Lipidomic profiling reveals metabolic signatures in psoriatic skin lesions

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Abstract

Psoriasis is a chronic immune-mediated inflammatory disease. Lipids play an important role in regulating the inflammatory response. However, the alteration of lipids involved in psoriasis particular in skin lesions remain unclear. Here, we performed the lipidomics to investigate lipid profiling in the skin lesions of the imiquimod-induced psoriasis-like dermatitis and psoriasis patients. The findings showed that ceramides phosphate (CerP) and ceramides were enriched in psoriatic lesions compared with controls from both psoriasis patients and psoriasis-like mouse model. Psoriasis patients were classified into two subtypes, the CC1 and CC2, by consensus clustering of these lipid signatures. The CC1 was characterized by the higher levels of CerP, uric acid, and more severe psoriasis, compared with CC2 subtype. Interestingly, ceramide-1-phosphate (C1P), dramatically enriched in CC1 subtype, facilitated imiquimod-induced psoriasis-like inflammatory responses. Mechanistically, C1P induced the expression of inflammatory factors and activated DNA replication and cell cycle signaling pathways in the primary keratinocytes. Inhibiting the production of C1P with ceramide kinase inhibitor effectively alleviated the imiquimod-induced psoriasis-like inflammation. Taken together, we described the landscape of lipids alteration and established lipids classification based on pattern of abundance of lipids in psoriatic skin lesions. Suppression of C1P pathway is a novel potential strategy for psoriasis treatment.

Introduction

Psoriasis is a chronic inflammatory disease mediated by genetic background, and it affects approximately 2–3% of the population [1]. Plaque psoriasis is the main type of psoriasis, and it is characterized by inflammatory erythema, scale, and thickening [2]. The pathological manifestations of psoriatic lesions are abnormal epidermal differentiation, hyperproliferation, angiogenesis, and increased T cells infiltration [1]. Based on these pathological manifestations, the pathogenesis of psoriasis is mainly focused on the interaction between keratinocytes and T cells [3]. Exogenous antigens stimulate the activation of dendritic cells, which secrete cytokines (IL-12 and IL-23) to activate T cells and induce Th1 and Th17 cell differentiation [4]. Activated T cells release inflammatory cytokines (IFN-γ, IL-17A, IL-17F, and IL-6), promote the abnormal differentiation and proliferation of keratinocytes, and increase the release of inflammatory cytokines (S100A8, S100A9, and IL-17C) and chemokines (CXCL1 and CXCL2), exacerbating local inflammation and promoting plaque formation [5].

Lipids play crucial roles in the skin related to both structural integrity and function [6]. The skin surface is covered with a mixture of sebocyte-derived sebum and keratinocyte-derived lipids, including triglycerides, cholesteryl esters, wax esters, and squalene [7]. Epidermal keratinocytes are supported by the extracellular lipid matrix, which is composed of approximately 45–50% ceramides, 25% cholesterol, and 10–15% free fatty acids [8,9]. Thus, lipids play a key role in cutaneous inflammation, especially when they are transformed into bioactive mediators [10]. These key bioactive lipids include eicosanoids, endocannabinoids, and sphingolipids [6]. Sphingolipids are a complex family of structural and mediator lipids, and sphingolipids exhibit great molecular diversity resulting from combinations of different

sphingoid bases and N-acylated fatty acids [11]. There are four derivatives of sphingosine, i.e., sphingosine-1-phosphate (S1P), ceramide (Cer), ceramide-1-phosphate (C1P), and sphingomyelin (SM). Keratinocyte growth is influenced by sphingolipids [12]. S1P affects COX-2 metabolism and induces PGE2 production [13,14]. Ceramides can induce apoptosis by increasing mitochondrial membrane permeability [15]. C1P has been shown to induce arachidonic acid release [16]. Sphingomyelins are active constituents of cell membranes and are involved in membrane trafficking [17]. Previous researchers have shown abnormal levels of bioactive lipids in psoriatic lesions [18,19]. Hence, exploring the lipid metabolomics in psoriatic lesions and the role of abnormal lipids in these lesions could contribute to understanding the pathogenesis of psoriasis.

In this study, we conducted metabolomic profiling of the skin lesions of the IMQ-induced psoriasis-like mouse model and psoriasis patients to explore the differential lipids in psoriatic lesions. The results show that the level of ceramides phosphate (CerP) and ceramides (Cer) are significantly altered in psoriasis, indicating that altered metabolisms of these lipids may play a vital role in the development of psoriasis. The levels of CerP and uric acid contribute to the development of various molecular subtypes of psoriasis. Furthermore, C1P facilitates the pathogenesis of psoriasis in the IMQ-induced psoriasis-like mouse model. C1P induces inflammatory factor expression and activates DNA replication and cell cycle signaling pathways in keratinocytes. Moreover, inhibiting the production of C1P with a ceramide kinase inhibitor attenuates IMQ-induced psoriatic inflammation. In conclusion, these results indicated abnormal lipid metabolism in the psoriatic lesions, providing new insight into the pathological role of lipids in the immune microenvironment of plaques.

Materials And Methods

Study population

Skin lesions were derived from 21 psoriasis patients (see Supplementary Tables 1). The diagnosis of psoriasis was confirmed and quantified by a dermatologist using the Psoriasis Area Severity Index (PASI) [20], ensuring that all psoriasis patients were treated in the same manner. None of the included study participants received any systemic anti-psoriasis treatments or topical therapy within 4 weeks before the collection of biopsy skin samples, including psoriatic lesions and non-lesions. These samples were obtained by collecting 3 mm surgical biopsies from representative lesions.

Mice

Six-week-old BALB/c female mice were used to establish the IMQ-induced psoriasis-like mouse model. The mice were bred and housed in the Department of Laboratory Animals, Central South University, and maintained under specific pathogen-free conditions. Before each experiment, the mice were randomly assigned to the control or treatment group. No sample-size estimation was performed before the experimentation.

