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Immune regulations of inflammatory skin diseases

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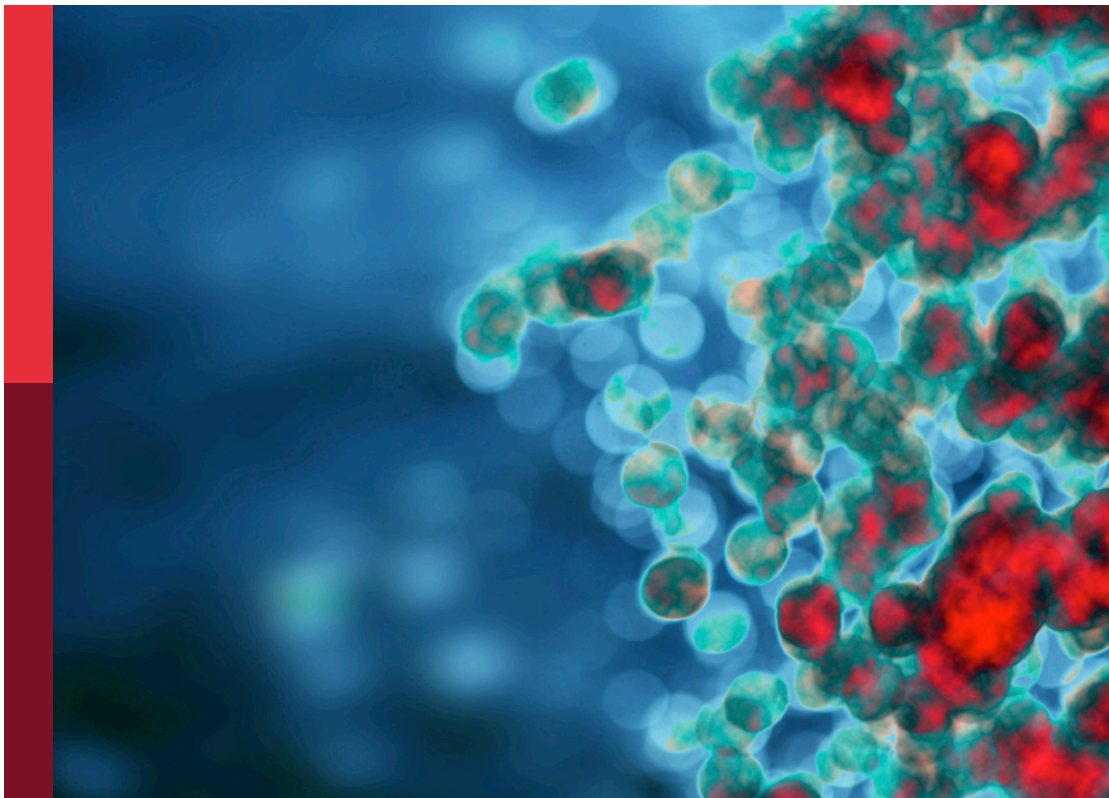

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Immune regulations of inflammatory skin diseases

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IL36G is associated with cutaneous antiviral competence in psoriasis

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Background: Psoriasis is a common inflammatory skin disease that has a great impact on patients' physical and mental health. However, the causes and underlying molecular mechanisms of psoriasis are still largely unknown.

Methods: The expression profiles of genes from psoriatic lesion samples and skin samples from healthy controls were integrated *via* the sva software package, and differentially expressed genes (DEGs) between psoriasis and healthy skin were screened by the limma package. Furthermore, GO and KEGG pathway enrichments for the DEGs were performed using the Clusterprofiler package. Protein–protein interaction (PPI) networks for the DEGs were then constructed to identify hub genes. scGESA analysis was performed on a single-cell RNA sequencing dataset *via* irGSEA. In order to find the cytokines correlated with the hub genes expression, single cell weighted gene co-expression network analyses (scWGCNA) were utilized to build a gene co-expression network. Furthermore, the featured genes of psoriasis found in suprabasal keratinocytes were intersected with hub genes. We then analyzed the expression of the intersection genes and cytokines in the integrated dataset. After that, we used other datasets to reveal the changes in the intersection genes' expression levels during biological therapy. The relationship between intersection genes and PASI scores was also explored.

Results: We identified 148 DEGs between psoriatic and healthy samples. GO and KEGG pathway enrichment analysis suggested that DEGs are mainly involved in the defense response to other organisms. The PPI network showed that 11 antiviral proteins (AVPs) were hub genes. scGSEA analysis in the single-cell transcriptome dataset showed that those hub genes are highly expressed in keratinocytes, especially in suprabasal keratinocytes. ISG15, MX1, IFI44L, and IFI27 were the characteristic genes of psoriasis in suprabasal keratinocytes. scWGCNA showed that three cytokines—IL36G, MIF, and

IL17RA—were co-expressed in the turquoise module. Only interleukin-36 gamma (IL36G) was positively correlated with AVPs in the integrated dataset. IL36G and AVPs were found co-expressed in a substantial number of suprabasal keratinocytes. Furthermore, we found that the expression levels of IL36G and the 4 AVPs showed positive correlation with PASI score in patients with psoriasis, and that these levels decreased significantly during treatment with biological therapies, but not with methotrexate.

Conclusion: IL36G and antiviral proteins may be closely related with the pathogenesis of psoriasis, and they may represent new candidate molecular markers for the occurrence and severity of psoriasis.

KEYWORDS

psoriasis, antiviral protein (AVP), IL36G, keratinocyte, treatment

Introduction

Psoriasis is a common immune-mediated inflammatory dermatosis, prevalent worldwide, that can have serious effects on patients' physical and mental health (1). The pathogenesis of psoriasis depends on genetic susceptibility, infection, immune abnormalities, and psychoneurotic factors, resulting in chronicity and leaving patients prone to the recurrence of erythematous plaques with adherent silvery scales (2). The main pathological characteristics of psoriatic lesions are hyperproliferation and aberrant differentiation of epidermal keratinocytes. Even though much is known about this condition, the multifactorial nature of its pathogenesis has not been studied thoroughly. In particular, the extent of the role infection plays in the pathogenesis of psoriasis remains controversial.

A previous study found that persistent subclinical streptococcal and staphylococcal infections may be responsible for not only recurrent acute guttate psoriasis, but also new episodes of chronic plaque psoriasis (3). Recent studies have demonstrated that the pathophysiology of psoriasis is closely associated with antimicrobial peptides (AMPs), including cathelicidin (LL-37), human β-defensins, S100 proteins, and lipocalin 2 (4–6). AMPs are an essential part of the innate immune system's defense against pathogen-based infections (7). Furthermore, it has been confirmed that infection is involved in the pathological mechanism of psoriasis. Levels of keratinocyte-derived antiviral proteins (AVPs) of 2–5-oligoadenylate synthase 2 (OAS2) were found to be significantly elevated in both the epidermis and serum of psoriasis patients (8). Gao et al. found that AVPs, such as ISG15, MX1, OAS2, OASL, and OAS3, were overexpressed in cultured human keratinocyte cells (HaCaT) stimulated by tumor necrosis factor-alpha (TNF-α) in a psoriasis cell model (9). Moreover, ISG15, RSAD2, IRF7, MX2, and TRIM22 have been found to be overexpressed in psoriatic skin. However, another study found no increased expression of AVPs in skin affected by atopic dermatitis, a chronic inflammatory disease similar to psoriasis (10). It is therefore of interest to determine the link between skin antiviral phenotype and the pathogenesis of psoriasis. Therefore, it is imperative to explore the roles of AVPs in the occurrence of psoriasis.

Herein, we merged five mRNA microarray datasets from the gene expression omnibus (GEO) database to screen for differentially expressed genes (DEGs) between psoriasis and healthy samples. Subsequently, enrichment analysis and protein–protein interaction (PPI) network analysis were performed to screen for the hub gene. We then explored the underlying mechanism of hub genes in a single-cell transcriptome. The workflow for this study is shown in Figure S1.

Material and methods

Data sources and preprocessing

We obtained the psoriasis transcriptome dataset from GEO, which included the GSE13355 dataset (58 psoriatic lesion samples and 58 non-lesion samples), the GSE30999 dataset (85 psoriatic lesion samples and 85 non-lesion samples), the GSE34248 dataset (14 psoriatic lesion samples and 14 non-lesion samples), the GSE41662 dataset (24 psoriatic lesion samples and 24 non-lesion samples), the GSE14905 dataset (33 psoriatic lesion samples and 28 non-lesion samples), and the GSE162183 dataset (including the lesions of 3 patients and similar regions from 3 healthy donors). Besides these datasets, the GSE85034 (30 psoriatic lesion tissues before and after adalimumab or

methotrexate (MTX) treatment), GSE51440 (39 psoriatic lesion tissues before and after guselkumab treatment), GSE117468 (41 psoriatic lesion tissues before and after brodalumab treatment and 44 psoriatic lesion tissues before and after ustekinumab treatment), GSE41664 (36 psoriatic lesion tissues before and after etanercept treatment), and GSE69967 (26 psoriatic lesion tissues before and after tofacitinib treatment) datasets are also included in our study (Table 1).

All patients whose samples were included in this study were diagnosed with psoriasis vulgaris. The list of cytokines was obtained from https://www.immport.org/resources/cytokineRegistry. During data integration, when a given gene expression was detected across all five datasets, the genes were preserved for further analysis. The expression profiles were integrated *via* the Combat function of the sva package (11) to remove the batch effects.

Analysis of differentially expressed genes

The DEGs between psoriasis and normal tissues were identified by the limma R software package. Moreover, |log2 fold change (FC) |≥ 2, with an adjusted *p*-value of < 0.05, was set as the threshold for differentially expressed genes selection.

GO and KEGG pathway enrichment analyses

The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed using the clusterProfiler software package (v 4.4.1) to explore the biological function of the DEGs. GO terms of the KEGG pathway with a false discovery rate (FDR) < 0.05 were considered to be significantly enriched.

Construction of the protein–protein interaction network and hub gene screening

The DEGs were imported into the STRING protein database (STRING11.5; https://string-db.org/) with a combined score >0.9 was adjusted to obtain the protein–protein interaction network. The plug-in CytoHubba (http://apps.cytoscape.org/cytohubba) was used to calculate the topological characteristic parameters of the nodes in the PPI network to screen out the hub genes. The degree method was used to explore the hub genes in the PPI network, and the screening condition was degrees ≥ 10.

Data processing for single-cell RNA sequencing

The GSE162183 expression matrices were obtained from the GEO database. Quality control and dimensionality reduction were performed by the Seurat software package (v4.1.1). For the initial QC step, we created Seurat objects for the normal and psoriasis groups and filtered out the cells that expressed < 200 genes. Genes expressed in fewer than 3 cells were also excluded. I gene expression profiles of the remaining cells were then normalized, and 2,000 highly variable genes from each sample were identified by the vst method. All genes were scaled, and the principal component analysis was conducted. The cells were clustered by unsupervised clustering (resolution = 0.5) and visualized by umap using the top 20 principal components. Cell-type annotation was performed using the singleR software package (v 1.8.1) and refined with manual annotation. Integration of all single-cell rank-based gene set enrichment analyses with the AVPs was performed by irGSEA (v 1.1.2), and the calculation of the enrichment scoring method was set as UCell.

TABLE 1 The GEO datasets included in the present study.

GEO accession	Diagnosis	Treatment	Lesional skin	Non-lesional	Platforms	Severity	PubMed ID
GSE13355	psoriasis vulgaris	None	58	58	GPL570	NA*	19169254
GSE30999	psoriasis vulgaris	None	85	85	GPL570	moderate to severe	22763790
GSE34248	psoriasis vulgaris	None	14	14	GPL570	NA	23308107
GSE41662	psoriasis vulgaris	None	24	24	GPL570	moderate to severe	23308107
GSE14905	psoriasis vulgaris	None	33	28	GPL570	NA	18648529
GSE162183	psoriasis vulgaris	None	3	3	GPL24676	NA	33958582
GSE85034	psoriasis vulgaris	Adalimumab 16 weeks	17	17	GPL10558	moderate to severe	27667537
GSE85034	psoriasis vulgaris	MTX 16 weeks	13	13	GPL10558	moderate to severe	27667537
GSE117468	psoriasis vulgaris	Brodalumab 12 weeks	41	41	GPL570	moderate to severe	31883845
GSE117468	psoriasis vulgaris	Ustekinumab 12 weeks	44	44	GPL570	moderate to severe	31883845
GSE41664	psoriasis vulgaris	Etanercept 12 weeks	36	36	GPL570	moderate to severe	23308107
GSE69967	psoriasis vulgaris	Tofacitinib 12 weeks	26	26	GPL570	moderate to severe	27059729

*NA, not available.

Single-nucleus consensus weighted gene coexpression network analysis

Single-nucleus consensus weighted gene coexpression network analysis (scWGCNA) is a systematic biological method used to construct a scale-free network based on single-cell gene expression profiles. In the present study, scWGCNA was performed on the expressions and clinical prototypes found in the GSE162183 dataset. First, we constructed metacells with k = 200, then a signed similarity matrix was created. The signed similarity matrix was then raised to power $\beta = 16$, varying between cell types, to emphasize strong correlations and reduce the emphasis of weak correlations on an exponential scale. The resulting adjacency matrix was then transformed into a topological overlap matrix. Modules were defined using specific module-cutting parameters, including a minimum module size of 25 genes, a deepSplit score of 2, and a merge Cut Height of 0.6. Module eigengenes (MEs) were identified using the package's ME function, and signed module membership (MM) was utilized to determine the correlation of MEs with individual genes. Gene significance (GS) was defined as the correlation between the gene expression level and the psoriasis. Modules with the strongest positive correlations with psoriasis were selected.

Statistical analysis

All statistical analyses were performed on R software (v4.1.2). Differences between groups were compared by the Mann-Whitney-Wilcoxon test. The correlation between immune infiltrating cells and core genes was measured by Pear'on's chi-square test. An adjusted p-value of < 0.05 was considered statistically significant.

Results

Differential gene expression analysis

The GSE13355, GSE30999, GSE34248, GSE41662, and GSE14905 datasets were merged into an integrated dataset, which contained 214 psoriatic lesion samples (LS) and 209 non-lesion samples (NL). Before the adjustment, the clustering of samples was largely driven by batch effect, which is due to merging the data from different datasets (Figure 1A). After applying ComBat, the batch effect was mitigated (i.e., samples from different datasets were mixed together) (Figure 1B). The gene differential expression in the integrated dataset was analyzed *via* the limma package. Based on standard threshold values for |log2 fold change (FC) |≥ 2, with an adjusted p-value of < 0.05, 148 DEGs were identified, 125 of which were up-regulated and 23 of which were down-regulated (Figures 1C, D) (Table S1).

Pathway enrichment analysis and protein–protein interaction analysis

To further investigate the biological functions of the DEGs, we performed a pathway enrichment analysis. The result of the GO enrichment analysis showed that DEGs were mainly related to defense responses to other organisms, to the antimicrobial humoral response, and to the response to virus (Figure 2A; Table S2). The KEGG enrichment analysis showed that DEGs were mainly associated with viral proteins interacting with cytokines and cytokine receptors, cytokine–cytokine receptor interactions, and the interleukin (IL)-17 signaling pathway (Figure 2B) (Table S3).

To screen for hub genes, we first constructed a protein–protein interaction (PPI) network with 32 nodes and 1347 interaction edges (Figure 2C). In the PPI network, a total of 11 genes (RSAD2, IFIT1, MX1, ISG15, IFI27, OAS3, OAS2, IFI6, IRF7, OAS1, and IFI44L) were identified as hub genes with degrees ≥ 10 (Table S4). All of those genes were AVPs, and were significantly upregulated in psoriatic lesions (Figure 2D).

GSEA of scRNA-seq

To investigate the expression of AVPs in psoriatic lesions at single-cell resolution, we collected tissue from 3 psoriasis patients and tissue of a similar region from 3 healthy donors and performed single-cell RNA sequencing (scRNA-seq). After standard data processing and quality control procedures, we obtained transcriptomic profiles for 24,234 cells. Principal cell clusters were classified using an unsupervised graph-based clustering strategy. Cells with similar profiles were annotated using the singleR package.

The cellular composition is shown in Figure 3A. Single-cell rank-based gene set enrichment analysis (GSEA) showed that the enrichment score was higher in keratinocytes (including suprabasal keratinocyte and basal keratinocyte) (Figure 3B). The AVP expression levels in cell populations of the psoriatic lesions and normal skin are shown in Figure S2. ISG15, MX1, IFI44L, and IFI27 were the characteristic psoriasis genes found in suprabasal keratinocytes (Table S5).

Use of scWGCNA to screen the cytokines correlated with AVP expression

The scWGCNA package was used to merge 200 cells into a metacell, which was then used to screen module genes associated with psoriasis in the dataset (GSE162183). A dendrogram of samples with clinical traits were clustered using the average linkage method and Pearson's correlation method (Figure 4A). We determined the soft threshold of 16 by calculating the scale-

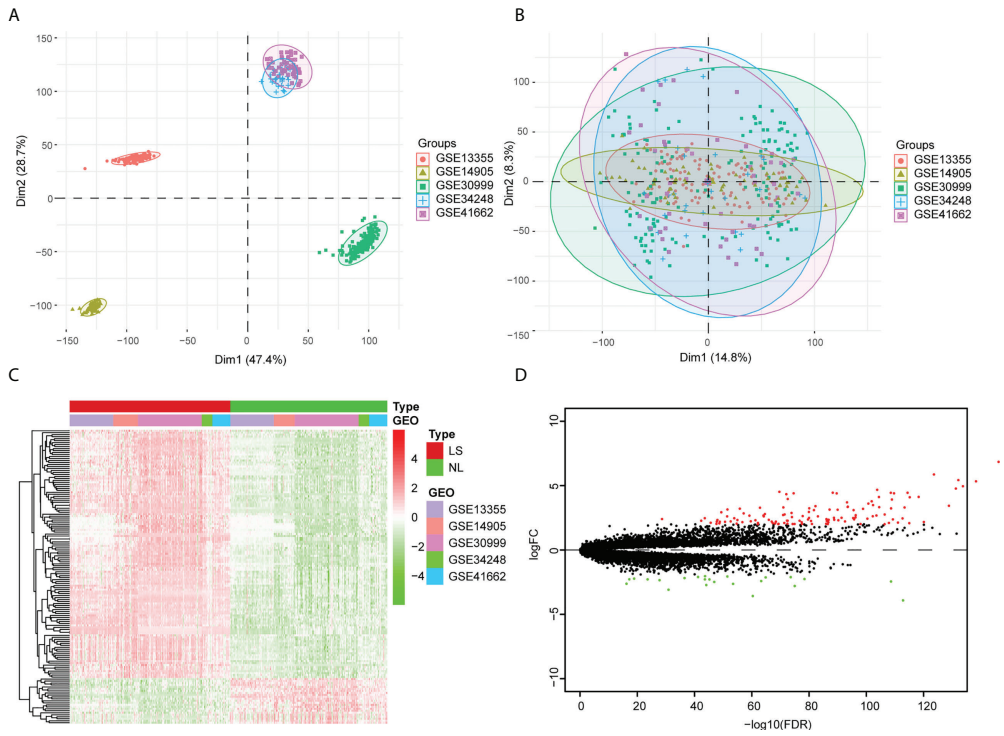

FIGURE 1
Analysis of the differentially expressed genes within the integrated dataset. **(A)** Principal component analysis (PCA) before batch effect adjustment. Samples from the different datasets cluster together. **(B)** PCA after batch effect adjustment. Samples from different datasets overlap. Red points: samples from GSE1335. Olive green points: samples from GSE14905. Spring green points: samples from GSE30999. Blue points: samples from GSE34248. Pink points: samples from GSE41662. **(C)** The DEG Volcano map shows genes in psoriatic lesion samples (LS) and non-lesion samples (NL). Up-regulated genes are in red; down-regulated genes are in green. **(D)** DEG expression heat map of skin tissue, with high expression in red and low expression in green.

free model fit and mean connectivity (Figures 4B, C). Different module genes in the dynamic tree cut were re-clustered through a topological similarity strategy. Eventually, seven modules developed after merging, and the turquoise module was found to be strongly correlated with psoriasis (Figure 4D). We then analyzed the correlation of each module with two clinical traits (normal and psoriasis). We found that AVPs were present in the turquoise module (cor = 0.39, $p = 2.5e^{-165}$) (Figure 4E) and therefore extracted cytokines from that module (Figure 4F; Table S6). We obtained three cytokines (MIF, IL17RA, and IL36G) and analyzed the relationship between those cytokines and the AVPs. Only interleukin-36 gamma (IL36G) was found to positively correlate with AVPs (Table S7).

High levels of AVPs and IL36G in suprabasal keratinocytes

Of all the IL36G-expressing cells in the scRNA-seq data, IL36G-expressing cells were mainly found in suprabasal keratinocytes (Figure 5A). IL36G was upregulated in psoriatic skin compared with normal skin (Figure 5B). The IL36G-expressing suprabasal keratinocytes from normal skin did not express any AVPs (Figure 5C). The IL36G that was highly expressed in the suprabasal keratinocytes from the psoriatic lesions was also highly co-expressed with AVPs (Figure 5D). Suprabasal keratinocytes from psoriatic lesions expressed more AVPs and IL36G than suprabasal keratinocytes from the control skin (Figures 5E, F).

Expression of AVPs and IL36G in patients with psoriasis before and after therapy

The expression levels of the AVPs and IL36G in psoriatic lesions were significantly decreased during treatment, especially at 12 weeks or 16 weeks of treatment. The downward trend was even more pronounced with biologic therapies (adalimumab, ustekinumab and brodalumab, tofacitinib, etanercept) than with MTX (Figures 6A–F). We therefore conclude that biological therapy is superior to other drugs in reducing the level of AVPs in psoriasis.

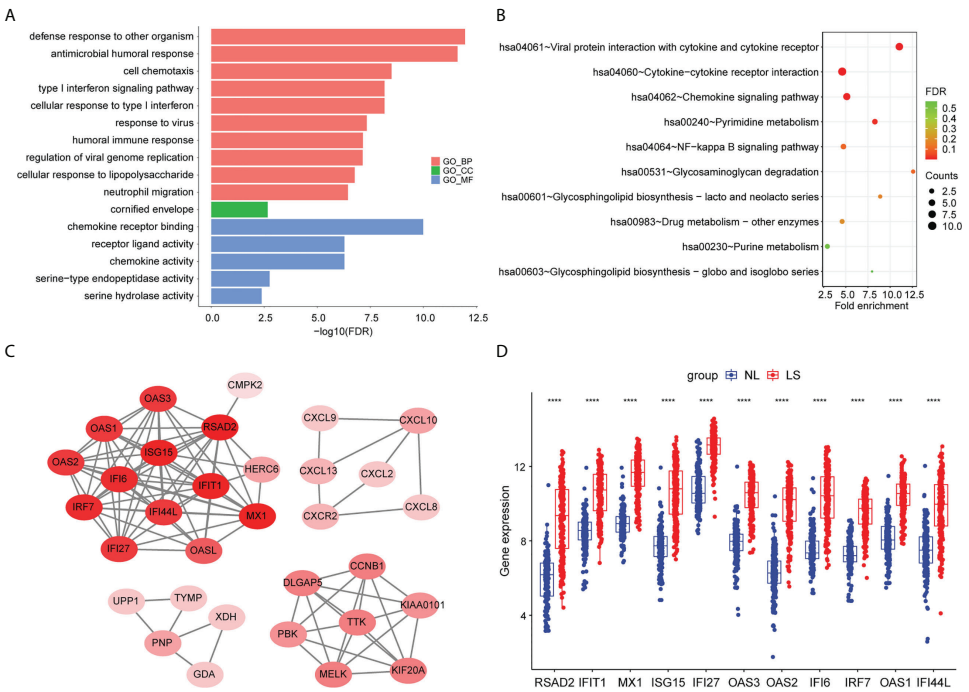

FIGURE 2
Pathway enrichment analyses and PPI network of DEGs. **(A)** Bar chart of differentially expressed genes' GO enrichment analysis. **(B)** Bubble chart of differentially expressed genes' KEGG enrichment analysis. The top 10 pathway enrichments are shown. **(C)** The PPI network of the DEGs. **(D)** Hub genes' expression between psoriatic lesion samples (LS) and non-lesion samples (NL). p values were calculated using a Wilcoxon signed-rank test. Asterisks corresponding to p values<0.05 (****< 0.0001).

The correlation between AVPs, IL36G expression levels, and psoriasis area severity index scores during therapy

In order to explore the association between the AVPs and IL36G expression levels in the context of psoriasis severity, we further investigated the relationship between levels of AVPs and IL36G and Psoriasis Area Severity Index (PASI) scores during psoriasis treatment. Levels of AVPs and IL36G were positively correlated with PASI scores during treatment with adalimumab, ustekinumab, brodalumab, MTX, and tofacitinib. These results therefore suggest that AVPs and IL36G play a role in the severity of psoriasis (Figures 7A–E).

Discussion

It is well accepted that infection with many different microorganisms or viruses, including β-haemolytic streptococci, *Staphylococcus aureus*, *Po`rphyromonas gingivalis*, *Candida albicans*, *Chlamydia psittaci*, HIV, and hepatitis C virus, is a common trigger or exacerbation factor for psoriasis (12). In particular, acute guttate psoriasis is generally thought to be caused by bacterial infection, such as streptococcal infection (13). Beyond the classical role of T helper cell 1 (Th1)/Th2 homeostasis and the IL17/IL23 axis, recently described AMPs have been found to play a role in the immunopathogenesis of psoriasis, further confirming the relationship between infection and psoriasis (4). Numerous high-throughput sequencing studies have suggested that the skin microbiome could play a role in the pathogenesis and therapeutic effect monitoring of psoriasis (14–16) and that AMPs could modify host microenvironments and regulate the colonization of microorganisms (17). It has been suggested that increased expression levels of AMPs could be involved in the pathogenesis of psoriasis (18). AMPs and AVPs are two important functional protein clusters mediating innate immune in psoriatic epidermis. However, the role of AVPs in the keratinocytes of psoriatic lesions remains unelucidated.

In this study, a series of bioinformatics analyses was performed on microarray and single-cell gene expression datasets. We found that the DEGs were mainly associated with a defense response to other organisms, with the type I interferon (IFN) signaling pathway, with the interactions between viral proteins and cytokines, and with the cytokine receptor and cytokine–cytokine receptor interaction pathway. The hub genes were AVPs (RSAD2, IFIT1, MX1, ISG15, IFI27, OAS3, OAS2, IFI6, IRF7, OAS1, and IFI44), which were highly

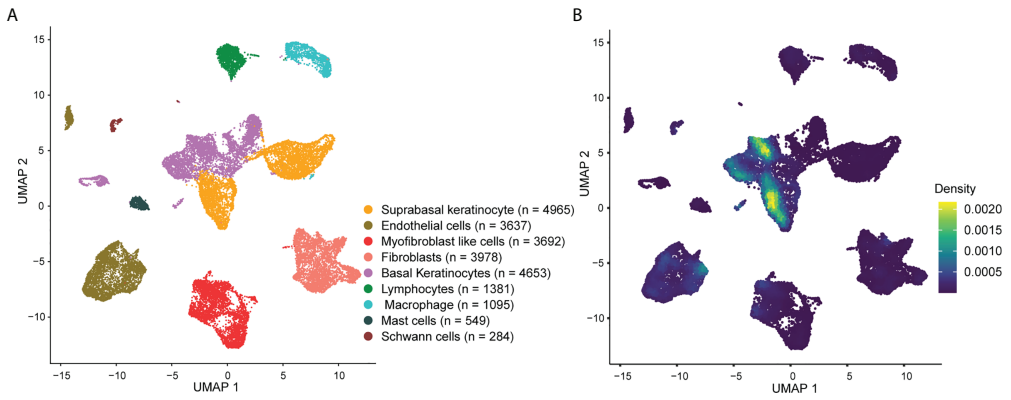

FIGURE 3
Overview of the single-cell landscape for psoriatic lesions. **(A)** A UMAP view and clustering analysis of combined single-cell transcriptome data from psoriasis and normal skin (*n* = 24234). Clusters are distinguished by different colors, with the general identity of each cell cluster shown on the right. **(B)** Density scatterplot of AVP gene set. UMAP, uniform manifold approximation and projection; AVP, antiviral protein.

expressed within the keratinocytes in psoriatic lesions. Moreover, IL36G may interplay with ISG15, MX1, IFI44L, and IFI27 in the execution of antiviral function within suprabasal keratinocytes.

Recent findings have demonstrated that the genes of AVPs were overexpressed in psoriatic lesions (8, 10, 19). Zhou et al. found that the keratinocyte-derived OAS2 expression level was positively correlated with PASI scores and decreased after therapy (8). Moreover, in TNF-α–induced HaCaT cells, ISG15 and MX1 were significantly up-regulated (9). Within this series of AVP genes, Mx proteins, such as MX1, are known for inhibiting negative-strand RNA viruses (4, 20).

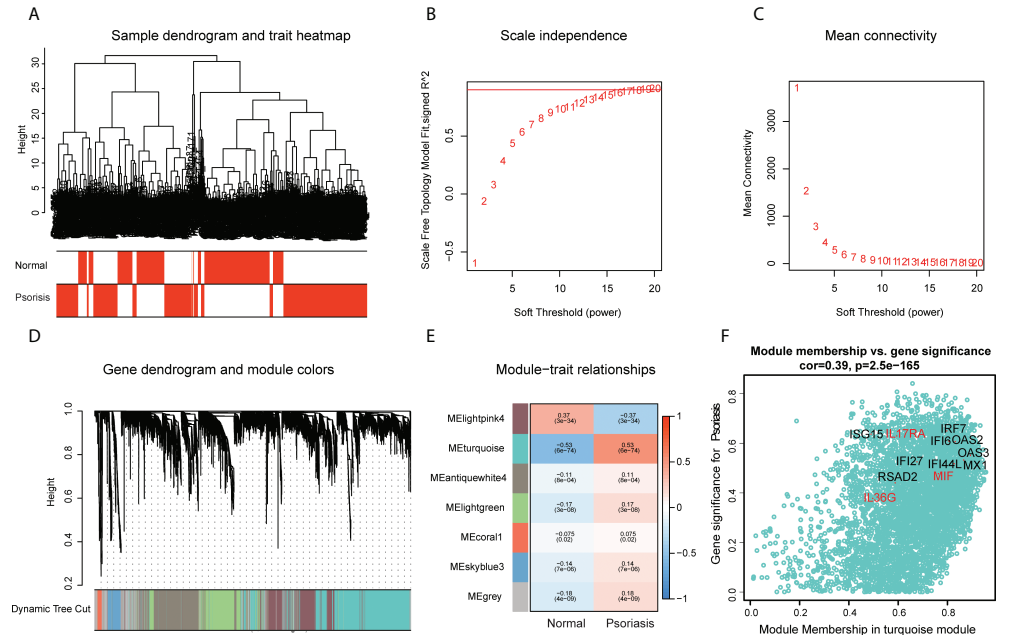

FIGURE 4
Construction of co-expression modules by the scWGCNA. **(A)** Clustering dendrogram of samples with trait heatmap. **(B)** The relationship between the scale-free fit index and various soft-thresholding powers. **(C)** The relationship between the mean connectivity and various soft-thresholding powers. **(D)** The cluster dendrogram of genes in GSE162183. Branches of the cluster dendrogram of the most connected genes gave rise to 7 gene co-expression modules. **(E)** Heatmap of the correlation between module eigengenes and clinical phenotype (normal and psoriasis). **(F)** Scatter plot describing the relationship between MM and GS in the turquoise module.

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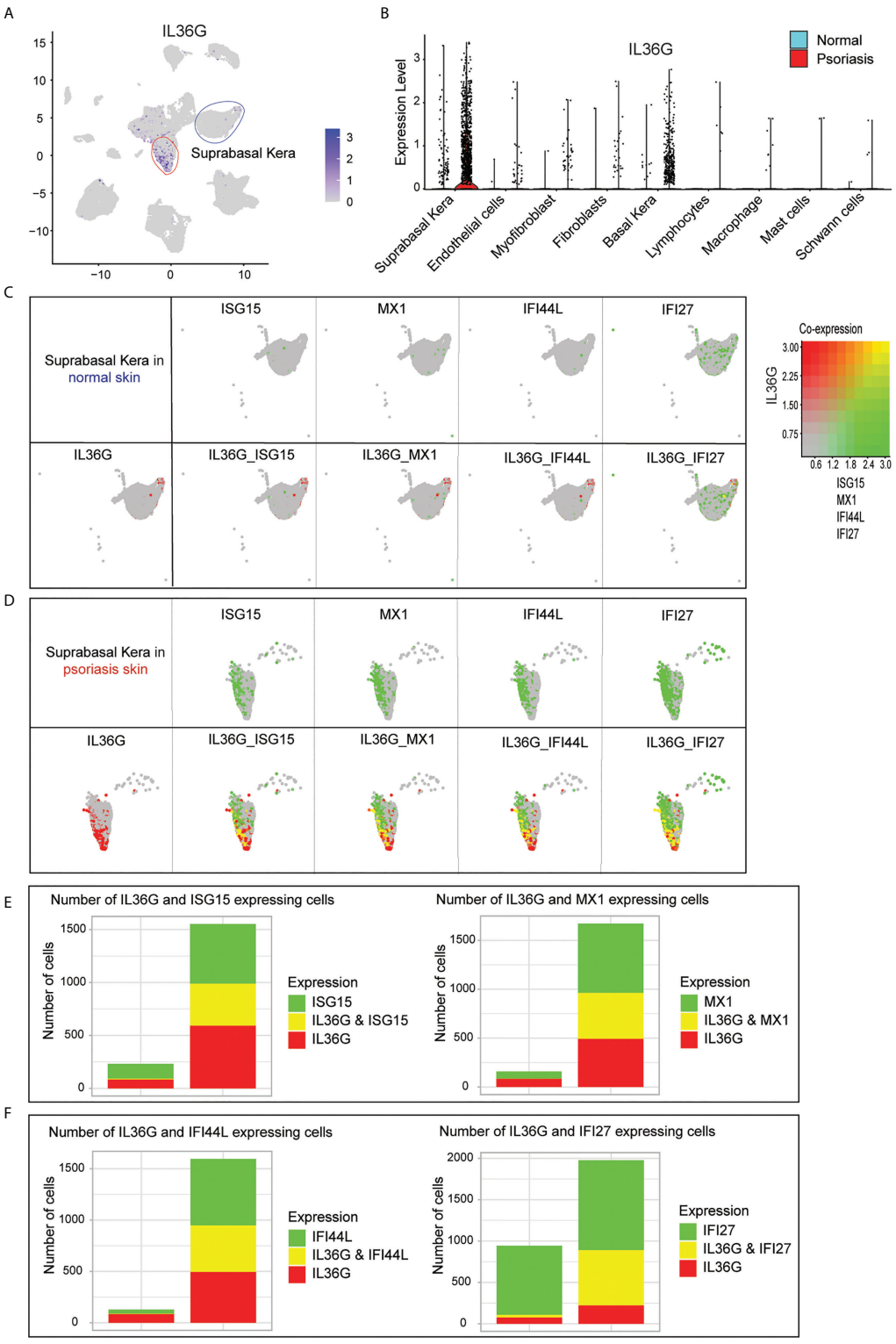

FIGURE 5
Expression of IL36G and AVPs in suprabasal keratinocytes of psoriatic lesions. **(A)** IL36G expression in total scRNA-seq data is visualized in low-dimensional space. **(B)** Violin plot of each cell cluster. **(C)** Co-expression of IL36G and AVPs in normal skin keratinocytes. **(D)** Co-expression of IL36G and AVPs in suprabasal keratinocytes of psoriatic lesions. **(E, F)** Cells with expression of ISG15, MX1, and IL36G **(E)** and IFI44L, IFI27, and IL36G **(F)** within suprabasal keratinocytes in normal and psoriatic skin are quantified by number of cells.

