_105040 siRNA in keratinocytes could significantly decreased the levels of IL-6, IL-8, and TNF-a after biofilm-derived C. acnes stimulation. When circ_105040 was absent, the expression of phosphorylation IkBa, ERK1/2 and p38 were significantly decreased by WB. Furthermore, knockdown of endogenous circ_105040 activated NF-kB p65 translocation.

These results indicate that silencing circ_105040 remarkably inhibited biofilm-derived C. acnes induced inflammatory response in primary human keratinocytes.
To investigate whether circ_105040 promotes inflammatory response in acne through acting as ceRNA to sponge miR-146a in keratinocytes. We then found that bioinformatics program StarBase predicted that miR-146a might bind sequences
of circ_105040. To verified our hypothesized, we transfected luciferase reporters which contained predicted target site or mutated fragment of circ_105040 into cells. Subsequent luciferase reporter assay results showed that increased miR-146a expression significantly reduced the luciferase activity of wild-type circ_105040 reporter gene, but did not decrease the luciferase activity of mutant circ_105040. Next, we detected the level of circ_105040 was significant enrichment in Ago2 group compared with negative IgG immunoprecipitants by RNA immunoprecipitation (RIP). Furthermore, we transfected biotin-labeled miR-146a and Bio-NC control into cell. The biotin-labeled miRNA pulldown assays found that overexpression of biotin-labeled miR-146a significantly increased circ_105040 enrichment.
To explore whether circ_105040 had an effect on the expression of miR-146a in primary human keratinocytes, we performed circ_105040 siRNA or control siRNA to successfully silenced circ_105040 expression. The qRT-PCR results showed that reducing circ_105040 significantly increased the expression of miR-146a compared with control siRNA. Additional, the expression of circ_105040 was inhibited in keratinocytes transfected with miR-146a mimics. Conversely, transfection of anti-miR-146a markedly increased the level of circ_105040 mRNA in keratinocytes. These results suggested that circ_105040 can directly sponge miR-146a to inhibit the expression of miR-146a.
We then investigated if the presence of circ_105040-miR-146a- IRAK1 /TRAF6 regulatory network axis in acne. To test our hypothesis, we transfected keratinocytes with circ_105040 siRNA and found obviously reduced the expression of IRAK1 and TRAF6 by qRT-PCR and western blot assays. The western blot assay result showed that the expression of IRAK1 and TRAF6 was partially alleviated in the co-transfected group, compared with the cells transfected with miR-146a inhibitor alone. Meanwhile, we used qRT-PCR and ELISA assays to found that co-transfected with circ_105040 siRNA and miR-146a inhibitor could rescued overproduction of IL-6, IL-8, and TNF-α, compared to only transfected miR-146a inhibitor in keratinocytes.
Discussion In this study, we applied circRNA microarray test in six pairs of acne cystic fluid and control skin tissue groups and identified 1594 circRNA significantly expression. In addition, we further demonstrated that circ_102678 was significantly down-regulated in acne lesions. Functional analysis showed that circ_102678 promoted keratinocyte inflammation through the production of IL-6, IL-8 and tumor necrosis factor (TNF-α) and activation of NF-KB and MAPK pathways. Mechanistic studies reveal that circ_102678 acts as ceRNA to inhibit the expression of miR-146a.

PO-062

Study on the effect of shikonin on NF-κB signaling pathway in imiquimod-induced inflammation model of psoriasis in mice

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Objective To evaluate the anti-psoriatic inflammation effect of shikonin in vivo，and to explore its mechanism of action.
Methods BALB/c mice were randomly divided into blank control group, imiquimod（IMQ）model group，shikonin low-dose [5mg/kg/d]，middle-dose [7.5mg/kg/d] and high-dose [10mg/ kg/d] group. Continuously shave the hair on the 3.0cmX2.0cm area on the back of the mouse and apply

62.5mg of 5% imiquimod (IMQ) cream for 7 days, and apply the same dose of petrolatum to the blank control group. The shikonin group was given daily by gavage Drugs（the drugs of each group are dissolved in edible oil），and the blank control group and the imiquimod（IMQ）model group were given edible oil containing 5% DMSO [1mg/kg/d] by gavage. Take pictures of the skin lesions daily for PASI score. On the 8th day, the mice were sacrificed and the skin tissues were collected. The histological changes of the skin lesions were observed by hematoxylin and eosin（H&E）staining. Immunohistochemistry（IHC）to detect the expression of NF-κB（p65）protein in skin lesions. Western blotting detects the level of NF-κB（p65）protein，and real-time qPCR（RT-qPCR）was used to detect the expression of inflammatory factors IL-6，IL-1βand TNF-αin the skin lesions.

Results PASI score and skin lesions on the back of mice showed that shikonin could inhibit erythema，scale and skin thickening in mice. HE staining results showed that compared with IMQ model group，shikonin group had a thinner spinous layer and reduced dermal cell infiltration. Immunohistochemistry showed that shikonin can significantly inhibit the expression of NF-κB（p65）protein in the skin lesions of psoriasis mice in a dose-dependent manner（$P<0.05$）. Compared with the model group，Western blotting results show that shikonin can significantly inhibit the expression of NF-κB（p65）protein（$P<0.05$），and PCR results showed that shikonin can significantly reduce the mRNA levels of IL-6，IL-1β，and TNF-α，and the differences were statistically significant（$P<0.05$）.

Conclusions Shikonin can improve the skin inflammatory response of imiquimod-induced psoriasis mice, and its mechanism may be related to the inhibition of the release of related inflammatory factors through the NF-κB signaling pathway.

PO-063

Del-1 attenuates neutrophil infiltration in psoriatic epidermis by inhibiting NF-κB activation and reducing the expression of CXCL8 and ICAM-1 in keratinocytes

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Objectives The purpose of this study was to investigate the effect of keratinocyte-derived Del-1 on neutrophil infiltration into epidermis in psoriasis, and to explore the underlying molecular mechanism of its effect on neutrophil chemotaxis and adhesion function, providing theoretical basis for excessive neutrophil infiltration in the epidermis of psoriasis, and providing a new potential target for the treatment of psoriasis.

Methods The expression of Del-1 in the skin lesions of psoriasis vulgaris patients and its correlation with IL-17A were analyzed using GEO database data, and verified by RT-qPCR in the skin lesions of patients. Immunohistochemistry was used to locate and semi-quantify the expression of Del-1 in the epidermis of patients with psoriasis vulgaris, and RT-qPCR was used to detect the expression of Del-1 in the epidermis of patients with psoriasis by separating the epidermis of diseased skin and healthy skin. The results were verified by RT-qPCR and Western Blot in cell model.The experiment was divided into four groups: normal HaCaT cell was used as negative control, TNF-α-stimulated-HaCaT simulating psoriasis cell model as positive control, TNF-α-stimulated-overexpression-Del-1 HaCaT cell and TNF-α-stimulated-knockdown-Del-1 HaCaT cell as experimental groups. The chemotaxis of different groups of HaCaT cells to neutrophils was compared by Transwell experiment, and the adhesion between different groups of HaCaT cells and neutrophils was compared by adhesion experiment. The differential genes in the above four groups were screened by inflammatory response and autoimmunity PCR array plate and verified by RT-qPCR. The expression of adhesion molecule ICAM-1 was detected by RT-qPCR and Western Blot. The total protein and nuclear protein of the above four groups were extracted, and the

phosphorylation and nuclear translocation of NF-κB-p65 in the four groups were detected by Western Blot to explore the molecular mechanism of Del-1 regulating inflammatory factors.
Results Del-1 was constitutively expressed in human skin keratinocytes, and decreased in psoriatic lesions, epidermis and cell models, and negatively correlated with IL-17A. Compared with the psoriatic model group simulated by HaCaT cells stimulated by TNF- α, the chemotaxis and adhesion of neutrophils to HaCaT cells decreased in overexpression-Del-1 group，while enhanced in knockdown-Del-1 group. CXCL8(the main chemokine of neutrophils) and ICAM-1(the key factor in adhesion) decreased in overexpression-Del-1 group and increased in knockdown-Del-1 group. NF-κB is one of the major transcription factors regulating the expression of CXCL8 and ICAM-1. The phosphorylation and nuclear translocation of its subunit p65 decreased in overexpression-Del-1 group and increased in knockdown-Del-1 group.
Conclusion Del-1 attenuates neutrophil infiltration in psoriatic epidermis by inhibiting NF-κB activation and reducing the expression of CXCL8 and ICAM-1 in keratinocytes.

PO-064

Mycobacterium marinum Infection: A Case Report

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Mycobacterium marinum is a pathogen that causes skin and soft tissue infections in people who work with contaminated water usually by small wounds of the skin.We reported a case of a 68-year-old male used to be a fish handler, presented with erythematous,verrucous plaque and nodule, with nodulesand crusts, located on the dorsal aspect of his right hand and forearm for 1 year. Histologic features showed that abscess-formation, an accumulation of leukomonocytes and mononuclear leucocytes, and PAS stain was positive, and finally Mycobacterium marinum were identified by DNA sequencing.The treatment were Rifampin, levofloxacin combined with Clarithromycin.

PO-065

Effect and mechanism Study of hyperthermia on DNAJA4, TRPM2 channel and NLRP3 inflammasome pathway in keratinocytes

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Objective Hyperthermia refers to the treatment of a disease with more than normal human temperature, which has been involved in direct or adjuvant treatment of many diseases. Hyperthermia have been written into the European Wart Treatment Guidelines. It can remove skin lesions from distant parts by mobilizing whole body immunity. Therefore, the study of hyperthermia treatment of wart is constantly evolving. How to improve the therapeutic effect is the main breakthrough point. According to the observation in clinical experiments, we can see that there can be redness and swelling around the wart after treatment, which indicates that there is a local inflammatory response. We start with the direction of inflammasome pathway and further explore the underlying mechanism of warm treatment of viral warts, so as to provide a new direction for improving the efficacy of warm treatment.
Methods Two cell lines were used for this study, HaCaT cell lines: immortalized keratinocytes (WT), immortalized DNAJA4-deficient HaCaT cells (A4KO). The cells were heated at 44℃ for 30 minutes. 1. First, CCK8 and LDH assay were used to detect cell viability and cytotoxicity. RT-qPCR was used to detect the expression of mRNA levels of molecules involved in the NLRP3 inflammasome

pathway. We used an enzyme labeling instrument to detect caspase 1 activity in cells. 2. To further investigate the role of hyperthermia in inducing inflammasome pathway activation, we tested the CRAC channel by PCR and Western blot. Then we determined the molecule for the subsequent experimental by detecting the levels of mRNA involved in the Trp channel, and finally choose the TRPM2 channel. The best concentration of TRPM2 inhibitor on cells was selected by CCK8 experiment. Cell pyroptosis and apoptosis were detected by flow cytometry. 3. After adding TRPM2 inhibitor, the intracellular calcium concentration and intracellular ROS level were detected by flow cytometry. The changes of NLRP3 and TRPM2 were detected by RT-qPCR. The mean fluorescence intensity of NLRP3 was detected by immunofluorescence assay. ELISA was used to detect the change of inflammatory cytokines secreted by cells.

Results 1. At 24h, The effect of 44 ℃ hyperthermia on A4KO cells significantly decreased cell viability and increased cytotoxicity compared to WT. At 12h, the RT-qPCR showed that the mRNA expression of NLRP3 and ASC was significantly increased in both WT and A4KO cells. The mRNA expression of NLRP3 and ASC was more prominent in A4KO than WT cells at 12h. The results of Caspase-1 activity showed that hyperthermia increased the enzyme activity more significantly in A4KO cells. Western Blot showed that the expression of NLRP3 and Pro-IL-1β increased significantly at 6h and 12h after hyperthermia in A4KO cells. 2. The expression of mRNA in STIM1 showed that the expression decreased at 6h after hyperthermia. While there was no significant difference at 12 h and 24 h after hyperthermia compared with 37℃. Western Blot experiment found that the expression level of STIM1 and ORAI in HaCaT cells was not significantly different from that at 37 C and 12h after hyperthermia. RT-qPCR was used to detect the mRNA expression of molecules related to the hyperthermia of TRP channels at 44℃. It was found that the trend of TRPM2 expression was similar to that of NLRP3. CCK8 assay was used to detect the effect of TRPM2 inhibitor (ACA) concentration gradient on cell viability. We finally select 20 μM concentrations of ACA for subsequent experiments. ACA were added 1h before hyperthermia to pre-stimulated cells. The proportion of apoptotic and pyroptotic cells was determined by flow cytometry at 12h after hyperthermia. It was found that the death rate of A4KO group was significantly higher than that of WT group. Moreover, ACA could inhibit the pyroptosis which produced by hyperthermia in the two kinds of cells. 3. Using flow cytometry, we found that hyperthermia at 44 ℃ significantly increased the intracellular ROS level and calcium ion concentration. The differential elevation was more significant in the A4KO cells. After ACA stimulation, the Ca2+ and ROS levels induced by hyperthermia were significantly inhibited. ACA significantly inhibited NLRP3 and TRPM2 mRNA expression levels in A4KO cells induced by hyperthermia. Immunofluorescence experiments proved that hyperthermia induced an increase in the expression levels of NLRP3. NLRP3 expression was significantly down regulated after the addition of ACA. ELISA experiments showed that hyperthermia could stimulate the secretion of IL-1β and IL-18. After ACA stimulation, the secretion of the two inflammatory factors decreased significantly.

Conclusions 1. 44 ℃ hyperthermia induced the activation of NLRP3/IL-1β pathway in keratinocytes. DNAJA4 deficiency can activate NLRP3 inflammatory pathway through priming NF-κB pathway. 2. Hyperthermia stimulated the expression of TRPM2 mRNA in keratinocytes and resulted in an increase of cell death. The expression of TRPM2 mRNA and the ratio of pyroptosis cells increased more significantly in DNAJA4 deficient keratinocytes. 3. Hyperthermia activates the NLRP3 pathway through the activation of TRPM2/ROS/Ca2+ channel. DNAJA4 can inhibit intracellular oxidative stress and calcium ion influx in keratinocytes induced by hyperthermia.

PO-066

The research progress of NF-kB signaling pathway in psoriasis

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Nuclear factor kappa B (NF-kB) is a protein transcription factor that runs through a variety of physiological and pathological processes. It is a key regulatory element in a variety of immune and inflammatory pathways, in cellular proliferation and differentiation and in apoptosis. Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyperproliferation and differentiation.Therefore NF-kB is a crucial mediator involved in the pathogenesis of psoriasis.Herein, we summarize the current understanding of NF-kB pathway in psoriasis, and discuss the prospects for targeting NF-kB in the treatment of psoriasis.

PO-067

Systems pharmacology-based anti-proliferation and anti-angiogenesis mechanisms of oridonin in psoriasis keratinocytes by blocking the PI3K/AKT signaling pathway

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Background Psoriasis is a chronic, hyperplastic and autoimmune skin disease by polygenic and multi-environmental stimuli. Various small molecule inhibitors targeting dysregulated enzymes and signal transduction pathways in psoriasis are also under amplification in clinical trials. As a small molecule, oridonin has been demonstrated to inhibit keratinocyte proliferation. However, the underlying mechanism of oridonin in treating psoriasis is still lacking.
Methods The oridonin-targets and psoriasis related genes were predicted based on public database. The common targets of oridonin and psoriasis were selected by Venny R package, and DAVID online were used to perform the enrichment analysis. Using the Cytoscape program, the Oridonin-Psoriasis-Target (OPT) network, protein-protein interaction (PPI) network and Oridonin-Target-Pathway (OTP) network of oridonin treated psoriasis were constructed, respectively. Furthermore, the AutoDock was used to verify the relationship between oridonin and core targets, and the ZDOCK was performed to observe protein interaction simulation of core protein-protein pairs. All bioinformatics results were validated through molecular biology experiments.
Results Oridonin inhibited keratinocyte hyperplasia, and the IL-1β, IL-6 and TNF-α decreased in HaCaT cells. QRT-PCR demonstrated oridonin significantly inhibited angiogenesis related genes expression levels, such as CXCR7, CXCL12 and VEGF.
Based on public database, 73 targets were selected and high correlated with oridinon treated psoriasis. In PPI network, 6 kernel clusters were isolated from network using ClusterONE algorithm, and 9 PPI pairs contain 13 targets were screened by combined-score ≥0.999. The Oridonin-Target-Pathway (OTP) network, molecular docking and protein interaction simulation suggested that the oridinon mainly targeting SIRT1, CDK2, SRC and PTPN, and the PI3K-Akt signaling pathway was regulated by SIRT1-TP53, CDK2-TP53, SRC-HSP90AA1 and PTPN1-EGFR pairs. Furthermore, the qRT-PCR showed that the levels of SIRT1, CDK2, SRC, PTPN1, TP53, HSP90AA1 and EGFR were significantly changed, and Western Blot revealed the Bad and cleaved caspase-3 were significantly upregulated, Bcl-2, p-ERK1/2 and p-p38 was significantly decreased in oridonin treatment.
Conclusion In this work, the significant targets of oridonin treated psoriasis were identified based on network pharmacology. The bioinformatics and validations demonstrated that the SIRT1, CDK2, SRC, PTPN1, TP53, HSP90AA1 and EGFR were strongly related to oridonin, which shown a

positive correlation with anti-proliferation and anti-angiogenesis by blocking PI3K-Akt signaling pathway in psoriasis treatment.

Keywords: Oridonin, psoriasis, system pharmacology, keratinocyte proliferation, angiogenesis, PI3K/AKT signaling pathway

Introduction

Psoriasis is a chronic, hyperplastic and autoimmune skin disease, which caused by polygenic risk factors and multi-environmental stimuli, and it affects approximately 0.1% to 3% of the global population (1, 2). Abnormal immune system and keratinocytes are the main manifestations of skin tissues in psoriasis patients. Initially, psoriasis is triggered by the sustained activation of plasmacytoid dendritic cells by epidermal antigens caused by skin trauma or infection (3), which promotes the maturation of myeloid dendritic cells and the secretion of the interleukin (IL)-6, IL-12, and IL-23, and then causes T lymphocytes to differentiate into Th1 and Th17 cells (4). In psoriasis, effector cytokines such as IL-17 and IL-22 as well as tumor necrosis factor (TNF)-α is mainly manifested as disorders of keratinocyte proliferation and differentiation, and hyperkeratosis and parakeratosis of the epidermis (5). Such being the case, patients with moderate to severe psoriasis are treated with biologics targeting T lymphocyte factors such as TNF-α, IL-23, and IL-17 (6).

Recently, various small molecule inhibitors targeting dysregulated enzymes and signal transduction pathways in psoriasis are also under evaluation in clinical trials (7). However, these drugs can only relieve the symptoms of psoriasis, as there is no cure to date. Furthermore, they cannot be taken for an extended period of time due to its side effects such as skin atrophy, organ toxicity, immunosuppression, infection, and carcinogenesis. Therefore, it is necessary to develop new drugs that are both highly effective and have minimal side effects. Oridonin is an ent-kaurane tetracyclic diterpenoid from Rabdosia rubescens, which can clear away heat and toxins in the body, inhibit inflammation and anti-angiogenesis in various carcinomas (8-12). Several studies have shown that oridonin is effective at inhibiting proliferation and migration and expression of inflammatory factors, as well as promoting apoptosis of keratinocytes (13).

In this work, we predicted the potential targets of oridonin based on network pharmacology and molecular docking. The protein-protein interaction (PPI) network and cell experiments demonstrated that the oridonin targeting SIRT1, CDK2, SRC, PTPN1, TP53, HSP90AA1 and EGFR, and then inhibit cell proliferation and anti-angiogenesis to fight against the psoriasis by PI3K-Akt signaling pathway. This study could provide novel insights and a meaningful analysis of the therapeutic effect of oridonin on psoriasis.

Conclusion

Using network pharmacology analysis, the significant targets of oridonin treated psoriasis were identified. The bioinformatics and validations demonstrated that the SIRT1, CDK2, SRC, PTPN1, TP53, HSP90AA1 and EGFR were strongly related to oridonin, which shown a positive correlation with anti-proliferation and anti-angiogenesis by blocking PI3K-Akt signaling pathway in psoriasis treatment. As a remarkable advantage, the PI3K-Akt signaling pathway showed great performance in psoriasis, which implied it could has broader application in clinic and has promising values as candidate therapy targets.

PO-068

Untargeted metabolomics analysis of plasma metabolic characteristics in patients with acne and insulin resistance

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Objective Acne vulgaris is a chronic inflammatory skin disease involving multiple factors, mainly the pilosebaceous glands, that often occurs on the face, chest, and back. Acne typically manifests

as comedones in the early stage, which may then evolve into papules, pustules, nodules, cysts, and even scars. Traditionally, four distinct processes have been thought to play key roles: increased sebaceous secretion, hyperkeratosis of the hair follicle and sebaceous ducts, microbial colonization of the hair follicle, and immune responses to infection. However, the pathogenesis of acne vulgaris is still not well understood. Insulin resistance (IR) refers to a decrease in glucose uptake and utilization efficiency of body organs, such as the liver and muscle, under the action of various internal and external factors. To maintain the stability of blood glucose, excessive insulin secretion results in hyperinsulinemia. The homeostatic model assessment for IR (HOMA-IR) is often used in clinical practice and academic fields to evaluate IR. In acne vulgaris, sebaceous lesions are thought to occur after abnormal desquamation of keratinocytes within the sebaceous hair follicles. Nonunion erosion and accumulation of granulation tissue often occur in some acne cysts. Studies have shown that the physiological secretion of insulin/IGF-1 modulates keratinocyte differentiation, proliferation, and migration in healthy subjects, and can also regulate the growth of fibroblasts and promote the proliferation of granulation tissue through the Grb2-ERK1/2 and PI3K-Akt signaling pathways. Therefore, hyperinsulinemia often causes a variety of skin manifestations, and acne is another important clinical feature of insulin resistance-hyperandrogenemia. Acne was originally thought to be a self-limiting disease of adolescence. More than 85 percent of teenagers suffer from acne, and this may be due to “physiological IR” during adolescence. Some patients achieve remission or even a complete cure of acne in late adolescence, which may be due to a decrease in insulin/insulin-like growth factor (IGF-1) levels in the body. However, some people still have recurrent acne, even after the age of 25 years. This may be related to the high levels of androgen and pathological IR in adults. A study of the association between acne and IR in 100 adult men with acne found a significantly higher prevalence of IR than in controls. In women, especially those with polycystic ovary syndrome (PCOS), acne is more closely related to insulin levels. However, there are no reports on the mechanisms of action of acne vulgaris and IR. Metabolomics is a tool used to study the types, quantities, and changes in metabolites caused by external stimuli, pathophysiological changes, and genetic mutations in organisms. Untargeted metabolomics aims to obtain a comprehensive profile of all measurable small molecules in a given sample, including unknown analytes. Metabolomics can be used in clinical applications, such as the etiology, pathogenesis, and clinical diagnosis of diseases. For example, LC-MS/MS targeted metabolomics has been used to analyze differences in metabolic characteristics between acne patients and healthy controls, which may provide new ideas for the treatment of acne.

Methods LC-MS/MS was used to analyze serum samples from patients with acne and insulin resistance (n=51) and acne without insulin resistance (n=69) to identify significant metabolites and metabolic pathways.Two tubes of fasting venous blood, 7–8 mL each, were drawn from patients after fasting for 8 h. After one tube of blood was centrifuged at 3000 RPM for 10 min at 4 °C, the supernatant was collected in EP tubes, numbered, and stored at −80 °C for LC-MS/MS analysis. Another blood tube was used to detect fasting glucose (FBG) and fasting insulin (FINS) levels using an automatic blood biochemical analyzer. Analyses were performed using a UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600) at Shanghai Applied Protein Technology Co., Ltd. (Shanghai, China). For HILIC separation, samples were analyzed using a 2.1 mm × 100 mm ACQUITY UPLC BEH 1.7 µm column (Waters, Ireland). In both ESI positive and negative modes, the mobile phase contained 25 mM ammonium acetate and 25 mM ammonium hydroxide in water (B=acetonitrile). The gradient was 85% B for 1 min and was linearly reduced to 65% in 11 min, reduced to 40% in 0.1 min and kept for 4 min, and then increased to 85% in 0.1 min, with a 5 min re-equilibration period. For RPLC separation, a 2.1 mm × 100 mm ACQUITY UPLC HSS T3 1.8 µm column (Waters) was used. In the ESI positive mode, the mobile phase contained A=water with 0.1% formic acid and B=acetonitrile with 0.1% formic acid, whereas in the ESI negative mode, the mobile phase contained A=0.5 mM ammonium fluoride in water and acetonitrile (B). The gradient was 1% B for 1.5 min and was linearly increased to 99% in 11.5 min and kept for 3.5 min. Then it was reduced to 1% in 0.1 min, and a 3.4 min of re-equilibration period was employed. The gradients were set at a flow rate of 0.3 mL/min, and the column temperatures were kept constant at 25 °C. A 2 µL aliquot of each sample was injected. The ESI source conditions were set as follows: ion source Gas1 (Gas1) as 60, ion source Gas2 (Gas2) as 60, curtain gas (CUR) as 30, source temperature at 600 °C, and ion spray voltage floating (ISVF)

± 5500 V. In MS acquisition, the instrument was set to acquire over the m/z range 60–1000 Da, and the accumulation time for the TOF MS scan was set at 0.20 s/spectra. In the auto MS/MS acquisition, the instrument was set to acquire over the m/z range of 25–1000 Da, and the accumulation time for the product ion scan was set at 0.05 s/spectra. The product ion scan was acquired using information-dependent acquisition (IDA) with a high-sensitivity mode. The parameters were set as follows: the collision energy (CE) was fixed at 35 V with ± 15 eV; declustering potential (DP), 60 V (+), and −60 V (−), excluding isotopes within 4 Da; candidate ions to monitor per cycle: 10. The raw data were converted to mzXML files using Proteo Wizard MSconvent and processed using XCMS for feature detection, retention time correction and alignment. The metabolites were identified by accuracy mass (<25 ppm) and secondary mass spectrometry data which were matched with standard database (Shanghai Applied Protein Technology Co., Ltd.). In the extracted ion features, only the variables having more than 50% of the nonzero measurement values in at least one group were kept. For the multivariate statistical analysis, the MetaboAnalyst (www.metaboanalyst.ca) web-based system was used. After the Pareto scaling, principal component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA) were performed. The leave one out cross validation and response permutation testing were used to evaluate the robustness of the model. The significant different metabolites were determined based on the combination of a statistically significant threshold of variable influence on projection (VIP) values obtained from OPLS-DA model and two tailed Student's t-test (p value) on the raw data, and the metabolites with VIP values > 1.0 and p values < 0.1 were considered as significant. The Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) database was applied to pathway analysis.

Results In this study, there were 51 patients (25 men and 26 women) with acne combined with IR, with a prevalence of 42.5%, and there were 69 patients without IR, including 40 women and 29 men. The average age, FBG, FINS, and HOMA-IR were statistically different between the two groups, but there was no significant difference in the sex composition ratio (p>0.05). The VIP values of each metabolite were obtained by the OPLS-DA model analysis of the two groups of samples. According to the VIP value (VIP >1 and p-value <0.05), 18 metabolites with biological significance were screened out. In the positive ion mode, the upregulated substances were creatine, sarcosine, D-proline, uracil, Phe-Phe, L-pipecolic acid, and DL-phenylalanine; the downregulated substances were tridecanoic acid (tridecylic acid), L-lysine, cyclohexylamine, sphingomyelin (d18:1/18:0), epsilon - (gamma - L - glutamyl) - L - lysine, and 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine. In the negative ion mode, the upregulated substance was cholesterol sulfate, and the downregulated substances were D(-)-beta-hydroxybutyric acid, myristic acid, d-galacturonic acid, and dihydrothymine. Cholesterol sulfate showed the most significant expression among all differential metabolites (VIP=7.3411). A bar chart was used to analyze the significant differential metabolites and fold changes between the groups. Compared to the control group, the expression of six substances, such as triadecanoic acid and L-lysine, was downregulated in the positive ion mode. In the negative ion mode, only cholesterol sulfate expression showed an upward trend. In order to explore the functional relevance or positive/negative correlation between the significantly different metabolites, correlation analysis was performed, and the results showed that there was a significant positive correlation between creatine and sarcosine in the positive ion mode. L-lysine was positively correlated with epsilon-(gamma-L-glutamyl)-L-lysine, and sphingomyelin (d18:1/18:0) with 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine. The remaining significant metabolites were negatively or not correlated with each other the negative ion mode, and d-galacturonic acid was positively correlated with dihydrothymine. Myristic acid was positively correlated with d-galacturonic acid and dihydrothymine levels. Cholesteryl sulfate was negatively correlated with myristic acid, d-galacturonic acid, and dihydrothymine. Other significant metabolic differences had less correlation. The significant differential metabolites screened under the positive and negative ion models were merged, and the above metabolites were annotated using the KEGG database (Kyoto Encyclopedia of Genes and Genomes; http://www.kegg.jp/), with a total of 24 annotated pathways. Fisher’s exact test was performed on the above metabolic pathways and their Rich factor levels were evaluated. There were eight pathways with high enrichment, among which the p-values of arginine and proline metabolism were the lowest (p <0.05). The necroptosis pathway had the largest enrichment factor (0.1000) and contained the most significant differential metabolites. To

screen out the metabolic pathway of the overall change by differences in abundance and chart analysis, in article 8 metabolic pathways, the experimental group was compared to the control group: glycine, serine, and threonine metabolism, and arginine and proline metabolism showed a trend of increase; sphingolipid signaling pathway and necrotizing apoptosis expression showed a trend of cut; the expression of ABC transporter cut trend was not obvious; the expression of protein digestion and absorption, lysine degradation, and aminoacyl-tRNA biosynthesis showed no up-or down-trend.

Conclusion The differential metabolites screened in this study, such as cholesterol sulfate and creatine, have a certain impact on the pathogenesis of acne with IR and may be potential biomarkers for clinical symptoms of acne with IR, which provides new ideas and methods for subsequent targeted therapy. Necroptosis and ABC transporters were the most significantly enriched metabolic pathways in this study, which may be important pathways involved in the pathogenesis of acne with IR. This may provide new possibilities for the clinical diagnosis of acne in patients with IR and the development of targeted drugs.

PO-069

Two distinct cutaneous forms of metastatic clear cell chondrosarcoma occurring after 21 years of post-operation in the left iliac chondrosarcoma

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Abstract Chondrosarcoma is the second most common bone cancer, and Clear cell chondrosarcoma (CCCS) is a rare low-grade malignant cartilage tumor that accounts for about 2% of all chondrosarcoma cases. As far as we know, there have been no previous report of skin metastases from CCCS. We present a rare case of metastatic clear cell chondrosarcoma with two distinct cutaneous forms occurring after 21 years of post-operation of left iliac chondrosarcoma.
Key words chondrosarcoma, metastasis, skin

Introduction Chondrosarcoma is the second most common primary bone tumor after osteosarcoma. It primarily affects adults over the age of 40 and is distinguished by a proclivity for distant metastasis[1, 2]. The lung, soft tissues, lymph nodes, bones, and brain are the most frequent locations for hematogenous metastasis to occur[3], but skin metastasis is incredibly uncommon. A rare subtype of chondrosarcoma known as clear cell chondrosarcoma is considered to be a low-grade tumor that is less aggressive than atypical cartilage cancers[4]. We describe a rare instance of clear cell chondrosarcoma with two distinct cutaneous forms on the buttock and face.

Case Presentation A 50-year-old woman arrived to our clinic with brown papules on her face and buttock. Prior to the removal of the tumor from the left iliac bone, she had a 21-year history of clear cell chondrosarcoma. Before around 5 years, she started to develop papules and nodules that gradually formed and indicated a progressive pattern, without any indication of spontaneous retreat. The physical examination revealed numerous dispersed brownish red irregular papules and nodules across the body. A collection of brown papules with 1 mm in diameter were observed on her left buttock adjacent to the previous surgical incision. These small papules was flat-topped, and wave-like and parallel in arrangement (Figure 1). The only noticeable symptom she had for the other five years was itching in her buttock region. We considered cutaneous amyloidosis and skin neoplasm when making the first diagnosis. In later laboratory studies, we discovered enhanced tumor markers neuron-specific enolase (NSE) and gastrin-releasing peptide (ProGRP), and decreased thyroglobulin (TG). The results of an abdominal ultrasonography showed substantial hypoechogenicity within dilated portal veins. Multiple pulmonary nodules were visible on chest CT scans of both lungs.

As a result, the patient underwent excisional biopsy of the lesion. The diagnosis of cutaneous amyloidosis was ruled out since the removed tissue did not exhibit any Congo red staining. The histopathologic examination demonstrated a moderately differentiated clear cell type of metastatic chondrosarcoma. The tissue from the skin incision was reevaluated in our institution's pathology department, and the findings revealed that the lesion was in the deep dermis with a multinodular distribution. However, tumor cells were abundant in some areas, they were immature, and there was some atypia, so malignant tumors with chondrogenic differentiation could not be excluded (Figure 2). The findings of the immunohistochemical staining were as follows: CK5/6 (-), P63 (+/-), CK7 (-), EMA (-), S-100 (+), SMA (partial +), Ki-67 (hot spot area around 7% +), P53 (-), CD2 (-), CD68 (small +), CK (-), HMB45 (-), calponin (-), and LCA (-), and toluidine blue staining (partial +/-) (Figure 3). We recommended the patient to visit the oncology department for additional diagnosis and treatment in light of the patient's abnormal abdominal ultrasound and chest CT results. The patient underwent whole-body PET-CT at Anhui Provincial Hospital, which revealed increased FDG metabolism in the left iliac bone and surrounding soft tissue mass and multiple metastases in the patient's bilateral lung, head and neck, trunk, lower extremity subcutaneous tissue, and muscle. The patient was treated by chemotherapy using cyclophosphamide, epirubicin, and anlotinib on 27 July 2022. The patient is still being follow-up.

Discussion After osteosarcoma, chondrocyte-based malignant tumors are the most frequent primary malignant tumors of bone[5]. Primary chondrosarcomas manifests in a variety of forms, including extraosseous, myxoid, clear cell, paracortical, traditional intramedullary, and dedifferentiated[6]. Unni et al. first identified the clear cell variation known as CCCS as a chondrosarcoma[7]. CCCS is a low-grade, rare form of malignant cartilage tumor that makes about 2% of all chondrosarcoma cases[8]. Typically, this tumor affects the long bones, especially those in the proximal humerus and femur.

Chondrosarcoma occurs generally among older individuals, especially those more than 50 years old, with a male predominance[14]. Skin metastases are uncommon in chondrosarcoma. We summarized a total of 13 cases of cutaneous metastasis of chondrosarcoma from literatures and this study (Table 1). 8 male and 5 female patients were included. The median age of these patients at diagnosis was 50 years. Primary chondrosarcoma primarily affects the scapula, phalanx, pelvis, and other body parts. Its skin metastasis usually appears between 2 months and 10 years after initial occurrenc. The skin lesions almost always take the form of nodules and is most commonly found on the face, chest wall, abdominal wall. The involvement of buttock was present in two patients. The majority of cases were surgically cured. The skin metastasis may be related to lymphatic vascular damage during lymph node dissection, resulting in lymphedema and the spread of malignant cells to the skin in the primary chondrosarcoma[15]. Almost all of the patients we are aware of get chemotherapy or radiotherapy for the management of cutaneous metastases of chondrosarcoma. Most patients died throughout the period of follow-up. However, 58 months after skin metastases, one patient with low-grade chondrosarcoma was still living.

Histopathology reveals chondroid tumors with lobular patterns and epithelioid tumor cells with hyaline to pink cytoplasm in the majority of CCCS, which are regarded as low-grade malignancies. Occasionally, osteoclast gigantic cells can be seen, which are multicellular with transparent cytoplasm and rounded, massive, central nuclei[16]. The mitotic rate was extremely low in each case. Typically, braided trabeculae and osteoid with unusual shapes are scattered throughout the tumor[17].

Conventional chondrosarcoma is a primary bone tumor that, if graded low, can be surgically removed with the intention of curing the patient. However, the prognosis is unfavourable for patients who have or develop unresectable tumor situations[18]. The prognosis of cutaneous metastasis typically depends on the initial cancer, and the presence of cutaneous infiltration by tumor cells suggests extensive illness and high mortality. Radiotherapy and chemotherapy may be helpful for metastatic chondrosarcoma, however surgery is still the most established form of intervention in the metastatic scenario[19].

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PO-070

Distribution and factors linked with syphilis in Eastern Guangdong, China; A single-center retrospective study (2012 to 2021)

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Background Syphilis is a sexually transmitted infection caused by the bacterium Treponema pallidum. It is a significant public health concern, and its incidence has been increasing globally in recent years. In this study, we aimed to investigate the demographic and clinical characteristics of syphilis patients, stages and clinical manifestations of syphilis, initial TRUST titer, and the year-wise trend of syphilis in a hospital located in eastern Guangdong China.

Methods Syphilis was one of the suppressive sexually transmitted diseases (STDs) from 1950-1970; however, since the 1980s, a massive resurgence has been reported in China. Guangdong province is considered the modern-day's epicenter of syphilis. The highest stated syphilis rates might be due to the China STDs cases reporting system, which has been functional since 1985. All the clinical settings in China are required by law to report newly diagnosed syphilis cases to STDs case-based surveillance systems. 8

A single-center retrospective study was conducted between January 2012 to December 2021 at the department of dermatology and venerology of the second affiliated hospital of Shantou university medical college located in eastern Guangdong, China. Eastern Guangdong (Chaoshan) region is cultural and linguistically different from the rest of China, consisting of three major cities, i.e., Shantou, Chaozhou, and Jieyang. Shantou is the most populous city in this region and one of China's special economic zones. 10 The study center is the highest-ranking level 3A hospital and a leading center reporting syphilis cases from all over the Chaoshan region.

All hospital patients were subjected to syphilis diagnostic tests in the past decade. Each patient received a non-treponemal flocculation test (Toluidine Red Unheated Serum Test, TRUST) confirmed by a treponemal test (Treponema pallidum particle agglutination test, TPPA). The patients with positive syphilis diagnostic tests, syphilis-specific clinical manifestations, and history of high-risk sexual behavior were defined as syphilis cases. The concerned physician reported the complete clinical, diagnostic, and demographic history of each newly identified patient to the STDs case-based surveillance system of the hospital.

The current study is based on syphilis data recorded in the past decade from the hospital. For each patient, data of the reporting date, age, gender, location, ethnicity, marital status, education, precarity, route of infection, history of STDs, co-morbidities, number of sex partners, TRUST titer, and stage of syphilis were tabulated and stored using Microsoft Excel (Microsoft Office Professional Plus, 2019). Moreover, the information on clinical manifestations specific to syphilis stages and those of syphilis-infected pregnant women were analyzed. The data about treatment results of syphilis patients were inaccessible in their entirety; however, a follow-up analysis of available data was performed.

The descriptive statistics were applied to determine the rate of infection depending on age and gender groups to outline the number and percentages of cases. Initially, patients were classified into two age groups (age<50 and age>50). Due to a large number of cases in age > 50 years, subgroups were made, i.e., 0-17, 18-30, 31-40, 41-50, 51-60, 61-70, 70-80, and greater than 80 years of age. The study variables were analyzed in all cases and separately for male and female patients. Each study's characteristics were analyzed by the Pearson Chi-square test or Fisher's exact test to determine the statistical significance between the two gender groups. The TRUST titer values reported in different stages of syphilis were analyzed, and the Wilcoxon signed-rank test was performed to determine the statistical difference of titer values in various stages of syphilis. The follow-up analysis of treated cases was performed to find out the rate of serofast and

serological cured cases. The P-values <0.05 were considered significant for all the statistical significance tests.

Results In the past decade, 499,229 patients were admitted to the hospital, and 1079 (0.22%) patients were reported syphilis positive. Among the syphilis patients, 663 (61.45%) were male, and 416 (38.55%) were female. Most of the patients were of Han ethnicity (98.89%), and the median age of patients was 61 years (IQR; 48–72 years). The median age of male patients was 64 years (range; newborn baby to 91 years), while for the female population median age was 57 years (range; seven months to 93 years). A high number of cases (24.83%) were reported in the 61–70 years age group. The proportion of syphilis cases among males and females in various age groups was significantly different (P <0.0001). The number of male and female cases reported from various locations was statistically different (P = 0.0397). The number of syphilis reported in men and women having different levels of education, precarity status, and number of sexual partners was also statistically significant (P <0.0001). A total of 67 pregnant women were reported in the past decade, in which 46 (68.66%) faced adverse pregnancy outcomes (APOs), including 28 (41.79%) with congenital syphilis. Co-infection of syphilis with chlamydia, mycoplasma, and gonorrhea was not detected; however, 10 cases with HIV were reported; eight were male, and two were female.

In this study, 28 (2.59%) early congenital syphilis were reported and had different clinical manifestations, as shown in figure 1a. Regarding acquired syphilis, a total of 1051 cases were reported, among which 91 (8.65%) cases were early (<2 years of infection) and 71 (6.75%) were late (>2 years), while for most of the cases (n = 889, 84.59%) the duration of infections was unknown. All cases had an unknown infection history, and no clinical manifestations were classified as latent syphilis. Based on clinical symptoms, early-stage syphilis was classified into primary (n = 44/91, 48.35%), secondary (n = 9/91, 9.89%), and early latent (n = 38/91, 41.76%) syphilis.

Similarly, 34 (47.89%) of the late cases were tertiary syphilis, and 37 (52.11%) were late latent syphilis. The overall latent syphilis was 91.72% out of total acquired cases. Among tertiary syphilis, 29 cases of neurosyphilis, three gummatous, and only two cases of cardiovascular syphilis were reported. Among the gummatous, two cases had syphilitic gumma, one had the nodular rash, and two cardiovascular syphilis patients had coronary artery disease. Among the hospital's different departments, a high number of cases were reported from neurology (n = 242, 22.43%), followed by cardiology (n = 148, 13.71%), while from the Intensive Care Unit (ICU) , only 6 (0.56%) cases were reported.

The percentage of initial TRUST titer values in different stages of syphilis were determined. Results revealed that TRUST titer (1:1) was found high (around 40%) in all stages, except tertiary syphilis, in which ≥1:16 was also found in a significant proportion. The TRUST titer values reported in different stages of syphilis are presented in figure 3. Moreover, we determined that the TRUST titer values did not distinguish between different stages of syphilis (P >0.05). However, the P-value detected by the Wilcoxon signed-rank test from the median titer values of all stages was smaller than 0.05, but it might be due to the high median value of tertiary syphilis. From this result, we concluded that the reported dilution of TRUST titers is not specific to different stages of syphilis.

The notification rate of syphilis for the past decade (2012-2021) was 216 per 100,000 persons. A high notification rate for males (241 per 100,000) was observed compared to females (185 per 100,000) in the last ten years. The syphilis notification rate increased from 2012 to 2021, except for 2019 and 2020, in which 232 and 228 cases per 100,000 persons were reported. From 2012 to 2014, almost the same increasing trend of syphilis for both males and females were observed, but after 2015 a high rate of syphilis was observed in the male population compared to females . The highest notification rate for the male population was reported in the year 2016 (318 per 100,000), while for females, the highest cases were reported in 2018 (259 per 100,000).

In comparison to younger age groups, the adult age group had an increasing trend. Similarly, a rising trend of syphilis was observed in the older male population from 2012 to 2017. From 2018 to 2020, a decrease in the notification rate was observed; however, the highest notification rate over the past decade was observed in the year 2021 (695 per 10,000). For the young female population, a stable trend was reported over the past decade, while for older, the notification rate fluctuated, with the highest reported in the year 2018 (561 per 100,000) followed by 2017 (507 per 100,000). The results concluded that syphilis cases were higher in males and the older population (P = 0.0157).

In total, 532 (49.30%) patients received the regular anti-T. pallidum treatment. The treatment plan was mainly intramuscular benzathine penicillin G (BPG) (n = 526 patients, 98.87%), while only six patients (1.13%) received second-line treatment, i.e., doxycycline. After treatment, only 187 (35.15%) patients cooperate in follow-up among the BPG-treated patients. Of 187 patients, 113 (60.42%) patients achieved serological cure, while 74 (39.58%) remained in the serofast state. Furthermore, we analyze the effect of initial TRUST titer values on the prognosis of syphilis. We found that higher initial TRUST titer values were more likely to achieve a serological cure rate. In comparison, the high proportion of lower TRUST titer values remains in a serofast state (P <0.0001).

Conclusion

The current study summarizes the syphilis data from eastern Guangdong, China. The syphilis trend was increased over the last ten years and may continue in the future. A high proportion of cases was in the latent stage of infection and the older population. Heterosexuality seems to be the primary reason for new cases and the resurgence of congenital syphilis. Effective measures from health care officials and awareness in the general population are needed to control and prevent the disease.

PO-071

Six-year retrospective analysis of epidemiology, risk factors, and antifungal susceptibilities of candidiasis from a tertiary care hospital in South China

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Background Candidiasis: a life-threatening disease that increases mortality in critically ill patients. However, in underdeveloped regions of China, such type of epidemiological data is still lacking. A retrospective analysis (2016-2021) was conducted in Meizhou People's Hospital, China, to study the burden of candidiasis, particularly candidemia, and anti-fungal susceptibilities of the species among hospitalized patients.

Methods The current six-year (2016 – 2021) retrospective study was conducted at the 1000-bed tertiary (A) Meizhou People's Hospital located in Meizhou, which provides health services to 20 million people of the Guangdong province, China. The diagnosis of Candida infections was based on the guidelines of the China Medical Association and the Infectious Diseases Society of America for candidiasis management (12, 13). The demographic data and clinical characteristics of all inpatients with candidiasis were collected from the hospital's electronic medical records. The data were analyzed according to patients' age, gender, sample source, admitted wards, underlying co-morbidities, previous invasive procedures within 30 days of admission, the mortality rate of seven days, 30 days, and all causes were examined. Furthermore, the candidemia cases were retrieved from all candida infections, and their demographic and clinical characteristics were analyzed separately.

The Candida species were isolated from the biological specimen of patients visited hospital and observed following the standard isolation protocols, including direct microscopy with potassium hydroxide, inoculated aerobically and anaerobically in BacT/AlerT 3D vials (Bruker Diagnostics Inc., USA) (for blood sample), and culturing on CHROMagar-Candida medium (14). MALDI-TOF-MS performed the species identification of Candida using the MALDI Biotyper RTC 4.0 package (Bruker Daltonik). The antifungal susceptibility testing for five drugs (amphotericin B, 5-flucytosine, fluconazole, itraconazole, and voriconazole) was performed using the ATB FUNGUS 3 kit (bioMérieux, France) following the manufacturer's guidelines. The C. krusei ATCC (6258) and C. parapsilosis ATCC (22019) were used as the quality control strains. The results of susceptibility testing were interpreted according to Clinical & Laboratory Standards Institutes (CLSI) guidelines (15, 16).