IMQ-induced psoriasis-like mouse establishment and measurement of skin scores and epidermal thickness

Six-week-old BALB/c female mice were used. Metabolomic profiling of the skin lesions of the IMQ-induced psoriasis-like mouse model was conducted as follows. A daily dose of 62.5 mg of 5% IMQ cream (Med-shine Pharmaceutical Co., Ltd.) was applied to the shaved backs of mice for 6 consecutive days. C1P treatment of the IMQ-induced psoriasis-like mouse model was as follows. A daily dose of 31.25 mg of 5% IMQ cream was applied to each of the ears of the mice, and two ears were treated. IMQ cream was applied for 6 consecutive days. The ears were intradermally injected with 1 mM C16 ceramide-1-Phosphate (d18:1/16:0) (Avanti Cat. 860533P) (10 µl) or vehicle control (normal saline) once per day for 6 consecutive days. The mice were sacrificed on Day 7. The clinical skin scores of the mice were determined beginning on Day 1 (the first day of IMQ treatment) and every other day until Day 7 using the modified psoriasis severity index score (PASI). The degree of skin erythema, induration, and scaling was classified as follows: 0, no symptoms, 1, mild, 2, moderate, 3, severe, or 4, very severe. Histological evaluation of mouse ears samples was performed by H&E staining. For each section, the epidermal thickness was measured from the stratum basal to the stratum granulosum using Image Pro-Plus. The average value of 7 random fields of view was calculated for each mouse.

LC-MS/MS based lipidomics of psoriatic skin

The lipids were extracted from skin samples following protocols as previously reported protocols [21]. In short, 10 mg skin samples were weighted with 0.5 ml of prechilled solution of dichloromethane/methanol (3:1) spiked in Lipid internal standard mix (SPLASH® LIPIDOMIX® Mass Spec Standard, Avanti, USA). The mixture was lysed using a TissueLyser by 50HZ for 10 minutes. After centrifuge the mixture at 10,000g for 10 min at 4 °C, the supernatant was moved into glass vials for LC-MS analysis. Equal amount supernatant from each sample was mixed as quality control samples (QCs). Lipids were separated by a CSH C18 column (1.7 µm 2.1*100 mm, Waters, USA) and analyzed by a QExactive mass spectrometer (Thermo Fisher Scientific, USA) for qualification and quantification. Mobile phase A consisted of 10 mM ammonium formate with 0.1% formic acid in water-ACN (40:60, v/v), and mobile phase B was 10 mM ammonium formate with 0.1% formic acid in ACN-IPA (10:90, v/v). The following gradient was used for elution: 0~2 min, 40%-43% B solution, 2~2.1 min, 43%~50% B solution, 2.1~7 min, 50%~54% B solution, 7-7.1 min, 54%~70% B solution, and 7.1-13 min, 70%~99% B solution with a flow rate of 0.35 mL/min. The mass spectrometric settings for positive/negative ionization modes were as follows: spray voltage, 3.8/−3.2 kV, aux gas heater temperature, 350 °C, and capillary temperature, 320 °C. The full scan range was 200–2000 m/z with a resolution of 70,000, and the AGC target for MS acquisitions was set to 3e6 with a maximum ion injection time of 100 ms. The top three precursors were selected for subsequent MSMS fragmentation with a maximum ion injection time of 50 ms and resolution of 17,500, and the AGC was 1e5. The stepped normalized collision energy was set to 15, 30, and 45 eV. QC samples were injected after every 10 sample injections followed previous description [22] for system quality control.

LipidSearch 4.1 SP2 software (Thermo Fisher, USA) was used for lipid identification and quantitation. The processing parameters for product ion search including precursor and product tolerance as 10 ppm, adduct ions of positive mode as +H and $+NH_4$, adduct ions of negative mode as -H, +HCOO and -2H. Data scaling and normalization were further processed using metaX [23].

Flow cytometry

Single-cell suspensions were prepared from the ear skin of mice. Skin lesions were dissected and digested with 2.0 mg/ml collagenase IV (Sigma-Aldrich Cat. V900893) and 1.0 mg/ml dispase II (Sigma-Aldrich Cat. D4693) for 60 min at 37°C. All the single-cell suspensions were filtered through 40-micron pores (BD Cat. 352340) and then were stained with fluorophore-conjugated antibodies. The cells were incubated with live/dead stain (Zombie Aqua™ Fixable Viability Kit, BioLegend Cat. 432102) and Fc block (BioLegend Cat. 101302). The cells were then washed and stained with antibodies specific for CD45 (30-F11, BioLegend Cat. 103116), CD4 (RM4-5, BioLegend Cat. 100510 or Cat. 100438), and CD25 (PC61, BioLegend Cat. 102012). For intracellular cytokine staining, the cells were stimulated for 5 hours with PMA (10 ng/ml, Sigma Cat. 79346), ionomycin (1 mg/ml, TOCRIS Cat. 56092-8-0), and GolgiPlug (1000×, BD Cat. 555029). For intracellular and intranuclear staining, the cells were fixed and permeabilized using the Foxp3/Transcription Factor Staining Buffer Kit (eBioscience Cat. 00-5523-00) according to the manufacturer's protocol. The cells were intracellularly stained with antibodies specific for IFN-γ (XMG1.2, BioLegend Cat. 505810), or IL-17A (TC11-18H10, BD Biosciences Cat. 559502), or Foxp3 (FJK-16s, eBioscience Cat. 12-5773-82). For analysis of cell proliferation, cells were prestained with 5 mM CFSE (eBioscience Cat. 65-0850-84) before activation, according to the manufacturer's instructions. Cell proliferation was evaluated by measuring CFSE dilution. All samples were acquired on a Cytek Dxp Athena flow cytometer. Analysis of acquired data was performed with FlowJo software. Analysis of the stained populations was performed by gating on single, live cells.