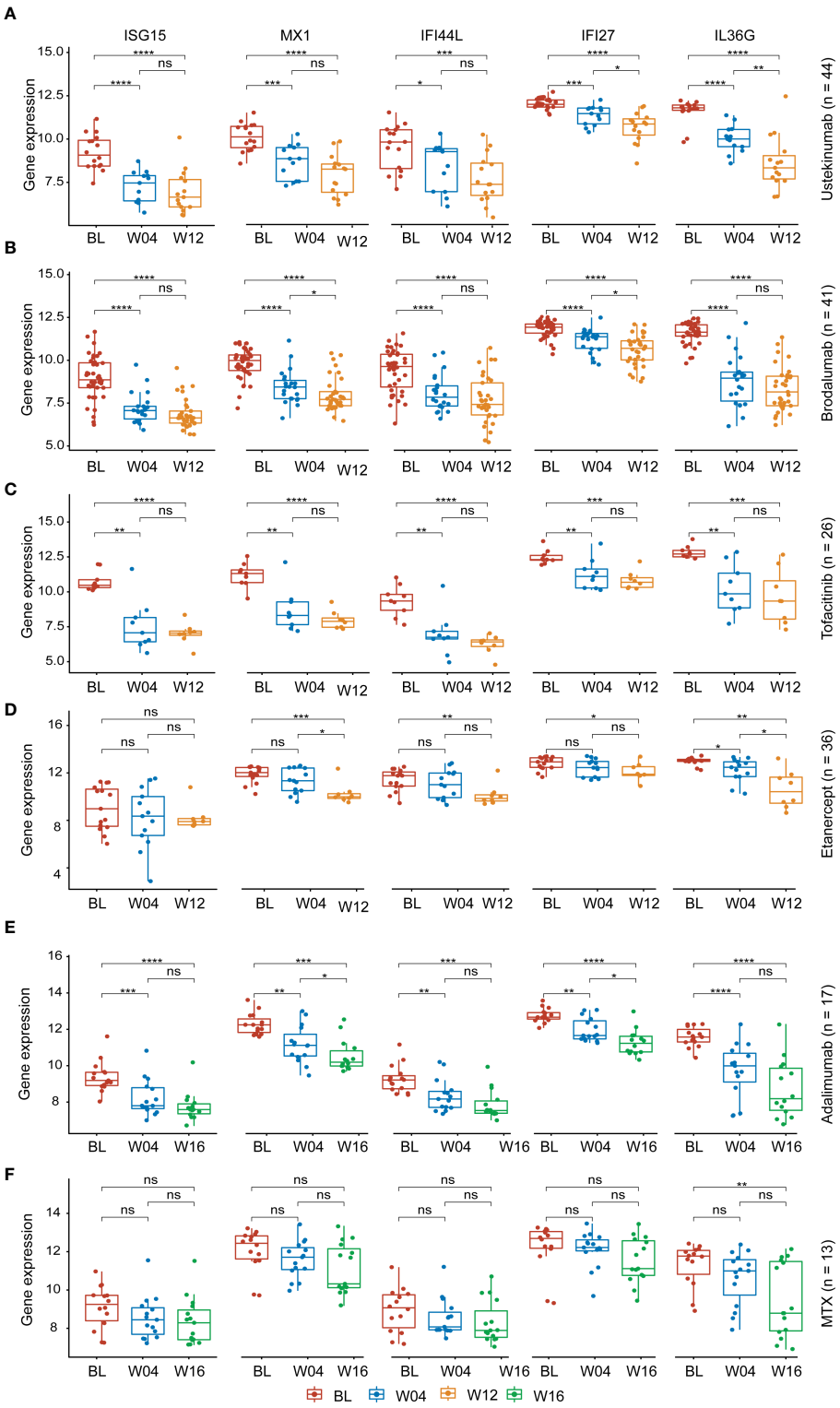

FIGURE 6
Expression of AVPs and IL36G during treatment. **(A)** Expression of AVPs and IL36G during ustekinumab treatment. **(B)** Expression of AVPs and IL36G during brodalumab treatment. **(C)** Expression of AVPs and IL36G during tofacitinib treatment. **(D)** Expression of AVPs and IL36G during etanercept treatment. **(E)** Expression of AVPs and IL36G during adalimumab treatment. **(F)** Expression of AVPs and IL36G during MTX treatment. MTX, methotrexate. p values were calculated using a Wilcoxon signed-rank test. Asterisks corresponding to p values<0.05 (*< 0.05, **< 0.01, ***< 0.001, ****< 0.0001) and those with p values> 0.05 as not significant "ns" (stat_compare_means function).

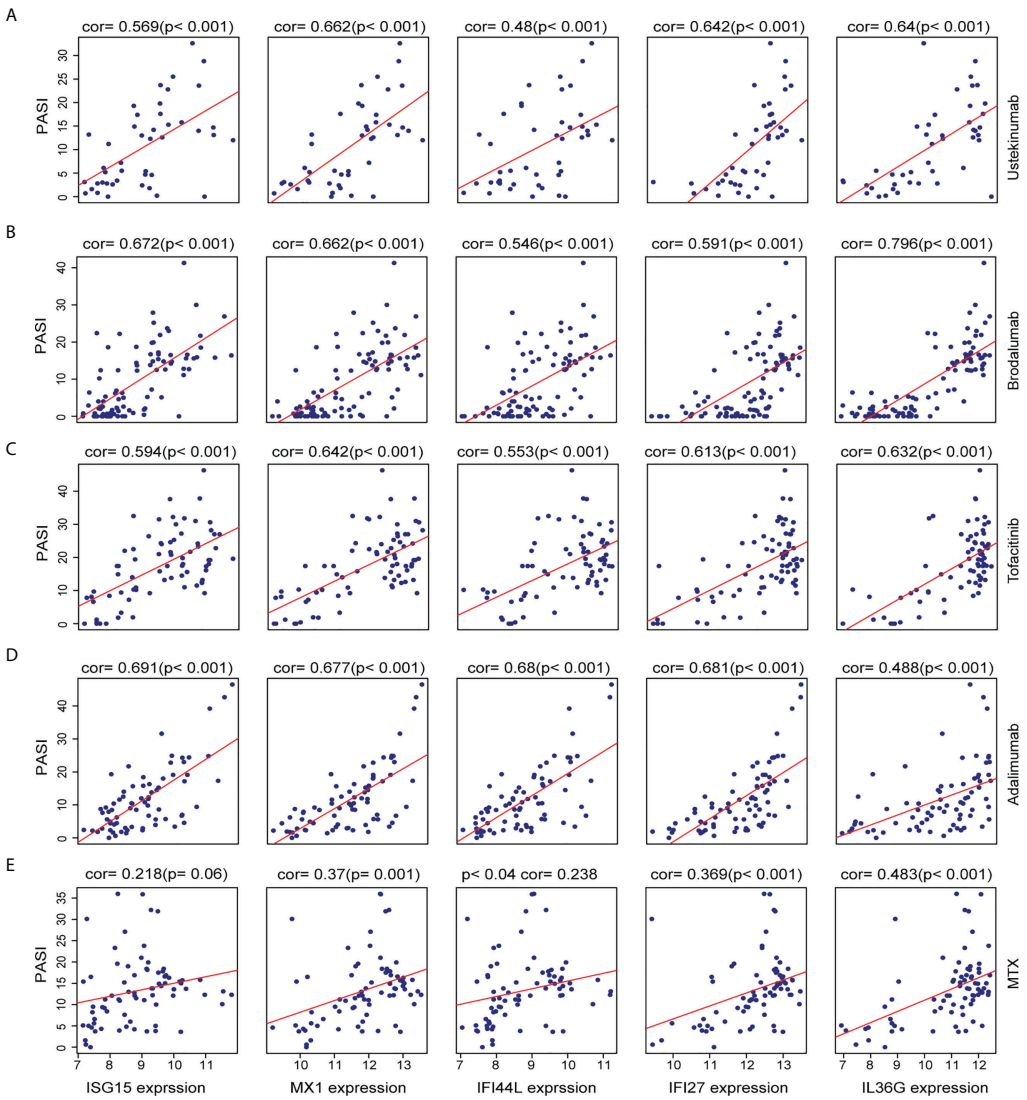

FIGURE 7
(A–E) The relationship between the expression of three biomarkers and PASI scores during psoriasis treatment. **(A)** Ustekinumab treatment. **(B)** Brodalumab treatment. **(C)** Tofacitinib treatment. **(D)** Adalimumab treatment. **(E)** Methotrexate treatment.

Interferon-stimulated genes (ISGs) are cellular products that mediate the type I interferon response against a wide range of invading viruses. Only a few ISGs, such as ISG15, IRF7, IFIT1, IFI27, IFI6, and IFI44L, have antimicrobial activity (21). ISG15 (interferon-stimulated gene product 15) participates in many antiviral signaling pathways to directly promote viral clearance (22). IFI27 (interferon α–inducible protein 27) is involved in the proliferation of skin keratinocytes, both in imiquimod-induced psoriasis-like skin and in HaCaT cells (23). Although extensive research confirms that the ISG family are involved in various bacterial and viral infections, the novel functions of AVPs in psoriatic keratinocytes need to be thoroughly investigated.

Although T cells are currently considered to be the main driver of psoriasis, keratinocytes play an important role in regulating inflammation and relapse (24). Epidermal keratinocytes secrete antimicrobial peptides, LL-37, and cytokines like TNF-α, IL6, and IL36 to activate Th1/Th17 cells, which interact with each other to regulate the proliferation and differentiation of keratinocytes (25). IL36G is a pro-inflammatory cytokine whose role in the pathogenesis of psoriasis has been extensively described (26–29). More noteworthy is that our findings showed that AVP genes were abundantly co-expressed with IL36G on psoriatic keratinocytes. Previous research has suggested that IL36G may be an alarmin

that signals viral infection (30). One study suggested that IL36G itself is a novel antiviral protein involved in the defense against influenza virus (28). Infectious agents may induce the primary skin barrier layers to produce large amounts of AVPs as a response. Another study found that Th17 cells derived IL29, but not type I IFN-mediated psoriatic keratinocyte is responsible for the expression of the AVPs (31). Whether upregulation of IL36G and AVPs expression in keratinocytes trigger psoriasis is currently unknown.

In addition, the expression levels of AVPs and IL36G were significantly positively correlated with PASI scores, and these levels decreased dramatically during biological treatment. These observations indicate that IL36G and AVPs may be related to the severity of psoriasis and could be used as an indicator for therapeutic efficacy. Research conducted by D'Erme et al. (26) also found that IL36G was closely associated with the disease activity of psoriasis. However, the exact production mechanism of IL36G and AVPs, and the regulatory relationship between them in the context of psoriasis, have still not been sufficiently investigated.

In summary, we demonstrated that the presence of IL36G may mediate the keratinocyte expression of AVPs such as MX1, ISG15, IFI27, and IFI44L in psoriasis vulgaris. The co-expression of AVP genes and IL36G was associated with psoriasis severity and therapeutic efficacy. Our results also suggest that infection factors and AVPs produced by keratinocytes may play an important role in the pathogenesis of psoriasis.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

Y-WL, H-MW, and Y-JC performed the data analysis and interpreted the results. NS and L-HY prepared the draft. R-JD, Y-QK, and Y-YL designed the research and revised the draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker J. Psoriasis. *Lancet* (2021) 397(10281):1301–15. doi: 10.1016/S0140-6736(20)32549-6

2. Rendon A, Schakel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci* (2019) 20(6):1475. doi: 10.3390/ijms20061475

3. Bartenjev I, Rogl Butina M, Potocnik M. Subclinical microbial infection in patients with chronic plaque psoriasis. *Acta Derm Venereol Suppl (Stockh)* (2000) 211):17–8. doi: 10.1080/00015550050500068

4. Ma JY, Shao S, Wang G. Antimicrobial peptides: Bridging innate and adaptive immunity in the pathogenesis of psoriasis. *Chin Med J (Engl)* (2020) 133(24):2966–75. doi: 10.1097/CM9.0000000000001240

5. Takahashi T, Gallo RL. The critical and multifunctional roles of antimicrobial peptides in dermatology. *Dermatol Clin* (2017) 35(1):39–50. doi: 10.1016/j.det.2016.07.006

6. Qian L, Chen W, Sun W, Li M, Zheng R, Qian Q, et al. Antimicrobial peptide ll-37 along with peptidoglycan drive monocyte polarization toward Cd14(High) Cd16(+) subset and may play a crucial role in the pathogenesis of psoriasis guttata. *Am J Transl Res* (2015) 7(6):1081–94.

7. Pahar B, Madonna S, Das A, Albanesi C, Girolomoni G. Immunomodulatory role of the antimicrobial ll-37 peptide in autoimmune diseases and viral infections. *Vaccines (Basel)* (2020) 8(3):517. doi: 10.3390/vaccines8030517

8. Zhou Y, Wang P, Yan BX, Chen XY, Landeck L, Wang ZY, et al. Quantitative proteomic profile of psoriatic epidermis identifies Oas2 as a novel biomarker for disease activity. *Front Immunol* (2020) 11:1432. doi: 10.3389/fimmu.2020.01432

9. Gao LJ, Shen J, Ren YN, Shi JY, Wang DP, Cao JM. Discovering novel hub genes and pathways associated with the pathogenesis of psoriasis. *Dermatol Ther* (2020) 33(6):e13993. doi: 10.1111/dth.13993

10. Raposo RA, Gupta R, Abdel-Mohsen M, Dimon M, Debbaneh M, Jiang W, et al. Antiviral gene expression in psoriasis. *J Eur Acad Dermatol Venereol* (2015) 29(10):1951–7. doi: 10.1111/jdv.13091

11. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* (2012) 28(6):882–3. doi: 10.1093/bioinformatics/bts034

12. Rademaker M, Agnew K, Anagnostou N, Andrews M, Armour K, Baker C, et al. Psoriasis and infection. a clinical practice narrative. *Australas J Dermatol* (2019) 60(2):91–8. doi: 10.1111/ajd.12895

13. Garritsen FM, Kraag DE, de Graaf M. Guttate psoriasis triggered by perianal streptococcal infection. *Clin Exp Dermatol* (2017) 42(5):536–8. doi: 10.1111/ced.13129

14. Wang H, Chan HH, Ni MY, Lam WW, Chan WMM, Pang H. Bacteriophage of the skin microbiome in patients with psoriasis and healthy family controls. *J Invest Dermatol* (2020) 140(1):182–90 e5. doi: 10.1016/j.jid.2019.05.023

15. Langan EA, Kunstner A, Miodovnik M, Zillikens D, Thaci D, Baines JF, et al. Combined culture and metagenomic analyses reveal significant shifts in the composition of the cutaneous microbiome in psoriasis. *Br J Dermatol* (2019) 181(6):1254–64. doi: 10.1111/bjd.17989

16. Stehlikova Z, Kostovcik M, Kostovcikova K, Kverka M, Juzlova K, Rob F, et al. Dysbiosis of skin microbiota in psoriatic patients: Co-occurrence of fungal and bacterial communities. *Front Microbiol* (2019) 10:438. doi: 10.3389/fmicb.2019.00438

17. Heimlich DR, Harrison A, Mason KM. Host antimicrobial peptides in bacterial homeostasis and pathogenesis of disease. *Antibiotics (Basel)* (2014) 3(4):645–76. doi: 10.3390/antibiotics3040645

18. Morizane S, Gallo RL. Antimicrobial peptides in the pathogenesis of psoriasis. *J Dermatol* (2012) 39(3):225–30. doi: 10.1111/j.1346-8138.2011.01483.x

19. Zou A, Jian Q. Cxcl10 and its related key genes as potential biomarkers for psoriasis: Evidence from bioinformatics and real-time quantitative polymerase chain reaction. *Med (Baltimore)* (2021) 100(38):e27365. doi: 10.1097/MD.0000000000027365

20. Verhelst J, Hulpiau P, Saelens X. Mx proteins: Antiviral gatekeepers that restrain the uninvited. *Microbiol Mol Biol Rev* (2013) 77(4):551–66. doi: 10.1128/MMBR.00024-13

21. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* (2011) 472(7344):481–5. doi: 10.1038/nature09907

22. Freitas BT, Scholte FEM, Bergeron E, Pegan SD. How Isg15 combats viral infection. *Virus Res* (2020) 286:198036. doi: 10.1016/j.virusres.2020.198036

23. Hsieh WL, Huang YH, Wang TM, Ming YC, Tsai CN, Pang JH. Ifi27, a novel epidermal growth factor-stabilized protein, is functionally involved in proliferation and cell cycling of human epidermal keratinocytes. *Cell Prolif* (2015) 48(2):187–97. doi: 10.1111/cpr.12168

24. Ni X, Lai Y. Keratinocyte: A trigger or an executor of psoriasis? *J Leukoc Biol* (2020) 108(2):485–91. doi: 10.1002/JLB.5MR0120-439R

25. Benhadou F, Mintoff D, Del Marmol V. Psoriasis: Keratinocytes or immune cells - which is the trigger? *Dermatology* (2019) 235(2):91–100. doi: 10.1159/000495291

26. D'Erme AM, Wilsmann-Theis D, Wagenpfeil J, Holzel M, Ferring-Schmitt S, Sternberg S, et al. Il-36gamma (Il-1f9) is a biomarker for psoriasis skin lesions. *J Invest Dermatol* (2015) 135(4):1025–32. doi: 10.1038/jid.2014.532

27. Keermann M, Koks S, Reimann E, Prans E, Abram K, Kingo K. Transcriptional landscape of psoriasis identifies the involvement of Il36 and Il36rn. *BMC Genomics* (2015) 16:322. doi: 10.1186/s12864-015-1508-2

28. Ovesen SK, Schulze-Osthoff K, Iversen L, Johansen C. Ikbzeta is a key regulator of tumour necrosis factor-a and interleukin-17a-Mediated induction of interleukin-36g in human keratinocytes. *Acta Derm Venereol* (2021) 101(2):adv00386. doi: 10.2340/00015555-3749

29. Traks T, Keermann M, Prans E, Karelson M, Loite U, Koks G, et al. Polymorphisms in Il36g gene are associated with plaque psoriasis. *BMC Med Genet* (2019) 20(1):10. doi: 10.1186/s12881-018-0742-2

30. Lian LH, Milora KA, Manupipatpong KK, Jensen LE. The double-stranded rna analogue polyinosinic-polycytidylic acid induces keratinocyte pyroptosis and release of il-36gamma. *J Invest Dermatol* (2012) 132(5):1346–53. doi: 10.1038/jid.2011.482

31. Wolk K, Witte K, Witte E, Raftery M, Kokolakis G, Philipp S, et al. Il-29 is produced by T(H)17 cells and mediates the cutaneous antiviral competence in psoriasis. *Sci Transl Med* (2013) 5(204):204ra129. doi: 10.1126/scitranslmed.3006245

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Corrigendum: IL36G is associated with cutaneous antiviral competence in psoriasis

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In the published article, there was an error in the author list. You-Wang Lu and Yong-Jun Chen contributed equally to this work and share first authorship. The corrected author list appears below.

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In the published article, there is an error in the title. Instead of "L36G is associated with cutaneous antiviral competence in psoriasis", it should be "IL36G is associated with cutaneous antiviral competence in psoriasis".

The authors state that these errors do not change the scientific conclusions of the article in any way. The original article has been updated.

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Interleukin-18 and IL-18BP in inflammatory dermatological diseases

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Interleukin (IL)-18, an interferon-γ inducer, belongs to the IL-1 family of pleiotropic pro-inflammatory factors, and IL-18 binding protein (IL-18BP) is a native antagonist of IL-18 *in vivo*, regulating its activity. Moreover, IL-18 exerts an influential function in host innate and adaptive immunity, and IL-18BP has elevated levels of interferon-γ in diverse cells, suggesting that IL-18BP is a negative feedback inhibitor of IL-18-mediated immunity. Similar to IL-1β, the IL-18 cytokine is produced as an indolent precursor that requires further processing into an active cytokine by caspase-1 and mediating downstream signaling pathways through MyD88. IL-18 has been implicated to play a role in psoriasis, atopic dermatitis, rosacea, and bullous pemphigoid in human inflammatory skin diseases. Currently, IL-18BP is less explored in treating inflammatory skin diseases, while IL-18BP is being tested in clinical trials for other diseases. Thereby, IL-18BP is a prospective therapeutic target.

KEYWORDS

IL-18 binding protein, IL-18, psoriasis, atopic dermatitis, rosacea, bullous pemphigoid, inflammatory dermatological disease

1 Introduction

Interleukin (IL)-18, first described as an interferon (IFN)-γ inducer, is a member of the interleukin-1 (IL-1) cytokine family. IL-1 cytokines and their receptors are linked to inflammation, fever, and immunostimulation (1). There are 11 known members of the IL-1 family termed IL-1α, IL-1β, IL-1 receptor antagonist (Ra), IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37, and IL-38, of which IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ are pro-inflammatory and IL-1Ra, IL-36Ra, IL-37, and IL-38 are considered anti-inflammatory (2). The IL-1 and its related family member IL-18 lack signal peptides and are not readily secreted, except for IL-1Ra. IL-1 family proteins are synthesized as the inactive precursors in the cytoplasm, and the precursors contain consistent conserved sequence A-X-D, where A is likely to be an aliphatic amino acid, X represents any amino acid, and D stands for aspartic acid (2, 3). Furthermore, like IL-1β, the IL-18 precursor is processed by the IL-1β converting enzyme (ICE, caspase-1) to produce mature and active IL-18 (4). The IL-1 family

members all have their corresponding receptors, and IL-1 and IL-18 receptors belong to the IL-1 receptor family (containing the Toll/IL-1 receptor (TIR) domain) (5). However, IL-18 activity can be neutralized, and the interaction with IL-18R is prevented by a natural inhibitor, IL-18 binding proteins (IL-18BP) (6).

Although discovered only 30 years ago, IL-18 has been reported as a versatile cytokine that causes a multitude of biological effects associated with infection, inflammation, and autoimmune processes, including inflammatory bowel diseases (7, 8), chronic liver disease (9), adult-onset Still's disease (10), hemophagocytic syndrome (11), rheumatoid arthritis (12), idiopathic thrombocytopenic purpura (13), cancer (14, 15), and cardiac diseases (16). Furthermore, IL-18 and IL-18BP are also implicated in developing psoriasis, atopic dermatitis (AD), lupus erythematosus (LE), and other inflammatory skin diseases. In the past years, researchers reviewed the roles of IL-18 and IL-18BP on a diversity of skin diseases (17, 18). However, with new advances and discoveries, to our knowledge, the detailed roles of IL-18 and its binding protein signaling in skin diseases have not yet been thoroughly investigated. This literature review will address the most recent updates regarding IL-18 and IL-18BP in psoriasis, atopic dermatitis, rosacea, and bullous pemphigoid and discuss the underlying impacts of IL-18 and IL-18BP and inflammatory disease in the skin.

2 IL-18 and IL-18BP

2.1 IL-18

IL-18, first termed IFN-γ inducible factor (IGIF), has profound effects on natural killer and T-helper (Th) cell activation (19, 20). Furthermore, IL-18 is expressed constitutively in keratinocytes, macrophages, Langerhans cells (LC), dendritic cells (DC), as well as epithelial cells in the lack of an inflammatory stimulus (17, 21, 22). *IL-18* gene is located on chromosomes 11 and 9 in humans and mice, respectively (23). Besides, IL-18 protein is expressed at high concentrations in human keratinocyte lysates and is released as an unprocessed 24-kDa form (21). This gene encodes IL-18 protein and exists as pro-IL-18, mainly localized in the cytoplasm. Precursor human IL-18 (prohIL-18) has 193 amino acids with 24-kDa molecular weight converted into an active 18-kDa monomer of 157 amino acids by the cysteine proteinase caspase1-mediated cleavage of an N-terminal fragment (24). Precursor murine IL-18 (mIL-18) has 192 amino acids, and mature mIL-18 is a polyfunctional cytokine with 157 amino acids (25).

IL-18 is composed of 12 chains (S1-S12), forming three twisted 4-strand β-folded sheets, one short α-helix (H1) and one 3_{10}-helix (H2). The three β-sheets fold over one another to form a β-trefoil fold. IL-18 shares sequence similarity (17%) with IL-1β of the IL-1 family, and both are structurally related as β-pleated sheet folded molecules (26). However, IL-18 and IL-1β show significant differences in the length and conformation of S3-S4, S4-S5, S7-S8, and S11-S12 segments (27). The essential residues of IL-18 and IL-1β make up sites I, II, III, and sites A, and B, respectively. Site I comprises Arg13, Asp17, Met33, Asp35, and Asp132 residues and is positioned on one side of the β-triplet folding central barrel, while site II consists of six residues (Lys4, Leu5, Lys8, Arg58, Met60, and Arg104) and is situated at the upper part of the β-barrel. Comparing the primary and tertiary structures of IL-18 and IL-1β indicates that sites I and II of IL-18 are in equivalent positions on the sequence and tertiary structure as sites A and B of IL-1β. Lys79, Lys84, and Asp98 are assembled at the bottom of the barrel, on the opposite side of site II, which is site III. Sites I and II are associated with IL-18Rα binding, whereas site III is involved in cellular responses (27, 28).

Pro-IL-18 processing, maturation, and activation of IL-18 are primarily mediated through canonical caspase-1 or IL-1β-converting enzyme induced by inflammasomes. Formation of inflammasomes, including members of leucine-rich-repeat-(LRR)-NOD-like receptors (NLR) family and the absence in melanoma 2 (AIM2) in recognition of microbial or danger signals, leads to the procaspase-1 cleavage into active caspase-1 enzyme, the intracellular cysteine protease, further cleaving pro-IL-18 into their mature molecule forms (32, 40). Moreover, NLRP6 (PYD domains-containing protein 6) can recruit caspase-1 and atypical caspase-11. Upon recruitment, caspase-11 is processed and stimulates caspase-1-dependent IL-18 secretion (39). NLR family CARD domain containing-4 (NLRC4) also can mediate IL-18 processing *via* caspase-1 in immune cells (46). In addition, other alternative pathways regulated by inflammatory cells can also cause the synthesis and secretion of active IL-18. For example, fas-ligand signaling can trigger caspase-8 in dendritic cells and macrophages, which leads to the processing and release of mature IL-18 (33). Furthermore, IL-18 binding to IL-3 can lead to the release of mast cell-induced chymase, and in turn, pro-IL-18 can be activated by chymase (34). And Granzyme B (GrB), a family member of serine proteases, converts proIL-18 into mature IL-18 in non-hematopoietic cells such as keratinocytes (35, 36). IL-18 activity is regulated at the transcriptional and promoter level (37), by post-translational modification (caspase-1 cleavage), and by binding of the endogenous inhibitor IL-18BP (29).

2.2 IL-18 receptor

After maturation and activation, IL-18 participates in several biological functions through the IL-18 receptor chains (IL-18R). IL-18 combines with the IL-18R which comprises two subunits, one for the IL-18 receptor conjugated α chain (IL-18Rα) and the other for the non-conjugated signaling chain (IL-18Rβ). Furthermore, IL-18R is a heterodimeric complex that is a member of the IL-1 receptor family (30). IL-18Rα is expressed by most cells, while only a few cells, like T cells, dendritic cells, and mast cells, express the IL-18Rβ chain (31).

The ligand-binding IL-18Rα has the same chromosomal location as IL-1R types I and II, of which ectodomain has three Ig-like domains (D1, D2, and D3) in a curved shape. The D1 and D2 domains are tightly aligned, forming the D1D2 module, connecting to the D3 domain *via* a Linker. Each of the three domains possesses a bilayer sandwich structure consisting of 6-9 β-strands and at least one intradomain disulfide bond. IL-18Rα has a similar structure to IL-1RI. The three domains of IL-18Rα form a grasping hand that wraps IL-18 inside (41). The IL-18Rβ (also known as IL-18 receptor accessory protein-like (AcPL) molecule) is associated with IL-1R accessory protein (42) and is required for signaling (43). IL-18Rβ also contains three Ig-like domains, similar to those of IL-18Rα, except for the disulfide bonds within the domain and the spatial arrangement of the three domains in the triplet compound. IL-18Rβ

D2 and D3 are closely coupled and directly adjacent to IL-18/IL-18Rα, whereas D1 is not relevant for the formation of the ternary compound. Further, the IL-18 and IL-18Rα complex concave surfaces, where the IL-18 site III and D2 of IL-18Rα are located, match the convex surfaces of IL-18Rβ-D2 (44). In a word, IL-18 activates the downstream signaling process by linking to the low-affinity IL-18Rα to form a signaling complex, producing an inactivated IL-18/IL-18Rα binary complex, which recruits the IL-18Rβ chain to develop a high-affinity complex that launches IL-18-dependent signaling pathway (45) (Figure 1A). In the presence of IL-18Rβ deletion, IL-18/IL-18Rα complex would not be a pro-inflammatory signal.

2.3 IL-18 binding protein

IL-18BP, with 40-kDa weight, belongs to the Ig superfamily and is a decoy receptor of IL-18. It resembles the extracellular structural domain of the immunoglobulin (Ig)-like receptor but without a transmembrane and cytosolic domain (4, 38). Previous studies have found that human and murine IL-18BP (huIL-18BP and muIL-18BP) are homologous to molluscum contagiosum virus (MCV) and poxviruses, including smallpox virus (47). Soluble IL-18BP is induced by IFN-γ and is constitutively expressed and secreted by the human spleen and leukocytes (4, 48). Efficient production of IL-18BP may counteract excess IL-18 in epithelial cells and antagonizes the pathological inflammation at the biological barrier (49). Four isoforms of human IL-18BP (IL-18BP a, b, c, and d) have been identified from the alternative mRNA splicing of the human gene, which is located on chromosome 11q13 and has no exon coding for a transmembrane domain. IL-18BP a and c both have intact Ig domains that neutralize mature IL-18, whereas the non-binding isoforms b and d lack a complete Ig domain at C-terminus, and their biological function is currently unknown (50). Usually, IL-18BPa represents IL-18BP due to its high affinity to IL-18 (51). There are two isoforms in mice, IL-18BPc and IL-18BPd, which possess the identical Ig domain and neutralize more than 95% of murine IL-18 (50).

Based on the resolved IL-18BP of ectromelia virus (ectv), ectvIL-18BP was concluded that it comprised of two 4-stranded β-sheets with a short c' strand, a helix (H1), and elongated flexible loops between the β-sheets (52). IL-18BP can balance IL-18-driven activation by binding mature IL-18, thereby blocking its interaction with the activated receptor IL-18R, and this affinity is higher than IL-18Rα (4). IL-18BP has been demonstrated to bind to IL-18 using a single Ig fold and interact with IL-18 in a manner closely resembling the D3 domain of IL-18Rα signaling, thus directly preventing the binding site II between IL-18 and IL-18Rα (41, 52–54). Furthermore, IL-18BP binds to IL-18 and further forms a blunting complex with IL-18Rα, preventing IL-18Rβ from activating cells (55, 56) (Figure 1B).

IL-18BP can also bind to IL-37, another anti-inflammatory cytokine of the IL-1 family. IL-37, binding to IL-18BP, forms a complex with IL-18Rβ, depriving it of the β-chain that forms a complex with IL-18Rα as a functional receptor, thereby enhancing the inhibition of IL-18 activity (57, 58) (Figure 1C). Besides, a recent study indicates that IL-37 can bind to IL-18Rα and act as a negative regulator, causing the loss of recruitment with IL-18Rβ (59) (Figure 1D). Under physiological conditions, plasma IL-18BP concentration is much higher than IL-18, preventing IL-18 from binding to IL-18Rα and IL-18Rβ. Therefore, the concentration of plasma IL-37 depends on IL-18BP. Indeed, IL-18BP has been reported to be a potential treatment for several diseases. IL-18BP is an immunoregulatory binding protein and natural product with attractive therapeutic effects for diseases that are mediated, in part, by IL-12, IFN-γ, or IL-18 itself (38). Furthermore, IL-18BP is protective against LPS-induced acute lung injury, which may be related to its regulation of NF-κB and nuclear

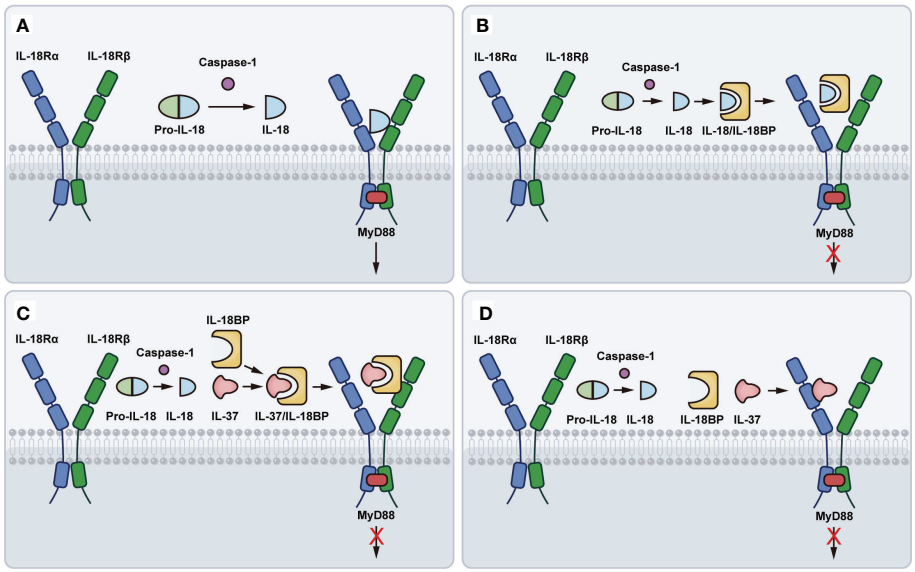

FIGURE 1
Schematic representation of IL-18 binding to IL-18R. **(A)** The pro-IL-18 is processed by the caspase-1 to produce mature and active IL-18. Mature IL-18 links to the low-affinity IL-18Rα to form an inactivated IL-18/IL-18Rα binary complex, which recruits the IL-18Rβ chain to develop a high-affinity complex that launches IL-18-dependent signaling pathway. **(B)** IL-18BP binds to IL-18 and further forms a blunting complex with IL-18Rα, preventing IL-18Rβ from activating cells. **(C)** IL-37 binding to IL-18BP, forms a complex with IL-18Rβ, thereby enhancing the inhibition of IL-18 activity. **(D)** IL-37 binds to IL-18Rα, causing the loss of recruitment with IL-18Rβ.

factor erythroid 2-related factor 2 (Nrf2) activity (60). In addition, downregulation of IL-18BP leads to elevated vascular cell adhesion molecule 1 (VCAM-1) expression, monocyte/macrophage adhesion, and accelerated atherosclerotic plaque formation in diabetic mice (60).