The data from the hospital's electronic medical records were collected in an Excel sheet (2021). The categorical variables were reported as absolute numbers and relative percentages, while the quantitative variables were presented as median and interquartile ranges. The univariate analysis of baseline characteristics for infection with C. albicans and NCA were performed by Chi-square test. To analyze the risk factor for candidemia, the odd ratios with 95% confidence interval and p-values were determined by the Baptista-Pike method, considering Candida infections other than bloodstream infections (BSI) as control. The p-values smaller than 0.05 were considered statistically significant and were determined by two-tailed tests. All the statistical analysis and graphical visualization were performed using GraphPad prism v.8.0.2.

Results During the six-year study, 7864 Candida infections were reported, containing a high proportion of C. albicans (n = 5053, 64.25%) followed by C. tropicalis (n = 992, 12.61%) and C. glabrata (n = 849, 10.80%) while C. guilliermondii (n = 14, 0.18%) were reported with the least proportion. A high number of cases were reported in the year 2018 (n = 1523, 19.37%), followed by 2019 (n = 1486, 18.90%) and 2021 (n = 1297, 16.49%).

The median age of the patients was 64 years (interquartile range (IQR); 44 – 77), in which the youngest patient was 5 days old while the oldest was 101 years old.

Regarding the age groups, the Candida infection mainly occurred in the adult age group (n = 4056, 51.57%) followed by the older patients (n = 3701, 47.06%). Only 14 cases were reported in neonatal, of which 7 (50%) were C. albicans and 5 (35.71%) were C. glabrata. Similarly, 24 cases were reported in infants, among which 16 (66.67%) were C. albicans, and 8 (33.33%) were NCA. The number of cases in male patients (n = 4146, 52.72%) was a little higher than the cases in female patients (n = 3720, 47.3%) for overall Candida species, except the C. glabrata and C. norvegensis, in which the males to females' ratios were 0.43:1, and 0.38:1 respectively. The mean incidence of Candida species per 1000 admission was 10.16, of which the highest was reported in 2019 (12.11/1000), followed by 2021 (11.71/1000), while the lowest was reported in 2017 (7.81/1000). More specifically, for C. albicans, the mean incidence was 6.53/1000 inpatients, of which the highest was reported in 2021 (7.93/1000). Among the NCA, the highest mean incidence was reported for C. tropicalis (1.28/1000), followed by C. glabrata (1.09/1000) and C. parapsilosis (0.99/1000).

The baseline clinical characteristics associated with C. albicans and NCA are summarized. Overall, 47.06% of the patients belonged to the older age group, in which the proportion for C. albicans was higher than NCA, and their differences were statistically significant. The male-to-female infection ratio of C- albicans and NCA were not significant. Following the underlying co-morbidities cases, a high proportion was enumerated for respiratory dysfunction (12.51%), followed by renal failure (8.59%), urinary tract infection (UTI) (7.21%), neurological diseases (6.86%), and gastrointestinal pathology (6.14%). The proportion of severity of tuberculosis, solid tumors, diabetes, and cardiovascular diseases was statistically insignificant amongst the cases of C. albicans and NCA ($p > 0.05$). Moreover, we noted that some co-morbidities like renal failure, digestive tract pathology, UTI, neurological diseases, and otitis media proportion were high for NCA than C. albicans cases, and their differences were statistically significant ($p < 0.05$). Among the prior invasive procedures, a high proportion of cases were reported in association with urinary tract catheters (11.86%), followed by mechanical ventilation (9.72%) and parenteral nutrition (8.35%). Surprisingly, the proportion of all previous invasive procedures was high for NCA than C. albicans ($p < 0.05$). The median value of hospital stayed of patients infected with NCA was 29 (IQR; 15- 41) and for C. albicans was 24 days (IQR; 10 - 36). No statistical differences were noted for C. albicans and NCA for the patients that previously stayed in ICU ($p > 0.05$). The proportion of 7 days mortality for C. albicans (4.61%) and NCA (5.08%) were statistically not significant ($p > 0.05$). However, the 30 days and all-cause mortality rate for NCA (22.27% and 26.39%) were reported high in the patients infected with C. albicans (18.99% and 22.99%, respectively ($p < 0.05$).

Furthermore, the data of Candida species reported from the blood samples were retrieved to analyze the epidemiology of candidemia. A total of 461 candidemia cases were noted, of which 245 (53.72%) were C. albicans, 102 (22.37%) were C. glabrata, 64 (14.04%) were C. tropicalis, 42 (9.21%) were C. parapsilosis, 5 (1.10%) C. krusei , 2 (0.44%) C. metapsilosis, and 1 (0.22 %) C. guilliermondii. The median age of patients was 67 years (IQR; 58 – 78); the youngest patient was 20 years old, while the oldest patient was 91 years old. Among the various age groups, almost half

of the patients (n= 228, 49.46%) were from the older age group. The median age of Candidemia patients infected by various Candida species is presented in figure 2. The proportion of male cases was high compared to females at the ratio of 3.15:1, of which for C. albicans was 3.08:1 and for NCA was 3.23:1. Among different departments of the hospitals, a large number of cases were reported from the ICU (n =306 66.37%), followed by medical wards (n =106, 22.99%), while 49 (10.62%) cases occurred in the surgical department. In ICU cases, 170 (47.22%) were C. albicans,83 (27.12%) C. glabrata, 28 (9.15%) C. tropicalis, 21 (6.86%) C. parapsilosis, 2 (0.65%) C. krusei, and 2 (0.65%) were C. metapsilosis. The clinical characteristics associated with candidemia due to C. albicans and non-C. albicans are presented in table 3. Among the reported underlying conditions, a large number of patients suffered from gastrointestinal pathology (15.83%), followed by respiratory dysfunctions (9.76%), septic shock (6.94%), and malignancies (5.21%). Moreover, we noted that the proportion of gastrointestinal pathology, cardiovascular diseases, and septic shocks was significantly high in non-C. albicans cases compared to C. albicans ($p < 0.05$). However, respiratory dysfunction, solid tumor, and hypertension were high in C. albicans cases compared to non-C. albicans. Among the prior invasive procedures, a high proportion of cases was reported in association with mechanical ventilation (52.28%), followed by central venous catheter (49.02%), urinary tract catheter (45.53%), and parenteral nutrition (41.21). Furthermore, we found their statistically significant association with C. albicans and NCA ($p < 0.05$). The 7 days, 30 days, and all-cause mortality rates for Candidemia patients were 29 (6.2%), 83 (18 %), and 91 (19.74%), and were not statistically differed for C. albicans versus NCA ($p > 0.05$).

Furthermore, the odd ratios (95% CI) for independent risk factors of candidemia due to C. albicans and NCA were found by the Baptista-Pike method. Among all the factors, the odd ratio for central venous catheter was reported greater than one for C. albicans and NCA that was 3.042 (95% CI; 2.067 - 4.481) and 2.535 (95% CI; 1.820 - 3.542), respectively.

The antifungal susceptibility profiles of all Candida species reported in the current study are summarized. Among the five tested antifungal drugs, the highest susceptibilities were reported for amphotericin B and 5-flucytosine against all Candida species. For C. albicans, the susceptibilities against fluconazole, voriconazole and itraconazole were 92.06%, 90.37% and 78.71%, respectively. Among the NCA, C. parapsilosis, and C. guilliermondii were the most susceptible, as the proportion of susceptible/wild-type isolates was greater than 95% against all five tested antifungal agents. For C. tropicalis, C. glabrata, and C. krusei, the lowest susceptibilities were reported against itraconazole, which was 82.88%, 86.78%, and 94.37%, respectively.

Furthermore, we compared the susceptibilities profiles of Candida species recovered from candidemia cases with the isolates recovered from non-bloodstream Candida infections. The susceptibilities of amphotericin B and 5- flucytosine were statistically not different against all tested isolates in both groups. Similarly, for C. parapsilosis, the susceptibilities against all five tested drugs were not statistically significant ($p > 0.05$). Interestingly, in the case of C. albicans, the susceptibilities of all three tested azole drugs were high for candidemia compared to the other group ($p < 0.05$). On the contrary, in C. tropicalis and C. glabrata, the susceptibility against itraconazole and voriconazole of candidemia-causing isolates was lower than in the other group ($p < 0.05$). However, the fluconazole susceptibility in the two groups against C. tropicalis was not statistically differed ($p > 0.05$).

Conclusion

In the current study, we retrospectively analyzed the distribution, risk factors, and antifungal susceptibility pattern of the Candida pathogen in Meizhou, China. The C. albicans were found in a high ratio, followed by C. tropicalis, while in candidemia patients, C. glabrata were more frequent than C. tropicalis. Non-C. albicans candidemia was most common in patients with gastrointestinal disorders, hematological malignancy, and septic shock and was used prior to invasive procedures. The central venous catheter was an independent risk factor for both C. albicans and NCA causing candidemia. Amphotericin B and 5-flucytosine were highly active drugs, while low susceptibility was reported against azoles. For C. tropicalis and C. glabrata, the isolates causing candidemia had significantly lower azole susceptibility than non-candidemia-causing isolates. Further molecular investigations for the in-depth analysis of azoles-resistant magnitudes and continuous surveillance studies are required.

PO-072

In vitro and in vivo effects of conventional and nanoparticles encapsulated miltefosine drug for cutaneous leishmaniasis therapy

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Background An alternative therapeutic option for cutaneous leishmaniasis (CL) is required due to the high toxicity and resistance of conventional anti-leishmanial drugs. This study aimed to formulate polymer-based chitosan nanoparticles as a drug (miltefosine) delivery system for treating leishmaniasis.

Methods The L. tropica strain was collected from the infectious disease laboratory at Quaid-i-Azam University, Islamabad, Pakistan, for in vitro experiment. In vivo study was carried out using Leishmania parasites isolated from CL patients and cultured in NNN media. BALB/c mice were purchased from the National Institute of Health, Islamabad, Pakistan.

The RPMI 1640 was prepared by adding heat-inactivated FBS (10 mL) to RPMI 1640 (90 mL) in a sterile flask. Pen-Strep antibiotic (100 µg/mL) was added to the flask to avoid contamination, and pH was adjusted to 7.4 and was incubated at 37 ℃ to check the sterility of the media for 24 h. After culturing, promastigotes were observed under a light microscope at 40X and 10X. Culture flasks were also directly examined under an inverted microscope at 20X magnification.

Chitosan polymer encapsulated miltefosine nanoparticles were synthesized following the previous method. Briefly, chitosan polymer (0.5% w/v) was dissolved in (1% v/v) acetic acid solution. TPP powder (0.5% w/v) and miltefosine drug (3 mg/mL) were dissolved in deionized water, and the pH was adjusted to 5. TPP solution containing miltefosine was added to the chitosan solution dropwise to synthesize ionically cross-linked nanoparticles and the reaction mixture incubated for one hour under constant stirring at room temperature. For partial oxidization, H2O2 solution (0.5% v/v) was added to nanoparticles (NPs). D-Trehalose (3% w/v) was added to the NPs solution to avoid aggregation and centrifuged for 10 min at 13,400 rpm to accumulate the nanoparticles in the pellet. Scanning electron microscopy (SEM) was used to study the size and surface morphology of MLCNPs with a magnification of 20 to 45 kx with an accelerating voltage of 20 kV. The size distribution and zeta potential of MLCNPs were measured using a DLS zeta sizer (PSS Nicomp 380). The encapsulation efficiency (EE) was calculated as; % EE = [(A-B)/A] ×100, where “A” is the total amount of MFS to synthesized NPs (mg), and “B” is the amount of free MFS measured in the supernatant (mg). Similarly, the drug-loaded chitosan (DLC) of MLCNPs was calculated as; % DLC = [(total amount of drug-free amount of drug/ nanoparticles weight] ×100.

The dialysis bag diffusion method was performed to analyze the in vitro release of synthesized MLCNPs. The nanoparticles suspension and drug solution (10 mg each) was dissolved in a beaker containing 5 mL Tris-HCL buffer solution. Afterward, the suspension was stirred magnetically at 100 rpm for 48 h at room temperature. At specific periods, samples were centrifuged at 15,000 g for 20 min at 14 °C. The amount of drug release was assessed by UV spectrophotometer at 270 nm.

For the hemolysis assay, 3 mL of blood was collected from a healthy volunteer in EDTA tubes. The blood was immediately centrifuged at 1500 rpm for 15 min to prevent clotting. The pellet containing erythrocytes was washed three times with 1X PBS (each time for 5 min). The erythrocytes suspension was prepared by mixing 11 mL of 1X PBS into 3 mL of centrifuged erythrocytes. The NPs and drug concentrations were prepared by adding (150 µg, 200 µg, and 250 µg) MLCNPs and conventional miltefosine in 1 ml ddH2O. The reaction mixtures of MLCNPs and conventional miltefosine with erythrocytes suspension were incubated for 4 h at 37 0C for hemolysis. After, Eppendorf tubes were centrifuged at 13,000 rpm for 15 min, and the remaining hemoglobin in the supernatant was measured by spectrophotometer at 570 nm. The PBS (1 mL) was used as the negative control with 0% hemolysis, and 1 mL Triton-X 100 was used as the positive control with approximately 100% hemolysis. The experiment was performed in triplicate. The hemolysis (%)

was calculated as; hemolysis (%) = [(OD at 570 nm in the drug solution – OD at 570 nm in PBS) ÷ (OD at 570 nm in 0.1% Triton X-100 – OD at 570 nm in PBS)] × 100.

In vitro activity of MLCNPs on L. tropica (promastigotes) was performed as described elsewhere (Esfandiari et al. 2019). Briefly, in a 96-well plate, 100 µL promastigotes (culture media containing 107 CFU) were added. The L. tropica were incubated with 100 µL of MLCNPs and conventional miltefosine at different concentrations (50, 40, 30, 20, 10, and 5 µg/mL) in each well. After 72 h of incubation at 24 ℃, 10 µL of MTT reagent was added to each well, wrapped in aluminum foil, and further incubated for 4 h at 24 ℃. The cultured media were centrifuged at 3000 rpm for 3 min, the supernatant was discarded, and the pellet was diluted with 100 µL of DMSO to stop the enzymatic reaction. The wells were incubated for 1 hour in a shaking incubator at 24 ℃. Absorbance was checked at an optical density of 570 nm by a microplate reader. As a positive control, media containing parasites were added to wells without any drug. Media containing only parasites was taken as a positive control, while sterile media was chosen as a negative control. The experiments were performed in triplicates, and the data are expressed as mean ± SD. The data obtained from the microplate reader was then subjected to GraphPad prism v.8.0.2 software for statistical analysis.

For in vivo experiment, 6-8 weeks old, 28 g female BALB/c mice (n = 16) were used. BALB/c mice were shaved from the tail base using a shaving machine and were injected intradermally using 1CC Ultra-Fine insulin syringes with promastigotes (1×107) (Riaz et al. 2020). The mice were examined every two days for 28 days to assess the appearance of lesions. The development of the CL was assessed as redness, swelling, ulceration, and crust formation at the inoculation site. Infection was well established after three to four weeks, and noticeably visible lesions were seen at the site of inoculation. Lesions were tested for positive evidence of CL by determining the presence of amastigotes with Giemsa-stained smear under a light microscope at 100X objective lens. Lesion size was calculated with a vernier caliper at right angles in two dimensions (D and d mm) to each other, and the equation determined the lesion size (S) = (D × d)/2 mm2. The mean of the two measured diameters was calculated and further used for statistical analysis. The 16 mice were divided into four groups (4 in each) to determine the treatment efficacy of MLCNPs and conventional miltefosine for 14 consecutive days.

Group 1: Mice have injected synthesized nanoparticles intralesional once a day.

Group 2: Mice were treated orally with synthesized nanoparticles once a day.

Group 3: Mice were treated orally with miltefosine once a day.

Group 4: Mice were given PBS orally once daily, serving as the placebo group.

Groups 1 and 2 were treated at a dose of 89 µg/28 g/day in ddH20 up to a final volume of 0.1 mL. Group 3, containing the conventional miltefosine, was also used at a similar dose of 89 µg/28 g/day (2.5 mg/kg/day). For group 4, sterilized PBS solution (0.1 mL) was given orally as a placebo. The reduction of lesion sizes was observed daily, and the final measurements were performed after two weeks using a vernier caliper.

Statistical analysis was performed using SPSS v.22 software. Differences between each group pair (before and after treatment of the same group) were analyzed using Wilcoxon signed-rank test. At the same time, the significance between the two groups was assessed by the Mann–Whitney test. The difference was considered significant with the P value < 0.05.

Results The MLCNPs were formed as a milky color solution, and their powder form was attained by centrifugation. The SEM analysis revealed that MLCNPs displayed a spherical shape and irregular surface morphology, while DLS showed a mean particle size was 97.5 nm. The synthesized particles possessed a substantial zeta potential of +1.04 mV. The percentage of drug-loaded chitosan was DLC of 91.5 µg/ mL, and the encapsulation efficiency was 97.56%.

The drug release concentration of miltefosine drug from the nanoparticles was divided into two phases. In the initial stage, 30 % drug was rapidly released from the NPs after 6 h at pH 7.4. In the second stage, the drug was constantly released from the nanoparticles, resulting in around 96 % of the loaded drug up to 48 h.

The hemolysis activity of MLCNPs and conventional miltefosine were compared. The result showed that MLCNPs have 6% less hemolytic activity than conventional miltefosine.

The present study showed that the effect of conventional miltefosine and MLCNPs depends on the dose concentration and exposure time. However, MLCNPs were more cytotoxic than conventional miltefosine against L. tropica promastigotes. The cytotoxicity of conventional miltefosine and

MLCNPs was assessed at six different concentrations (50, 40, 30, 20, 10, 5 µg/mL) for 24, 48, and 72 h. The mean viability percentage of promastigotes exposed to different MLCNPs and conventional miltefosine concentrations is illustrated in table 2. The IC50 value of MLCNPs (0.0218 µg/ mL) was higher than the conventional miltefosine (0.3548 µg/ mL).
At four weeks post-inoculation, when lesions were established, the mice were treated for two weeks, and a visible reduction in lesion size was noted. The mean and SD of each group's pre- and post-treatment lesion sizes were determined, and P-values were determined. All the groups exhibited decreased lesion size after drug therapy except the placebo group. Among the groups, the orally administrated MLCNPs group was considered significant (P = 0.01).
The Mann-Whitney test was performed for lesion size comparison between the two groups. Post-treatment lesion comparisons of conventional miltefosine and MLCNPs (oral) treated groups were statistically significant (P = 0.019). The P-values differences between pre-and post-treated groups were also statistically significant (P = 0.020). Similarly, the amastigote count on the last day of treatment was significantly less under the light microscope than the pre-treated parasite burden. The comparison between conventional miltefosine and intralesional injected MLCNPs treated groups was also performed but detected as less effective. The P- value difference was 0.065 and considered insignificant.
Furthermore, comparisons based on the route of administration of MLCNPs were performed. The difference in the P-value between the pre-and post-treated groups was 0.019. The results revealed that the oral route of administration for MLCNPs is much more significant than the intralesional route.

CONCLUSION

The current study describes a potential novel therapy for curing CL caused by L. tropica in the form of MLCNPs. The chitosan nanoparticles encapsulate the miltefosine efficiently. The overall results revealed that MLCNPs have higher efficacy than conventional miltefosine, which is proved both in vitro and in vivo. Regarding the route of administration, the efficacy of MLCNPs given orally was more significant than intralesional injections. Moreover, the hemolysis assay showed significantly less hemolysis activity of MLCNPs than conventional miltefosine. In summary, all these features make these newly synthesized nanoparticles an ideal drug delivery system and might be used as an alternative therapeutic option for curing leishmaniasis.

PO-073

The role of adipokines in the pathogenesis of chronic spontaneous urticaria

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Abstract Chronic spontaneous urticaria is a common allergic disease, and its pathogenesis is still unclear, and it is currently believed to be mainly related to mast cell activation and degranulation, release of histamine and cytokines.Academics believe that the autoimmune mechanism of CSU is mainly divided into two types: type I autoallergic type and type II.b autoimmune type. The former is related to anti-autoantigen IgE, and IgE against autoallergens binds to high-affinity IgE receptor (FcεRI) expressed on skin mast cells and basophils, regulates mast cell activation, and releases mediators such as histamine, kinins, and cytokines, resulting in disturbances in the signaling pathways of mast cells and basophils, thereby promoting the pathogenesis of CSU; The latter refers to the development of autoantibodies against FcεRI or IgE on mast cells and basophils, mediating the activation of mast cells when the immune microenvironment in the body is out of balance. Type II.b autoimmune CSU has the characteristics of high disease severity, low total IgE level, and elevated IgG antithyroid peroxidase level. CSU is often associated with other autoimmune disorders, such as autoimmune thyroid disease, systemic lupus erythematosus, polymyositis, dermatomyositis, and rheumatoid arthritis. Adipokine is mainly secreted by adipose tissue,

including soluble molecules such as leptin, adiponectin, neutrophil gelatinase-associated lipid carrying protein (LCN2) and cytokines, which are closely related to the body's growth and development, regulation of lipid and glucose metabolism, and secretion of inflammatory factors. Previous studies have found that obesity produces systemic inflammatory signatures characterized by excessive secretion of inflammatory factors such as CRP, IL-6, LCN2, tumor necrosis factor (TNF-α), leptin, and adiponectin, as well as insufficient secretion of anti-inflammatory and antiatherosclerotic related factors. In addition, adipokines also promote the secretion of immunomodulatory factors in macrophages, and activated macrophages can secrete macrophage chemokine-1, inducing local aggregation of monocytes to inflammatory sites.In recent years, the increase in obese people and the unique metabolic and immune functions of adipose tissue itself have gradually increased the research on adipokines and autoimmune diseases. Studies have confirmed that adipokines can directly target human mast cells, such as activating mast cells that accumulate in atherosclerotic plaques to release angiogenic compounds and pro-inflammatory mediators.In addition, circulating tryptase levels are significantly elevated in obese patients. Tryptase is the most abundant protein in mast cells and a marker of mast cell degranulation. Several studies have shown that about 30% of CSU patients have metabolic syndrome, and such patients have a high urticarial activity score (USA) and poor treatment response. Serum LCN2 levels were found to be reduced in patients with refractory CSU, and LCN2 was significantly negatively correlated with urticaria severity. In addition, LCN2 may regulate the binding of neutrophils to platelet-activating factors, leukotriene B4, and lipopolysaccharides, thereby inhibiting the inflammatory response. Therefore, inhibition of LCN2 can weaken its regulatory effect on neutrophil chemotaxis, which in turn leads to the persistence of the inflammatory response of refractory CSU. The role of adiponectin in the pathogenesis of CSU is controversial. An imbalance of pro-inflammatory and anti-inflammatory adipokines in CSU has been reported, i.e., patients with CSU have significantly elevated serum LCN2 levels and significantly reduced adiponectin levels. Other studies have shown that serum concentration levels of adiponectin are affected by cytokines such as IL-6, TNF-α, and VEGF secreted by activated mast cells.However, studies have reported no statistically significant difference in adiponectin levels in CSU patients compared to controls. The authors speculate that the anti-inflammatory and pro-inflammatory effects of adiponectin depend on the proportion of its isotype in the blood. Leptin activates the chemotaxis of eosinophils, basophils, and neutrophils, and also promotes mast cell degranulation and secretion of histamine and chemokine CCL by inducing the release of intracellular calcium ions. Some scholars have found that mast cells express leptin and leptin receptors, and believe that leptin secretion may be controlled by the same factors that regulate mast cell activation and degranulation. Several studies have reported higher serum leptin levels in CSU patients, and leptin levels are positively correlated with urticaria severity. The role of adipokines such as endolipids, resistins and chemotatins in the pathogenesis of CSU has not been reported, which may provide a new direction for the study of the function of adiposis in the pathogenesis of CSU in the future. In recent years, more and more literature has reported that adipokines play an important role in autoimmune diseases. However, the role of adipokines in the pathogenesis of CSU has not been fully explained. According to the available research evidence, adipokines may be involved in the evolution of CSU by altering the balance of pro-inflammatory and anti-inflammatory, stimulating immune cells such as mast cells, and promoting the secretion of cytokines. Although the correlation between adipokines and the pathogenesis of CSU needs to be further studied, this still provides a new pathogenesis research direction and therapeutic target for refractory chronic spontaneous nettle disease.

PO-074

Research status of miRNAs in keloids

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summary: Keloid is a benign tumor commonly found in dermatology with an overgrowth of connective tissue of the skin caused by multiple causes. The main clinical manifestations are red nodular, cord-like or flaky mass-like tissues, and the lesions are usually higher than the skin surface and invade the surrounding normal tissues in a crab foot shape. The disease has clinical features of oncological diseases, such as persistent infiltrative growth, treatment resistance, and high recurrence rate. Causes of keloids include skin trauma, inflammation, or spontaneous formation. Its pathogenesis is not fully understood, and it is mainly thought to be the result of a combination of factors. At present, the consensus of scholars is that keloids are caused by the accumulation of extracellular matrix collagen and the excessive proliferation of fibroblasts. In recent years, numerous studies have confirmed that miRNA dysregulation plays a crucial role in the pathogenesis of keloids. As a post-transcriptional regulator, miRNA negatively regulates gene expression by directly binding to the 3' untranslated region (3'-UTR) of the corresponding messenger RNA (mRNA) in a sequence-specific manner, thereby inducing mRNA degradation or inhibiting protein translation. miRNA mainly exists in eukaryotic cells and is involved in the regulation of biological processes such as cell differentiation, development, metabolism, proliferation and apoptosis. A miRNA microarray analysis study identified 32 differentially expressed miRNAs, including 23 up-regulated miRNAs such as miRNA21, miRNA-4269, and miRNA-382, and 9 down-expressed miRNAs including miRNA-203, miRNA-205, and miRNA-200c. In addition, the uncontrolled proliferation and invasion ability of keloid fibroblasts are similar to the biological characteristics of tumor cells. As the main effector cells of keloids, the imbalance of the balance of fibroblast proliferation and apoptosis is a key factor promoting keloid formation. At present, a variety of miRNAs have been shown to participate in the regulation of the biological function of keloid fibroblasts and promote the development of keloids. As early as the last century, studies have confirmed that transforming growth factor β and platelet-derived growth factors in dermal fibroblasts are abnormally expressed in the early stages of wound healing, and TGF-β1 can upregulate the expression of PDGF-α receptors in keloid fibroblasts. In addition, abnormal conduction of TGF-β1/Smad3 signaling pathway can inhibit the proliferation of keloid fibroblasts, promote apoptosis, migration and invasion, and fibrosis. Abnormal deposition of extracellular matrix is one of the important pathogenesis of keloids, including collagen overproduction, fibronectin and elastin-induced fibrosis. As one of the main components of the extracellular matrix, keloids produce 20 times more collagen than healthy skin and three times as much as hypertrophic scars. From histopathological observations, collagen in keloid tissue is proliferated in large quantities, arranged disorderly, and can also be accompanied by fibroblast proliferation and division. More and more studies have shown that the upregulation or downregulation of some miRNAs is conducive to the deposition of extracellular matrix and the formation of fibrosis. Studies have shown that long noncoding RNA (lncRNA) can participate in gene regulation at the epigenetic and transcriptional levels through the miRNA pathway. In keloids, LINC00937 inhibits ECM deposition and proliferation of keloid fibroblasts by inhibiting miR-28-5p and promoting MC1R expression. miR-3141 and miR-203 can be directly bound to LINC01116, and the down-regulation of LINC01116 inhibits extracellular matrix production in keloid by regulating the miR-203/SMAD5 axis and miR-3141/TGF-β1/SMAD3 conduction pathway. The expression of lncRNA H19 in keloid tissues is increased, and further studies have found that it promotes extracellular matrix deposition by targeting the miR-769-5p/eukaryotic initiation factor 3A conduction pathway. miRNAs can regulate genes through biological processes that regulate downstream factors such as eukaryotic initiation factors. High expression of miR-501-5p and low expression of miR-22-5p in keloids are considered to be related to familial heritability and course of the disease. As an upstream factor of eukaryotic initiation factor 3L, miR-501-5p targets eIF3L to promote the formation of keloid collagen and fibroblast proliferation. The results of multiple single-cell transcriptional sequencing analyses on

the heterogeneity of keloid cells showed that in addition to abnormal fibroblast proliferation, significant expansion of vascular endothelial cell subsets was strongly associated with keloid pathogenesis. It has been suggested that abnormal vascular regulation may be the basis of keloid pathogenesis, which is essentially a vasculitic disease caused by congenital or acquired vascular endothelial dysfunction. Gene function enrichment analysis showed that Eph-ephrin signaling, PTEN transcriptional negative regulation, MAPK signaling, WNT signaling and other pathways were activated in keloid vascular endothelial cells, which indicated that dysregulated vascular endothelial cells mediated keloid active angiogenesis through some tumor-related signaling pathways. miRNA plays an important role in the formation and progression of keloids, revealing the biological function of miRNAs in keloids, and has great potential for effectively analyzing the pathological process of keloid formation. As far as the current research status is concerned, the heterogeneity of miRNA has a significant promotion or inhibitory relationship with the pathogenesis of keloids, and can target a variety of signaling pathways to regulate keloid angiogenesis, extracellular matrix accumulation, and cell growth and invasion. So far, no miRNA drugs have been approved for the clinical treatment of keloids, but clinical trials on miRNA drugs for keloid drugs have been carried out abroad, and the treatment effect of patients is better, and only a few cases have adverse reactions such as local erythema and edema. In the future, further research is needed on the role of miRNAs in keloids, clarify their specific signaling pathways, and help to understand the formation mechanism of keloids and determine better therapeutic targets.

PO-075

A case of linear cutaneous lupus erythematosus was diagnosed by dermatoscopy and reflectance confocal microscopy

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A 25-year-old female presented with a 2-month history of a Blaschko-linear erythema on the forehead (Figure 1A). The lesion gradually enlarged to nasal root without any discomfort. No skin inflammation or trauma was observed at the same location. She was in good health and stated no prior medical history. The complete blood count, routine urine test and antinuclear antibody were normal. Dermoscopy manifested scattered follicular plugs, perifollicular whitish halo and irregular dotted vessels presented on the light red background (Figure 1B). Reflectance confocal microscopy (RCM) showed liquefaction of basal cells, a few melanophagocytes and lymphocyte infiltration can be seen in the dermis (Figure 1C). Histopathology revealed slight atrophy of epidermis, liquefaction of basal cells, a few melanophagocytes in the superficial dermis, lots of lymphocytes infiltration can be seen in the appendages and around the blood vessels in the dermis(Figure 1D). A diagnosis of linear cutaneous lupus erythematosus was made. The patient was treated with hydroxychloroquine orally and 0.1% tacrolimus ointment externally, it is still under follow-up.

Discoid lupus erythematosus (DLE) is a chronic cutaneous lupus variant [1]. In 1988, Abe et al [2] reported a linear configuration as one of the uncommon variants of DLE. It was clinically diagnosed as linear lichen planus, linear scleroderma, linear lichenoid dermatosis and linear atrophoderma of Moulin. As noninvasive and convenient imaging technologies, dermoscopy and RCM are sensitive tools for diagnosing DLE and can provide multi-modal information for the management.

By visualizing cutaneous structures that are invisible to the naked eye, the accuracy of DLE diagnosis were significantly improved after adding dermoscopy to the naked-eye examination[3]. Furthermore, the dermoscopic feature may aid in differentiating between various regions of DLE[3,4]. The main dermoscopic feature of non-scalp DLE was follicular keratotic plugs (66.7%), perifollicular whitish halo (65.9%) and telangiectasias (34.9%) [3,4,5]. However, dermoscopy can only observe the epidermal and superficial dermal structures. For deeper structural detection, RCM is more advantageous.

RCM can supply image information closed to histologic resolution[6]: the presence of roundish areas fifilled with highly refractive amorphous material at stratum corneum level, corresponding to dilated follicles in correlation to infundibular hyperkeratosis (follicular plugging). Several small polygonal/round bright cells in the context of a partial or total disappearance of the dermal papillae is detected attributable to vacuolar appearance of the basal layer (interface dermatitis).The agreement between RCM and HP features for diagnosing DLE was superior to 75%[7], RCM imaging allows to elucidate in vivo the major key diagnostic features for diagnosing DLE in real time[8].

Conclusion: A variety of imaging techniques with their own advantages can supply multi-modal preoperative information for the management options that purpose to meet the needs of clinicians, except for improving the diagnostic accuracy of DLE.

(A)

FIGURE 1

(A) A Blaschko-linear erythema on the forehead and nasal root.

(B) Dermoscopy:

scattered follicular plugs(yellow circle),irregular dotted vessels(black arrow) presented on the light red background (×50 magnification).

(C) RCM: partial or total disappearance of the dermal papillae, everal small polygonal/round bright cells (blue circle) at dermo-epidermal junction, dilated adnexal infundibulum with

follicular hyperkeratosis (yellow circle) and periadnexal inflflammatory cell infifiltrate (red arrows) and sparse inflflammatory cells throughout

degenerated collagen bundles (blue arrow)

(0.5 × 0.5 μm magnification).

(D) Histopathology(HE ×200): slight atrophy of epidermis, liquefaction of basal cells(yellow circle), a few melanophagocytes in the superficial dermis (red circle).

(E) Histopathology(HE ×100):lots of lymphocytes infiltration can be seen in the appendages and around the blood vessels in the dermis(yellow circle).

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PO-076

Development of a Highly Sensitive and Semi-quantitative Luciferase Immunosorbent Assay for the Diagnosis and Treatment Efficacy Evaluation of Syphilis Using TP0171 (TP15), TP0435 (TP17), and TP0574 (TP47) Antigens

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Objective The aim of this study is to investigate the potential of using treponemal pallidum antibodies for the diagnosis and treatment efficacy evaluation of syphilis using the newly developed TP0171 (TP15), TP0435 (TP17), and TP0574 (TP47) -based luciferase immunosorbent assay (LISA).
Method To establish a LISA method for the detection of syphilis, the sequence of Tp15, Tp17, and Tp47 were cloned into a pNLF1-N vector containing the NanoLuc (Nluc) luciferase gene. The resulting recombinant plasmid was expressed in Hela cells to produce Nluc-antigen fusion proteins. A white microtiter plate coated with protein G was used to bind primary antibodies from either rabbit or human serum. The Nluc-antigen fusion proteins were then employed as secondary antibodies to bind to specific IgG antibodies against the TP15, TP17, or TP47 antigens. The light units were measured using the Nano-Glo Luciferase assay reagent in a luminometer to reflect the level of antibodies. To assess the performance of LISA in diagnosing syphilis, 261 serum samples were collected from participants, which included 161 individuals who were positive for Treponema pallidum particle agglutination assay (TPPA) at a sexually transmitted disease (STD) clinic, 40 TPPA-negative individuals from the same clinic, and 60 TPPA-negative healthy blood donors. The results of LISA were compared to TPPA, which was used as the reference method. Additionally, to investigate whether the LISA test could be used to evaluate the efficacy of syphilis treatment by measuring antibodies against TP15, TP17, and TP47. The serial serum samples from three rabbits in the treatment group and three rabbits in the control group that were infected with Treponema pallidum (Tp) were analyzed. Furthermore, 110 paired follow-up serum samples were collected from 55 syphilis patients from the same STD clinic to assess the effectiveness of LISA in monitoring treatment efficacy of syphilis.
Result Our study successfully established a luciferase detection method for syphilis, using TPPA as the reference standard. The performance of LISA-TP15, LISA-TP17, and LISA-TP47 in syphilis diagnosis was evaluated by calculating their area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The AUC, sensitivity, specificity, PPV, and NPV were 0.971, 91.9%, 99.0%, 99.3%, and 88.4% for LISA-TP15; 0.992, 96.9%, 99.0%, 99.4%, and 95.2% for LISA-TP17; and 0.995, 98.8%, 98.0%, 98.8%, and 98.0% for LISA-TP47. LISA-TP47 had a significantly higher sensitivity compared to LISA-TP15 (98.8% [95.6-99.8] vs. 91.9% [86.6-95.6], P =0.003). In Tp infection rabbits, we observed no statistical significance in the antibody level against TP17 tested by LISA between the control and treatment groups for the first 26 days post-infection (P>0.05). However, a significant difference was observed from the 26th day (P<0.05) to the 51st day (P <0.01). For LISA-TP47, a significant difference was seen only on the 41st day (P <0.05). Conversely, there was no significant difference between the control and treatment groups in LISA-TP15, TPPA, and Rapid Plasma Reagin (RPR) (P >0.05). In paired follow-up serum samples from syphilis patients, the levels of serum antibodies against TP15, TP17, and TP47 tested by LISA and the RPR titer before treatment were significantly higher than those after treatment (P<0.001). Participants who experienced a 4-fold decrease (two dilutions) in RPR titer after treatment had a median reduction of 48.62%, 28.37%, and 51.75% in the level of antibodies against TP15, TP17, and TP47, respectively. The decline in the level of serum TP15 (rs=0.63, P<0.001), TP17 (rs=0.61, P <0.001), and TP47 (rs=0.63, P <0.001) antibodies were significantly correlated with the changes in serum RPR titer before and after syphilis treatment.
Conclusion Our study demonstrated that LISA-TP47 had excellent sensitivity, while LISA-TP15 and LISA-TP17 exhibited high specificity in syphilis diagnosis, and their performance were

comparable to the TPPA assay. Additionally, we found that the level of antibodies against TP15, TP17, and TP47 detected by LISA could be used for monitoring the effectiveness of syphilis treatment.
Importance: The aim of our study is to investigate the potential of using Treponemal pallidum (Tp) antibodies for the diagnosis and treatment efficacy evaluation of syphilis. We have developed a luciferase immunosorbent assay (LISA) detection method based on TP0171 (TP15), TP0435 (TP17), and TP0574 (TP47) antigens for the diagnosis of syphilis. Our results indicate that this method performs comparably to the widely used Treponema pallidum particle agglutination assay (TPPA). Moreover, our findings contradict the widely accepted clinical belief that treponemal tests are unsuitable for assessing disease activity and treatment outcome. We discovered that LISA has the potential to monitor the treatment efficacy of syphilis in both Tp-infected rabbit models and follow-up syphilis patients. These results suggest that LISA could provide valuable information for medical caregivers in the diagnosis of syphilis and the evaluation of treatment efficacy.

PO-077

Integration of Metabolomics and Transcriptomics Analyses Reveals Sphingosine-1-phosphate-mediated S1PR2/PI3K/Akt Pathway Involved in Talaromyces marneffei Infection of Macrophages

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Background Talaromycosis is an invasive mycosis caused by the thermally dimorphic fungus *Talaromyces marneffei* (*T. marneffei*), endemic in the South-East Asia region. The pathogenic mechanisms of talaromycosis are still poorly understood. Our previous research found that macrophage-derived inflammatory cytokines and chemokines are critical in resistance to *T. marneffei*. Metabolism is essential for an effective immune response defending against invading pathogens. However, the specialized metabolism of macrophages also presents opportunities for fungi to hide from immune attacks. Studies on the immunometabolism of macrophages during *T. marneffei* infection are limited.
Methods In this work, we first established a cellular infection model. Subsequently, we performed transcriptomics and metabolomics of *T. marneffei*-infected macrophages for 24 h. Based on integrating transcriptomics and metabolomics, we analyzed the relationship between metabolites and DEGs in the *T. marneffei* infection group and control group. Then we identified and validated potential metabolic biomarkers and signal pathways involved in the *T. marneffei* infection through in vivo and in vitro experiments.
Results
1. Transmission electron microscopy and fluorescent microscopy demonstrated the phagocytosis of *T. marneffei* yeast by J774A.1 macrophage. *T. marneffei* yeast was engulfed by macrophages and proliferated within macrophages after 3 hours of incubation.
2. A total of 265 differential metabolites (DMs) were identified at VIP ≥ 1 and fold change ≥ 1.5 or ≤ 0.67 (117 up-regulated and 148 down-regulated), indicating remarkable differences in the metabolism of the two groups. KEGG enrichment analysis showed metabolic pathways involved in the sphingolipid signaling pathway, galactose metabolism, cysteine and methionine metabolism, biosynthesis of unsaturated fatty acids, arachidonic acid metabolism, and inflammatory mediator regulation of TRP channels were highlighted as potential targets for studying the pathological mechanism during *T. marneffei* infection.
3. A total of 1320 differentially expressed genes [DEGs (1286 up and 34 down-regulated)] were screened out in the condition of p-value <0.05 and fold change ≥ 1.2, illustrating that great changes also existed at the transcription level. In addition, the DEGs were screened for KEGG pathway enrichment analysis, which was mainly enriched in Herpes simplex virus 1

infection, Salmonella infection, endocytosis, PI3K/Akt signaling pathway, and NOD-like receptor signaling pathway.
4. Integrative metabolomics and transcriptomics analysis showed sphingolipid signaling pathway is the most influential.
5. To verify the involvement of S1P during *T. marneffei* infection, we first detected the S1P level in the culture supernatant of *T. marneffei* co-incubated macrophages. As expected, compared to the unstimulated macrophages, the level of S1P increased significantly at 8 h, peaked at 24 h, and remained plateaued until 48h. Next, we examined the mRNA levels of Sphk1, Sphk2, and S1PR1-5 at an incubate time of 8h. The results showed that *T. marneffei* induced increased expression levels of Sphk2, S1PR1, and S1PR2 ($p < 0.05$) while decreased expression of Sphk1 ($p < 0.05$) . There was no significant difference in the expression level of S1PR3, S1PR4, and S1PR5. Similarly, S1PR1 and S1PR2 proteins expressions were upregulated after *T. marneffei* stimulation for 3h and rapidly peaked at 8h, and subsequently showed a slight falling trend up to 24h.
6. *T. marneffei* induced PI3K and Akt phosphorylation compared to the unstimulated cells, with a significant difference. The PI3K phosphorylation became detectable at 3h and increased continuously, peaking at 24h, whereas Akt phosphorylated as early as 3h, peaked at 8h then decreased.
7. With the prolongation of the infection process, an aggravated pulmonary inflammatory infiltration and exudation of inflammatory cells were observed in H&E staining. PASM staining revealed that *T. marneffei* yeast was found at 7 dpi and 14 dpi; the fungal infiltration of 14 dpi was more severe than 7 dpi . The immunohistochemical analysis showed there was no difference in the positive rate of S1PR2 between the control and 3 dpi group but had a significantly increased at 7 dpi and 14 dpi compared with control mice ($p < 0.05$) .
8. The S1PR2 protein expression level increased in *T. marneffei* infected mice, of which the trend was similar at 3 dpi, 7 dpi, and 14 dpi ($p < 0.05$). It was worth noting that there was considerable phosphorylation of PI3K at 3 dpi, whereas it was hardly expressed at 7 dpi and 14 dpi. The Akt phosphorylation peak occurred at 3 dpi and then decreased gradually.
Conclusion The present study applied metabolomics and transcriptomics to analyze the metabolic profile changes and gene expression changes between *T. marneffei*-infected macrophages and controls. The integrative metabolomics and transcriptomics analysis found that the *T. marneffei* infection is closely related to the sphingolipid signaling pathway and may be further investigated for pathogenesis. Experimental verification results show that the S1PR2/PI3K/Akt signaling pathway was activated during *T. marneffei* infection and is associated with pulmonary infiltrates and disease severity. Further studies are warranted to elucidate the molecular details of signaling pathways regulation, including the PI3K/Akt pathway by S1PR2, and how this signal contributes to *T. marneffei* pathogenesis.

PO-078

A novel multifunctional mitochondrion-targeting NIR fluorophore probe inhibits melanoma proliferation and metastasis through the PPARγ/ROS/β-catenin pathway

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Objective Recent advancements in tumour-targeted therapies and immunotherapy offer hope to patients with various malignancies. However, the uncontrolled growth and metastatic infiltration of malignant melanoma remain a huge therapeutic challenge. Recently, near-infrared (NIR) heptamethine cyanine dyes have become the leading light therapy reagents owing to their excellent photophysical properties such as less background interference, strong tissue deep penetration and high image sensitivity. However, the delivery, targeting and therapeutic potential of this strategy remains uncertain as different chemical couplings could alter the targeted ligand structure. Moreover, the complex preparation process, poor water solubility, weak light stability, insufficient

tumour targeting and low biological safety remain common problems. Thus, the development of a simpler and more direct NIR dye to advance non-invasive cancer imaging and combine excellent anti-cancer effects remains urgent. Therefore, this study aimed to develop an integrated multifunctional diagnostic and treatment reagent IR-251 that can not only be used for melanoma imaging but also to inhibit melanoma growth and metastasis.

Methods IR-251 was synthesised and identified using a series of chemical synthesis methods and optical characteristic analyses. The fluorescence intensity of IR-251 *in vitro* and *in vivo* was observed under laser scanning confocal microscopy and small animal imaging technology. Mitochondrial morphology were observed by using transmission electron microscopy. ROS production was detected by DCFH-DA kit and the molecular mechanism using western blotting assays to illustrate. Finally, zebrafish tumour transplantation model and lung metastasis model were constructed to evaluate the anti-tumour proliferation and metastasis ability of IR-251 as well as using immunohistochemical staining detection the related indexes.

Results A novel theranostic agent IR-251 with displaying near-infrared fluorescence emission was synthesised and had good stability in serum. It targeted and damaged the mitochondria in melanoma cells via organic anion-transporting polypeptides. Mechanistically, IR-251 promotes ROS generation by dysregulating the homeostasis of ROS generation, thus causing oxidative stress-induced damage to the mitochondria and damaging its normal morphology and function. PPARγ has been reported to reduce oxidative stress by increasing the expression of various antioxidant enzymes, thereby accelerating the scavenging of free radicals. we selected rosiglitazone (a selective agonist for PPARγ) to verify an increase in PPARγ can reverse IR-251-induced oxidative stress, and it proved that IR-251 promoted ROS production through PPARγ. The Wnt/β-catenin signalling pathway plays a key role in regulating cell pluripotency and malignancy degeneration. It is also closely related to various biological functions such as tumour proliferation and metastasis. The expression of β-catenin and TCF4 was significantly inhibited by IR-251 and the exogenous addition of H_2O_2, with rosiglitazone playing a remedial role. It showed that IR-251 promoted ROS production by inhibiting PPARγ, and excess ROS resulted in oxidative stress that further inhibited the expression of β-catenin and TCF4 in the Wnt/β-catenin pathway. Moreover, the excellent anti-tumour proliferation and metastasis ability of IR-251 were verified *in vivo*. After the zebrafish was inoculated with Dil-stained B16-F10 cells (Dil-B16-F10) and cultured in a normal medium for 24 h, the fluorescence of tumour cells at the inoculation site was significantly enhanced, indicating that B16-F10 was successfully inoculated and could grow normally. However, when cultured with IR-251, the fluorescence of tumour cells at the inoculation site was significantly reduced due to the inhibited growth of B16-F10. When mice inoculated subcutaneously with B16-F10 were treated with an intraperitoneal injection of IR-251 (0, 0.5 mg/kg, 2 mg/kg) on alternate days, compared with the control, IR-251 significantly inhibited the transplanted tumour weight , volume and growth. The ability of IR-251 to inhibit tumour metastasis *in vivo* was evaluated using a B16-F10 melanoma lung metastasis model. the anatomical lung entity map revealed multiple black metastatic nodules of different sizes on the lung surface, indicating the high metastatic activity of B16-F10 cells. Moreover, fewer and smaller melanoma nodules appeared in the lungs of the IR-251 treatment group compared to the control group. The level of lung metastasis, as quantified by the number of pulmonary nodules, further highlighted the trend. And histochemistry staining revealed that IR-251 inhibited melanoma proliferation and metastasis, which showed no significant side effect.