Cell culture

Human primary keratinocytes (KCs) were isolated from the foreskin of healthy donors obtained after circumcision. The skin samples were cut into small pieces and then incubated in dispase II (Sigma-Aldrich Cat. D4693, 2 mg/mL) overnight at 4 °C. The next day, the epidermis was removed from the dermis, subsequently digested with 0.05% trypsin for 10 min at 37 °C and filtered through 40-micron filters (BD Cat. 352340). After centrifugation at 1000 rpm for 5 min, the cells were cultured in the serum-free basal medium supplemented with growth factors (PromoCell Cat. C-20011). The medium was refreshed every day, and the cells were sub-cultured according to the cell fusion. Mouse naïve $CD4^+$ T cells were obtained from total mouse splenocytes by magnetic cell sorting with the $CD4^+CD62L^+$ T Cell Isolation Kit following the manufacturer's instructions (Miltenyi Biotech Cat. 130-106-643).

Keratinocyte proliferation

Keratinocyte proliferation was analyzed by CCK8 cell proliferation assay using the Cell Counting Kit-8 (CCK-8) (Bimake, B34302). In brief, keratinocytes were seeded into 96-well plates at 5000 cells/well and

cultured for 24, 48, or 72 hours in the presence of different concentrations of C1P or vehicle control. Then CCK8 (10 μl) mixed with 100 μl medium was added to each well, and plates were incubated at 37 °C for 2 hours. Then, the absorbance was measured at 450 nm using an automated microplate reader (Beckman, USA). Five replicates per sample were analyzed.

Quantitative Real-Time PCR (RT-qPCR)

RNA was extracted from cells using TRIpure Reagent (Bioteke Cat. RP1001) according to manufacturer's instructions. RNA was converted to cDNA using HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme Cat. R223-01), and gene expression was determined by RT-qPCR using the UltraSYBR One-Step RT-qPCR Kit (CWBIO Cat. CW0659) on a 7500 Fast thermocycler (Applied Biosystems). The relative expression of target genes was confirmed using the quantity of target gene/quantity of *β-Actin*. The fold change of gene expression was calculated by $2^{-(\Delta Ct\ experimental\ group - \Delta Ct\ control\ group)}$, which normalized to the control group. All primer sequences used for RT-qPCR were as follows: *IL-6*: forward, 5' -CACTGGTCTTTTGGAGTTTGAG -3', and reverse, 5' -GGACTTTTGTACTCATCTGCAC -3', *IL-1β*: forward, 5' -CTCCACCTCCAGGGACAGGATATG -3', and reverse, 5' - TCATCTTTCAACACGCAGGACAGG -3', *IL-17A*: forward, 5' - GAGATATCCCTCTGTGATCTGG -3', and reverse, 5' - GACAGAGTTCATGTGGTAGTCC -3', *β-Actin*: forward, 5' - AGAGCTACGAGCTGCCTGAC -3', and reverse, 5' - AGCACTGTGTTGGCGTACAG -3'. All primers were purchased from Sangon Biotech.

In vitro mouse T cell differentiation

Purified mouse naïve $CD4^+$ T cells were stimulated with plate-bound anti-CD3 (5 μg/ml, eBioscience Cat. 145-2c11) and anti-CD28 (2 μg/ml, BD Cat. 553294) in RPMI 1640 (BI Cat. 01-100-1ACS), supplemented with 10% FBS (Gibco Cat. 16140071), 1000× 2-mercaptoethanol (Gibco Cat. 21985023), 100× HEPES Buffer (Gibco Cat. 15630080), 100× sodium pyruvate (Gibco Cat. 11360-070), 100× nonessential amino acid solution (Gibco Cat. 11140050) and 100× penicillin/streptomycin nystatin solution (Geneview Cat. B103010106). To assess Th1 cells polarization, naïve $CD4^+$ T cells were activated with IL-12 (20 ng/ml, R&D Systems Cat. 419-ML-010) in the presence of anti-IL-4 (5 μg/ml, BioXcell Cat. BE0045). To assess Th17 cells differentiation, naïve $CD4^+$ T cells were stimulated with IL-6 (30 ng/ml, R&D Systems Cat. 406-ML-005), IL-1β (10 ng/ml, PeproTech Cat. 211-11B-10), IL-23 (20 ng/ml, R&D Systems Cat. 1887-ML-010), and TGF-β (2 ng/ml, R&D Systems Cat. 7666-MB-005) in the presence of anti-IFN-γ (10 μg/ml, BioXcell Cat. BE0054) and anti-IL-4 (10 μg/ml, BioXcell Cat. BE0045). We cultured the cells culture in 96-well plates in a total volume of 0.2 ml/well of culture medium with 2×10^5 naïve $CD4^+$ T cells. For C1P stimulation, C1P (Avanti Cat. 860533P) was dissolved at a concentration of 1 mM in normal saline and stored at -20 °C until use. The cells were incubated at 37 °C for 3 days under polarization conditions in the presence of C1P or vehicle control.

Statistical analysis

Metaboanalyst [24] was used for biomarker analysis. All figures were drawn using corresponding R packages. All quantified lipids were clustered into time patterns as follows. The mean abundance level for each lipid was determined at each of the three time points (N0, Q3 and Q6). Each row was then centered and clustered using k-means clustering with different cluster numbers on a Euclidean distance matrix using the R package TCseq (version 1.10.0). Appropriate cluster number was determined by assessing the stability of the clustering in terms of within-cluster sums of squares over 100 iterations of the clustering.

Clustering was performed using consensus clustering algorithm implemented in ConsensusClusterPlus R package [25] with subsampling psoriasis samples over 1000 iterations and 10 as maximum k (number of clusters) using differential lipids identified between psoriasis and control tissue samples. Before classification, samples were z-score normalized followed by lipid expression z-score normalization. Clustering results were evaluated visually in the resulting consensus matrix (one matrix per each k) as well as quantitatively using the cumulative distribution function of the area under the curve for each k.

Statistical analyses were performed on GraphPad Prism 8.0 software. Data are expressed as means ± SEM. A Student's t test was used to compare two conditions, and an analysis of variance (ANOVA) with Bonferroni or Newman-Keuls correction was used for multiple comparisons. The level of significance was defined as $p < 0.05$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. The error bars depict the SEM.