3 Signaling and function of IL-18 and IL-18BP

The signaling transduction pathways of IL-18 are similar to IL-1β. Analogous to the IL-1β receptor IL-1R, IL-18R includes a Toll/IL-1 receptor (TIR) domain in the cytosolic region, so the signal into the cell is first mediated by a molecule known to be an adaptor of TLRs and IL-1R, myeloid differentiation 88 (MyD88), which docks with the IL-18R complex and leads to the recruitment and phosphorylation of IL-1 receptor-associated kinases (IRAKs). Both IRAK1 and IRAK4 are implicated in IL-18 signaling in Th1 and NK cells (61–63). The IRAKs bind to another adaptor molecule, TNF receptor-associated factor 6 (TRAF6), which associates with a kinase called TAK1 (transforming growth factor-β-activated kinase 1), the kinase that in turn phosphorylates nuclear factor kappa B (NF-κB)-induced kinase (NIK). Moreover, NIK activates the I kappa B (IκB) kinase (IKK), which phosphorylates IκB, causing IκB ubiquitination and rapid degradation. Transcription factor NF-κB is then free and migrates into the nucleus, where it combines with specific regulatory sequences (κB sites) in the promoter regions of multiple inflammatory genes (*e.g.*, IFN-γ, IL-8, IL-1β, TNF-α) (64–68). Furthermore, the phosphorylation of TRAF6 also associates with apoptosis signal-regulating kinase 1 (ASK1) *via* TAK1, and interfaces with ASK1 causing the activation of mitogen-activated protein kinases (MAPK)-related signaling downstream, p38 mitogen-activated protein kinase (p38MAPK), jun kinase (JNK), phosphoinositide 3-kinase (PI3K)/AKT, extracellular regulated protein kinases (ERK), resulting in the activation of AP-1 transcription factor (65, 69) (Figure 2A).

Besides, IL-18 can enhance Th1 responses and is essential for IFN-γ production (70). Conversely, the IFN-γ-induced soluble IL-18BP inhibits the biological activity of IL-18, thereby reducing IFN-γ production and thus suppressing the Th1 immune responses. It is readily speculated that a dysregulation of the IL-18/IL-18BP balance may contribute to chronic inflammation driven by the T-helper type I cytokine. Indeed, IL-18 has the opposite impacts on Th1 and Th2 activation balance (71). Mature IL-18 with IL-12 or IL-15 induces differentiation of naive T cells into Th1 cells. However, administration of IL-18 alone mainly results in elevating IgE expression levels and enhanced IL-4 and IL-13 production by mast cells, basophils, NK cells, and $CD4^+$ T cells, driving a Th2 response (72–74). Furthermore, IL-18 and IL-2 promote IL-13 production in NK cells and T cells. And IL-18 induces histamine release from basophils in concert with IL-13 (75) (Figure 2B). IL-18 enhances Fas Ligand (FasL) on natural killer (NK) cells as well, thereby facilitating FasL-mediated NK cytotoxicity (29).

Furthermore, IL-18 can promote allergic inflammation through mast cells and basophils to enhance the production of critical factors that drive atopic inflammation (76). This evidence suggests the pleiotropic function of this cytokine in promoting immune responses and inflammation, with IL-18 acting as a key bridge between innate and adaptive immunity. IL-18 has been found to have two biological functions, first as a co-stimulator of Th1 cytokine production, together with IL-2, IL-12, microbial agents, or mitogens to promote the production of IFN-γ. In addition, IL-18 directly induces tumor necrosis factor (TNF)-α, IL-1β, CXC, and other chemokines, Fas ligand, and vascular adhesion molecules, and NF-κB nuclear translocation, initiating cytokine cascade responses accompanied by the expression of several pro-inflammatory markers, possibly as a contributor to inflammation (6, 56, 77).

3.1 IL-18 and IL-18BP in keratinocytes

Keratinocytes (KC) represent makeup 95% of the epidermis and are considered to play a critical role in skin inflammation and immune response. In human KC, pro-IL-18 is constitutively

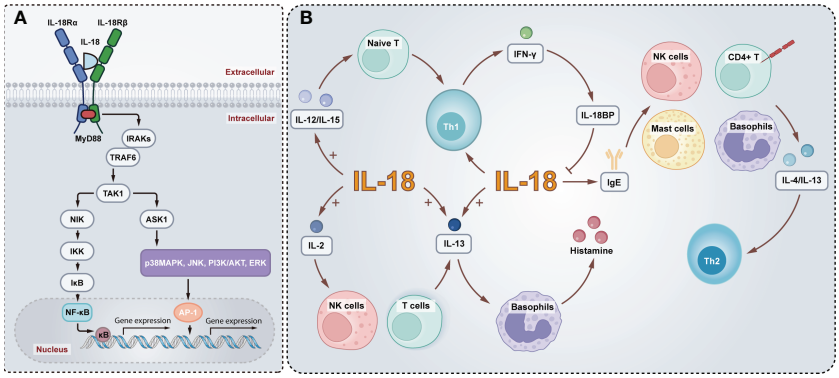

FIGURE 2
Schematic model of the IL-18 signaling pathways and biological functions. **(A)** Active IL-18 is secreted extracellular, and activation of IL-18R recruits MyD88 into the TIR domain and anchors IRAKs, which bind to TRAF6 associating with TAK1. TAK1 in turn phosphorylates NIK. This is followed by the activation of IKK, phosphorylation of IκB, and finally nuclear translocation of NF-κB, which regulates the transcription of inflammatory genes. In addition, the TRAF6 activates ASK1 through TAK1, and contributes to activate p38MAPK, JNK, PI3K/AKT and ERK, leading to the activation of AP-1. **(B)** IL-18 enhances Th1 production of IFN-γ which in turn induces IL-18BP inhibits IL-18. Mature IL-18 with IL-12 or IL-15 induces differentiation of naive T cells into Th1 cells. Alternatively, IL-18 alone elevates IgE and actives mast cells, basophils, NK cells, and $CD4^+$ T cells to produce IL-13 and IL-4, driving a Th2 response. Furthermore, IL-18 and IL-2 promote IL-13 production in NK cells and T cells and, in conjunction with IL-13, induce histamine release from basophils.

expressed (22), and compared to monocytes, PBMC, or leukocytes, keratinocytes produce large amounts of pro-IL-18 (78). It has been found that in human keratinocyte cell line HaCaT, ultraviolet B (UVB) irradiation time- and dose-dependently promotes the production of IL-18, which is selectively mediated through reactive oxygen intermediates (ROI) production and activator protein-1 (AP-1) activation (79). IL-18R is also present in keratinocytes, signifying that IL-18, released from keratinocytes, acts in an autocrine or paracrine manner on surrounding keratinocytes (80). Besides, INF-γ significantly upregulates IL-18BPa mRNA expression in HaCaT cells, while IL-18 mRNA expression levels were not affected (81).

In addition to their barrier function, epidermal keratinocytes can sense harmful pathogens through pattern recognition receptors (PRRs). Besides Toll-like receptors (TLRs), epidermal keratinocytes also have nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), which is an intracellular PRR (82). Inflammasomes are large, complex proteins consisting of NLR, the adapter protein- ASC containing a carboxy-terminal caspase-recruitment domain of the apoptosis-associated speck-like protein, and caspase-1, an inducer of IL-1β and IL-18 that promotes inflammation and inflammatory cell death. NLR interacts directly with caspase-1 *via* caspase activation and recruitment domain (CARD) or by the ASC linking NLR to caspase-1 (83). There are five PRRs (NLRP1, NLRP3, NLRC4, pyrin, and AIM2) that form various inflammasomes. In addition, other members of the NLR family and the PYHIN family, including NLRP6, NLRP7, NLRP12, and IFI16 (γ-interferon-inducible protein 16), can also be found form different inflammasomes (84). Upon detecting danger signals, inflammasomes initiate the immune response by activating caspase-1 to cleave precursors of IL-1β and IL-18, releasing mature IL-1β and IL-18. Dai et al. found that cytoplasmic double-stranded RNA (dsRNA) is significant in mediating the activation of NLRP3 inflammasomes, causing the release of mature IL-1β and IL-18 and that cytosolic dsRNA-activated protein kinase (PKR) is a potent factor in human primary keratinocytes through dsRNA-mediated activation of NLRP3 inflammasomes (82). Besides, Papale et al. revealed that NLRP12 expression was inversely related to IL-18 production in KC (85). Zhang et al. demonstrated that activation of AIM2 by PKR upregulates the production of mature IL-1β and IL-18 in human keratinocytes (86). Fenini et al. found that NLRP1 inflammasomes have an essential function in UVB perception and subsequent secretion of IL-1β and IL-18 from keratinocytes (87). Moreover, Galbiati et al. speculated that contact allergens could induce oxidative stress, which activates inflammasome and releases high mobility group protein B1 (HMGB1), further activating TLR4 which leads to the neo-synthesis of IL-18 in human KC (88). The IFN-γ-induced chemokines CXCL9 (chemokine C-X-C motif ligand 9), CXCL10, and CXCL11 recruit Th1 cells by binding to CXCR3 (chemokine C-X-C motif receptor 3) on the cell surface (89) and significant infiltration of Th1 cells are seen in inflammatory skin diseases. In keratinocytes, IL-18 enhances the mRNA expression of CXCL9, CXCL10, and CXCL11 induced by IFN-γ. Further, it is suggested that IFN-γ activates STAT1 *via* JAK1/JAK2 and/or p38MAPK, leading to CXCL9, CXCL10, and CXCL 11 production. In addition, IFN-γ can also affect the activation of IRF-1 (interferon regulatory factor-1), which is only responsible for CXCL11 through p38MAPK, while IL-18 enhances this activation through PI3K/AKT and MEK/ERK. IL-18 induces NF-κB activity through MEK/ERK and PI3K/AKT pathways, thus enhancing IFN-γ-induced CXCL9 secretion (75).

In human dermal fibroblasts (HDF), IL-18 regulates transcription factor Ets-1 activation *via* the ERK pathway, thereby reducing collagen expression and inhibiting TGF-β-induced collagen production. Therefore, IL-18 exerts antifibrotic activities in dermal fibroblasts (90). The IL-18 expression level is reduced, indicating that IL-18 is closely associated with hypertrophic scar formation in hypertrophic scar fibroblasts and hypertrophic scar tissue. Recent studies have revealed that IL-18 facilitates apoptosis in hypertrophic scar fibroblasts by enhancing the production of Fas ligand (FasL), and rhIL-18 upregulates Caspase-3, Caspase-8, Caspase-9, and FADD (FAS-associated death domain) expression in a dose-dependent manner (91). FasL is a ligand for Fas that binds specifically to Fas, and FADD recognizes the death domain (DD), which in turn forms a death-inducing signal complex (DISC) with caspase-8, further activating caspase-3 and delivering it to the cytoplasm, thereby launching the caspase cascade and apoptosis (92). Consequently, rhIL-18 can inhibit cell proliferation in hyperplastic scars by increasing FasL expression to initiate apoptosis, and it is a promising target for the therapy of hyperplastic scars.

3.2 IL-18 and IL-18BP in immune cells

The keratinocytes are exposed to external irritation and release of IL-18, which acts on surrounding keratinocytes and affects immune cells (DC, LC, macrophages, among others) in the skin, thus causing an inflammatory response. Langerhans cells, a population of antigen-presenting and myeloid-derived immature dendritic cells, reside in the epidermis and are the first to encounter skin pathogens, and LC are activated into mature DC in response to various stimuli. IL-18 induces LC migration and DC accumulation, with mature LC migrating to skin-draining lymph nodes where they deliver antigens to $CD4^+$ T cells and regulate the adaptive immune response. In addition, IL-18 acts synergistically with IL-12 to stimulate Th1 production of INF-γ. IL-18 does not induce Th1 differentiation but upregulates IL-12Rβ expression, thereby enhancing IL-12-mediated Th1 development (93, 94).

Neutrophils are participants in innate immunity and are responsible for bacterial killing when pathogens invade, influencing inflammatory and immune responses by producing large numbers of cytokines. In parallel to phagocytic and microbicidal features, neutrophils are essential in initiating and/or amplifying inflammatory and immune responses (95). Unstimulated neutrophils secrete IL-18BP and IL-18, a neutrophils activator, inducing the expression and release of multiple inflammatory cytokines in neutrophils (96, 97). Fortin et al. revealed that IL-18 induced the production of IL-8, CCL3/Mip-1α, CCL4/Mip-1β, and CCL20/Mip-3α from human neutrophils and affected the expression of IL-8, Mip-1α, and Mip-1β through the p38MAPK, ERK, PI3K/AKT, and IKK/NF-κB pathways (96).

In human peripheral blood mononuclear cells (PBMC), mature IL-18 induces IL-8 production. Furthermore, IL-18BP inhibits IL-12-induced IFN-γ generation in PBMC. IL-18 stimulation results in IL-8 synthesis increased in macrophages. IL-18 induces IFN-γ synthesis by T-cells and natural killer cells, whereas IL-12, mitogens, or microbial agents, are necessary for IL-18 to induce the production of IFN-γ (72).

4 IL-18 and IL-18BP in inflammatory dermatological diseases

To date, many reports revealed the dysregulation of IL-18 in inflammatory skin diseases, and IL-18 has been speculated to be a possible marker for diverse disorders. Here, the topic mainly focuses on psoriasis, atopic dermatitis, bullous pemphigoid, and rosacea; other disorders, including cutaneous lupus erythematosus, dermatomyositis, and allergic contact dermatitis, are also reviewed. IL-18 inhibitors and IL-18BP drugs in clinical trials and preclinical studies for the treatment of inflammatory skin diseases or related diseases were summarized in Table 1.

4.1 Psoriasis

Psoriasis is a common cutaneous inflammatory disease with different variants, including plaque psoriasis, pustular psoriasis, guttate psoriasis, and erythrodermic psoriasis (100). The pathogenesis of genetics and immunity in psoriasis is widely accepted (101). Triggers from exogenous or endogenous environments in genetically susceptible individuals activate the immune system and activate cell networks, including keratinocytes, plasmacytoid dendritic cells, macrophages, and T cells, primarily the Th1 and Th17 pathways (102, 103) (Figure 3).

Many reports have focused on the significance of IL-18 in psoriasis. Patients with psoriasis had significantly higher levels of IL-18 in the lesions and in the serum compared to healthy controls (104). In previous studies, serum or plasma IL-18 concentration has been correlated with the severity of psoriasis and the Psoriasis Area and Severity Index (PASI), and therefore, IL-18 may be considered a possible biomarker of psoriasis (105–107). Recently, Deepti Verma et al. revealed that patients with psoriasis (without treatment) exhibited high plasma IL-1β and IL-18 levels, while normal plasma levels of IL-1β and IL-18 in psoriasis patients treated with anti-TNF agents (108). Moreover, Niu et al. explored the possible pathological mechanisms of IL-18 in psoriasis and reported that IL-18 might exacerbate pronounced inflammation and impact pathological features to cause micro-abscesses and scale formation through upregulating pro-inflammatory cytokines and reducing protective cytokines in a mouse model of imiquimod-induced psoriasis (109). Furthermore, a recent study found that recombinant mouse (rm) IL-18 synergized with rmIL-23 to induce prominent inflammation, upregulate the levels of IFN-γ and CXCL9, and enhance psoriasis-like epidermal hyperplasia, indicating that IL-18 might cooperate with IL-23 to induce a Th1 immune reaction, thereby aggravating psoriatic inflammation (110). Besides, IL-18 has been shown to facilitate the production and maintenance of Th17 cells, and Zhang et al. found that IL-18-neutralizing antibody could block the Th17 immune response in the psoriasis-like mouse model (46, 111). These pieces of evidence indicate that IL-18-mediated T cell response may hold an implicated role in psoriasis, and the inhibition of IL-18 can be a potential therapy for psoriasis.

4.2 Atopic dermatitis

Atopic eczema or atopic dermatitis (AD) is the most common inflammatory skin disorder. The primary pathogenesis of AD includes the skin barrier defect and immune dysregulation, especially innate and Th2 immune response (112). IL-18 is a marker for innate immune activation, and many reports have indicated elevated serum levels of IL-18 in AD patients (113–115). Restriction fragment length polymorphism suggested that GG genotype was correlated significantly with elevated serum IL-18 levels, and the G allele of the IL-18 gene (rs 187238) was a risk factor for AD (116). GG genotype of IL-18 in AD patients was related to elevated levels of IgE and pruritus. GG genotype and G-allele in the -137 position of IL-18 increased the risk of AD in the Polish population (117, 118). Besides, Inoue Y et al. revealed consistently upregulated IL-18 levels in the horny layer of skin lesions in AD patients than in healthy controls, and IL-18 levels in the horny layer of patients colonized with *Staphylococcus aureus* (*S. aureus*) were significantly higher compared with those who were not (119). Moreover, McAleer MA et al. measured multiple cytokines and

TABLE 1 Drugs targeting IL-18 and IL-18BP for inflammatory dermatological diseases and related diseases.

Drug	Target	mechanism	Status	Drug category	Indication
Tadekinig alfa (AB2 Bio SA)/r-hIL-18BP/ recombinant Interleukin-18 Binding Protein	IL-18	IL-18 inhibitor	Phase III	recombinant human IL-18 binding protein	Macrophage Activation Syndrome/Immune System Diseases/Adult-Onset Still's Disease (98, 99)
CMK-389	IL-18	IL-18 inhibitor	Phase II	Biological products	Atopic Dermatitis/Pulmonary Sarcoidosis
Camoteskimab/AEVI-007/CERC-007/ MEDI-2338/	IL-18	IL-18 inhibitor	Phase I	Monoclonal antibody	Adult-Onset Still's Disease/Autoimmune Diseases/
ST-067	IL-18	IL-18 agonist	Phase II	Biological products	Melanoma/Carcinoma/Non-Small Cell Lung Cancer
APB-R3/long acting IL-18 binding protein (Aprilbio)	IL-18	IL-18 inhibitor	Drug discovery	Fusion protein	Adult-Onset Still's Disease/Rheumatoid Arthritis/ Inflammatory Bowel Diseases
Tadekinig alfa (Yeda Pharma)	IL-18	IL-18 inhibitor	Phase II (terminated)	recombinant human IL-18 binding protein	Psoriasis/Rheumatoid Arthritis/Crohn Disease/ Autoimmune Diseases
AMP18P1RA/IL-18bp-Fc-IL-1ra	IL-18, IL-1R1	IL-18 Inhibitor, IL-1R1 Antagonist	Preclinical (progression-free)	Fusion protein	Psoriasis/Myocardial Ischemia/Neoplasm Metastasis

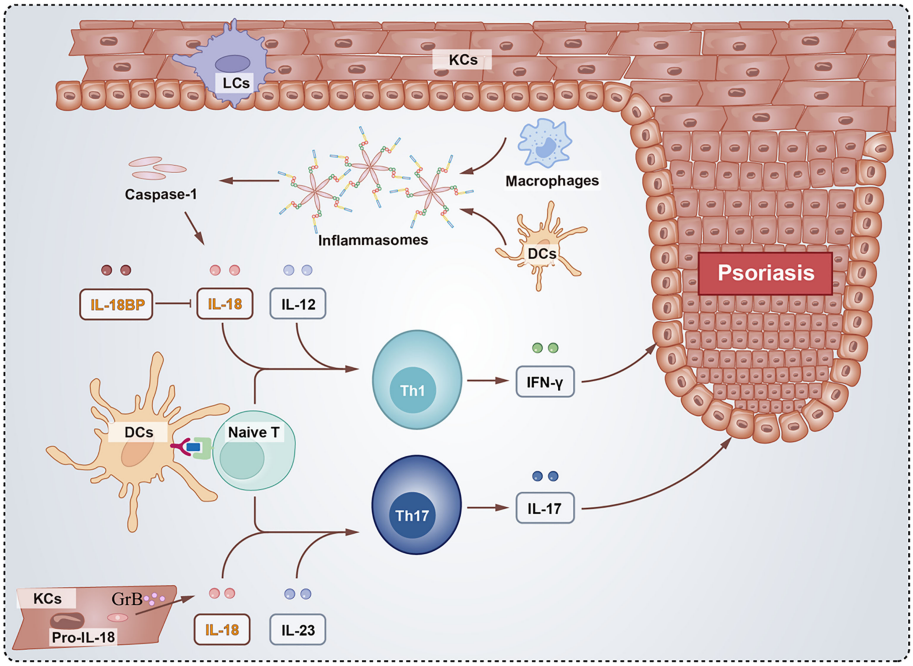

FIGURE 3
Schematic of the role of IL-18 and IL-18BP in psoriasis. The activated inflammasomes directly recruit and cleave procaspase-1 into caspase-1, whose proteolysis activate the pro-inflammatory cytokine IL-18. Besides, proIL-18 were cleaved by GrB in KCs. Inflammatory cytokines including IL-12 and IL-23 produced by Naïve T together with IL-18 facilitate the differentiation of Th1 and Th17 cells, respectively. The Th1 cells secrete IFN-γ and Th17 cells secrete IL-17 both contributing to psoriasis. However, IL-18BP binds to IL-18 blocking the development of psoriasis.

chemokines in the stratum corneum and plasma from AD and healthy children (120). As far as these cytokines are concerned, IL-18 was the most significantly elevated biomarker in the AD skin compartment (121, 122). Furthermore, IL-18 showed one of the strongest associations with barrier function and AD severity (120). AD skin lesions have many skin-infiltrating cells, including $CD8^+$ T cells, which can release granzyme B to cleave pro-IL-18, thus triggering inflammation activation (36). Additionally, *S. aureus*, particularly important in the pathogenesis of AD, can secrete staphylococcal enterotoxin B, which promotes IL-18 production (123). Konishi et al. reported that IL-18 played an essential role in developing AD-like dermatitis in specific transgenic mice (124). Recently, Chen JL et al. pointed out that IL-18 knockout mice reduced aggravated AD–like lesions compared to AD–like mice induced by MC903 (125). Studies have implicated that the pathogenesis of AD is mediated in part by Th2, which promotes IL-4 and IL-13 production and induces IgE (126). IL-18 enhances Th1 and Th2 responses, respectively (127); thus, IL-18 may induce IgE in AD. IL-18 increases IL-4, IL-13, and histamine levels by activating mast cells and basophils. IgE and mast cells can promote the production of a variety of inflammatory mediators (125, 128, 129). Hence, IL-18 may help evaluate the progression of AD, and IL-18 inhibition may be a prospective therapeutic target for AD.

4.3 Rosacea

Rosacea is a classical inflammatory dermatosis with various clinical manifestations, including erythema, telangiectasias, papules, pustules, phymatous changes in the central face, and even ocular involvement (130). Indeed, external stimuli, innate and acquired immune dysfunction combined with neurovascular dysregulation play critical roles in the pathophysiological mechanisms for rosacea (131). Among these factors, the cathelicidin LL-37 activation pathway is the best understood and most classical pathway. Under triggers such as UV radiation, demodex folliculorum, increased Toll-like receptor (TLR) 2 causes the activation and release of matrix metalloproteases (MMPs) and kallikrein 5 (KLK5), which leads to the LL-37 cleavage from its precursor. In addition, a range of pro-inflammatory conditions, including NF-κB activation, leukocyte chemotaxis, and influence on vascular endothelial growth factor (VEGF) and angiogenesis, are induced by activated LL-37 (132). Moreover, recent studies illustrated the critical roles of mast cells and Th1/Th17 cells on rosacea (133–136).

The usefulness of IL-18 in rosacea is not yet clear. Firstly, Salamon et al. found elevated serum concentration of IL-18 in rosacea patients by ELISA (137). However, Casas C et al. obtained epidermal mRNA from lesional samples by scratching the skin surface and found that compared with the control group, the rosacea group showed significantly decreased expression of IL-18 no matter the subtype (138). In 2015, Kim M et al. found that IL-18 expression in rosacea patients was upregulated by immunohistochemical staining of skin biopsies (139). They also revealed that treatment with recombinant erythroid differentiation regulator 1 (Erdr1), a negatively regulated conserved factor of IL-18, resulted in significant improvement in a rosacea-like BALB/c mouse model and downregulation of IL-18. TLR2 or TLR4 can increase IL-18 expression in different cell types; TLR2 and TLR4 can activate and then facilitate IL-18 maturation and secretion (140). IL-18 is a critical modulator of $CD8^+$ T-cell activation and is a mediator of angiogenesis due to its ability to induce

endothelial tube formation (141). Moreover, increased mast cells in rosacea may be associated with IL-18. Whether the IL-18 plays a role in rosacea through angiogenesis or mediating inflammation needs further study.

4.4 Bullous pemphigoid

Bullous Pemphigoid (BP) is one of the most common bullous autoimmune skin diseases. It is characterized by symmetric tense bullae on urticarial, erythematous, or normal skin, with histopathology of subepidermal blisters, inflammatory infiltrates, especially eosinophils in the dermis, and direct immunofluorescence showing deposition of IgG and C3 along the basement membrane region (142). BP180 is critical in the autoimmunity of bullous pemphigoid (143, 144). The dysregulation of IL-18 levels was first reported by Fang et al., who found elevated levels of IL-18 in serum, blister fluid, and lesional skin in BP patients, and a positive correlation between the serum IL-18 levels and the titers of anti-BP180-NC16A autoantibody. More importantly, they reported a dramatic decrease in mRNA expressions of the NLRP3-caspase-1-IL-18 axis components and the serum IL-18 level in BP patients after effective treatment (145). Similarly, Esmaili et al. illustrated that serum IL-18 levels were higher in untreated BP patients admitted for the first time than in control and treated BP patients (146). Besides, they also found a weak significant correlation between BP180 with IL-18 levels. Recently, Margaroli C et al. revealed that blister fluid from BP patients was enriched in multiple inflammatory factors, including IL-18 (147). Although there is no in-depth research on the relationship between IL-18 and BP, high levels of IL-18 in serum, blister fluid, and lesional skin in patients with BP indicated that IL-18 might be a key biomarker in BP.

4.5 Others

Lupus erythematosus is a chronic, multisystemic, genetically and environmentally induced disease with abnormal immune regulation. The patients may have cutaneous lupus erythematosus (CLE) and/or systemic lupus erythematosus (SLE) (148). CLE patients have a substantial amount of apoptotic keratinocytes in their lesions and epidermal keratinocytes, which are thought to be the target cells for immune damage (149). Wang et al. indicated that IL-18 might trigger inflammation in CLE, causing a high TNF-α response and a low IL-12 response, promoting cytokine imbalance and providing a pro-apoptotic microenvironment for keratinocytes. Thus, IL-18BP may play a vital value in the clinical therapy of CLE (149). Dermatomyositis (DM) is an autoimmune disease that affects the skin, muscles, and lungs and is characterized by a skin rash and is associated with prominent muscle weakness (150, 151). DM lesions are histologically similar to CLE lesions and are frequently confusing to distinguish. Ekholm et al. found that endothelial progenitor cells in DM, as in SLE, are characterized by phenotypic and functional abnormalities, which may be triggered by the type I IFN/IL-18 axis (152). Besides, Tsoi et al. confirmed the presence of high type I IFNs in DM skin, including IFN-κ. Their team has found that IL-18 was the only elevated cytokine in DM lesions, and that IL-18 combined with the expression of LCE2D (late cornified envelope 2D), LCE1B (late cornified envelope 1B), KRT80 (keratin 80) and TPM4 (Tropomyosin 4) to clearly differentiate DM from CLE lesions (151). Hance, IL-18 can be a valuable biomarker for DM disease activity.

Allergic contact dermatitis is also a common clinical skin condition, and since allergens are often unavoidable, improving the inflammation caused by re-exposure to allergens becomes the primary treatment. IL-18 significantly contributed to the induction of contact hypersensitivity (CHS) by increasing the recruitment of IFN-γ producing T cells to inflammation foci. Wang et al. reported that during the induction phase of CHS, LC migrating into the lymph nodes (LN) produced large amounts of functional IL-18, which acted synergistically with IL-12 to induce IFN-γ production and significantly contributed to the initiation of CHS (93). In addition, it was shown that plasma levels of free IL-18 and IL-18BP were significantly higher in eczema patients than in healthy controls and that the IL-18BP/IL-18 molar concentration ratio was reduced (153). IL-18BP not only reduced symptoms after exposure to 2,4-dinitrofluorobenzene (DNFB) but also significantly reduced inflammation in mice with previously untreated CHS (154); taken together, IL-18BP is a candidate for the therapeutic indication of allergic contact dermatitis and CHS.

5 Conclusion and perspective

IL-18, a member of the IL-1 family, plays a critical role in the pathogenesis of some skin inflammatory diseases. However, the specific signaling mechanism of IL-18 in inflammatory-related diseases (*e.g.* in rosacea and bullous pemphigoid) remains to be further explored. The IL-18 natural blockers, IL-18BP, has been applied successfully in an emergency case of auto-inflammatory HPS/macrophage activation syndrome. Moreover, the protective properties of recombinant IL-18BP were characterized. Overexpression of IL-18BPc in mice reduces the incidence and severity of encephalomyelitis in mice and decreases Th17 responses, and transfection of IL-18BPd-expressing plasmids *in vivo* improves murine atherosclerosis (155, 156). IL-18 opponent and IL-18BP agonist couple are promising candidates for therapy of diverse medical conditions, including acute and chronic inflammation. Accordingly, IL-18BP has shown potential therapeutic capacity in cutaneous inflammation.

Author contributions

XYW and LW drafted the manuscript. XW and LZ edited the figures and tables. GH and XJ contributed to the critical reading and correction of the review. All authors contributed to the writing of the manuscript and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Boraschi D, Italiani P, Weil S, Martin MU. The family of the interleukin-1 receptors. *Immunol Rev* (2018) 281:197–232. doi: 10.1111/imr.12606

2. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev* (2018) 281:8–27. doi: 10.1111/imr.12621

3. Klück V, Liu R, Joosten LAB. The role of interleukin-1 family members in hyperuricemia and gout. *Joint Bone Spine* (2021) 88:105092. doi: 10.1016/j.jbspin.2020.105092

4. Novick D, Kim SH, Fantuzzi G, Reznikov LL, Dinarello CA, Rubinstein M. Interleukin-18 binding protein: A novel modulator of the Th1 cytokine response. *Immunity* (1999) 10:127–36. doi: 10.1016/s1074-7613(00)80013-8

5. Akira S. The role of IL-18 in innate immunity. *Curr Opin Immunol* (2000) 12:59–63. doi: 10.1016/s0952-7915(99)00051-5

6. Dinarello CA. Novel targets for interleukin 18 binding protein. *Ann Rheum Dis*. (2001) 60(Suppl 3):iii18–24. doi: 10.1136/ard.60.90003.iii18

7. Leach ST, Messina I, Lemberg DA, Novick D, Rubenstein M, Day AS. Local and systemic interleukin-18 and interleukin-18-binding protein in children with inflammatory bowel disease. *Inflammation Bowel Dis* (2008) 14:68–74. doi: 10.1002/ibd.20272

8. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, et al. Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: Blocking interleukin 18 attenuates intestinal damage. *Gut* (2002) 50:812–20. doi: 10.1136/gut.50.6.812

9. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Vogel W, et al. Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. *J Clin Immunol* (2002) 22:331–7. doi: 10.1023/a:1020600230977

10. Girard C, Rech J, Brown M, Allali D, Roux-Lombard P, Spertini F, et al. Elevated serum levels of free interleukin-18 in adult-onset still's disease. *Rheumatol (Oxford)* (2016) 55:2237–47. doi: 10.1093/rheumatology/kew300

11. Mazodier K, Marin V, Novick D, Farnarier C, Robitail S, Schleinitz N, et al. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. *Blood* (2005) 106:3483–9. doi: 10.1182/blood-2005-05-1980

12. Shao XT, Feng L, Gu LJ, Wu LJ, Feng TT, Yang YM, et al. Expression of interleukin-18, IL-18BP, and IL-18R in serum, synovial fluid, and synovial tissue in patients with rheumatoid arthritis. *Clin Exp Med* (2009) 9:215–21. doi: 10.1007/s10238-009-0036-2

13. Shan NN, Zhu XJ, Wang Q, Wang CY, Qin P, Peng J, et al. High-dose dexamethasone regulates interleukin-18 and interleukin-18 binding protein in idiopathic thrombocytopenic purpura. *Haematologica* (2009) 94:1603–7. doi: 10.3324/haematol.2009.007708

14. Fung KY, Nguyen PM, Putoczki TL. Emerging roles for interleukin-18 in the gastrointestinal tumor microenvironment. *Adv Exp Med Biol* (2020) 1240:59–72. doi: 10.1007/978-3-030-38315-2_5

15. Fabbi M, Carbotti G, Ferrini S. Context-dependent role of IL-18 in cancer biology and counter-regulation by IL-18BP. *J Leukoc Biol* (2015) 97:665–75. doi: 10.1189/jlb.5RU0714-360RR

16. O'Brien LC, Mezzaroma E, Van Tassell BW, Marchetti C, Carbone S, Abbate A, et al. Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure. *Mol Med* (2014) 20:221–9. doi: 10.2119/molmed.2014.00034

17. Lee JH, Cho DH, Park HJ. IL-18 and cutaneous inflammatory diseases. *Int J Mol Sci* (2015) 16:29357–69. doi: 10.3390/ijms161226172

18. Fenini G, Contassot E, French LE. Potential of IL-1, IL-18 and inflammasome inhibition for the treatment of inflammatory skin diseases. *Front Pharmacol* (2017) 8:278. doi: 10.3389/fphar.2017.00278

19. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* (2003) 73:213–24. doi: 10.1189/jlb.0602313

20. Smith VP, Bryant NA, Alcamí A. Ectromelia, vaccinia and cowpox viruses encode secreted interleukin-18-binding proteins. *J Gen Virol* (2000) 81:1223–30. doi: 10.1099/0022-1317-81-5-1223

21. Mee JB, Alam Y, Groves RW. Human keratinocytes constitutively produce but do not process interleukin-18. *Br J Dermatol* (2000) 143:330–6. doi: 10.1046/j.1365-2133.2000.03759.x

22. Naik SM, Cannon G, Burbach GJ, Singh SR, Swerlick RA, Wilcox JN, et al. Human keratinocytes constitutively express interleukin-18 and secrete biologically active interleukin-18 after treatment with pro-inflammatory mediators and dinitrochlorobenzene. *J Invest Dermatol* (1999) 113:766–72. doi: 10.1046/j.1523-1747.1999.00750.x

23. Nolan KF, Greaves DR, Waldmann H. The human interleukin 18 gene IL18 maps to 11q22.2-q22.3, closely linked to the DRD2 gene locus and distinct from mapped IDDM loci. *Genomics* (1998) 51:161–3. doi: 10.1006/geno.1998.5336

24. Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T, et al. Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. *J Biol Chem* (1997) 272:26595–603. doi: 10.1074/jbc.272.42.26595

25. Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* (1995) 378:88–91. doi: 10.1038/378088a0

26. Bazan JF, Timans JC, Kastelein RA. A newly defined interleukin-1? *Nature* (1996) 379:591–1. doi: 10.1038/379591a0

27. Kato Z, Jee J, Shikano H, Mishima M, Ohki I, Ohnishi H, et al. The structure and binding mode of interleukin-18. *Nat Struct Biol* (2003) 10:966–71. doi: 10.1038/nsb993