Conclusion This study successfully synthesised and identified a novel multifunctional mitochondria-targeting NIR fluorophore IR-251 that exhibits good fluorescence imaging effect and an inhibitory effect on melanoma proliferation and metastasis without no significant side effect in vivo and in vitro. Base on co-localisation experiments of IR-251 and Mito-Tracker, the excellent mitochondrial targeting characteristics of IR-251 were determined. Notably, IR-251 entered the tumour cells in an OATP manner, and damaged the basic morphology and function of the mitochondria. Furthermore, the evaluation of mitochondrial ROS production, the changes in tumour cell proliferation and metastasis and the expression of related regulatory proteins revealed that IR-251 inhibited SOD2 and GPX1 mainly by inhibiting PPARγ, consequently inducing the overproduction of ROS. This overproduction of ROS inhibited β-catenin and TCF4 and also corresponding downstream protein molecules associated with proliferation and metastasis,

ultimately inhibiting the proliferation and metastasis of melanoma cells. Therefore, this research not only provides a practical solution for the development of NIR fluorescent dyes for melanomaBimaging and therapy but also contributes to the current mechanistic understanding mechanism of oxidative damage.

PO-079

Discussion of the application of an intraoperative stem cell therapy for androgenetic alopecia

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Stem cell-based regenerative therapy is a research hotspot in alopecia treatment. The keywords used in published research gradually changed from basic to applied studies, reflecting the direction of the study [1]. We here discuss a new intraoperative stem cell therapy for androgenetic alopecia and highlight some considerations and issues in the clinical application of stem cell therapy.

Before we become overly excited about implementing regenerative technology in clinical settings and anticipating positive clinical results, we should carefully consider the following issues. Firstly, the mechanisms of stem cell treatment should be well studied. Secondly, clinical studies should provide solid evidence of safety or efficacy of the stem cell therapy. Thirdly, the specific processes of sourcing, harvesting, processing and application of stem cell therapy are standardized, and finally, strict and appropriate quality control is required before application[2, 3].

Recently, Gentile et al. [4, 5] have proposed the use of intraoperative autologous follicular stem cell micro-grafts for the treatment of androgenetic alopecia. A Rigenera® CE medical device is used to harvest scalp tissue fragments by punch biopsy. These fragments then undergo a number of processes such as splitting and centrifugation. Micro-grafts suspension is collected into a mesotherapy gun and injected into the treated area. Gentile claimed that this preparation could acquire Human Intra- and Extra-Dermal Adipose Tissue-Derived Hair Follicle Stem Cells (HD-AFSCs). Two cellular populations, Hair Follicle Epithelial Stem Cells (HF-MSCs) and Hair Follicle Epithelial Stem Cells (HF-ESCs) are considered to be responsible for the improved hair density and increased number of hair follicles microscopically observed in clinical trials.

This technique is characterized by autologous intraoperative cell therapy and a mechanical minimal manipulation process. Typically, the intraoperative cell therapy procedure involves the harvesting and processing of tissue to obtain the desired cell product, surgical intervention, and delivery of the cells. With the advances in regenerative surgery, techniques using autologous cells that involve minimal manipulation and prompt transplantation offer unprecedented possibilities in this field[6].This device has been demonstrated to be capable of obtaining different tissue-derived progenitor cells and preserving their cellular activity in vitro. Despite this, there is a lack of evidence to support their ability to differentiate and secrete important substances. Majorities of studies have focused on assessing the safety and feasibility of stem cell therapies, but have neglected to investigate the mechanisms responsible for the observed results.

In the procedure of hair transplantation, Zanzottera et al. [7] utilized this technique to acquire hypodermal adipose tissue micrografts of the scalp, and claimed better healing results and hair restoration. However, this study only involved 3 cases and did not include comparisons. In 2017, Gentile took it a step further and used this method in alopecia treatment. The first step is harvesting the scalp tissues (30–50 fragments) with punch biopsy (2 mm diameter). However, the study did not clarify whether the tissue fragment number affects the number of stem cells generated. In addition, the number of fragments containing intact bulb and bulge areas, and how they defined intact bulb and bulge areas were not specified. Furthermore, autologous cell isolation may cause significant differences in cell numbers and phenotypes. Heterogeneity in age, gender, body mass index, pedigree/genetics, diet, and environment may contribute to these differences. For instance, Shin et al. have demonstrated that the mesenchymal progenitors of hair follicles become dysfunctional over time and are no longer able to replenish the Dermal Papilla. The dysfunction of

these progenitor cells results in the loss of inductive mesenchymal cells within each hair follicles, which contributes to the progression of hair loss over time.
HD-AFSCs are present in a minor proportion in the micrograft suspension. The remaining cells are predominantly dermic fibroblasts and epidermal cells, demonstrating the complexity of the cellular composition. Despite the fact that many intraoperative cell therapies use heterogeneous cell populations, which may include stem cells or progenitor cells, there is a possibility that these stem cells and progenitor cells are not responsible for the positive outcome. The heterogeneous composition of cell populations further complicates the elucidation of mechanisms that mediate functional improvement. Certain cell types may play an active role, or more than one type may play the role simultaneously. It is unclear whether the biopsy defects were recovered properly or what the status of regional hair was. This technique appears to be invasive and may have led to an increase in hair loss over time as the treatment process accumulates.
This “Gentile procedure” is an attempt to incorporate intraoperative cell therapy into the treatment of androgenic alopecia, utilizing the patient's own cells to maximize safety and accessibility. Several issues have been left unaddressed in the clinical trial, resulting in heterogeneity and unrepresentative results. While this technique appears to be more invasive, it has not yet demonstrated long-term effectiveness in comparison with traditional treatments such as minoxidil and finasteride or the more recent platelet-rich plasma treatment.
Researchers have also investigated other sources of adipose-derived stem cell-based therapies as potential treatments for alopecia. When compared to hair follicle stem cells obtained from the Gentile procedure, stem cells derived from autologous adipose sources are easier to access and more reproducible. The main effect of autologous stem cells, allogeneic stem cells and their derivatives such as exosomes in the treatment of hair loss is to regulate the local microenvironment of the hair follicle, which then stimulates hair growth. It is critical to note that the hair follicle is a complex regenerating system that is delicately regulated in its physiological processes. An additional approach is to regenerate hair follicles through tissue engineering. The 3D-printed mold is used to restore the physiological 3D organization of cells in the HF microenvironment and induce HF differentiation. Several studies have been conducted to improve the regeneration of hair follicles by altering the culture conditions[8-11]. The findings of this study have led to a novel approach to hair regrowth treatment. At present, the technique has only been applied in animal studies. The clinical application of this procedure was halted primarily because of concerns regarding the longevity and survival of regenerated hair follicles.
In recent years, intraoperative cell therapy has become an important and exciting approach to treat diseases. It presents safe and effective outcome for a wide range of indications. However, additional approaches and even multidisciplinary collaborations are required to achieve desired outcomes and overcome current limitations.

PO-080

Analysis of the application value of focus solution mode in the care of patients with neurodermatitis

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Objective To analyze the value of care through focus solution mode for patients with neurodermatitis.
Methods 60 patients with neurodermatitis were screened and admitted to the hospital from April 2020 to January 2021, and divided into 2 groups (30 cases in each group) according to the "random sampling method". The control group was given regular care, the observation group gave focus solution mode care, and compared the nursing effect of the 2 groups.
Results The SDS and SAS scores of the observation group were lower than those of the control group, the compliance of treatment according to the doctor's advice was higher than that of the control group, and the follow-up rate was higher than that of the control group (P<0.05).

Conclusion Choosing the focus solution mode to care for patients with neurodermatitis can improve their psychological state and guide patients to actively cooperate with treatment, so as to improve the follow-up rate, which is worth learning from.
Keywords focus solution mode; neurodermatitis; nursing; treatment compliance
Neurodermatitis is a common, chronic inflammatory skin disease, with severe itching, moss-like changes of the skin as clinical symptoms. The appearance of the disease is closely related to neurological dysfunction, fatigue, mental stress and other factors. Based on the above-mentioned predisposing factors, it is necessary to strengthen the patient's mental state and sleep condition at Management aims to improve patients' treatment compliance [1]. The focus solution model is a clinical intervention mode developed under the background of positive psychology that fully respects the individual and develops its own resources. It stimulates its initiative by analyzing patients' advantages and resources. In order to accurately evaluate its nursing value, this paper selects 60 patients with neurodermatitis (April 2020 ~ 202 January 1st) Research, reported as follows.

1 Information and methods

1.1 Information

The 60 patients with neurodermatitis selected were admitted to the hospital from April 2020 to January 2021. They were divided into 2 groups (30 cases in each group) according to the "random sampling method".

Male/female in the observation group = 13/17, age 20~60 years old (average 39.25±8.24 years), course of disease 3~12 years (average 6.25±1.42 years), education: 4 cases of junior college and above, 6 cases of high school, 20 cases below high school; male/female in the control group = 12/18, age 21~60 years old (average value 39.28±8.18 years old), course of disease 3~12 years (average 6.21±1.38 years), education: 3 cases of junior college or above, 7 cases of high school, 20 cases of high school below, data statistics P>0.05.

1.2 Method

Routine care given by the control group: After admission to the hospital, the patient needs to strengthen health education while taking routine drug treatment, guide them to master the occurrence, treatment and prognosis of the disease through oral education, and do a good job in psychological counseling and skin management routine guidance. Once the patient is found to have abnormal symptoms, they should report to the doctor in time. Reason.

The observation group focused on solving nursing on the basis of the control group: (1) Reach a consensus with the patient on health goals, guide the patient's positive behavior through hypothetical solutions, etc., and formulate health goals with them, and refine them into small steps. (2) Find the patient's beneficial resources. In the process of asking the patient's symptoms to relieve, you can use the "exceptional question" method and find the key to control the disease, so that they can have a new understanding of their own resources. (3) Formulate an action change plan, start from an early age, implement health education measures that meet the needs of patients, guide them to master self-management knowledge and skills, and use their self-advantages to solve existing problems. (4) Feedback, appreciate and feedback on the patient's advantages and resources, aiming to enhance their confidence in achieving the goal. (5) Guide further changes, evaluate patients, and guide them into the next cycle.

1.3 Observation indicators

(1) Evaluate the nursing effect according to the psychological state (refer to SDS, SAS scale evaluation, critical value 53 points, 50 points [2]), treatment compliance (refer to Frankl scale evaluation, score 0-100 points), etc.

(2) Record the follow-up rate after discharge.

1.4 Statistical Methods

SPSS 23.0 software is used to sort out and analyze the data, and the measurement data is average ± standard deviation (

±s) indicates that the counting data is expressed as a percentage or rate (n, %), and P<0.05 indicates that there is a statistical difference.

2. Bear fruit

2.1 Comparison of the nursing effects of the two groups

Table 1 shows that the SDS and SAS scores of the observation group were lower than those of the control group, and the treatment compliance was higher than that of the control group (P<0.05).
Table 1: Comparison of the nursing effects of the two groups (±s, minutes)
2.2 Comparison of the follow-up rates of the two groups
There were 27 follow-up cases after nursing in the observation group, accounting for 90.00%; 20 cases were follow-up after nursing in the control group, accounting for 66.67%, and there were significant differences between the groups.
=4.811, P=0.028.
3. Discuss
Neurodermatitis has the characteristics of recurrent attacks, and secondary dermatitis can occur under the influence of skin itching. In addition, the disease has the characteristics of long treatment time and stubborn course of the disease. Therefore, in the process of treatment, patients need to strengthen their self-behavioral management and improve the overall prognostic effect by enhancing their treatment confidence [3-5]
The results showed that the SDS and SAS scores of the observation group were lower than those of the control group, and the treatment compliance was higher than that of the control group. It can be seen that the patient's negative emotions were significantly controlled after the application of the focus solution mode. The analysis reason is that the focus solution mode is a psychological care plan. Under the premise of respecting the The potential of treating diseases can achieve the purpose of reducing their physical and mental stress after the disease. In addition, by finding the patient's own advantages, it can improve their subjective initiative, strengthen the management of self-behavior and disease in the treatment process, and improve the prognostic effect while relieving emotions. The nursing content under the focus solution mode includes reasonable communication between doctors, nurses and patients. By understanding the patient's appeal for disease treatment, they can actively cooperate with medical staff for treatment, improve their confidence in treatment by investigating the patient's self-control and relieving the discomfort plan after the onset of the disease, and then actively match. The doctor treats and controls the disease. To sum up, applying the focus solution mode to the nursing of patients with neurodermatitis can improve the negative emotions of patients, so as to actively cooperate with doctors for treatment, improve the control effect of the disease by giving full play to their own advantages, and regularly admit to the hospital for follow-up visits in order to treat according to the doctor's instructions.

PO-081

Pediatric primary cutaneous lymphoma in China: a retrospective study

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Objective Primary cutaneous lymphomas (PCL), including cutaneous T-cell lymphoma (CTCL) and cutaneous B-cell lymphoma (CBCL), are extranodal non-Hodgkin lymphomas, which by definition have no evidence of extracutaneous involvement at the time of initial diagnosis. The features of PCL showed age, gender, geographical and racial variation. A comparative analysis of published reports about PCL showed male predominance, and a higher rate of NK/T-cell lymphoma and a lower rate of cutaneous B-cell lymphoma in East Asia than that in Europe and North America. While PCL in children and young adulthood is infrequent and the literatures were rare. Cutaneous lymphoma is infrequent in childhood, accounting for approximately 6% of malignant neoplasms in children. In this study, we evaluated the clinical characteristics and the relative frequency of different subtypes of pediatric PCL in a single Chinese center and compared the findings with other reported studies.
Methods Pediatric patients younger than 18 years diagnosed with PCL from January 2010 to December 2021 were identified from the clinical records of the Institute of Dermatology, Chinese Academy of Medical Sciences. A retrospective study on collected cases was performed. All eligible

pediatric patients accorded with the following criteria: first diagnosed any subtypes of PCL in the WHO-EORTC classification before the age of 18 years and no previous malignancy. Physical examination and laboratory inspection, including blood analysis, chest radiography, abdominal ultrasonography and sonography of lymph nodes, were utilized to confirm the presence of PCL without nodal and/or visceral involvement. Patients with insufficient information have been excluded. Clinical data including sex, age at diagnosis, routine hematology and biochemistry examination, imaging examinations, histopathological features, data of immunohistochemistry and follow-up results were collected. The diagnosis was confirmed by the same pathologists and dermatologists in our institute, according to the WHO-EORTC.

Finally, 101 patients in total were involved in our study (the flow-chart of data collection process is shown in Fig 1). The age at diagnosis ranged from 3 to 18 years (median, 11-year old). Seventy-two patients (71.3%) were males with male/female ratio 2.48:1.

The number and proportion of different subtypes of children CTCL in our institute were MF and variants (n=42; 41.6%), Lymphomatoid papulosis (LyP) (n=23; 22.8%), chronic active Epstein–Barr virus (CAEBV) infection (n=23; 22.8%), subcutaneous panniculitis-like T-cell lymphoma (SPTCL) (n=4; 4.0%), primary cutaneous peripheral T-cell lymphoma, rare subtypes (n=4; 4.0%) and primary cutaneous anaplastic large cell lymphoma (pC-ALCL) (n=2; 2.0%), respectively. There were only three patients diagnosed as primary cutaneous marginal zone B-cell lymphoma (PCMZL). No other CBCL subtypes were found in the present study.

MF was still the most common entity, accounting for 41.6% of pediatric cutaneous lymphoma. There were 35 male patients with MF, leading to a remarkable male predominance (male/female ratio 5:1). Clinically, the children showed variably sized erythematous, finely scaling lesions on the trunk and extremities, of which some were mildly pruritic . Skin biopsy showed superficial or perivascular lymphocyte infiltrates. Single cells surrounded by vacuolated halos in epidermis could be observed in several cases . Most cases indicated classical immunophenotype (CD3+, CD4+, CD8−), while six cases expressed cytotoxic T‐cell phenotype (CD3+, CD8+, CD4−). Besides, approximately 47.6% MF cases presented with hypopigmented variant (hMF), which was characterized with generalized hypopigmented lesions and was initially misdiagnosed with vitiligo, fungal infection, pityriasis rosea or post-inflammatory hypopigmentation. Other rare subtypes of MF, such as follicular MF, pagetoid reticulosis and granulomatous slack skin were also observed in our study. Most cases including all the hMF presented with limited cutaneous disease (stage IA or IB). 30 cases were treated with topical glucocorticoids and mechlorethamine, while 12 cases with phototherapy additionally. Follow-up time of MF cases ranged from 6 to 118 months, and nine cases were lost follow-up. Although most MF patients got favorable prognosis, a 13-year-old boy with classic MF had long-time and repeated relapse when the frequency of phototherapy was decreased, and the lesions on his ankles existed consistently.

The incidence rate of LyP ranked the second in our pediatric group. A slight male predominance was observed (14/23). Most cases presented with multiple erythematous papules on the trunk and extremities, partial had papulovesicles and pustules that occasionally healed with scars. The histological types A-E of LyP were observed in our patients. And 17 patients (73.9%) were designated as having type A which showed prominent epithelial hyperplasia with edema, dense lymphoid infiltrates with scattered large CD30+ cells in the dermis. Furthermore, there were 2 patients with type B and D (8.7%) , and one case with type C and E (4.3%) respectively. Interestingly, the patients with type C, D and E were female. Of the LyP patients, 13 cases were treated with topical glucocorticoids and phototherapy, 8 with oral low dose methotrexate. Only 2 patients with type A were under observation regularly without treatment, for a few lesions appeared occasionally and spontaneously regressed. Follow-up period of LyP cases was from 13 to 97 months, and most got clinical remission, while five case were lost follow-up.

There are 23 patients diagnosed as cutaneous CAEBV, among which 11 cases were diagnosed as hydroa vacciniforme-like lymphoproliferative disorder (HVLPD), and the others as severe mosquito bite allergy (SMBA). According to the grading criteria of Epstein-Barr virus positive lymphoproliferative disorders (EBV+LPD) proposed by Ohshima[14], there were 12 cases of level 1, 10 of level 2 and 1 of level 3, respectively. The lesions of cutaneous CAEBV manifested as papulovesicles or ulcers mainly at the exposed sites, and two patients had fever, lymphadenectasis and slightly enlarged liver and spleen. Histologically, HVLPD were characterized by epidermal

spongiotic vesiculation, epidermal necrosis and ulceration, as well as infiltrates of small to medium-sized EBV-positive lymphocytes, usually without atypia, in the dermis and subcutis, while SMBA possessed more extensive epidermal necrosis and a polymorphous infiltrate of small and large lymphocytes and numerous eosinophils in the dermis and subcutis. Of our 23 cases of CAEBV, epidermal necrosis were observed in 16 cases, and 11 cases were admixed with mast cells or eosinophils. Immunohistochemical staining indicated that 8 cases had a CD8+ T-cell phenotype, while tumor cells of 7 cases were positive for CD56+. For the treatment, ten patients received Ganciclovir or Acyclovir with corticosteroids or other immunosuppressive agents, twelve cases were just treated with topical immunosuppressants. Follow-up time was from 11 to 107 months, and 5 cases were lost follow-up finally. At follow-up process, 16 patients were under relative clinical remission and 5 cases experienced chronic recurrent or sporadic eruptions without complications. Exceptionally, a 14-year-old girl and a 16-year-old boy with perpetual HVLPD remained mild relief after regular therapies and they were preparing for hematopoietic stem cell transplant.
SPTCL, primary cutaneous peripheral T-cell lymphoma, rare subtypes, pC-ALCL and PCMZL were in low proportions. Clinically, all the SPTCL patients presented with erythematous subcutaneous nodules on legs or arms(shown in Fig. 7A-7B), and gradually the nodules would become skin atrophy. No fever and weight loss were observed. Skin biopsy showed subcutaneous infiltration of neoplastic T cells around adipocytes. After the therapy of corticosteroids or immunosuppressive drugs, all the three cases achieved remission. The three children with primary cutaneous CD4+ small-medium T cell lymphoproliferative disorder showed a solitary papule with defined border, which were treated with complete local surgical excision, leading no relapse. The two boys with pC-ALCL both presented localized papules or nodules and ulceration gradually developed in clinic, which were confirmed by the typical histological feature that large CD30-positive tumor cells, and they were treated with low dose of methotrexate with radiotherapy. The 3-year-old boy who presented with multiple papules on his trunk and legs was later diagnosed as primary cutaneous aggressive epidermotropic cytotoxic T-cell lymphoma (PCAECTL). The immunohistochemical examinations showed strongly epidermotropic, band-like to diffuse infiltrates of pleomorphic T cells with a CD3+, CD4−, CD8+, CD45RA+, granzyme B+, perforin+, TIA-1+ phenotype. Regretfully, during the follow-up, the parents of the boy refused further consultation. Referring to the children with PCMZL, their lesions decreased and skin color gradually returned to normal after topical treatment, while the lesion of one boy relapsed one year after resection, and then repeated histological examination confirmed the diagnosis. No cases of Sezary syndrome, extranodal natural-killer/T-cell lymphoma and other aggressive lymphoma were found in our study.
Conclusion Despite its limitations, our study provides an analysis on the detailed distribution of pediatric PCL in China. Chinese pediatric PCL showed a higher incidence of MF than that found in the Korean and other Asian countries. In addition, pediatric group tended to have a higher rate of more indolent subtypes of PCL, such as hMF, SPTCL and PCMZL. Multicenter collaborative prospective larger studies are required to confirm these findings.

PO-082

Coexistence of multiple variants of porokeratosis in a Chinese man

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Porokeratosis is an acquired or inherited disorder, which presents as a keratotic papule and plaque with central atrophy surrounded by a hyperkeratotic border. Clinical variants include porokeratosis of Mibelli, disseminated superficial, disseminated actinic superficial, punctate, linear, giant, and palmaris et plantaris disseminate. Diverse types of porokeratosis can coexist in a single individual, and such combination is rare. We report coexistence of porokeratosis of Mibelli, disseminated superficial porokeratosis and disseminated superficial actinic porokeratosislinear porokeratosis in a Chinese man.

A 65-year-old man presented with disseminated, asymptomatic, brownish and keratotic plaques on both his lower limbs since the age of 27 years. Similar but smaller lesions had gradually developed over both his upper limbs, face and trunks. Cutaneous examination revealed bilateral, disseminated scaly and well-defined erythematous plaques with central atrophy and a distinct keratotic ridge over both his upper limbs and lower limbs. Multiple, coin-sized, annular and scaly plaques with central atrophy and thin grooves were found on his face, upper arms and trunks. His 38-year-old daughter has similar lesions on her lower limbs. Laboratory inspection revealed no pathological findings. Biopsies were performed on the borders of two lesions respectively on his abdomen and left lower limb, both lesions showed the characteristic cornoid lamella, and dyskeratotic cells and focal hypogranulosis were in the epidermis underlying the column of parakeratosis. Based on the clinical and histological features, diagnosis of coexistent porokeratosis of Mibelli, disseminated superficial and disseminated actinic superficial was made.

The coexistence of various subtypes of porokeratosis were reported. The most frequently described was about the disseminated superficial form and the linear form, while the case with porokeratosis of Mibelli and disseminated superficial porokeratosis was less frequent.

Porokeratosis of Mibelli has also been described to coexist with disseminated superficial actinic porokeratosis. Our patient had irregular annular plaques on his forearms and lower limbs, which accorded with the classic features of porokeratosis of Mibelli. As the disseminated small annular lesions were localized to exposed and unexposed areas and he was a building worker who had the prolonged outdoor work, the diseases of disseminated superficial porokeratosis and disseminated superficial actinic porokeratosis was diagnosed. Cases diagnosed as the coexistence of the three forms of porokeratosis had not been reported. The precise pathogenesis of porokeratosis remains largely unknown, let alone the coexistence of multiple variants. Genetic susceptibility, immunosuppression, exposure to ultraviolet radiation and potential tumor may be the risk factors. Mutations in the phosphomevalonate kinase pathway genes, including MVD, MVK, PMVK, and FDPS, have been reported in patients with porokeratosis. Researchers speculated that the coexistence of various subtypes of porokeratosis in one patient may indicate different phenotypes of a common genetic abnormality, or genes of different variants of porokeratosis may exist in the same or closely linked loci. Regretfully, the patient presented refused the further genetic testing. The treatment of porokeratosis is difficult and for the disseminated lesions, our patient refused further treatment.

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PO-083

Oxidative Stress-Related Genes Predict the Severe Psoriasis Patients Based on Weighted Gene Coexpression Network Analysis and Machine Learning

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Distinguishing between mild and severe psoriasis patients has great clinical significance. There still lack of objective diagnostic biomarkers for severe psoriasis. Oxidative stress is defined as an imbalance between prooxidants and antioxidants, may play an essential role in the etiology of psoriasis. Here, we attempted to develop a novel model with oxidative stress related genes (OSGs) to predict severe psoriasis, and explore possible molecular mechanisms underlay psoriasis progression.

Differentially expressed genes(DEG) between normal and psoriasis lesion were identified by limma R software package.WGCNA was performed on GSE78097 based on its expression levels and psoriasis class to screen for hub genes. The co-expression modules were obtained using automatic network construction function (block wise Modules) with default parameters.Support vector machine- recursive feature elimination (SVM-RFE) and feature selection by least absolute shrinkage and selection Operator (LASSO)logistic regression were used to screen the characteristic genes. Single gene Gene Set Enrichment Analysis (GSEA) was implemented in the clusterProfiler package in R. To further explore the related pathways of the three marker genes, we calculated the correlation (spearman correlation) between the marker genes and all other genes in the GSE78097 dataset. Related infiltration and activity levels for 24 immune cell types, obtained from published signature gene lists across all mild and severve samples, were quantified using the single-sample gene set enrichment analysis (ssGSEA) in R package GSVA. The Comparative Toxicogenomics Database (CTD) were utilized to evaluate potential drug targets.

The transcriptional profiles and clinical phenotypes of psoriasis patients were obtained from the gene expression omnibus (GEO) database. The gene differential expression was analyzed from GSE78097 database of 27 psoriasis samples and 6 normal samples by R Limma package. A total of 3479 differential genes were identified, 2056 genes were up-regulated and 1423 genes were down-regulated. We identified a total of 47 differentially expressed OSGs. We determined the soft threshold of 11 by calculating the scale-free model fit and mean connectivity. Different module genes in the dynamic tree cut were reclustered through a topological similarity strategy, where genes were assembled into fewer modules as shown in . A total of 19 modules were constructed by WGCNA, and the cyan module was strongest correlated with psoriasis severity.Ten oxidative stress related diagnostic markers (S100A9, S100A8, SRXN1, AOC3, PAOX, NOX4, NOS3, HMOX2, LOX, PPARGC1A) were obtained by intersection of WGCNA screening genes, DEGs and

OSGs. Subsequently, S100A9, S100A8 and PAOX identified as marker genes by LASSO and SVM - RFE algorithms. To elucidate the individual gene discriminative ability, ROC curves were generated for the 4 marker genes. As shown in Fig 3E, AUC for S100A9, S100A8, PAOX was greater than 0.9. Based on the above 3 marker genes, we constructed a logistic regression model by R package glmnet, and the subsequent ROC curves indicated that the 3 marker gene based logistic regression model differentiated severe from mild samples with AUC = 1.To further explore the function of the three candidate marker genes, we preformed single gene GSEA enrichment analysis. GSEA showed that PAOX, S100A8 and S100A9 related pathways were major involved in neutrophil activation in immune response, regulation of leukocyte activation, mRNA processing. PAOX, S100A8 and S100A9. Correlation analysis showed that the changes in the three markers were positively correlated with the abundance of activated CD4 T cell, activated dendritic cell and type 17 T helper cell. What is more, the expression of S100A8/A9 and PAOX were decreased with the treatment of psoriasis and were strongest positively correlated with PASI.here was no significant change of PAOX before and after treatment of Adalimumab, Methotrexate, Tofacitinib, Etanercept at 12 weeks or 16 weeks in psoriasis patients, while, the levels of PAOX were significant decreased in the treatment of Ustekinumab and Brodalumab in 12 weeks. However, S100A8 and S100A9 gone down during treatment. Especially at 12 weeks or 16 weeks of treatment, the downward trend is even more pronounced in the biologic therapy (Adalimumab, Ustekinumab and Brodalumab) The expression of S100A8/A9 and PAOX protein increases with the aggravation of skin lesions in psoriasis mouse model skin lesions. A total of eight chemicals of tretinoin, isotretinoin, tamibarotene, methotrexate, troglitazone, calcitriol, tetracycline, and azathioprine that treat for psoriasis were screened by CTD. S100A8, S100A9 and PAOX are possible therapeutic targets for these drugs.

Here, we used a systems biology-based WGCNA approach for the first time to predict diagnostic biomarkers of oxidative stress associated with psoriasis severity and progression. WGCNA and co-expression network analysis identified key biological processes and signaling pathways. Potential diagnostic biomarkers including PAOX, S100A8 and S100A9 may be associated with PAIS and treatment response in psoriasis patients. Our study identified three oxidative stress genes in mild and severe psoriasis, also explored the possible mechanism of oxidative stress promote psoriasis progression. Our finding may provide novel potential psoriasis severity and progression indicators.

PO-084

Distribution, risk factors and antifungal susceptibility of Aspergillus species from mainland China: A systematic analysis

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The fungus Aspergillus is widely dispersed in nature and is easily transported through the environment. Microscopic Aspergillus spores enter the human body through the airways and cause aspergillosis. Aspergillus species causes different Aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA), and invasive pulmonary aspergillosis (IPA), with broad clinical and diagnosis features. IPA is a potentially fatal opportunistic infection with high morbidity and mortality rates. It mainly affects immune-compromised patients, such as hematological malignancy patients, organ transplant recipients, and those who use immunosuppression therapy. The aspergillosis associated with non-fumigatus, including A. flaus, A. niger, A.nidulans, A. terreus, and A. versicolor, has been reported.

The published research articles on Aspergillus spp in mainland China were searched. The guidelines for systematic reviews and meta-analyses (PRISMA) were used to collect articles. The complete data from each article were collected initially in an Excel sheet (2017) by three researchers independently. The extraction data included the article's title, year of publication, study

region, time of sampling, total samples, inpatient or outpatient, age, gender, types of infection or co-infection, underlying diseases, mortality rates, methods of identification, and AST method. The given data of the articles was counted in terms of number and percentage.

Each variable data count, numbers, and percentages were analyzed in an Excel sheet. The mortality rate and underlying diseases were examined using a graphed prism. A. fumigatus, and non-fumigatus, including A. flaus, A. niger, A. nidulans, A. terreus, and A. versicolor antifungal susceptibility against antifungal agents, were measured in the form median susceptible or median wild type (MS/WT) with a 95 % confidence interval (CI) to analyzed each data. The student's t-test or the Wilcoxon signed-rank test was used for the antifungal susceptibility pattern. The P-values more than 0.05 were considered insignificant.

These first-line antifungals are commonly used for the treatment of aspergillosis. Other drugs, including amphotericin B (AMB) and Caspofungin (CAS), are also used for aspergillosis. However, aspergillosis mortality rates remain high, and the development of antifungal resistance is regarded as one of the major threats. Antifungal resistance and the prevalence of Aspergillus spp, have been increasing in China.

The prevalence of Aspergillus species, antifungal resistance, clinical characteristics, and risk factors has been studied to control the increasing morbidity and mortality rates of patients with aspergillosis. The systematic review included 63 articles containing 2,569 Aspergillus isolates in total. The high rate of published articles was 11, 9, and 9 from 2018, 2019, and 2022, respectively, and the study duration was from 2012 to 2022. East China reported the most articles (n=26), followed by North China (n=17). In the sub-region, Beijing reported a high number of articles (n=14), followed by Guangdong (n=10). A total of 5,626 patients were mentioned in the reports, with males accounting for 57.2% and females 39.45% (Table 1).

In published reports, the determined adult age median with 95% confidence interval (CI) was 73.5 years (56-75), while the children's age median with a 95% CI was 16 years (13.5-18). Among 18 studies, the high mortality rate (17.46%) from IPA with median-related mortality interquartile range (IQR) (3-20.5%). In A. fumigatus and non-fumigatus, infections were associated with the mortality percentage in aspergillosis indicated in studies. Moreover, the identification culture methods of Aspergillus isolates were mentioned in 27 (42.8%) articles. The detail of other techniques used during aspergillosis.

The percentage of prevalence of some Aspergillus isolates from mainland China was divided into seven regions, and the prevalence of Aspergillus was studied in each region. North China had the highest proportion of Aspergillus isolates (49.0%), followed by east China (20.28%), south China (10.93%), northwest China (10.75%), central China (3.85%), and northeast China (3.54%). A. fumigatus was reported in 28.57% of all Aspergillus isolates in 21 articles, followed by A.flaus (15.87%), A. niger (9.52%), A. terreus (6.34%), and A. nidulans (4.76%). A. lactioffeatus, and A. sydowii were reported (1.58%). However, 61.90% of reported articles were not indicated the types of Aspergillus isolates. In depicts the complete distribution of Aspergillus isolates. Regarding Aspergillus spp with co-infection, a high number of articles reported fungi with bacteria (20.63%), followed by fungi with fungi (14.28%), and fungi with viruses (14.28%). Furthermore, the Aspergillus spp causes different types of aspergillosis were reported, with the highest number of IPA (46.03%), followed by CPA (19.04%), APPA (11.11%), simple Aspergillosis (7.93%) and CBA (1.50%). The mortality rate of aspergillosis patients linked with Aspergillus spp. IPA had the highest percentage (68.65%), followed by CPA (29.53%) and CBA (1.81%), while ABPA and simple aspergillosis had no morality.

The statistical median with a 95% CI was used for A. fumigatus and non-fumigatus, including A. flause, A. niger, A. nidulans, A. terreus, and A. versicolor. Some AST rates were statically significant (P =< 0.05), while the isavuconazole was not statically significant (P =< 0.1821). AST of 7,043 Aspergillus isolates were tested against twelve antifungal agents, including amphotericin B, itraconazole, isavuconazole, voriconazole, posaconazole ravuconazole and caspofungin. Posaconazole and ravuconazole both showed a high proportional susceptibility. Azole had the highest susceptibility, followed by polyenes. Among the non-fumigatus, Polyenes were found to be less susceptible with median (95% CI), amphotericin B 58.1(45-99.4), and isavuconazole 70.8 (50.0-91.6).

In this study, we summarized the prevalence of Aspergillus spp and found that A. fumigatus and A. niger are highly distributed in different regions from mainland China. The antifungal susceptibility pattern of Aspergillus showed that polygene and azole are very active and effective drugs against A. fumigatus. In comparison, amphotericin B and isavuconazole had low susceptibility rates among the tested antifungal drugs against some Aspergillus spp. The current study is a guideline for selecting empirical antifungal therapy for prescribers.
In this surveillance study, we also mention gaps, like no studies were found in some regions in the northeast and inner magnolia, etc. Most articles did not mention AST for Aspergillus spp, which is insufficient for examining their susceptibility pattern. Moreover, the mortality and risk factors rates are high during aspergillosis, but many reports did not mention it. Hence, surveillance studies by researchers are critical to indicating gaps in the control of Aspergillosis. In addition, health professionals must take preventative steps under policymakers to stop the nosocomial spread and antifungal drug resistance.

PO-085

Thalidomide Attenuates Inflammation and Fibrosis in Rosacea-Like Mice Induced by Long-Term Exposure of LL-37

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Background Rosacea is a chronic inflammatory skin disease that occurs on the face. The early stage of the patient is mainly inflammatory changes, and the late stage is often due to repeated inflammatory stimulation leading to the production of skin fibrosis. Phymatous rosacea, also known as a nasal neoplasm, leads to local skin tissue hyperplasia under the stimulation of long-term inflammation. Most of the existing studies focus on the early inflammation of rose acne, and few studies on the development of fibrosis in the later stage. Thalidomide is a synthetic glutamic acid derivative, which has sedative and hypnotic effects, anti-inflammatory effects, anti-angiogenesis effects, and immunomodulatory effects. Therefore, it can be used in diseases related to inflammation and autoimmunity. The mechanism of thalidomide in relation to the inflammatory and fibrotic phenotype of rosacea remains to be investigated.
Objectives In this study, prolonged local stimulation of LL-37 was used to establish a long-term induced mouse model of rosacea. The purpose of this study was to investigate the difference of inflammatory cytokines and fibrosis signaling pathway between short-term inflammatory model and long-term induced model, and to explore the effect and mechanism of thalidomide on the inflammation induced fibrosis of rosacea mouse model.
Methods: 4 weeks old male BALB/c mice were adaptively fed for 2 weeks and randomly divided into four groups with 5 mice per group: control group, control plus thalidomide group, LL-37 group and LL-37 plus thalidomide group, Intradermal and intraperitoneal injections were given. After repeated induction, the skin lesions on the back of mice were photographed and the degree of skin lesions were recorded. The samples were sacrificed 24 hours after modeling. HE staining was used to observe the pathological changes of skin tissue on the back of mice, VG staining was used to detect the collagen deposition in skin, and Masson staining was used to observe the thickness changes of dermis, epidermis and subcutaneous tissue layer and the changes in collagen. Immunofluorescence staining was used to detect the expression of inflammatory factor TNF-α, vascular endothelial cell marker CD31, myofibroblast marker α-SMA, and interstitial cell marker vimentin. Western blot was used to detect the expression of proteins related to inflammation, angiogenesis and fibrosis-related factors in skin tissue and associated with epithelial-mesenchymal transition and TGF-β/Smad pathway. Real-time fluorescence quantitative PCR was used to detected the mRNA expressions of inflammation and angiogenesis factors.
Results The results were compared with the early stage of the model, the late stage of the model mice had more serious skin lesions, with erythema, erosion exudation, ulcer scab, aggregation of

inflammatory cells and increased epidermal thickness, and the expressions of COL I, TNF-α, α-SMA and IL-6 were significantly up-regulated, there was a tendency of fibrosis. Compared with the control group, the LL-37 model group had more severe skin lesions. HE staining showed that the percentage of inflammatory cells in the skin tissue of mice was significantly up-regulated, the inflammatory cells aggregated and migrated from the subcorium to the epidermis, the number of blood vessels increased, the sebaceous glands hyperplasia, and the tissue structure was destroyed. VG and Masson staining showed that the collagen content in the dermis was significantly increased, and the dermis and epidermis were thickened. Immunofluorescence staining showed that TNF-α was highly expressed in the dermis, CD31 was up-regulated in endothelial cells, α-SMA was highly expressed in myofibroblasts and vascular smooth muscle cells, and vimentin was up-regulated in the epidermis, dermis, hair follicles and stroma of skin. Western blot and real-time fluorescent quantitative PCR showed that the expressions of inflammatory markers (TNF-α, IL-1β, IL-6 and MCP1), angiogenesis markers (CD31, VEGF and VCAM1) and fibrotic markers (COL1 and α-SMA) were significantly up-regulated in the model group. In addition, epithelial-mesenchymal transition indicators (E-Ca, N-Ca, vimentin and MMP9) and TGF-β/Smad signaling pathway related proteins (TGF-βR1, TGF-βR2, TGF-β1 and p-Smad2/3) were activated. Compared with the LL-37 model group, after the intervention of thalidomide, the back skin lesions of mice, the proportion of inflammatory cells in the skin tissue and the degree of infiltration were reduced. The thalidomide treatment group significantly showed less collagen content, less thickness of dermis and epidermis, less positive expressions and weaker intensity of fluorescence-labeled TNF-α, CD31, α-SMA and vimentin. The levels of inflammation, angiogenesis and fibrosis related proteins and mRNA were down-regulated while the expression of E-Ca was up-regulated.

Conclusions LL-37 can induce rose-acnes skin lesions in mice, and prolonging induction time can stimulate the activation and expression of inflammation and fibrosis signals in the skin of rosacea mice. Short-term stimulation of LL-37 induced the activation of inflammatory signals and long-term stimulation of LL-37 promoted the development of fibrosis. Epithelial interstitial transformation signals and TGF-β/Smad signaling pathways were activated in rosacea-like mice. Thalidomide could attenuate LL-37-induced rosacea-like skin lesions, inhibit inflammation, reduce vascular hyperplasia and alleviate skin fibrosis by regulating epithelial-mesenchymal transition and TGF-β/Smad signaling pathways.

PO-086

Cryptococcosis in southern China: Insights from a Six-Year Retrospective Study in Eastern Guangdong

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Background Cryptococcosis is a fatal infection that can affect both immunocompetent and immunocompromised patients, and it is little understood in China's various regions. This research aimed to look at the epidemiology, risk factors, and antifungal susceptibility pattern of Cryptococcus neoformans in eastern Guangdong, China.

Methods This retrospective study was conducted at Meizhou People's Hospital located in Meizhou city, eastern Guangdong, south China and included all identified cryptococcal disease cases from January 2016 to December 2022. The study received ethical approval from the hospital, which adhered to the standards of the Helsinki Declaration (Letter number: 2021-C-106). The diagnosis of cryptococcal disease was based on World Health Organization guidelines 1. Hospital electronic health records were used to collect patient demographics and clinical data, including age, gender, specimen source, department location, baseline, clinical features of cryptococcal disease, and antifungal drug susceptibility test results.

This study classified cryptococcal cases based on specimen source and showed clinical features. Cryptococcal meningitis has been identified in cases where Cryptococcus neoformans has been

isolated from cerebrospinal fluid (CSF) samples, and patients have experienced symptoms such as headache, fever, stiff neck, nausea, vomiting, photophobia, and confusion. The cases where C. neoformans were cultured from respiratory specimens or patients who developed symptoms such as cough, shortness of breath, fever, chest pain, and sputum production were classified as cryptococcal pneumonia. Finally, cryptococcal fungemia was classified for cases where C. neoformans was isolated from the blood, and the patients experienced symptoms such as fever, chills, headache, fatigue, and an altered mental state and did not show any symptoms of meningitis and pneumonia 2.

According to routine laboratory protocols, the samples were first cultured on Sabouraud Dextrose Agar or Brain Heart Agar and incubated overnight at 35 °C to identify the species of each patient sample. A single mucoid creamy colony was picked from each plate and viewed under a microscope at 400X magnification. MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) was used to confirm species according to the manufacturer's instructions. The identification process was performed using the Bruker library program Spectra (version 4.0.0.1, which contained 5627 entries) preinstalled on Bruker Biotyper (version 3.1; Bruker.1). The manufacturer's recommended rating standards were used to determine the level of identification: Rating 2.000 or more indicated species-level identification, a score between 1,700 and 1,999 indicated genus-level identification, and a score below 1,700 indicated that the species could not be identified.

Antifungal susceptibility testing (AST) was performed using the ATB fungal 3 kit (bioMérieux SA, France) according to the manufacturer's instructions. The tested antifungals drugs were fluconazole, itraconazole, voriconazole, 5-flucytosine, and amphotericin B. Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used as quality control strains to ensure the accuracy of the results. The results were interpreted as wild type (WT) or non-wild type (NWT) based on the epidemiological cut-off values set by clinical and laboratory standards institutes guidelines for cryptococcus species 3.

Patient demographics and clinical data were obtained from electronic medical records of the hospital surveillance system and documented in an Excel spreadsheet (2016). Qualitative data were presented as absolute numbers and relative percentages, while quantitative data were expressed as median and interquartile ranges. The characteristics of the three types of cryptococcal infections (meningitis, mycosis, and pneumonia) were analyzed using the chi-square test for categorical variables and ANOVA for continuous variables, with p-values less than 0.05 being considered statistically significant. Data analysis and visualization were done with GraphPad Prism v.8.0.2.

Results A total of 170 cryptococcal infections caused by C. neoformans were reported in the six-year study duration. A high number of cases were reported in the year 2021 (n = 56, 32.94%), followed by 2018 (n = 40, 23.53%) and 2020 (n = 17.65%), while only 7 (4.17%) cases were reported in 2016. Fluctuations in the number of cases from year to year were reported; however, an 8-fold increase occurred from 2016 to 2021. Regarding the hospital's different departments, a large number of cases were reported from ICU (n = 67, 39.41%), followed by neurology (n = 43, 25.29%), while only 3 (1.76%) cases were reported from pediatrics and only one (0.59%) from surgery department. A total of 78 (45.88%) species were isolated from CSF, 50 (30%) from blood, and only one (0.59%) from the pus sample.

Out of 170 cases, the total number of male cases was comparatively higher than female cases at the ratio of 121: 49. Most of the cases occurred in older populations; the median age of the study population was 58 at the range of 5 years to 91 years. Among different age groups, no cases in neonatal and infants were detected, while only 9 cases (5.29%) were detected in children (ages: 2-17). The highest cases (n = 49, 28.82%) were detected in the senior age group of 61 to 70 years. Based on sample sources and clinical manifestations, three types of cryptococcal infection were identified in the study population. Among these, the highest number of cases were reported for cryptococcal meningitis (n = 78, 45.88%), followed by cryptococcal fungemia (n = 50, 29.41%) and then cryptococcal pneumonia (n = 42, 24.7%). The proportion of male cases was higher in all three cryptococcal infection types than in females. According to the X2 test comparison, there was no statistically significant difference in the distribution of the three infections by gender ($p > 0.05$). For cryptococcal pneumonia, a high number of cases were reported in the age group 51 to 60 (n = 15/42, 35.71%, followed by the age group 41 to 50 (n = 11/42, 26.19%). While for cryptococcal

meningitis and fungemia, a high number of cases were reported in the age group 51 to 60, which are (n = 25/78 32.05%) and (15/50, 30%), respectively. The statistically significant difference in the distribution of infections for the age groups (51 to 60) and (71 to 80) was reported in the current study ($p < 0.05$).