Results

Identification lipid profiling in IMQ-induced psoriasis-like dermatitis

The IMQ-induced psoriasis-like mouse model, which mimics the phenotype, pathogenesis, and systemic inflammation of psoriasis in humans' psoriasis [26], was used to explore lipid dysregulation during the development of psoriasis. The quality of data acquisition was evaluated by principal component analysis (PCA) with QC samples. PCA showed that the QC samples were clustered together, with an average coefficient of variation less than 10% (Supplementary Fig. 1a), these results indicated that the whole LC-MS analysis satisfied the required qualitative conditions [27]. A total of 1064 lipids were quantified in all the mouse samples, and all the lipid species were annotated in Supplementary Fig. 1b and Supplementary Table 1, these results indicated that the untargeted lipid profiling provided wide-coverage and highly sensitive data for this psoriasis study. The differential analysis of Q3 vs. N0, Q6 vs. N0, and Q6 vs. Q3, which using fold change >=2 and adjusted p-value less than 0.01 as criteria, revealed differential lipid levels in each stage of psoriasis-like mouse model. A total of 346 and 407 lipids were upregulated in the N0 group compared with the Q3 and Q6 groups, 384 and 305 lipids were upregulated in the Q3 group compared with the N0 and Q6 groups, while 432 and 306 lipids were upregulated in the Q6 group compared with the N0 and Q3 groups. The abundance of all the quantified lipids is shown in the heatmap in Fig. 1a, and the fold changes and adjusted p-value are shown.

Considering that the IMQ-induced psoriasis-like mouse model reflects the development of psoriasis phenotypes, we further explored time series-dependent changes in lipids levels with the TCseq package [28]. As shown in Fig. 1b, all the quantified lipids could cluster into 8 clusters based on abundance patterns, with 89 lipids in Cluster1, 125 lipids in Cluster 2, 182 lipids in Cluster 3, 216 lipids in Cluster 4, 46 lipids in Cluster 5, 48 lipids in Cluster 6, 179 lipids in Cluster 7, and 179 lipids in Cluster 8. Importantly, the levels of the lipids enriched in Cluster 3 were dramatically increased after the treatment with IMQ for 6 days compared with the control and IMQ treatment for 3 days, indicating that the lipids enriched in Cluster 3 play a vital role in the development of psoriasis. The annotation of lipid species in each cluster revealed that the lipids in Cluster 3 mainly included ceramides phosphate (CerP), dimethylphosphatidylethanolamine (dMePE), lysodimethylphosphatidylethanolamine (LdMePE), monogalactosylmonoacylglycerol (MGDG), and ceramides (Cer) (Fig. 1c), which indicated that these lipids might be involved in the pathogenesis of psoriasis. For downregulated lipid in cluster 1, 4 and 7, we annotated them by their lipid species as shown in Figure 1c and could find that they were enriched in Cholesteryl Ester (ChE), Triglyceride (TG) and Lyso-phosphatidylethanolamine (LPE).

Identification lipid profiling in psoriatic lesions and non-lesions of psoriasis patients

To validate the role of the differential lipids identified in the IMQ-induced psoriasis-like mouse model in the development of psoriasis, 21 psoriatic lesions and non-lesions from psoriasis patients were used for lipid profiling. The demographic and clinical characteristics of the psoriasis patients (n = 21) enrolled in this skin lesion analysis are presented in Table 1. The quality of the experiment was evaluated by principal component analysis (PCA) with QC samples. The PCA showed no significant difference between the two groups, however, the pooled QC samples clustered together (Supplementary Fig. 2a) with average coefficient of variation less than 10%, indicating that the authenticity of LC-MS is qualified. A total of 882 lipids were quantified (Supplementary Fig. 2b). Partial least squares discriminate analysis (PLS-DA), a supervised multivariate data analysis method, was conducted to examine differences between the psoriasis samples and healthy control samples, and the PLS-DA model results clearly distinguished psoriatic and healthy control skin samples as shown in Supplementary Fig. 2c with cross-validation for 200 times (Supplementary Fig. 2d). Differentially expressed lipids (Supplementary Table 2) with fold change above 2 and adjusted p-value less than 0.01 were identified by t-test, and these differentially expressed lipids included 59 upregulated and 71 downregulated lipids in psoriasis patients compared with healthy controls (Fig. 2a). The annotation of these lipids to assess their ratio of dysregulation among corresponding lipid species revealed that most ceramides were upregulated in the psoriatic skin lesions (Fig. 2b). The total differential lipids are shown in the cloudplot of Fig. 2c. The abundance of CerP, Cer, phosphatidylethanol (PEt), and (O-acyl)-1-hydroxy fatty acid (OAHFA) was increased in psoriatic lesion compared with non-lesions, while the levels of monogylcosylceramide (CerG1), triglyceride (TG), and phosphatidylethanolamine (PE) were decreased in psoriatic lesion compared with non-lesions (Fig. 2b-c). According to the heatmap (Supplementary Fig. 2e), there was a significant difference in the expression of CerP, Cer, and CerG1 between the psoriatic lesions group and the non-lesions group (Fig. 2d). Furthermore, the levels of CerP and ceramides were significantly increased in psoriatic lesions

compared with non-lesions (Fig. 2d), which was similar to the results of the metabolomic profiling of the skin lesions of the IMQ-induced psoriasis-like mouse model (Supplementary Fig. 2f and Supplementary Fig. 3). We applied LASSO modeling to these differential lipids identified in both the mouse model and human skin lesions, and variables with selection frequencies above 50% were used to classify psoriasis to healthy controls, including CerP (d16:0/21:1+O), Cer (d18:1/18:2), Cer (d18:0/16:0), and Cer (d18:0/24:0) (Fig. 2e). The AUC of the above four lipids mentioned above was 90.1% (Fig. 2f). Therefore, these results indicated that disordered lipid metabolism occurred in psoriatic lesions and that CerP and Cer might play an important role in the development of psoriasis.