28. Kimura T, Kato Z, Ohnishi H, Tochio H, Shirakawa M, Kondo N. Expression, purification and structural analysis of human IL-18 binding protein: a potent therapeutic molecule for allergy. *Allergol Int* (2008) 57:367–76. doi: 10.2332/allergolint.O-08-546

29. Pourcet B, Gage MC, León TE, Waddington KE, Pello OM, Steffensen KR, et al. The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms. *Sci Rep* (2016) 6:25481. doi: 10.1038/srep25481

30. Debets R, Timans JC, Churakowa T, Zurawski S, de Waal Malefyt R, Moore KW, et al. IL-18 receptors, their role in ligand binding and function: Anti-IL-1RAcPL antibody, a potent antagonist of IL-18. *J Immunol* (2000) 165:4950–6. doi: 10.4049/jimmunol.165.9.4950

31. Hoshino K, Tsutsui H, Kawai T, Takeda K, Nakanishi K, Takeda Y, et al. Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. *J Immunol* (1999) 162:5041–4. doi: 10.4049/jimmunol.162.9.5041

32. Maroni L, Agostinelli L, Saccomanno S, Pinto C, Giordano DM, Rychlicki C, et al. Nlrp3 activation induces il-18 synthesis and affects the epithelial barrier function in reactive cholangiocytes. *Am J Pathol* (2017) 187:366–76. doi: 10.1016/j.ajpath.2016.10.010

33. Bossaller L, Chiang PI, Schmidt-Lauber C, Ganesan S, Kaiser WJ, Rathinam VA, et al. Cutting edge: FAS (CD95) mediates noncanonical IL-1β and IL-18 maturation *via* caspase-8 in an RIP3-independent manner. *J Immunol* (2012) 189:5508–12. doi: 10.4049/jimmunol.1202121

34. Omoto Y, Tokime K, Yamanaka K, Habe K, Morioka T, Kurokawa I, et al. Human mast cell chymase cleaves pro-IL-18 and generates a novel and biologically active IL-18 fragment. *J Immunol* (2006) 177:8315–9. doi: 10.4049/jimmunol.177.12.8315

35. Akeda T, Yamanaka K, Tsuda K, Omoto Y, Gabazza EC, Mizutani H. CD8+ T cell granzyme b activates keratinocyte endogenous IL-18. *Arch Dermatol Res* (2014) 306:125–30. doi: 10.1007/s00403-013-1382-1

36. Omoto Y, Yamanaka K, Tokime K, Kitano S, Kakeda M, Akeda T, et al. Granzyme b is a novel interleukin-18 converting enzyme. *J Dermatol Sci* (2010) 59:129–35. doi: 10.1016/j.jdermsci.2010.05.004

37. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: A possible role of polymorphisms in expression regulation. *J Neuroimmunol* (2001) 112:146–52. doi: 10.1016/s0165-5728(00)00407-0

38. Dinarello CA. Targeting interleukin 18 with interleukin 18 binding protein. *Ann Rheum Dis* (2000) 59(Suppl 1):i17–20. doi: 10.1136/ard.59.suppl_1.i17

39. Hara H, Seregin SS, Yang D, Fukase K, Chamaillard M, Alnemri ES, et al. The NLRP6 inflammasome recognizes lipoteichoic acid and regulates gram-positive pathogen infection. *Cell* (2018) 175:1651–1664.e1614. doi: 10.1016/j.cell.2018.09.047

40. Wilson JE, Petrucelli AS, Chen L, Koblansky AA, Truax AD, Oyama Y, et al. Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis *via* DNA-PK and akt. *Nat Med* (2015) 21:906–13. doi: 10.1038/nm.3908

41. Wei H, Wang D, Qian Y, Liu X, Fan S, Yin HS, et al. Structural basis for the specific recognition of IL-18 by its alpha receptor. *FEBS Lett* (2014) 588:3838–43. doi: 10.1016/j.febslet.2014.09.019

42. Greenfeder SA, Nunes P, Kwee L, Labow M, Chizzonite RA, Ju G. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem* (1995) 270:13757–65. doi: 10.1074/jbc.270.23.13757

43. Born TL, Thomassen E, Bird TA, Sims JE. Cloning of a novel receptor subunit, AcPL, required for interleukin-18 signaling. *J Biol Chem* (1998) 273:29445–50. doi: 10.1074/jbc.273.45.29445

44. Tsutsumi N, Kimura T, Arita K, Ariyoshi M, Ohnishi H, Yamamoto T, et al. The structural basis for receptor recognition of human interleukin-18. *Nat Commun* (2014) 5:5340. doi: 10.1038/ncomms6340

45. Dinarello CA, Novick D, Kim S, Kaplanski G. Interleukin-18 and IL-18 binding protein. *Front Immunol* (2013) 4:289. doi: 10.3389/fimmu.2013.00289

46. Martinon F, Pétrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* (2006) 440:237–41. doi: 10.1038/nature04516

47. Xiang Y, Moss B. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. *Proc Natl Acad Sci U S A.* (1999) 96:11537–42. doi: 10.1073/pnas.96.20.11537

48. Veenstra KG, Jonak ZL, Trulli S, Gollob JA. IL-12 induces monocyte IL-18 binding protein expression *via* IFN-gamma. *J Immunol* (2002) 168:2282–7. doi: 10.4049/jimmunol.168.5.2282

49. Bachmann M, Pfeilschifter J, Mühl H. Epigenetic regulation by CpG methylation splits strong from retarded IFNγ-induced IL-18BP in epithelial versus monocytic cells. *Biochim Biophys Acta Gene Regul Mech* (2018) 1861:191–9. doi: 10.1016/j.bbagrm.2018.01.020

50. Kim SH, Eisenstein M, Reznikov L, Fantuzzi G, Novick D, Rubinstein M, et al. Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. *Proc Natl Acad Sci U S A.* (2000) 97:1190–5. doi: 10.1073/pnas.97.3.1190

51. Mühl H, Bachmann M. IL-18/IL-18BP and IL-22/IL-22BP: Two interrelated couples with therapeutic potential. *Cell Signal* (2019) 63:109388. doi: 10.1016/j.cellsig.2019.109388

52. Krumm B, Meng X, Li Y, Xiang Y, Deng J. Structural basis for antagonism of human interleukin 18 by poxvirus interleukin 18-binding protein. *Proc Natl Acad Sci U S A.* (2008) 105:20711–5. doi: 10.1073/pnas.0809086106

53. Krumm B, Meng X, Xiang Y, Deng J. Crystallization of interleukin-18 for structure-based inhibitor design. *Acta Crystallogr F Struct Biol Commun* (2015) 71:710–7. doi: 10.1107/s2053230x15006871

54. Krumm B, Meng X, Xiang Y, Deng J. Identification of small molecule inhibitors of interleukin-18. *Sci Rep* (2017) 7:483. doi: 10.1038/s41598-017-00532-x

55. Dinarello CA. Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. *Am J Clin Nutr* (2006) 83:447s–55s. doi: 10.1093/ajcn/83.2.447S

56. Liu Z, Wyffels L, Barber C, Hui MM, Woolfenden JM. A (99m)Tc-labeled dual-domain cytokine ligand for imaging of inflammation. *Nucl Med Biol* (2011) 38:795–805. doi: 10.1016/j.nucmedbio.2011.02.012

57. Cavalli G, Dinarello CA. Suppression of inflammation and acquired immunity by IL-37. *Immunol Rev* (2018) 281:179–90. doi: 10.1111/imr.12605

58. Bufler P, Azam T, Gamboni-Robertson F, Reznikov LL, Kumar S, Dinarello CA, et al. A complex of the IL-1 homologue IL-1F7b and IL-18-binding protein reduces IL-18 activity. *Proc Natl Acad Sci U S A.* (2002) 99:13723–8. doi: 10.1073/pnas.212519099

59. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, et al. IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nat Immunol* (2015) 16:354–65. doi: 10.1038/ni.3103

60. Zhang LM, Zhang J, Zhang Y, Wang L, Fei C, Yi ZW, et al. Interleukin-18 binding protein attenuates lipopolysaccharide-induced acute lung injury in mice *via* suppression NF-κB and activation Nrf2 pathway. *Biochem Biophys Res Commun* (2018) 505:837–42. doi: 10.1016/j.bbrc.2018.09.193

61. Carroll HP, Paunovic V, Gadina M. Signalling, inflammation and arthritis: Crossed signals: the role of interleukin-15 and -18 in autoimmunity. *Rheumatol (Oxford)* (2008) 47:1269–77. doi: 10.1093/rheumatology/ken257

62. Kanakaraj P, Ngo K, Wu Y, Angulo A, Ghazal P, Harris CA, et al. Defective interleukin (IL)-18-mediated natural killer and T helper cell type 1 responses in IL-1 receptor-associated kinase (IRAK)-deficient mice. *J Exp Med* (1999) 189:1129–38. doi: 10.1084/jem.189.7.1129

63. Suzuki N, Chen NJ, Millar DG, Suzuki S, Horacek T, Hara H, et al. IL-1 receptor-associated kinase 4 is essential for IL-18-mediated NK and Th1 cell responses. *J Immunol* (2003) 170:4031–5. doi: 10.4049/jimmunol.170.8.4031

64. Hirooka Y, Nozaki Y. Interleukin-18 in inflammatory kidney disease. *Front Med (Lausanne)* (2021) 8:639103. doi: 10.3389/fmed.2021.639103

65. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev* (2001) 12:53–72. doi: 10.1016/s1359-6101(00)00015-0

66. Murphy JE, Robert C, Kupper TS. Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. *J Invest Dermatol* (2000) 114:602–8. doi: 10.1046/j.1523-1747.2000.00917.x

67. Robinson D, Shibuya K, Mui A, Zonin F, Murphy E, Sana T, et al. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. *Immunity* (1997) 7:571–81. doi: 10.1016/s1074-7613(00)80378-7

68. Puren AJ, Fantuzzi G, Gu Y, Su MS, Dinarello CA. Interleukin-18 (IFNgamma-inducing factor) induces IL-8 and IL-1beta *via* TNFalpha production from non-CD14+ human blood mononuclear cells. *J Clin Invest* (1998) 101:711–21. doi: 10.1172/jci1379

69. Virtue A, Wang H, Yang XF. MicroRNAs and toll-like receptor/interleukin-1 receptor signaling. *J Hematol Oncol* (2012) 5:66. doi: 10.1186/1756-8722-5-66

70. Chudnovskiy A, Mortha A, Kana V, Kennard A, Ramirez JD, Rahman A, et al. Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell* (2016) 167:444–456.e414. doi: 10.1016/j.cell.2016.08.076

71. Xu D, Trajkovic V, Hunter D, Leung BP, Schulz K, Gracie JA, et al. IL-18 induces the differentiation of Th1 or Th2 cells depending upon cytokine milieu and genetic background. *Eur J Immunol* (2000) 30:3147–56. doi: 10.1002/1521-4141(200011)30:11<3147::Aid-immu3147>3.0.Co;2-j

72. Yoshimoto T, Takeda K, Tanaka T, Ohkusu K, Kashiwamura S, Okamura H, et al. IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and b cells: synergism with IL-18 for IFN-gamma production. *J Immunol* (1998) 161:3400–7. doi: 10.4049/jimmunol.161.7.3400

73. Son YI, Dallal RM, Mailliard RB, Egawa S, Jonak ZL, Lotze MT. Interleukin-18 (IL-18) synergizes with IL-2 to enhance cytotoxicity, interferon-gamma production, and expansion of natural killer cells. *Cancer Res* (2001) 61:884–8.

74. Hoshino T, Yagita H, Ortaldo JR, Wiltrout RH, Young HA. *In vivo* administration of IL-18 can induce IgE production through Th2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on CD4+ T cells. *Eur J Immunol* (2000) 30:1998–2006. doi: 10.1002/1521-4141(200007)30:7<1998::Aid-immu1998>3.0.Co;2-u

75. Kanda N, Shimizu T, Tada Y, Watanabe S. IL-18 enhances IFN-gamma-induced production of CXCL9, CXCL10, and CXCL11 in human keratinocytes. *Eur J Immunol* (2007) 37:338–50. doi: 10.1002/eji.200636420

76. Wang J, Zhang H, Zheng W, Xie H, Yan H, Lin X, et al. Correlation of IL-18 with tryptase in atopic asthma and induction of mast cell accumulation by IL-18. *Mediators Inflamm* (2016) 2016:4743176. doi: 10.1155/2016/4743176

77. Kämpfer H, Kalina U, Mühl H, Pfeilschifter J, Frank S. Counterregulation of interleukin-18 mRNA and protein expression during cutaneous wound repair in mice. *J Invest Dermatol* (1999) 113:369–74. doi: 10.1046/j.1523-1747.1999.00704.x

78. Companjen AR, van der Velden VH, Vooys A, Debets R, Benner R, Prens EP. Human keratinocytes are major producers of IL-18: predominant expression of the unprocessed form. *Eur Cytokine Netw* (2000) 11:383–90.

79. Cho D, Seung Kang J, Hoon Park J, Kim YI, Hahm E, Lee J, et al. The enhanced IL-18 production by UVB irradiation requires ROI and AP-1 signaling in human keratinocyte cell line (HaCaT). *Biochem Biophys Res Commun* (2002) 298:289–95. doi: 10.1016/s0006-291x(02)02433-6

80. Koizumi H, Sato-Matsumura KC, Nakamura H, Shida K, Kikkawa S, Matsumoto M, et al. Distribution of IL-18 and IL-18 receptor in human skin: Various forms of IL-18 are produced in keratinocytes. *Arch Dermatol Res* (2001) 293:325–33. doi: 10.1007/s004030100237

81. Mühl H, Kämpfer H, Bosmann M, Frank S, Radeke H, Pfeilschifter J. Interferon-gamma mediates gene expression of IL-18 binding protein in nonleukocytic cells. *Biochem Biophys Res Commun* (2000) 267:960–3. doi: 10.1006/bbrc.1999.2064

82. Dai X, Tohyama M, Murakami M, Sayama K. Epidermal keratinocytes sense dsRNA *via* the NLRP3 inflammasome, mediating interleukin (IL)-1β and IL-18 release. *Exp Dermatol* (2017) 26:904–11. doi: 10.1111/exd.13334

83. Kolb R, Liu GH, Janowski AM, Sutterwala FS, Zhang W. Inflammasomes in cancer: a double-edged sword. *Protein Cell* (2014) 5:12–20. doi: 10.1007/s13238-013-0001-4

84. Tartey S, Kanneganti TD. Differential role of the NLRP3 inflammasome in infection and tumorigenesis. *Immunology* (2019) 156:329–38. doi: 10.1111/imm.13046

85. Papale A, Kummer E, Galbiati V, Marinovich M, Galli CL, Corsini E. Understanding chemical allergen potency: Role of NLRP12 and blimp-1 in the induction of IL-18 in human keratinocytes. *Arch Toxicol* (2017) 91:1783–94. doi: 10.1007/s00204-016-1806-8

86. Zhang M, Qi Y, Li H, Cui J, Dai L, Frank JA, et al. AIM2 inflammasome mediates arsenic-induced secretion of IL-1 β and IL-18. *Oncoimmunology* (2016) 5:e1160182. doi: 10.1080/2162402x.2016.1160182

87. Fenini G, Grossi S, Contassot E, Biedermann T, Reichmann E, French LE, et al. Genome editing of human primary keratinocytes by CRISPR/Cas9 reveals an essential role of the NLRP1 inflammasome in UVB sensing. *J Invest Dermatol* (2018) 138:2644–52. doi: 10.1016/j.jid.2018.07.016

88. Galbiati V, Papale A, Galli CL, Marinovich M, Corsini E. Role of ROS and HMGB1 in contact allergen-induced IL-18 production in human keratinocytes. *J Invest Dermatol* (2014) 134:2719–27. doi: 10.1038/jid.2014.203

89. Flier J, Boorsma DM, Bruynzeel DP, Van Beek PJ, Stoof TJ, Scheper RJ, et al. The CXCR3 activating chemokines IP-10, mig, and IP-9 are expressed in allergic but not in irritant patch test reactions. *J Invest Dermatol* (1999) 113:574–8. doi: 10.1046/j.1523-1747.1999.00730.x

90. Kim HJ, Song SB, Choi JM, Kim KM, Cho BK, Cho DH, et al. IL-18 downregulates collagen production in human dermal fibroblasts *via* the ERK pathway. *J Invest Dermatol* (2010) 130:706–15. doi: 10.1038/jid.2009.302

91. Le X, Wu WW. The therapeutic effect of interleukin-18 on hypertrophic scar through inducing fas ligand expression. *Burns* (2021) 47:430–8. doi: 10.1016/j.burns.2020.07.008

92. Aguirre A, Shoji KF, Sáez JC, Henríquez M, Quest AF. FasL-triggered death of jurkat cells requires caspase 8-induced, ATP-dependent cross-talk between fas and the purinergic receptor P2X(7). *J Cell Physiol* (2013) 228:485–93. doi: 10.1002/jcp.24159

93. Wang B, Feliciani C, Howell BG, Freed I, Cai Q, Watanabe H, et al. Contribution of langerhans cell-derived IL-18 to contact hypersensitivity. *J Immunol* (2002) 168:3303–8. doi: 10.4049/jimmunol.168.7.3303

94. Chang JT, Segal BM, Nakanishi K, Okamura H, Shevach EM. The costimulatory effect of IL-18 on the induction of antigen-specific IFN-gamma production by resting T

cells is IL-12 dependent and is mediated by up-regulation of the IL-12 receptor beta2 subunit. *Eur J Immunol* (2000) 30:1113–9. doi: 10.1002/(sici)1521-4141(200004)30:4<1113::Aid-immu1113>3.0.Co;2-p

95. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol* (2006) 6:173–82. doi: 10.1038/nri1785

96. Fortin CF, Ear T, McDonald PP. Autocrine role of endogenous interleukin-18 on inflammatory cytokine generation by human neutrophils. *FASEB J* (2009) 23:194–203. doi: 10.1096/fj.08-110213

97. Robertson SE, Young JD, Kitson S, Pitt A, Evans J, Roes J, et al. Expression and alternative processing of IL-18 in human neutrophils. *Eur J Immunol* (2006) 36:722–31. doi: 10.1002/eji.200535402

98. Gabay C, Fautrel B, Rech J, Spertini F, Feist E, Kötter I, et al. Open-label, multicentre, dose-escalating phase II clinical trial on the safety and efficacy of tadekinig alfa (IL-18BP) in adult-onset still's disease. *Ann Rheum Dis* (2018) 77:840–7. doi: 10.1136/annrheumdis-2017-212608

99. Kiltz U, Kiefer D, Braun J, Schiffrin EJ, Girard-Guyonvarc'h C, Gabay C. Prolonged treatment with tadekinig alfa in adult-onset still's disease. *Ann Rheum Dis* (2020) 79:e10. doi: 10.1136/annrheumdis-2018-214496

100. Armstrong AW. Psoriasis. *JAMA Dermatol* (2017) 153:956–6. doi: 10.1001/jamadermatol.2017.2103%JJAMADermatology

101. Boehncke WH, Schön MP. Psoriasis. *Lancet* (2015) 386:983–94. doi: 10.1016/s0140-6736(14)61909-7

102. Hugh JM, Weinberg JM. Update on the pathophysiology of psoriasis. *Cutis* (2018) 102:6–12.

103. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: A review. *Jama* (2020) 323:1945–60. doi: 10.1001/jama.2020.4006

104. Arican O, Aral M, Sasmaz S, Ciragil P. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm* (2005) 2005:273–9. doi: 10.1155/mi.2005.273

105. Flisiak I, Klepacki A, Chodynicka B. Plasma and scales levels of interleukin 18 in comparison with other possible clinical and laboratory biomarkers of psoriasis activity. *Biomarkers* (2006) 11:194–200. doi: 10.1080/13547500600565735

106. Gangemi S, Merendino RA, Guarneri F, Minciullo PL, DiLorenzo G, Pacor M, et al. Serum levels of interleukin-18 and s-ICAM-1 in patients affected by psoriasis: preliminary considerations. *J Eur Acad Dermatol Venereol* (2003) 17:42–6. doi: 10.1046/j.1468-3083.2003.00647.x

107. Forouzandeh M, Besen J, Keane RW, de Rivero Vaccari JP. The inflammasome signaling proteins ASC and IL-18 as biomarkers of psoriasis. *Front Pharmacol* (2020) 11:1238. doi: 10.3389/fphar.2020.01238

108. Verma D, Fekri SZ, Sigurdardottir G, Bivik Eding C, Sandin C, Enerbäck C. Enhanced inflammasome activity in patients with psoriasis promotes systemic inflammation. *J Invest Dermatol* (2021) 141:586–595.e585. doi: 10.1016/j.jid.2020.07.012

109. Niu XL, Huang Y, Gao YL, Sun YZ, Han Y, Chen HD, et al. Interleukin-18 exacerbates skin inflammation and affects microabscesses and scale formation in a mouse model of imiquimod-induced psoriasis. *Chin Med J (Engl)* (2019) 132:690–8. doi: 10.1097/cm9.0000000000000140

110. Shimoura N, Nagai H, Fujiwara S, Jimbo H, Yoshimoto T, Nishigori C. Interleukin (IL)-18, cooperatively with IL-23, induces prominent inflammation and enhances psoriasis-like epidermal hyperplasia. *Arch Dermatol Res* (2017) 309:315–21. doi: 10.1007/s00403-017-1735-2

111. Zhang W, Guo S, Li B, Liu L, Ge R, Cao T, et al. Proinflammatory effect of high-mobility group protein B1 on keratinocytes: an autocrine mechanism underlying psoriasis development. *J Pathol* (2017) 241:392–404. doi: 10.1002/path.4848

112. Sullivan M, Silverberg NB. Current and emerging concepts in atopic dermatitis pathogenesis. *Clin Dermatol* (2017) 35:349–53. doi: 10.1016/j.clindermatol.2017.03.006

113. Zedan K, Rasheed Z, Farouk Y, Alzolibani AA, Bin Saif G, Ismail HA, et al. Immunoglobulin e, interleukin-18 and interleukin-12 in patients with atopic dermatitis: correlation with disease activity. *J Clin Diagn Res* (2015) 9:Wc01–05. doi: 10.7860/jcdr/2015/12261.5742

114. Trzeciak M, Gleń J, Bandurski T, Sokołowska-Wojdyło M, Wilkowska A, Roszkiewicz J. Relationship between serum levels of interleukin-18, IgE and disease severity in patients with atopic dermatitis. *Clin Exp Dermatol* (2011) 36:728–32. doi: 10.1111/j.1365-2230.2011.04113.x

115. Kou K, Aihara M, Matsunaga T, Chen H, Taguri M, Morita S, et al. Association of serum interleukin-18 and other biomarkers with disease severity in adults with atopic dermatitis. *Arch Dermatol Res* (2012) 304:305–12. doi: 10.1007/s00403-011-1198-9

116. Gohar MK, Atta AH, Nasr MM, Hussein DN. Serum thymus and activation regulated chemokine (TARC), IL- 18 and IL-18 gene polymorphism as associative factors with atopic dermatitis. *Egypt J Immunol* (2017) 24:9–22.

117. Trzeciak M, Gleń J, Rębała K, Bandurski T, Sikorska M, Nowicki R. Coexistence of 2282del4 FLG gene mutation and IL-18 -137G/C gene polymorphism enhances the risk of atopic dermatitis. *Postepy Dermatol Alergol* (2016) 33:57–62. doi: 10.5114/pdia.2015.48050

118. Novak N, Kruse S, Potreck J, Maintz L, Jenneck C, Weidinger S, et al. Single nucleotide polymorphisms of the IL18 gene are associated with atopic eczema. *J Allergy Clin Immunol* (2005) 115:828–33. doi: 10.1016/j.jaci.2005.01.030

119. Inoue Y, Aihara M, Kirino M, Harada I, Komori-Yamaguchi J, Yamaguchi Y, et al. Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with staphylococcus aureus colonization. *Br J Dermatol* (2011) 164:560–7. doi: 10.1111/j.1365-2133.2010.10145.x

120. McAleer MA, Jakasa I, Hurault G, Sarvari P, McLean WHI, Tanaka RJ, et al. Systemic and stratum corneum biomarkers of severity in infant atopic dermatitis include markers of innate and T helper cell-related immunity and angiogenesis. *Br J Dermatol* (2019) 180:586–96. doi: 10.1111/bjd.17088

121. Lyubchenko T, Collins HK, Goleva E, Leung DYM. Skin tape sampling technique identifies proinflammatory cytokines in atopic dermatitis skin. *Ann Allergy Asthma Immunol* (2021) 126:46–53.e42. doi: 10.1016/j.anai.2020.08.397

122. Andersson AM, Sølberg J, Koch A, Skov L, Jakasa I, Kezic S, et al. Assessment of biomarkers in pediatric atopic dermatitis by tape strips and skin biopsies. *Allergy* (2022) 77:1499–509. doi: 10.1111/all.15153

123. Suwarsa O, Adi S, Idjradinata P, Sutedja E, Avriyanti E, Asfara A, et al. Interleukin-18 correlates with interleukin-4 but not interferon-γ production in lymphocyte cultures from atopic dermatitis patients after staphylococcal enterotoxin b stimulation. *Asian Pac J Allergy Immunol* (2017) 35:54–9. doi: 10.12932/ap0787

124. Konishi H, Tsutsui H, Murakami T, Yumikura-Futatsugi S, Yamanaka K, Tanaka M, et al. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. *Proc Natl Acad Sci U S A.* (2002) 99:11340–5. doi: 10.1073/pnas.152337799

125. Chen JL, Niu XL, Gao YL, Ma L, Gao XH, Chen HD, et al. IL−18 knockout alleviates atopic dermatitis−like skin lesions induced by MC903 in a mouse model. *Int J Mol Med* (2020) 46:880–8. doi: 10.3892/ijmm.2020.4630

126. Sugiyama A, Hata S, Suzuki K, Yoshida E, Nakano R, Mitra S, et al. Oral administration of paramylon, a beta-1,3-D-glucan isolated from euglena gracilis z inhibits development of atopic dermatitis-like skin lesions in NC/Nga mice. *J Vet Med Sci* (2010) 72:755–63. doi: 10.1292/jvms.09-0526

127. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* (2001) 19:423–74. doi: 10.1146/annurev.immunol.19.1.423

128. Voss GT, de Oliveira RL, Davies MJ, Domingues WB, Campos VF, Soares MP, et al. Suppressive effect of 1,4-anhydro-4-seleno-D-talitol (SeTal) on atopic dermatitis-like skin lesions in mice through regulation of inflammatory mediators. *J Trace Elem Med Biol* (2021) 67:126795. doi: 10.1016/j.jtemb.2021.126795

129. Yoshimoto T, Mizutani H, Tsutsui H, Noben-Trauth N, Yamanaka K, Tanaka M, et al. IL-18 induction of IgE: Dependence on CD4+ T cells, IL-4 and STAT6. *Nat Immunol* (2000) 1:132–7. doi: 10.1038/77811

130. Johnson SM, Berg A, Barr C. Recognizing rosacea: Tips on differential diagnosis. *J Drugs Dermatol* (2019) 18:888–94.

131. Ahn CS, Huang WW. Rosacea pathogenesis. *Dermatol Clin* (2018) 36:81–6. doi: 10.1016/j.det.2017.11.001

132. Holmes AD, Steinhoff M. Integrative concepts of rosacea pathophysiology, clinical presentation and new therapeutics. *Exp Dermatol* (2017) 26:659–67. doi: 10.1111/exd.13143

133. Mascarenhas NL, Wang Z, Chang YL, Di Nardo A. TRPV4 mediates mast cell activation in cathelicidin-induced rosacea inflammation. *J Invest Dermatol* (2017) 137:972–5. doi: 10.1016/j.jid.2016.10.046

134. Wang L, Wang YJ, Hao D, Wen X, Du D, He G, et al. The theranostics role of mast cells in the pathophysiology of rosacea. *Front Med (Lausanne)* (2019) 6:324. doi: 10.3389/fmed.2019.00324

135. Choi JE, Werbel T, Wang Z, Wu CC, Yaksh TL, Di Nardo A. Botulinum toxin blocks mast cells and prevents rosacea like inflammation. *J Dermatol Sci* (2019) 93:58–64. doi: 10.1016/j.jdermsci.2018.12.004

136. Buhl T, Sulk M, Nowak P, Buddenkotte J, McDonald I, Aubert J, et al. Molecular and morphological characterization of inflammatory infiltrate in rosacea reveals activation of Th1/Th17 pathways. *J Invest Dermatol* (2015) 135:2198–208. doi: 10.1038/jid.2015.141

137. Salamon M, Sysa-Jedrzejowska A, Lukamowicz J, Lukamowicz M, Swiatkowska E, Wozniacka A. [Concentration of selected cytokines in serum of patients with acne rosacea]. *Przegl Lek* (2008) 65:371–4.

138. Casas C, Paul C, Lahfa M, Livideanu B, Lejeune O, Alvarez-Georges S, et al. Quantification of demodex folliculorum by PCR in rosacea and its relationship to skin innate immune activation. *Exp Dermatol* (2012) 21:906–10. doi: 10.1111/exd.12030

139. Kim M, Kim KE, Jung HY, Jo H, Jeong SW, Lee J, et al. Recombinant erythroid differentiation regulator 1 inhibits both inflammation and angiogenesis in a mouse model of rosacea. *Exp Dermatol* (2015) 24:680–5. doi: 10.1111/exd.12745

140. Koch KN, Hartung ML, Urban S, Kyburz A, Bahlmann AS, Lind J, et al. Helicobacter urease-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma. *J Clin Invest* (2015) 125:3297–302. doi: 10.1172/jci79337

141. Park CC, Morel JC, Amin MA, Connors MA, Harlow LA, Koch AE. Evidence of IL-18 as a novel angiogenic mediator. *J Immunol* (2001) 167:1644–53. doi: 10.4049/jimmunol.167.3.1644

142. Schmidt E, Zillikens D. Pemphigoid diseases. *Lancet* (2013) 381:320–32. doi: 10.1016/s0140-6736(12)61140-4

143. Liu Y, Li L, Xia Y. BP180 is critical in the autoimmunity of bullous pemphigoid. *Front Immunol* (2017) 8:1752. doi: 10.3389/fimmu.2017.01752

144. Nakama K, Koga H, Ishii N, Ohata C, Hashimoto T, Nakama T. Clinical and immunological profiles of 14 patients with bullous pemphigoid without IgG autoantibodies to the BP180 NC16A domain. *JAMA Dermatol* (2018) 154:347–50. doi: 10.1001/jamadermatol.2017.5465

145. Fang H, Shao S, Cao T, Lei J, Dang E, Zhang J, et al. Increased expression of NLRP3 inflammasome components and interleukin-18 in patients with bullous pemphigoid. *J Dermatol Sci* (2016) 83:116–23. doi: 10.1016/j.jdermsci.2016.04.009

146. Esmaili N, Mortazavi H, Ansari MS, Nasimi M, Ardalanrad Y, Teimourpour A. Serum levels of interleukin 18 and interleukin 10 in Iranian patients with bullous pemphigoid and their correlation with BP180-NC16a and BP230. *Iran J Allergy Asthma Immunol* (2020) 19:102–4. doi: 10.18502/ijaai.v19i1.2423

147. Margaroli C, Bradley B, Thompson C, Brown MR, Giacalone VD, Bhatt L, et al. Distinct compartmentalization of immune cells and mediators characterizes bullous pemphigoid disease. *Exp Dermatol* (2020) 29:1191–8. doi: 10.1111/exd.14209

148. Robinson ES, Werth VP. The role of cytokines in the pathogenesis of cutaneous lupus erythematosus. *Cytokine* (2015) 73:326–34. doi: 10.1016/j.cyto.2015.01.031

149. Wang D, Drenker M, Eiz-Vesper B, Werfel T, Wittmann M. Evidence for a pathogenetic role of interleukin-18 in cutaneous lupus erythematosus. *Arthritis Rheumatol* (2008) 58:3205–15. doi: 10.1002/art.23868

150. Chen M, Quan C, Diao L, Xue F, Xue K, Wang B, et al. Measurement of cytokines and chemokines and association with clinical severity of dermatomyositis and clinically amyopathic dermatomyositis. *Br J Dermatol* (2018) 179:1334–41. doi: 10.1111/bjd.17079

151. Tsoi LC, Gharaee-Kermani M, Berthier CC, Nault T, Hile GA, Estadt SN, et al. IL18-containing 5-gene signature distinguishes histologically identical dermatomyositis and lupus erythematosus skin lesions. *JCI Insight* (2020) 5(16):e139558. doi: 10.1172/jci.insight.139558

152. Ekholm L, Kahlenberg JM, Barbasso Helmers S, Tjärnlund A, Yalavarthi S, Zhao W, et al. Dysfunction of endothelial progenitor cells is associated with the type I IFN pathway in patients with polymyositis and dermatomyositis. *Rheumatol (Oxford)* (2016) 55:1987–92. doi: 10.1093/rheumatology/kew288

153. Hu Y, Wang J, Zhang H, Xie H, Song W, Jiang Q, et al. Enhanced expression of IL-18 and IL-18BP in plasma of patients with eczema: Altered expression of IL-18BP and IL-18 receptor on mast cells. *Mediators Inflamm* (2017) 2017:3090782. doi: 10.1155/2017/3090782

154. Plitz T, Saint-Mézard P, Satho M, Herren S, Waltzinger C, de Carvalho Bittencourt M, et al. IL-18 binding protein protects against contact hypersensitivity. *J Immunol* (2003) 171:1164–71. doi: 10.4049/jimmunol.171.3.1164

155. Millward JM, Løbner M, Wheeler RD, Owens T. Inflammation in the central nervous system and Th17 responses are inhibited by IFN-gamma-Induced IL-18 binding protein. *J Immunol* (2010) 185:2458–66. doi: 10.4049/jimmunol.0902153

156. Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, et al. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res* (2001) 89:E41–45. doi: 10.1161/hh1901.098735

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Cutaneous manifestations associated with immune checkpoint inhibitors

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Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that block key mediators of tumor-mediated immune evasion. The frequency of its use has increased rapidly and has extended to numerous cancers. ICIs target immune checkpoint molecules, such as programmed cell death protein 1 (PD-1), PD ligand 1 (PD-L1), and T cell activation, including cytotoxic T-lymphocyte-associated protein-4 (CTLA-4). However, ICI-driven alterations in the immune system can induce various immune-related adverse events (irAEs) that affect multiple organs. Among these, cutaneous irAEs are the most common and often the first to develop. Skin manifestations are characterized by a wide range of phenotypes, including maculopapular rash, psoriasiform eruption, lichen planus-like eruption, pruritus, vitiligo-like depigmentation, bullous diseases, alopecia, and Stevens-Johnson syndrome/toxic epidermal necrolysis. In terms of pathogenesis, the mechanism of cutaneous irAEs remains unclear. Still, several hypotheses have been proposed, including activation of T cells against common antigens in normal tissues and tumor cells, increased release of proinflammatory cytokines associated with immune-related effects in specific tissues/organs, association with specific human leukocyte antigen variants and organ-specific irAEs, and acceleration of concurrent medication-induced drug eruptions. Based on recent literature, this review provides an overview of each ICI-induced skin manifestation and epidemiology and focuses on the mechanisms underlying cutaneous irAEs.