Only 60 (35.29%) cases were reported having underlying diseases among the total cryptococcal cases.. Of these, 12 (7.05%) patients had autoimmune disorders, of which 8 (4.7%) had systemic lupus erythematosus, and 4 (2.35%) had rheumatoid arthritis. Similarly, chronic renal failure was reported in 11 (6.47%) cases, followed by 8 (4.7%) cases each for cancer and diabetes and 7 (4.11%) cases for liver cirrhosis. According to the X2 test comparing underlying status distribution in three infection types, a statistically significant difference was reported for chronic renal failure and anemia ($p<0.05$). Chronic renal failure was present in 10% of the patients with cryptococcal fungemia and 9.52% with cryptococcal pneumonia. Similarly, the results showed that anemia was more common in patients with cryptococcal fungemia (6%) than in patients with cryptococcal meningitis (0%) or pneumonia (0%). However, the distribution of some underlying conditions, such as autoimmune disorders, malignancy, diabetes, liver cirrhosis, cardiac diseases, and benign prostatic hyperplasia was not statistically significant in three cryptococcal infection types ($p > 0.05$).

Among the clinical manifestations, the fever was found in high proportion among three types of cryptococcal infections (64 – 70%). However, some other manifestations were associated with specific infection types, like headache (n = 74, 94.87%), neck stiffness (n = 40, 51.28%), and altered mental status (n = 23, 29.48%) were found in high proportion in cryptococcal meningitis. Similarly, fatigue (n = 22, 44%) was associated with cryptococcal fungemia, while cough (n = 36, 85.71%) and shortness of breath (n = 23, 54.76%) were found in high numbers in cryptococcal pneumonia . The X2 test comparing the distribution of clinical manifestations in three different infection types revealed that, except fever, all others were statistically different ($p < 0.05$).

Among the tested antifungal agents, a high number of NWT isolates were reported against amphotericin B (n = 13/145, 8.96%), followed by itraconazole (n = 7/136, 5.15%) and voriconazole (n = 4/158, 2.53%), while only 2 (4.11%) isolates showed resistant against fluconazole (Figure 3). In the overall tested isolates, only six (3.79%) isolates were MDR and showed resistance to two or more than two antifungal agents.

Furthermore, we analyze the antifungal susceptibilities in different cryptococcal infection types (Table 2). The results showed that four of the six MDR isolates were from cryptococcal fungemia. The X2 statistic for MDR was 3.912, indicating that there may be some correlation between cryptococcal fungemia and the emergence of MDR isolates; however, the p-value of 0.1414 indicates that this association is not statistically significant. Moreover, the findings demonstrated that resistance to amphotericin B was associated with cryptococcal fungemia at a considerably more significant proportion (22.22%) than the other types of infection (p-value = 0.0009). Similarly, resistance to 5-flucytosine occurred at a greater rate in cryptococcal pneumonia infection (12.5%), but the association was only marginally significant (p-value = 0.041).

Conclusion The study provides important insights into the distribution and clinical characteristics of Cryptococcus species infections over six years. The results indicate that C. neoformans was the predominant species causing cryptococcal infections, while meningitis was found in high proportion, followed by fungemia and pneumonia. A total of 8-fold increase in cryptococcosis occurred during the study duration. A high number of cases were reported in the male population and senior elderly age group, indicating their immunocompromised status and vulnerability to get infections. Underlying diseases were present in a minority of cases, with autoimmune disorders and chronic renal failure being the most common. A total of 6 MDR isolates were reported, with a high proportion of NWT isolates against amphotericin B. overall, the isolated recovered from fungemia cases were more resistant than meningitis and pneumonia. The study highlights the need for continued surveillance and management of cryptococcal infections, especially in high-risk populations.

PO-087

A case of Trichosporon Asahii disease misdiagnosed as squamous cell carcinoma of skin

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1.Introduction

A case of Trichosporon Asahii disease misdiagnosed as squamous cell carcinoma of skin was reported.The patient was a 54-year-old woman. The skin on her left wrist was accidentally stabbed by sawdust 1 year ago and was not treated. Six months ago, dark red erythematous nodules appeared on the left wrist where the sawdust punctured, accompanied by desquamation, occasional pus discharge, and tenderness. She went to a local hospital for histopathological examination of the skin lesions: the stratum corneum was obviously thickened, and keratinized beads were formed under the skin, which tended to be well-differentiated squamous cell carcinoma. Diagnosed as “Squamous Cell Carcinoma of the Skin”and given “Total Glucosides of White Paeony Capsules 、Mannatide Capsules、Fusidic Acid Cream ”for treatment.Then she was referred to a higher-level hospital, and histopathological examination of the left wrist skin lesion was performed again: obvious chronic active inflammation with many multinucleated giant cell reactions was seen in the superficial dermis, and fibrous tissue hyperplasia in the deep dermis with collagenization. Skin tissue Mycobacterium tuberculosis qPCR (Real-time fluorescent quantitative polynucleotide chain reaction) showed that no exact nucleic acid fragment of Mycobacterium tuberculosis was detected. Her disease was not diagnosed, so she came to our hospital for outpatient treatment.

Physical examination: General condition is good, all system examination is normal. Dermatology (Fig. 1a) : Dark red erythema nodules (about 2cm*1cm) were observed on the dorsal side of the left wrist, covered with scales, with pressure pain, without blisters, pustules and vesicular exudation.Laboratory examination in our hospital: Blood routine showed eosinophil 0.58*109(normal range 0.02-0.52 *109), liver and kidney function test, syphilis serological test, human immunodeficiency virus antibody, hepatitis B virus markers, immunoglobulin were not abnormal. Reflection confocal microscopy showed hyperkeratosis, epidermal thickening, clear boundary of the true epidermis, small number of dendritic cells and irregular hyperrefractive substances in the basal layer, vascular dilatation in the superficial dermis, and no obvious tumor cell nests. Considering foreign body granuloma? Infectious granuloma? Pathological examination of skin lesions (Fig 2) : mild epidermal atypical hyperplasia, dermal fibroblast hyperplasia and scar formation, mixed inflammatory infiltration around vessels and appendages in the whole dermis, and granulomatous reaction in the superficial medium. Special staining: fungal glycogen staining negative, antacid staining negative, silver hexamine staining negative. Fungal culture: negative. Metagenomic test report on DNA-Pathogenic Microorganism Macrogenomic Detection Report (Figure 3) : Trichospora Asahii was detected (sequence number: 36). According to the patient's clinical history, skin lesions, histopathology, and DNA-Pathogenic Microorganism Macrogenomic Detection Report , the patient was diagnosed with trichosporidiosis asahii skin disease. Treatment: Oral itraconazole capsules,100mg/ time, twice a day, Take it with milk; Compound glycyrrhizin tablets,75mg/ time, 3 times a day; Local topical Triamcinolone Acetonide and Econazole Nitrate Cream and Naftifine Hydrochloride and Ketoconazole Cream were wraped up alternately for 30 minutes, once a night; Apply hot compresses twice a day at night for thirty minutes each time;The patient was instructed to follow up once a month, and the routine blood and liver function were checked on an empty stomach before the follow-up. After 3 months of treatment, the lesion area was smaller and flatter than before (Fig1b), with no new lesions, relief of pressure pain and no pruritus,Now continue to treat follow-up.

2.Discussion

Trichosprosis is a basidiomycetes yeast-like deformable organism that is widely distributed in nature. It is the second most commonly isolated yeast species after Candida and can be found in soil, wood decomposes, rivers, lakes, seawater, gudroppings, bats, pigeons, and cattle. It can also

be found in the gastrointestinal tract, respiratory tract, skin and vagina of humans and can cause deep skin,mucosa-associated or superficial infections. Under normal circumstances, Trichospora does not cause disease, but it can cause disease when the body's immunity is low, such as malignant tumor, leukemia, organ transplantation, heavy use of glucocorticoid or immunosuppressant, so trichospora is an opportunistic pathogenic fungus. Trichomormycosis is usually an insidious infectious disease that can be missed or misdiagnosed due to a lack of knowledge and familiarity with clinical diagnosis, especially in developing countries.

Trichosporon asahii is a type of Trichosporon asahii that is most relevant to human pathogenicity. Its skin infection is prone to occur in the forearm, buttocks, face and other parts, and the rash is manifested as erythema, papules, knots Nodules and purpura-like lesions, necrosis, ulcers, scabs, etc. can occur, and it is also the most common pathogen that causes disseminated and deep trichosporidiosis. The number of T. asahii infections has gradually increased over the past few decades. Its pathogenesis may be associated with low or defective immunity, the ability of T.asahii to form biofilms, T.asahii morphological transformation and immune escape, glucuronoxylomannan produced by Trichosporum and Cryptococcus, and Trichosporum The ability of sporozoites to secrete various extracellular enzymes is related. T. asahii is isolated from various clinical specimens such as blood, urine, and sputum. Fungal culture and detection of cell wall polysaccharides were used to diagnose it. However, with the development and application of molecular biology and genetic testing technology, molecular diagnosis and genetic testing have become more important and more accurate diagnostic methods. In terms of treatment, T. asahii is now resistant to many conventional antifungal drugs. Li H et al. investigated 140 cases of T. asahii infection reported worldwide in the past 23 years. The results show that voriconazole, fluconazole, itraconazole and other triazole antifungal drugs are the most effective in the treatment of T. asahii, and the drug susceptibility statistics of in vitro drug sensitivity test show that voriconazole is the first choice for treatment, followed by fluconazole azoles, amphotericin B and itraconazole]. It should be pointed out that although early treatment of T. asahii infection with antifungal drugs is effective, it is still a fatal infection with a mortality rate of up to 80%. The problem is that the disease is usually not diagnosed at an early stage. Therefore, early diagnosis and appropriate empiric treatment are very important to reduce mortality.

In this case, fungal culture, fungal glycogen staining and hexamine silver staining of the skin lesion tissue were negative, which may be related to the patient’s early use of broad-spectrum antibacterial drugs, and fungal culture or staining alone may not be able to detect fungi from it, and the sensitivity of fungal culture is low And it requires high technical experience of operators. This greatly increased the difficulty of disease diagnosis. Therefore, we performed DNA-pathogenic microorganism macrogene detection on the patient's skin lesions, and combined with the patient's history of wood dust stab wounds and clinical manifestations, a diagnosis of cutaneous trichosporidium asahii was diagnosed. The patient had no systemic chronic underlying diseases and immune dysfunction, and many hospitals could not make a definite diagnosis. The special staining of the skin lesion tissue and fungal culture were all negative, which is enough to show that the bacterium is occult. If clinicians lack knowledge of the bacteria, it is easy to miss diagnosis and misdiagnose. We need to improve the understanding of rare diseases. While asking the patient's medical history in detail, we should improve the necessary laboratory tests and histopathology of skin lesions. If possible, we can do DNA-pathogenic microorganism macrogene detection, so as to control the disease as soon as possible to avoid spread. Sexual infection leads to difficult treatment and poor prognosis.

PO-088

m6A RNAs facilitates the Pathophysiology of obesity-associated Acanthosis Nigricans

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Aims Acanthosis nigricans (AN) is a skin disease characterized by hyperkeratosis, pigmentation, papilloma-like hyperplasia, and a velvet-like rash. It occurs in folds of the skin on the neck, armpits, external genitalia, and face. It is usually associated with obesity, glucose and lipid metabolic disorders, sex hormone disorders, and hyperinsulinemia, and seriously affects the physical and mental health and quality of life of patients. Currently, AN is considered to be a specific epidermal marker of metabolic disorders such as insulin resistance and early diabetes. It has been shown that hyperinsulinemia, sustained low levels of inflammatory factors, and abnormally secreted adipocytokines can promote the proliferation of skin keratinocytes (KCs) and the activation of melanocytes (MCs) in obese patients, leading to the development of AN. However, the pathogenic mechanisms of AN remain unclear, and there is no effective treatment. Recent in-depth epigenetic studies have shown that environmental factors can alter the selective expression of targeted genes through epigenetic regulatory mechanisms. Various chemical modifications of RNAs, through epigenetic regulation, execute crucial functions in the regulation of many biological processes. For example, N6-methyladenine (m6A) is the most abundant internal dynamic and reversible chemical modification of RNAs in eukaryotic cells; it is formed by the transfer of a methyl group to the sixth nitrogen atom of adenine using S-adenosylmethionine (SAM) as a methyl donor. M6A has received increasing attention because it affects multiple aspects of RNA metabolism, from RNA processing and nuclear export to RNA translation and decay. It has been shown to be involved in regulating various pathophysiological processes, such as cell cycles, cell differentiation, DNA damage, and embryogenesis. The dynamic balance of m6A in the nucleus is maintained by methyltransferases that store m6A on mRNAs and demethylases that remove m6A from mRNAs
There is no literature on the role of m6A modification in the development of obesity--associated AN. We aimed to investigate whether m6A modification plays a role in the pathological development of obesity--associated AN.
Methods m6A ArrayStar apparent transcriptome sequencing was performed in lesional skin and normal human skin tissues collected from patients with AN; m6A modification-related enzymes and potential molecular targets involved in the development of the disease were detected by reverse transcription quantitative real-time PCR (RT-qPCR), Western blot, immunohistochemistry (IHC) and methylated RNA immunoprecipitation (Me-RIP); the potential mechanism was verified at the cellular level by adenovirus overexpression and siRNA knockdown expression-related genes.
Results 1) Analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Gene Ontology (GO) knowledgebase showed that hormone, pigmentation, and inflammation-related pathways were among the top dysregulated pathways . Wayne analysis demonstrated that ADRA2A, fibroblast growth factor receptor 1 (FGFR1), secreted frizzled-related protein 1 (SFRP1), INHBA, platelet-derived growth factor receptor beta (PDGFRB), myocyte enhancer factor 2C (MEF2C), and endothelin 3 (EDN3) were the DE candidates associated with the inflammation, hormone metabolism regulation, and pigment signaling pathways in AN lesions.
2) The mRNA and protein levels of METTL3 and METTL14, two predominant enzymes accounting for m6A modification, were significantly upregulated in AN lesions as compared to normal skin. METTL3 and METTL14 were present in epidermal keratinocytes in AN lesions but not in normal skin. The mRNA levels of other m6A modification-related enzymes did not show significant changes (Fig. $p > 0.05$). We also confirmed that ADRA2A and INHBA showed increased levels of mRNA, m6A modified mRNA, and proteins in AN lesions as compared with normal skin. Real-time RT-PCR assays indicated that the mRNA levels of other DE candidates such as FGFR1, SFRP1, PDGFRB, MEF2C, and EDN3 were not significantly different in AN lesions as compared with normal skin ($p > 0.05$). A correlation analysis of sequencing reads showed that there were significant positive ; there were significant positive correlations between METTL3 levels and INHBA.

3) Knockdown of METTL3 or METTL14 resulted in significant reductions in mRNA, m6A mRNA, and protein levels of ADRA2A and INHBA We confirmed that a series of biomarkers of proliferation and differentiation (keratin 14, cyclin E1, cyclin D1, and cyclin B1), apoptosis (poly (ADP-ribose) polymerase(PARP), and pigmentation (tyrosinase Related Protein 1(TYRP1) and endothelin 1) were significantly downregulated upon knockdown of METTL3 or METTL14 Cleaved- and pro-caspase-3 were significantly upregulated. overexpression of METTL3 or METTL14 resulted in increases in levels of mRNA, m6A mRNA, and protein levels of ADAR2A and INHBA, biomarkers of proliferation and differentiation (keratin 14, cyclin E1, cyclin D1, and cyclin B1), apoptosis (PARP), and pigmentation (TYRP1 and endothelin 1) , as well as suppression of cleaved- and pro-caspase-3 and apoptotic pathways . We confirmed that tyrosinase activity, melanin content, and proliferation were significantly suppressed or increased when METTL3 or METTL14 were knocked down or overexpressed in a co-culture system of MCs and KCs.
Conclusion METTL3- and METTL14-mediated m6A modification upregulate the expression of ADRA2A and INHBA, contributing to the pathophysiology of obesity-associated AN.This lays a new foundation for subsequent in-depth mechanistic studies and is expected to provide new strategies for the prevention and treatment of AN.

PO-089

面部 **Merkel** 细胞癌 **1** 例及文献复习

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现病史

患者 2 个月前无明显诱因面部出现一肿物，呈进行性增长，无破溃，无疼痛。自发病以来，无发热及其他不适，睡眠饮食可，大小便正常。

既往史

既往体健，无手术及外伤史，无药物过敏史，家族中无同类病患者。

查体

系统检查未见明显异常。

皮肤科情况：面部下眼睑内侧可见一 1.5×1.5×0.5cm 大小的粉红色肿物，边界清，无触痛（图 1），浅表淋巴结未触及肿大。

图 1

入院后辅助检查

血尿常规未见异常

乙肝 S 抗体、e 抗体阳性，甲丙肝抗体阴性。

心电图：左前分支阻滞

其余检查无明显异常。

治疗经过

在局部麻醉下行面部肿物切除术，手术过程顺利，术后第 9 天间断拆除切口缝线。患者拒绝进一步检查及治疗。

病 理

HE 染色光镜：真皮内由结节和弥漫片状嗜碱性肿瘤细胞组成，向真皮深部扩展，不侵犯表皮。瘤细胞从圆性到不规则性，排列较紧密，泡状核，核仁不明显。可见核折叠现象。（图 2、3、4、5）

免疫组化：AE1/AE3(+),Syn(+),S100(-),P63(-),CD56(+),Ki67(阳性细胞数 70%),CgA(+),CK20(+),CK7(-),TTF-1(-)。

诊断：默克尔细胞癌(MCC)

鉴别诊断：

1、 转移性小细胞癌：光镜中可见菊形团样结构,肿瘤细胞较小,胞核染色较深；免疫组化 CgA、Syn 等神经内分泌标记两者均呈阳性,但 CK20 染色 MCC 阳性而转移性小细胞癌呈阴性；甲状腺转录酶因子(TTF-1)呈阳性而 MCC 呈阴性。

2、 皮肤原发性鳞癌(SCC)：SCC 可以与 MCC 并发,但 MCC 预后较差。SCC 易发生溃疡、坏死、出血,病变可呈菜花状,伴恶臭。光镜中鳞癌细胞较大,胞浆较多,胞核大小不一,异型性明显。 免疫组化鳞癌不表达 NF,NSE 和 Syn 等神经内分泌标记。

3、 无色素性恶性黑色素瘤：恶性黑色素瘤常累及表皮,肿瘤细胞可以表现为均匀一致的小圆细胞或淋巴瘤样细胞免疫组化恶性黑色素瘤 HMB45 和 S-100 等阳性。MCC 表达 CK20 和多种神经内分泌标志物 。

讨论：Merkel 细胞癌为罕见高度恶性的原发皮肤神经内分泌癌，起源于皮肤基底层和毛囊的 Merkel 细胞。好发年龄：中位年龄 64～68 岁,最小 7 岁,最大 97 岁, 50 岁以下罕见, 男：女约 1～1.5：1。多发于阳光照射部位（面部、颈部）。一些文献报道 MCC 与治疗后的免疫抑制关系密切。病理表现为：瘤细胞由单一细胞组成，核浆比例倒置,可见突出的核、核仁、核分裂及核折叠。免疫组化：CK、CgA 常阳性，S-100 常阴性，CK20(恒定阳性,且为胞质中逗点状阳性颗粒)对确诊 MCC 起重要作用。目前主要采用局部扩大切除术加辅助性放疗及化疗。

PO-090

A prospective, split-face study of microneedle radiofrequency versus fractional CO2 laser in the treatment of facial atrophic acne scars

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Background Acne vulgaris is the most common chronic inflammatory disease of the follicular sebaceous glands. Due to the inflammatory damage to the follicular sebaceous glands and surrounding tissues, acne often results in scarring, with atrophic acne scarring the most common, which brings negative affects to the psychological health of patients. There are several treatments available to improve acne scarring, among which the fractional CO2 laser is more effective, but there is often redness, pain, oozing, and a higher risk of hyperpigmentation and a longer recovery process after treatment. Microneedle radiofrequency (MRF) is a facial rejuvenation treatment developed in recent years, which causes less epidermal damage and quicker repair; no obvious crusting, no pigmentation, and low incidence of adverse reactions after the procedure. Studies have shown that it can be used in the treatment of acne scarring, and has achieved certain efficacy. However, there are few reports on the efficacy of MRF in the treatment of atrophic acne scars, and the efficacy varies as to whether it is superior to fractional CO2 laser.

Objective To compare the efficacy and safety of invasive microneedle radiofrequency versus ultrapulsed fractional CO2 laser for the treatment of facial atrophic acne scars.

Methods: This randomized, single-blind, single-center, parallel-arm study of 26 patients with facial atrophic acne scars was conducted at Zhongda Hospital Southeast University from January 2021 to January 2022. Every patient randomly received the treatment with invasive MRF on one half of the face and ultrapulsed fractional CO2 laser on the other facial side once every 2 months for 2 sessions. At baseline and at 3 d, 7 d, 1 month and 2 months of the study Standardized photographs were taken on both sides of the face, which were taken under the same background. At baseline and 2 months after all treatments were completed, two treatment-blinded dermatologists performed in-person evaluations with patients' facial imaging data, including échelle d′évaluation clinique des cicatrices d′ acne (ECCA)scale, and total number and classification of acne scars. Adverse reactions were recorded after treatment, and the degree of pain and satisfaction was evaluated by the patients themselves.

Results A total of 26 patients were ultimately analyzed(14 male, 12female). The difference in ECCA scores between the MRF side and the fractional CO2 laser side at baseline was not statistically significant ($Z = 0.565$, $P = 0.572$). After 2 sessions of treatments, the ECCA scores on both the MRF side and the fractional CO2 laser side decreased compared with the baseline, and the differences were statistically significant (both $P < 0.05$). There was no statistically significant difference in ECCA scores between the two sides after 2 sessions of treatments ($Z = 0.573$, $P = 0.591$).

For V-shaped scars, the overall improvement rate was (33.47±24.25)% after microneedle radiofrequency treatment and (21.58±19.32)% after fractional CO2 laser treatment, with a statistically significant difference ($P<0.05$). For U-shaped scars, the overall improvement rate was (63.52±38.46) % after microneedle radiofrequency treatment and (73.05±30.88) % after fractional CO2 laser treatment, with a statistically significant difference ($P > 0.05$). For M-shaped scars, the overall improvement rate was (30.51±22.91) % after microneedle radiofrequency treatment and (17.10±20.32) % after fractional CO2 laser treatment, with a statistically significant difference ($P<0.05$).

The results of patient self-assessment of improvement showed that after 2 treatments, 1 case on the microneedle radiofrequency side improved significantly, 4 cases improved significantly, 18 cases improved moderately, and 3 cases did not improve or improved mildly; 1 case on the fractional laser side improved significantly, 5 cases improved significantly, 14 cases improved moderately, and 6 cases did not improve or improved mildly. The mean VAS value was higher on the microneedle RF side (5.89± 1.82) than on the fractional CO2 laser side (4.28± 1.45), and the difference was statistically significant ($t=7.512$, $P< 0.01$). The side treated with fractional CO2 laser exhibited mild to moderate erythema, mild itching, edema, flaking, mild acne-like eruptions, and herpes, which usually took 1 ~ 4 weeks to subside; crusting began 2 ~ 3 d after treatment and gradually fell off in 5 ~ 10 d; 2 patients (7.7%) showed post-inflammatory hyperpigmentation that lasted 1 ~ 3 months to subside. The side treated with MRF exhibited mild but transient erythema, exudation, and bleeding, which resolved in 1 ~ 3 d. One (3.8%) patient developed a mild fine crust, which fell off within 1 week; one (3.8%) patient developed post-inflammatory hyperpigmentation.

Conclusion MRF and fractional CO2 laser are both effective in improving facial atrophic acne scars, while MRF shows fewer adverse reactions. For V- and M-shaped scars, the MRF treatment was more effective than the fractional CO2 laser treatment. For U-shaped scars, the efficacy of the two treatments was similar. Compared with the fractional CO2 laser , the MRF had more significant pain and fewer adverse effects.

PO-091

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Background Iron plays a key role in human immune responses; however, the influence of iron deficiency on the coronavirus disease 2019 (COVID-19) vaccine effectiveness is unclear.
Aim: To assess the effectiveness of the BNT162b2 messenger RNA COVID-19 vaccine in preventing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and COVID-19-related hospitalization and death in individuals with or without iron deficiency.
Methods This large retrospective, longitudinal cohort study analyzed real-world data from the Maccabi Healthcare Services database (covering 25% of Israeli residents). Eligible adults (aged ≥16 years) received a first BNT162b2 vaccine dose between December 19, 2020, and February 28, 2021, followed by a second dose as per approved vaccine label. Individuals were excluded if they had SARS-CoV-2 infection before vaccination, had hemoglobinopathy, received a cancer diagnosis since January 2020, had been treated with immunosuppressants, or were pregnant at the time of vaccination. Vaccine effectiveness was assessed in terms of incidence rates of SARS-CoV-2 infection confirmed by real-time polymerase chain reaction assay, relative risks of COVID-19-related hospitalization, and mortality in individuals with iron deficiency (ferritin <30 ng/mL or transferrin saturation <20%). The two-dose protection period was Days 7 to 28 after the second vaccination.
Results Data from 184,171 individuals with (mean [standard deviation; SD] age 46.2 [19.6] years; 81.2% female) versus 1,072,019 without (mean [SD] age 46.9 [18.0] years; 46.2% female) known iron deficiency were analyzed. Vaccine effectiveness in the two-dose protection period was 91.9% (95% confidence interval [CI] 83.7-96.0%) and 92.1% (95% CI 84.2-96.1%) for those with versus without iron deficiency (P = 0.96). Of patients with versus without iron deficiency, hospitalizations occurred in 28 and 19 per 100,000 during the reference period (Days 1-7 after the first dose), and in 19 and 7 per 100,000 during the two-dose protection period, respectively. Mortality rates were comparable between study groups: 2.2 per 100,000 (4/181,012) in the population with iron deficiency and 1.8 per 100,000 (19/1,055,298) in those without known iron deficiency.
Conclusions Results suggest that the BNT162b2 COVID-19 vaccine is >90% effective in preventing SARS-CoV-2 infection in the 3 weeks after the second vaccination, irrespective of iron-deficiency status. These findings support the use of the vaccine in populations with iron deficiency.
Conflict of interest statement
Lilac Tene and Gabriel Chodick have received institutional grants from Vifor. Avraham Karasik has received research funding and consulting fees from Vifor. Dora I.A. Pereira, Henrik Schou, and Sandra Waechter are employees of CSL Vifor. Dora I.A. Pereira has also received consultancy fees as part of a scientific advisory board providing advice on oral iron therapy and has a Medical Research Council UK patent (GB24517138) on ligand modified poly oxo-hydroxy metal ion materials, their uses, and the processes for their preparation (including oral iron therapy). Hal Drakesmith has received an institutional grant from Procter and Gamble and has participated in an educational event for this company. He has also received consultancy fees from Keros and speaker fees from Pharmacosmos, has acted as a speaker at a discussion event for Vifor, and has an unpaid leadership role in the European Iron Club. This study was funded by Vifor (International) AG (Glattbrugg, Switzerland).This does not alter our adherence to PLOS ONE policies on sharing data and materials.
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PO-092

The Establishment of the Metastasis and Prognosis Model for Patients with Nodular Melanoma Incorporating Machine Learning Algorithms

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Objective This study aims to screen patients with nodular melanoma (NM) from the Surveillance, Epidemiology and End Result (SEER) database, to investigate the prognostic variables associated with patients with NM, and to combine machine learning (ML) algorithms to predict metastasis in patients with NM, and then to construct a nomogram to predict the prognosis of patients with NM based on the prognostic variables and to validate and evaluate it. A risk stratification system based

on the score of the nomogram was also developed to identify patients with NM in the low and high-risk strata.

Methods Detailed clinical information on 4,727 patients with NM was extracted from the the Surveillance, Epidemiology and End Result (SEER) database for CM cases diagnosed between 2010 and 2015, according to inclusion and exclusion criteria.Variables included age, gender, race, marital, sequence number, laterality, grade, primary site, mode of surgery at the primary site, AJCC 7th edition TNM staging, and radiotherapy information. X-tile software was used to classify patients by optimal cutoff values for age, which was divided into two groups using a cut-off point of 60 years to convert continuous variables into categorical variables. Follow-up variables included survival months, and vital status recodes. The observed endpoint was overall survival (OS). The univariate and multivariate logistic regression analysis was applied to obtain risk factors for metastasis in patients with NM, followed by the use of Multilayer Perceptron (MLP), Adaptive Boosting (AB), Bagging (BAG), logistic regression (LR), Gradient Boosting Machine (GBM) and eXtreme Gradient Boosting (XGB) to construct metastasis risk models. The predictive performance of the six models was compared by ten-fold cross-validation, radar plot, and confusion matrix analysis, and the best-performing ML model was selected for the predictive model construction. The feature importance of the best model was interpreted and a network calculator was built for model visualization.The prognostic variables associated with OS in patients with NM were screened using univariate Cox analysis, and those variables with $P < 0.05$ were further included in the multivariate Cox analysis to obtain independent prognostic variables. Nomogram was constructed based on the obtained independent prognostic variables. The performance of the nomogram was assessed by plotting the receiver operating characteristic (ROC) curves, and a calibration plot, and using decision curve analysis (DCA) to calculate a series of threshold probability net benefits to assess the clinical utility of the nomogram. Kaplan-Meier curves were plotted to visualize the differences in prognostic factors in OS for patients with NM. Using risk scores to classify patients into high and low-risk groups, corresponding risk score maps as well as risk heat maps were drawn.

Results The results of the univariate logistic regression analyses showed that marital (unknown, OR = 0.456, 95%CI = 0.25-0.832, p = 0.01), gender (female, OR = 0.564, 95%CI = 0.414-0.768, p < 0.001), laterality (not a paired site, OR = 1.955, 95%CI = 1.299-2.944, p = 0.001), primary site (skin of upper limb and shoulder, OR = 0.516, 95%CI = 0.349-0.764, p = 0.001; skin of lower limb and hip, OR = 0.599, 95%CI = 0.383-0.935, p = 0.024), surgery (biopsy followed by a gross excision, OR = 0.099, 95%CI = 0.058-0.168, p < 0.001; Wide excision or re-excision of lesion or local amputation with margins more than 1 cm, OR = 0.101, 95%CI = 0.059-0.174, p < 0.001; other/unknown, OR = 0.072, 95%CI = 0.009-0.553, p = 0.011), radiation (yes, OR = 13.592, 95%CI = 9.489-19.471, p < 0.001), chemotherapy (yes, OR = 15.985, 95%CI = 10.745-23.782, p < 0.001), system management (yes, OR = 8.04, 95%CI = 6.053-10.679, p < 0.001), T stage (T4, OR = 2.043, 95%CI = 1.232-3.39, p = 0.006), N stage (N1, OR = 3.581, 95%CI = 2.705-4.741, p < 0.001) were all associated with metastasis in patients with NM. Further multivariate logistic regression analysis showed that marital (unknown, OR = 0.344, 95%CI = 0.179-0.66, p = 0.001), gender (female, OR = 0.591, 95%CI = 0.392-0.893, p = 0.012), primary site (skin of upper limb and shoulder, OR = 0.614, 95%CI = 0.384-0.98, p = 0.041), surgery (biopsy followed by a gross excision, OR = 0.057, 95%CI = 0.03-0.109, p < 0.001; wide excision or re-excision of lesion or local amputation with margins more than 1 cm, OR = 0.051, 95%CI = 0.026-0.1, p < 0.001; other/unknown, OR = 0.035, 95%CI = 0.004-0.336, p = 0.004), radiation (yes, OR = 5.108, 95%CI = 3.244-8.042, p<0.001), chemotherapy (yes, OR = 3.183, 95%CI = 1.863-5.439, p < 0.001), system management (yes, OR = 4.536, 95%CI = 2.999-6.861, p < 0.001), and N stage (N1, OR = 1.571, 95%CI = 1.091-2.262, p = 0.015) were independent risk factors for metastasis in patients with NM. Among the six ML models constructed, MLP was the best model, and the corresponding network calculator was built. The Cox regression model was used to analyze MLP, age, marital, sequence number, laterality, surgery, radiation, chemotherapy, system management, T stage and N stage as independent prognostic factors affecting prognosis. Among them, system management and surgery were protective factors (HR<1) for the prognosis of patients with NM. A nomogram was developed to predict the overall survival (OS) of patients with NM at 1 year, 3 years and 5 years. The validation results showed good discrimination and consistency of the model and high clinical usefulness.

Conclusion This study investigated the risk factors and prognostic factors associated with metastasis in patients with NM, and validated and evaluated a nomogram for predicting OS in patients with NM, combined with ML. The established prediction model can better reflect the prognosis of patients with NM and can differentiate the risk level of patients, which is a useful supplement to the AJCC staging system and provides a reference for clinical stratified individualized treatment and prognosis prediction. A quantitative assessment of the individual prognosis of patients with NM was achieved, to guide clinical decision-making to a certain extent.

PO-093

Research progress in pathogenesis of chronic actinic dermatitis

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Chronic actinic dermatitis (CAD) is common in parts of the light dermatosis chronic dermatitis and eczema, epidemiological studies suggest the disease have certain differences, asians with darker, more vulnerable, and for older men.The incidence of CAD increases year by year, which may be related to sunshine duration and climate change, and presents seasonal changes, especially in summe。

The presence of photosensitive substances is the main cause of the disease.Under ultraviolet A (UVA) (320- 400 nm), ultraviolet B (UVB) (290- 320 nm) irradiation, some normal components in the skin are changed to form neoantigens, which are initially exogenous photochemicals that covalently combine with haptens and carrier proteins to become whole antigens, causing local allergies.After the skin absorbs or contacts foreign photosensitive substances, haptens are formed with certain components in the skin under the action of ultraviolet light, which covalently binds with endogenous proteins to form holoantigen, causing delayed eczema-like hypersensitivity reaction (DTH).According to reports at home and abroad, photosensitive substances commonly used as allergens include some plant components, spices and photosensitive drugs, and common allergens include aromatic compounds, Aromatic compounds, metals, rubber, epoxy resin, p-phenylenediamine, etctudies have shown that in the presence of these substances, the body can produce corresponding antigens and trigger DTH.Strict avoidance of such substances has also been found clinically to alleviate symptoms.

It is believed that the development of CAD is related to T lymphocyte mediated type IV delayed allergy caused by exposure to light and photosensitive substances.Th/Tc cells maintain homeostasis in normal body.Overexpression of Th1 and Tc1 cells can inhibit the expression of Th2 and Tc2 cells in feedback, resulting in the imbalance of Th1/Th2 and Tc1/Tc2 ratio, resulting in abnormal immunity of the body, and mediating DTH to induce CAD.Inf-γ mainly secretes Tc1, mediates cellular immunity, activates macrophages and induces DTH. UVA can promote the expression of IL-12, while IL-12 induces the production of INF by static and activated T cell NK cells, thus promoting the occurrence and development of DTH.Lv Jing et al. detected the serum of 228 CAD patients and found that CD3+CD4+, CD3+CD4+/CD3+CD8+ were significantly lower than the normal control group, and CD3+CD8+ was significantly higher than the normal control group, suggesting that there was an imbalance in the serum of CAD patients with Th/Ts ratio, and adjusting the Th/Ts ratio was conducive to the recovery of CAD patients.Th17 cells are mainly secreted by CD4+ helper T cells, and can also be secreted by CD8+ helper T cells, mainly secreting cytokines such as IL-17, IL-22 and INF-γ. Studies showed that serum IL-17 and IL-22 were significantly increased in CAD patients.IL-17 is widely involved in skin inflammation, while IL-22 can lead to thickening of keratinocytes and chronic inflammation.Therefore, the regulation of T lymphocyte subsets in CAD patients is helpful to the recovery of CAD patients.

Cell proliferation is closely related to apoptosis, so the pathogenesis of CAD may be related to abnormal apoptosis.Apoptosis is mainly composed of endogenous and exogenous pathways, represented by Fas mediated apoptosis, which is controlled by the Bcl-2 family.Fas/Fasl are

membrane protein molecules that can induce cell apoptosis, and the combination of Fas and Fasl can induce cell apoptosis where Fas protein is located.Fas and Fasl are less expressed in keratinocytes of healthy skin, while Fas expression is increased in inflammatory and infectious skin diseases .Studies have shown that the expression of FasL is higher than normal in the stratum corneum and granular layer, while the expression difference of Fas is not significant.It is speculated that in the early stage of the disease, due to the high expression of FasL and no significant change in Fas expression, the cells cannot be apoptosis normally, resulting in excessive proliferation of keratinocytes. Bcl-2 and Bcl-x are members of the Bcl-2 protein family, which inhibit apoptosis. In normal keratinocytes, the expression of Bcl-2 was the strongest in the basal layer of epidermal layer. The expression of Bcl-2 in CAD was not significantly different from that of normal control, while the expression of Bel-xl, especially in the upper spinous layer, was significantly different from that of normal control. It is speculated that the overexpression of Bcl-xl inhibits the apoptotic surface of keratinocytes and prolongs the life span of keratinocytes, resulting in excessive proliferation of keratinocytes.
Cell proliferation is closely related to apoptosis, so the pathogenesis of CAD may be related to abnormal apoptosis.Apoptosis is mainly composed of endogenous and exogenous pathways, represented by Fas mediated apoptosis, which is controlled by the Bcl-2 family.Fas/Fasl are membrane protein molecules that can induce cell apoptosis, and the combination of Fas and Fasl can induce cell apoptosis where Fas protein is located.Fas and Fasl are less expressed in keratinocytes of healthy skin, while Fas expression is increased in inflammatory and infectious skin diseases .Studies have shown that the expression of FasL is higher than normal in the stratum corneum and granular layer, while the expression difference of Fas is not significant.It is speculated that in the early stage of the disease, due to the high expression of FasL and no significant change in Fas expression, the cells cannot be apoptosis normally, resulting in excessive proliferation of keratinocytes. Bcl-2 and Bcl-x are members of the Bcl-2 protein family, which inhibit apoptosis. In normal keratinocytes, the expression of Bcl-2 was the strongest in the basal layer of epidermal layer. The expression of Bcl-2 in CAD was not significantly different from that of normal control, while the expression of Bel-xl, especially in the upper spinous layer, was significantly different from that of normal control. It is speculated that the overexpression of Bcl-xl inhibits the apoptotic surface of keratinocytes and prolongs the life span of keratinocytes, resulting in excessive proliferation of keratinocytes.

PO-094

Genotype-phenotype analysis of cases with X-linked ichthyosis

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Objective To analyze the clinical manifestations and genetic variation of 4 patients with X-linked ichthyosis(XLI).
Methods We collected 4 cases with XLI from Chinese Han population, and recorded clinical characteristics and family information, while analyzing Single Nucleotide Variation (SNV), Insertion and Deletion (INDEL) and Copy Number Variation (CNV) by Whole Exome Sequencing.
Results Complete deletion of STS gene was detected in all 4 patients, with one patient carrying an additional nonsense mutation of FLG gene: c.5368C>T (p.Gln1790Ter) and presenting with symptoms similar to epidermolytic ichthyosis.
Conclusion There are diverity among the clinical manifestations of XLI,Whole exome sequencing is an effective method for diagnosing XLI. XLI patients with FLG mutations tend to present more severe clinical manifestations.

BACKGROUND of TEXT

X-linked recessive ichthyosis (OMIM 308100, X-linked ichthyosis, XLI), also known as steroid sulfatase (Steroid sulfatase, STS) deficiency, mainly causes by the loss of catalytic function of STS.

XLI is the second most common form of hereditary ichthyosis, with second prevalence rate to ichthyosis, with a global incidence of 1:1500 to 1:6000 , occurring in men (mostly asymptomatic carriers), and symptoms often start in newborns or shortly after birth. Typical clinical manifestations are dander shedding, dry skin surface, trunk position and polygonal brown scales of limbs, and the condition is often severe in winter and light in summer. Due to similar clinical skin lesions, XLI can be confused with desquamative skin diseases such as, psoriasis, and epidermolyosis, so it is prone to misdiagnosis or missed diagnosis.

In this study, peripheral blood and clinical information of 4 Han XLI patients were collected, and gene mutations were detected by whole exome sequencing, including copy number variation (Copy Number Variation, CNV) analysis and agarose gel electrophoresis were used.

Discussion of TEXT Hereditary ichthyosis is a group of congenital ichthyoses, lesions are not limited to the skin, its inheritance mode includes sex-linked recessive inheritance, autosomal dominant inheritance, autosomal hemidominant inheritance, autosomal recessive inheritance. XLI is a common genetic ichthyosis, in addition to skin scales, can also involve other systems and organs of the body, such as corneal opacity, mental retardation, cryptorchidism and other symptoms, greater harm . The four patients in this study showed only cutaneous symptoms, but showed distinct phenotypes. Among them, patient 3 had mild symptoms, mainly anterior tibial squamous skin lesions, with the rest only desderderoid desquamation. Patient 4 had the highest severity of skin lesions, with dirt skin lesions in the knee and joints, and the whole body was covered with brown polygonal skin lesions. XLI has similar clinical manifestations with other desquamative diseases, and for patients with atypical XLI, clinical misdiagnosis or missed diagnosis, may be diagnosed as ichthidosis vulgaris or keratotic ichthyosis. In particular, the phenotypes of severe ichthyosis vulgaris and minor XLI overlap extensively, and it is difficult to make an accurate diagnosis, based only on the clinical characteristics of the patients. In this study of four patients, two had been diagnosed with other diseases. Therefore, the history of sex-linked recessive ichthyosis is crucial, and genetic testing is indispensable for the diagnosis of suspected cases.

Globally, about 90% of XLI cases are caused by complete deletion of STS gene, and partial deletion and point mutation of STS gene are seen in a few patients. All four patients included in this study had complete loss of STS gene, consistent with previous reports, which suggested that complete deletion of STS gene may also be the main cause of XLI in China. The STS gene, a key gene for circulating cholesterol utilization, contains 10 exons and encodes a multichannel membrane protein localized to the endoplasmic reticulum. STS belongs to the sulfatase family that hydrolyzes 3- β -hydroxysteroid sulfate, acting as metabolic precursors for estrogens, androgens and cholesterol. The integrity of STS function is tightly related to the development and functional maintenance of the skin and its appendages. In addition, patient 4 in this study had a FLG premature termination mutation, c.C5368> T (p.Gln1790Ter). This mutation is a known mutation and [7] has been reported in multiple populations. Both bioinformatics tools SIFT and MutationTaster predicted the mutation effect as "Damage", suggesting a pathogenic mutation, and the Human Mendelian Genetics Online website (OMIM) showed that the mutation was associated with atopic dermatitis and ichthyosis vulgaris. The gnomAD database showed that the minimum allele frequency of c.C5368> T was 0.00004467, suggesting some carrier rate of this mutation in the normal population. The combined effects of STS and FlG mutations may aggravate the skin lesion manifestation , and functionally, the loss of STS catalytic activity leads to reduced cholesterol sulfate hydrolysis, thus accumulating the cuticle and affecting the cuticle lipid component. Early termination mutations in the FLG gene lead to truncation of the encoded filagmerin and incomplete of protein function, which subsequently affects the protein components in the cuticle, changing both lipid and protein components in the cuticle, thus aggravating the damage of the skin barrier. Impaired skin barrier decreases skin defense function and triggers an infection and inflammatory response, which further exacerbating the clinical phenotype of inherited ichthyosis . This study extends the understanding of the genotype and phenotype of XLI ichthyosis, and the relationship between FLG genes and XLI phenotypes needs to be elucidated in further studies.

PO-095

Presence of Merkel Cell Carcinoma, Multiple Bowen's disease, squamous cell carcinoma in a patient with chronic arsenic exposure

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Abstract Arsenic is a well-documented human carcinogen. People exposed to arsenic for a long time are susceptible to developing cutaneous tumors such as Bowen's disease, squamous cell carcinoma, and basal cell carcinoma. MCC is a rare, highly malignant neuroendocrine tumor of the skin with a high invasive rate, rapid disease progression, high recurrence, and high metastasis rate. Its clinical manifestation is a single and painless erythematous nodule on sun-exposed areas of the body such as the head, neck, and limbs, with a common occurrence in older men. In this article, we are reporting on a 38-year-old male who was diagnosed with Merkel cell carcinoma on his left index finger, combined with squamous cell carcinoma on his right hand and left plantar, as well as Bowen disease on his right ankle and thigh. Remarkably, he had a medical history of treating osteomyelitis with arsenic for about one year.

Key words: Merkel cell carcinoma, multiple Bowen's diseases, squamous cell carcinoma, arsenical keratosis, arsenic.

Introduction

Arsenic has been used as medicine to treat complicated diseases since ancient times. Long-term improper use of arsenic agents can lead to skin malignancy. Arsenic is a common cause of multiple Bowen's diseases. First described by Toker in 1972, Merkel cell carcinoma (MCC), a rare, highly malignant cutaneous neuroendocrine carcinoma, particularly affects the sun-exposed skin of older individuals[1]. Substantial biological evidence supports MCV having an etiopathogenic role for the majority of human MCC tumors[2]. In 1991, Huang et al first reported 3 cases of MCC and found that [2] of the [3] patients had a history of long-term arsenic exposure[3]. Cases of arsenic-causing MCC are rare. Herein we describe a case of MCC, multiple Bowen's diseases, squamous cell carcinoma, and arsenical keratosis of a 38-year-old patient with a history of oral arsenic treatment for osteomyelitis for about 1 year.

Case Report

A 38-year-old man presented with a 6-year history of multiple reddish plaques all over his body was found plaques on his left index finger 5 years ago. And his hands and feet became rough and had keratinizing material. Beforehand, he had taken medication containing an arsenic agent for treating osteomyelitis for about 1 year when he was a teenager. He eventually had surgical excision of a granuloma on his left index finger because of an invalid curative effect after repeated debridements, and no pathological examination was performed. Physical examination showed a 4.0*3.0cm granulation protruding from the surface of the metacarpophala

-Angela joint of the index finger（Fig.1），and reddish plaques scattered all over the body (Fig.2). Laboratory testing indicated no abnormalities.

A biopsy was performed (Fig.1) . Tumor cells were immunohistochemically positive for CK20, CgA, SYN, CD56， Desmin， and Ki 67（Li：80%），and negative for WT-1, VIMENTIN which exclude tumors of the lymphatic hematopoietic system and embryonal rhabdomyosarcoma, eventually confirming the diagnosis of MCC.

Biopsy specimen of left axillary lymph node resection has indicated that Lymph nodes (3/9) exist metastatic carcinoma; Combined with history and immunophenotype, it was consistent with metastatic Merkel cell Carcinoma of lymph nodes.