Classification of psoriasis subtypes based on lipid profiling

Psoriasis patients were classified into two groups, namely, the CC1 (n=8) and CC2 (n=13) groups (Fig. 3a), by consensus clustering of all differential lipids between psoriatic skin lesions and non-lesions controls [29]. The clinical characteristics of the CC1 and CC2 groups are shown in the Supplementary Table 3. The distribution of the abundance of all the differential lipids related to these two molecular subtypes of psoriasis is shown in Fig. 3b, the results showed that the levels of 8 cermides, 3 glucosylceramide (CerG1), 1 cholesterol ester (ChE), 1 lysophosphatidylcholine (LPC), 4 diacylglycerol (DG), 2 phosphatidylcholines (PC), 10 phosphatidylethanolamine (PE), 2 phosphatidylglycerol (PG), 2 sphingomyelin (SM) and 35 triglyceride (TG) were decreased in CC1, while the level of CerP(d16:0/20:1+O) was increased in CC1, as shown in Fig. 3b. Although there was no significant difference in BMI or waist-to-hip ratio between the two groups, the expression of CerP in the CC1 group was significantly higher than that in the CC2 group (Fig. 3c), which was similar to the results of the skin lesions of the IMQ-induced psoriasis-like mouse model. The level of CerP after the treatment of IMQ for 6 days was significantly higher than that after control treatment and IMQ treatment for 3 days (Fig. 3d). Interestingly, the epidermal thickness was significantly higher in the CC1 group than in the CC2 group (Fig. 3e), indicating that CerP might play an important role in the development of psoriasis. In addition, the level of uric acid in the CC1 group was significantly higher than that in the CC2 group (Fig. 3f). To investigate the relationship between uric acid and disease severity of psoriasis, we enrolled 937 psoriasis patients and measured the uric acid levels in plasma. The clinical characteristics of the validation cohorts are summarized in Table 2. The results showed that the level of uric acid was positively correlated with the PASI score of psoriasis (Fig. 3g). The high uric acid group showed a higher PASI score than that the normal uric acid group (Fig. 3h). Therefore, these results showed that lipid disorders play a crucial role in the pathogenesis of psoriasis. The high level of CerP in the psoriatic skin lesions and the high level of uric acid suggest more severe psoriasis.

C1P facilitates IMQ-induced psoriasis-like dermatitis in mouse model

To investigate the role of CerP in the development of psoriasis, we subcutaneously injected ceramide-1-phosphate (C1P) into the ears of IMQ-treated mice once per day for six consecutive days, and the mice were sacrificed on day 7. Compared with vehicle treatment, C1P treatment significantly aggravated psoriasis-like pathological progression (Fig. 4a), including the PASI score and epidermal thickness (Fig.

4b-d). Given that Th1 and Th17 cells are well documented to play crucial roles in psoriasis [1], we examined immune cell infiltration in the IMQ-induced psoriasis-like mouse model. Consistent with previous studies, IMQ treatment alone induced Th1 and Th17 infiltration into skin lesions (Fig. 4e-h), while C1P treatment significantly exacerbated the IMQ-induced inflammatory response, including the Th1 and Th17 responses (Fig. 4e-h). There was no significant difference in the body weights of the C1P-treated mice and vehicle control (Supplementary Fig. 4a). These results indicated that C1P promotes IMQ-induced psoriasis-like inflammation.

Hyperproliferation and production of inflammatory factors by keratinocytes (KCs) play a key role in the pathogenesis of psoriasis [30]. To study the role of C1P in the proliferation of KCs, we treated KCs isolated from the human foreskin with different concentrations of C1P. The results showed that C1P had no significant effect on the proliferation of KCs (Fig. 5a). However, the mRNA expression of inflammatory factors, including *IL-6*, *IL-1β*, and *IL-17A*, was dramatically increased in KCs after treatment with C1P (Fig. 5b). To further understand the molecular mechanism underlying these effects of C1P, RNA sequencing (RNA-seq) was performed on KCs treated with the vehicle control or C1P for 12 hours. The results showed that the expression of 1171 genes were increased, whereas the expression of 756 genes was decreased. Gene set enrichment analysis (GSEA) analysis identified the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and we found that the top 10 enriched pathways included DNA replication and the cell cycle (Fig. 5c-d). Furthermore, the KEGG pathway enrichment analysis also indicated that the DNA replication and the cell cycle signaling pathways were activated in C1P-treated KCs (Fig. 5e), suggesting that C1P not only increases the expression of inflammatory factors in keratinocytes but also affects DNA replication and the cell cycle.

In addition, abnormal function of $CD4^+$T cells, including increased numbers and inflammatory cytokines production of Th1 and Th17 cells, is involved in the pathogenesis of psoriasis [1]. Therefore, we investigated the role of C1P in the proliferation and differentiation of $CD4^+$T cells. Mouse $CD4^+$T cells were isolated and activated by anti-CD3 and anti-CD28 Abs in the presence of different concentrations of C1P, and the results showed that C1P had no effect on the proliferation of $CD4^+$ T cells in vitro (Supplementary Fig. 5a-b). Then, we isolated naïve $CD4^+$ T cells and cultured them under Th1- or Th17-polarizing conditions upon C1P treatment. Our results showed that C1P did not affect Th1 and Th17 differentiation (Supplementary Fig. 5c-f). These findings showed that C1P facilitates the pathogenesis of psoriasis by activating keratinocytes.