KEYWORDS

CTLA-4, cutaneous manifestation, epidemiology, immune checkpoint inhibitors, immune-related adverse events, PD-1, PD-L1

1 Introduction

Immune checkpoint inhibitors (ICIs), which are monoclonal antibodies that block key mediators of tumor-mediated immune evasion, were initially approved for treating patients with unresectable malignant melanoma (MM) in 2014, and their frequency of use has rapidly increased in numerous cancers. ICIs target immune checkpoint molecules, such as programmed cell death protein 1 (PD-1), PD ligand 1 (PD-L1), and T cell activation, including cytotoxic T-lymphocyte associated protein-4 (CTLA-4) (1). Anti-PD-1 agents

(cemiplimab, dostarlimab nivolumab, pembrolizumab, and tislelizumab), anti-PD-L1 agents (atezolizumab, avelumab, and durvalumab), and anti-CTLA4 agents (ipilimumab and tremelimumab) have been approved by the Food and Drug Administration and European Medicines Agency (Table 1). PD-1 is an immune checkpoint receptor expressed on antigen-stimulated T-cells, and PD-L1 is a ligand of PD-1 (2). In contrast, CTLA-4 is an inhibitory receptor expressed on the surface of activated T cells that prevents the binding of CD28 to CD80 and CD86, which are stimulatory receptors. Blockage of PD-1 and/or CTLA-4 can lead to the stimulation and augmentation of anti-tumor effects *via* the activation of tumor-specific cytotoxic T-cells and inhibition of regulatory T cells (Tregs) (2). These treatments constitute one of the most effective strategies for anti-cancer therapy (3).

However, alterations in the immune system induced by these drugs can lead to various immune-related adverse events (irAEs) specific to ICI treatment. IrAEs can affect multiple organs such as the skin, thyroid gland, adrenal glands, pituitary gland, gut, liver, and lungs (4, 5). Among these, cutaneous irAEs are the most common and often the first to develop (6). To achieve the most favorable outcomes for patients with cancer, an early and accurate diagnosis of irAEs is essential for management, including discontinuation of ICIs and/or the addition of immunosuppressive agents such as systemic corticosteroids. Therefore, dermatologists should be aware of various types of cutaneous irAEs, regardless of whether they are common or rare. In contrast, the mechanism of cutaneous irAEs remains unclear; however, several hypotheses have been proposed based on recent findings.

In this review, we focus on the clinical presentations, mechanisms, and management of various cutaneous irAEs.

2 The function of PD-1 and CTLA-4

PD-1 is an inhibitory receptor expressed on the surface of activated T and B cells that induces and maintains peripheral tolerance against self-reactive T cells (7, 8). PD-1 interacts with PD-L1 and PD-L2, which are expressed on antigen-presenting cells (APCs) and tumor cells, resulting in the suppression of T-cell activation and tumor-mediated immune evasion. Inhibition of PD-1 enhances T cell effector function and activation of B cells and natural killer cells, while PD-1 blockade inhibits the suppressive function of Tregs in anti-tumor immunity. Furthermore, PD-L1 and PD-L2 play different roles in the immune response (9). In APCs, stimulation with interferon (IFN)-γ and interleukin (IL)-17A strongly induces PD-L1 expression, whereas PD-L2 expression is induced by stimulation with IL-4. PD-L1 plays an important role in Th1 and Th17 type immunity, while PD-L2 is associated with Th2 type immunity. Therefore, PD-1 blockade may shift the immune balance toward a Th1/Th17 response (9). Furthermore, a recent study revealed that the binding of PD-1, PD-L2, and PD-1–PD-L1 triggered the clustering of PD-1 with T cell receptor (TCR), resulting in the formation of TCR-PD-1-PD-L2 signalosomes. This signalosome suppresses T-cell responses. Similar to the effect of anti-PD-L1 agents, PD-L2 blockade may exert anti-tumor effects, although no therapeutic agents target PD-L2 (10). In the future, anti-PD-L2 agents are expected to be used to treat all types of cancer. CTLA-4 is expressed on the surfaces of activated T cells and Tregs. It can bind to B7 molecules (CD80/86) on APCs with a higher affinity and impede CD28 and B7 binding, suppressing T-cell activation by reducing IL-2 and IL-2 receptor expression (11, 12). Moreover, CTLA-4 expression in Tregs mediates immune inhibitory effects (13). CTLA-4 blocking impedes the binding of CTLA-4 to B7 and induces the binding of CD28 and B7 to reactivate T cells. It also decreases the immune-inhibitory effects of Tregs and further reduces the number of Tregs in tumor tissues *via* antibody-dependent cellular cytotoxicity (ADCC) (14).

Tumors and the tumor microenvironment (TME) express multiple inhibitory pathways and related molecules, resulting in T-cell dysfunction and immune escape. Although the blockage of PD-1 and/or CTLA-4 can promote the activation of T cells and exert an effective anti-tumor function, the exuberant activation of self-reactive T cells with the resultant autoimmunity is presumed to be an irAE (8).

TABLE 1 Immune checkpoint inhibitors approved by the Food and Drug Administration and European Medicines Agency.

Immune checkpoint inhibitor	Trade name®	Target for immunotherapy
Ipilimumab	Yervoy®	CTLA-4
Nivolumab	Opdivo®	PD-1
Pembrolizumab	Keytruda®	PD-1
Cemiplimab	Libtayo®	PD-1
Dostarlimab	Jemperli®	PD-1
Tislelizumab	No data	PD-1
Atezolizumab	Tecentriq®	PD-L1
Avelumab	Bavencio®	PD-L1
Durvalumab	Imfinzi®	PD-L1

CTLA-4, cytotoxic T-lymphocyte antigen 4.
PD-1, programmed cell death 1.
PD-L1, programmed cell death ligand 1.

3 Mechanisms of cutaneous irAEs

The pathophysiological mechanisms of ICI-induced cutaneous irAEs are mainly unknown; however, skin manifestations are thought to occur *via* several immunological mechanisms. The proposed mechanisms include (1) activation of T cells against common antigens in tumor cells and normal tissues, (2) increased release of inflammatory cytokines and antibodies associated with immune-related effects in specific tissues and organs, (3) association with specific human leukocyte antigen (HLA) variants and organ-specific irAEs, and (4) acceleration of concurrent medication-induced drug eruptions.

3.1 Activation of T cells against common antigens in target tumor cells and normal tissues

This mechanism is involved in the cross-reactivity between antigens on ICI-targeting tumor cells and self-antigens in normal tissues. Several studies have shown an association between the appearance of vitiligo, a cutaneous irAE, and the response to treatment with ICIs in patients with melanoma (15–18). Vitiligo is associated with cross-reactivity between melanoma-related antigens and the melanocytes in normal tissues, both of which are possible targets of ICI-induced immune responses (19). In addition, the onset of bullous pemphigoid (BP) may be caused by cross-reactivity between the skin basement membrane and the targeting of BP180 on tumor cells (20). In the analysis of patients with non-small cell lung cancer (NSCLC) treated with anti–PD-1 agents, T cells that recognize both lung tumor tissues and antigens in normal skin simultaneously target both organs. These antigens can stimulate CD4+ and CD8+ T-cells *in vitro*. Furthermore, antigen-specific T cells detected in the peripheral blood were found in the skin lesions and lung tumor tissues of patients treated with an anti-PD-1 agent. Therefore, T-cell clones can interfere with autoimmunity-related skin toxicity in patients with NSCLC treated with anti–PD-1 agent, as well as with tumor regression in patients who respond well to treatment (21).

These reports indicate that the development of cutaneous irAEs is associated with the blockade of common antigens that are co-expressed on both tumor cells and the dermo-epidermal junction and/or other parts of the skin. However, not all tumor tissues have potent neoantigens, and tissue-specific antigens can, in principle, support strong anti-tumor T cell responses with autoimmunity as a toxic skin effect.

3.2 Increased release of proinflammatory cytokines and antibodies *via* activation of T cells and B cells

This mechanism may involve various immune cells, such as T and B cells. The blockade of PD-1 and CTLA-4 enhances Th1 and Th17 cell activity (22, 23). Th17 cells produce IL-17A and IL-22, which encourage neutrophil recruitment and the proliferation of epidermal keratinocytes. Thus, ICIs can promote a secondary increase in pro-inflammatory cytokines *via* Th17 cells, resulting in the exacerbation or induction of psoriasis. At the onset of ICI-induced lichen planus (LP), anti-PD-1 agents increase T cell proliferation and IFN-γ and IL-2 production in patients with oral LP (24). It has been shown that nivolumab treatment upregulated granzyme B and IFN-γ in the responding lesions in patients with metastatic melanoma (25). PD-1 is also expressed on major human B-cell subsets, including naive and memory B cells, and the expression of PD-L1 is induced by TLR9 activation. Blockade of the PD-1/PD-L1 pathway increases B-cell activation, proliferation, and production of disease-specific autoantibodies, such as anti-BP180 antibody which is involved in BP (26). These reports suggest that the increased release of proinflammatory cytokines, such as IL-2, IL-17A, and IL-22, and B cell activation with the production of autoantibodies by ICIs treatment are associated with immune-related damage in specific tissues and organs.

3.3 The association with HLA variants and the specific irAEs

Specific HLA variants can serve as useful markers of autoimmune diseases. Indeed, the frequency of specific HLA is higher in patients with irAEs than in healthy controls (27, 28). Among cutaneous irAEs, 102 patients with metastatic cancer who received ICIs treatment were significantly associated with HLA-DRB1*11:01 and pruritus (OR = 4.53, X_2 1,95 = 9.45, $P < 0.01$) (29). This result indicates that HLA-DRB1*11:01 may be a useful predictive marker for the development of pruritus in patients treated with ICIs, suggesting a genetic etiology for irAEs. However, the specific mechanism underlying HLA-associated irAEs remains unclear. Meanwhile, a large cohort study of 530 patients who received ICIs revealed that irAEs in particular organs and tissues might be associated with certain HLA types (HLA-DRB3*01:01 and thrombocytopenia, HLA-DPB1*04:02 and hypokalemia/hyponatremia, leukopenia and anemia, HLA-A*26:01 and bilirubin elevation); however, HLA heterogeneity has no significant influence on the occurrence of irAEs. In contrast, organ-specific irAEs are strongly involved in multiple HLA variants (27, 28). The molecular mimetic process is tissue-specific, so HLA that presents certain self-peptides may only be associated with some subtypes of irAE. Therefore, HLA types might be useful biomarkers in irAE risk assessments, but it is difficult to identify the association between HLA variants and the occurrence of irAEs. Heterogeneity-inducing mechanisms, such as epitope spread, loss of self-tolerance, and increased inflammatory cytokines, influence the different subtypes of irAEs (30). Further research is required to identify the role of HLA in risk assessment and the occurrence of irAEs.

3.4 Allergic mechanisms

MPR, LP/LP-like eruptions, and SJS/TEN/DIHS/DRESS caused by ICIs are partly considered to involve type IV hypersensitivity

reactions. ICIs can induce cutaneous reactions through type IV hypersensitivity. In contrast, patients treated with ICIs develop cutaneous irAEs from other concomitant medications, which may resemble the clinical presentation of cutaneous irAEs (31). The administration of ICIs may have triggered an immune response to concomitant medications that were previously tolerated, resulting in the induction of cutaneous irAEs. Indeed, in a retrospective study, 80% of patients who developed lichenoid eruptions after treatment with anti-PD-1/PD-L1 agents simultaneously consumed drugs previously reported to induce lichenoid eruptions (32). The detailed mechanisms remain unclear, but it is speculated to be due to the enhancement of inflammatory response *via* the activation of the immune system, including T cells and APCs, and the inhibition of the suppressive function of Tregs by PD-1 blockage (9, 32). Thus, ICIs may accelerate concurrent medication-induced drug eruptions.

4 Epidemiology of cutaneous irAEs

The incidence of cutaneous irAEs ranges from 30 to 60% in patients treated with ICIs (33–37). In contrast, the frequency of cutaneous irAEs differed according to the ICI administered. Anti-CTLA-4 monotherapy has a higher incidence of cutaneous irAEs (44-59%) than anti-PD-1 (34-42%) and anti-PD-L1 monotherapy (up to 20%), whereas combination therapy with anti-PD-1 and anti-CTLA-4 agents has the highest incidence (59–72%) (38, 39). In the severity analysis, cutaneous irAEs were observed in approximately 25% of patients treated with anti-CTLA-4 agents, of which 2.4% were grade 3 and 4 (severe to life-threatening) (40). The incidence of cutaneous irAEs of grades 3 and 4 is much higher during treatment with anti-PD-L1 monotherapy (7.2%) than with anti-PD-1 monotherapy (2.3%) or anti-CTLA-4 monotherapy (4.7%) (41). In combination therapy, anti-PD-L1 and anti-CTLA-4 therapies were associated with the highest incidence (14.5%) compared to anti-PD-1 and anti-CTLA-4 therapies (5.4%) (41). Furthermore, the prevalence of cutaneous irAEs depends on the type of cancer that is treated with ICIs; cutaneous irAEs are more likely to occur in MM than in NSCLC (odds ratio [OR] 1.8, 95% confidence interval [CI] 1.4-2.3) and renal cell carcinoma (RCC) (OR 1.6, 95% CI 1.2-2.1) (42). Different tumor types have different incidences and severities of cutaneous irAEs, even though the same ICIs are used for treatment. The reasons for this observation are not clear, but the TME, immune infiltrate, adaptive immune response, and neoantigen formation may be affected by histology and therefore explain the different skin toxicities (43, 44).

5 Severity of cutaneous irAEs

The American Society of Clinical Oncology has established a grading system for the severity of cutaneous irAEs. This system provides appropriate guidelines for the management of cutaneous irAEs according to the involvement of body surface area (BSA) and additional manifestations. Cutaneous irAEs were classified based on histologic and clinical severity and the percentage of BSA involvement (Table 2) (45).

6 Clinical presentation

6.1 Maculopapular rash

MPR is the most frequent cutaneous irAE and occurs relatively early (39, 46). Rashes reported as irAEs often include MPR. A typical MPR presents as faint erythematous macules and papules that coalesce into plaques. Rashes are generally observed in the trunk and extremities, whereas flexural skin, scalp, palms, and face are rarely involved (Figure 1A). Histopathological features revealed superficial perivascular dermatitis with infiltration of CD4-predominant T cells and eosinophils (47, 48). The incidence rate of MPR differed for each of the ICIs. Anti-CTLA-4 agents are associated with a higher risk of MPR than anti-PD-1/anti-PD-L1 agents. Approximately 49–68% of patients receiving anti-CTLA-4 agents can develop MPR of any grade, compared to 20% of patients receiving anti- PD1/PDL-1 agents (40, 48, 49). Skin rashes are widespread; however, almost all patients present with self-limiting MPR, which can be treated with ICIs. However, MPR sometimes occurs as an early manifestation of severe cutaneous irAEs such as the initial presentation of BP, Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), or drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS). The patient should be carefully followed-up for blister formation, mucositis, epidermal detachment, high fever, or swollen lymph nodes from a few days to a week after the onset of such eruptions. In mild cases, MPR is usually treated with topical corticosteroids, emollients, and oral antihistamine drugs. However,

TABLE 2 Common Terminology Criteria for Adverse Events.

Grade	Representative Conditions
1	Asymptomatic with macules/papules covering majorly <10% of BSA
2	Macules/papules covering 10% to 30% of BSA can be symptomatic as well as asymptomatic.
3	About >30% of BSA is covered. The appearance of macules/papules with or without symptoms.
4	It is the most severe cutaneous response and can be life-threatening, such as SJS, TEN, and bullous dermatitis involving about >30% of BSA. Intensive care should be taken for proper management.

BSA, body surface area.
SJS, Stevens-Johnson Syndrome.
TEN, toxic epidermal necrolysis.

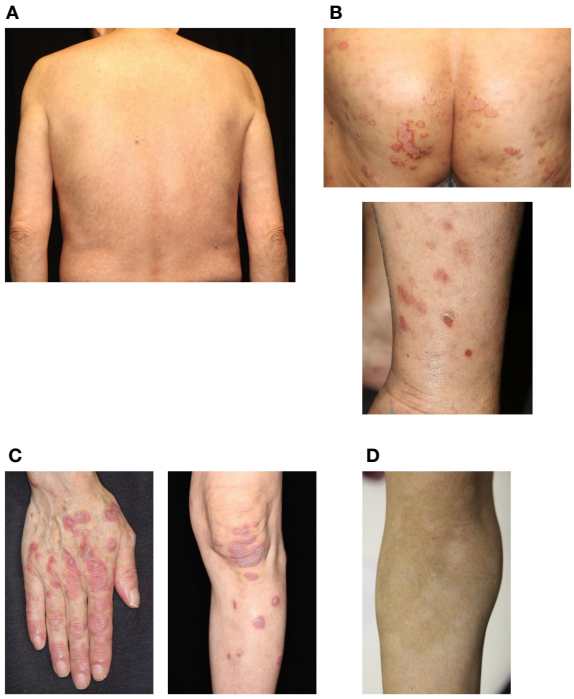

FIGURE 1
Common cutaneous irAEs. **(A)** Maculopapular rash. Erythematous macules and papules on the trunk. **(B)** Psoriasiform eruption. Scaly keratotic erythema plaques on the gluteal region and lower limbs. **(C)** Lichen planus-like eruption. Erythematous patches with scale and hyperkeratosis on the hands and lower limbs. **(D)** Vitiligo. Depigmented macules developing into plaques on the forearm. irAEs, immune-related cutaneous adverse events. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

systemic corticosteroids (0.5-1 mg/kg/day) are administered in approximately 20% of MPR, and it is estimated that some cases are refractory to treatment with symptomatic therapy (50). Systemic corticosteroids should be considered in patients with systemic symptoms such as fever or widespread erythema multiforme.

6.2 Xerosis and eczematous conditions

Xerosis is often observed in patients treated with anti-PD-1 agents. Various types of eczematous dermatitis are often induced by xerosis, particularly during winter. Clinical manifestations often include itchy, poorly demarcated, and erythematous macules and papules that coalesce into plaques and patches on the trunk and extremities, whereas seborrheic dermatitis-like lesions are observed on the face (51). Patients with xerosis are encouraged to moisten their entire body using topical emollients. Eczematous dermatitis is treated with topical corticosteroids, calcineurin inhibitors, and oral antihistamine drugs, depending on the eczematous condition.

6.3 Psoriasis/psoriasiform eruption

Psoriasiform dermatitis induced by ICIs can be divided into two types: new-onset psoriasis (*de novo* psoriasis) and worsening existing psoriasis (reactivated psoriasis) (52). Data from the European Network for Cutaneous Adverse Events to Oncologic Drugs revealed that of the 115 ICI-induced psoriasis cases, 30% had reactivated psoriasis, and 70% had *de novo* psoriasis (52). The patients received either anti-PD-1 (86.1%) or anti-PD-L1 (13.9%) agents (53). The typical manifestation appears as scaly erythematous plaques with well-defined borders on the trunk and extremities, while in some cases, palms/soles are involved, and small-sized rashes present as guttate-type psoriasis (Figure 1B) (54). Histopathological features resemble spontaneous psoriasis, which shows parakeratosis, loss of the granular layer, acanthosis with elongation of the rete ridges, and perivascular lymphocytic infiltration. However, lichenoid features, spongiosis, and infiltration of eosinophils can be observed in ICI-induced psoriasis (55, 56). ICIs augment Th1 and Th17 activities, resulting in the production of IL-17, which plays an important role in the pathogenesis of psoriasis (22, 23). Interestingly, patients with reactivated psoriasis tend to develop cutaneous findings early after initiation of ICI therapy compared with those with *de novo* psoriasis (50 days *vs.* 91 days) (57). Regardless of the type of psoriasis, ICI-induced psoriasis is treated with topical corticosteroids, vitamin D3 analogs, and narrowband ultraviolet B phototherapy (50, 57). If lesions persist despite these treatments, systemic treatments such as methotrexate, apremilast, retinoids, and biologics can be administered (50, 57–59). However, biologics, particularly tumor necrosis factor-α inhibitors, are contraindicated in patients with active malignancy. In contrast, the use of infliximab for other life-threatening irAEs has been reported, and data on its use in ICI-induced psoriasis are purely descriptive. Hence, IL-17 or IL-23 inhibitors may be preferable.

6.4 Lichen planus/lichen planus-like eruption

LP/LP-like eruptions are more often observed in patients treated with anti-PD-1/PD-L1 agents than in those treated with CTLA-4 agents (60, 61). Clinically, LP/LP-like eruptions are observed in 0.5–6% of patients treated with ICIs (48, 62, 63). In a single-institution cohort study, LP/LP-like eruptions affected 17% of patients with metastatic melanoma treated with anti-PD-1 agents (64). Rashes generally show itchy, red to violaceous, flat-topped papules or plaques on the extremities and trunk (Figure 1C) (32, 60, 64). Interestingly, unlike spontaneous LP, ICI-induced LP-like eruptions are rarely observed in the oral mucosa (60). Histopathological features included hyperkeratosis, interface changes with dense band-like superficial infiltration of lymphocytes, and keratinocyte apoptosis in the basal layer of the epidermis. Unlike typical LP, epidermal spongiosis, parakeratosis, eosinophils, and necrosis are observed in ICI-induced LP-like eruptions (60, 61, 64). Furthermore, gene expression profiling and immunohistochemical staining showed upregulation of toll-like receptor (TLR) 2 and TLR4 and increased CD14+ and CD16+ monocytes in patients with lichenoid dermatitis. Thus, the innate immune response may be involved in the onset of LP/LP-like eruptions *via* the activation of CD14/TLR signaling (65). The treatment of LP/LP-like eruptions mainly consists of topical corticosteroids, which allow the continuation of ICIs therapy. In intractable cases, systemic treatment, including oral corticosteroids, retinoids, cyclosporine, and narrowband ultraviolet B phototherapy, has also been reported to be effective in intractable cases (38, 66).

6.5 Vitiligo

Vitiligo is an autoimmune disease characterized by loss of melanocyte function in the epidermis. ICI-induced vitiligo is frequently observed in melanoma patients, whereas other cancers are less commonly reported. Vitiligo affects 2–9% of patients with melanoma treated with anti-CTLA-4 agents and 7–11% of those treated with anti-PD-1 agents or combination therapy (38). Unlike idiopathic vitiligo, the clinical features of ICI-induced vitiligo are characterized by its occurrence in photo-exposed areas, which consist of flecked macules that coalesce into patches without koebnerization (Figure 1D) (67). In the treatment with anti-PD-1 agents, depigmentation is induced by the activation of CD8+ cytotoxic T cells against melanoma-associated antigens (MART-1/MelanA, gp100, and tyrosinase-related proteins 1 and 2) shared by normal melanocytes and melanomas (18, 68). Unfortunately, no definitive treatment for ICI-induced vitiligo has been reported, and most cases do not improve after ICI discontinuation. However, the onset of depigmentation during ICI therapy is significantly associated with favorable results in melanoma (69, 70).

6.6 Bullous diseases

Autoimmune bullous diseases are rare cutaneous irAEs characterized by autoantibodies against basement membrane proteins BP180 and BP230 (71–76). The incidence of autoimmune bullous diseases, including BP, bullous lichenoid dermatitis, and linear IgA bullous dermatosis, is approximately 1% in patients administered anti-PD-1/PD-L1 agents (77). The clinical manifestations of ICI-induced BP include tense bullae and erythematous erosions on the trunk and extremities, while involvement of the mucous membrane is less frequent (Figure 2) (77, 78). The histopathological features of ICI-induced BP are similar to those of spontaneous BP, which shows subepidermal blistering with eosinophilic infiltration and linear deposition of complement component 3 (C3) and immunoglobulin G (IgG) in the basement membrane zone on direct immunofluorescence (73). Blockade of the PD-1/PD-L1 pathway enhances B cell activation, resulting in the production of autoantibodies such as anti-BP180 antibody and inflammatory cytokines. Moreover, basement membrane components are also expressed in cancers and in the production of autoantibodies against different epitopes (cross-reactivity), thus causing ICIs to induce the development of BP (20). Mild cases are generally treated with a combination of high-potency topical corticosteroids and doxycycline with or without niacinamide (79). Severe cases typically require systemic corticosteroids (0.5–1.0 mg/kg/day prednisone) that are slowly tapered over the course of 1–2 months and the addition of rituximab, which is a B cell-depleting anti-CD20 antibody (77, 79, 80). The development of BP is linked to favorable tumor response to anti-PD-1 agents and decreased mortality (81); however, other studies did not support this finding (20).

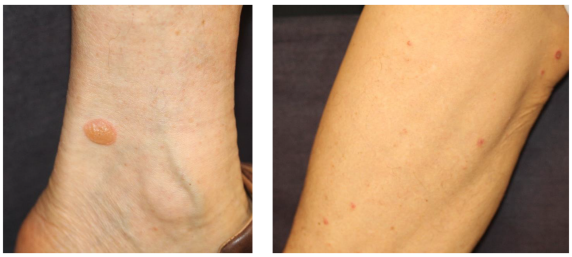

FIGURE 2
Bullous pemphigoid eruption. Multiple small tense bullae on extremities. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

6.7 Alopecia

Alopecia is less frequent but represents a significant proportion of irAEs. The incidence of ICI-induced alopecia is approximately 1–2% (82–84). ICI-induced alopecia shows a phenotype similar to alopecia areata, in which hair follicles are impaired by CD8+ T-cells (85). The clinical manifestations of alopecia vary, ranging from well-circumscribed patches or diffuse hair loss on the scalp to alopecia universalis (Figure 3) (86). Histopathological examination revealed a perifollicular lymphocytic inflammation. The hair follicle dermal sheath expresses PD-L1, and PD-1 blockade directly induces alopecia areata, alopecia totalis, or alopecia universalis *via* CD4+ and CD8+ T cell–mediated immune response (85). Alopecia is usually treated with intralesional and topical corticosteroids as well as systemic immunomodulators (86, 87).

6.8 Pruritus

Pruritus is the second most common cutaneous irAE associated with ICIs. The overall incidence of pruritus is reported to be 13–20% (38, 88). Symptoms are commonly grade 1 or 2 in severity, with high-grade pruritus rarely reported (<2% of patients) (46, 88). In another report, pruritus affected 14–47% of patients, of whom the highest incidence was observed in patients receiving anti-CTLA-4 agents (25–36%) and combination therapy (33–47%) (38). The onset of pruritus is associated with a specific HLA. In an analysis of 102 patients receiving anti-PD-1, anti-CTLA-4, or combination therapy, a significant correlation was found between HLA-DRB1*11:01 and pruritus (29). Pruritus often complicates other skin changes such as erosions, ulcerations, hyperpigmentation, or prurigo nodules, but it can also occur independently of any other skin changes. Pruritus is commonly observed on the trunk and scalp, whereas the face, soles, anterior neck, and genitalia are rarely involved (38, 89). Pruritus is mainly treated with oral antihistamine drugs and emollients with or without topical corticosteroids (35, 90). In grades 3 and 4 severity, severe itchiness affects the quality of life and sometimes requires discontinuation of ICIs (35, 90).

6.9 Scleroderma

ICI-induced scleroderma-like lesions are rare cutaneous irAEs (Figure 4). Terrier et al. summarized 10 cases (5 males and 5 females), which consisted of 6 cases of systemic sclerosis (2 limited and 4 diffuse types) and 4 cases of morphea (2 localized and 2 generalized types) (91). The ICIs administered to these 10 patients included nivolumab in 4 cases and pembrolizumab in 6 cases (91). Interestingly, sclerotic skin changes were observed more rapidly with pembrolizumab than with nivolumab. This result indicates that inhibition of PD-1/PD-L1 may be associated with the onset of scleroderma-like lesions. Indeed, blockade of the PD-1/PD-L1 pathway induces macrophage polarization to the profibrotic M2 type, resulting in excessive production of extracellular matrix proteins *via* fibroblast activation (92). Th17 is also involved in the pathogenesis of systemic sclerosis, and blockage of PD-1 leads to a shift in the immune balance toward a Th1/Th17 response (9). Regarding the treatment of scleroderma-like lesions, six patients with generalized skin lesions were treated with high-dose corticosteroids, and almost all showed improvement in skin thickness (92).

6.10 SJS/TEN/DIHS/DRESS

Severe cutaneous drug eruptions, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), are rare with ICIs treatment, but life-threatening cutaneous adverse drug reactions are characterized by high fever, widespread detachment of the epidermis, and erosions, and mucositis. However, the incidence of ICI-induced SJS/TEN remains unknown. To date, 20 cases of SJS/TEN have been reported, including 12, six, and two cases associated with the use of pembrolizumab, nivolumab, and atezolizumab, respectively (37). The clinical and histopathological features of ICI-induced SJS/TEN were similar to those of SJS/TEN caused by other drugs (Figure 5). It has been speculated that PD-L1 is typically undetectable in epidermal keratinocytes, but ICI treatment increases PD-L1 expression, which induces apoptosis of PDL-1 expressing keratinocytes by activated cytotoxic CD8+ T cells

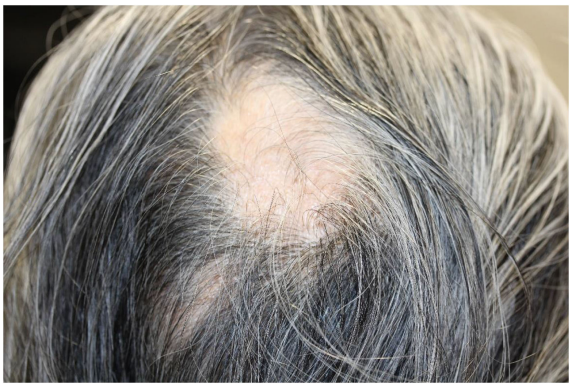

FIGURE 3
Alopecia areata. Circumscribed patches of hair loss on the parietal region. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

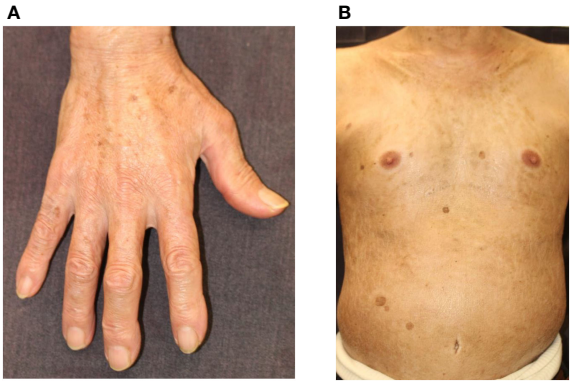

FIGURE 4
Scleroderma. **(A)** Skin thickening and hardening causing the stiffness of the fingers and hands. **(B)** Skin thickening and hardening with pigmentation on the trunk. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

(93). In addition, ICI-induced skin damage shows a similar gene expression profile as SJS/TEN from other causative drugs, with increased expression of inflammatory chemokines, cytotoxic mediators (perforin and granzyme B), and apoptosis-promoting molecules (Fas Ligand) (94, 95). Furthermore, the dysfunction of Tregs and enhancement of co-stimulatory factors are associated with SJS/TEN pathogenesis. Degranulation of CTL and NK cells, which induces apoptosis of keratinocytes, has been implicated in the association between CD49/NKG2C and HLA-E. Additional implicated factors include Fas/FasL, PD-L1-expressing T cells and epidermal cells, and CD40/CD40L interactions at the dermal-epidermal junction (96, 97). In the management of SJS/TEN, it should be discontinued. Treatment with high-dose corticosteroids (0.5-1.0 mg/kg/day prednisone) is recommended because the mechanism of adverse events involves T-cell immunodirected toxicity. In addition to systemic corticosteroids, adjuvant therapies based on a combination of immunosuppressive agents, such as oral cyclosporine, intravenous immunoglobulin (IVIG), and/or plasmapheresis therapy, have been proposed (98). However, the effect of immunosuppressive agents on cancer is unknown; therefore, the decision to use immunosuppressive agents should be made in consultation with the oncologist in charge. The mortality rates of SJS and TEN are 10% and 50%, respectively (94).

Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) can also be induced by ICIs but are rare cutaneous irAEs. In the three published cases of ICI-induced DIHS/DRESS, the causative ICIs consisted of one case of use of nivolumab and one case of use of ipilimumab (99–101). Patients developed renal and hepatic involvement, although steroid-responsive multiorgan dysfunction could have occurred due to either DIHS/DRESS or direct ICIs toxicity. For the management of DIHS/DRESS, discontinuation of ICIs and administration of systemic corticosteroids (1.0 mg/kg/day prednisone) a required. In contrast to SJS/TEN, systemic corticosteroids should be tapered slowly over at least 6–8 weeks to reduce the risk of recurrence.

7 Time to onset of cutaneous irAEs

The time from the initiation of ICIs treatment to the onset of irAEs is generally weeks to months. Among the various irAEs,

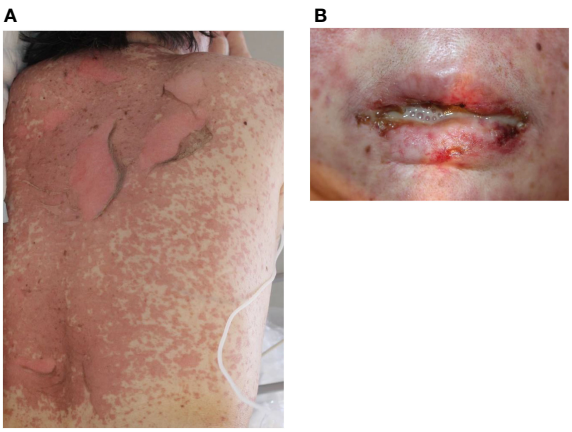

FIGURE 5
Toxic epidermal necrolysis. **(A)** Erythematous macules, bullae, and erosions on the trunk. **(B)** Hemorrhagic erosion of the lip. Written informed consent was obtained from the individual(s) for the publication of any identifiable images or data included in this article.

cutaneous irAEs are the earliest complications to develop (47, 102). However, the duration from the initiation of ICIs to the onset of cutaneous irAEs differs depending on the type of skin rash. Figure 6 summarizes the time to the onset of each cutaneous irAE. MPR occurs within the first 3–6 weeks of ICIs treatment initiation (40, 48, 49). Similar to MPR, psoriasiform rash appears three weeks after the initiation of ICIs treatment (54, 57). In contrast, other studies have shown that the time from ICIs treatment initiation to psoriasis development ranges from 5– to 12 weeks (53, 103). These differences may be due to *de novo* or reactivated psoriasis (57). The onset of LP/LP-like eruptions occurs later than that of psoriasiform rash and MPR, with an onset ranging from 6 to 12 weeks (34, 48). In another report, LP/LP-like eruptions occurred anywhere from 3 to 52 weeks after the initiation of ICIs treatment (32). The occurrence of vitiligo is characterized by late onset, with the time to development varying from seven weeks to several months (median onset time of approximately 26 weeks) after ICIs initiation (46, 104, 105). The onset of alopecia is also delayed, occurring within 12–24 weeks of the initiation of ICIs treatment (83). The time to the onset of BP eruptions also varied according to previous reports. Previously, BP eruptions had been reported to occur at 13-15 weeks (48), but more recent studies have reported that they can occur anywhere between 3– and 80 weeks after ICI therapy initiation (71, 72, 78). Pruritus can develop 1– to 27 weeks after initiating ICIs (106). Since the onset of ICI-induced scleroderma is very rare, the detailed time from ICI initiation to onset is unknown. However, in 10 published cases, the time from ICIs therapy initiation to scleroderma onset ranged from 6– to 48 weeks (91, 107–110). The onset of severe cutaneous adverse reactions, including SJS, TEN, and DIHS/DRESS, varies from one – to 20 weeks (94). The average onset time is reported to be 8.9 weeks for SJS and 5.4 weeks for TEN; however, they can occur within a week of the first administration of ICIs (111). Interestingly, different types of skin eruptions can develop in the same individual at different times, depending on the type of lesion.