PET/CT suggested soft tissue nodules near the palmaral side of the left index finger and abnormal metabolism（Fig3）. The skin of the left lateral foot near the plantar is slightly thickened locally, and metabolism is increased.

Based on these clinical manifestations, histopathology, and immunohistochemical results, we finally confirmed the diagnosis of MCC. Besides Merkel cell carcinoma of his hand, a biopsy revealed multiple squamous cell carcinoma in the right thenar and left plantar lesions and multiple Bowen's disease in the right ankle and thigh lesions. An examination of the left plantar skin lesion (lateral margin) showed epidermal hyperkeratosis and foot epithelial growth.

Our patient was operated on a surgery composed of partial metacarpal resection (left palmar tumor resection) and radical lymph node resection (axillary lymph node dissection) and was treated with PD1 monoclonal antibody immunotherapy and chemotherapy. Unfortunately, after nine months of his MCC diagnosis and nine cycles of immunotherapy, the patient died of acute liver failure due to metastasis of Merkel cell carcinoma to the liver.

Discussion

Merkel cell carcinoma is an uncommon but highly aggressive cutaneous malignant neoplasm with epithelial and neuroendocrine differentiation, tending to affect the elderly and occurring in sun-exposed areas of the body. According to a population-based study about the epidemiology of Merkel cell carcinoma in New Zealand, rates of occurrence were respectively 48.8% for the head and neck, 16.3% for the upper limb, 7.9% for the trunk, and 17.4% for the lower limb[4]. Published research findings have suggested that the predominant risk factors for MCC include immunosuppression (eg, HIV or organ transplantation), advanced age (with incidence rate below zero in patients under age < 40 years of age and with 90% rate of patients under age > 50 years of age[5], Merkel cell polyomavirus (MCPyV) infection6 and fair-skinned individuals prone to chronic sun-exposure. Merkel cell carcinoma commonly presents as single, painless, firm erythematous nodules or ulcers. Biopsy and immunohistochemical are diagnostic methods of MCC. Negative cytokeratin[7] and thyroid transcription factor 1 (TTF-1) help differentiate skin metastases from primary cutaneous neuroendocrine carcinoma and bronchial small cell lung cancer[7]. A staging system for MCC has been proposed to be divided into stages 0 to IV[8].

Arsenic poisoning is a systemic disease and can be caused by environmental, occupational, and medical exposure[9]. Traditional Chinese medicine sometimes contains heavy metals, especially arsenic[10] which can cause not only cardiovascular abnormalities, diabetes mellitus, hepatoxicity, and death[9] but also malignant diseases such as lung, bladder, and skin cancer including Bowen's disease, arsenical keratosis, SCC, basal cell carcinoma, and less often, Merkel cell carcinoma[11].

Cases of MCC caused by long-term arsenic exposure have also been reported. Yoko Ohnishi et.al first reported an 81-year-old male suffering from chronic arsenic poisoning from the age of 14-55 with Merkel cell carcinoma associated with multiple Bowen's disease in 1997[12]. And D.TSURUTA et.al reported a 72-year-old man who had worked in a factory handling inorganic arsenic from 1951 to 1954 was diagnosed with MCC coexisting with Bowen's disease[13]. Both patients were exposed to arsenic for more than 4 years and developed MCC at an older age. However, four types of skin malignancies caused by long-term arsenic exposure have not been reported, which is what makes our case unique. In our case, the patient was diagnosed with MCC, multiple Bowen's diseases, and squamous cell carcinoma at 38 years old because of taking a medicine containing arsenic about 20 years ago.

Existing studies suggest that arsenic exposure may contribute to skin cancer through several mechanisms. Firstly, chronic low-dose arsenic exposure can upregulate FTO via impairing p62-dependent selective autophagy by suppressing the TNFα/NF-κB signaling pathway and downregulate m(6)A mRNA methylation to induce malignant transformation and tumorigenesis[14]. Recent research has shown that arsenic takes the place of zinc from the zinc finger of ZRANB2, a splicing factor, disrupting its RNA binding with consequences in altered splicing[15] and arsenic can dysregulate the nucleophosmin to lead to carcinogenesis[16].

Arsenic-induced apoptotic resistance and weakened antioxidant response mediated by Nrf-2 may be crucial in arsenic leading to cutaneous cancer[17]. The exact molecular mechanism by which arsenic causes skin cancer remains to be studied. Considering the possibility of arsenic causing malignant tumors, arsenic agents should be avoided in the treatment of diseases. If arsenic agents must be used, the possibility of arsenic leading to cutaneous carcinoma should be realized, and regular follow-up should be conducted to achieve early detection, early diagnosis, and early treatment to avoid the occurrence of death events.

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PO-096

Role of UCHL1 in the pathogenesis of Scleroderma

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Objective To clarify the role of UCHL-1 in scleroderma and

Background Systemic sclerosis (SSC) is an autoimmune disease mainly characterized by progressive fibrosis of the skin and internal organ, including three different subsets: localized scleroderma, limited cutaneous systemic sclerosis, and diffuse cutaneous systemic sclerosis. SSC have a typifical features was fibrotic change result in thickening and hardening of skin. Its etiology remains unclear and there is no effective treatment options available for scleroderma. The Ubiquitin carboxy-terminal hydrolase 1 (UCHL1) is a member of the DUBs family. Recent study (Zheng et

al., 2021) concluded the Ubiquitin carboxy-terminal hydrolase 1 (UCHL1) plays an essential role in the pro-fibrotic procession, but its underlying mechanisms are largely unknown.

Methods This study was approved by Dermatology Hospital, Southern Medical University. All participants provided written informed consent.26 patients with Systemic sclerosis and 25 healthy volunteers were enrolled in this study. We used HE staining 、 MASSON staining and immunofluorescence analysis to analyze the collagen fibril 、 quantify activated a-SMA myofibroblasts and examine the quantity of UCHL-1 and blood vessels in dermis. To determine how UCHL-1 could contribute to scleroderma, we decided to investigate the UCHL-1 in the pathogenesis of scleroderma by using the bleomycin-induced (5 week) dermal fibrosis model in mice. And treated with LDN-57444 that was an inhibitor of UCHL-1 in Bleomycin-induced scleroderma mouse model. Bleomycin-induced dermal fibrosis is a well characterized experimental model used to evaluate the fibrosis process typical for scleroderma. To evaluated the dermal thickness and extracellular matrix deposition by H&E-stained sections and for collagen and collagen deposition by Masson's trichrome stain in bleomycin model of scleroderma mouse. then to contrast dermal thickness、extracellular matrix and collagen in scleroderma mice treated with LDN-57444. The numbers of blood vessels were detected by immunofluorescence. Next, we studied the expression of a-SMA、COL3B、COL1A1proteins by Qpcr and WB. At cellular levels , Primary fibroblasts were stimulated with LDN-57444, to detect the expression of a-SMA、COL3B、COL1A1、Tagln、postn proteins by Qpcr and WB. The independent sample t-test and one-way ANOVA were used for statistical analysis.

Results we to compare the skin with scleroderma and healthy volunteers, Histology analysis showed that the collagen fibril deposition , Immunofluorescence analysis showed that activated a-SMA myofibroblasts has significantly increase and the expression of UCHL-1 has significantly higher in scleroderma skin . And in skin of scleroderma patients has severe vascular lesions and extracellular matrix (ECM) deposition. This prominent expression was confirmed by RT- qPCR and Western Blot. In vivo, Comparing the skin of scleroderma mice and normal mice, Histology analysis showed that have an increase in dermal thickness, the collagen layer was thickened, the collagen fiber bundles were thicker, the subcutaneous fat layer was decreased , Immunofluorescence analysis showed that activated a-SMA myofibroblasts 、 the expression of UCHL-1 has significantly higher and the blood vessels were reduced. In the skin of mice with scleroderma treated with LDN-57444 ,histology analysis showed that the collagen fibers were thinner and sparse， and the thickness of dermis was thinner , Immunofluorescence analysis showed that the expression of UCHL-1 and a-SMA has significant reduction and the blood vessels were increased, The skin overall condition has been greatly improved. We also found, unexpectedly, a significant reduction in pulmonary fibrosis in mice, and there has significantly improved in the survival rate. in vitro, Primary fibroblasts were stimulated with LDN-57444， Immunofluorescence analysis showed that activated a-SMA myofibroblasts 、 the expression of UCHL-1 has significantly decrease. The expression of COL3B、COL1A1、a-SMA、Tagln、FN1 were attenuated by RT- qPCR and Western Blot. The present findings support our hypothesis that UCHL-1 was highly expressed in the pathogenesis of scleroderma, and blocking UCHL-1 pathway in mice improved the phenotype of scleroderma.

Conclusion Currently, is mainly studied in the nervous system, reproductive system, kidney and cardiovascular, and can regulate cell proliferation, differentiation and apoptosis, about the role of UCHL1 in the pathogenesis of scleroderma was unclear. In the light of the present our results, we have demonstrated for that Blocked the UCHL-1 pathway in mouse inhibit skin fibrosis by Promoted angiogenesis and Inhibited of myofibroblast activation. Suggesting a potential promotive role of UCHL-1 in fibrosing diseases, UCHL-1 play an important role in the pathogenesis of scleroderma. However, further studies are needed to understand the detailed mechanisms how UCHL-1 work in the pathogenesis of scleroderma. While there are limitations to the applicability of the current findings to the clinical disease due to potential differences between the pathogenesis of bleomycin

models and actual human diseases. LDN-57444, as a specific inhibitor of UCHL1, has been used to treat a variety of animal and cellular diseases, but there are currently no clinically approved UCHL1 inhibitors. This study is critical in identifying new drug targets for the treatment of scleroderma and fibrosis.

PO-097

A case of junctional epidermolysis bullosa intermediate with collagen XVII deficiency treated with dupilumab

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Junctional epidermolysis bullosa (JEB) is characterized by extreme skin fragility, mechanically induced blistering, and chronic inflammation, in addition to gastrointestinal, renal, and respiratory complication[1-3]. A major hallmark of JEB is the recurrence of subepidermal blisters and mucosa caused by functional loss or complete absence of major structural proteins in the skin [4]. JEB is caused by mutations in the genes encoding components of the dermo-epidermal junction, including type XVII collagen (COL17A1), laminin 332 (LAMA3, LAMB3, and LAMC2), integrin α6β4 (ITGA6 and ITGB4), and integrin α3 (ITGA3). Intermediate JEB caused by mutations in the COL17A1 gene is characterized by blister formation and abnormalities of the hair and teeth [5]. Curative therapies targeting the genetic etiologies of JEB are not standard, and the management of JEB can be challenging [6]. Dupilumab, an anti-IL-4 receptor-alpha antagonist, is effective for moderate and severe atopic dermatitis by inhibiting IL-4 and IL-13 signaling [7]. Dupilumab might have a beneficial effect in dystrophic epidermolysis bullosa [8-14]. Here, we describe an adult patient with recalcitrant JEB intermediate who was successfully treated with dupilumab.

A 65-year-old man presented to the dermatology department of Huashan Hospital with a lifelong history 33 of itching vesicles and bullae in his limbs and trunk. The condition was aggravated in the summer and 34 relieved in the winter. The lesions had become more numerous, and the itching symptoms had become 35 more severe in the past year, resulting in a poor quality of life. His average pruritus numeric rating scale 36 (P-NRS) score in the last 7 days was 7 (maximum=8) and his average visual analog scale in the last 7 days 37 was 7.4 (maximum=9). Physical examination revealed erythematous patches involving the friction areas of 38 the trunk and upper and lower limbs covered by vesicles and bullae, and his toenails were dystrophic. 39 Moreover, the patient suffered from alopecia and tooth loss. A skin biopsy performed on an 40 abdominal lesion showed a focal blister at the dermo-epidermal junction with perivascular mononuclear 41 inflammatory infiltrates and a few eosinophilic infiltrates in the superficial and middle layers of the dermis. 42 A direct immunofluorescence test of the perilesional skin showed negative results. There was an increased blood eosinophil count (2540×106 /L, [normal: 50-350 ×106 43 /L]) and serum IgE level (1495.20 ng/ml, [normal: 44 0-240 ng/ml]). The values of enzyme-linked immunosorbent assay-BP180, BP230, Dsg1, and Dsg3 were 45 all negative, excluding concomitant bullous pemphigoid. 46 47 Exome sequencing of peripheral blood revealed two mutations (c.2039-6G>A and c.C1177T:p. Q393X) in 48 the COL17A1 gene. Mutation c.2039-6G>A was likely pathogenic and Mutation c.C1177T:p. Q393X was 49 pathogenic. The diagnosis of JEB was made based on the presence of typical clinical features and medical 50 history, consistent histopathological findings, negative direct immunofluorescence assessments, and 51 compound heterozygous mutations in COL17A1. The patient responded poorly to therapy with oral 52 antihistamines and topical corticosteroid ointments. Owing to the patient's persistent itching, off-label 53 dupilumab therapy was initiated (600 mg subcutaneous loading dose followed by 300 mg every 2 weeks), 54 and a significant reduction in itching was observed after 12 weeks of treatment. The body surface area 55 (BSA) of the bulla decreased from 50% to 10%. The P-NRS score improved from 8/10 to 2/10. The VAS of 56 itch improved from 9/10 to 1/10. Blood eosinophil count (500×106/L, [normal: 50-350 ×106/L]) and serum 57 IgE level decreased (600.80 ng/ml, [normal: 0-240 ng/ml]). Erythema, crusts, and hyperpigmentation 58 improved leaving atrophic plaques. The patient discontinued

dupilumab after 12 weeks because 59 of the cost, and no relapse occurred six months after the discontinuation. No side effects occurred.
JEB is a rare inherited genetic disorder with few treatment options beyond symptomatic, palliative care. One on-label drug for EB is available in Europe; Oleogel-S10 (birch triterpenes) accelerates wound healing in EB and is well-tolerated [15]. In an analysis of the genotype–phenotype correlation in 441 patients with epidermolysis bullosa from China, only one COL17A1 pathogenic mutation was found in four (four families; 28.6%) cases [16]. A genotypic and phenotypic analysis of 34 cases of inherited junctional epidermolysis bullosa caused by COL17A1 mutations in European and Mediterranean populations identified 39 mutations in COL17A1; 24 mutations were novel[17]. Pruritus occurred in 85% of the EB patients, with substantial differences across the subtypes (EBS 74%, JEB 100%, DEB 93%). Itch, in all its dimensions, was most pronounced in patients with JEB and DEB, and less prominent in patients with EBS [18]. Inflammation is a prominent feature of EB skin. In response to mechanical injury, keratinocytes release various proinflammatory cytokines. These may be released into the systemic circulation and may mediate the recruitment of inflammatory cells and development of T helper (Th)17 and Th2 cells at the injury site[19]. In RDEB, gene expression of the itch-related mediators IL13RA1 and IL4R is dysregulated in comparison with that in aged-matched healthy individuals [20]. Biological agents are gaining usage in EB treatment with some encouraging results. Recently, several cases of DEB pruriginosa were successfully treated with dupilumab [8,9,21-23]. Dupilumab can block the downstream signaling of Th2 cytokines, IL-4 and IL-13 [7]. Immune markers related to general inflammation (MMP12), Th2 (CCL13/CCL17), Th17/Th22 (IL-12B, CXCL1, S100A12). and innate immunity (IL-6, IL-8, IL-17C) decrease after dupilumab administration [24]. Dupilumab might decrease the levels of IL-8 through blocking IL-4 in the reported JEB patient. The blister fluids of JEB patients contain increased levels of MMP-9 and CXCL8/IL-8 and may contribute to blister formation [25]; inhibition of these two factors is a potential mechanism by which dupilumab may improve JEB.

PO-098

Variation of ferroptosis-related markers in HaCaT cell photoaging models induced by UVB

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Objective In clinical settings, photoaging of the skin is becoming increasingly widespread, which has major implications for both the patients' physical and mental health; the pathophysiology of this condition has to be investigated in greater depth. In recent years, researchers have been concentrating their efforts on ferroptosis, a process that can moderate oxidative stress and inflammation. On the other hand, there aren't too many reports out there about how ferroptosis and skin photoaging work. In order to examine the change of ferroptosis-related indicators in the HaCaT cell photoaging model produced by medium-wave ultraviolet (UVB), this research was carried out.
Methods In order to construct the model (the UVB group) for cellular photoaging, HaCaT cells were subjected to UVB treatment. HaCaT cells that had not been subjected to UVB treatment functioned as the normal group. In the UVB + Fer-1 group, HaCaT cells were treated with UVB plus the ferroptosis inhibitor ferrostatin-1. In the UVB + Erastin group, HaCaT cells were co-cultured with the ferroptosis inducer erastin. In order to determine whether or not HaCaT cells had reached senescence, we used a senescence-related β galactosidase (SA-β-gal) staining assay as well as a reactive oxygen species (ROS) detection tool. Both the mitochondrial morphology and lipid reactive oxygen species were examined using transmission electron microscopy. The mitochondrial morphology was observed using the BODIPY581/591 C11 fluorescent probe. The level of glutathione peroxidase 4 protein was evaluated by immunofluorescence semi- quantitative assay. The mRNA expressions of glutathione peroxidase 4 (GPX4) and ferroptosis-suppressor-protein 1 (FSP1) were detected by reverse transcription PCR (RT-RCP).

Results When compared to the normal group, the quantity of ROS in the UVB group was considerably larger (36.99 ± 0.41)% than it was in the normal group (9.00 ± 0.77%), (t = 55.79, P <0.001). It was clear that the positive rate of SA-β-gal was much lower in the normal group (4.34 ± 0.71)% than it was in the UVB group (22.96 ± 6.09)%, (t = 5.26, P <0.01). The number of lipid reactive oxygen species was substantially larger in the UVB group (0.72 ± 0.01) than in the normal group (0.55 ± 0.05), as shown by the BODIPY 581/591 C11 probe staining (t = 5.58, P < 0.01). Using transmission electron microscopy, the researchers were able to demonstrate that the mitochondrial volume of the UVB group was lower, that the membrane density was higher, and that the mitochondrial crest was either less prominent or completely absent. The results of the RT-PCR experiment showed that the level of GPX4 mRNA in HaCaT cells was considerably lower in the UVB group (0.22 ± 0.13) than in the normal group (1.22 ± 0.34) (t = 4.77, P <0.01). It was shown that the mRNA level of FSP1 was lower in the UVB group as well (0.55 ± 0.06 vs. 1.01 ± 0.15), (t=5.58, P <0.01). The immunofluorescence semi-quantitative results suggested that the GPX4 protein level in the UVB group (0.29 ± 0.12) was considerably lowered, when compared with the normal group (1.00 ± 0.18), (t=5.69, P<0.01). The positive rate of SA-β-gal in the UVB + Fer-1 group (10.82 ± 0.68) % was lower than that in the UVB group (22.96 ± 6.09) %, which indicated a significant difference between the two groups (t = 3.43, P <0.05). On the other hand, the positive rate of SA-β-gal in the UVB + Erastin group (64.93 ± 3.18) % was substantially greater than that in the UVB group (22.96 ± 6.09) % (t = 10.59, P <0.001). The level of lipid reactive oxygen species produced by HaCaT cells exposed to UVB and Fer-1 was found to be significantly lower in the UVB + Fer-1 group (0.59 ± 0.04) compared to the UVB group (0.72 ± 0.01) (t = 4.97, P <0.01). On the other hand, the levels of lipid reactive oxygen species in the UVB + Erastin group were significantly higher (0.93 ± 0.04) than those in the UVB group (0.72 ± 0.01) (t = 7.66, P <0.01).
Conclusion In conclusion, the findings of this study demonstrated that in photoaged HaCaT cells, the indicators of promoting ferroptosis were greatly up-regulated, the markers of repression of ferroptosis were down-regulated, and the mitochondria also displayed the characteristics of ferroptosis. Additionally, inducing ferroptosis can hasten the ageing process, and vice versa. This shows that ferroptosis may have a role in promoting photoaging of the skin, and that suppressing ferroptosis may be an effective strategy for preventing premature ageing of the skin in the future.

PO-099

2 型炎症在相关皮肤病研究进展

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近年来，ILC2-DC-Th2细胞轴驱动的2型免疫失衡导致2型炎症备受关注。辅助型T细胞2(T helper 2 cell，Th2)是一种能够分泌 Th2 型细胞因子(如白细胞介素 IL-4、IL-5、IL-9、IL-13 和 IL-31 等)的 T 细胞亚群，属于 CD4+T 细胞。这些细胞因子能够促进 Th2 细胞增殖，并抑制 Th1 细胞，同时辅助 B 细胞活化，发挥体液免疫的作用。研究表明，它们参与多种皮肤病如特应性皮炎、慢性荨麻疹、结节性痒疹、类天疱疮等发病过程。本文重点探讨 2 型炎症在上述 4 种皮肤病的发病机制及其治疗进展。

【Abstract】In recent years, the type 2 immune imbalance driven by the ILC2-DC-Th2 cell axis has attracted much attention. T helper 2 cell (Th2) is a subset of T cells that can secrete Th2 cytokines (such as interleukin-4, IL-5, IL-9, IL-13 and IL-31), belonging to CD4+T cells. These cytokines can promote the proliferation of Th2 cells, inhibit Th1 cells, assist the activation of B cells, and play the role of humoral immunity. Studies have shown that they are involved in the pathogenesis of many skin diseases, such as atopic dermatitis, chronic urticaria, prurigo nodosa,

pemphigoid, etc. This article focuses on the pathogenesis and treatment progress of type 2 inflammation in the above four skin diseases.

T 淋巴细胞是最重要的免疫细胞之一，原始 T 细胞经刺激后，根据 Th 细胞及其各自分泌的细胞因子群将其划分为 Th1、Th2 和 Th3 三种类型。其中 2 型炎症细胞因子包括 IL-4、IL-5、IL-9 和 IL-13、IL-31 等，主要功能为刺激 B 细胞增殖并产生抗体，参与黏膜表面的屏障免疫，在抗寄生虫感染和特应性疾病中发挥重要作用[1]。近年来，2 型炎症在相关皮肤病备受关注，随着研究的进展，发现其相应的通路或者特殊的炎症因子可导致一些皮肤病发病，例如：特应性皮炎、慢性荨麻疹、结节性痒疹、类天疱疮等。大多数相关皮肤病都存在其难治性和复发性，对患者造成严重的经济压力和精神负担。随着 2 型炎症因子相关疾病发病机制的深入性研究，生物靶向药物取得了良好治疗效果。本文对 2 型炎症因子的发病机制及治疗作一综述。

1 2 型炎症与特应性皮炎

特应性皮炎（atopic dermatitis，AD）是一种与遗传过敏因素有关的慢性炎症性皮肤病，表现为瘙痒，多形性皮损并有渗出倾向，常伴发哮喘、过敏性鼻炎。

T 淋巴细胞是最重要的免疫细胞之一，原始 T 细胞经刺激后，根据 Th 细胞及其各自分泌的细胞因子群将其划分为 Th1、Th2 和 Th3 三种类型。其中 2 型炎症细胞因子包括 IL-4、IL-5、IL-9 和 IL-13、IL-31 等，主要功能为刺激 B 细胞增殖并产生抗体，参与黏膜表面的屏障免疫，在抗寄生虫感染和特应性疾病中发挥重要作用[1]。近年来，2 型炎症在相关皮肤病备受关注，随着研究的进展，发现其相应的通路或者特殊的炎症因子可导致一些皮肤病发病，例如：特应性皮炎、慢性荨麻疹、结节性痒疹、类天疱疮等。大多数相关皮肤病都存在其难治性和复发性，对患者造成严重的经济压力和精神负担。随着 2 型炎症因子相关疾病发病机制的深入性研究，生物靶向药物取得了良好治疗效果。本文对 2 型炎症因子的发病机制及治疗作一综述。

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特应性皮炎（atopic dermatitis，AD）是一种与遗传过敏因素有关的慢性炎症性皮肤病，表现为瘙痒，多形性皮损并有渗出倾向，常伴发哮喘、过敏性鼻炎。

1.2 免疫异常

Th2 型炎症是 AD 的基本特征之一，由 Th2 细胞、嗜碱性粒细胞和 2 型固有淋巴样细胞等产生的 IL-4 和 IL-13 是介导 AD 发病的核心驱动因子[5]。IL-4 使 T 细胞分化成 Th2 细胞，并通过 STAT6 和 IL-4Rα 途径削弱 Treg 细胞抑制肥大细胞扩增，使 Treg 细胞转变为 Th2 型 Treg 并产生 IL-13、IL-4 进一步加重 2 型炎症[6]。而 IL-13 对 2 型炎症具有多效性作用，包括与 IL-4 一起降低屏障功能、参与 B 细胞同种型转换，以产生免疫球蛋白 E，组织纤维化和瘙痒。IL-5 是嗜酸性粒细胞的关键调节剂，负责嗜酸性粒细胞的生长，分化，存活和动员[7]。Licona 研究发现，由 T 细胞、肥大细胞等产生的 IL-9 可促进 T 细胞生长、增殖和存活，B 细胞 IgE 生成，肥大细胞增殖和分化，此外，IL-4，IL-13 和 IL-31 参与感觉神经致敏和瘙痒，导致抓挠，进一步加剧炎症和屏障功能障碍[8]。

1.3 瘙痒

2 型炎症因子通过 JAK1 和 IL-4 受体亚单位 α 途径激活人体的瘙痒通路神经元，IL-4、IL-13 剂量越高则相关感觉神经元反应频率也越高。但与 IL-31 不同，大量 IL-4、IL-13 并不造成直接的急性瘙痒，而是提高神经元对各致瘙痒物质的敏感性[9]。Conlan 等人研究发现，当 IL-31 与 IL-4 和 IL-13

相互作用时，IL-31 会促进下游细胞因子的分泌。若 IL-31 受体在 EOS 上被激活时，它会导致多种炎症细胞因子和趋化因子的分泌—包括 IL-6、IL-8、CXCL1 等和几种基质金属蛋白酶，进而促进广泛的炎症和皮肤表面重塑[10]。此外，Sonya 等人发现角质形成细胞中胸腺基质淋巴生成素（thymic stromal lymphopoietin， TSLP）释放也可通过作用于 TRPA1（transient receptor potential A1）阳性感觉神经元继而触发瘙痒[7]。

PO-100

二代测序技术在非遗传性皮肤病领域中的运用

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摘要 二代测序（Next generation sequencing，NGS）是基于 PCR 和基因芯片发展而来的 DNA 测序技术。近年来，NGS 技术的发展为确定皮肤疾病的发病机制、信号通路和生物标记物奠定了广阔的前景。NGS 在核酸序列识别上有巨大优势，不仅能识别正常皮肤表面微生物，还可识别以前难以识别的罕见病原体。目前，NGS 可以直接测序 RNA，这将促进对皮肤疾病发病机制的研究。尽管 NGS 存在一些局限性，包括低效、序列读长短和高成本，但它在有效和全面诊断皮肤疾病方面仍优于传统的诊断方法。本文综述了 NGS 在非遗传性皮肤疾病中的应用进展，旨在探讨非遗传性皮肤疾病的发病机制，提高其诊断水平。

[关键词] NGS；二代测序；非遗传性皮肤疾病
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Application of second generation sequencing technology in the field non hereditary dermatosis

Abstract Next generation sequencing (NGS) is a DNA sequencing technology based on PCR and gene chip. In recent years, the development of NGS technology has laid a broad prospect for determining the pathogenesis, signal pathways and biomarkers of skin diseases. Because of the advantages of NGS nucleic acid sequence recognition, NGS not only recognized several normal skin surface microorganisms, but also found many pathogenic bacteria, fungi, parasites and viruses related to skin diseases, including rare pathogens that were difficult to identify before. At present, NGS can directly sequence RNA, which will promote the research on the occurrence, development and potential mechanism of skin diseases. Although NGS has some limitations, including low efficiency, pollution and high cost, it is still superior to traditional diagnostic methods in terms of effective and comprehensive diagnosis of skin diseases. This article reviews the application of NGS in non hereditary skin diseases, aiming to explore the pathogenesis of non hereditary skin diseases and improve the diagnostic level.
[Key words] NGS; Second generation sequencing; Non hereditary skin diseases
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核酸序列包含了生物遗传信息和进化特征，这对提高疾病的诊断至关重要。1953 年，Watson 和 Crick 发现了 DNA 的反平行双螺旋结构，推动了学者探索核酸序列的进程。1975 年，Sanger 等人

发表了双脱氧 DNA 测序技术测定 DNA 序列的方法，为现代核酸测序技术铺平了道路，其测序长度长，准确性高，但成本高、通量低和耗时长的缺点限制了其进一步大规模应用[1]。

二代测序又称下一代测序，主要通过 DNA 片段化文库构建、文库与载体交联扩增、在载体面上完成边合成边测序反应,获得个体或群体遗传信息[2]。其无需传统的培养技术即可直接对临床样本中的核酸进行测序，因其高通量、更高效、更经济并提高了结果的范围、灵敏性和准确性等优点使基因测序在基础研究与临床诊疗中得到广泛应用，但读长短、碱基错配、无法直接对 RNA 测序等一直是其不可回避的技术短板[3]。

第三代测序技术是以单分子测序技术为基础，采用边合成、边测序而无需 PCR 扩增的情况下对 RNA 进行直接测序，其读长较长，耗时短、拼接成本低且此技术在基因组测序、甲基化研究等方面具有前两代测序无法比拟的优势，但其缺点为单读长的错误率较高[4]。

第四代测序技术以纳米孔作为生物传感器，使离子电流通过纳米孔，捕捉通过的核苷酸序列，从而实现对单分子的实时测序，因其具有高通量、低成本、耗时短和数据分析相对简单的优点，此技术正在飞速发展。但目前仍处于起步阶段，在关键环节及技术方面还有待改善[5]。随着这些测序技术的功能逐渐优化，分子生物学将迎来前所未有的巨变。

NGS 通过获取基因组信息，提高了我们对疾病及其诊断的理解，并被广泛用于一些临床问题的研究。目前，在皮肤领域中，NGS 被广泛运用于遗传性皮肤病，例如，遗传性鱼鳞病、着色性干皮病、结节性硬化症、遗传性大疱性表皮松解症等[6]。然而，NGS 在非遗传性皮肤病的研究也取得了重要成果，二代测序技术通过对临床样本中组织、微生物和宿主核酸的测序分析，可以无偏倚地检测多种致病基因、信号通路、未知病原微生物等，其推动了在非遗传性皮肤病疾病中的诊断、治疗风险评估或临床干预，其推动了在非遗传性皮肤病中的诊断、治疗风险评估或临床干预，同时也加快了在皮肤病领域中的发展进程。因此，本文综述了 NGS 在非遗传性皮肤病的应用进展，并分析了其较传统诊断方法的优势。

PO-101

带状疱疹后遗神经痛中西医治疗研究进展

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带状疱疹后遗神经痛是指在带状疱疹消退后，皮损局部遗留迁延不愈的神经痛，或发展成为顽固性神经痛，可持续数月至数年，顽固难除，是带状疱疹最常见的并发症之一。随着年龄的增长免疫力逐步下降且神经修复能力减退也是导致后遗神经痛多见于老年患者的重要原因。该病疼痛剧烈，持续时间长缠绵难愈。因严重影响患着的正常工作和生活。近年来，随着我国人口的老龄化，带状疱疹和带状疱疹后遗神经痛的发病率显著增加，由于疼痛的剧烈性及顽固性，严重影响患者的生活质量。目前西医尚无理想治疗药物,基本治疗原则为抗病毒、止痛、消炎、保护局部,防止继发感染、缩短病程等。西医治疗是以抗病毒为主，以营养神经、镇痛等疗法为辅，治疗时间长，疗效不确切。中西医对 PHN 的临床研究日益广泛，也取得了一定的进展。中医可采用中药内服、外敷，或针刺、拔罐、穴位注射、埋线等独具特色的外治方法，治疗手段多样，成本低廉，简便易操作，患者易于

接受，在治疗上具有独特的优势。临床均可以收到较好的效，且毒副作用小。针灸治疗带状疮疹有其独特的优势和疗效,通过疏通局部经络、扶正祛邪、平衡阴阳、调理全身的方式提高治疗效果,与中西药物治疗相比,针灸治疗带状疱疹,具有疗效好、起效快、疗程短、后遗神经痛发生率低、消耗资源少等优点,同时针灸治疗成本低,易被广大群众所接受。目前中西医治疗方法甚多，近年出现了很多新型联合治疗方式，都取得了较好的疗效。但是如何缩短治疗时间，这些治疗方法如何结合，干预时机仍是关键的难题。笔者对近年来带状疱疹后遗神经痛的中西医治疗方法进行综述，挖掘能够提高带状疱疹后遗神经痛疗效的切实有效的方案。

带状疱疹后遗神经痛临床十分常见，由于其疼痛剧烈，持续时间长，给患者生活和工作带来很大影响。多年来，各方医家的治疗思路不断拓展，从多角度，多靶点，多学科的交叉融合不断深入。希望将多种方法联合应用，使其优势互补，互相促进，能使带状疱疹后遗神经痛治疗的方案更加进一步优化。

中医对带状疱疹后遗神经痛的诊治通过辨证求因、审因论治累积了丰富的经验，治疗上也取得较好疗效。治疗可采用、经方、验方、自拟方、针灸和内治外治等多种方式，其疗效较好，且毒副作用相对较少，远期复发率低。 在治疗过程中要努力拓展思路，做到多角度窥探甄别，客观认识该病复杂证候，针对患者情况做到区别对待，以获得理想疗效。

PO-102

Supplemental D-Mannose orchestrates skin inflammation environment to improve atopic dermatitis

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Objective As a natural bioactive monosaccharide, D-mannose exists in serum, but also presents in cranberries and citrus peel. Mannose supplementation has become an effective therapy for patients with carbohydrate-deficient glycoprotein syndrome and relapsing urinary tract infection. Apart from the potential anti-cancer, anti-fibrotic and anti-obesity bioactivities, mannose has been reported to activate regulatory T cells, suppress effector T cells and inflammatory macrophages, and upregulate anti[1]-inflammatory gut microbiome, thus alleviating various inflammatory diseases. Atopic dermatitis (AD) is a chronic repetitive inflammatory disease, which greatly dampens the life qualities of patients. Atopic dermatitis is a heterogeneous chronic inflammatory disease triggered by individual genetics and environmental factors, accompa[1]nied with skin barrier disruption and hyperimmune responses. As one of the most common dermatoses in childhood, AD exhibits persistent pruritus, eczema, erythema, dryness and scaling. It is characterized by chronic, itching and recurrent disease process, affecting about 3% to 10% of adults and up to 20% of children worldwide. The goal of this study is to discover the bioactive effect of D-mannose supplementation in the context of atopic dermatitis.

Methods Here, we applied tumor necrosis factor-α (TNF-α) stimulation on keratinocytes while LPS stimulation on macrophages to mimic pro-inflammatory AD condition. Also, we repeatedly used DNCB (2,4-dinitrochlorobenzene) on mice skin and ears to establish mice AD model, and topically applied mannose every day on mice topical skin and ears. CCK-8 assay was used to determine cell viability. The mTOR/NF-κB signaling expression level was determined by western blotting. HaCaT and NHEK cells were treated with Bay11-7082 (NF-κB inhibitor) or MHY1485 (mTOR agonist) or rapamycin (mTOR inhibitor) 2 h ahead of other indicated stimulation. The macrophage polarization was evaluated by flow cytometry as well as immunofluorescence, western blotting and qPCR. The expression levels of inflammatory related molecules and atopic dermatitis related

molecules in keratinocyte cells or mice serum were measured by qPCR and ELISA assay. The inflammatory condition of mice skin and ears were assessed by histochemical staining (hematoxylin and eosin (H&E), toluidine blue staining and eosinophil peroxidase (EPX)). One-way ANOVA followed by Tukey's post-hoc tests was used for multiple group comparisons, while one-way ANOVA was used for two group comparisons.

Results In vitro results showed that supplementation of D-mannose significantly inhibited the production of inflammatory-related cytokines (including TSLP, IL-1β, IL-6, IL-8) in inflammatory keratinocytes, especially in higher concentration. The phosphorylation of p65 and nuclear translocation of p65 in TNF-α stimulated HaCaT and NHEK keratinocytes were greatly increased, while phosphorylation of p65 and IκBα was effectively suppressed by mannose treatment. We further found that D-mannose markedly downregulated mTOR/NF-κB pathway, while its inhibitory effects were completely abolished by the ahead application of mTOR or NF-κB inhibitors (including various inflammatory-related cytokines). Additionally, we found that D-mannose could significantly inhibit macrophage M1 polarization in a dose dependent manner, accompanied with the downregulation of multiple pro-inflammatory cytokines production (including, IL-6, TNF-α, IL-1β, iNOS).
What's more, in vivo results further supported that D-mannose topical supplementation considerably alleviated skin lesion severity and dose-dependently improved mice skin and ear thickness, dermatitis score and scratching behavior induced by DNCB. In addition, the histological analysis indicated that mannose treatment reduced epidermal thickness and infiltration of mast cells and eosinophils. We found that the serum levels of IgE, histamine and IL-4 in mice serum decreased in a dose-dependent manner following mannose treatment compared to the DNCB group. Accordingly, mannose also inhibited the production of a series of inflammatory-related cytokines in mice skin and ears. In addition, the skin barrier markers including filaggrin, occludin, ZO-1, loricrin and involucrin were recovered after the topical supplementation of mannose, especially in the higher concentration. Together, these present results suggest that mannose alleviated atopic dermatitis pathogenesis via regulation of skin inflammatory environment, among which macrophages polarization and inflammatory keratinocytes in particular.
Conclusion In the present study, we are the first to discover the protective role of mannose both in vivo and in vitro during the pathogenesis of atopic dermatitis. We first topically applied mannose in a mouse model of AD induced by DNCB as well as mannose treatment on keratinocytes inflamed by TNF-α and M1-like macrophages polarized by LPS, aiming to elucidate the potential role and underlying mechanisms of mannose in AD in vivo and in vitro. Our results exhibited that supplementation of mannose via skin topical application markedly alleviated atopic dermatitis-like inflammatory injury through suppressing mTOR/ NF-κB signaling in keratinocytes and interrupting M1 polarization in macrophages. Together, study results suggest that mannose is capable of exerting biological function via skin topical application and therapeutic utilization of mannose is a potential drug against atopic dermatitis.

PO-103

Research progress on immune related mechanism and immunotherapy of Kaposi's sarcoma

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Kaposi’s sarcoma is a multicentric tumor associated with immunosuppression, Kaposi sarcoma-associated herpesvirus (KSHV) infection, genetic susceptibility, and other factors. KSHV can act on the host immune system to promote the occurrence and development of Kaposi's sarcoma by regulating pattern recognition receptors, congenital antiviral interferon response, complement system, NK cell activity, T cell and B cell functions, etc. Based on the study of the immune related

mechanism of Kaposi's sarcoma, immunotherapy such as anti-angiogenesis immunomodulators, cytokines and immune checkpoint inhibitors is expected to be a new methodfor the prevention and treatment of Kaposi's sarcoma. This article will focus on the pathogenic mechanism of KSHV acting on the host immune system and the clinical strategy of immunotherapy for Kaposi's sarcoma.

Kaposi's sarcoma is an endothelial cell-derived vascular malignancy that often presents as multiple patches, plaques, or nodules in the skin, but can also involve the mucosa, lymph nodes, and internal organs. Kaposi's sarcoma is classified into four categories based on clinical and epidemiologic features: classic Kaposi's sarcoma, AIDS-related (also known as epidemic) Kaposi's sarcoma, medical-derived (also known as immunosuppressive) Kaposi's sarcoma, and endemic Kaposi's sarcoma. Kaposi sarcoma-associated herpes virus (KSHV) is a gamma herpesvirus that causes Kaposi's sarcoma, destroys the host immune system, causes lifelong latent infection, and contributes to tumor development. This article will focus on the pathogenic mechanism of KSHV on the host immune system and the clinical strategy of immunotherapy for Kaposi's sarcoma, with the aim of providing new ideas and references for the future research and treatment of Kaposi's sarcoma.

Kaposi's sarcoma has been associated with Kaposi's sarcoma-associated herpesvirus infection, immune dysfunction, genetic susceptibility, and environmental factors. Immune dysfunction appears to be a common factor in the development of all strains of Kaposi's sarcoma. For example, epidemic Kaposi's sarcoma usually results from severe immune compromise, classic Kaposi's sarcoma often occurs in middle-aged and older men with immune compromise, and Kaposi's sarcoma of medical origin is often a complication of long-term immunosuppressive therapy. A significant negative correlation between CD4 cell counts and Kaposi's sarcoma has been shown, suggesting that immunodeficiency plays an important role in the development of tumors. In addition, there may be a relationship between host genetic factors and KSHV susceptibility, which may influence the host's intrinsic immunity to viral infection. At the same time, KSHV can modulate the host immune response through multiple mechanisms, establishing a delicate balance between activation and suppression of the immune response to regulate the host's innate and specific immune defenses to establish persistent infection and contribute to tumor development.KSHV infection activates the host innate antiviral immune response and affects the intrinsic immune response by modulating pattern recognition receptors (PRR), blocking the action of interferon, evading the complement system and the antiviral activity of NK cells, thus achieving a mutual balance between the host immune defense system and viral action.

The main target cells of KSHV (B cells and endothelial cells) express large amounts of PRRs, which activate the host's intrinsic immune response and induce the production of a series of antiviral factors such as interferons and pro-inflammatory cytokines. Aberrant expression of PRRs has been shown to release multiple proliferative, anti-apoptotic and angiogenic factors that promote Kaposi's sarcoma development.

KSHV can evade the adaptive immune response through a variety of mechanisms.B cells are an important component of the adaptive immune system, and KSHV can act on B cells to evade the immune response.KSHV K1 and K15 inhibit B cell receptor (BCR) transduction signaling and inhibit B cell activation[. B cells, mast cells, and other cells may be the main reservoir of KSHV in Kaposi's sarcoma and may be a source of KSHV reactivation, shedding, and dissemination, where KSHV can establish latent infection in memory B cells and can be reactivated when the body is under pathophysiologic conditions such as decreased immunity, oxidative stress, and viral co-infection, and where mast cells synthesize and secrete histamine, which stimulates viral replication and transmits infection. MHC-I is essential for the presentation of viral antigens from CD8+ T cells to the TCR, and KSHV K3 and K5 cause downregulation of MHC-I, evade T cell immunity, and increase the risk of Kaposi's sarcoma. KSHV also induces the secretion of pro-inflammatory cytokines such as interleukin (IL)-1α and IL-6, which contribute to the differentiation of monocytes into tumor-associated macrophages, promote angiogenesis, and suppress T-cell responses and anti-tumor immune responses. KSHV contributes to persistent viral infection and evasion of host detection by infecting monocytes and suppressing latent cellular immune recognition and downregulating the expression of costimulatory receptors for adaptive immune responses. In addition, Poppe et al. first reported the detection of Ab-dependent cell cytotoxicity (ADCC) activity

in KSHV seropositive patients, but no link has been found between ADCC and Kaposi's sarcoma status or disease progression.

Current treatment cannot eradicate KSHV, and the goal of anti-Kaposi's sarcoma therapy is to slow the progression of the disease and relieve the patient's symptoms. During treatment, the criteria for disease spread, the restrictive or disseminated nature of the lesion and the evolution of the disease should be taken into account, among which the recovery of the patient's immune status and the manifestation of comorbidities should be taken into account. Pomalidomide is the most potent immunomodulator against Kaposi's sarcoma to date, with the ability to improve immune surveillance by counteracting vascular proliferation, inhibiting programmed death ligand 1 (PD-L1) expression in tumor cells, enhancing the activation of T cells and NK cells, and restoring the expression of immune markers. In a single-center phase I/II study of pomalidomide in 22 patients with Kaposi's sarcoma, Pomalidomide was administered orally at 5 mg/d for 21 days and stopped for 7 days in a 28-day treatment cycle, with an overall efficacy rate of 73% after 36 weeks of treatment, regardless of HIV infection and stage of disease progression. Ramaswami et al. reported a trial of 28 patients with Kaposi's sarcoma treated with the same dosage of pomalidomide as in the above study until disease progression or unacceptable side effects, showing an overall effectiveness of 71% with pomalidomide, including 67% and 80% in 18 patients with AIDS-related and 10 patients with classic Kaposi's sarcoma, respectively. These studies suggest that pomalidomide is an effective drug for the treatment of Kaposi's sarcoma. A phase II single agent trial (NCT03601806) is currently underway to evaluate the efficacy of pomalidomide in the treatment of sub-Saharan AIDS-related Kaposi's sarcoma. A Phase I trial (NCT04902443) to confirm the potential immunosynergistic effects of pomalidomide and nabumab in combination for the treatment of viral-induced cancers and to confirm safe dosing and a Phase I clinical trial (NCT02659930) of pomalidomide in combination with liposomal adriamycin for the treatment of advanced or refractory Kaposi's sarcoma are also ongoing.

Interferon is a cytokine that exerts a strong direct antiproliferative as well as antiviral effect. Interferon-α has been shown to have a direct antiviral effect on KSHV, but its efficacy depends largely on the patient's cellular immune status, with remission rates of up to 45% in patients with CD4+ T-cell counts >400/μl and 45% in patients with CD4+ T-cell counts <200/μl.The remission rate of interferon treatment is up to 45% in patients with CD4+ T-cell counts >400/μl and only 7% in patients with CD4+ T-cell counts <200/μl. Interferon-α is approved for the treatment of patients with AIDS-related Kaposi's sarcoma, but fewer studies have been performed in patients with classic Kaposi's sarcoma. 2019 European guidelines state that low-dose interferon-α can be used as first-line therapy in patients with classic Kaposi's sarcoma aged <70 years with normal cardiac function, and that 71% to 100% of patients with classic Kaposi's sarcoma treated with interferon-α have high long-term partial remission rates . The long-term partial remission rate is high. A retrospective study by Qu Yuanyuan et al. described an overall efficacy of 68.42% in 19 patients with classic Kaposi's sarcoma treated with interferon (6 million U, every other day intramuscularly) for at least 3 to 6 months. After 9 months of treatment, no tumor was detected and the original lesion symptoms had improved. It is important to note that interferon-alpha is not used for rapidly progressive or visceral Kaposi's sarcoma due to the long response time and is not usually recommended after organ transplantation to avoid a high risk of rejection.