Topical application of ceramide kinase inhibitor (NVP 231) attenuates IMQ-induced psoriatic dermatitis

Ceramide kinase (CERK) regulates the phosphorylation of ceramide and produces C1P [31]. To investigate whether inhibiting the production of C1P could be a new strategy for the treatment of psoriasis, we treated the IMQ-induced psoriasis-like model mice with the CERK inhibitor (NVP 231). We first treated IMQ-induced psoriasis-like model mice with topical NVP 231 creams once per day. After the IMQ-induced model mice were treated with topical NVP 231 creams for 6 days, the redness, scaling, and thickness of the ear skin were significantly alleviated, the PASI score was also significantly decreased (Fig. 6a-b). In

addition, the histological evaluation showed a significant decrease in epidermal thickness after treatment with the topical NVP 231 creams (Fig. 6c-d). Topical NVP treatment improved the IMQ-induced weight loss of the mice (Supplementary Fig. 6a). To determine the effect of topical NVP 231 creams on dermal immune cells in the IMQ-induced psoriasis-like mouse model, we examined the percentages of Th1, Th17, MDSCs, and Tregs in the ear skin lesions. The results showed that topical NVP 231 alleviated inflammatory cell infiltration in the IMQ-induced psoriatic inflammation, and the infiltration of Th1, Th17, and MDSCs in skin lesions was significantly decreased (Fig. 6e-f and Supplementary Fig. 6b-e), while the percentage of Tregs was increased after topical NVP 231 treatment (Supplementary Fig. 6f-g). Similarly, intraperitoneal injection of NVP 231 effectively alleviated the IMQ-induced psoriasis-like phenotype and epidermal thickness (Supplementary Fig. 7a-d) but had no effect on the body weight (Supplementary Fig. 7e). Therefore, these data indicated that the CERK inhibitor effectively alleviated IMQ-induced psoriasis-like dermatitis, including reducing epidermal thickening and dermal inflammatory cells infiltration (Fig. 6g).

Discussion

Lipids have long been known to play a key role in immune regulation, and their activity in cutaneous inflammation is particularly prominent [32]. To explore the role of abnormal lipids in the pathogenesis of psoriasis, skin lesions of the IMQ-induced psoriasis-like mouse model were used for lipid profiling. IMQ is a toll-like receptor (TLR)7 agonist, and topical application of IMQ-induced psoriasis-like dermatitis, including the psoriasis-like phenotype and IL-23/Th17 signaling pathways activation [33]. Our results showed that with the application of IMQ, ceramides phosphate and ceramides are significantly enriched in skin lesions. Similarly, metabolomic profiling of psoriatic lesions and non-lesions of psoriasis patients showed that the abundance of ceramides phosphate and ceramides were increased in psoriatic lesions compared with non-lesions. These data indicated that IMQ-induced psoriasis-like skin lesions have lipids abnormalities that are similar to those of skin lesions of psoriasis patients, suggesting that the IMQ-induced mouse model is suitable for use to study psoriasis. More importantly, disordered metabolism of lipids, mainly ceramides phosphate and ceramides, plays an important role in inducing psoriatic inflammation.

The cutaneous immune system is regulated by mediators, such as cytokines, and bioactive lipids [6,34]. Previous researchers found that bioactive lipid mediators may contribute to the pathophysiology of psoriasis. Psoriatic lesions were rich in arachidonic acid metabolites compared with adjacent non-lesions and healthy control, suggesting that psoriatic skin has a disease-specific phenotype-specific profiles of omega-6 fatty acid–oxidized derivatives [19]. In this study, through metabolomic profiling, we found that ceramides phosphate and ceramides levels were increased in psoriatic lesions compared with control from both psoriasis patients and the psoriasis-like mouse model. LASSO modeling identified four lipids, including CerP (d16:0/21:1 + O), Cer (d18:1/18:2), Cer (d18:0/16:0), and Cer (d18:0/24:0). These above four lipids showed good AUC values (90.1%). These data indicated that CerP and Cer might play an important role in the development of psoriasis.

Previous studies have shown that metabolic abnormalities, especially obesity, are associated with higher incidence, prevalence, and severity of psoriasis [35]. Obesity affects the efficacy of biological agents used for the treatment of psoriasis. In this study, we found that psoriasis patients could be classified into two groups, the CC1 and CC2 groups, by consensus clustering of differential lipids. The level of CerP in the CC1 group was significantly higher than that in the CC2 group. Consistently, the epidermal thickness was significantly higher in the CC1 group than in the CC2 group. Moreover, although there was no significant difference in BMI or waist-to-hip ratio between the two groups, the level of uric acid in the CC1 group was significantly higher than that in the CC2 group. It was confirmed that the level of uric acid was positively correlated with the PASI score of the psoriasis patients in the validation cohorts. Importantly, it is well known that uric acid levels increase with obesity due to insulin resistance reducing the ability of the kidney to eliminate uric acid [36,37]. Therefore, uric acid may be a more sensitive index for metabolic molecular subtypes of psoriasis, which provides evidence for the screening and management of metabolic psoriasis patients.

Ceramides phosphate and ceramides are derivatives of sphingosine. Sphingolipids are bioactive lipid components of cell membranes [38]. Ceramide is the central building block of sphingolipid biosynthesis, and ceramide is processed to form sphingolipids with different structures and functions. Ceramide is an integral component of the stratum corneum lipid matrix, and the topical application of Cer to impaired skin is useful for repairing the skin barrier [39]. However, researchers found that the ratios of Cer [NP]/[NS], Cer [NH]/[NS], Cer [NP]/[AS], Cer [NH]/[NS], Cer [NDS]/[AS], Cer [AH]/[AS] and Cer [EOP]/[AS] were significantly different between psoriasis patients and healthy control subjects [40]. In addtion, the relative levels of Cer [NS], Cer [NP], Cer [AS], Cer [ADS], Cer [AP], and Cer [EOS] tended to be increased in the keratinocytes of psoriasis patients, whereas Cer [NDS] tended to be expressed at lower levels in psoriasis patients than in healthy subjects [41]. A significant decrease in the percentage of long-chain Cer was observed in psoriasis-like murine epidermis and human psoriatic lesions [42]. Therefore, changes in the profiles and metabolism of ceramides are associated with psoriasis. In this study, we found that the levels of ceramides, including Cer (d18:1/18:2), Cer (d18:0/16:0), and Cer (d18:0/24:0), are enriched in psoriatic lesions. Therefore, these results provide insights into the ceramide profile associated with the development of psoriasis.