8 Association with cutaneous irAEs and prognosis

Patients who develop irAEs are generally considered to have a high anti-tumor response. In patients with malignant melanoma treated with nivolumab, overall survival was improved in patients who developed irAEs and in those with a higher number of irAEs (16). Interestingly, in a large cohort analysis of the association between cutaneous irAEs and survival rates within six months of anti-PD-1/PD-L1 therapy, the incidence of cutaneous irAEs correlated with decreased mortality (112). Furthermore, cutaneous irAEs often precede irAEs in other organs and are expected to be biomarkers for the development of secondary irAEs and overall survival. Thompson et al. examined the clinical types of cutaneous irAEs and other organ irAEs and found an association between mucosal lesions and overall irAEs as well as psoriasis-like skin rash and endocrine organ irAEs (113). In a retrospective study, lichenoid and spongiotic dermatitis were identified as biomarkers of favorable tumor response in patients receiving anti-PD- 1/PD-L1 therapy (114). Another study focused on the histopathology of cutaneous irAEs and their prognosis. Hirotsu et al. showed associations between vacuolar lesions and pneumonia, psoriasis-like histology and musculoskeletal and multiple other organ irAEs, and bullous histology and ipilimumab–nivolumab combination therapy (114). Spongiform changes and lichenoid reactions are associated with progression-free survival and decreased mortality, whereas vacuolar degeneration and superficial perivascular dermatitis increase the risk of mortality (115).

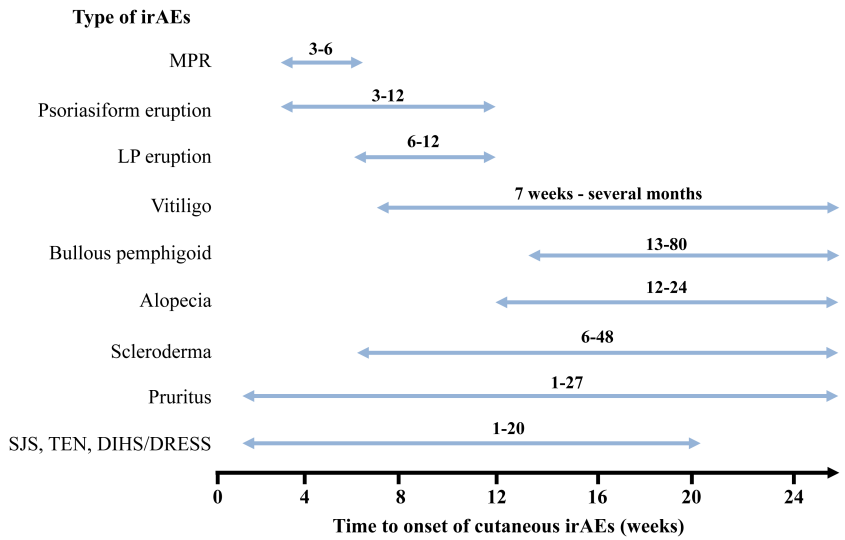

FIGURE 6
Time to onset of cutaneous immune-related cutaneous adverse events. MPR, Maculopapular rash; SJS, Stevens-Johnson Syndrome; TEN, toxic epidermal necrolysis; DIHS, Drug-induced hypersensitivity syndrome; DRESS, Drug reaction with eosinophilia and systemic symptoms; irAEs, immune-related cutaneous adverse events.

9 Conclusions

ICIs have been approved for many advanced malignancies and will be developed and used for more malignancies in the future. Although cutaneous irAEs are the most common adverse events, most cases are mild (grades 1 and 2), allowing continuous treatment with ICIs. In contrast, rare and severe cutaneous irAEs such as SJS/TEN and DIHS/DRESS should be carefully considered for discontinuation of ICIs and treatment. To achieve the most favorable outcomes for patients with cancer, early and accurate diagnosis of irAEs is essential to implement management steps, including discontinuation of ICIs and/or addition of immunosuppressive agents such as systemic corticosteroids. Furthermore, as recent studies have shown, a detailed diagnosis of cutaneous irAEs may provide useful information regarding patient prognosis and biomarkers for predicting subsequent irAEs and their risk factors. Therefore, dermatologists should be aware of many types of cutaneous features, whether they are common or rare, treatment strategies for cutaneous irAEs, and their mechanisms of action.

Author contributions

YY contributed to determining the content of each section and edited the manuscript. TW collected data and wrote each section of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol* (2015) 33:1974–82. doi: 10.1200/JCO.2014.59.4358

2. Carlino MS, Larkin J, Long GV. Immune checkpoint inhibitors I melanoma. *Lancet* (2021) 398:1002–14. doi: 10.1016/S0140-6736(21)01206-X

3. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK, et al. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. *Front Pharmacol* (2017) 8:561. doi: 10.3389/fphar.2017.00561

4. Arnaud-Coffin P, Maillet D, Gan HK, Stelmes JJ, You B, Dalle S, et al. A systematic review of adverse events in randomized trials assessing immune checkpoint inhibitors. *Int J Cancer* (2019) 145:639–48. doi: 10.1002/ijc.32132

5. Gu L, Khadaroo PA, Su H, Kong L, Chen L, Wang X, et al. The safety and tolerability of combined immune checkpoint inhibitors (anti-PD-1/PD-L1 plus anti-CTLA-4): a systematic review and meta-analysis. *BMC Cancer* (2019) 19:559. doi: 10.1186/s12885-019-5785-z

6. Jin KT, Wang SB, Ying XJ, Lan HR, Lv JQ, Zhang LH, et al. Immune-mediated adverse effects of immune checkpoint inhibitors and their management in cancer. *Immunol Lett* (2020) 221:61–71. doi: 10.1016/j.imlet.2020.02.008

7. Okazaki T, Honjo T. PD-1 and PD-1 ligands: From discovery to clinical application. *Int Immunol* (2007) 19:813–24. doi: 10.1093/intimm/dxm057

8. Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. *Cell Immunol* (2014) 290:72–9. doi: 10.1016/j.cellimm.2014.05.006

9. Tanaka R, Ichimura Y, Kubota N, Saito A, Nakamura Y, Ishitsuka Y, et al. Differential involvement of programmed cell death ligands in skin immune responses. *J Invest Dermatol* (2022) 142:145–54.e8. doi: 10.1016/j.jid.2021.06.026

10. Takehara T, Wakamatsu E, Machiyama H, Nishi W, Emoto K, Azuma M, et al. PD-L2 suppresses T cell signaling *via* coinhibitory microcluster formation and SHP2 phosphatase recruitment. *Commun Biol* (2021) 4(1):581. doi: 10.1038/s42003-021-02111-3

11. Alegre ML, Frauwirth KA, Thompson CB. T-Cell regulation by CD28 and CTLA-4. *Nat Rev Immunol* (2001) 1:220–8. doi: 10.1038/35105024

12. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyteassociated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U.S.A.* (2003) 100:8372.e7. doi: 10.1073/pnas.1533209100

13. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, et al. Immunologic self-tolerance maintained by CD25(þ)CD4(þ) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* (2000) 192:303.e10. doi: 10.1084/jem.192.2.303

14. Zhang P, Xiong X, Rolfo C, Du X, Zhang Y, Yang H, et al. Mechanism- and immune landscape-based ranking of therapeutic responsiveness of 22 major human cancers to next generation anti-CTLA-4 antibodies. *Cancers (Basel)* (2020) 12:284. doi: 10.3390/cancers12020284

15. Lo JA, Fisher DE, Flaherty KT. Prognostic significance of cutaneous adverse events associated with pembrolizumab therapy. *JAMA Oncol* (2015) 1:1340–1. doi: 10.1001/jamaoncol.2015.2274

16. Freeman-Keller M, Kim Y, Cronin H, Richards A, Gibney G, Weber JS. Nivolumab in resected and unresectable metastatic melanoma: Characteristics of immune-related adverse events and association with outcomes. *Clin Cancer Res* (2016) 22:886–94. doi: 10.1158/1078-0432.CCR-15-1136

17. Teulings HE, Limpens J, Jansen SN, Zwinderman AH, Reitsma JB, Spuls PI, et al. Vitiligo-like depigmentation in patients with stage III–IV melanoma receiving immunotherapy and its association with survival: A systematic review and meta-analysis. *J Clin Oncol* (2015) 33:773–81. doi: 10.1200/JCO.2014.57.4756

18. Hua C, Boussemart L, Mateus C, Routier E, Boutros C, Cazenave H, et al. Association of vitiligo with tumor response in patients with metastatic melanoma treated with pembrolizumab. *JAMA Dermatol* (2016) 152:45–51. doi: 10.1001/jamadermatol.2015.2707

19. Huang SK, Okamoto T, Morton DL, Hoon DS. Antibody responses to melanoma/melanocyte autoantigens in melanoma patients. *J Invest Dermatol* (1998) 111:662–7. doi: 10.1046/j.1523-1747.1998.00354.x

20. Tsiogka A, Bauer JW, Patsatsi A. Bullous pemphigoid associated with anti-programmed cell death protein 1 and anti-programmed cell death ligand 1 therapy: A review of the literature. *Acta Derm Venereol* (2021) 101:adv00377. doi: 10.2340/00015555-3740

21. Berner F, Bomze D, Diem S, Ali OH, Fässler M, Ring S, et al. Association of checkpoint inhibitor-induced toxic effects with shared cancer and tissue antigens in non-small cell lung cancer. *JAMA Oncol* (2019) 5:1043–7. doi: 10.1001/jamaoncol.2019.0402

22. Dulos J, Carven GJ, van Boxtel SJ, Evers S, Driessen-Engels LJ, Hobo W, et al. PD-1 blockade augments Th1 and Th17 and suppresses Th2 responses in peripheral blood from patients with prostate and advanced melanoma cancer. *J Immunother* (2012) 35:169–78. doi: 10.1097/CJI.0b013e318247a4e7

23. Sarnaik AA, Yu B, Yu D, Morelli D, Hall M, Bogle D, et al. Extended dose ipilimumab with a peptide vaccine: Immune correlates associated with clinical benefit in patients with resected high-risk stage IIIc/IV melanoma. *Clin Cancer Res* (2011) 17:896–906. doi: 10.1158/1078-0432.CCR-10-2463

24. Zhou G, Zhang J, Ren XW, Hu JY, Du GF, Xu XY. Increased B7-H1 expression on peripheral blood T cells in oral lichen planus correlated with disease severity. *J Clin Immunol* (2012) 32:794–801. doi: 10.1007/s10875-012-9683-2

25. Anegawa H, Otsuka A, Kaku Y, Nonomura Y, Fujisawa A, Endo Y, et al. Upregulation of granzyme b and interferon-γ mRNA in responding lesions by treatment with nivolumab for metastatic melanoma: A case report. *J Eur Acad Dermatol Venereol* (2016) 30:e231–2. doi: 10.1111/jdv.13567

26. Thibult ML, Mamessier E, Gertner-Dardenne J, Pastor S, Just-Landi S, Xerri L, et al. PD-1 is a novel regulator of human b-cell activation. *Int Immunol* (2013) 25:129–37. doi: 10.1093/intimm/dxs098

27. Inaba H, Ariyasu H, Iwakura H, Ueda Y, Kurimoto C, Uraki S, et al. Comparative analysis of human leucocyte antigen between idiopathic and anti-PD-1 antibody induced isolated adrenocorticotropic hormone deficiency: A pilot study. *Clin Endocrinol (Oxf)* (2019) 91:786–92. doi: 10.1111/cen.14082

28. Kobayashi T, Iwama S, Sugiyama D, Yasuda Y, Okuji T, Ito M, et al. Anti-pituitary antibodies and susceptible human leukocyte antigen alleles as predictive biomarkers for pituitary dysfunction induced by immune checkpoint inhibitors. *J Immunother Cancer* (2021) 9:e002493. doi: 10.1136/jitc-2021-002493

29. Hasan Ali O, Berner F, Bomze D, Fässler M, Diem S, Cozzio A, et al. Human leukocyte antigen variation is associated with adverse events of checkpoint inhibitors. *Eur J Cancer* (2019) 107:8–14. doi: 10.1016/j.ejca.2018.11.009

30. Jiang N, Yu Y, Zhang M, Tang Y, Wu D, Wang S, et al. Association between germ-line HLA and immune-related adverse events. *Front Immunol* (2022) 13:952099. doi: 10.3389/fimmu.2022.952099

31. Okiyama N, Tanaka R. Immune-related adverse events in various organs caused by immune checkpoint inhibitors. *Allergol Int* (2022) 71:169–78. doi: 10.1016/j.alit.2022.01.001

32. Shi VJ, Rodic N, Gettinger S, Leventhal JS, Neckman JP, Girardi M, et al. Clinical and histologic features of lichenoid mucocutaneous eruptions due to anti-programmed cell death 1 and anti-programmed cell death ligand 1 immunotherapy. *JAMA Dermatol* (2016) 152:1128–36. doi: 10.1001/jamadermatol.2016.2226

33. Ellis SR, Vierra AT, Millsop JW, Lacouture ME, Kiuru M. Dermatologic toxicities to immune checkpoint inhibitor therapy: A review of histopathologic features. *J Am Acad Dermatol* (2020) 83:1130–43. doi: 10.1016/j.jaad.2020.04.105

34. Geisler AN, Phillips GS, Barrios DM, Wu J, Leung DYM, Moy AP, et al. Immune checkpoint inhibitor-related dermatologic adverse events. *J Am Acad Dermatol* (2020) 83:1255–68. doi: 10.1016/j.jaad.2020.03.132

35. Quach HT, Johnson DB, LeBoeuf NR, Zwerner JP, Dewan AK. Cutaneous adverse events caused by immune checkpoint inhibitors. *J Am Acad Dermatol* (2021) 85:956–66. doi: 10.1016/j.jaad.2020.09.054

36. Malviya N, Tattersall IW, Leventhal J, Alloo A. Cutaneous immune-related adverse events to checkpoint inhibitors. *Clin Dermatol* (2020) 38:660–78. doi: 10.1016/j.clindermatol.2020.06.011

37. Bhardwaj M, Chiu MN, Sah SP. Adverse cutaneous toxicities by PD-1/PD-L1 immune checkpoint inhibitors: Pathogenesis, treatment, and surveillance. *Cutan Ocul Toxicol* (2022) 20:1–18. doi: 10.1080/15569527.2022.2034842

38. Sibaud V. Dermatologic reactions to immune checkpoint inhibitors: Skin toxicities and immunotherapy. *Am J Clin Dermatol* (2018) 19:345–61. doi: 10.1007/s40257-017-0336-3

39. Collins LK, Chapman MS, Carter JB, Samie FH. Cutaneous adverse effects of the immune checkpoint inhibitors. *Curr Probl Cancer* (2017) 41:125–8. doi: 10.1016/j.currproblcancer.2016.12.001

40. Minkis K, Garden BC, Shenhong W, Pulitzer MP, Lacouture ME. The risk of rash associated with ipilimumab in patients with cancer: A systemic review of the literature and meta-analysis. *J Am Acad Dermatol* (2013) 69:e121–8. doi: 10.1016/j.jaad.2012.12.963

41. El Osta B, Hu F, Sadek R, Chintalapally R, Tang SC. Not all immune-checkpoint inhibitors are created equal: Meta-analysis and systematic review of immune-related adverse events in cancer trials. *Crit Rev Oncol Hematol* (2017) 119:1–12. doi: 10.1016/j.critrevonc.2017.09.002

42. Khoja L, Day D, Wei-Wu Chen T, Siu LL, Hansen AR. Tumour- and class-specific patterns of immune-related adverse events of immune checkpoint inhibitors: A systematic review. *Ann Oncol* (2017) 28:2377–85. doi: 10.1093/annonc/mdx286

43. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* (2014) 515:568–71. doi: 10.1038/nature13954

44. Khoja L, Kibiro M, Metser U, Gedye C, Hogg D, Butler MO, et al. Patterns of response to anti-PD-1 treatment: An exploratory comparison of four radiological response criteria and associations with overall survival in metastatic melanoma patients. *Br J Cancer* (2016) 115:1186–92. doi: 10.1038/bjc.2016.308

45. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American society of clinical oncology clinical practice guideline. *J Clin Oncol* (2018) 36:1714–68. doi: 10.1200/JCO.2017.77.6385

46. Sibaud V, Meyer N, Lamant L, Vigarios E, Mazieres J, Delord JP. Dermatologic complications of anti-PD-1/PD-L1 immune checkpoint antibodies. *Curr Opin Oncol* (2016) 28:254–63. doi: 10.1097/CCO.0000000000000290

47. Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol* (2012) 30:2691–7. doi: 10.1200/JCO.2012.41.6750

48. Curry JL, Tetzlaff MT, Nagarajan P, Drucker C, Diab A, Hymes SR, et al. Diverse types of dermatologic toxicities from immune checkpoint blockade therapy. *J Cutan Pathol* (2017) 44:158–76. doi: 10.1111/cup.12858

49. Bottlaender L, Amini-Adle M, Maucort-Boulch D, Robinson P, Thomas L, Dalle S. Cutaneous adverse events: A predictor of tumour response under anti-PD-1 therapy for metastatic melanoma, a cohort analysis of 189 patients. *J Eur Acad Dermatol Venereol* (2020) 34:2096–105. doi: 10.1111/jdv.16311

50. Coleman E, Ko C, Dai F, Tomayko MM, Kluger H, Leventhal JS. Inflammatory eruptions associated with immune checkpoint inhibitor therapy: A single-institution retrospective analysis with stratification of reactions by toxicity and implications for management. *J Am Acad Dermatol* (2019) 80:990–7. doi: 10.1016/j.jaad.2018.10.062

51. Yamamoto T. Skin manifestation induced by immune checkpoint inhibitors. *Clin Cosmet Investig Dermatol* (2022) 15:829–41. doi: 10.2147/CCID.S364243

52. Matsumura N, Ohtsuka M, Kikuchi N, Yamamoto T. Exacerbation of psoriasis during nivolumab therapy for metastatic melanoma. *Acta Derm Venereol* (2016) 96:259–60. doi: 10.2340/00015555-2212

53. Nikolaou V, Sibaud V, Fattore D, Sollena P, Ortiz-Brugués A, Giacchero D, et al. Im- mune checkpoint-mediated psoriasis: A multicenter European study of 115 patients from the European network for cutaneous adverse event to oncologic drugs (ENCADO) group. *J Am Acad Dermatol* (2021) 84:1310–20. doi: 10.1016/j.jaad.2020.08.137

54. Ohtsuka M, Miura T, Mori T, Ishikawa M, Yamamoto T. Occurrence of psoriasiform eruption during nivolumab therapy for a patient with mucosal melanoma. *JAMA Dermatol* (2015) 151:797–9. doi: 10.1001/jamadermatol.2015.0249

55. Ruiz-Bañobre J, Abdulkader I, Anido U, Leon L, Lopez-Lopez R, Garcıa-Gonzalez J. Development of *de novo* psoriasis during nivolumab therapy for metastatic renal cell carcinoma: immunohistochemical analyses and clinical outcome. *APMIS* (2017) 125:259–63. doi: 10.1111/apm.12658

56. Balak DM, Hajdarbegovic E. Drug-induced psoriasis: clinical perspectives. *Psoriasis (Auckl)* (2017) 7:87–94. doi: 10.2147/PTT.S126727

57. Bonigen J, Raynaud-Donzel C, Hureaux J, Kramkimel N, Blom A, Jeudy G, et al. Anti-PD1-induced psoriasis: A study of 21 patients. *J Eur Acad Dermatol Venereol* (2017) 31:e254–7. doi: 10.1111/jdv.14011

58. Lidar M, Giat E, Garelick D, Horowitz Y, Amital H, Steinberg-Silman Y, et al. Rheumatic manifestations among cancer patients treated with immune checkpoint inhibitors. *Autoimmun Rev* (2018) 17:284–9. doi: 10.1016/j.autrev.2018.01.003

59. Fattore D, Annunziata MC, Panariello L, Marasca C, Fabbrocini G. Successful treatment of psoriasis induced by immune checkpoint inhibitors with apremilast. *Eur J Cancer* (2019) 110:107–9. doi: 10.1016/j.ejca.2019.01.010

60. Tetzlaff MT, Nagarajan P, Chon S, Huen A, Diab A, Omar P, et al. Lichenoid dermatologic toxicity from immune checkpoint blockade therapy: A detailed examination of the clinicopathologic features. *Am J Dermatopathol* (2017) 39:121–9. doi: 10.1097/DAD.0000000000000688

61. Schaberg KB, Novoa RA, Wakelee HA, Kim J, Cheung C, Srinivas S, et al. Immunohistochemical analysis of lichenoid reactions in patients treated with anti-PD-L1 and anti-PD-1 therapy. *J Cutan Pathol* (2016) 43:339–46. doi: 10.1111/cup.12666

62. Phillips GS, Wu J, Hellmann MD, Postow MA, Rizvi NA, Freites-Martinez A, et al. Treatment outcomes of immune-related cutaneous adverse events. *J Clin Oncol* (2019) 37:2746–58. doi: 10.1200/JCO.18.02141

63. Kaunitz GJ, Loss M, Rizvi H, Ravi S, Cuda JD, Bleich KB, et al. Cutaneous eruptions in patients receiving immune checkpoint blockade: clinicopathologic analysis of the nonlichenoid histologic pattern. *Am J Surg Pathol* (2017) 41:1381–9. doi: 10.1097/PAS.0000000000000900

64. Hwang SJE, Carlos G, Wakade D, Byth K, Kong BY, Chou S, et al. Cutaneous adverse events (AEs) of anti-programmed cell death (PD)-1 therapy in patients with metastatic melanoma: a single-institution cohort. *J Am Acad Dermatol* (2016) 74:455–61.e1. doi: 10.1016/j.jaad.2015.10.029

65. Curry JL, Reuben A, Szczepaniak-Sloane R, Ning J, Milton DR, Lee CH, et al. Gene expression profiling of lichenoid dermatitis immune-related adverse event from immune checkpoint inhibitors reveals increased CD14+ and CD16+ monocytes driving an innate immune response. *J Cutan Pathol* (2019) 49: 627–36. doi: 10.1111/cup.13454

66. Fixsen E, Patel J, Selim MA, Kheterpal M. Resolution of pembrolizumab-associated steroid-refractory lichenoid dermatitis with cyclosporine. *Oncologist* (2019) 24:e103–5. doi: 10.1634/theoncologist.2018-0531

67. Larsabal M, Marti A, Jacquemin C, Rambert J, Thiolat D, Dousset L, et al. Vitiligo-like lesions occurring in patients receiving anti-programmed cell death-1

therapies are clinically and biologically distinct from vitiligo. *J Am Acad Dermatol* (2017) 76:863–70. doi: 10.1016/j.jaad.2016.10.044

68. Quaglino P, Marenco F, Osella-Abate S, Cappello N, Ortoncelli M, Salomone B, et al. Vitiligo is an independent favourable prognostic factor in stage III and IV. *Ann Oncol* (2010) 21:409–14. doi: 10.1093/annonc/mdp325

69. Lommerts JE, Bekkenk MW, Luiten RM. Vitiligo induced by immune checkpoint inhibitors in melanoma patients: an expert opinion. *Expert Opin Drug Saf* (2021) 20:883–8. doi: 10.1080/14740338.2021.1915279

70. Quach HT, Dewan AK, Davis EJ, Ancell KK, Fan R, Ye F, et al. Association of antiprogrammed cell death 1 cutaneous toxic effects with outcomes in patients with advanced melanoma. *JAMA Oncol* (2019) 5:906–8. doi: 10.1001/jamaoncol.2019.0046

71. Hanley T, Papa S, Saha M. Bullous pemphigoid associated with ipilimumab therapy for advanced metastatic melanoma. *JRSM Open* (2018) 9:2054270418793029. doi: 10.1177/2054270418793029

72. Kuwatsuka Y, Iwanaga A, Kuwatsuka S, Okubo Y, Murayama N, Ishii N, et al. Bullous pemphigoid induced by ipilimumab in a patient with metastatic malignant melanoma after unsuccessful treatment with nivolumab. *J Dermatol* (2018) 45:e21–2. doi: 10.1111/1346-8138.14043

73. Naidoo J, Schindler K, Querfeld C, Busam K, Cunningham J, Page DB, et al. Autoimmune bullous skin disorders with immune checkpoint inhibitors targeting PD-1 and PD-L1. *Cancer Immunol Res* (2016) 4:383–9. doi: 10.1158/2326-6066.CIR-15-0123

74. Biolo G, Caroppo F, Salmaso R, Alaibac M. Linear bullous lichen planus associated with nivolumab. *Clin Exp Dermatol* (2019) 44:67–8. doi: 10.1111/ced.13700

75. Zumelzu C, Alexandre M, Le Roux C, Weber P, Guyot A, Levy A, et al. Mucous membrane pemphigoid, bullous pemphigoid, and anti-programmed death-1/programmed death-ligand 1: A case report of an elderly woman with mucous membrane pemphigoid developing after pembrolizumab therapy for metastatic melanoma and review of the literature. *Front Med (Lausanne)* (2018) 5:268. doi: 10.3389/fmed.2018.00268

76. Haug V, Behle V, Benoit S, Kneitz H, Schilling B, Goebeler M, et al. Pembrolizumab-associated mucous membrane pemphigoid in a patient with merkel cell carcinoma. *Br J Dermatol* (2018) 179:993–4. doi: 10.1111/bjd.16780

77. Siegel J, Totonchy M, Damsky W, Berk-Krauss J, Castiglione FJr., Sznol M, et al. Bullous disorders associated with anti-PD-1 and anti-PD-L1 therapy: A retrospective analysis evaluating the clinical and histopathologic features, frequency, and impact on cancer therapy. *J Am Acad Dermatol* (2018) 79:1081–8. doi: 10.1016/j.jaad.2018.07.008

78. Fässler M, Rammlmair A, Feldmeyer L, Suter VGA, Gloor AD, Horn M, et al. Mucous membrane pemphigoid and lichenoid reactions after immune checkpoint inhibitors: common pathomechanisms. *J Eur Acad Dermatol Venereol* (2020) 34:e112–5. doi: 10.1111/jdv.16036

79. Damsky W, Kole L, Tomayko MM. Development of bullous pemphigoid during nivolumab therapy. *JAAD Case Rep* (2016) 2:442–4. doi: 10.1016/j.jdcr.2016.05.009

80. Ridpath AV, Rzepka PV, Shearer SM, Scrape SR, Olencki TE, Kaffenberger BH. Novel use of combination therapeutic plasma exchange and rituximab in the treatment of nivolumab-induced bullous pemphigoid. *Int J Dermatol* (2018) 57:1372–4. doi: 10.1111/ijd.13970

81. Nelson CA, Singer S, Chen T, Puleo AE, Lian CG, Wei EX, et al. Bullous pemphigoid after anti-PD-1 therapy: A retrospective case-control study evaluating impact on tumor response and survival outcomes. *J Am Acad Dermatol* (2022) 87:1400–2. doi: 10.1016/j.jaad.2019.12.068

82. Jaber SH, Cowen EW, Haworth LR, Booher SL, Berman DM, Rosenberg SA, et al. Skin reactions in a subset of patients with stage IV melanoma treated with anti-cytotoxic T-lymphocyte antigen 4 monoclonal antibody as a single agent. *Arch Dermatol* (2006) 142:166–72. doi: 10.1001/archderm.142.2.166

83. Zarbo A, Belum VR, Sibaud V, Oudard S, Postow MA, Hsieh JJ, et al. Immune-related alopecia (areata and universalis) in cancer patients receiving immune checkpoint inhibitors. *Br J Dermatol* (2017) 176:1649–52. doi: 10.1111/bjd.15237

84. Guidry J, Brown M, Medina T. PD-1 inhibitor induced alopecia areata. *Dermatol Online J* (2018) 24:130/30qt2vj8b7cv. doi: 10.5070/D32412042449

85. Lacouture M, Sibaud V. Toxic side effects of targeted therapies and immunotherapies affecting the skin, oral mucosa, hair, and nails. *Am J Clin Dermatol* (2018) 19(Suppl 1):31–9. doi: 10.1007/s40257-018-0384-3

86. Antoury L, Maloney NJ, Bach DQ, Goh C, Cheng K. Alopecia areata as an immune-related adverse event of immune checkpoint inhibitors: A review. *Dermatol Ther* (2020) 33:e14171. doi: 10.1111/dth.14171

87. Strazzulla LC, Wang EHC, Avila L, Lo Sicco K, Brinster N, Christiano AM, et al. Alopecia areata: an appraisal of new treatment approaches and overview of current therapies. *J Am Acad Dermatol* (2018) 78:15–24. doi: 10.1016/j.jaad.2017.04.1142

88. Apalla Z, Papageorgiou C, Lallas A, Delli F, Fotiadou C, Kemanetzi C, et al. Cutaneous adverse events of immune checkpoint inhibitors: A literature review. *Dermatol Pract Concept* (2021) 11:e2021155. doi: 10.5826/dpc.1101a155

89. Phillips GS, Freites-Martinez A, Wu J, Chan D, Fabbrocini G, Hellmann MD, et al. Clinical characterization of immunotherapy-related pruritus among patients seen in 2 oncodermatology clinics. *JAMA Dermatol* (2019) 155:249–51. doi: 10.1001/jamadermatol.2018.4560

90. Wu J, Lacouture ME. Pruritus associated with targeted anticancer therapies and their management. *Dermatol Clin* (2018) 36:315–24. doi: 10.1016/j.det.2018.02.010

91. Terrier B, Humbert S, Preta LH, Delage L, Razanamahery J, Laurent-Roussel S, et al. Risk of scleroderma according to the type of immune checkpoint inhibitors. *Autoimmun Rev* (2020) 19:102596. doi: 10.1016/j.autrev.2020.102596

92. Kurasawa K, Hirose K, Sano H, Endo H, Shinkai H, Nawata Y, et al. Increased interleukin-17 production in patients with systemic sclerosis. *Arthritis Rheum* (2000) 43:2455–63. doi: 10.1002/1529-0131(200011)43:11<2455::AID-ANR12>3.0.CO;2-K

93. Vivar KL, Deschaine M, Messina J, Divine JM, Rabionet A, Patel N, et al. Epidermal programmed cell death-ligand 1 expression in TEN associated with nivolumab therapy. *J Cutan Pathol* (2017) 44:381–4. doi: 10.1111/cup.12876

94. Chen CB, Wu MY, Ng CY, Lu CW, Wu J, Kao PH, et al. Severe cutaneous adverse reactions induced by targeted anticancer therapies and immunotherapies. *Cancer Manag Res* (2018) 10:1259–73. doi: 10.2147/CMAR.S163391

95. Goldinger SM, Stieger P, Meier B, Micaletto S, Contassot E, French LE, et al. Cytotoxic cutaneous adverse drug reactions during anti-PD-1 therapy. *Clin Cancer Res* (2016) 22:4023–9. doi: 10.1158/1078-0432.CCR-15-2872

96. Hsu YO, Lu KL, Fu Y, Wang CW, Lu CW, Lin YF, et al. The roles of immunoregulatory networks in severe drug hypersensitivity. *Front Immunol* (2021) 12:597761. doi: 10.3389/fimmu.2021.597761

97. Caproni M, Torchia D, Schincaglia E, Volpi W, Frezzolini A, Schena D, et al. The CD40/CD40 ligand system is expressed in the cutaneous lesions of erythema multiforme and stevens-Johnson syndrome/toxic epidermal necrolysis spectrum. *Br J Dermatol* (2006) 154:319–24. doi: 10.1111/j.1365-2133.2005.07023.x

98. Saw S, Lee HY, Ng QS. Pembrolizumab-induced stevens-Johnson syndrome in non-melanoma patients. *Eur J Cancer* (2017) 81:237–9. doi: 10.1016/j.ejca.2017.03.026

99. Lu J, Thuraisingam T, Chergui M, Nguyen K. Nivolumab associated DRESS syndrome: a case report. *JAAD Case Rep* (2019) 5:216–8. doi: 10.1016/j.jdcr.2018.11.017

100. Voskens CJ, Goldinger SM, Loquai C, Robert C, Kaehler KC, Berking C, et al. The price of tumor control: an analysis of rare side effects of anti-CTLA-4 therapy in metastatic melanoma from the ipilimumab network. *PLos One* (2013) 8:e53745. doi: 10.1371/journal.pone.0053745

101. Mirza S, Hill E, Ludlow SP, Nanjappa S. Checkpoint inhibitor associated drug reaction with eosinophilia and systemic symptom syndrome. *Melanoma Res* (2017) 27:271–3. doi: 10.1097/CMR.0000000000000326

102. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* (1995) 3:541–7. doi: 10.1016/1074-7613(95)90125-6

103. Voudouri D, Nikolaou V, Laschos K, Charpidou A, Soupos N, Triantafyllopoulou I, et al. Anti-PD1/PDL1 induced psoriasis. *Curr Probl Cancer* (2017) 41:407–12. doi: 10.1016/j.currproblcancer.2017.10.003

104. Guida M, Strippoli S, Maule M, Quaglino P, Ramondetta A, Chiaron Sileni V, et al. Immune checkpoint inhibitor associated vitiligo and its impact on survival in patients with metastatic melanoma: An Italian melanoma intergroup study. *ESMO Open* (2021) 6:100064. doi: 10.1016/j.esmoop.2021.100064

105. Postow MA. Managing immune checkpoint-blocking antibody side effects. *Am Soc Clin Oncol Educ Book* (2015) 35:76–83. doi: 10.14694/EdBook_AM.2015.35.76

106. Hofmann L, Forschner A, Loquai C, Goldinger SM, Zimmer L, Ugurel S, et al. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. *Eur J Cancer* (2016) 60:190–209. doi: 10.1016/j.ejca.2016.02.025

107. Shenoy N, Esplin B, Barbosa N, Wieland C, Thanarajasingam U, Markovic S. Pembrolizumab induced severe sclerodermoid reaction. *Ann Oncol* (2017) 28:432–3. doi: 10.1093/annonc/mdw543

108. Barbosa NS, Wetter DA, Wieland CN, Shenoy NK, Markovic SN, Thanarajasingam U. Scleroderma induced by pembrolizumab: A case series. *Mayo Clin Proc* (2017) 92:1158–63. doi: 10.1016/j.mayocp.2017.03.016

109. Tjarks BJ, Kerkvliet AM, Jassim AD, Bleeker JS. Scleroderma-like skin changes induced by checkpoint inhibitor therapy. *J Cutan Pathol* (2018) 45:615–8. doi: 10.1111/cup.13273

110. Cho M, Nonomura Y, Kaku Y, Nakabo S, Endo Y, Otsuka A, et al. Scleroderma-like syndrome associated with nivolumab treatment in malignant melanoma. *J Dermatol* (2019) 46:e43–4. doi: 10.1111/1346-8138.14492

111. Maloney NJ, Ravi V, Cheng K, Bach DQ, Worswick S. Stevens-Johnson syndrome and toxic epidermal necrolysis-like reactions to checkpoint inhibitors: a systematic review. *Int J Dermatol* (2020) 59:e183–8. doi: 10.1111/ijd.14811

112. Tang K, Seo J, Tiu BC, Le TK, Pahalyants V, Raval NS, et al. Association of cutaneous immune-related adverse events with increased survival in patients treated with anti-programmed cell death 1 and anti-programmed cell death ligand 1 therapy. *JAMA Dermatol* (2022) 158:189–93. doi: 10.1001/jamadermatol.2021.5476

113. Thompson LL, Krasnow NA, Chang MS, Yoon J, Li EB, Polyakov NJ, et al. Patterns of cutaneous and non-cutaneous immune-related adverse events among patients with advanced cancer. *JAMA Dermatol* (2021) 157:577–82. doi: 10.1001/jamadermatol.2021.0326

114. Min Lee CK, Li S, Tran DC, Zhu GA, Kim J, Kwong BY, et al. Characterization of dermatitis after PD-1/PD-L1 inhibitor therapy and association with multiple oncologic outcomes: A retrospective case-control study. *J Am Acad Dermatol* (2018) 79:1047–52. doi: 10.1016/j.jaad.2018.05.035

115. Hirotsu KE, Scott MKD, Marquez C, Tran AT, Rieger KE, Novoa RA, et al. Histologic subtype of cutaneous immune-related adverse events predicts overall survival in patients receiving immune checkpoint inhibitors. *J Am Acad Dermatol* (2022) 87:651–3. doi: 10.1016/j.jaad.2021.11.050

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An updated review of the immunological mechanisms of keloid scars

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Keloid is a type of disfiguring pathological scarring unique to human skin. The disorder is characterized by excessive collagen deposition. Immune cell infiltration is a hallmark of both normal and pathological tissue repair. However, the immunopathological mechanisms of keloid remain unclear. Recent studies have uncovered the pivotal role of both innate and adaptive immunity in modulating the aberrant behavior of keloid fibroblasts. Several novel therapeutics attempting to restore regulation of the immune microenvironment have shown variable efficacy. We review the current understanding of keloid immunopathogenesis and highlight the potential roles of immune pathway-specific therapeutics.