The immune checkpoint receptor PD-1 inhibits tumor-specific immune response through interaction with its ligand PD-L1. PD-L1 in KSHV-infected human monocytes increases transcriptional and translational levels in a dose-dependent manner and contributes to immune escape. Studies have shown that anti-PD-1 antibodies (pablizumab or nabolutumab) show significant antitumor activity and low toxicity in Kaposi's sarcoma patients.The phase II trial (NCT03469804) showed that PD-L1 expression may be a predictor of immunotherapy efficacy in classic or endemic Kaposi's sarcoma.Saller et al. Saller et al. reported a case of advanced classic Kaposi's sarcoma that had failed to respond to multiple lines of chemotherapy and had a partial response after 30 weeks of pablizumab treatment. Another patient with disseminated Kaposi's sarcoma with chronic atopic dermatitis (AD) with chronic CD8+ lymphopenia did well overall after 6 months of treatment with pablizumab 2 mg/kg every 3 weeks, and AD also improved after treatment. The study by Cesmeci et al. described a case of an 82-year-old man with metastatic Kaposi's sarcoma who was treated with nabulizumab 3 mg/kg every 2 weeks for 24 weeks.The patient's health status improved, and

FDG-PET and upper gastrointestinal endoscopy showed resolution of the Kaposi's sarcoma lesion. All of these studies demonstrated the effectiveness of pablizumab or nabritumomab in the treatment of Kaposi's sarcoma.
Prophylactic vaccination allows the body to develop specific immunity to prevent specific diseases. Although KSHV vaccines can prevent Kaposi's sarcoma, the ability of KSHV to evade the host immune system makes the development of such a vaccine challenging. gpK8.1, gB and gH/gL glycoproteins of KSHV are associated with viral entry mechanisms into host cells and are potent vaccine targets for the induction of neutralizing antibodies against viral infection. It has been shown that the KSHV gpK8.1, gB and gH/gL glycoproteins can be incorporated into the surface of viral particles to induce the production of potent antibodies against KSHV and can be used as prophylactic vaccine candidates. A multi-epitope vaccine targeting the key KSHV glycoproteins involved in viral entry is currently under development
Kaposi's sarcoma is an opportunistic tumor associated with KSHV and immune dysregulation that antagonizes the host immune response through pattern recognition receptor expression, interferon signaling, complement activation, antigen presentation, and immune cell recruitment; however, the understanding of the mechanisms involved in KSHV immune regulation also provides a window for clinical intervention. As the immune system is better understood, new therapeutic tools will emerge. Currently, immunotherapies such as anti-hematopoietic immunomodulators, cytokines and immune checkpoint inhibitors have yielded good results, however, due to the low incidence of Kaposi's sarcoma, which is mostly disseminated, there are few experimental studies on immunotherapy and more studies are needed to verify the efficacy and safety of these drugs, while a vaccine against KSHV still needs to be further investigated. In addition, research on the immune-related mechanisms of Kaposi's sarcoma may provide new ideas for better immunotherapy regimens in the future.

PO-104

Epidermal Growth Factor Receptor Inhibitor-induced Erosive Pustular Dermatosis of the Scalp is Associated with HLA-C*15:02

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Epidermal growth factor receptor inhibitors (EGFRIs) are widely used to treat various types of malignancies. One of the common adverse reactions is cutaneous toxicity, mostly presenting acneiform eruptions, paronychia and xerosis. Erosive pustular dermatosis of the scalp (EPDS) is a rare cutaneous adverse reaction that develops during treatment with EGFRIs. The pathogenesis of EGFRIs-induced EPDS is poorly understood. Here we present three cases of EPDS induced by EGFRIs. LTA4H, METAP1, BID, SMAD1, PRKRA, YES1 and EGFL7 were significantly upregulated in EGFRIs-stimulated peripheral blood mononuclear cell (PBMC) cultures and validated in the lesions. All the proteins colocalized with CD4 and CD8 T-cell expression. Next generation-based HLA typing showed all patients carried HLA-C*15:02 and modelling studies showed that afatinib and erlotinib bound well within the E/F pockets of HLA-C*15:02. Moreover, T-cells were preferentially activated with EGFRIs in individuals carrying HLA-C*15:02. The case series revealed that EGFRIs-induced EPDS may be mediated by drug-specific T-cells.

PO-105

A case of linear IgA bullous derma

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The female patient,64 years old, was hospitalized in the First Affiliated Hospital of Hebei North University for "erythema, blisters and pruritus of trunk and limbs for 4 months, aggravated for 1 week". Specialty: The rash is generalized throughout the body, with symmetrical distribution on the upper body. Annular and semi-annular dense arrangements of rice grains to dark red spots the size of apricot pits can be seen on the trunk, limbs flexion and buttocks, and some of them are fused into patches. The upper part of the blister ruptured with reddish-brown blood scab. The blisters of rice grains to mung bean size in circular arrangement can be seen at the macular edge near the popliteal fossa on the flexion side of both upper limbs. Most of them are ulcerated and obliterated, and are covered with reddish-brown blood scab. Pathological report: Acantholysis, subepidermal blister formation, infiltration of neutrophils, eosinophils and monocytes in the superficial dermis, neutrophilic microabscess in the dermis papillae. Direct immunofluorescence: Basement membrane with IgA linear positive .Diagnosis : Linear IgA bullous derma .Treatment : Hydroprednisone 50mg once/day; Sodium thiosulfate 0.64g once/day; Calcium chloride 0.90g once/day; Topical halometasone cream/compound polymyxin B ointment 2 times/day.

PO-106

Human umbilical cord mesenchymal stem cells can promote the repair of back skin wounds in mice, which may be related to the activation of classical Wnt/β-catenin pathway

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Objective To explore the regulatory role of human umbilical cord mesenchymal stem cell-derived exosomes (hucMSC-Exos) in back wound repair of C57BL/6 mice and its possible mechanism.
How Human umbilical cord mesenchymal stem cell lines were cultured in vitro, and then the supernant was collected, hucMSC-Exos was collected by ultra-fast centrifuge method, and the extract was identified by transmission electron microscopy, nano particle size analyzer and western blot. The extract was identified as hucMSC-Exos. After 6 weeks of female C57BL/6 mice were fed for 1 week, The full-layer skin tissue of 1.5x1.5cm was cut off from the central area of the back of mice and applied with Vaseline gauze of appropriate size. After 3 days, the mice were randomly divided into two groups: control group and hucMSC-Exos (200ng/ml) group were injected once a day under uniform wound scab from the 3rd day of modeling to the 14th day. Mice in the control group were injected with 100μl phosphate buffer daily. Mice in the hucMSC-Exos group were injected with 100μl exosome solution (200ng/ml) every day. On days 0, 3, 5, 7, 10, 12 and 14, the wound healing was observed and recorded with photographs. On day 14, the whole skin of the central scar area was cut and total RNA was extracted. The relative expression levels of classic Wnt/β-catenin pathway genes such as Wnt5a, Lrp5, β-catenin, Lef1 and Dkk1 were detected by RT-PCR.
Results The typical saucer-like structure was observed under transmission electron microscopy, the average diameter of the extracted substance was 154.4nm, and the expression of TSG101, CD9 and HSP70 on the surface was detected by western blot, which confirmed that the extract was exosome. On day 14, the wound healing rate of hucMSC-Exos(200ng/ml) group was higher

than that of control group, and the difference was statistically significant (P < 0.05). The results of RT-PCR in scar tissue showed that the relative expressions of Wnt5a (P < 0.05), Lrp5 (P < 0.05), β-catenin (P < 0.01) and Lef1 (P < 0.05) in hucMSC-Exos (200ng/ml) group were up-regulated. The relative expression of DKK1 was decreased (P < 0.05).
Conclusion hucMSC-Exos (200ng/ml) can promote the repair of back skin wound in C57BL/6 mice, which may be related to the activation of classic Wnt/β-catenin pathway.

PO-107

Research Progress in studies of the S100 protein family members in psoriasis

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Psoriasis, commonly known as psoriasis, is an immune-mediated polygenic chronic inflammatory skin disease whose typical clinical manifestations is red or bright red patches covered with white or silver white scales.In addition to the skin involvement, it can also affect the scalp, nails, palarpal toes, cardiovascular, joints, etc. About 20% -30% of patients will complicated with arthritis, and increase the risk of metabolic syndrome and coronary atherosclerotic heart disease, which brings great economic and psychological burden to patients, and seriously reduces the quality of life of patients.The treatment of psoriasis is difficult and easy to relapse, and there is no complete cure at present.The current research view suggests that the pathogenesis of psoriasis involves abnormal innate and adaptive immune responses, in which T cells, keratinocytes, dendritic cells play a central role in the development and development of psoriasis.However, some of them specifically expressed molecules in psoriasis diseased tissues, including protein and nucleic acid sequences, and their functions and molecular mechanisms of action remain unclear.Studies have found that the S100 protein family in psoriasis skin, in the inflammatory cell response, keratinocyte differentiation and angiogenesis, greatly increased the risk of psoriatic arthritis, coronary atherosclerotic heart disease.Relevant clinical and experimental data show that the expression level of S100 protein will change accordingly with the severity of psoriasis, and inhibiting the expression of S100 protein can improve the skin thickness and vascularization of psoriasis, suggesting that S100 protein is a reliable biodiagnostic marker of psoriasis.Based on this, this paper reviews the role of some members of the S100 protein family in psoriasis, in order to provide a new direction for the diagnosis, treatment and prevention of psoriasis.

PO-108

A study on the efficacy and safety of ustekinumab and secukinumab in the treatment of psoriasis vulgaris.

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Introduction Psoriasis is a chronic autoimmune disease characterized by recurrent flare-ups. IL-17 inhibitor secukinumab and IL-12/23 inhibitor ustekinumab have demonstrated efficacy in the treatment of psoriasis in adult patients. However, there is a lack of comparative studies on their effectiveness and safety.
Objective This study aims to investigate the efficacy and safety of secukinumab and ustekinumab in real-world clinical settings for patients with psoriasis.
Methods: We included patients with moderate to severe plaque psoriasis who received treatment with secukinumab and ustekinumab in our department from July 2020 to April 2022 and followed them up for 24 weeks. Patient demographics and treatment characteristics were assessed.

Results A total of 30 patients with moderate to severe plaque psoriasis were included in this study. Systemic inflammation in all patients was controlled within 2 weeks, with a significant decrease in serum inflammatory cytokine levels within 4 weeks. The proportion of patients achieving PASI 50 and PASI 100 was higher for secukinumab-treated patients at week 2 compared to ustekinumab-treated patients. From week 4 to week 24, 52 patients achieved PASI 100, while 9 patients achieved PASI 75 due to minor relapse. The quality of life of psoriasis patients significantly improved within 48 weeks. Good treatment responses with secukinumab were not associated with gene mutations. All patients were followed up for 24 weeks, and no serious adverse events (AEs) were observed.
Conclusion Secukinumab demonstrated faster improvement in PASI scores in psoriasis patients at week 2, but there was no significant difference in long-term efficacy compared to ustekinumab. Both medications were well-tolerated with no serious adverse events (AEs) reported

PO-109

Exploring Treatment Strategies for Folliculitis Based on Skin Microbiome Regulation

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Folliculitis is a common skin disease that usually presents as a red, painful, and itchy skin lesion around the hair follicle. Folliculitis can be divided into two types: acute and chronic, with the latter being more difficult to cure. In recent years, researchers have found that the skin microbiome is an important factor affecting the development of folliculitis. This article will review the relationship between the skin microbiome and folliculitis, investigate the role of the skin microbiome in the pathogenesis of folliculitis, and explore new treatment strategies based on the regulation of the skin microbiome.

The skin microbiome refers to the microbial communities that live on the surface of the human skin and inside hair follicles, including bacteria, fungi, and viruses. The skin microbiome is closely related to human health as it can help maintain the healthy state of the skin, but it can also cause various skin diseases. In recent years, more and more research has shown that the skin microbiome is closely related to the development of folliculitis.

One study found that there were significant differences in the composition of the skin microbiome between patients with folliculitis and healthy individuals. The skin microbiome of folliculitis patients contained more pathogenic bacteria such as Staphylococcus and Pseudomonas, while the skin microbiome of healthy individuals contained more beneficial bacteria such as Streptomyces and Staphylococcus aureus. This indicates that an imbalance in the skin microbiome may be an important factor in the development of folliculitis.

It is also known that the skin microbiome can affect the development of folliculitis in several ways, including:

1 Cause inflammatory response

Some pathogenic bacteria in the skin microbiome can cause inflammatory reactions that cause symptoms like redness, swelling and pain in the skin around the hair follicles. These pathogenic bacteria can exacerbate the symptoms of folliculitis by causing inflammatory responses in ways such as releasing toxins and activating immune cells.

2 Disturbing of the skin barrier function

Some pathogenic bacteria in the skin microbiome can destroy the skin barrier function and make the skin vulnerable to stimulation by the external environment. These pathogenic bacteria can destroy the skin barrier by breaking down the sebum and cuticle, which leads to the occurrence and development of folliculitis.

3 Affect the immune system

The skin microbiome can affect its function by its direct action on the skin immune system. Some studies have shown that some beneficial bacteria can improve the symptoms of folliculitis by

regulating the response of the immune system and promoting the proliferation and repair of skin cells.

4 Interference with the hair follicle cell cycle

The skin microbiome may promote the occurrence and development of folliculitis by interfering with the hair follicle cell cycle. For example, it was found that the skin microbiome of patients with folliculitis contains more pathogenic bacteria such as staphylococci and Staphylococcus aureus, and these bacteria may cause inflammation and damage of hair follicles by interfering with the division and differentiation of hair follicle cells.

5 Affect the internal environment of the hair follicle

The skin microbiome can affect the pH, oxygen level and nutrient content of the environment, and then affect the health and the degree of inflammation. For example, the skin microbiome of folliculitis patients may have excessive pathogenic bacteria in Staphylococcus and P. aeruginosa, which can consume oxygen and nutrients in hair follicles, leading to insufficient nutrition and hypoxia of hair follicles, and thus promote the occurrence and development of folliculitis.

With the intensive study of the relationship between skin microbiome and folliculitis, novel therapeutic strategies based on the regulation of skin microbiome are also gradually proposed. One treatment is a skin microbiome modulator, a drug or compound that regulates the balance of the skin microbiome. For example, some antibiotics, antifungal drugs, and probiotics can all regulate the balance of the skin microbiome to treat folliculitis. In addition, the researchers found that some natural products and plant extracts, which also regulate the skin microbiome, are known as chamomile, tea tree essential oil and Salvia miltiorrhiza. Another treatment is skin microbiome transplantation, a treatment for transplanting the skin microbiome of healthy people to the skin of patients with folliculitis. This method can restore the balance of the patient's skin microbiome and thus the treatment of folliculitis. Currently, skin microbiome transplantation has been used to treat some skin diseases, such as refractory papilloma virus infection and refractory contact dermatitis. Although this method has certain risks and limitations, the researchers believe that with technological advances and improved safety, skin microbiome transplantation is expected to be an effective treatment for follicusitis. The third treatment is individualized treatment, an approach that targets the unique condition and skin microbiome characteristics of each patient. This method can develop a targeted treatment plan through the analysis and evaluation of patients' skin microbiome, which can improve the effect of treatment and reduce the side effects. For example, researchers can analyze the patient's skin microbiome to determine which bacteria are pathogenic and which are beneficial, and then develop targeted drugs or compounds. In addition, receiving treatment with probiotics is also feasible. Through oral or topical probiotics, it can increase the content of beneficial bacteria in the skin microbiome, regulate the response of the immune system, and promote the proliferation and repair of skin cells, thus improving the balance of the skin microbiome and alleviating the symptoms of folliculitis.

The skin microbiome plays an important role in skin health and disease. For folliculitis, a common skin disease, some novel microbiome treatment strategies can be developed based on the regulation of skin microbiome, including skin microbiome modulators, skin microbiome transplantation and individualized therapy. These treatment strategies are targeted and personalized, which can improve the therapeutic efficacy and reduce the side effects. However, it should be noted that a comprehensive analysis and evaluation of the patients' skin microbiome is required before the treatment to ensure the safety and efficacy of the treatment. In the future, we can expect that such personalized and precise treatment strategies can be widely used in the field of skin health and disease, bringing better treatment results and quality of life for patients.

PO-110

Photodynamic Therapy for Skin Diseases: Exploring the Use of Novel Photosensitizers and Clinical Applications

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Photodynamic therapy (PDT) is a therapeutic technology based on photosensitizer and laser light source, widely used in cancer, skin diseases and other fields. In recent years, with the development of new photosensitizers and the continuous improvement of PDT technology, its application in the treatment of skin diseases has become one of the research hotspots. This paper will discuss the application of photodynamic therapy in the treatment of skin diseases, including the development of new photosensitizers and the clinical application of photodynamic therapy.

New photosensitizer is an important direction for the development of PDT technology, and its choice and properties directly affect the therapeutic effect. In recent years, new photosensitizers have been developed, including ALA, ZnPcTAPP, MALME, etc., which have potential applications in the treatment of skin diseases.5-ALA is a commonly used photosensitizer, which is widely used in PDT technology. However, 5-ALA has a short half-life and a low light absorption rate, which limits its efficacy in clinical applications. To overcome these shortcomings, researchers have begun to explore novel ALA derivatives. One study reported a novel ALA derivative (ALA-BF3) with higher photosensitivity and photostability that can be used to treat a variety of skin diseases, such as acne, psoriasis and non-melanin skin cancer. ZnPcTAPP Is a novel photosensitizer with good water solubility and cytotoxicity and can be used in the treatment of skin diseases. One study reported the clinical effect of using ZnPcTAPP-PDT for melanoma, which significantly reduces tumor size and number while preserving the integrity of the surrounding normal tissue. Another study reported the clinical effect of using ZnPcTAPP-PDT for dermatophytic infections, which significantly reduced the extent and number of infectious lesions while improving the quality of life of patients. MALME is a novel photosensitizer with high photosensing activity and good cytotoxicity that can be used in the treatment of various skin diseases. One study reported the clinical effect of using MALME-PDT for psoriasis, which significantly reduces the extent and number of lesions in psoriasis while improving the quality of skin and the patient's quality of life. Another study reported the clinical effect of using MALME-PDT for non-melanin skin cancer, which significantly reduces the number of cancer cells and the range of lesions while preserving the integrity of the surrounding normal tissue. Overall, the research and development of new photosensitizers provide more options for the clinical application of PDT technology.

Acne is a common skin disease, and PDT technology has promising applications in its treatment. ALA-BF3 has higher light sensitivity and photostability. In clinical trials, researchers used ALA-BF3 as a photosensitizer to kill acne pathogens and abnormal cells by light exposure, thus reducing the degree and number of acne lesions. The results showed that ALA-BF3 was 70% effective in treating acne, and there was no significant discomfort or side effects during the treatment. This indicates that ALA-BF3 has great potential application in the treatment of acne and can be one of the important options for PDT technology in the treatment of skin diseases.

Facial hemangiomas are a common vascular skin disease, and PDT technology has certain application value in its treatment. A study reported a new photosensitizer, manganese tetramethylpyridyl porphyrin (MnTmPyP), which has good prospects for the treatment of facial hemangiomas. In the clinical trial of MnTmPyP-PDT for the treatment of facial hemangiomas, MnTmPyP was used as a photosensitizer to kill hemangioma cells through photodynamic action, achieving the goal of treatment. The results showed that MnTmPyP-PDT had an effective rate of up to 90% in treating facial hemangiomas, and there were no obvious discomfort or side effects during the treatment process. In addition, PDT technology also has other advantages in the treatment of facial hemangiomas. For example, PDT technology has the characteristics of non-invasiveness, selectivity, and repeatability, which can kill hemangioma cells without damaging normal tissue, reducing the risk of treatment and complications. Moreover, PDT technology can

also be used for local treatment of facial hemangiomas, avoiding the trauma and pain of overall surgery.

Psoriasis is a common chronic inflammatory skin disease, and the PDT technology also has some application value in its treatment. Many studies have shown that the PDT technique can improve the symptoms and conditions of psoriasis by inhibiting the inflammatory response and reducing the cuticular hyperplasia. One study reported a novel photosensitizer, —— methionoppionic acid (MAL), which is promising in the treatment of psoriasis. In clinical trials of MAL-PDT for psoriasis, the researchers used MAL as a photosensitizer by killing psoriasis pathogens and abnormal cells with light exposure. The results show that MAL-PDT was more than 70% effective in treating psoriasis, with no significant discomfort and side effects during the treatment. In addition to MAL, aminoacetylpropionic acid (ALA) is also a commonly used photosensitizer, which also has a certain application value in the treatment of psoriasis. One study reported a novel ALA derivative (ALA-BF3) with higher photosensitivity and photostability that could be used to treat a variety of skin diseases, including psoriasis. In clinical trials of ALA-BF3-PDT for psoriasis, the researchers used ALA-BF3 as a photosensitizer to kill psoriasis pathogens and abnormal cells by illumination. The results showed that ALA-BF3-PDT was 85% effective in treating psoriasis, and there was no significant discomfort and side effects during the treatment.

Although remarkable progress and achievements have been achieved in PDT technology in the treatment of skin diseases, some limitations and challenges remain. For example, its treatment depth is affected by many factors, such as photosensitizer type, light source type, light dose, and so on, which requires individualized adjustment. In addition, PDT technology is less effective in the treatment of malignant tumors, and it requires comprehensive treatment combined with other treatment methods. Therefore, in practical application, the patient's condition and treatment plan need to be considered comprehensively in order to achieve the best treatment effect.

In the future, with the in-depth study of PDT technology, we can foresee that the application of PDT technology in the treatment of skin diseases will be more extensive and in-depth development. For example, the continuous development and optimization of new photosensitizers, light sources and light doses can further improve the therapeutic efficacy and safety of PDT technology. Moreover, with the in-depth understanding of the mechanism of PDT technology, we can also foresee that the application of PDT technology in other disease fields will get more attention and application.

PO-111

Skin Microbiome and Acne: Exploring Mechanisms and New Approaches with Skin Microbiome Modulators

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Acne is a common chronic skin disease characterized by hyperplasia of hair follicle sebaceous glands and inflammation. Although the pathogenesis of acne is not yet fully understood, research has suggested that the skin microbiome may play a key role in the development and progression of this disease.The skin microbiome refers to the microbial community on the human skin, including bacteria, fungi, and viruses, among others. They form a symbiotic relationship between microorganisms and hosts, which is crucial for maintaining skin health and immune homeostasis.

In recent years, increasing evidence has suggested that the skin microbiome is closely related to the pathogenesis and development of acne. Certain microorganisms in the skin microbiome, such as Propionibacterium acnes and Staphylococcus aureus, may participate in the pathogenesis of acne, such as promoting follicular keratinization, inducing inflammation of hair follicle sebaceous glands, and so on. In addition, the interaction between the skin microbiome and the immune system of acne patients may also play an important role in the development of acne.

Research on the mechanisms linking the skin microbiome and acne is still in its infancy, but some important findings have been made. Studies have shown that propionia bacteria may promote the keratinization process of hair follicles by secreting some metabolites, such as fatty acids and

aromatic compounds, which can lead to hair follicle obstruction and excessive hyperplasia of sebaceous glands. In addition, propionia bacteria may also contribute to the development of acne through mechanisms such as causing inflammatory responses. Meanwhile, S. aureus has also been shown to have a role in the production of acne. —— S. aureus may cause the inflammatory response of acne by causing exotoxin production and activating Toll-like receptors, thus promoting the development of acne. In addition to propionibacterium and Staphylococcus aureus, other microorganisms may also be involved in the development of acne. And promote the development of acne. In addition to propionibacterium and Staphylococcus aureus, other microorganisms may also be involved in the development of acne. For example, studies have shown that microorganisms such as Cutibacterium acnes (formerly known as acnes) and Staphylococcus epidermidis may participate in the development of acne through mechanisms such as regulating the immune response and affecting skin barrier function.

As we have seen, the skin microbiome plays an important role in the pathogenesis of acne. However, we still need to thoroughly study the specific mechanisms to better develop and apply relevant therapeutic strategies.Certainly, it is evident that the amount of Propionibacterium acnes in the skin of acne patients is significantly increased, and other bacterial groups such as Staphylococcus aureus and Pseudomonas aeruginosa are also closely associated with the development of acne. These microorganisms may be involved in acne development through various pathways, such as stimulating excessive growth of hair follicle sebaceous glands and affecting the process of hair follicle keratinization. Additionally, metabolites of some bacteria may also impact the secretion of sebaceous glands and intensify inflammatory reactions.

Apart from directly affecting the physiological processes involved in acne development and immune system regulation, the skin microbiome may also be involved in acne development through immune modulation. The interaction between the skin microbiome and the immune system plays a crucial role in acne development. For instance, the number of T cells in acne patients is significantly increased, and these T cells may become overactivated, leading to an exacerbation of inflammatory reactions. Furthermore, some microbial groups such as Propionibacterium acnes may also increase inflammatory reactions by stimulating immune signal pathways, such as Toll-like receptors and ribosomal receptors.

Furthermore, the skin microbiome may also be involved in acne development by influencing gene expression. Some studies suggest that there are differences in gene expression between the skin microbiomes of acne patients and those without acne. For example, the expression levels of certain genes in the skin of acne patients may be influenced by microorganisms such as Propionibacterium acnes, which further impacts the pathological and physiological processes of the skin.

In recent years, with the intensive study of the skin microbiome, novel therapeutic strategies based on microbiome regulation have become a focus of microbiome. As a new treatment method, the skin microbiome modulator has made some progress in the research and development and clinical application.Skin microbiome modulators refer to drugs that prevent or treat diseases by regulating the balance of skin microbiome. These drugs can work through various mechanisms such as inhibiting bacterial growth, regulating skin microbiome balance, or improving skin immune environment.Skin microbiome modulators that inhibit bacterial growth mainly include antibiotics, antimicrobial peptides, etc. These drugs can directly inhibit the growth of acne-causing bacteria, thereby reducing inflammation. Clinical evidence shows that antibiotics can significantly reduce the incidence and lesion area of acne, but long-term use may lead to the risk of drug resistance and microbiome imbalance, so caution is needed.Skin microbiome modulators that regulate skin microbiome balance mainly include probiotics, prebiotics, herbal extracts, etc. These drugs can reduce the occurrence of acne by regulating the balance of skin microbiome. Research shows that using an emulsion containing probiotics can significantly reduce acne inflammation and lesion area. Similarly, some herbal extracts such as tea tree oil and white willow bark extract have been proven to have a certain therapeutic effect on acne.Skin microbiome modulators that improve skin immune environment include glucocorticoids, vitamin D, etc. These drugs can also relieve acne inflammation by regulating the function of the immune system. However, these drugs may also affect the balance of skin microbiome, so caution is needed when using them.

Meanwhile, skin microbiome modulators can also be used in combination with traditional treatments. Some patients with acne are treated with oral antibiotics or topical drugs. This combination therapy

may have better therapeutic effects, but may also increase the risk of adverse effects. Therefore, attention should be paid to monitoring adverse reactions and controlling the dosage when combining skin microbiome modulators and traditional treatments.

In conclusion, hyperactivity of sebaceous glands and bacterial overgrowth are one of the main causes in the pathogenesis of acne. And the skin microbiome modulators can reduce the occurrence of acne by regulating the balance of the skin microbiome. Some skin microbiome modulators, such as probiotics, prebiotics and herbal extracts, have been proved to inhibit the growth of acne pathogens, reduce the inflammatory response and lesion area of acne. In addition to skin microbiome modulators, we have also learned about other treatments for acne, such as topical drugs, oral drugs, laser therapy, etc. These treatments all have their advantages and disadvantages, which need to be selected and applied according to the specific circumstances. Deep into the relationship between the skin microbiome and acne pathogenesis and the application of skin microbiome modulators in the treatment of acne have important implications for the prevention and treatment of acne. However, it should be noted that acne is a disease caused by multiple factors, and its treatment needs to consider a variety of factors, including individual differences, living habits, eating habits and so on. We hope that there will be more research and clinical practice in the future to provide more effective and safe methods for the prevention and treatment of acne.

PO-112

The Role of Skin Microbiome in Atopic Dermatitis Pathogenesis and Potential Therapeutic Strategies: A Review

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In recent years, with the deepening of microbiome research, people have gradually recognized the important role of the microbial community on the skin in the maintenance of skin health and pathogenesis. To better understand the development of this field, this paper will review the relevant literature over the last decade and introduce some cutting-edge perspectives.

1 The relationship between the skin microbiome and eczema

1.1 Composition and balance of the skin microbiome

The skin microbiome refers to the microbial community on the skin surface and its accessory organs, including bacteria, fungi, viruses, and parasites. The composition of the normal skin microbiome and its balance are essential for skin health. Common skin symbiotic bacteria mainly include Staphylococcus epidermidis, Propionibacterium acnes, etc. On healthy skin, these microorganisms interact with skin cells to maintain skin barrier function and immune balance.

1.2 Imbalance of eczema and the skin microbiome

Eczema is a common chronic skin inflammatory disease, manifested by skin itching, redness, swelling, exudation, scab, etc. The study found that the skin microbiome of eczema patients diffdifferent from those of healthy people. The number of pathogenic bacteria represented by Staphylococcus aureus and Candida albicans was significantly increased on the skin of eczema patients, while the number of commensal bacteria was relatively reduced. This microbial imbalance may be closely related to the onset of eczema.

2 the role of the skin microbiome in the pathogenesis of eczema

2.1 Impaired skin barrier function

Skin microbiome imbalance may lead to the impairment of skin barrier function. Studies have shown that S. aureus on the skin of eczema patients produces a series of proteases and lipases that disrupt the junctions between keratinocytes, leading to skin barrier damage.

2.2 Abnormal immune response

Skin microbiome imbalance may also cause abnormal immune responses. For example, S. aureus can activate the local skin immune response by inducing keratinocytes to produce inflammatory

cytokines, such as TNF- α, IL-6, etc. Moreover, Candida albicans on the skin of eczema patients can also further aggravate skin inflammation by inducing IL-17 production in Th 17 cells.

2.3 Microbe and host interactions

Complex interactions exist between the skin microbiome and skin cells. On the one hand, skin cells can inhibit the growth of pathogenic bacteria by secreted antimicrobial peptides and acidic environment; on the other hand, commensal bacteria on the skin can inhibit the growth of pathogenic bacteria by competing for nutrients and producing antibacterial substances. In patients with eczema, this interaction may be disrupted, causing an imbalance of the skin microbiome.

3 New therapeutic strategy based on skin microbiome regulation

3.1 Skin microbial transplantation

Skin microbiological transplantation is a novel therapeutic strategy to restore skin microbiome balance by inoculation of microorganisms of healthy people with their skin onto the skin of patients with eczema. Clinical studies in recent years have found that skin microbial transplantation has a certain effect on improving the condition of eczema. However, this treatment still faces many challenges, such as individual differences in microbiome, persistence of transplantation effect.

3.2 Probiotics and prebiotics

Probiotics refer to active microorganisms that can have beneficial effects on the host, such as Lactobacillus, Bifidobacterium, etc. Prebiotics are non-digestive food ingredients that can promote the growth of probiotics, if oligosaccharides, inulin, etc. Studies have found that oral probiotics and prebiotics have a certain effect on improving the condition of eczema. This may be related to the regulation of probiotics on the gut microbiome and the effects on the immune system.

3.3 The antimicrobial peptide

Antimicrobial peptides are a class of small molecule peptides with broad-spectrum antibacterial activity, such as defensins, epidermal antimicrobial proteins, etc. Recent studies have found that antimicrobial peptides have strong inhibitory effects on pathogenic bacteria on the skin of eczema patients. Therefore, antimicrobial peptides may become novel drugs for treating eczema.

4 Conclusion

The skin microbiome plays an important role in maintaining skin health and the pathogenesis of eczema. Future studies are needed to further reveal the relationship between the skin microbiome and eczema in order to provide new targets for the prevention and treatment of eczema. Moreover, novel therapeutic strategies based on skin microbiome modulation also deserve further investigation in order to provide more options for clinical treatment.

PO-113

Target binding and sequencing analysis of the RNA binding protein S100A4 in HaCat cells.

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Objective S100A4 is a multifunctional RNA binding protein (RNA-binding proteins, RBP), specifically distributed in cells and tissues of vertebrates, can participate in transcriptional and post-transcriptional regulation, affect gene expression, play an important role in a variety of inflammatory skin diseases, such as allergy, systemic sclerosis, psoriasis. Having shown that S100A4 is significantly upregulated in the dermal tissue of psoriasis patients and is able to participate in the pathogenesis of psoriasis by affecting epidermal thickness, cell proliferation and neovascularization, it is still unclear by what way S100A4 affects psoriasis.This study aims to clarify the function of RNA binding protein S100A4 in human immortalized keratinocyte cell line (HaCat), in order to lay a theoretical basis for further exploring the mechanism of S100A4 regulation of psoriasis.

Methods Using antibodies to S100A4 in HaCat cells, RNA UV cross-linked immunoprecipitation combined with high-throughput sequencing (RNA immunoprecipitation and deep sequencing, RIP-seq) to obtain the high-throughput sequencing data, HISAT2 aligned the resulting reads to the reference genome, Then the binding peak motif of S100A4 in HaCat cells obtained by two software

programs piranha and ABLIRC, Finally identified the target RNA bound by S100A4 by binding peaks, For the G0 functional analysis of the peak-related genes, To explore the molecular function of S100A4-bound RNA and the biological processes involved.
Results The target RNA of S100AA4 in HaCat cells was significantly enriched in CDS and intron regions, but also in non-coding regions, such as 5 ′ UTR, 3 ′ UTR and Nc _ exon.The S100A4-bound RNA was significantly enriched at the transcription start site and the translation start site, indicating that S100A4 binds RNA and may affect co-transcription or translation processes.The results of further GO function analysis of genes associated with S100A4 binding peaks indicate that these genes are mainly enriched in related pathways such as transcriptional regulation, intracellular response, translation and initiation.
Conclusion In HaCat cells, S100A4 specifically binds certain RNA and participates in the transcription, translation and alternative splicing processes of related genes.We speculate that S100A4 may regulate the cell proliferation and inflammatory response processes in psoriasis by affecting the transcription, translation and alternative splicing pathway of target RNA related genes, but these functions have not been further verified. Therefore, our next step is to carry out related functional experiments in cells, animal models and clinical samples to clarify the specific molecular mechanism of S100A4 regulating the occurrence and development of psoriasis, so as to further expand the molecular network of psoriasis and provide new ideas for the development of more effective treatments.

PO-114

Hemoporfin-mediated photodynamic therapy-caused skin barrier damage and triggered dermatitis in Port-Wine Stains

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Background Hemoporfin-mediated photodynamic therapy (PDT) is a high efficacy treatment alternative for port-wine stains (PWS) patients, and PDT also induced eczematous dermatitis in treated areas. However, the effect of PDT treatment on the prevalence and risk of dermatitis in patients with PWS have not been reported.
Objectives To assess the association between PDT and dermatitis, and the mechanism of PDT-triggered dermatitis in PWS patients.
Methods Clinical images were used to calculate the percentage of dermatitis in treated areas. The arithmetic mean roughness (Ra), the average depth of roughness (Rz) and the mean square roughness (Rq) were used to assess the change of surface roughness. Transepidermal water loss (TEWL), stratum corneum hydration (SCH) and total lipid content (TLC) were used to analyze the skin barrier function.
Results After treatment, we found that 27.15% (139/512) of PWS patients developed dermatitis on the treated areas, and the percentage of dermatitis was closely related to the treatment times and age of the patient. Moreover, the skin surface roughness of Ra, Rz, Rq and the skin barrier function of TEWL and TLC of these treated areas all were significantly increased ($p<0.05$), and the SCH was markedly decreased ($p<0.05$).
Conclusions PDT caused skin barrier damage in treated area, which may lead to increased permeability of the epidermis and trigger dermatitis in PWS patients.

PO-115

Altered sumoylation affects the proliferation, migration, and apoptosis of keloid fibroblasts.

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Objective Recent studies have highlighted the involvement of sumoylation in the development and progression of fibrosis. The objective of this study was to investigate the impact of sumoylation on the biological behavior of keloid fibroblasts.
Methods Immunohistochemistry (IHC) was used to examine the expression of sumoylation-related proteins in keloid tissue samples. Human keloid fibroblasts (HKF), isolated from keloid tissues, were subjected to siSENP1 interference or treated with 2-D08, a sumoylation inhibitor. Flow cytometry and BrdU assay were employed to analyze apoptosis and proliferation of HKFs, respectively. Cell migration was evaluated using a scratch assay.
Results IHC analysis revealed significantly increased expression levels of SUMO1 and SUMO2/3 in keloid tissues compared to normal skin samples. Conversely, the expression of SENP1, a protein involved in sumoylation regulation, was notably decreased in keloid samples. Treatment with 2-D08 significantly inhibited cell proliferation and migration while inducing fibroblast apoptosis. RNA interference targeting SENP1 (siSENP1) resulted in increased proliferation and migration, and decreased apoptosis of HKFs.
Conclusion The findings of this study indicate elevated expression of sumoylation-related proteins in keloid tissues. Altered sumoylation processes have notable effects on the proliferation, migration, and apoptosis of HKFs, suggesting that regulation of sumoylation could be a potential strategy for keloid therapy.

PO-116

The Exploration of Evodiamine's Interventional Effects and Mechanisms on Pruritus in a Mouse Psoriasis Model

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Objective Psoriasis (PS) is a common, immune-mediated, chronic, inflammatory skin disease with a complex pathogenesis and a prolonged course that is difficult to cure completely. PS patients have obvious itching symptoms in addition to skin damage. The mechanisms of PS itch have not been fully elucidated, and there is a lack of clinically pertinence drugs and methods to treat PS itch. Pruritus affects up to 80% of PS patients. It has been shown that Evodiamine (EVO) is one of the effective active ingredients extracted from the traditional anti-itch Chinese medicine evodia, which has an anti-itch effect, but whether it has an inhibitory effect on PS pruritus is unclear and the relevant mechanism has not been reported. Therefore, in this study, we used Imiquimod (IMQ) to induce C57BL/6J mice to construct the PS itch model to investigate the mechanism of the effect of EVO on PS itch and to provide some reference for the clinical application of EVO in the treatment of PS.
Methods Forty 6-8 week-old SPF grade C57BL/6J mice were randomly divided into blank group, model group, Evo-7.5 group, Evo-15 group, and Evo-30 group. The hair on the back of the mice was removed to fully expose the skin before the experiment. For 7 days, IMQ was applied to the back skin of the mice in the model group and each dosing group daily. At the same time, 0.2 ml of solvent, 0.2 ml of solvent, 0.2 ml of low concentration EVO suspension, 0.2 ml of medium concentration EVO suspension and 0.2 ml of high-concentration concentration EVO suspension were given to the mice by intragastric administration in the blank group, model group, Evo-7.5 group, Evo-15 group, and Evo-30 group respectively. The mice were observed daily for skin damage on the back, scored, and also observed twice for pruritic behavior. Blood was taken on the last day of the experiment, and the levels of pruritus-associated inflammatory cell molecules TNF-α, IL-23, and IL-17A in the serum were determined by enzyme-linked immunosorbent assay

(ELISA). The mice were executed by cervical dislocation, and the skin of the mice was removed from the back. The skin specimens were stained with Hematoxylin and Eosin (HE) to observe the histopathological changes. The expression levels of TRPV1, TRPV3, TRPV4, and the pruritus-related inflammatory molecules SP, NGF, and CGRP in the skin lesions of mice were observed by western blot (WB). The expression levels of TRPV1, TRPV3, TRPV4, SP, NGF, and CGRP in mouse skin lesion tissues were observed by Real-time quantitative reverse transcription (qRT-PCR). In order to take stock of the effects of EVO on excitation, exploratory behavior, limb movement, and coordination of mice, twenty-four mice were randomly divided into four groups: Blank, Evo-7.5, Evo-15, and Evo-30.Then the mice were observed in the open-field, suspension, and bar-turner experiments. The molecular docking technique was used to verify whether EVO could bind stably to residues of TRPV1, TRPV3, and TRPV4. Finally, the data from the above sections were statistically analyzed and summarised.

Results

(1) EVO inhibited the pruritic behaviour of the mouse PS itch model and improved the condition of PS skin damage in mice.

(2) EVO may reduce pruritus and inhibit inflammation by inhibiting the expression of pruritus-related inflammatory molecules TNF-α, IL-23 and IL-17A in the serum of PS mice, the expression levels of pruritus-related molecules SP, NGF and CGRP in the skin, and the expression of pruritus-related ion channels TRPV1, TRPV3 and TRPV4.

(3) EVO did not inhibit excitation and exploratory behaviour, limb locomotion and coordination in mice, and it can be excluded that EVO exerts its antipruritic effect through central sedation.

PO-117

She-Chuang-Si-Wu-Tang reduces inflammation and itching in a mouse model of psoriasis

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Aim of the study

to evaluate the effects of SSWT on imiquimod-induced psoriasis in a mouse model and explored the mechanisms of alleviating inflammation and inhibiting itch.

Materials and methods

Mouse model of psoriasis was established using IMQ. The administration of the drug was continuous for seven days and the experiment was conducted over an eight-day period. Upon completion of the experiment, the PASI of the mice was assessed, their spontaneous gripping behavior was observed and their epidermal thickness was examined through hematoxylin-eosin staining (H&E). Additionally, enzyme-linked immunosorbent assay (ELISA) and Quantitative RT-PCR methodologies were employed to determine the levels of IL-23, IL-17A, IL-17F and IFN-γ in the serum of mice, as well as the mRNA expressions of these cytokines in the skin of the same animals. Western Blot was employed to assess the levels of proteins related to the signal pathway (p38, p-p38, ERK1/2, p-ERK1/2, JNK, p-JNK, STAT3, p-STAT3) on the IL-23/IL17 axis, while High Performance Chromatography (HPLC) was used to analyze the components of SSWT.

Results

The results showed that SSWT reduced imiquimod-induced psoriasis-like skin inflammation, skin thickness, Psoriasis Area and Severity Index (PASI) score, inhibited spontaneous scratching behaviors in mice, and did not affect locomotion, exploration, or motor coordination. The levels and mRNA of IFN-γ, IL-23, IL-17A, and IL-17F in the dorsal skin of the SSWT-treated group were lower than those in the model group. Furthermore, SSWT significantly inhibited the phosphorylation of P38, ERK1/2, JNK, and STAT3. Additionally, five chemical components, namely osthole, matrine, caffeic acid, oxymatrine and ferulic acid, were identified in SSWT.

Conclusion

These results demonstrated that SSWT attenuated imiquimod-induced psoriasis-like skin lesions by inhibiting inflammatory cytokines production, and phosphorylation of the MAPK/STAT3 pathway. SSWT may be an effective herbal formula for the treatment of psoriasis.

PO-118

Spatial metabolomics to discover hypertrophic scar relevant metabolic alterations and potential therapeutic strategies

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Objective Being mild and non-toxic, bioactive metabolites show good pharmacokinetics and pharmacodynamics, and their role in drug development has attracted much attention. Mapping hypertrophic scar and surrounding normal skin tissues' metabolic remodeling spatially can fundamentally improve our understanding of scar formation, facilitates the development of advanced therapeutic strategies.
Methods We used matrix-assisted laser desorption/ionization (MALDI), a mass spectrometry imaging-based spatial metabolomics, to hierarchically visualize the metabolic heterogeneity in hypertrophic scar and surrounding normal skin tissues. Bioinformatics were performed to analyze the differential metabolites. 6 available metabolites were selected to check their effects on hypertrophic scar fibroblasts.
Results A total of 1631 metabolites were identified. Top 4 identified classes were benzene and substituted derivatives, heterocyclic compounds, amino acid and its metabolites, and glycerophospholipids. 22 metabolites were upregulated and 66 metabolites were downregulated in hypertrophic scar tissues. Top 4 altered metabolites were glycerophospholipids, glycerolipids, benzene and substituted derivatives, and heterocyclic compounds between hypertrophic scar and surrounding normal skin tissues. We then selected 6 available metabolites, analyzed their spatial characteristics and added them into the cell culture medium of primary hypertrophic scar fibroblasts respectively to check their actions. The results revealed that 1-pyrrolidinecarboxamide, glycerol trioleate and Lyso-PAF C-16 inhibited expressions of COL1A1, COL1A2 and ACTA2 at specific concentrations. We also analyzed the potential bound proteins of these 88 altered metabolites based on bioinformatic websites.
Conclusion This study mapping tissue metabolites architecture provides highly integrated picture of hypertrophic scar heterogeneity. And it is expected to provide new therapeutic clues for hypertrophic scar from the perspective of bioactive metabolites.

PO-119

m6A methyltransferase METTL3 promotes B cell differentiation in systemic lupus erythematosus

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Background Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by excessive B cell activation, abnormal differentiation, and abundant autoantibody production. Methyltransferase like 3 (METTL3), a prominent writer of the post-transcriptional N6-methyladenosine (m6A) modification, is considered to have a potential connection with B-cell-mediated adaptive immunity. However, the role of METTL3 in B cells of SLE has not been

studied. In this work, we investigated the mechanism of METTL3 on B cell activation, differentiation, and SLE pathogenesis.
Methods We performed flow cytometry to detect the expression of METTL3 in B-cell subsets from peripheral blood mononuclear cells (PBMCs) of SLE patients and healthy controls (HCs), and global RNA m6A modification was quantified in CD19+ B cells. The activity of the METTL3 enzyme was inhibited using a catalytic inhibitor to evaluate the role of METTL3 on B cell differentiation in vitro. Sheep red blood cell (SRBC)-immunized mouse model was established in CD19cre METTL3ff mice to investigate the function of the METTL3 in B cells. RNA-seq was applied to identify pathways and targeted gene signatures.
Results Compared with HCs, the mean fluorescence intensity (MFI) of METTL3 was enhanced in several major B-cell subsets of SLE patients, including total CD19+B cells, CD19+CD20+IgD+CD27- Naive B cells, CD19+CD20+IgD-CD27+ Memory B cells, CD19+CD20-CD38+ Plasma cells. Among them, the MFI of METTL3 in CD19+CD20-CD38+ Plasma cells from SLE patients was positively correlated with the SLEDAI score. Consistent with this, Naive B cell activation expressed higher METTL3 with IgM stimulation. Global m6A modification level in CD19+ B cells is significantly increased in SLE patients, in which active SLE was higher than that of inactive SLE. catalytic inhibition of METTL3 suppressed B-cell terminal differentiation in vitro. Moreover, METTL3 knockdown in CD19+ B cells in mice of the SRBC model decreased the proportion of memory B cells and plasma cells meanwhile increasing naive B cells in the spleen, and reducing antibody production, including IgG1、IgM、IgGH+L、IgG3、IgG2a、IgG2b in serum. Further, RNA-seq revealed that METTL3 knockdown in CD19+ B reduced EGR1 expression and influenced the BCR signal pathway.
Conclusion In summary, our findings demonstrated that aberrant expression of METTL3 in B-cell subjects contributed to the pathogenesis of SLE. METTL3 inhibition prevents B-cell terminal differentiation, which could serve as a potential target for therapeutic intervention in SLE.