Ceramide can be phosphorylated by ceramide kinase (CERK) to generate ceramide-1-phosphate (C1P) [31,43], which is involved in the development of metabolic inflammation [44,45]. Ceramide-1-phosphate (C1P) plays an important role in regulating cell survival, migration, apoptosis, and autophagy, participates in proinflammation processed and is involved in inflammasome assembly/activation, which can stimulate group IVA cytosolic phospholipase A2α and subsequently increase the levels of arachidonic acid and proinflammatory cytokines [43,46,47]. Similar to our study, we found that the level of ceramides phosphate was highly increased in the psoriatic lesions and skin lesions from the IMQ-induced psoriasis-like mouse model compared with the control groups. Importantly, C1P facilitates the pathogenesis of psoriasis, exacerbating the severity of disease and the infiltration of Th1 and Th17 cells into the skin lesions of the IMQ-induced psoriasis-like mouse model. Furthermore, C1P induced the expression of *IL-6*, *IL-1β*, and *IL-*

17A and activated DNA replication and cell cycle signaling pathways in the primary keratinocytes, as shown by RNA-seq. These data indicated that C1P is involved in the pathogenesis of psoriasis by activating keratinocytes. Interestingly, inhibiting the production of C1P with the ceramide kinase inhibitor NVP231 can effectively alleviate the IMQ induced psoriasis-like inflammation, suggesting that inhibiting the synthesis of C1P may be a novel therapy for the treatment of psoriasis.

In summary, our data suggest that the abundance of ceramides phosphate is enriched in psoriatic lesions. C1P aggravates psoriasis-like inflammation by activating keratinocytes, as a consequence, suppression of C1P pathway by topical application is a novel potential strategy for psoriasis treatment.

Declarations

Ethical Approval and Consent to participate

The use of human specimens was approved by the ethics committees of Xiangya Hospital of Central South University, Changsha, Hunan, China, and informed consent was obtained from all subjects. All animal the experiments were performed according to the Animal Care and Use Committee guidelines of Xiangya Medicine School of Central South University.

Consent for publication

Not applicable.

Availability of data and materials

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Cong Peng (pengcongxy@csu.edu.cn). The accession numbers for the sequencing data in this paper are OEP003183. Supplementary information accompanies the manuscript on the Experimental & Molecular Medicine website (http://www.nature.com/emm/).

Competing interests

The authors have declared that no conflict of interest exists.

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Authors' contributions

P.L., X.C., and C.P. conceived the project and wrote the manuscript. P.L. and G.H. designed, performed experiments, and analyzed the data. L.L., C.C., and B.Y. helped with mouse experiments. W.Z., Y.K., J.L., M.C., and J.S. diagnosed with plaque psoriasis, provided patient samples and information. P.L. and C.P. edited the manuscript. L.L., X.C. and C.P. supervised the work.

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Tables

Table 1. Demographics and clinical characteristics of the participants (n=21)

Characteristics	
Age, years, mean ± SEM (range)	43.00 ± 7.774 (15-72)
Sex	
Male	14
Female	7
Course of the disease, years, mean ± SEM (range)	
Age at onset of psoriasis	36.18 ± 7.940 (8-56)
Duration of psoriasis	7.02 ± 6.223 (0.17-30)
Disease process	
Progressive phase	19
Stable phase	2
Regressive phase	0
Work status, n (%)	
Student	3
Employed	15
Retired or Unemployed	3
Family history [1]	
No	19
Yes	2
BMI [2], mean ± SEM	22.95 ± 4.689
PASI [3], mean ± SEM	10.73 ± 5.046
Epidermal thickness [4], µm, mean ± SEM	749.2 ± 198.3
DLQI [5], mean ± SEM	8.23 ± 5.880

1: Family history, family history of plaque psoriasis.

2: BMI, body mass index

3: PASI, psoriasis area and severity index.

4: Epidermal thickness, the epidermal thickness was measured from the stratum basale to the stratum granulosum

5: DLQI: dermatology life quality index [48].

Table 2. Demographics and clinical characteristics of the validation cohorts (n=937)

Characteristics	
Age, years, mean ± SEM (range)	41.68 ± 6.914 (8-84)
Sex	
Male	634
Female	303
Course of the disease, years, mean ± SEM (range)	
Age at onset of psoriasis	33.23 ± 6.701 (1-82)
Duration of psoriasis	6.12 ± 3.144 (0.08-40.42)
Disease process	
Progressive phase	810
Stable phase	87
Regressive phase	40
Work status, n (%)	
Student	98
Employed	638
Retired or Unemployed	201
Family history [1]	
No	770
Yes	167
BMI [2], mean ± SEM	26.71 ± 37.56
PASI [3], mean ± SEM	11.48 ± 5.398
DLQI [4], mean ± SEM	6.31 ± 1.976

1: Family history, family history of plaque psoriasis.

2: BMI, body mass index

3: PASI, psoriasis area and severity index.

4: DLQI: dermatology life quality index [48].

Figures

Figure 1

Identification lipid profiling in IMQ-induced psoriasis dermatitis

(a) Heatmap of all quantified lipids in IMQ-induced mice model at the time points of initial, 3 days, 6 days (n = 7). The differential information was annotated in the right panel with adjusted p values, fold changes between each compared two groups and lipid classes. **(b)** Time series clustering of lipids with different abundance patterns across the three stages of psoriasis-like mouse model. **(c)** Heatmap of ratios based on the lipid numbers enriched in each cluster vs total lipid numbers for each lipid species.

Figure 2

Identification lipid profiling in psoriatic lesions and non-lesions of psoriasis patients

(a) Volcanic map of differential lipids in psoriatic lesions and non-lesions of psoriasis patients. Red points represent significantly differential lipids. **(b)** Barplot based on ratio of lipid numbers for different lipid species up-regulated or down-regulated in psoriasis group. **(c)** Cloud plot of differential lipids annotated with fold changes and lipid species in psoriatic lesions and non-lesions of psoriasis patients. **(d)** Heatmap of all Cer, CerG1, and CerP in psoriatic lesions and non-lesions of psoriasis patients. **(e)** LASSO frequency distribution of differential ceramides and CerP. **(f)** ROC of lipid signatures combined with CerP (d16:0/21:1+O), Cer (d18:1/18:2), Cer (d18:0/16:0), and Cer (d18:0/24:0) to distinguish psoriasis from healthy controls.