KEYWORDS

keloid, scar, immunity, macrophages, T lymphocytes, cytokines, signal transduction

1 Introduction

Keloid is a type of pathological scarring unique to human skin. The disorder is characterized by dysregulated fibroproliferation with excessive production of extracellular matrix (ECM) and extension beyond the initial wound (1). Keloid scars are often disfiguring, profoundly impair the quality of life and cause immense physical and mental distress of affected individuals, especially in those with symptomatic (pruritic,

painful) and/or hyperpigmented scars (2–4). Limited epidemiological data suggested a female predominance, and a higher prevalence among people of darker skin complexion, such as those of African and Asian descents (5, 6). The prevalence of excessive scarring in Black, Asians and Caucasians was recently reported at 2.4%, 1.1% and 0.4%, respectively (6). Association between excessive scarring and other systemic conditions including hypertension (7–9), vitamin D deficiency (10, 11), and atopic dermatitis (12, 13) has been suggested. A recent cohort of the UK biobank found atopic dermatitis significantly associated with excessive scarring across ethnic groups (6). Hypertension in Blacks and vitamin D deficiency in Asians also showed significant association with keloid formation (6).

In practice, several preventive and therapeutic therapies are used to manage keloids. Application of silicone gel sheets, topical corticosteroids, and intralesional corticosteroids are frequently utilized in individuals with a history of excessive scarring after trauma or surgeries (14). For established keloids, nonsurgical management commonly involves intralesional corticosteroids (e.g., triamcinolone acetonide) (5). Laser-assisted topical steroid application is a novel alternative with better reported aesthetic outcome (15). Intralesional injection of botulinum toxin A, 5-fluorouracil, verapamil, bleomycin, and interferon (IFN)-α2b are less common measures with varying efficacy (16, 17). Other methods include laser therapy (18), and intralesional cryosurgery (19). Monotherapy with radiation is less preferred due to the requirement of large radiation doses (14). Successful surgical management of keloids hinges on the ability to minimize dermal tension (20). Body site-specific techniques have been proposed (14). The high postsurgical recurrence rate can be ameliorated with adjunctive radiation and/or local corticosteroids (14). There are also anecdotal reports with tissue-engineered allografts (21) and platelet-rich plasma (22). The associated adverse effects of established therapies could be significant, especially with long-term or repeated treatment. Intralesional corticosteroids, one of the most frequent methods in both prophylactic and therapeutic management of keloids, is associated with skin hypo-/hyper-pigmentation, atrophy, and telangiectasia.

The pathogenesis of the exuberant scarring remains incompletely understood. No single determining pathway has been identified. Instead, roles of several transcription factors, growth factors, cytokines, ECM proteins, and their associated regulators/effectors have been implicated in experimental studies. The dysregulated molecular profile causes imbalance within and across stages of tissue repair. Wound healing consists of an overlapping sequence of hemostasis, inflammation, proliferation and re-epithelization, and remodelling (23, 24). At the inflammation stage, the innate immune system is activated in response to the damage-associate molecular patterns (DAMPs) and other danger signals (23, 24). Cell debris are removed *via* phagocytosis of neutrophils (23, 24). Macrophages are later recruited. In addition to phagocytosis, macrophages play an important role in the resolution of inflammation, setting the stage for proliferation (23, 24). The proliferation phase is characterized by migration of keratinocytes, angiogenesis, and formation of granulation tissue (23, 24). Remodelling ensues with replacement of collagen III with collagen I and regression of blood vessels (23, 24). Across the stages, there is a complex interplay between immune cells and fibroblasts. Moreover, the outcome of subsequent stages is closely associated with the integrity and functionality of prior events (23). Hence, excessive scarring could arise as primary dysfunction of the remodelling phase or secondary to an exaggerated inflammatory response (23, 25).

The etiology of keloids is likely multifactorial and hinges on a constellation of factors, including genetic predisposition (26–34), inflammation (35–39), mechanical stress (40–43), tissue hypoxia (44–48), delayed-type hypersensitivity (49), and metabolic dysfunction (50, 51). Familial cases of autosomal dominant inheritance with incomplete clinical penetrance and variable expression have been described (52–54). Several immune pathway-associated susceptible genotypes have been identified, including polymorphisms of interleukin (IL)-6 and transforming growth factor (TGF)-β receptors (26–28, 55–57). Moreover, immune cell infiltration is a hallmark of keloid tissue. Preferential recruitment of immune cells modulates the process of skin repair *via* interaction with keloid fibroblasts (58, 59). Since the 1970s, the immunological aspect of keloid formation has been proposed (60–63), and a potential role for autoimmunity was frequently evoked in early reports (60, 63). With the advent of novel technologies and laboratory methods, the interest in the immunological landscape of keloid formation has led to vigorous investigations over the past two decades.

The reticular dermis has been proposed as the main locale of chronic inflammation underscoring the formation of keloid scars with upregulation of various proinflammatory cytokines, including IL-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α (35). Interestingly, there appeared to be a concomitant excess of regulatory cell types and cytokines (64, 65). Study of keloid histology demonstrated altered expression of ECM molecules with increased type I/III collagen ratio, and a hypercellular dermis with increased numbers of fibroblasts, mast cells and macrophages, as well as varying presence of lymphocytes (1, 36, 64, 66–75). Keloid tissue also harbored a higher percentage of mesenchymal stem cells, and the amount of which was found to be correlated with disease recurrence (76). The role of myofibroblasts is less defined. A recent report found that myofibroblasts, a key feature of cultured fibroblasts in several reports, are not characteristic of keloid lesion *in vivo* (77). The concept of keloid microenvironment has been frequently evoked to describe the complex cellular and molecular interplay that gives rise to and sustains keloidogenesis. Recent technologies, such as identification of differentially expressed genes *via* examination of RNA sequencing data sets (74, 75, 78–80), have led to more extensive analysis of keloid tissue. A skewed T helper (Th) 2 phenotype was recently characterized (81–85), along with a potential co-susceptibility of keloids and atopic dermatitis (6, 12, 86). Moreover, even in the absence of comorbid atopic dermatitis, both lesional and non-lesional skin of patients with chronic keloids exhibit Th2 predominance (81). These features of heightened immune response were validated in a recent transcriptomic study, in which a globally elevated expression of several immune pathways over the entire integument of keloid

patients was seen, especially the Th2 and Janus kinase (JAK) 3 pathways. Increased expression of T cell, regulatory T cell (Treg), and dendritic cell (DC) markers was also observed, along with the expression of the innate, Th1- and Th17/Th22-signaling pathways (85). The change in cellular composition and function is accompanied by increased levels of IL-6, IL-10, IL-17, TGF-β, and TNF (38, 39, 48, 78, 85, 87–94). Increase in IL-4, IL-13, IL-18, granulocyte colony-stimulating factors, and granulocyte-monocyte colony-stimulating factors, were also observed (39, 82–85, 87, 88). On the other hand, reduced expression of potential anti-inflammatory mediators, such as IL-34 and IL-37, has been reported (85, 87, 95, 96). Single-cell RNA sequencing and spatial transcriptomics (72, 97–99), as well as epigenetics (34, 100) are emerging fields utilized to reveal potential pathophysiological features of keloids. One single-cell RNA sequencing study identified a distinct macrophage-centered communication regulatory network that may favor transition and proliferation of M2 macrophages (72). In addition to the local characteristics, corresponding abnormalities in the cytokine profile have been identified in the peripheral blood of keloid patients (39). Serum soluble human leukocyte antigen-E (sHLA-E) was recently identified as a potential biomarker of keloid occurrence and recurrence (101). Furthermore, aberrant immune cell composition and activity are increasingly recognized in the non-lesional skin of keloid patients (81, 82, 85). Reports on the involvement of humoral immunity were less consistent. Anti-hnRNPA2B1, an autoantibody against RNA-associated proteins, was found to be significantly elevated in the serum of keloid patients (73). The same study also showed deposition of immunoglobulins (IgA, IgM) and complements (IgA, IgM, C3 and C1q) *via* immunofluorescence in keloid skin tissue (73). These findings suggest a systemic pathological process underscoring the development of keloids, such that the risk-benefit of repeated local therapy for susceptible individuals is called into question. Further studies are required to elucidate the origin of the keloid-prone immunological signatures.

In this review, current understanding of keloid immunopathogenesis is discussed, with highlights of potential pathway-targeted therapeutics.

2 The roles of immune cells in keloid formation

2.1 Mast cells as profibrotic mediators

Mast cells cluster in tissue exposed to the external environment. In human skin, mature mast cells are abundant near the vasculature, lymphatics, nerves, and fibroblasts, and play a crucial role in wound healing by initiating inflammation, facilitating re-epithelialization, and inducing angiogenesis (68). It has been postulated that mast cells contribute to profibrotic chronic inflammation as well as to the common symptoms (pruritus and erythema) associated with keloid scars (Figure 1). Silicone gel sheeting has been shown to reduce mast cell infiltration in keloid lesions and thus provide symptomatic relief (102).

Increased intralesional and perilesional mast cells can be observed in keloid tissue, both perivascularly and within abnormal collagen bundles (36, 103). Degranulated mast cells are frequently seen in contact with active fibroblasts, indicating the presence of cell–cell interaction (67, 103). Attenuation of such cellular crosstalk has been achieved by blockading the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway using green tea extract (polyphenol

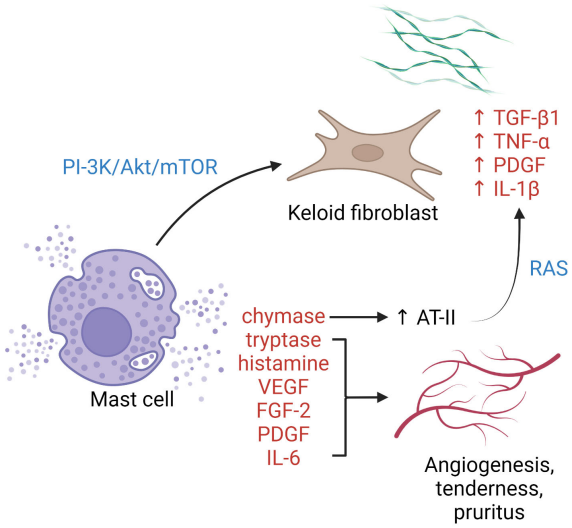

FIGURE 1
The role of mast cells in keloid pathogenesis. Degranulated mast cells crosstalk with activated keloid fibroblasts *via* the PI3K/Akt/mTOR pathway, leading to more collagen synthesis. Enzymes, growth factors, and cytokines released upon mast cell degranulation contribute to activation of the RAS, upregulation of keloid fibroblasts, angiogenesis, and cutaneous symptoms. *AT-II*, angiotensin II; *FGF-2*, fibroblast growth factor 2; *IL*, interleukin; *PDGF*, platelet-derived growth factor; *PI3K/Akt/mTOR*, phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin pathway; *RAS*, renin–angiotensin system; *TGF-β*, transforming growth factor-β; *TNF-α*, tumor necrosis factor-α; *VEGF*, vascular endothelial growth factor.

EGCG), with a corresponding reduction in type I collagen production (104). Several pro-angiogenic factors are released by mast cells, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2, platelet-derived growth factor, IL-6, tryptase, and chymase. Tryptase, a serine protease, is one of the most potent inducers of tissue angiogenesis. Tryptase-positive mast cell density and keloid angiogenesis are positively correlated (105). The use of transdermal tryptase inhibitors for hypertrophic scars and keloids has been described with symptomatic benefit (105). Mast cell chymase expression and activity are heightened in keloid tissue. The enzyme is profibrotic and stimulates fibroblast proliferation and collagen synthesis *via* the TGF-β1/Smad signaling pathway (106). Mast cell-derived chymase enhances angiotensin II expression, leading to local activation of the renin-angiotensin system and upregulation of TGF-β1, TNF-α, platelet-derived growth factor, and IL-1β in keloid fibroblasts (107). Chymase inhibitors have been shown to possess antifibrotic property in skin (108), cardiovascular system (109), and liver (110) in animal models. Other means of mast cell antagonization, e.g., with mast cell stabilizers (111) or tyrosine kinase inhibitors (112), have not been tested in keloids.

2.2 Macrophage polarization and chronic inflammation

M1 (classically activated, CD68-positive) and M2 (alternatively activated, CD163-positive) are two well-established macrophage subgroups. The two phenotypes possess opposing properties, with the former exerting a pro-inflammatory effect and the latter an anti-inflammatory effect (113). An imbalance between M1 and M2 macrophages has been described in several chronic inflammatory conditions such as rheumatoid arthritis (114). Normal wound healing is characterized by an orchestrated transition from M1-predominant early inflammatory stages to M2-predominant restitution (115). Dysregulation of this process leads to either prolonged inflammation with delayed wound closure or increased scarring. M2 macrophages are disproportionally elevated in keloid lesions (69–72, 74), in part due to local enrichment of Th2 cytokines. Although not yet verified, M2 dominance has also been linked to macrophage sensitivity to mechanical signals, including skin tension and stiffness (42). M2 macrophages initiate wound closure *via* secretion of TGF-β1, a potent inducer of both fibroblast proliferation and their differentiation into myofibroblasts (115). Moreover, M2 macrophages induce transcription factor forkhead box P3 (FOXP3) expression in circulating CD3+ T cells, contributing to the formation of Tregs (64) (Figure 2). Interestingly, while M2 predominance is clearly present, expression of both M1 (inducible nitric oxide synthase [iNOS], IL-12)- and M2 (IL-10, TGF-β)-associated genes and proteins is enhanced keloid lesions compared to normal skin (64).

2.3 Tregs-derived TGF-β1 and collagen expression

The numbers of Tregs are increased in keloid lesions (64, 65). Tregs proliferate after cellular contact with dermal fibroblasts in the presence of IL-15 in chronically inflamed skin (116). In keloids, they promote preferential accumulation of collagen III in the presence of anti-CD3/CD28 (65). In patients with multiple keloid scars, the local infiltration of Tregs was found to be coupled with a reduction in circulating CD4+ CD25high FOXP3+ Tregs (117). Whether the apparent excess of local Tregs is pathogenic or merely represents a response to inflammation remains unclear. TGF-β1 and IL-10 are key cytokines secreted by Tregs and exert an autocrine effect (118, 119). The former mediates elaboration of matrix proteins and stimulates the production of IL-6 by mast cells (120), while the latter downregulates proinflammatory macrophages and promotes B cell activation and immunoglobulin secretion (121) (Figure 3). Interestingly, IL-10, rather than IFN-γ, antagonizes the TGF-β1 effect on keloid fibroblasts (93, 122). In muscle, Tregs are known to accumulate at injured sites and modulate the polarization of M1 macrophage to M2 macrophage (123). It is likely that they assume a similar coordinating role in wound healing. Further investigations are required to determine the extent to which Tregs alter the

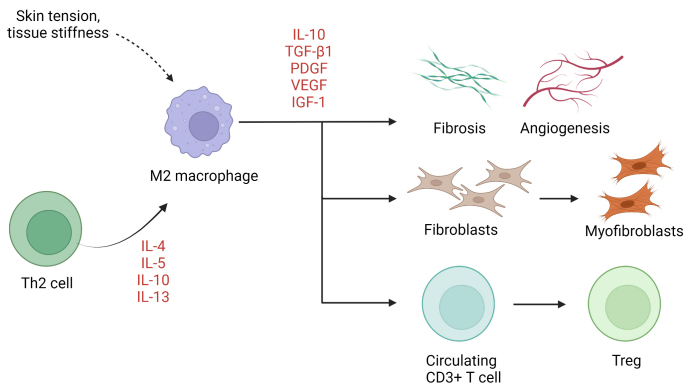

FIGURE 2
The role of macrophages in keloid pathogenesis. M2 macrophages predominate in keloids, resulting in activation of keloid fibroblasts, Treg differentiation, and fibrosis and angiogenesis. *IGF-1*, insulin-like growth factor 1; *IL*, interleukin; *PDGF*, platelet-derived growth factor; *TGF-β1*, transforming growth factor β1; *Th*, helper T cells; *Treg*, regulatory T cell; *VEGF*, vascular endothelial growth factor.

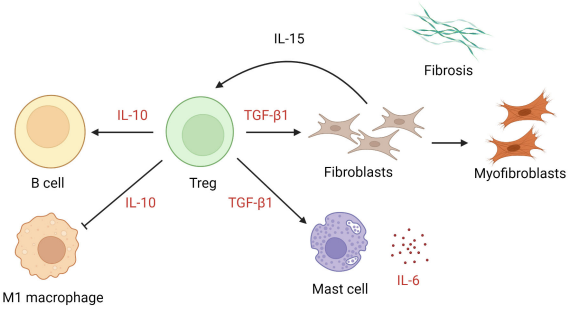

FIGURE 3
The role of regulatory T cells in keloid pathogenesis. Tregs exert effects through the action of IL-10 and TGF-β1, leading to suppression of M1 macrophages, activation of keloid fibroblasts, and mast cell production of IL-6. *IL*, interleukin; *TGF-β1*, transforming growth factor β1; *Tregs*, regulatory T cells.

balance between M1 and M2 macrophages and contribute to keloidogenesis.

2.4 Chronic stimulation and exhaustion of CD8+ T cells

In normal skin, the majority of T cells are CD45RO+ memory T cells. The same holds true in keloids, with a significantly higher proportion of effector memory CD8+ T cells (T_{EM}) and CD103 +CD8+ resident memory T cells (T_{RM}) (117). T_{RM} are known to trigger an exaggerated inflammatory response to stimuli (124). Keloid memory T cells are less adept at producing TNF-α and more prone to generating IFN-γ (117). FOXP3+ CD8- memory T cells are also defective with decreased IL-10 secretion, resulting in exuberant but dysregulated T cell responses in keloids (117). Further adding to the dysregulation, the expression of granzyme B+ CD8+ cytotoxic T cell is downregulated in keloids, a feature presumably related to the characteristic uncontrolled growth. A recent single-cell RNA study discovered that chronic antigenic stimulation in keloids result in enhanced surface NKG2A expression on CD8+ T cells and natural killer (NK) cells (125, 126), with resultant suppression of cytotoxic T cells *via* the NKG2A-soluble human leukocyte antigen-E (sHLA-E) axis (101). IL-15 (127) and TGF-β (128) were implicated in this process. The enhanced expression of the NKG2A/CD94 complex on CD8+ cytotoxic T cells is correlated with progression of keloids. The level of sHLA-E reflects clinical response to intralesional therapy (triamcinolone and 5-fluorouracil) and predicts recurrence risk (101). Furthermore, the degree of sHLA-E elevation could differentiate keloid scars from certain malignant mimics, with the former exhibiting significantly higher levels of sHLA-E (101). Monalizumab, a humanized anti-NKG2A IgG4 monoclonal antibody, exerts an antitumor effect by unleashing both cytotoxic T cells and NK cells (125). The agent has been tested in clinical trials as part of the immunotherapeutic regimens for advanced solid organ cancers, such as recurrent/metastatic squamous cell carcinoma of the head and neck (129), unresectable stage III non-small-cell lung cancer (130), and recurrent gynecologic malignancies (131). Further studies are required to determine the therapeutic potential of NKG2A/CD94 blockade for keloids.

2.5 Dendritic cells

Dermal infiltration of factor XIIIa (FXIIIa)-positive DCs is increased in keloid scars comparing to hypertrophic scars and mature scars (132, 133). These potent antigen-presenting cells are thought to take part in the pathogenic epidermal–dermal interactions in keloids (132), and DC-derived TGF-β could contribute to the differentiation of Tregs. RNA sequencing study confirmed increase of DC markers CD80 and CD86, as well as markers typical of atopic DCs (OX40L+, FCεR1+) in both lesional and nonlesional skin of keloid patients (85). Unlike in atopic dermatitis, where DCs have been linked to mast cell activation and Th2, Th17 and Th22 differentiation (134, 135), the exact action of DCs in keloids is less clear.

2.6 Natural killer cells

Flow cytometric analyses of keloid single-cell suspensions have shown an unusually high number of NK cells (79). Although their role in keloidogenesis is less well described, NK cells express the surface NKG2A/CD94 complex and thus are implicated in the NKG2A-sHLA-E axis (101). Therefore, it is possible that NK cell activity is relatively suppressed in the TGF-β-rich, chronically inflamed keloid milieu, and that a phenomenon paralleling uncontrolled cancerous growth due to NK and cytotoxic T cell exhaustion is likely present.

3 Key cytokine pathways in keloid formation

Keloids are characterized by dysregulation of multiple signaling pathways and associated cytokines. The best described are IL-6/IL-17, IL-4/IL-13, canonical and non-canonical TGF-β1, and JAK/STAT signaling (Figure 4).

3.1 The essential role of IL-6 in inflammation

IL-6 signals through the JAK1–signal transducer and activator of transcription (STAT) 3 pathway and the extracellular signal-regulated kinase (ERK) 1/2–mitogen-activated protein kinase (MAPK) pathway. Both pathways have been implicated in keloid ECM gene expression and collagen synthesis (90, 92). IL-6 and the soluble IL-6 receptor (sIL-6R) are essential for collagen production (136). Similar to patients with systemic sclerosis (137), patients with keloids show elevated IL-6 in the serum and skin (38, 55, 90). In addition, IL-6 polymorphisms have been associated with susceptibility to keloid formation across populations (55–57).

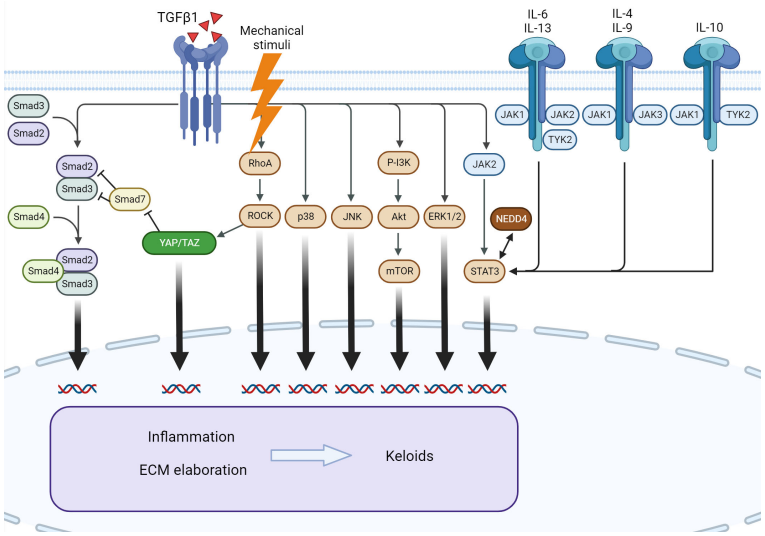

FIGURE 4
Signaling pathways involved in keloid formation. Several downstream pathways of TGF-β1 participate in keloid pathogenesis. Mechanical stimuli exert an effect through Rho/ROCK and YAP/TAZ, leading to the modulation of canonical TGF-β1 signaling. The interleukin family of cytokines and NEDD4 exert profibrotic action *via* STAT3, independent of TGF-β1. *ERK*, extracellular signal-regulated kinase; *IL*, interleukin; *JAK*, Janus kinase; *JNK*, c-Jun N-terminal kinase; *mTOR*, mammalian target of rapamycin; *NEDD4*, neural precursor cell expressed, developmentally downregulated 4; *PI3K*, phosphatidylinositol-3-kinase; *ROCK*, RhoA/Rho-associated protein kinase; *STAT3*, signal transducer and activator of transcription 3; *TGF-β1*, transforming growth factor β1; *TYK2*, tyrosine kinase 2; *YAP/TAZ*, Yes-associated protein/transcriptional coactivator with PDZ-binding motif.

IL-6 is pivotal to the transition from acute to chronic inflammation via initiation of a profibrotic state (138–140). Specifically, the cytokine modulates the fibrogenic crosstalk between fibroblasts and keratinocytes by inducing proinflammatory cytokines (IL-1β and TNF-α) in monocytes *via* MAPK and nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signaling (141). Keratinocyte growth factor production by fibroblasts is enhanced, and the activated keratinocytes in turn produce oncostatin M, triggering STAT3 signaling in dermal fibroblasts (141). IL-6 production is increased in response to enhanced TGF-β1 signaling as a downstream effector (via PI3K and p38-MAPK) (142), and in turn it enhances TGF-β1 production by macrophages (64), creating a positive feedback loop. IL-6 is also crucial to Th2 and M2 macrophage polarization by initiating IL-4 secretion by CD4+ T cells) and upregulating IL-4 receptors (IL-4R) on macrophages (141). Of note, several experimental therapies for keloids and other forms of cutaneous fibrosis directly or indirectly antagonize IL-6. Examples include corticosteroids, verapamil, angiotensin receptor blocker/angiotensin converting enzyme inhibitors, tocilizumab, pirfenidone, and ultraviolet A (87, 141). TNF-α-stimulated gene-6 (TSG-6), a protein suppressed in keloid fibroblasts, has been shown to attenuate IL-1β, IL-6, and TNF-α when intradermally injected into hypertrophic scars (143). The IL-17/IL-6 axis is crucial to sustaining a cytokine-rich, chronically inflamed niche, augmented by an autocrine loop with increased differentiation of Th17 and subsequent heightened secretion of IL-6 (38). IL-17-mediated enhancement of stromal cell-derived factor-1 (SDF-1) in keloid fibroblasts further reinforces Th17 differentiation *via* STAT3 mediation (89). This hyperinflammatory milieu is the most prominent perilesionally (89). Through upregulation of hypoxia-inducible factor-1α (HIF-1α) and STAT3, IL-17 impairs autophagy of both normal and keloid fibroblasts, resulting in increased necroptosis and fibrosis. Antagonization of IL-17 *via* HIF-1α or SDF-1α suppression has been demonstrated *in vitro* (48, 89).

3.2 The role of type 2 immunity: IL-4/IL-13

Several studies have investigated the association between keloids and other conditions characterized by the Th2 response. The results are variable, with some studies reporting a positive correlation with atopic dermatitis (6, 12, 13, 81). Th2 immunity is involved in normal wound healing as well as various fibrotic conditions (144). IL-4 and IL-13 are key Th2 cytokines that have wide-ranging influence across cell types as their receptors are commonly present (144). The binding of IL-4 and IL-13 to their cognate receptors activates the IL-4Rα/STAT6 signaling pathway, a TGF-β-independent profibrotic mechanism (145). Both IL-4 and IL-13 independently participate in normal and pathogenic healing. Topical IL-4 significantly accelerates the rate of fibrotic tissue formation, whereas IL-4 antisense oligonucleotides attenuate the healing process in animal models (146). In mouse models of systemic sclerosis, anti-IL-4 monoclonal antibodies prevent progression of cutaneous fibrosis by reducing dermal collagen deposition (147). On the other hand, IL-13 has been shown to directly contribute to fibroblast proliferation and differentiation. IL-13 enhances the expression of type I collagen, α-smooth muscle actin (α-SMA), and other essential proteins involved in fibrogenesis. Furthermore, tissue inhibitors of metalloproteinases are attenuated while matrix metalloproteinases are upregulated in keloid fibroblasts treated with IL-13 (83).

The expression of IL-4, IL-13 and their respective receptors is enhanced in keloid scars (81–83, 85, 144, 145). Regression of

chronic keloids has been achieved with Th2-targeting therapy with dupilumab (an anti-IL-4Rα agent) in a case report (81). Others showed variable efficacy (148, 149). Molecular profiling of keloids with RNA sequencing demonstrated a significant increase in Th2 expression in both lesional and non-lesional skin of keloid patients (85). The relative dominance of the Th2 response has been attributed to the anti-apoptotic effect conferred to CD4+ T cells by IL-4 (39). IL−4− and IL−13−activated macrophages (M2 macrophages) are critical to resolving inflammation during wound repair (150, 151). In chronic inflammation, these cytokines have been shown to upregulate miR-142-5p and suppress miR-130a-3p in macrophages, leading to a sustained profibrogenic phenotype (152). Human dermal fibroblasts treated with IL-4 and IL-13 exhibit drastically elevated levels of periostin mRNA with enhanced secretion (82). Periostin is an important promoter of RhoA/Rho-associated protein kinase (ROCK) pathway-mediated TGF-β1 secretion impliacted in pathological scarring (82, 153). In systemic sclerosis, periostin is correlated with skin disease severity (154), and the serum level of IL-13 recflects severity of both skin fibrosis (155). The clinical utility of these biomarkers in keloids remains to be determined.

3.3 The pivotal role of TGF-β1

TGF-β1 is one of the most studied mediators of fibrosis. It is frequently implicated in keloid pathogenesis. TGF-β1 has a wide range of cellular sources, including fibroblasts, monocytes, T cells, and platelets (156). As a key regulator of fibrogenesis, this pleiotropic cytokine plays a pivotal role in various cutaneous and solid organ fibrotic disorders, as well as in tumorigenesis *via* induction of cancer-associated fibroblasts (156). Several monoclonal antibodies, small molecule inhibitors, small interfering RNAs (siRNAs), and antisense oligodeoxynucleotides targeting TGF-β1 signaling are currently under development (156).

The induction by TGF-β1 of various growth factors, including connective tissue growth factor (CTGF) and VEGF, is crucial to the maintenance of the ECM. Moreover, TGF-β1 exerts an autocrinal effect that downregulates dipeptidyl peptidase-4 (DPP4) expression, contributing to a chronically inflamed state with elevated levels of the extracellular C-X-C motif chemokine ligand 12 (CXCL12) (157). Fibroblasts in keloids are considerably sensitive to TGF-β compared to those in hypertrophic scars (158, 159). These abnormal fibroblasts are able to overcome Fas-mediated apoptosis when augmented by TGF-β1 (160). TGF-β1-induced smooth muscle actin (SMA) expression in keloid fibroblasts contributes to increased cell rigidity, a phenomenon common to both keloids and scleroderma (161). SMA expression is linked to wound contracture, and the process can be inhibited with treatment with recombinant human decorin, TNF-like weak inducer of apoptosis (TWEAK), and SB-431542, a novel specific inhibitor of TGF-β1 receptor kinase (162). TGF-β1 is also capable of upregulating C-MYC and its downstream splicing regulator polypyrimidine tract-binding protein—a key factor in tumorous growth—in keloid fibroblasts (163). Furthermore, altered interaction between TGF-β isoforms at the receptor level in keloid fibroblasts has been described (164–167). The ratio of these isoforms may cause a tendency to fibrosis (168). Accordingly, a novel truncated type II TGF-β receptor has been designed as an anti-scarring agent (169, 170). Both canonical and non-canonical TGF-β1 signaling are implicated in modulating the keloid keratinocytes to possess a metabolic profile similar to those undergoing epithelial–mesenchymal transition with increased invasiveness (45, 171–173).

3.3.1 Modulators of the TGF-β1/Smad pathway

The canonical TGF-β signaling is enhanced in keloids, and strategic targeting of TGF-β1/Smad has been shown to retard keloid fibroblasts *in vitro* or in animal models (Supplemental Table 1). Upstream modulators of the TGF-β1/Smad pathway, including activating transcription factor 3 (174), CR6-interacting factor 1 (175), NLR family CARD domain containing 5 (NLRC5) (176), and nuclear receptor subfamily 3, group C, member 1 (NR3C1), are overexpressed in keloid fibroblasts. HIF-1α and high temperature requirement factor A1 activate the TGF-β1/Smad pathway and promote keloid formation (46, 177). S100A4, a small, calcium-binding protein involved in skin and solid organ fibrosis, is upregulated in keloid fibroblasts and inhibited by calcimycin (178). Syndecan-1, a cell surface proteoglycan highly expressed in wounds, also enhances the pathway in keloids (179). Post-translational sumoylation amplifies TGF-β1/Smad signal transduction in keloids (47).

MicroRNAs are small regulatory RNAs capable of altering post-translational gene expression. In keloid fibroblasts, the anti-fibrotic regulators miR-200c (180), miR-92b (181), miR-1224-5p (182), and miR-133a-3p (183) are expressed at low levels and pro-fibrotic miR-21is overexpressed, altering the activity of the TGF-β1/Smad pathway (184). Peroxisome proliferator-activated receptor-γ agonists have been shown to induce miR-92b expression and thus lower TGF-β1 expression in keloids (181). MicroRNA expression is modulated by long-noncoding RNAs. In keloids, fibroblast behavior is altered in the presence of different long-noncoding RNAs (180, 185–188). For example, LINC01116 contributes to a pro-fibrotic state in keloid tissue *via* editing of miR-3141 (185). In addition, the BMP and activin membrane-bound inhibitor (189), Dickkopf-3 (190), and the receptor for activated C-kinase 1 (191) attenuate TGF-β1-induced fibrosis; all are downregulated in keloid fibroblasts. Smad-7 provides negative feedback to the TGF-β1/Smad system. The molecule is suppressed due to a marked increase in the level of TGF-β inducible early gene-1 in keloids (192). Downregulation of TRAF3IP2 in keloid fibroblasts by FOXO4 attenuates the growth of keloid scars (193). IL-37 is a broad inhibitor of innate inflammation and regulator of TGF-β (194, 195). Recent studies have uncovered its role in modulating several metabolic pathways and a potential role in reversing trained immunity (196). As seen in idiopathic pulmonary fibrosis (197), lower serum levels of IL-37 were found to indicate higher keloid severity (95).