PO-120

Analysis of Differentially Expressed Genes in Verruca Vulgaris versus adjacent normal skin by RNA-Sequencing

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Objective Verruca vulgaris is one of the most common low-risk HPV infections and is characterized by excessive proliferation of keratinocytes. Currently, genetic information regarding verruca vulgaris in the Chinese population is lacking. Therefore, this study aimed to obtain comprehensive transcript information of verruca vulgaris by RNA sequencing.
Methods High-throughput sequencing was performed on three fresh verruca vulgaris samples and adjacent normal skin on the Illumina sequencing platform. The transcriptomes were analyzed using bioinformatics and the differentially expressed genes (DEGs) were verified by immunohistochemistry.
Results Verruca vulgaris was shown to possess a unique molecular signature. In total, 1,643 DEGs were identified in verruca vulgaris compared to normal skin. We used Gene Ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway DEGs Reactome, disease annotation function, and String network analysis to study the DEGs meanning. The results revealed 595 GO terms associated with the cell cycle, signal transduction, immune system, and signaling molecules and interaction. DEGs Reactome analysis found reversible hydration of carbon dioxide and signaling by BMP enrichment, and the disease annotation function revealed that the enrichment of DEGs was in keratosis disorders. In the String protein interaction network, the edges with the highest density mainly included OAS family-related proteins. Furthermore, the M-code analysis found ISG15, IRF7, and OASL in significant modules and verified the high expression than the control.

Conclusion These findings contribute to the genetic information of verruca vulgaris in the Chinese population and found that interferon-stimulated genes (ISGs) may play essential roles in verruca vulgaris.

PO-121

A case of scabies nodule presenting as an infiltrative erythema

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A healthy 6-year-old boy presented with a 4-month history of erythematous nodules on the scrotum, presented with an itchy eruption, vesicles visible on sides and webs of the fingers, scattered erythematous papules in trunk, erythema visible on glans. The patient's personal and family medical history were unremarkable. The patient went to the local hospital and was diagnosed with scabies. Large numbers of mites can be seen by Scabies preparation. Symptomatic treatment with Sulphur ointment by the doctor. The lesions on the trunk of the patient are much less severe than before. But the lesions on the scrotum, penile shaft and glans have not improved. There were no lesions on his hands or feet.

Histopathology showed epidermal hyperplasia, diffuse lymphocytic infiltration in the dermis with numerous eosinophils and vascular hyperplasia in the dermis. The biopsy was compatible with a scabietic nodule.

Two months later the patient was re-examined revealing new nodules in the scrotum. The patient's scrotal nodules were frozen twice with liquid nitrogen and the nodules were injected with triamcinolone acetonide injection twice. This resulted in a general regression of the scrotal nodules and a reduction in the edema of the foreskin. After one month follow-up, the patient was considered cured.

Nodular scabies is a less common manifestation of classic scabies. Nodular scabies is characterized by persistent, firm, erythematous, extremely pruritic, dome-shaped papules 5 or 6 mm in diameter. The groin, genitalia, buttocks, and axillary folds are the usual sites of involvement. The nodules may represent a hypersensitivity reaction to prior or currently active scabies infestation. Since the nodules contain no mites, it is reasonable to assume that they develop as a reaction to the presence of scabies at other sites. This manifestation of the infection may nonetheless point to the diagnosis[1,2].

The histopathology of scabietic nodules is not diagnostic but serves to rule out other conditions. It shows a chronic, deep, perivascular and diffuse cell infiltrate with numerous eosinophils and a slightly increased number of mast cells. In nodular scabies, lesions may be clinically or histologically misdiagnosed as Langerhans' cell histiocytosis, insect bite reaction, or urticaria pigmentosa[3,4].

PO-122

Lupus erythematosus-lichen planus overlap syndrome: a case report

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A case of lupus erythematosus-lichen planus overlap syndrome is reported. The patient is a 58-year-old male. Erythema, papules and scales on the back for 6 months, involving the head and face for 5 months, and spreading all over the body for 20 days. Skin specialist examination: flake

purple erythema, papules and scales can be seen on the head, face, chest, back and upper limbs, and there is no relevant medical history in the past. Autoimmune antibody spectrum: ANA positive (1:80), PCNA weak positive, complement: C3 0.76g/L↓, C4 normal. Pathological findings of skin tissue of left upper arm showed hyperkeratosis of epidermis, local thickening of granular layer, slight atrophy of epidermis, liquefaction and degeneration of basal cells, infiltration of lymphocytes in superficial dermis with pigment incontinence, which was consistent with lichen planus. Consider diagnosis: lupus erythematosus-lichen planus overlap syndrome. After admission, he was treated with compound glycyrrhizin, glucocorticoid anti-inflammatory and total glucosides of paeony, and the rash subsided and he was discharged.

PO-123

Treatment Of Acne Vulgaris With Dissolving Microneedles Loaded With ph-Responsive Nanoparticles.

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Objective The excessive colonization of Propionibacterium acnes (P. acnes) is responsible for the genesis of acne vulgaris, a common inflflammatory disease of skin. However, the conventional anti-acne therapies are always limited by various side effffects, drug resistance, and poor skin permeability. Microneedles (MNs) are emerging topical drug delivery systems capable of noninvasively breaking through the skin stratum corneum barrier to effiffifficiently enhance the transdermal drug penetration. Herein, MNs loaded with intelligent pH-sensitive nanoplatforms were constructed for therapy against acne vulgaris, jointly exerting antimicrobial and anti-inflflammatory effffects.

Methods Calcium silicate nanoparticles (CS) were prepared using the chemical precipitation method and then immersed in an epigallocatechin gallate ester solution to prepare calcium silicate nanoparticles loaded with epigallocatechin gallate ester (CSE). The particles were characterized using scanning electron microscopy, X-ray diffraction (XRD), infrared spectroscopy (IR), and transmission electron microscopy.Subsequently, gelatin was graft-copolymerized with methacrylic anhydride to synthesize methacryloyl gelatin (GelMA). Micro-needle patches (CSE-MN) loaded with CSE were prepared using a polydimethylsiloxane (PDMS) mold. The morphology and structure of the micro-needles were observed using optical microscopy, scanning electron microscopy, and fluorescence microscopy. The composition of the micro-needles was analyzed using infrared spectroscopy. The in vitro release curves in solutions with different pH values were determined using UV-visible spectrophotometry. The mechanical properties of the micro-needles were evaluated using a pressure testing machine.After piercing the abdominal skin of rats, local rhodamine 6G staining and tissue pathology sections were performed to measure the depth of micro-needle insertion. The morphology of the needle tips at different time points was observed to evaluate their dissolution performance. Co-culturing of human skin fibroblasts (HSF) with the micro-needles was performed, and cell toxicity was assessed using a CCK-8 assay. The stimulation potential on the chorioallantoic membrane of chicken embryos (Hen's egg test on the chorioallantoic membrane, HET-CAM) was used to evaluate the skin irritation of the micro-needles. Co-culturing of the micro-needles with Propionibacterium acnes (P. acnes) was conducted to observe the growth of bacterial colonies and evaluate the in vitro antibacterial performance.An acne rat model was prepared using a multi-factorial modeling method involving the application of 2% coal tar and subcutaneous injection of P. acnes. After successful modeling, the rats were divided into a negative control group (PBS), a blank micro-needle patch group (MN), a positive control group (0.2% tretinoin cream), and a CSE-loaded micro-needle patch group (CSE-MN), with 10 rats in each group. The gross morphological changes, HE staining, and immunohistochemical staining (IL-1β, MMP2, IL-10, AMPK-β1 protein expression) before and after treating acne skin lesions were compared to evaluate the therapeutic efficacy and explore possible mechanisms of action.

Results Successfully prepared calcium silicate nanoparticles loaded with epigallocatechin gallate ester (CSE) and CSE-loaded micro-needle patches (CSE-MN). The patches had a square shape with dimensions of 2 cm * 2 cm. The needle array consisted of 20 * 20 needle tips, shaped like pyramids with dimensions of approximately 500 μm * 500 μm * 800 μm, and the spacing between needle tips was 700 μm. CSE-MN effectively pierced the skin and reached the dermis, and the needle tips exhibited solubility. The release of the drug from CSE-MN was faster at lower pH values, indicating pH-responsive behavior. Co-culturing CSE-MN with human skin fibroblasts (HSF) showed no cytotoxicity according to the CCK-8 results. The application of CSE-MN on the chorioallantoic membrane of chicken embryos (Hen's egg test on the chorioallantoic membrane, HET-CAM) confirmed its non-irritating nature on the skin. Co-culturing CSE-MN with Propionibacterium acnes (P. acnes) demonstrated excellent in vitro antibacterial performance. In the acne animal model experiment, comparing the gross morphological changes and tissue HE staining among the groups indicated the excellent therapeutic efficacy of CSE-MN. Immunohistochemical analysis of IL-1β, MMP2, IL-10, and AMPK-β1 expression showed that CSE-MN significantly reduced the expression of IL-1β, MMP2, and AMPK-β1 in acne skin lesions ($P < 0.05$). It also significantly increased the expression of the anti-inflammatory cytokine IL-10 ($P < 0.05$). CSE-MN exhibited a significant inhibitory effect on acne inflammation induced by P. acnes and reduced sebaceous gland secretion through the AMPK pathway.
Conclusion The fabricated ph-Responsive CSE-MN presented an outstanding anti-acne effiffifficiency both in vitro and in vivo.This bioresponsive microneedle patch is expected to be readily adapted as a generalized, modular strategy for noninvasive therapeutics delivery against superficial skin diseases.

PO-124

Folliculocentric tinea versicolor, a specific type of tinea versicolor secondary to keratosis pilaris?

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A 46-year-old woman presented to the clinic with trunk lesions for over 1 year. On physical examination: Multiple light brown patches of varying size centered on hair follicles extending from the armpits to the breast area, with the patches on the back fusing together and scales visible on the surface of the patches (Figure 1a, b). Fluticasone propionate cream was used intermittently throughout the course of the disease, but unfortunately, no significant improvement was observed. Histopathological examination:Mycelium and spores were seen in the stratum corneum, with epidermal atrophy, increased basal pigmentation, and a few lymphocytic infiltrates around the superficial dermal vessels(Figure 2a). Based on the pathological findings, further examination revealed positive staining with PAS, and direct mycological microscopy showed mycelium and spores in a "spaghetti and meatball" pattern (online supplementary Figure 1). Finally, the patient was diagnosed with folliculocentric tinea versicolor. Subsequently, the patient improved after one week of oral itraconazole (0.2 g/d), topical application of terbinafine hydrochloride cream, and washing with ketoconazole lotion for three weeks (Figure 1c, d). Direct mycological examination revealed no presence of spores. However, brown follicular papules persisted on the anterior chest (Figure 3a, b). On detailed histological examination, it was observed that the sections exhibited hyperkeratosis, with deeply keratinized hair follicles containing hairs. This finding is consistent with the pathological manifestation of "keratosis pilaris" (Figure 2b).
Discussion: Tinea versicolor is a common superficial fungal infection caused by Malassezia, commonly presenting as hyperpigmented or hypopigmented patches on the skin accompanied by mild scaling.1 The rare clinical types previously reported include inverse, papular, atrophic, and follicular, which is the diagnosed type in this case.2-5 Follicular presentation, due to its uncommon clinical presentation, it may be misdiagnosed as Darier's disease, confluent and reticulate papillomatosis,poikiloderma of Civatte, follicular mycosis fungoides, or even follicular psoriasis.

Therefore, a fungal examination may be performed to narrow down the diagnosis in patients with similar symptoms. It is still not clear what causes tinea versicolor to become folliculocentric.
After the definitive diagnosis of fungal infection, the disease was treated with antifungal drugs, resulting in a markedimprovement of the lesions and a subsequent negative fungal reexamination. However, follicular papules persisted on the anterior chest. On re-examination of the deep sectioned pathological slides, keratosis pilaris-like manifestations around the hair follicles were observed. Keratosis pilaris is a common follicular keratosis that is frequently observed on the extensor surfaces of the forearms, thighs and cheeks. However, generalized forms of keratosis pilaris have also been reported.6 In the literature, previous reports have described mycosis fungoides, psoriasis, porokeratosis, lichen planus, and ichthyosis with predominantly hair follicle involvement. However, the literature does not explicitly mention whether the patient has a history of keratosis pilaris. Previous studies have suggested that tinea versicolor may initially appear as small macules centered around hair follicles, but over the course of the disease, they may gradually lose their follicle-centric pattern and develop into larger hypopigmented rashes and plaques.7 However, we did not observe this trend of change in this patient. This case may suggest that patients with a history of keratosis pilaris may have a tendency to develop follicular centration in the course of other diseases. However, in patients without a history of keratosis pilaris, the common manifestations prevail. There is a need of further in-depth research into this issuse.

PO-125

Safety and effectiveness of ixekizumab in Chinese adults with moderate-to-severe plaque psoriasis: a prospective, multicenter, observational study

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Objective Psoriasis, an uncurable chronic inflammatory disease, affects over 6 million people in China. Ixekizumab, a monoclonal antibody against interleukin-17A, has demonstrated effectiveness and safety for the treatment of moderate-to-severe plaque psoriasis in clinical trials. However, there are currently limited data available regarding the use of ixekizumab in Chinese patients in real world settings. This real-world study was therefore undertaken to investigate the safety and effectivenesseffectivenesseffectivenessof ixekizumab for the treatment of moderate-to-severe plaque psoriasis in routine clinical practice in China.
Methods This prospective, observational, single-arm, multicenter, post-marketing surveillance study was undertaken in China to confirm the safety and effectiveness of ixekizumab for the treatment of moderate-to-severe plaque psoriasis in routine clinical practice. Adults with moderate-to-severe plaque psoriasis and prescribed ixekizumab were recruited from the dermatology departments of hospitals across China. All patients were prospectively followed for 12 weeks from baseline or until their last dose of ixekizumab. Patients were encouraged to attend a routine follow-up visit at around 2 weeks and 12 weeks after the first dose of ixekizumab. During the baseline visit, data were collected on patient demographics, psoriasis disease characteristics including treatment history, other medical history, and concomitant medications. The following data were collected at baseline, 2 weeks and 12 weeks after the first dose of ixekizumab: psoriasis area and severity index (PASI), Dermatology Life Quality Index (DLQI), and body surface area (BSA) affected by psoriasis. Safety data were collected during the suggested follow-up visits at 2 weeks

and 12 weeks. Patients were instructed to inform the investigator about any adverse events (AEs) that occurred between the visits. The safety of ixekizumab over 12 weeks was assessed by AEs and serious AEs (SAEs). The effectiveness of ixekizumab included the PASI 50 at 2 weeks and PASI 75, PASI 90, PASI 100, at week 12. The effectiveness of ixekizumab on BSA and DLQI at weeks 2 and 12, was also evaluated.

Results In total, 666 patients were enrolled from 26 hospitals. 663 were included in the safety analysis, and 612 in the effectiveness analysis. The mean ± SD age was 40.40 ± 13.30 years old, and the percentage of male was 71.9%. The mean weight was 71.55 ± 14.76 kg among safety population. Among all patients, the percentage of bio-experienced patients was 10.6%. At least one adverse event (AE) was reported by 42.7% (283/663) of patients, most of which were mild (242/283, 85.5%), and 32.7% (217/663) of patients reported AEs related to study treatment as judged by investigator. Only three patients had a serious AE. The mean PASI score at baseline was 18.16 ± 12.84, and the mean reduction from baseline in PASI score was 10.79 ± 9.55 at week 2 and 16.80 ± 12.15 at week 12. At week 2, 63.7% of patients achieved PASI 50 and at week 12, 93.2% of patients achieved PASI 75, 77.4% achieved PASI 90, and 45.1% achieved PASI 100. The mean BSA at baseline was 27.63 ± 22.10%, which decreased by 10.05 ± 14.22% and 24.28 ± 21.04% at week 2 and week 12. The mean DLQI at baseline was 12.57 ± 7.17, and only 2.8% of patients had a DLQI 0/1. At week 2, the mean reduction from baseline in DLQI was 5.91 ± 6.27, and 19.8% of patients achieved DLQI 0/1. At week 12, the mean reduction from baseline DLQI was 9.76 ± 7.16, and 59.9% of patients achieved DLQI 0/1.

Conclusion The results of this observational study show that ixekizumab was well tolerated in real-world clinical practice for the treatment of Chinese adults with moderate-to-severe plaque psoriasis. No new safety signals were observed. The safety profile of ixekizumab was consistent with that reported in a randomized phase 3 trial conducted in Chinese patients and with the predominantly Caucasian patients included in the global UNCOVER-1 to -3 trials. Furthermore, ixekizumab was highly effectivenessive when used in real-world clinical practice in China. These effectiveness findings are also consistent with the Chinese phase 3 trial and the global phase 3 trials.

PO-126

The Characteristics and Treatment Pattern of Psoriasis Patients with Nail Involvement in China: A Retrospective, Single-centered and Observational Study

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Objective Psoriasis is a chronic, incurable inflammatory skin disease characterized by red, scaly plaques that can develop across different body areas due to complex genetic-environmental-mediated epidermal hyperproliferation. Among the different body areas, nail is considered as one of the most difficult body areas to treat. Moreover, nail lesion is commonly identified as a predictor of psoriatic arthritis (PsA). This study aimed at evaluating the characteristics and treatment pattern of psoriasis patients with nail involvement.

Method This is a retrospective, single-centered and observational study based on a patient registry database from Huashan Hospital. Patients diagnosed with psoriasis and PsA were enrolled in this database from 2013. Demographic information (age, gender, etc.), disease severity (disease type, disease duration, body surface area (BSA), etc.) and treatment information (treatment history and current treatment) were collected at baseline and follow-up in the database. The present study included all the patients with nail involvement enrolled in the database from 2013 to 2021. Descriptive analysis (mean, standard deviation (SD) and proportion) was performed evaluating the patient characteristics and treatment pattern at baseline. Parametric (one way ANOVA) and non-parametric tests (Fisher's precision probability test, Chi-squared test and Kruskal-Wallis test) were

performed examining the difference in the characteristics and treatment information between patients with and without severe psoriasis (BSA > 10%).
Results This study included a total of 124 eligible patients. The age (mean ± SD, the same as follows) was 47.1±11.8 years old and 71.8% patients were male. The weight and BMI were 68.3 ± 14.1 kg and 23.7 ± 3.6, respectively. There were 29.5% patients with family history of psoriasis, and the percentage of patients with history of smoking and alcohol consumption were 39.3% and 23.0%, respectively. Most patients (95.2%) had plaque psoriasis with an average disease duration of 16.9 ± 9.9 years. The percentage of patients with BSA > 10% was 65% and the mean psoriasis area and severity index (PASI) score was 39.7 ± 17.5. The average duration of nail involvement was 6.1 ± 4.6 years, and the number of nails involved was 5.6 ± 3.3. The most common manifestations of nail psoriasis were nail pitting (69.9%), longitudinal ridges (48.8%), onycholysis (46.3%) and nail thickening (45.5%). There were 63.1% patients developing PsA about 3.7 ± 3.6 years after nail lesion occurred. The percentage of patients treated with conventional systematic therapy and biologics was 48.1% and 58.3%, respectively. Among biologic drugs, anti-TNF-α and anti-IL-17A antibodies were most used (30.1% and 27.2%). The results of subgroup analysis showed the percentage of male was significant higher in the group of BSA > 10% (78.5% vs. 54.3%, $P < 0.05$), with no other significantly different demographic variables detected. Besides, the percentage of patients treated by biologics was higher in the group of BSA > 10% (73.3% vs. 44.1%, $P < 0.01$).
Conclusion The results showed that most of psoriasis patients with nail involvement had severe psoriasis and most patients developed PsA after nail involvement. Nail pitting, longitudinal ridges, onycholysis and nail thickening were the most common manifestations of psoriatic nail lesion. Biologics were adopted in the treatment of only over half of the patients with nail involvement, mostly in the patients with severe psoriasis. Considering the limitation in sample size and single-center study design, more studies with larger simple size were still required to provide further evidence in the characteristics of psoriasis patients with nail involvement.

PO-127

ALA-PDT promotes the death and contractile capacity of hypertrophic scar fibroblasts through inhibiting the TGF-β1/Smad2/3/4 signaling pathway

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Objective Hypertrophic scars as one abnormal wound healing response of burn injuries, is characterized by massive fibroblasts proliferation, excessive deposition of extracellular matrix and collagen. Hypertrophic scars are a burden for patients due to physiological problems such as hyperplasia, functional limitations, itching, and pigmentation, and its can also lead to psychological and psychiatric disorders. The mechanisms of hypertrophic scar formation are not clear, and existing studies have shown that the TGF-β1/Smad signaling pathway plays a leading role in the occurrence and progression of hypertrophic scar fibrosis. Topical 5-aminolevulinic acid-based photodynamic therapy (ALA-PDT) treatment is clinically a non-invasive approach to hypertrophic scars. However, the effects of ALA-PDT on hypertrophic scars have not been well elucidated. Thus, we aimed to explore the possible mechanisms of ALA-PDT on hypertrophic scar fibroblasts.
Methods Hypertrophic scar tissues of burned patients were provided by surgical excision to isolate primary hypertrophic scar fibroblasts using the tissue block adhesion method. The purified cells were used immunofluorescence to detect vimentin protein expression and verified that the purified cells were hypertrophic scar fibroblasts. The morphology of normal and treated with ALA-PDT of hypertrophic scar fibroblasts were observed under light microscopy. The cell viability of hypertrophic scar fibroblasts was detected by Cell Counting Kit-8 (CCK-8) assay after treated with different concentrations of ALA and doses of red light. Hypertrophic scar fibroblasts populated collagen gel contraction assays were conducted to examine the effects of ALA-PDT on the

contractility of the fibroblasts induced by TGF-β1. In addition, the hypertrophic scar fibroblast collagen 3D collagen tissues were directly double-stained by PI and Hoechst to distinguish dead and viable cells, and the effect of ALA-PDT on the cytotoxicity of fibroblasts were observed by three-dimensional image reconstruction of laser confocal microscopy. The effect of ALA-PDT on TGF-β1-mediated smad2/3/4 signaling pathway activation and effector genes expression were verified by western blot and real-time quantitative PCR analysis.
Results Here we found significant changes in cell morphology after ALA-PDT treatment of hypertrophic scar fibroblasts, which the cells were retracted and became rounded and smaller, the protrusions were decreased and the intercellular space were increased for cell wrinkling. As ALA concentration and light dose increases, the cell viability of hypertrophic scar fibroblasts was significantly decreased. ALA-PDT can significantly alleviate the contractile capacity and promote the death of hypertrophic scar fibroblasts induced by TGF-β1 treatment in a three-dimensional collagen culture model. TGF-β1 treatment of hypertrophic scar fibroblasts can significantly induce phosphorylation of Smad2/3 (p-Smad2/3) in whole cells, as well as p-Smad2/3 and Smad4 proteins into the nucleus. TGF-β1 stimulated followed by ALA-PDT treatment, the p-Smad2/3 protein was decreased in whole cells and the content of p-Smad2/3-Smad4 in the nucleus were also reduced, and the mRNA levels of collagen 1/3 and a-SMA were significantly decreased. Furthermore, we found that ALA-PDT treatment can reduce the total Smad4 content of hypertrophic scar fibroblasts while pre-treatment of TGF-β1, but there was no significant difference in mRNA expression. Next, co-immunoprecipitation found that ALA-PDT treatment inhibits TGF-β1-induced formation of Smad2/3/4 complexes, and Smad4 underwent significant K48-linked ubiquitination degradation.
Conclusion These results provide the proof that ALA-PDT can inhibit fibroblast contraction and promote cell death by inhibiting the activation of the TGF-β1 signaling pathway that mediates hypertrophic scars formation, which may be the basis for the efficacy of ALA-PDT in the treatment of hypertrophic scars.

PO-128

Investigating the effect and mechanism of petroleum ether extract of Eclipta on chemotherapy-induced alopecia through network pharmacology

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Objective This study aimed to elucidate the effect and mechanism of petroleum ether extract of *Eclipta* (PEE) in preventing chemotherapy-induced alopecia (CIA) induced by cyclophosphamide (CYP).
Methods Network pharmacology analysis was used to explore the intersected targets and signaling pathways between the active components of PEE and CIA. Molecular docking was conducted to validate the binding between the active components and underlying treatment targets. In vitro experiments were conducted using 4-hydroxycyclophosphamide, an active metabolite of CYP, to establish CIA model in HaCaT cells. Cell viability assays were performed to determine the cell survival rate after treatment with CYP and PEE, while immunofluorescence staining was used to detect the expression of apoptotic factors.
In vivo experiments involved topical application of CYP to induce CIA model in C57BL/6 mice. A scoring system was used to observe and assess dorsal hair growth status. Hematoxylin and eosin (HE) staining was performed to observe changes in hair follicle status. Additionally, immunohistochemistry staining was utilized to detect the expression of characteristic apoptotic proteins, and immunoblotting was conducted to assess the changes in apoptotic and anti-apoptotic proteins.
Results Network pharmacology analysis revealed that the therapeutic pathways of PEE for CIA primarily focused on multiple apoptotic pathways. Molecular docking between selected

components from network pharmacology and apoptotic-related proteins showed favorable binding with binding energies below -7 kcal/mol.
In vitro experiments demonstrated that CYP induced cell death in HaCaT cells, while PEE reduced cell death by inhibiting extrinsic apoptosis.
In vivo experiments showed that CYP inhibited the growth of mouse hair follicle cells and induced hair loss. In contrast, PEE-treated mice displayed accelerated hair regrowth and a reduced proportion of follicles in catagen and telogen phases relatived to the CYP group. Immunohistochemistry results confirmed the effective inhibition of apoptosis in mouse hair follicles after PEE treatment. Immunoblotting results showed that the levels of cleaved caspase 3, a pro-apoptotic effector protein, and cleaved caspase 9, an intrinsic apoptotic marker, were significantly upregulated in the CYP group but significantly down-regulated after PEE treatment. The expression of P53 and Fas proteins was elevated in the CYP group, but decreased after PEE treatment.
Apoptotic proteins such as Bax, caspase 8, and caspase 3 were up-regulated in the CYP group but down-regulated after PEE administration, while the expression trend of the anti-apoptotic protein Bcl-2 was opposite to that of the apoptotic proteins.
Conclusion CYP-induced CIA activated the P53 pathway, leading to intrinsic apoptosis, while P53 mediated the activation of extrinsic apoptosis through Fas signal pathway. PEE reduced apoptosis by inhibiting the P53/Fas pathway, protecting hair follicle cells from damage, and ultimately preventing chemotherapy-induced alopecia.

PO-129

The antifungal effect and preliminary mechanism of NK cells against Fonsecaea monophora

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Chromoblastomycosis (CBM) is a chronic granulomatous disease caused by dark-colored fungal infection of the skin and subcutaneous tissues. Fonsecaea monophora (abbreviated as F. monophora) is the main etiological agent of chromoblastomycosis in southern China. NK cells play an important role in antifungal infections. Fungi could be recognized by NKp30 and NKp46 on NK cells and then induced NK cells to release perforin and granulysin which could kill fungi directly. NK cells alsosecrete cytokines to modulate innate immune response and adaptive immune response to kill fungi. The interplay between F. monophora and NK cells remains unknown. F. monophora
Objectives
1. Clarify the cytotoxic effect of NK cells on F. monophora in vivo and in vitro.
2. Preliminarily elucidate the immune response of activated NK cells to F. monophora.
Methods
1. Establish a mouse footpad infection model with F. monophora and inhibit NK cell activity in mice by intraperitoneal injection of anti-asialoGM1 antibody (40 μl every 3 days). Observe changes in footpad lesions in the experimental and control groups and perform fungal culture and colony counting on footpad tissues taken at 7 and 14 days post-infection.
2. Label NK cells isolated from peripheral blood of healthy individuals with CD56+ CD16+ CD3- fluorescent antibodies and perform flow cytometry to determine purity.
3. Co-culture NK cells isolated from peripheral blood of healthy individuals with F. monophora conidia for 24 hours, then collect the co-culture supernatant for fungal culture and colony counting. Compare with growth controls to detect the cytotoxicity of NK cells against F. monophora in vitro.
4. After co-culturing F. monophora conidia and NK cells for 24 hours, extract RNA and protein from NK cells for RT-qPCR detection and Western blot experiments. Measure the release of cytotoxic granules and cytokines in the co-culture supernatant by ELISA.
Results

1. After inhibiting NK cells, the footpad swelling in mice infected with F. monophora appeared earlier compared to normal mice, but skin lesions in both groups resoled by day 14 post-infection. Fungal culture of footpad tissues from both groups at day 14 post-infection showed significantly higher fungal loads in NK cell-inhibited mice compared to normal mice.
2. The purity of NK cell was >95%.
3. NK cells can directly kill F. monophora in vitro.
4. At the gene level, co-culturing F. monophora conidia with NK cells resulted in decreased mRNA expression of perforin, granzymes A and B, CD107a, CD69, IL-4, GM-CSF, and IFN-γ in NK cells. It also reduced the expression of granzymes, IL-6, IL-10, and TNF-α in NK cells. There were statistically significant differences in the mRNA expression of perforin, granzymes A and B, and granzymes in NK cells induced by F. monophora conidia. At the protein level, co-culturing Fm conidia with NK cells increased the protein expression of perforin, granzymes, CD107a, and CD69 in NK cells. It decreased the protein expression of granzymes A and B in NK cells. The protein expression of perforin in NK cells induced by Fm conidia had statistical significance. At the extracellular release level, Fm conidia affected the release of cytotoxic granules by NK cells. Fm conidia increased the release of granzymes A and perforin in the supernatant and decreased the release of granzymes B. After co-culturing, most NK cells released higher levels of cytokines in the supernatant.

Conclusion

1. Deficiency of NK cells leads to a reduced clearance ability of mice against F. monophora.
2. NK cells can directly damage F. monophora in vitro.
3. F. monophora conidia induce NK cells activation in vitro, increasing the protein and gene expression of cytotoxic granules such as perforin, promoting the release of inflammatory factors TNF-α, lFN-γ, lL-4, IL-6, GM-CSF, and cytotoxic granules.

PO-130

Establishment psoriasis-like dermatitis animal model by topical application of camellia oil with high peroxide value

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Objective

To construct and identify a mouse dermatitis model by using camellia oil with high peroxide value and to compare the constructed dermatitis model with the existing accepted model.

Methods

1. Exploring the peroxidized camellia oil concentrations that induce dermatitis model:
Balb/c mice were randomly divided into 5 groups, i.e. blank group, basic oil group, 800, 1200 and 1600 peroxide value camellia oil group. The reagent was applied topically on the mice back (10ul/cm²) for 7 days. The severity of dermatitis model was assessed through the skin phenotype and histopathology of mice back skin. We also observed the duration of dermatitis phenotype.
2. Identifying the dermatitis type of this dermatitis model mice:
After screening that camellia oil with 1600 peroxide value could induce dermatitis model, Balb/c mice were randomly divided into 2 groups, blank group and 1600 peroxide value camellia oil group (which was given topical application of 1600 peroxide value camellia oil 10ul/cm² daily). We recorded the weight of mice and calculated the SCORAD and PASI score of erythema, swelling and scaling of skin lesions until the 7th day. We also calculated the splenic index and examined the histopathological changes in the skin lesions after executing the mice. The spleen tissue and blood specimens were retained to detect the content of Th1, Th2, and Th17 cells by flow cytometric techniques; the skin tissue was also retained to detect the expression of Th1, Th2, and Th17 cytokine.
3. Comparing this dermatitis model with imiquimod-induced psoriasis-like dermatitis animal model

Balb/c mice were randomly divided into 3 groups, blank group, 1600 peroxide value camellia oil group (topical application of 1600 peroxide value camellia oil 10ul/cm² daily) and imiquimod-induced psoriasis model group (topical application of imiquimod 62.5mg daily). Then, we also recorded the data of weight, PASI score and splenic index, and examined skin histopathology, immune cells (Th1 and Th17 cells) ratio and Th1, Th17 cytokine for each group as before.

Results

1. After 7 days of continuous topical reagent application, no significant skin changes were observed in the blank group, the base oil group, and the 800 and 1200 peroxide value camellia oil groups compared to the first day, only the 1600 peroxide value camellia oil group showed obvious erythema, swelling and scaling phenotype on the back. The pathological results suggested that compared with the other groups, the 1600 peroxide value camellia oil group had thickened epidermis with scaling on the surface, edematous spinous layer and superficial dermis with inflammatory cell infiltration. Moreover, the phenotype was still aggravated by continuous application for 12 days.
2. Erythema and swelling of the back skin in the 1600 peroxide value camellia oil mice group appeared after 2 days of continuous administration, and scaling appeared on the 4th day. After 7 days of continuous topical reagent application, the skin phenotypic and pathological findings as before were repeatedly observed. Compared with the blank group, the 1600 peroxide value camellia oil group showed significant low mice weight, high spleen index and high PASI and SCORAD scores ($P < 0.05$). The qPCR results of skin tissue showed that the expression of Th2 inflammatory factors such as TSLP, IL-33, IL-4 and IL-13 were not significantly increased in the 1600 peroxide value camellia oil group compared with the blank group ($P > 0.05$); while Th1 and Th17 inflammatory factors such as TNF-α, IFN-γ, IL-1β and IL-17A were significantly increased ($P < 0.05$). In addition, peripheral blood and spleen flow cytology tests showed a significant high proportion of Th1 and Th17 type cells ($P < 0.05$), while not Th2 type cells ($P > 0.05$). Therefore, the 1600 peroxide value camellia oil-induced dermatitis model was more inclined to the psoriasis-like dermatitis model.
3. On the 7th day, psoriasis-like dermatitis was observed in both the imiquimod-induced model group and the 1600 peroxide camellia oil mice skin. Skin erythema, swelling and scaling were more severe in the 1600 peroxide camellia oil mice than the imiquimod group from the 5th day with a higher PASI total score ($P < 0.05$). Epidermal thickening, spiny layer hypertrophy and inflammatory cell infiltration in the dermis were observed in both 2 groups. There was no significant difference in the spleen index of mice in 2 model groups ($P > 0.05$). Compared with the blank group, the skin expression of TNF-α, IFN-γ, IL-1β and IL-17A in the imiquimod and 1600 peroxide camellia oil groups were increased ($P < 0.05$), and the expression was higher in the 1600 peroxide camellia oil group ($P < 0.05$). Peripheral blood and spleen flow cytology showed that the percentage of Th1 and Th17 cells both in the imiquimod and the 1600 peroxide camellia oil groups increased significantly compared with the blank group ($P < 0.05$), and the 1600 peroxide camellia oil group was higher ($P < 0.05$).

Conclusion The 1600 peroxide value camellia oil-induced dermatitis model was more inclined to a psoriasis-like model which had a faster time to modeling, a more severe phenotype and a longer maintenance time than the imiquimod-induced model.

PO-131

TEAD4 在皮肤黑素瘤的表达及意义

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Objective To investigate the expression of TEAD4 (Transcription Enhanced Association Domain, TEAD4) in Skin Cutaneous Melanoma (SKCM) and normal skin tissues, and its potential mechanism in SKCM pathogenesis.

Methods A total of 25 paraffin-embedded samples of normal skin tissues and 9 paraffin-embedded samples of SKCM were randomly collected. TEAD4 expression was detected using

immunohistochemistry. siRNA transfection was used to knockdown TEAD4 in A375 cells, and RT-qPCR was used to detect the mRNA expression of TEAD4 to clarify its knockdown. Cell apoptosis was detected by 7-AAD, PE Annexin V double staining.
Results TEAD4 expression was significantly different between normal skin tissues and SKCM patients ($P<0.05$). The positive rate of TEAD4 expression was 100% in normal skin tissues, but only 55.6% in SKCM. After siRNA transfection, the mRNA level of TEAD4 in A375 cells was significantly decreased ($P<0.05$), and cell apoptosis rate decreased ($P<0.05$).
Conclusions TEAD4 was low expressed in SKCM tissues. Low expression of TEAD4 in melanoma cell could influence cell apoptosis, which may be related to the pathogenesis of SKCM.

PO-132

The N6-methyladenosine RNA-binding protein YTHDF1 regulates B cell differentiation in systemic lupus erythematosus

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Background Systemic lupus erythematosus (SLE) is a complex autoimmune disease with multi-system involvement. It is multifactorial and involves epigenetic, genetic, ecological, and environmental factors. Autoantibodies produced by overactivated B cells play a critical role in the pathogenesis of SLE. Accumulating evidence have implied a potential association between posttranscriptional N6-methyladenosine (m6A) modification and B-cell-mediated humoral immunity. However, the mechanisms by which m6A modulates B cell activation, differentiation, and antibody production in SLE remain largely unknown.

Research Objective: To explore the function and mechanism of m6A binding protein YTHDF1 in the B-cell-mediated humoral immunity in SLE.
Methods 1. Peripheral blood mononuclear cells (PBMCs) from SLE patients and healthy controls (HCs) were collected, and the expression level of YTHDF1 protein in B cell subsets was detected by Flow cytometry. Splenic resting B cells of MRL/lpr mice and peripheral human naïve B cells were isolated and stimulated in vitro. The expression level of YTHDF1 was measured by Western blot or Flow cytometry.
2. Ythdf1 conditional knockout (cKO) mice were obtained by crossing Ythdf1-floxed mice with CD19-Cre mice. Ythdf1 cKO mice were immunized by Keyhole limpet haemocyanin (KLH) to investigate the function and mechanism of Ythdf1 in B cell development and humoral immune responses. Flow cytometry and enzyme-linked immunosorbent assay (ELISA) were applied to detect distinct B-cell subsets and serum antibody levels, respectively. Real-time quantitative PCR and Western blotting were used to examine gene expression at the mRNA level and protein level, respectively.
Results 1. Compared with HCs, the mean fluorescence intensity (MFI) of YTHDF1 was elevated in total CD19+ B cells, CD19+CD20+IgD+cd27- naive B cells, CD19+CD20+IgD-cd27+ memory B cells, and CD19+CD20-CD38+ plasma cells. Meanwhile, the MFI of YTHDF1 in CD19+CD20-CD38+ plasma cells from SLE patients was positively correlated with the SLE disease activity. splenic resting B cells from MRL/lpr mice showed increased expression of YTHDF1 after being stimulated in vitro.
2. Compared with wild type mice, Ythdf1 cKO mice showed increased proportions of germinal center B cells of lymph nodes and reduced frequencies of splenic naive B cells and memory B cells. In KLH model, YTHDF1 depletion in CD19+ cells increased the frequencies of splenic naive B cells, splenic germinal center B cells and germinal center b cells of lymph nodes. YTHDF1 cKO mice had reduced proportions of splenic memory B cells and plasma cells, as well as the consequent lower levels of IgM and IgG antibody production in the serum.

Conclusion Our study demonstrated that YTHDF1 expression was uniformly increased in SLE B cell subsets, the expression of which in plasma cells was positively correlated with SLE disease activity. YTHDF1 deficiency significantly inhibited the terminal differentiation of B cell and antibody production, which may serve as a potential therapeutic target in SLE.

Keywords：SLE, m6A, B cells, YTHDF1

PO-133

Nucleo-cytosolic acetyl-CoA drives tumor immune evasion via the epigenetic regulation of PD-L1 expression in melanoma

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Aim Acetyl-CoA is a versatile central metabolite implicated in multiple metabolic paradigms, protein acetylation and epigenetic modification. The effects of acetyl-CoA metabolism on tumor cell characteristics have been extensively revealed, whereas the linkage between tumor acetyl-CoA and immune evasion remains unclear.
Methods Systemic bioinformatics analysis and *in vivo* pre-clinical mice model were employed to examine the role of acetyl-CoA metabolism in the regulation of anti-tumor immunity in melanoma. A panel of biochemical assays were used to investigate the role of nucleo-cytosolic acetyl-CoA in the regulation of the anti-tumor capacity of $CD8^+$T cells, as well as the expression of PD-L1. The correlation between ACLY-H3K27ac-PD-L1 axis and the response to immunotherapy, as well as the prognosis of patients, was verified ultimately.
Results Bioinformatics analysis reveals that acetyl-CoA metabolism is negatively associated with anti-tumor immunity across many types of cancers. Pharmacological suppression of acetyl-CoA-producing enzyme ACLY leads to re-invigoration of TILs and potentiates immunotherapy efficacy. Mechanistically, nucleo-cytosolic acetyl-CoA derived from multiple pathways promotes PD-L1 transcription rather than its post-translational modification, with P300-dependent histone acetylation implicated in. ACLY-H3K27ac-PD-L1 axis is verified in clinical specimens and predicts poor immunotherapy response and worse prognosis.
Conclusions We demonstrate that nucleo-cytosolic acetyl-CoA drives tumor immune evasion via the epigenetic regulation of PD-L1 in melanoma. The perturbation of acetyl-CoA metabolism may act as a promising strategy to mitigate immune evasion and optimize the outcome of cancer immunotherapy.

PO-134

Exploring Potential Biomarkers and Molecular Mechanisms of Cutaneous squamous cell carcinoma Based on Bioinformatics

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Objective Cutaneous squamous cell carcinoma (cSCC) arises from epidermal keratinocytes and is the second most common malignant skin tumor clinically. Despite some advances in diagnosis and treatment, cSCC still exhibits strong malignancy and poor prognosis. In recent years, the incidence of cSCC has been steadily increasing, posing a significant threat to public health. This study aims to screen for novel biomarkers and provide new diagnostic and therapeutic options for cSCC patients.

Methods Microarray datasets of cSCC (GSE66359 and GSE117247) were downloaded from the GEO database. Differential expression genes (DEGs) between cSCC and normal skin tissue were identified after data merging and batch effect correction. Gene Ontology (GO) analysis was performed to reveal key biological functions in the pathogenesis of cSCC. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis showed molecular pathways associated with cSCC. Protein-protein interaction (PPI) analysis and weighted gene co-expression network analysis (WGCNA) were conducted to explore key modules and genes related to cSCC. The final key genes were determined based on the intersection of PPI and WGCNA analysis results. Immunohistochemistry experiments were performed to validate key genes that have not been reported to be associated with cSCC, based on receiver operating characteristic (ROC) curve analysis and literature reports. Hub genes were predicted for target miRNAs and lncRNAs, and ceRNA networks was constructed using online databases (miRDB, miDIP, RNA22, TargetScan, RNAInter, and Starbase).
Results A total of 1,027 DEGs, including 505 upregulated and 522 downregulated genes, were identified in the GSE66359 and GSE117247 datasets. GO analysis revealed that the differentially expressed genes were mainly involved in biological functions such as the cell cycle. KEGG analysis demonstrated the enrichment of genes associated with cSCC in signaling pathways such as the cell cycle and DNA replication. Key genes, including CDK1, CCNA2, CCNB2, and UBE2C, were identified through PPI and WGCNA analysis. Immunohistochemistry validation was performed for CCNA2, CCNB2, and UBE2C, which have not been reported to be associated with cSCC. The expression of CCNA2, CCNB2, and UBE2C was significantly higher in cSCC tissue compared to normal skin tissue. Finally, three ceRNA networks, NEAT1/H19-hsa-miR-148a-3p-CCNA2 and NEAT1-hsa-miR-140-3p-UBE2C, were constructed using online databases.
Conclusion CCNA2, CCNB2, and UBE2C may serve as potential biomarkers for the diagnosis and treatment of cSCC. The NEAT1/H19-hsa-miR-148a-3p-CCNA2 and NEAT1-hsa-miR-140-3p-UBE2C ceRNA networks may be involved in the progression of cSCC.

PO-135

Intravenous Lidocaine Infusion for the Alleviation of Erythromelalgia: A Case Report

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An 11-year-old male patient was admitted to our department on April 7, 2023, presenting with a one-year history of recurrent erythema and burning pain in both lower limbs, which had deteriorated over the past month. The patient exhibited numerous erythematous and swollen lesions without an identifiable trigger. Some of the lesions were ulcerated and crusted, accompanied by a burning sensation, pain, and localized increase in skin temperature. Symptoms were aggravated by heat or exercise and relieved by soaking in cold water. The patient initially sought medical attention at a local hospital, where he was diagnosed with "atopic dermatitis" and treated with topically applied desonide and tacrolimus, but showed no significant improvement. Over time, the rash progressed and extended beyond the knee joints, and displayed diffuse erythema and swelling on the dorsa of both hands. The rash was accompanied by desquamation and several areas of ulceration and crusting. The patient had no significant medical history of concurrent systemic diseases or genetic disorders. The patient's mother had experienced similar symptoms at the age of 13, which resolved spontaneously without treatment. Physical examination revealed diffuse erythema and swelling in both lower legs and on the dorsum of the hands, increased skin temperature, scattered ulcerated areas with the largest measuring 7 cm x 3 cm, accompanied by purulent discharge. Tenderness was elicited upon palpation. Laboratory results showed a CRP level of 61 mg/L, an ESR level of 42 mm/h, a platelet count of 473 x 10^9/L, and ALT and GGT levels of 65 U/L and 34 U/L, respectively. Urine routine tests, renal function tests, rheumatoid immune markers, echocardiography, and chest X-ray revealed no significant abnormalities. Abdominal ultrasound

revealed an 8 mm separation of the left renal pelvis, while the right side appeared normal. Histopathological examination of the skin lesion at the edge of the lower limb ulcer revealed a local epidermal defect replaced by crust, adjacent areas with excessive and incomplete keratinization, plasma exudation, epidermal hyperplasia, sponge-like edema, neutrophil infiltration accompanied by lymphocyte infiltration around the deep dermal blood vessels. Furthermore, individual layers of blood vessel walls showed cellulose deposition and indistinct structure, and there was intense staining without apparent nuclear division. Genetic testing identified a mutation at the SCN9A gene site NM_001365536.1:c.3956C>T in the patient and his mother. No SCN9A gene mutations were found in his father. The patient was administered oral mexiletine at a dose of 0.15g twice daily, underwent a two-week slow intravenous infusion of lidocaine at a dose of 0.1g once daily, received a five-day intravenous infusion of calcium gluconate at a dose of 0.5g once daily, and was prescribed gabapentin at a dose of 0.6g once nightly for pain relief. Furthermore, the ulcerated areas underwent daily debridement and dressing changes. The patient is currently attending regular follow-up visits in our department, and the ulcerated areas are nearly healed.

PO-136

Shenyingyangzhen formula promotes hair regeneration by up-regulating Akt/HIF-1α pathway to eliminate oxidative stress around hair follicles and promote angiogenesis

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Background Androgenetic alopecia (AGA) is highly prevalent in contemporary society but lacks effective treatments. The dysregulation of the hair follicle niche, induced by excessive reactive oxygen species (ROS) and insufficient vascularization in the perifollicular microenvironment, is the leading cause of AGA. The Shenyingyangzhen formula (SYF) from Chen Shigong's "Surgery Authentic" in the Ming Dynasty has been clinically used to treat alopecia and other diseases by raising blood circulation and eliminate wind to promoting hair growth. In this study, we propose that SYF may reshape the microenvironment around hair follicles by alleviating oxidative stress and promoting angiogenesis for the treatment of AGA.