Figure 3

Classification of psoriasis subtypes based on lipid profiling

Psoriasis patients' subtypes based on lipids. **(a)** The heat map corresponding to the consensus matrix for 2 molecular subtypes obtained by applying consensus clustering. **(b)** The heatmap of all differential

lipids related with CC1 and CC2, which was generated using the heat map function in R with subtypes, BMI, uric acid, thickness, PASI score and DLQI score as the annotations. CerP(d16:0/20:1+O) was annotated as specifically upregulated lipid in CC1. **(c)** The abundance of CerP(d16:0/20:1+O) in skin lesions of psoriasis patients in CC1 and CC2 group. **(d)** The abundance of CerP(d38:1+O) in skin lesions of IMQ-induced mice model at the time points of initial, 3 days, 6 days (n = 6). **(e)** The epidermal thickness in skin lesions of psoriasis patients in CC1 and CC2 group. **(f)** The uric acid in plasma of psoriasis patients in CC1 and CC2 group. **(g)** The uric acid (UA) in plasma of psoriasis patients in validation cohorts. **(h)** The PASI score of psoriasis patients in UA. normal and UA. high group. UA. normal: uric acid <= 430. UA. high: uric acid >430.

Figure 4

C1P facilitates IMQ-induced psoriasis-like dermatitis in mouse model

Intradermal administration of 10 μl C1P (1 mM) or 10 μl normal saline (Vehicle) once per day for 6 consecutive days during the application of IMQ in mice (BALB/c) ear. Eight mice in each group were sacrificed on Day 7 to conduct experiments. **(a-b)** Phenotypic presentation and PASI score of Control (Ctr) and IMQ-induced mouse model ear skin treated with vehicle or C1P. **(a)** Phenotypic presentation. **(b)** PASI score (n = 8). **(c)** H&E staining of mice in **a** (n = 8). Scale bars, 100 μm. **(d)** Epidermal thickness of mice in **a** (n = 8). **(e-h)** Flow cytometric analysis of cell suspensions from the ear skin in each group (n = 8) treated with IMQ for 6 days. **(e)** Representative Flow cytometry plots of Th1 ($CD4^+IFN\text{-}\gamma^+$). **(f)** Flow cytometric statistical data of Th1 ($CD4^+IFN\text{-}\gamma^+$). **(g)** Representative Flow cytometry plots of Th17 ($CD4^+IL\text{-}17A^+$). **(h)** Flow cytometric statistical data of Th17 ($CD4^+IL\text{-}17A^+$). Data are presented as the mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Significant differences were evaluated using Two-tailed Student's t test (**b**) or One-way ANOVA with Bonferroni's post hoc test (**d, f and h**).

Figure 5

The effect of C1P on transcriptional expression profiling in KCs

(a) The proliferation of KCs in the presence of different concentration of C1P. KCs were treated with vehicle control or different concentration of C1P for 24, 48, or 72 hours. CCK8 assay was performed to detect KCs viability of C1P treatment as described in *Methods*. Data are presented as the mean ± SEM. The results are representative of at least 3 independent experiments with 5 samples per group in each. **(b)** The expression of inflammatory factors was raised in KCs after being treated with C1P. KCs were isolated from the foreskin of healthy donors, then stimulated with C1P (1 μM) or vehicle control for 6 or 12 hours.

Total RNA was extracted from KCs, and the expression of *IL-6*, *IL-1β*, or *IL-17A* was performed by RT-qPCR. The data are representative of at least 3 independent experiments with 3 samples per group in each. Data are presented as the mean ± SEM. *p < 0.05. Significant differences were evaluated using One-way ANOVA with Bonferroni's post hoc test. (**c-e**) Transcriptome profiling in the C1P-treated KCs. KCs were treated with vehicle control or C1P (1 μM) for 12 hours (n = 3). Total RNA was extracted from KCs and were used for whole-genome transcriptome analysis. (**c**) DNA replication enriched by GSEA were identified from RNA-seq data of C1P-treated KCs compared with vehicle control. Normalized enrichment score (NES) and Normalized p-value (p) are shown in the plot. (**d**) Cell cycle enriched by GSEA were identified from RNA-seq data of C1P-treated KCs compared with vehicle control. Normalized enrichment score (NES) and Normalized p-value (p) are shown in the plot. (**e**) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway showing differentially expressed genes (DEGs) expression in C1P-treated KCs relative to vehicle control. p value cutoff = 0.01.

Figure 6

Topical application of ceramide kinase inhibitor (NVP 231) attenuates IMQ-induced psoriatic inflammation

Topical administration CERK inhibitor (NVP 231) cream or vehicle cream once per day for 6 consecutive days during the application of IMQ in mice (BALB/c) ear. Six mice in each group were sacrificed on Day 7 to conduct experiments. **(a-b)** Phenotypic presentation and PASI score of Control (Ctr) and IMQ-induced mouse model ear skin treated with vehicle or NVP 231 cream. **(a)** Phenotypic presentation. **(b)** PASI score (n = 6). **(c)** H&E staining of mice in **a** (n = 6). Scale bars, 100 μm. **(d)** Epidermal thickness of mice in **a** (n = 6). **(e-f)** Flow cytometric analysis of cell suspensions from the ear skin in each group (n = 6) treated with IMQ for 6 days. **(e)** Flow cytometric statistical data of Th1 ($CD4^+IFN\text{-}\gamma^+$). **(f)** Flow cytometric statistical data of Th17 ($CD4^+IL\text{-}17A^+$). **(g)** Schematic illustration of ceramides phosphate and ceramides were enriched in psoriatic lesions as well as their role in lipid subtypes and in the pathogenesis of psoriasis. Data are presented as the mean ± SEM. *p < 0.05, **p < 0.01, ****p < 0.0001. Significant differences were evaluated using Two-tailed Student's t test (**b**) or One-way ANOVA with Bonferroni's post hoc test (**d, e and f**).

Supplementary Files

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- SupplementaryTable3.xls
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