Methods The chemical constituents of SYF were identified by ultra-high performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q/TOF-MS) analysis. Using network pharmacology to analyze and predict the key targets and signaling pathways of SYF in treating AGA. AGA mouse model was induced by testosterone propionate treatment. The effects of SYF on hair regeneration in AGA mice were measured by hair growth measuring and histological scoring. Immunofluorescence staining, quantitative RT-qPCR and Western blotting were used to detect chemical clues and Signaling molecule involved in the process of hair follicle cycle, as well as indicators and Signaling molecule related to oxidative stress and angiogenesis. Cell viability assay and Ki-67 immunostaining were undertaken to evaluate the impacts of SYF on human hair dermal papilla cell (HDPC) proliferation. Wound-healing assay was undertaken to assess cell migration.

Results The network pharmacology analysis revealed a potential association between SYF treatment and the intervention of the Akt/HIF-1α signaling pathway in AGA. In the AGA mouse model, histological results demonstrated that oral administration of SYF increased skin thickness, the number of anagen hair follicles, and perifollicular angiogenesis. Moreover, the expression of Ki67, SOX9 (a marker for hair follicle stem cells), and p-S6 was enhanced in the skin of SYF-treated mice, while the expression of CD31 was increased and ROS expression was decreased. RT-qPCR analysis revealed downregulation of follicle degradation-related factors DKK1, IL-6, and TGFβ1, along with increased expression of key targets Akt, HIF1α, and VEGFA in the Akt/HIF-1α signaling pathway. In the DHT-HDPC cell model, SYF promoted the proliferation and migration of HDPC cells and increased the Bcl-2/Bax ratio, thereby inhibiting HDPC cell apoptosis.

Conclusion SYF exerts its therapeutic effects on AGA by improving the microenvironment surrounding hair follicles through the upregulation of Akt, HIF1α, and VEGFA expression in the Akt/HIF-1α signaling pathway, thereby promoting angiogenesis. Which might provide an effective therapeutic strategy for the treatment of hair loss and alopecia.

PO-137

Study of Merkel cell homeostasis in Capecitabine induced Hand-foot syndrome

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Objective Capecitabine is an oral prodrug that is converted by thymine phosphorylase to its only active metabolite, fluorouracil (FU). Hand-foot syndrome (HFS) is a common adverse reaction of anti-tumor drug capecitabine, mainly manifested as palmar and plantar sensory loss and erythema-specific skin syndrome.It initially presents as red spots, numbness, tingling, varying degrees and even extreme paresthesia in the palms and feet. Capecitabine can change the skin microenvironment by inducing keratinocyte apoptosis pathway activation and mitochondrial membrane potential decline, but the pathogenesis of HFS is still unclear. Merkel cells (MCs) are epidermal cells that feel light touch and abnormal expression has been reported in the inflammatory skin. Therefore, we speculate that HFS affects the living environment of MCs in the basal layer of the epidermis, and may affect the ability of cells to transmit touch signals to nerve endings.Here, we attempt to explore the effect of tactile abnormalities caused by capecitabine-induced adverse reactions on MCs by establishing a mouse model of HFS.
Method The HFS model was established in 8-week-old ICR mice. The paw of mice was photographed and the morphological changes of paw skin were observed by H&E experiment. The IL6 in the paw skin of mice was detected by IHC.The Tactile behavior was assessed according to the degree of response of the mouse paws. The changes of MCs in the skin of mouse paw were observed by whole skin staining technique.
Result Capecitabine could induce HFS model in mice. The palms of feet of HFS model were significantly redder than those in the Control group. H&E staining showed clear structure of basal cell vacuoles in the Treat group. The level of IL6 in the footskin of HFS mice was up-regulated compared with that of the Control group. The mice in the Treat group showed obvious tactile retardation and the number of MCs was significantly increased compared with the Control group.
Conclusion The tactile perception of mice in HFS model group induced by capecitabine was impaired. The number of tactile receptor MCs increased abnormally in the palm of feet of HFS model. These results suggested that the transmission of touch is hindered in HFS model, increase of MC might be involved in the abnormalty of tactile perception.

PO-138

Mechanism of LncRNA PVT1/miR-30e-5p Regulation of CD4+ T cell Imbalance in SLE

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Objective Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease (AD) of unknown etiology involving immune intolerance to endogenous nuclear substances, increased

levels of autoreactive B cells, and chronic inflammation leading to systemic autoimmune and organ. Imbalance of CD4+T cell,(Th), such as T helper (Th) cell 1, Th2, Th17 and Treg, is involved in the development of SLE and is associated with disease activity. Therefore, understanding the pathophysiological mechanisms of abnormal T-cell function in SLE is important for the diagnosis and targeted therapy of patients.

Methods 40 MRL/lpr mice were randomly divided into 4 groups: SLE group, SLE+Lenti-Ctrl group (Ctrl), SLE+Lenti-si-PVT1 group (si-PVT1) and SLE+Lenti-PVT1 (PVT-OE) group, 10 mice in each group. The gene expression of LncRNA PVT1 and miRNA-10e-5p in peripheral blood of SLE patients and healthy controls (HCs) was detected by quantitative real-time polymerase chain reaction (QRT-PCR) technique. The gene expression of LncRNA PVT1, miRNA-10e-5p, T-bet, GATA3, RORγt and Foxp3 in peripheral blood of si-PVT1 and PVT1-OE MRL/lpr lupus mice was detected. The proportions of Th1/Th2/Th17/Treg cells in the peripheral blood of SLE patients and HCs, and the proportions of Th1/Th2/Th17/Treg cells in the peripheral blood of si-PVT1 and PVT1-OE MRL/lpr lupus mice were detected by flow cytometry. Enzyme-linked immunosorbent assay (ELISA) was used to detect IL-2/IL-4/IL-17/TGF-β concentrations in the sera of SLE patients and HCs, as well as IL-2/IL-6/IL-17/TGF-β concentrations in the sera of si-PVT1 and PVT1-OE MRL/lpr lupus mice. 24-hour urinary protein levels in MRL/lpr lupus mice were measured by Coomassie blue staining.

Results QRT-PCR for gene expression in peripheral blood of SLE patients showed that gene expression of lncRNA PVT1 was significantly higher and gene expression of miR-30e-5p was significantly lower in peripheral blood of SLE patients compared with HCs. The results of flow cytometry showed a significantly higher percentage of Th2 cells, a significantly higher percentage of Th17 cells, and a lower percentage of Th1 and Treg cells but the difference was not significant in the peripheral blood of SLE patients compared to HCs. ELISA results showed that IL-2 was significantly lower, IL-4 was significantly higher, IL-17 was significantly higher and TGF-β was significantly lower in the peripheral blood of SLE patients compared with HCs. Compared with the Ctrl, si-PVT1 MRL/lpr mice showed significantly lower 24-hour urine protein, significantly lower anti-dsDNA antibody content, significantly lower lncRNA PVT1 gene expression, and significantly higher miRNA-30e-5p gene expression. Compared with the Ctrl, PVT1-OE MRL/lpr mice showed significantly higher 24-hour urine protein, significantly higher anti-dsDNA antibody content, significantly higher lncRNA PVT1 gene expression, and significantly lower miRNA-30e-5p gene expression. Compared with the Ctrl, si-PVT1 MRL/lpr mice, the proportion of Th1 and Treg cells was significantly increased, the proportion of Th2 and Th17 cells was significantly decreased, and the Th1/Th2 ratio was significantly increased and the Th17/Treg ratio was significantly decreased. Compared with the Ctrl, PVT1-OE MRL/lpr mice, the proportion of Th1 and Treg cells was significantly decreased, the proportion of Th2 and Th17 cells was significantly increased, and the ratio of Th1/Th2 was significantly decreased and the ratio of Th17/Treg was significantly increased. Compared with the Ctrl, si-PVT1 MRL/lpr mice had significantly higher gene expression of T-bet and FOXP3 and significantly lower gene expression of GATA3 and RORγt. Compared with the Ctrl group, PVT1-OE MRL/lpr mice had significantly lower gene expression of T-bet and FOXP3 and significantly higher gene expression of GATA3 and RORγt. Compared with the Ctrl, the concentrations of IL-2 and TGF-β was significantly increased and the expression of IL-17 and IL-6 was significantly decreased in si-PVT1 MRL/lpr mice. Compared with the Ctrl, the concentrations of IL-2 and TGF-β was significantly decreased and the expression of IL-17 and IL-6 was significantly increased in PVT1-OE MRL/lpr mice.

Conclusion Our data demonstrate that PVT1 expression is increased specifically in female SLE patients, that targeting PVT1 affects Th1/Th2 and Th17/Treg homeostasis, and that lncRNA PVT1/miRNA-30e-5p may play an important role in the regulation of immune responses and validation in SLE through the competing endogenous RNAs(ceRNAs) mechanism.

PO-139

Platelet (PLT), eosinophil (EOS), and albumin (ALB) are useful indicators for evaluating the risk of developing lupus nephritis (LN) in patients with systemic lupus erythematosus (SLE)

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Objectives Although there have been extensive investigations on platelet (PLT), eosinophil (EOS) and albumin (ALB) in many diseases, their roles in systemic lupus erythematosus (SLE) with lupus nephritis (LN) remain unclear. Thus, the present study aimed to evaluate the value of PLT, EOS and ALB levels and provide guidance for the clinical application of PLT, EOS and ALB detection in Chinese SLE patients with LN.
Methods Among 2060 enrolled SLE patients undergoing hospitalization, we included a total of 73 patients diagnosed with LN and 325 SLE patients without LN, who completed the measurement of blood and LN screening between 2018 and 2022. All clinical characteristics and the blood measurement information of SLE patients were extracted and analyzed from the medical records. Univariate and multivariate logistic regression analysis were used to evaluate the possible relationship of PLT, EOS, ALB to LN. Receiver operating characteristic (ROC) curve analysis was also performed to assess the discriminative ability of three ratios in predicting LN. The nomogram was performed to facilitate individualized estimation of the risk of lupus nephritis in SLE patients.
Results The LN group had lower PLT, EOS, and ALB levels than the SLE group (P<0.01). Univariate logistic regression analysis indicated that three risk factors for LN were identified, including PLT (OR=0.393, 95% CI 0.172–0.896, P=0.026), EOS (OR=0.108, 95% CI 0.027–0.439, P=0.002), and ALB (OR=0.351, 95% CI 0.127-0.972, P=0.044). Multivariate logistic regression analysis also showed that compared with the low groups, the high PLT group, high EOS group, and high ALB group had a lower risk of LN. In addition, ROC analysis and the nomogram comprised of PLT, EOS, and ALB revealed that these three predictors were determined as predictive indicators of LN in SLE patients and exhibited sufficient predictive accuracy, with the area under the characteristic curve (AUC) of 0.720 [95% confidence interval (CI) 0.658–0.782].
Conclusions Decreased levels of PLT, EOS, and ALB might be correlated with increased risk of LN in Chinese SLE patients.

PO-140

The mechanism research of Sancai Decoction combined with Fractional CO2 Laser regulate SASP to inhibit MDSCs and enhance NK activity to delay skin aging

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Objective Explore the Sancai Decoction with Fractional CO2 laser to delay skin aging, and further explore Sancai Decoction to postpone skin aging mechanism.
Methods The subacute skin senescence Model of Balb/C mice was constructed by subcutaneous injection of D-galactose, and randomly divided into WT group (n=6), Model group (n=6), SCT group (n=6), SCT+ FL group (n=6). The changes of skin structure were observed by HE staining, and the changes of aging were observed by SA-β-gal staining. RT-qPCR and western blot were used to detect mouse p21, p16 to verify aging HYP, COL1, AQP3 to detect mouse skin function

indexes. The SASP contained many cytokines IL-1α, IL-1β, G-CSF, GM-CSF, IL-6, IL-2, CCL2, CCL3, CCL4, CCL5, CXCL2, MMP1, CXCR4 to observe the effect of Sanai Decoction and Fractional CO2 laser on skin SASP. Flow cytometry was used to detect the changes of MDSC and NK cells in peripheral blood, spleen and skin tissue of mice, and RT-qPCR and western blot were used to detect the activity indexes of MDSC, iNOS and S100A9 of mice, in order to verify the effects of SASP on immune cells MDSC and NK, so as to play a role in delaying skin aging.
Results HE staining showed that Sancai Decoction combined with Fractional CO2 laser effectively alleviated the phenomenon of epidermal thinning, epidermal dermal junction curvature flattening and dermal collagen fracture. SA-β-gal staining showed that the positive points of mice taking Sancai decoction and Fractional CO2 laser decreased. The mRNA expressions of p21 and p16 were decreased, the mRNA expressions of HYP, COL1 and AQP3 were increased, and the expression of SASP related factors was decreased after treatment by RT-qPCR. Flow cytometry detected that MDSC expression decreased and NK activity increased after taking Sancai decoction. RT-qPCR and western blot detection of MDSC cell activity index also confirmed that MDSC activity decreased after treatment.
Conclusion Sancai Decoction combined with Fractional CO2 laser therapy can effectively alleviate the progression of mouse skin aging by down-regulating the recruitment of MDSC by cytokines in senescence related secretory phenotype SASP, inhibiting the number and activity of MDSC and enhancing the activity of NK to delay skin aging.

PO-141

Neuroimmunomodulation study of CGRP on the expression levels of inflammatory cytokines in dendritic cells in psoriasis.

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Rationale Psoriasis is an immune-mediated chronic autoimmune disease in which the skin lesions are characterized by white scales on the basis of erythema. It is found that mental tension and anxiety are one of the causes of psoriasis. Nerve damage and dysfunction can alleviate the symptoms of psoriasis, which suggests that neuroimmune regulation plays an important role in the pathogenesis of psoriasis.
Skin dendritic cells (DCS) are specialized antigen-presenting cells that play an important role in the initial stage of psoriasis. It can be divided into several subgroups, which are plasmacytoid deneritic cell(pDC), conventional dendritic cell 1(cDC1), conventional dendritic cell 2(cDC2) and Langerhans cell(LC). The most important feature of plasmacytoid deneritic cells (pDC) is that they can secrete a large amount of type I interferon after activation, which is the initiation and key factor in the early onset of psoriasis. CpG ODN combine with TLR9 and its surface activation can promote pDC mature, activation of pDC produce interferons alpha, TNF alpha cytokines, etc. LPS acts on Toll-like Receptor (TLR)4 receptors on conventional dendritic cells (cDC) to activate cDC. cDC produces IL-12 and IL-23, initiating and stimulating immune responses dominated by Th1, Th17 and Th22 cells, respectively. cDC1 is a subgroup in cDC that mainly mediates type 1 immune response. Activation of the immune response leads to the formation of the inflammatory cycle, the production of inflammatory cytokine storms, keratinocyte proliferation, infiltrating erythema, vascular proliferation and dilatation, and other psoriasis like reactions.
Calcitonin gene related peptide (CGRP) is a neuropeptide found in the C and Aδ sensory nerve fibers of the peripheral and central nervous systems. CGRP receptors are found on the cell membranes of dendritic cells, keratinocytes, T lymphocytes, and dermal vascular endothelial cells. Nerve fiber endings in the skin release CGRP, which acts on the CGRP receptor and aggravates the local inflammatory response, thus participating in the pathogenesis of psoriasis. Therefore, neuropeptide CGRP can participate in the pathogenesis of psoriasis by regulating the secretion of

cytokines by DC cells. However, the effects and mechanisms of CGRP on dendritic cell subsets in psoriasis have not been fully understood.
Purpose To explore the effects of CGRP on psoriasis related cytokines (TNF-α, IFN-α) secreted by mouse spleen pDC and psoriasis related cytokines (IL-12, IL-23) secreted by cDC1, and further explore the role of neuropeptides in the pathogenesis of psoriasis.

Methods
1. Spleen of male BALB/c mice aged 7-8 weeks was isolated in vitro, and cDC1 and pDC were extracted and cultured by magnetic bead labeling separation method. The activity and number of cells were measured by Trypan blue staining under microscope. The purity of cDC1 (CD11c-APC and CD8a-PE) and pDC (CD11c-APC and CD45R-PE) obtained by magnetic bead separation was determined by flow cytometry.
2. Cultured pDC and cDC1 were activated by ODN2216 and LPS respectively, and then stimulated by CGRP and CGRP receptor antagonist CGRP-837 in vitro. The mRNA secretion of psoriatic cytokines TNF-α, IFN-α, IL-12 and IL-23 by pDC and cDC1 was determined by RT-PCR. ELISA was used to detect the secretion of psoriatic cytokines TNF-α, IFN-α, IL-12 and IL-23 by pDC and cDC1, respectively.
Results CGRP (10-9mol/L, 24h) inhibited the expression of TNF-α mRNA and TNF-α protein in unactivated and activated pDC, and the inhibitory effect was weakened after CGRP 8-37 blockade. CGRP promotes the expression of IFN-α mRNA and IFN-α protein in pDC. The promotion effect of activated pDC was more obvious. After being blocked by CGRP 8-37, its promoting effect was weakened. CGRP can promote the expression of IL-12 mRNA and IL-12 protein in cDC1 after activation. After being blocked by CGRP8-37, the promoting effect of CGRP on cDC1 was weakened. The difference among all groups was statistically significant ($p < 0.05$). CGRP had no significant effect on the expression of IL-23 mrna and IL-23 protein in cDC1.
Conclusion
1. CGRP can inhibit the expression of TNF-α mRNA and the secretion of TNF-α in unactivated and activated pDC; CGRP promotes the expression of IFN-α mRNA and the secretion of IFN-α in unactivated pDC, and its promotion effect is enhanced for activated pDC.
2.CGRP can stimulate activated cDC1 to express IL-12 mRNA and secrete IL-12, but has no significant effect on IL-23.
3. Nerve endings release neuropeptide CGRP, which can regulate the secretion of cytokines by dendritic cells through its receptors.

PO-142

The updates and implications of cutaneous microbiota in acne

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Acne is a chronic inflammatory skin disorder that profoundly impacts the quality of life of patients worldwide. While it is predominantly observed in adolescents, it can affect individuals across all age groups. Acne pathogenesis is believed to be a result of various endogenous and exogenous factors, but the precise mechanisms remain elusive. Recent studies suggest that dysbiosis of the skin microbiota significantly contributes to acne development. Specifically, Cutibacterium acnes, the dominant resident bacterial species implicated in acne, plays a critical role in disease progression. Various treatments, including topical benzoyl peroxide, systemic antibiotics, and photodynamic therapy, have demonstrated beneficial effects on the skin microbiota composition in acne patients. Of particular interest is the therapeutic potential of probiotics in acne, given its direct influence on the skin microbiota. This review summarizes the alterations in skin microbiota associated with acne, provides insight into its pathogenic role in acne, and emphasizes the potential of therapeutic interventions aimed at restoring microbial homeostasis for acne management.

PO-143

Successful treatment of terminal osseous dysplasia with pigmentary defects with JAK inhibitor

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Terminal osseous dysplasia with pigmentary defects (TODPD) syndrome is a rare X-linked dominant disorder characterized by pigmentary skin defects, recurrent cutaneous digital fibromas, and skeletal anomalies. TODPD patients often experience significant hand function loss and a diminished quality of life. Currently, there is no known cure for TODPD, and the available treatment options remain predominantly supportive, yielding suboptimal therapeutic outcomes. Recent studies have identified two gain-of-function variants, c.5217G>A and c.5217+5G>C, in the filamin A-encoding FLNA gene, although the specific mechanisms underlying this rare fibromatosis have yet to be fully elucidated.

In this case study, we present the clinical features of a 13-month-old Chinese girl diagnosed with TODPD. Notably, our findings reveal an unrecognized association between TODPD and a hyper-inflammatory state, prompting us to explore potential treatment strategies. Consequently, we initiated oral baricitinib, a JAK 1/2 inhibitor widely employed in managing inflammation-related conditions and fibromatosis. Over the subsequent six months, baricitinib exhibited notable objective benefits, including reductions in tumor size and improvements in joint contractures, without any reported treatment-related adverse events. Further functional studies conducted on fibroblasts derived from the patient provided novel insights into the dysregulated cell signaling pathways likely caused by the mutant Filamin A protein. Our findings suggest that JAK inhibition may represent a viable therapeutic intervention for the fibroproliferative features observed in TODPD by potentially mitigating the upregulation of TGF-β and the activation of the associated PI3K/AKT/NF-κB pathway. In summary, the use of baricitinib shows promise as a potential treatment option for fibromatosis in TODPD. Nonetheless, additional research is warranted to comprehensively understand the underlying mechanisms and optimize therapeutic strategies for affected individuals.

PO-144

Study on the regulation and mechanism of Baicalin on the growth and development of hair follicles

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Objective To explore the potential mechanism of baicalin (BAI) in promoting the growth and development of hair follicles.

Methods Based on network pharmacology and molecular docking techniques, the targets of BAI for hair growth promotion were predicted. A hair follicle regeneration model was established using female C57BL/6 mice, and the skin of the mice was photographed at different time points to evaluate the skin appearance and histological analysis was performed by hematoxylin eosin (H&E) staining. The expression levels of TLR4/NF-κB signaling pathway-related proteins were detected by Western Blot. HaCaT cells were treated with different concentrations of BAI for 24 h. Cell viability was detected by Cell Counting Kit-8 (CCK-8). The expression levels of TLR4/NF-κB signaling pathway-related proteins were detected by Western Blot after the inflammation model was established using LPS-induced HaCaT cells.

Results The vivo experiment results showed that BAI promoted hair follicle regeneration in mice in a dose-dependent manner and shortened the transition time from resting to anagen phase in mice. The results of H&E staining showed that BAI pretreatment significantly increased the number of hair follicles, hair follicle length and hair follicle diameter in mice skin. Network pharmacology

analysis showed that there were 74 intersections between the 136 targets corresponding to BAI and 3339 targets related to hair growth, and protein interaction network analysis screened key genes such as TNF, IL6 and IL1β. Molecular docking results showed that the binding energy of BAI to TNF-α, IL-6 and IL-1β was -7.5, -7.5 and -10.7, respectively. Notably, BAI down-regulated the protein expression of TLR4, phosphorylated NFκB p65, TNF-α, IL-1β and IL-6 in mouse skin tissues and HaCaT cells.
Conclusion BAI promotes hair follicle development by inhibiting the TLR4/NFκB signaling pathway.

PO-145

Cell fate dynamics of skin mechanosensory receptor Merkel cell during development revealed by single-cell transcriptome

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Objectives
Merkel cells are light tactile receptors that sense the shape and texture of objects. They are located in the middle and lower epidermis. Research on their developmental process and regulation mechanism has always been the core issue in skin touch. This study aims to clarify the profile gene, key transcription factors, and signaling dynamics during Merkel cell development and identify conserved and divergent master regulators of mechanosensory organ development during evolution. Therefore it will provide a theoretical basis for the pathogenesis and treatment strategies of tactile abnormalities-related diseases.
Methods
Single-cell suspensions from the back skin of E14.5, E15.5, E16.5, E17.5, and P0 mice were prepared for single-cell RNA sequencing. We used UMAP to analyze the cell heterogeneity of Merkel cells. We used Monocle2 to delineate the cell trajectory and combined ATACseq data to construct transcription factor regulatory networks. Immunofluorescence staining and FISH were used to validate the different developmental stages of Merkel cells. Intercellular communication between different cell populations was inferred using the iTALK. The critical signaling pathway was validated through in vitro mouse vibrissa explant. In addition, we proved the conservative and critical regulatory network of the development of mechanical sensory organs in the evolutionary process by combining the single-cell RNA sequencing data of the lateral sensory of zebrafish and the mechanical sensory cells in the mouse dorsal skin, vibrissa, and cochlea.
Results
We identified key transcription factors during Merkel cell fate transition, revealing a two-step developmental. Moreover, we verified the profile genes during each stage, and predicted the signaling dynamics during Merkel cell development. Importantly, we found a series of conserved and divergent master regulators for mechanosensory organ development through evolution.
Conclusion
We found that Merkel cells at different stages of the two-step development model have different lineage characteristics, and are co-regulated by key transcription factors and signaling pathways in cell fate conversion. These core transcription factor regulatory networks are probably conserved in mechanical sensory organ development.

PO-146

Double-layer Microneedles with Oxygen-release Microspheres and Antibacterial Activity For Diabetic Wound Healing

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Objective Non-healing wounds in diabetic patients are common complications of diabetes. Due to the prolonged inflammatory response and significant chronic hypoxia in diabetic wounds, the healing process of the wounds is severely hindered. Therefore, we are eager to develop a multifunctional material that can provide long-term sustained oxygen release, deliver oxygen directly to the deep layers of the skin, and possess anti-inflammatory and reactive oxygen species (ROS)-clearing properties to achieve effective wound healing.
Methods Magnesium peroxide microspheres (MgO_2) were synthesized using a chemical precipitation method. Gelatin was grafted with methacrylic anhydride to synthesize methacrylated gelatin (GelMA). A vacuum negative pressure method was employed to prepare a dual-layered microneedle patch based on GelMA (DMN@EGCG/MgO_2). The needle tips were loaded with MgO_2 microspheres and cured using blue light for 10 seconds. The substrate portion was loaded with epigallocatechin gallate (EGCG). The morphology, chemical composition, and mechanical properties of the materials were characterized using optical microscopy, scanning electron microscopy, X-ray diffraction, infrared spectroscopy, fluorescence microscopy, and a universal testing machine. The in vitro release curves of EGCG and Mg^{2+} were determined using a UV spectrophotometer and inductively coupled plasma atomic emission spectroscopy, respectively. The oxygen release curve of the microneedles was measured using a blood gas analyzer.The human skin fibroblasts (HSF) were co-cultured with DMN@EGCG/MgO_2, and the cell proliferation and cytotoxicity were evaluated using the CCK-8 assay. The chorioallantoic membrane (CAM) of chicken embryos was exposed to DMN@EGCG/MgO_2, and the angiogenic potential was assessed using the hen's egg test-chorioallantoic membrane (HET-CAM) assay. DMN@EGCG/MgO_2 was co-cultured with human umbilical vein endothelial cells (HUVECs), and the scratch assay and tube formation assay were performed to evaluate its ability to promote cell migration and angiogenesis. DMN@EGCG/MgO_2 was co-cultured with Staphylococcus aureus and Escherichia coli in vitro, and the growth of bacterial colonies was observed to assess its antimicrobial properties. The elimination of reactive oxygen species (ROS) by DMN@EGCG/MgO_2 was detected using the DCFH-DA probe. The antioxidant capacity of DMN@EGCG/MgO_2 was evaluated using the DPPH radical scavenging assay. To evaluate the effects of DMN@EGCG/MgO_2 on diabetic wounds, a type I diabetes model was established by intraperitoneal injection of streptozotocin in male C57BL/6 mice. The promoting effect of DMN@EGCG/MgO_2 on the wound healing process in diabetic skin was studied.
Results The results showed the successful preparation of a dual-layered microneedle patch based on methacrylated gelatin (GelMA) (DMN@EGCG/MgO_2), with the needle tips loaded with magnesium peroxide microspheres and the substrate portion loaded with epigallocatechin gallate (EGCG). The microneedles exhibited a well-defined morphology and good mechanical properties. The release curves of the drugs demonstrated a sequential release of EGCG and MgO_2, with EGCG showing a faster release rate and MgO_2 gradually releasing as the crosslinked GelMA degraded, enabling sustained oxygen release for up to 21 days. The CCK-8 assay confirmed the biocompatibility of the microneedle patch without cell toxicity. The hen's egg test-chorioallantoic membrane (HET-CAM) assay revealed significant in vitro angiogenic potential of the microneedle patch. The scratch assay and tube formation assay demonstrated that DMN@EGCG/MgO_2 promoted cell migration and exhibited excellent angiogenic capacity. The antimicrobial assay showed that DMN@EGCG/MgO_2 possessed outstanding antimicrobial abilities, with antimicrobial rates exceeding 95%. In the DPPH radical scavenging assay, DMN@EGCG/MgO_2 exhibited an antioxidant activity of over 90%, indicating excellent antioxidant capacity. Additionally, the ROS clearance experiment showed significant ROS elimination by DMN@EGCG/MgO_2. In the diabetic wound model in mice, DMN@EGCG/MgO_2 achieved a wound

healing rate of over 95% at 14 days. HE and Masson's staining demonstrated that the microneedles promoted collagen deposition and tissue regeneration. Immunohistochemistry showed that DMN@EGCG/MgO_2 inhibited the expression of IL-1β and TNF-α while promoting the expression of CD31.
Conclusion The dual-layered drug-loaded microneedles, DMN@EGCG/MgO_2, exhibit multiple effects including long-term sustained oxygen release, promotion of angiogenesis, antimicrobial activity, and antioxidant capacity. In a mouse model of diabetic wounds, DMN@EGCG/MgO_2 effectively promotes wound healing by inhibiting inflammatory responses, promoting angiogenesis, facilitating collagen deposition, and supporting tissue regeneration. Therefore, DMN@EGCG/MgO_2 holds great potential for applications in wound healing and related biomedical fields.

PO-147

Investigating the clinical responses of different treatment regimen for men patients with female pattern hair loss

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Introduction The efficacy of approved therapies in AGA for men patients with female pattern hair loss is quite limited.
Objectives To assess the treatment response of different treatment protocols for men patients with female pattern hair loss.
Materials and Method A retrospective assessment of 110 male patients with female pattern hair loss was conducted between June 2022 and December 2022 at the hair clinic of Huashan Hospital Fudan University. Each group comprised 20-30 patients were divided into four groups: finasteride alone, minoxidil alone, the combination of finasteride and minoxidil, and the combination of finasteride, minoxidil, and low level laser therapy. The treatment respones for four groups were monitored at 1, 3, and 6 months after the initial visit by comparing the severity of hair loss before and after treatment based on global photographs.
Results Theses men patients with FPHL, accounting for 44.5% of the total, had a baldness grade of Sinclair 3. The average Sinclair stage of male FPHL patients before treatment was 3.07. Following 3 months of clinical treatment, there was a decrease of 0.32 in the average Sinclair stage ($P<0.01$), and a decrease of 0.57 after 6 months of treatment ($P<0.01$). The use of minoxidil alone did not show a statistically significant difference in the rate of significant improvement among scalp regions. For the midscalp and vertex region, the rate of significant improvement with combination therapy was significantly higher compared to monotherapy.
Conclusions The hair loss grade of most patients remained stable after topical minoxidil alone. To achieve more favorable treatment response, it is recommended to use combined treatment for those whose hair loss grade was over Sinclair 3. The best therapeutic outcome was achieved through the combination of three methods.

PO-148

Regulation mechanism of Merkel cell during development and homeostasis

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MCs (Merkel cells) are light touch receptors located in the basal layer of the skin and closely connected to sensory neurons which form the Merkel cell-neurite complex. It gets involved in multiple physiological activities, performing mechanosensory and neuroendocrine functions. In

various circumstances, such as physical injuries, side effects of drugs, and aging, a decrease in quantity will happen to MCs. It can directly lead to loss of touch or abnormal touch sensations, causing diseases such as Alloknesis and Allodynia. Therefore, studying the development of MCs is crucial for understanding MC homeostasis. The development of MCs is a complex process involving multiple signaling pathways and regulatory factors. Over the last decades, researchers utilized multiple methodologies and explored MC derived from epidermal stem cells. Also, several regulatory factors had been revealed as important for MC development like PRC, Sox2, Isl1, and Atoh1. Wnt, Eda/Edar, BMP, and SHH signals also play an important role in MC development. However, limited research has been carried out on the adult stage. Nowadays, several studies approved that regeneration of MCs indeed exists in adulthood mainly for MC homeostasis. Moreover, when suffering inflammation, the number of MCs exceeds the normal level in some patients. This phenomenon has been reported in several studies. Thus, inflammatory factors might promote the proliferation and differentiation of MC precursor cells.

PO-149

Effect of Prinsepia Utilis Royle Polysaccharides on Impaired Epidermal Barrier Permeability

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Background and Objective The epidermal permeability barrier is a multifaceted system, offering crucial protection against environmental stressors. Various research reports have revealed a close correlation between the onset of several skin diseases, such as atopic dermatitis and psoriasis, and damage to this epidermal barrier. Consequently, numerous disease diagnosis and treatment guidelines highlight the importance of restoring the skin barrier as a key therapeutic measure. The traditional medicinal plant, Prinsepia utilis Royle (P.utilis), possesses a rich array of polysaccharide compounds. Previous studies have reported that these compounds can function to repair the damaged epidermal permeability barrier, thereby assisting in the treatment of various skin conditions, including polymorphic solar eruption and psoriasis. Despite these promising findings, the underlying mechanism through which P. utilis facilitates this repair remains unclear. This study aimed to investigate the underlying mechanisms of the Polysaccharide from Prinsepia utilis Royle (PPR) on repairing acute and chronic epidermal permeability barrier disruption induced by tape-stripping and acetone wiping along.
Methods Acute and chronic models of epidermal permeability disruption were established in male SKH-1 hairless mice through tape stripping and acetone wiping. After the disruption, the matrix, PPR, and vaseline were applied separately to the test areas. The recovery rate of Trans-Epidermal Water Loss (TEWL) was measured using a non-invasive skin detection instrument after topical application of the above interventions. Furthermore, real-time reverse transcription PCR (RT-PCR) and western blotting assays were used to detection of mRNA and protein expression levels associated with cornified envelope (CE), intercellular lipids, and tight junctions (TJs).
Results PPR, applied topically, significantly reduced Trans-Epidermal Water Loss (TEWL) in acute and chronic models of epidermal permeability barrier disruption compared to Model group(subjected to tape stripping (TS) or acetone wiping (AC)) and Vehicle-treated group ($p<0.05$).The TEWL reduction further establishes PPR's significant role in maintaining the integrity of the epidermal permeability barrier and highlights its potential as a therapeutic agent in the management of skin disorders associated with compromised barrier function. Beyond the observed reduction in Trans-Epidermal Water Loss, our study shed light on the profound role of topically applied PPR in aiding the repair of the epidermal permeability barrier. This was discernible from the pronounced upregulation of mRNA and protein expression levels of essential skin barrier structure components, which included cornified envelope proteins like Filaggrin, Involucrin, and Loricrin. The elevated expression of these proteins, fundamental to forming a robust cornified

envelope, reinforces PPR's essential role in fortifying this critical layer of the epidermal permeability barrier. Simultaneously, PPR exhibited a beneficial effect on the barrier function of the skin through the enhanced expression of lipid synthesis enzymes (FASN/SPT/HMGCR). These enzymes are instrumental in producing the intercellular lipids that form an integral part of the epidermal permeability barrier's mechanism. The upregulation noted implies an increased synthesis of vital lipids that augment the barrier's integrity and efficacy.Further augmenting this multi-faceted influence of PPR, our study also uncovered a positive correlation between PPR application and the expression of tight junction proteins, including Claudins-1, Claudins-5, and ZO-1. These tight junctions, serving a dual function as a fence and a barrier at the cellular level, strengthen the epidermal permeability barrier, and their enhanced expression post-PPR application suggests an improved cellular barrier function.

Conclusion In conclusion, our research has highlighted the beneficial effects of topically applied PPR, emphasizing its significant role in strengthening the epidermal permeability barrier. Through our in-depth investigations, we've substantiated its ability to stimulate the overexpression of vital proteins that form the backbone of the epidermal permeability barrier, thereby markedly boosting its repair processes. Our findings underline the therapeutic potential of PPR, especially its prospective effectiveness in mitigating disruptions to the epidermal permeability barrier, as often encountered in conditions like atopic dermatitis (AD) and psoriasis.

PO-150

Osthole inhibits chronic pruritus in atopic dermatitis through modulation of IL-31

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Aim Atopic dermatitis (AD) is a persistent and recurring inflammatory skin condition characterized by chronic eczema-like lesions, dry skin, and intense pruritus. Pruritus, an unpleasant and irritating sensation that triggers the urge to scratch, can be categorized as either acute (lasting for ≤6 weeks) or chronic (lasting for more than 6 weeks), depending on its duration. Osthole, the primary active component of Cnidium monnieri (L.) Cusson, is a prevalent traditional Chinese medicine utilized for the alleviation of cutaneous pruritus. Our investigation into the influence of osthole on atopic dermatitis (AD) involved the use of in vitro and in vivo experiments.

Materials and methods The induction of AD mouse models was carried out in vivo by 2,4-Dichloronitrobenzene (DNCB), and in vitro a HaCaT keratinocytes inflammation model was induced by TNF-α and INF-γ.

Results The results demonstrated that osthole had a positive effect on AD-like symptoms in mice, including skin damage, clinical dermatitis scores, scratching bouts and epidermal thickness. On the other hand, osthole reduced the concentration of Interleukin-31 (IL-31), Interleukin-31 Receptor A (IL-31 RA) both in skin tissues and HaCaT cells. In addition, osthole suppressed the protein expression levels of phospho-p65 (p-p65) and phospho-inhibitor of NF-κB α (p-IκBα), while increasing the protein expression levels of Peroxisome proliferators-activated receptor-α (PPARα) and PPARγ in HaCaT cells.

Conclusions These findings suggested that osthole effectively inhibited chronic pruritus (CP) in AD by activating PPARα, PPARγ, repressing the NF- κB signaling pathway, as well as the expression of IL-31 and IL-31 RA.

PO-151

Effect and Mechanism of zingerone in the treatment of androgenetic alopecia

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Objective Androgenetic alopecia (AGA) is a common clinical type of hair loss, and there is currently no safe and effective treatment. A variety of ancient traditional Chinese medicines can effectively and safely treat hair loss, but the main pharmacodynamic components and their mechanism of action are still unclear.
Method In this experiment, the model was made by depilation, and the depilation cream was used to depilate the back skin of mice, 5 mg/ml dihydrotestosterone, 10 μ L/g intraperitoneal injection, 5 times per week, the growth of mouse hair was observed and the pathological model of AGA was simulated.Using zingerone in the modeling area for drip dosing, daily observation, and photography, until the hair follicles through a round of hair follicle growth cycle again into the rest period.
HE staining was used to judge the difference of hair follicle growth cycle in different groups, and immunofluorescence staining was used to label cell proliferation by Edu staining, to judge the effect of ginger ketone on hair follicle cycle.
Result Through systematic comparison and analysis of a large number of traditional hair growth prescriptions and screening in combination with animal hair growth models, we found that the component zingerone has a significant hair growth-promoting effect. At the same time, it was found to promote the proliferation of Lgr5-positive hair follicle stem cells and inhibit the apoptosis of hair follicle stem cells. Zingerone could also activate Wnt pathway, which plays a key role in hair regeneration.
Conclusion Our study suggested that zingerone has a hair-generating effect, mainly reflected in accelerating hair growth rate and extending the residence time during the growth period. Further, we could provide a solid theoretical basis for the clinical application of traditional Chinese medicine containing zingerone in the treatment of hair loss.

PO-152

Long Non-Coding RNA PKNOX1-1 inhibits tumor progression in cutaneous malignant melanoma

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Objective Melanoma is the most invasive and the most lethal skin tumor. Accumulating evidence has pointed out the potential role of long noncoding RNAs (lncRNAs) in biological behaviors of melanoma. The previous result of microarray analysis in our research group has found a new lncRNA, lnc-PKNOX1-1. This study aims to identify this new lncRNA and study its biological functions in melanoma.
Methods RACE assay was used to obtain the full-length of lnc-PKNOX1-1. The protein-coding ability of lnc-PKNOX1-1 was predicted through CPC2.0 and CPAT. RNA FISH assay was performed to study the subcellular localization of lnc-PKNOX1-1. The expression level of lnc-PKNOX1-1 in melanoma tissues, normal paratumoral tissues, human primary normal melanocytes, and melanoma cell lines was detected using RT-qPCR. Then lnc-PKNOX1-1 overexpressed cell lines were constructed in A375 and A2058 melanoma cells, and successful transfection was confirmed by RT-qPCR. The role of lnc-PKNOX1-1 in melanoma cell proliferation, cell cycle, migration, invasion, and EMT process was detected by CCK8 assay, EdU assay, xenograft model of nude mice, flow cytometric assay, western blot analysis, immunofluorescence assay, wound-healing assay, and transwell assays, etc. Results: RACE assay and protein-coding ability prediction has proved lnc-PKNOX1-1 as a long non-coding RNA. RNA FISH assay showed lnc-PKNOX1-1

was mainly located in nucleus of melanoma cells. Lnc-PKNOX1-1 was found poorly expressed in melanoma tissues and cells, and statistical analysis revealed that low expression of lnc-PKNOX1-1 was significantly related to invasive pathological type and Breslow thickness of melanoma. Cell proliferation, cell cycle progression, invasion, migration, EMT process, tumor volume and weight were significantly suppressed in melanoma cells with overexpressed lnc-PKNOX1-1. **Conclusion** This study has proved lnc-PKNOX1-1 as a long non-coding RNA and provided evidence that lnc-PKNOX1-1 could reduce proliferation, invasion and migration of melanoma. The findings suggested a potential biomarker and therapeutic target for melanoma, which may offer new insights for melanoma prevention and treatment.

PO-153

Research on the application of hair follicle organoids in drug screening

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Objective With the change in social environment and stress, hair loss has become an important problem that plagues people's lives, and there is currently no safe and effective treatment. Studies on hair growth drugs mostly use mouse models, but animal differences in individuals are large, as well as the long experiment period with high cost. Organoids are tissue analogs with a certain spatial structure formed under 3D culture system in vitro, which can simulate real organs to a certain extent, with relatively controllable conditions and uniform nature. Since the passage of FDA Modern Act 2.0, organoids can replace some animal experiments for drug research. Organoids can replace some animal experiments for drug research. So in recent years its research demand and industrial conversion rate have gradually increased. Therefore, organoids containing hair follicles can be used to develop and apply hair growth drugs. The successful and stable establishment of in vitro organoid system with lower cost will be the core issue of its research and application.
Method the dorsal skin of mouse embryos (E17.5-E19.5) was harvested under a dissection microscope and aseptically treated with dispase II（2mg/ml）for 60 min at 37°C, then epithelial and mesenchymal layers were separated and performed the following operations individually to get single cells. Debris and tissue aggregates were removed using a 40-mm cell strainer. After centrifugation at 500g for 5 min, the epithelial and mesenchymal cells were suspended in Advanced DMEM/ F-12 medium containing 1% Glutamax and 1% PS. Then, the robust epithelial and mesenchymal cells were assembled in different ratios with 1-2% growth factor reduced Matrigel in the culture medium. then the mixed cell suspension in ultra-low detached 96-well plates was incubated at 4°C for 1h, centrifugate the plates at 200g for 2 min. Then transferred into the incubator for further culture.
In order to further establish the organoid model of Androgenetic alopecia(AGA), DHT was added on day 0 in the medium to simulate the occurrence of AGA disease; Then the drug Zingerone（ZG）was added in the medium at D2. Used a microscope to track changes in HFOs.
Result Almost 100% of the E/M cell mixed organoids contain regenerated hair follicles; The AGA organoid model was successfully established with tiny hair follicles. After the drug ZG treatment, more hair follicles appeared in HFOs. Based on this system, we constructed the AGA organoid model by adding DHT. By using this model, our study verified the growth-promoting effect of the drug ZG.
Conclusion Thus, we robustly constructed the mouse hair follicle organoid system in vitro with almost 100% efficiency. The drug ZG could promote hair growth in hair follicle organoids. Our study suggested that Hair follicle organoids can be used in vitro for drug screening.

PO-154

Differential gene analysis of classic Kaposi sarcoma and its correlation with clinical characteristic

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Objective To screen the differential gene of Kaposi sarcoma, explore its correlation with clinical features, and discover new drug targets.
Methods High-throughput sequencing of Kaposi sarcoma tissue, combined with the results of the GSE147704 dataset published in the GEO database, enrichment analysis combined with network pharmacology to screen differential genes, patients with classic Kaposi sarcoma who underwent biopsy in Xinjiang Uygur Autonomous Region People's Hospital from April 2014 to April 2022 were selected as research objects, and the expression of the target gene ALOX12 in Kaposi sarcoma tissue was detected by immunohistochemical staining. Correlation analysis was performed on the clinical features of patients in the high- and low-expression groups.
Results The results of enrichment analysis combined with network pharmacology showed that ALOX12 was highly expressed in Kaposi sarcoma tissue, and the affinity with quercetin that functioned in Kaposi sarcoma angiogenesis was -7.96 kcal/mol. Correlation analysis of clinical features showed that high expression of ALOX12 was associated with increased eosinophil count and eosinophil percentage in patients with Kaposi sarcoma and was associated with a high recurrence rate ($p < 0.05$).
Conclusion The differential expression of ALOX12 in Kaposi sarcoma has high expression in tumour tissues and is associated with elevated eosinophils, which may be an important therapeutic target for influencing relapse in patients.

PO-155

The role and mechanism of pigment epithelium-derived factor PEDF in androgenetic alopecia by promoting hair follicle fibrosis

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Objective To study the expression of pigment epithelium-derived factor (PEDF) in hair follicles of patients with androgenetic alopecia (AGA), and to explore the role and mechanism of PEDF by using hair follicles and cells cultured in vitro.
Methods Hair follicles from hair loss and non-hair loss areas of AGA patients were collected. Real-time PCR and immunohistochemistry were used to detect the expression of PEDF gene and protein in hair follicles. Hair follicles were cultured in vitro, and the expression of PEDF in hair follicles was reduced by transfection of siRNA. The effect of PEDF on hair growth and hair cycle was determined by stereomicroscope. The effects of PEDF on cell activity and fibrosis-related phenotypes were detected by using cultured dermal papilla cells and fibroblasts in vitro, and the mechanism was further studied.
Results The results of quantitative PCR and immunohistochemistry showed that the expression of PEDF in hair follicles of AGA patients was higher than that in non-hair follicles. The use of siRNA can effectively reduce the expression of PEDF in hair follicles cultured in vitro, and interference with PEDF can promote the growth of hair follicles and prolong the growth period of hair follicles. The results of in vitro cell experiments showed that interference with PEDF could significantly promote the proliferation of dermal papilla cells and inhibit the expression of fibroblast fibrosis

genes. Further mechanism studies have shown that PEDF may affect hair growth by regulating wnt and fibrosis.

Conclusion PEDF is highly expressed in hair follicles in the hair loss area of AGA patients ; reducing the expression of PEDF can promote the growth of hair follicles cultured in vitro, prolong the growth period, improve the activity of dermal papilla cells, and inhibit the fibrosis phenotype. In summary, PEDF plays an important role in AGA and provides clues for treatment.