3.3.2 Non-canonical TGF-β pathways

Several non-canonical TGF-β pathways are involved in keloid formation. These include the MAPK (94, 198, 199), ERK 1/2 (44, 200) phosphatidylinositol-3-kinase (P-I3K)/AKT (44, 104, 200–202), c-Jun amino-terminal kinase (94), p38 mitogen-activated protein kinase (p38/MAPK) (94, 203–205), and Rho-like (82) signaling pathways. The multi-kinase inhibitor sorafenib induces cell arrest of keloid

fibroblasts by blockade of the intracellular TGF-β/Smad and MAPK/ERK pathways (206). JUN (an oncogene encoding the c-Jun protein) initiates fibrosis *via* CD36 in both human and murine hypertrophic scar fibroblasts, and the blockade of CD36 exerted an anti-scarring effect in the murine model (207).

3.3.3 Bridge to mechanical transduction: Reciprocal cross-regulation with the integrin and Yes-associated protein/transcriptional coactivator with PDZ-binding motif pathways

Crosstalk between TGF-β and mechanical transduction pathways is increasingly recognized. Among these pathways, the integrin pathway (79, 142) and the Hippo/Yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) pathway (43, 208, 209) are the most recognized. In addition, TGF-β interacts with Wnt/β-catenin activity in dermal fibroblasts, upregulating ECM genes (210, 211). YAP/TAZ are important actors in cellular mechanical transduction. These transcriptional factors are regulated mostly by cell–cell adhesion and cell–ECM attachment *via* integrins (212). Conditions that cause stiffening of the ECM, such as inflammation, lead to a lower threshold of YAP/TAZ activation (212). IL-6 is also known to activate YAP through gp130 signaling (212). Activated YAP/TAZ translocate into keloid fibroblast nuclei, a step required for wound healing (208). In liver cirrhosis, YAP/TAZ contribute to tissue fibrosis *via* enhanced SMA expression, promoting the transformation of fibroblasts into myofibroblasts. YAP/TAZ are also implicated in the sustained profibrotic transcriptional profile of idiopathic pulmonary fibrosis (213). Targeted knockdown of YAP or TAZ has been shown to significantly inhibit the activity and induce apoptosis of keloid fibroblasts (208). Inhibition of Rho/Rho kinase signaling, a major upstream regulator of YAP/TAZ, also attenuates fibroblast activity (82, 214). Manipulation of the YAP/TAZ-associated pathways could potentially reduce keloid scarring. A recent study identified a subpopulation of dermal Engrailed-1 lineage-negative fibroblasts in cell transplantation and transgenic mouse models that could give rise to scar-forming Engrailed-1 lineage-positive fibroblasts during adult wound healing (215). The process is initiated by canonical mechanotransduction signaling and depends on YAP (215). Inhibition or knockout of YAP prohibits Engrailed-1 activation, favoring scarless (regenerative) wound healing *via* Engrailed-1 lineage-negative fibroblasts (215). Verteporfin, a small-molecule YAP inhibitor, has been proposed as a potential novel agent for promoting regenerative skin healing without compromising the healing process (216).

3.4 Janus kinase/signal transducers and activators of the transcription pathway

STAT3 is highly expressed and phosphorylated in keloid tissue with increased activation of JAK2 (217). Moreover, STAT3 activity is correlated with fibroblast proliferation and migration, as well as collagen deposition, mainly due to dysregulated secretion of cytokines resulting from altered epithelial–mesenchymal interactions (218). Attenuation of such activity can be achieved with JAK2/STAT3 inhibitors or STAT3 siRNA (217, 219). Cytokines enriched in the keloid microenvironment, especially IL-6 and OSM, are strong activators of the JAK/STAT system. Various Th2- and Th17-cytokines, including IL-4, IL-10, IL-13, and IL-17, also signal through JAK/STAT (85, 87). IL-6-specific hyperactivation of STAT3 has been shown to be profibrotic due to the induction of Gremlin (a bone morphogenetic protein [BMP] antagonist), which in turn sustains canonical TGF-β signaling (136). Recently, RNA sequencing analyses confirmed robust expression of JAK3 in keloid tissue (85), and positioned STAT3 in a feedforward loop regulating a myriad of downstream target genes involved in keloidogenesis (220). From a metabolic viewpoint, keloids exhibit accelerated glycolysis reminiscent of Warburg metabolism, a unique adaptive state presumably induced by JAK2/STAT3 (50, 221). Interestingly, an *in vivo* study demonstrated regulation of keloid fibroblast activity at the cost of a worsened hyperglycolytic state with JAK1/2 blockade (222). Epigallocatechin-3-gallate (EGCG), a green tea extract, has been found to possess chemopreventive properties, including suppression of STAT3 signaling, potentially inhibiting keloid growth (219). ASC-J9, an inhibitor of STAT3 phosphorylation, has shown efficacy in suppressing keloid fibroblasts (223). AG490, a selective JAK2/STAT3 inhibitor, and STAT3–specific decoy oligodeoxynucleotides are also beneficial *in vitro* (224). Oral small-molecule JAK inhibitors are effective in treating skin and pulmonary diseases of systemic sclerosis (225). In a case report, tofacitinib, a pan-JAK inhibitor, facilitated control of keloid scar (226).

STAT3 was recently discovered as a transcription factor for the neural precursor cell expressed, developmentally downregulated 4 (NEDD4) gene (227). NEDD4 encodes a ubiquitin ligase involved in protein degradation and has been associated with susceptibility to keloids (30, 32, 33, 228–231). NEDD4 transcript variant 3 is overexpressed in keloid skin and is responsible for heightened activation of NF-κB *via* interaction with receptor interacting protein, an adaptor protein (29). NF-kB is more prominent in keloids than in normal skin and contributes to impaired apoptosis of fibroblasts (37, 232). Aspirin may potentially prevent this effect (232). NEDD4 regulates cell contact inhibition and T cell factor/β-catenin transcriptional activity (231). It is also linked to fibronectin and type 1 collagen expression (231). A positive feedback loop between STAT3 and NEDD4 has been described (29), and silencing of NEDD4 also attenuates STAT3 (29, 227), making NEDD4 a potential therapeutic target in keloids.

4 Other potential therapeutics

Fibroblast activation protein (FAP), a membrane-bound enzyme with structural similarity to DPP4, is found almost exclusively on activated fibroblasts and myofibroblasts under pathological conditions (233), making it a potential target for selective inhibition. Similar to DPP4, FAP upregulates extracellular CXCL12 (234). In addition to its enzymatic activity against the ECM (and thus its association with lesion invasiveness (234, 235), the molecule is likely pluripotent with immunomodulatory properties (234). The FAP expression level is enhanced in keloid fibroblasts (234, 235) and FAP modulation has been shown to attenuate the invasiveness of scars (235). As a marker of pathological fibroblast activation, FAP is a novel subject of interest in

solid tumors and connective tissue disorders. Previous studies have shown that FAP chimeric antigen receptor-T cell therapy may be limited by systemic toxicity as FAP is also expressed on multipotent bone marrow stromal cells (236, 237). On the other hand, FAP-inhibiting radiopharmaceuticals have shown theranostic promise in various malignancies and other disorders characterized by tissue fibrosis, such as systemic sclerosis (238), rheumatoid arthritis (239), and IgG4-related disease (240). Targeted photodynamic therapy with an anti-FAP photosensitizer exhibits a dose-dependent therapeutic effect on skin fibroblasts of patients with systemic sclerosis (238).

Additional pathway abnormalities, such as Notch and Toll-like receptor signaling pathways, have been implicated in keloid pathogenesis (51, 241, 242). Human adipose-, amnion-, bone marrow- and Wharton's jelly-derived mesenchymal stem cells have been shown to inhibit proliferation, migration, and synthesis of keloid fibroblasts *in vitro*, presumably though paracrine effects (243–249). The TGF-β1/Smad and TGF–β2/Smad3 pathways, Notch-1, and cyclooxygenase-2/prostaglandin E2 cascade were all implicated (243–245). Further investigations are warranted to evaluate the *in vivo* effects of these pathways.

5 Current challenges and future direction in keloid research

Even with modern technologies, several factors complicate our understanding of keloidogenesis. The lack of an ideal animal model has impeded experimental investigations, and the examination of the nature of keloid scars is limited by sample size. Moreover, the lack of standardization of the site of tissue sampling complicates the interpretation of study results. We previously reported that the inflammatory activity within a keloid scar is most vigorous at the periphery, corresponding to the gradational change in skin tension (31). The gene signatures also varied at the leading edge, center and top of keloid lesions (250). Theoretically, anti-inflammatory measures would be most beneficial at the initial inflammatory stage of wound healing and at the periphery of the scar. Anti-fibrotic therapy, on the other hand, ameliorates the later stages and the more central part of the lesion before scar maturation. To allow for timely and appropriate (i.e., without compromising the healing process) modulation of immune pathways, the mechanisms regulating the transition and spatio-temporal overlap across stages need to be better understood. Studies focusing on explicating the cellular and molecular processes of wound healing, could be of immense value to our understanding and management of keloid disorder.

6 Conclusions

The keloid microenvironment is characterized by an exuberant inflammatory response to mechanical and non-mechanical stimuli, resulting in a complex interplay between various hyperactivated immune components with an ultimately profibrotic cytokine profile and signaling. Manipulation of isolated elements or pathways has shown variable efficacy, mostly in an experimental setting. Keloid is increasingly characterized by an inflammatory process, and local treatment might be insufficient for long-term control. Newer biologics and small molecule drugs allow for more specific and systemic targeting of immune pathways. For both approved and experimental drugs, a critical issue is the timing of intervention, as premature suppression of either inflammation or fibrosis could impair wound healing. Further investigations to disentangle the delicate process of wound healing are thus crucial for a more targeted management of keloids.

Author contributions

Conceptualization, C-CL, C-HT, and C-BC; methodology, C-CL, C-HT, and C-BC; resources, C-HT, W-HC, and C-BC; writing—original draft preparation, C-CL, C-HT, and C-BC; writing—review and editing, C-CL, C-HT, C-HC, Y-CY, W-HC, and C-BC; visualization, C-CL, C-HT, and C-BC; supervision, C-HT, W-HC, and C-BC; project administration, C-HT, C-HC, Y-CY, W-HC, and C-BC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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References

1. Jumper N, Paus R, Bayat A. Functional histopathology of keloid disease. *Histol Histopathol* (2015) 30(9):1033–57. doi: 10.14670/hh-11-624

2. Sitaniya S, Subramani D, Jadhav A, Sharma YK, Deora MS, Gupta A. Quality-of-Life of people with keloids and its correlation with clinical severity and demographic profiles. *Wound Repair Regener* (2022) 30(3):409–16. doi: 10.1111/wrr.13015

3. Lu W, Chu H, Zheng X. Effects on quality of life and psychosocial wellbeing in chinese patients with keloids. *Am J Transl Res* (2021) 13(3):1636–42.

4. Bijlard E, Kouwenberg CA, Timman R, Hovius SE, Busschbach JJ, Mureau MA. Burden of keloid disease: A cross-sectional health-related quality of life assessment. *Acta Derm Venereol* (2017) 97(2):225–9. doi: 10.2340/00015555-2498

5. Davis SA, Feldman SR, McMichael AJ. Management of keloids in the united states, 1990-2009: An analysis of the national ambulatory medical care survey. *Dermatol Surg* (2013) 39(7):988–94. doi: 10.1111/dsu.12182

6. Ung CY, Warwick A, Onoufriadis A, Barker JN, Parsons M, McGrath JA, et al. Comorbidities of keloid and hypertrophic scars among participants in uk biobank. *JAMA Dermatol* (2023) . 159(2):172–81. doi: 10.1001/jamadermatol.2022.5607

7. Rutherford A, Glass DA2nd. A case-control study analyzing the association of keloids with hypertension and obesity. *Int J Dermatol* (2017) 56(9):e187–e9. doi: 10.1111/ijd.13618

8. Adotama P, Rutherford A, Glass DA2nd. Association of keloids with systemic medical conditions: A retrospective analysis. *Int J Dermatol* (2016) 55(1):e38–40. doi: 10.1111/ijd.12969

9. Snyder AL, Zmuda JM, Thompson PD. Keloid associated with hypertension. *Lancet* (1996) 347(8999):465–6. doi: 10.1016/s0140-6736(96)90042-2

10. El Hadidi HH, Sobhi RM, Nada AM, AbdelGhaffar MMM, Shaker OG, El-Kalioby M. Does vitamin d deficiency predispose to keloids *Via* dysregulation of koebnerisin (S100a15)? a case-control study. *Wound Repair Regener* (2021) 29(3):425–31. doi: 10.1111/wrr.12894

11. Yu D, Shang Y, Luo S, Hao L. The taqi gene polymorphisms of vdr and the circulating 1,25-dihydroxyvitamin d levels confer the risk for the keloid scarring in chinese cohorts. *Cell Physiol Biochem* (2013) 32(1):39–45. doi: 10.1159/000350121

12. Kwon HE, Ahn HJ, Jeong SJ, Shin MK. The increased prevalence of keloids in atopic dermatitis patients with allergic comorbidities: A nationwide retrospective cohort study. *Sci Rep* (2021) 11(1):23669. doi: 10.1038/s41598-021-03164-4

13. Lu YY, Lu CC, Yu WW, Zhang L, Wang QR, Zhang CL, et al. Keloid risk in patients with atopic dermatitis: A nationwide retrospective cohort study in taiwan. *BMJ Open* (2018) 8(7):e022865. doi: 10.1136/bmjopen-2018-022865

14. Ogawa R. The most current algorithms for the treatment and prevention of hypertrophic scars and keloids: A 2020 update of the algorithms published 10 years ago. *Plast Reconstr Surg* (2022) 149(1):79e–94e. doi: 10.1097/prs.0000000000008667

15. Abd El-Dayem DH, Nada HA, Hanafy NS, Elsaie ML. Laser-assisted topical steroid application versus steroid injection for treating keloids: A split side study. *J Cosmet Dermatol* (2021) 20(1):138–42. doi: 10.1111/jocd.13521

16. Wu W, Zhao Y, Chen Y, Zhong A. Comparing the efficacy of multiple drugs injection for the treatment of hypertrophic scars and keloid: A network meta-analysis. *Aesthetic Plast Surg* (2022). doi: 10.1007/s00266-022-03163-4

17. Lee JH, Kim SE, Lee AY. Effects of interferon-Alpha2b on keloid treatment with triamcinolone acetonide intralesional injection. *Int J Dermatol* (2008) 47(2):183–6. doi: 10.1111/j.1365-4632.2008.03426.x

18. Bouzari N, Davis SC, Nouri K. Laser treatment of keloids and hypertrophic scars. *Int J Dermatol* (2007) 46(1):80–8. doi: 10.1111/j.1365-4632.2007.03104.x

19. Har-Shai Y, Mettanes I, Zilberstein Y, Genin O, Spector I, Pines M. Keloid histopathology after intralesional cryosurgery treatment. *J Eur Acad Dermatol Venereol* (2011) 25(9):1027–36. doi: 10.1111/j.1468-3083.2010.03911.x

20. Ogawa R, Akaishi S, Huang C, Dohi T, Aoki M, Omori Y, et al. Clinical applications of basic research that shows reducing skin tension could prevent and treat abnormal scarring: The importance of Fascial/Subcutaneous tensile reduction sutures and flap surgery for keloid and hypertrophic scar reconstruction. *J Nippon Med Sch* (2011) 78(2):68–76. doi: 10.1272/jnms.78.68

21. Osswald SS, Elston DM, Vogel PS. Giant right plantar keloid treated with excision and tissue-engineered allograft. *J Am Acad Dermatol* (2003) 48(1):131–4. doi: 10.1067/mjd.2003.48

22. Jones ME, Hardy C, Ridgway J. Keloid management: A retrospective case review on a new approach using surgical excision, platelet-rich plasma, and in-office superficial photon x-ray radiation therapy. *Adv Skin Wound Care* (2016) 29(7):303–7. doi: 10.1097/01.Asw.0000482993.64811.74

23. Landén NX, Li D, Ståhle M. Transition from inflammation to proliferation: A critical step during wound healing. *Cell Mol Life Sci* (2016) 73(20):3861–85. doi: 10.1007/s00018-016-2268-0

24. Reinke JM, Sorg H. Wound repair and regeneration. *Eur Surg Res* (2012) 49 (1):35–43. doi: 10.1159/000339613

25. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: Molecular and cellular mechanisms. *J Invest Dermatol* (2007) 127(3):514–25. doi: 10.1038/sj.jid.5700701

26. Bayat A, Bock O, Mrowietz U, Ollier WE, Ferguson MW. Genetic susceptibility to keloid disease and transforming growth factor beta 2 polymorphisms. *Br J Plast Surg* (2002) 55(4):283–6. doi: 10.1054/bjps.2002.3853

27. Bayat A, Bock O, Mrowietz U, Ollier WE, Ferguson MW. Genetic susceptibility to keloid disease and hypertrophic scarring: Transforming growth factor Beta1 common polymorphisms and plasma levels. *Plast Reconstr Surg* (2003) 111(2):535–43. doi: 10.1097/01.Prs.0000041536.02524.A3

28. Bayat A, Bock O, Mrowietz U, Ollier WE, Ferguson MW. Genetic susceptibility to keloid disease: Transforming growth factor beta receptor gene polymorphisms are not associated with keloid disease. *Exp Dermatol* (2004) 13(2):120–4. doi: 10.1111/j.0906-6705.2004.00165.x

29. Fujita M, Yamamoto Y, Jiang JJ, Atsumi T, Tanaka Y, Ohki T, et al. Nedd4 is involved in inflammation development during keloid formation. *J Invest Dermatol* (2019) 139(2):333–41. doi: 10.1016/j.jid.2018.07.044

30. Nakashima M, Chung S, Takahashi A, Kamatani N, Kawaguchi T, Tsunoda T, et al. A genome-wide association study identifies four susceptibility loci for keloid in the japanese population. *Nat Genet* (2010) 42(9):768–71. doi: 10.1038/ng.645

31. Tsai CH, Ogawa R. Keloid research: Current status and future directions. *Scars Burn Heal* (2019) 5:2059513119868659. doi: 10.1177/2059513119868659

32. Velez Edwards DR, Tsosie KS, Williams SM, Edwards TL, Russell SB. Admixture mapping identifies a locus at 15q21.2-22.3 associated with keloid formation in african americans. *Hum Genet* (2014) 133(12):1513–23. doi: 10.1007/s00439-014-1490-9

33. Zhu F, Wu B, Li P, Wang J, Tang H, Liu Y, et al. Association study confirmed susceptibility loci with keloid in the chinese han population. *PloS One* (2013) 8(5): e62377. doi: 10.1371/journal.pone.0062377

34. Liu S, Yang H, Song J, Zhang Y, Abualhssain ATH, Yang B. Keloid: Genetic susceptibility and contributions of genetics and epigenetics to its pathogenesis. *Exp Dermatol* (2022) 31:1665–75. doi: 10.1111/exd.14671

35. Ogawa R. Keloid and hypertrophic scars are the result of chronic inflammation in the reticular dermis. *Int J Mol Sci* (2017) 18(3):606. doi: 10.3390/ijms18030606

36. Bagabir R, Byers RJ, Chaudhry IH, Müller W, Paus R, Bayat A. Site-specific immunophenotyping of keloid disease demonstrates immune upregulation and the presence of lymphoid aggregates. *Br J Dermatol* (2012) 167(5):1053–66. doi: 10.1111/j.1365-2133.2012.11190.x

37. Messadi DV, Doung HS, Zhang Q, Kelly AP, Tuan TL, Reichenberger E, et al. Activation of nfkappab signal pathways in keloid fibroblasts. *Arch Dermatol Res* (2004) 296(3):125–33. doi: 10.1007/s00403-004-0487-y

38. Zhang Q, Yamaza T, Kelly AP, Shi S, Wang S, Brown J, et al. Tumor-like stem cells derived from human keloid are governed by the inflammatory niche driven by il-17/Il-6 axis. *PloS One* (2009) 4(11):e7798. doi: 10.1371/journal.pone.0007798

39. Nangole FW, Ouyang K, Anzala O, Ogengo J, Agak GW. Multiple cytokines elevated in patients with keloids: Is it an indication of auto-inflammatory disease? *J Inflammation Res* (2021) 14:2465–70. doi: 10.2147/jir.S312091

40. Ogawa R, Okai K, Tokumura F, Mori K, Ohmori Y, Huang C, et al. The relationship between skin Stretching/Contraction and pathologic scarring: The important role of mechanical forces in keloid generation. *Wound Repair Regener* (2012) 20(2):149–57. doi: 10.1111/j.1524-475X.2012.00766.x

41. Song H, Liu T, Wang W, Pang H, Zhou Z, Lv Y, et al. Tension enhances cell proliferation and collagen synthesis by upregulating expressions of integrin αvβ3 in human keloid-derived mesenchymal stem cells. *Life Sci* (2019) 219:272–82. doi: 10.1016/j.lfs.2018.12.042

42. Zhou B, Gao Z, Liu W, Wu X, Wang W. Important role of mechanical microenvironment on macrophage dysfunction during keloid pathogenesis. *Exp Dermatol* (2022) 31(3):375–80. doi: 10.1111/exd.14473

43. Feng F, Liu M, Pan L, Wu J, Wang C, Yang L, et al. Biomechanical regulatory factors and therapeutic targets in keloid fibrosis. *Front Pharmacol* (2022) 13:906212. doi: 10.3389/fphar.2022.906212

44. Zhang Q, Oh CK, Messadi DV, Duong HS, Kelly AP, Soo C, et al. Hypoxia-induced hif-1 alpha accumulation is augmented in a co-culture of keloid fibroblasts and human mast cells: Involvement of Erk1/2 and pi-3k/Akt. *Exp Cell Res* (2006) 312 (2):145–55. doi: 10.1016/j.yexcr.2005.10.006

45. Ma X, Chen J, Xu B, Long X, Qin H, Zhao RC, et al. Keloid-derived keratinocytes acquire a fibroblast-like appearance and an enhanced invasive capacity in a hypoxic microenvironment in vitro. *Int J Mol Med* (2015) 35(5):1246–56. doi: 10.3892/ijmm.2015.2135

46. Lei R, Li J, Liu F, Li W, Zhang S, Wang Y, et al. Hif-1α promotes the keloid development through the activation of tgf-β/Smad and Tlr4/Myd88/Nf-κb pathways. *Cell Cycle* (2019) 18(23):3239–50. doi: 10.1080/15384101.2019.1670508

47. Lin X, Wang Y, Jiang Y, Xu M, Pang Q, Sun J, et al. Sumoylation enhances the activity of the tgf-β/Smad and hif-1 signaling pathways in keloids. *Life Sci* (2020) 255:117859. doi: 10.1016/j.lfs.2020.117859

48. Lee SY, Lee AR, Choi JW, Lee CR, Cho KH, Lee JH, et al. Il-17 induces autophagy dysfunction to promote inflammatory cell death and fibrosis in keloid fibroblasts *Via* the Stat3 and hif-1α dependent signaling pathways. *Front Immunol* (2022) 13:888719. doi: 10.3389/fimmu.2022.888719

49. Fong EP, Bay BH. Keloids – the sebum hypothesis revisited. *Med Hypotheses* (2002) 58(4):264–9. doi: 10.1054/mehy.2001.1426

50. Li Q, Qin Z, Nie F, Bi H, Zhao R, Pan B, et al. Metabolic reprogramming in keloid fibroblasts: Aerobic glycolysis and a novel therapeutic strategy. *Biochem Biophys Res Commun* (2018) 496(2):641–7. doi: 10.1016/j.bbrc.2018.01.068

51. Onoufriadis A, Hsu CK, Ainali C, Ung CY, Rashidghamat E, Yang HS, et al. Time series integrative analysis of rna sequencing and microrna expression data reveals key biologic wound healing pathways in keloid-prone individuals. *J Invest Dermatol* (2018) 138(12):2690–3. doi: 10.1016/j.jid.2018.05.017

52. Marneros AG, Norris JEC, Olsen BR, Reichenberger E. Clinical genetics of familial keloids. *Arch Dermatol* (2001) 137(11):1429–34. doi: 10.1001/archderm.137.11.1429

53. Clark JA, Turner ML, Howard L, Stanescu H, Kleta R, Kopp JB. Description of familial keloids in five pedigrees: Evidence for autosomal dominant inheritance and phenotypic heterogeneity. *BMC Dermatol* (2009) 9:8. doi: 10.1186/1471-5945-9-8

54. Chen Y, Gao JH, Liu XJ, Yan X, Song M. Characteristics of occurrence for han chinese familial keloids. *Burns* (2006) 32(8):1052–9. doi: 10.1016/j.burns.2006.04.014

55. Abdu Allah AMK, Mohammed KI, Farag AGA, Hagag MM, Essam M, Tayel NR. Interleukin-6 serum level and gene polymorphism in keloid patients. *Cell Mol Biol (Noisy-le-grand)* (2019) 65(5):43–8. doi: 10.14715/cmb/2019.65.5.7

56. Zhu XJ, Li WZ, Li H, Fu CQ, Liu J. Association of interleukin-6 gene polymorphisms and circulating levels with keloid scars in a chinese han population. *Genet Mol Res* (2017) 16(2):gmr16029110. doi: 10.4238/gmr16029110

57. Tosa M, Watanabe A, Ghazizadeh M. Il-6 polymorphism and susceptibility to keloid formation in a japanese population. *J Invest Dermatol* (2016) 136(5):1069–72. doi: 10.1016/j.jid.2016.01.019

58. Larouche J, Sheoran S, Maruyama K, Martino MM. Immune regulation of skin wound healing: Mechanisms and novel therapeutic targets. *Adv Wound Care (New Rochelle)* (2018) 7(7):209–31. doi: 10.1089/wound.2017.0761

59. Wilgus TA. Immune cells in the healing skin wound: Influential players at each stage of repair. *Pharmacol Res* (2008) 58(2):112–6. doi: 10.1016/j.phrs.2008.07.009

60. Yagi KI, Dafalla AA, Osman AA. Does an immune reaction to sebum in wounds cause keloid scars? beneficial effect of desensitisation. *Br J Plast Surg* (1979) 32(3):223–5. doi: 10.1016/s0007-1226(79)90037-7

61. Bloch EF, Hall MGJr., Denson MJ, Slay-Solomon V. General immune reactivity in keloid patients. *Plast Reconstr Surg* (1984) 73(3):448–51. doi: 10.1097/00006534-198403000-00020

62. Smith CJ, Smith JC, Finn MC. The possible role of mast cells (Allergy) in the production of keloid and hypertrophic scarring. *J Burn Care Rehabil* (1987) 8(2):126–31. doi: 10.1097/00004630-198703000-00008

63. Kazeem AA. The immunological aspects of keloid tumor formation. *J Surg Oncol* (1988) 38(1):16–8. doi: 10.1002/jso.2930380106

64. Jin Q, Gui L, Niu F, Yu B, Lauda N, Liu J, et al. Macrophages in keloid are potent at promoting the differentiation and function of regulatory t cells. *Exp Cell Res* (2018) 362(2):472–6. doi: 10.1016/j.yexcr.2017.12.011

65. Chen Y, Jin Q, Fu X, Qiao J, Niu F. Connection between t regulatory cell enrichment and collagen deposition in keloid. *Exp Cell Res* (2019) 383(2):111549. doi: 10.1016/j.yexcr.2019.111549

66. Wilgus TA, Wulff BC. The importance of mast cells in dermal scarring. *Adv Wound Care (New Rochelle)* (2014) 3(4):356–65. doi: 10.1089/wound.2013.0457

67. Arbi S, Eksteen EC, Oberholzer HM, Taute H, Bester MJ. Premature collagen fibril formation, fibroblast-mast cell interactions and mast cell-mediated phagocytosis of collagen in keloids. *Ultrastruct Pathol* (2015) 39(2):95–103. doi: 10.3109/01913123.2014.981326

68. Ud-Din S, Wilgus TA, Bayat A. Mast cells in skin scarring: A review of animal and human research. *Front Immunol* (2020) 11:552205. doi: 10.3389/fimmu.2020.552205

69. Li X, Wang Y, Yuan B, Yang H, Qiao L. Status of M1 and M2 type macrophages in keloid. *Int J Clin Exp Pathol* (2017) 10(11):11098–105.

70. Seoudy WM, Mohy El Dien SM, Abdel Reheem TA, Elfangary MM, Erfan MA. Macrophages of the M1 and M2 types play a role in keloids pathogenesis. *Int Wound J* (2022) 20(1):38–45. doi: 10.1111/iwj.13834

71. Xu X, Gu S, Huang X, Ren J, Gu Y, Wei C, et al. The role of macrophages in the formation of hypertrophic scars and keloids. *Burns Trauma* (2020) 8:tkaa006. doi: 10.1093/burnst/tkaa006

72. Feng C, Shan M, Xia Y, Zheng Z, He K, Wei Y, et al. Single-cell rna sequencing reveals distinct immunology profiles in human keloid. *Front Immunol* (2022) 13:940645. doi: 10.3389/fimmu.2022.940645

73. Jiao H, Fan J, Cai J, Pan B, Yan L, Dong P, et al. Analysis of characteristics similar to autoimmune disease in keloid patients. *Aesthetic Plast Surg* (2015) 39(5):818–25. doi: 10.1007/s00266-015-0542-4

74. Xia Y, Wang Y, Xiao Y, Shan M, Hao Y, Zhang L. Identification of a diagnostic signature and immune cell infiltration characteristics in keloids. *Front Mol Biosci* (2022) 9:879461. doi: 10.3389/fmolb.2022.879461

75. Yin X, Bu W, Fang F, Ren K, Zhou B. Keloid biomarkers and their correlation with immune infiltration. *Front Genet* (2022) 13:784073. doi: 10.3389/fgene.2022.784073

76. Nang'ole WF, Omu A, Ogeng'o JA, Agak GW. Do mesenchymal stem cells influence keloid recurrence? *Stem Cells Cloning: Adv Appl* (2022) 15:77–84. doi: 10.2147/SCCAA.S373551

77. Hahn JM, McFarland KL, Combs KA, Powell HM, Supp DM. Myofibroblasts are not characteristic features of keloid lesions. *Plast Reconstr Surg Glob Open* (2022) 10 (11):e4680. doi: 10.1097/gox.0000000000004680

78. Shan M, Liu H, Song K, Liu S, Hao Y, Wang Y. Immune-related gene expression in skin, inflamed and keloid tissue from patients with keloids. *Oncol Lett* (2022) 23 (2):72. doi: 10.3892/ol.2022.13192

79. Li Y, Li M, Qu C, Li Y, Tang Z, Zhou Z, et al. The polygenic map of keloid fibroblasts reveals fibrosis-associated gene alterations in inflammation and immune responses. *Front Immunol* (2021) 12:810290. doi: 10.3389/fimmu.2021.810290

80. Yuan B, Miao L, Mei D, Li L, Hu Z. A signature of genes featuring Fgf11 revealed aberrant fibroblast activation and immune infiltration properties in keloid tissue. *Emerg Med Int* (2022) 2022:4452687. doi: 10.1155/2022/4452687

81. Diaz A, Tan K, He H, Xu H, Cueto I, Pavel AB, et al. Keloid lesions show increased il-4/Il-13 signaling and respond to Th2-targeting dupilumab therapy. *J Eur Acad Dermatol Venereol* (2020) 34(4):e161–e4. doi: 10.1111/jdv.16097

82. Maeda D, Kubo T, Kiya K, Kawai K, Matsuzaki S, Kobayashi D, et al. Periostin is induced by il-4/Il-13 in dermal fibroblasts and promotes Rhoa/Rock pathway-mediated tgf-β1 secretion in abnormal scar formation. *J Plast Surg Handb Surg* (2019) 53(5):288–94. doi: 10.1080/2000656x.2019.1612752

83. Oriente A, Fedarko NS, Pacocha SE, Huang SK, Lichtenstein LM, Essayan DM. Interleukin-13 modulates collagen homeostasis in human skin and keloid fibroblasts. *J Pharmacol Exp Ther* (2000) 292(3):988–94.

84. Shan M, Liu H, Hao Y, Meng T, Feng C, Song K, et al. Il-4 and Ccr7 play an important role in the development of keloids in patients with a family history. *Am J Transl Res* (2022) 14(5):3381–94. doi: 10.3390/ijms23168862

85. Wu J, Del Duca E, Espino M, Gontzes A, Cueto I, Zhang N, et al. Rna sequencing keloid transcriptome associates keloids with Th2, Th1, Th17/Th22, and Jak3-skewing. *Front Immunol* (2020) 11:597741. doi: 10.3389/fimmu.2020.597741

86. Hajdarbegovic E, Bloem A, Balak D, Thio B, Nijsten T. The association between atopic disorders and keloids: A case-control study. *Indian J Dermatol* (2015) 60(6):635. doi: 10.4103/0019-5154.169144

87. Zhang D, Li B, Zhao M. Therapeutic strategies by regulating interleukin family to suppress inflammation in hypertrophic scar and keloid. *Front Pharmacol* (2021) 12:667763. doi: 10.3389/fphar.2021.667763

88. Do DV, Ong CT, Khoo YT, Carbone A, Lim CP, Wang S, et al. Interleukin-18 system plays an important role in keloid pathogenesis *Via* epithelial-mesenchymal interactions. *Br J Dermatol* (2012) 166(6):1275–88. doi: 10.1111/j.1365-2133.2011.10721.x

89. Lee SY, Kim EK, Seo HB, Choi JW, Yoo JH, Jung KA, et al. Il-17 induced stromal cell-derived factor-1 and profibrotic factor in keloid-derived skin fibroblasts *Via* the Stat3 pathway. *Inflammation* (2020) 43(2):664–72. doi: 10.1007/s10753-019-01148-1

90. Ghazizadeh M, Tosa M, Shimizu H, Hyakusoku H, Kawanami O. Functional implications of the il-6 signaling pathway in keloid pathogenesis. *J Invest Dermatol* (2007) 127(1):98–105. doi: 10.1038/sj.jid.5700564

91. Uitto J. Il-6 signaling pathway in keloids: A target for pharmacologic intervention? *J Invest Dermatol* (2007) 127(1):6–8. doi: 10.1038/sj.jid.5700604

92. Tosa M, Ghazizadeh M, Shimizu H, Hirai T, Hyakusoku H, Kawanami O. Global gene expression analysis of keloid fibroblasts in response to electron beam irradiation reveals the involvement of interleukin-6 pathway. *J Invest Dermatol* (2005) 124(4):704–13. doi: 10.1111/j.0022-202X.2005.23592.x

93. Shi CK, Zhao YP, Ge P, Huang GB. Therapeutic effect of interleukin-10 in keloid fibroblasts by suppression of tgf-β/Smad pathway. *Eur Rev Med Pharmacol Sci* (2019) 23(20):9085–92. doi: 10.26355/eurrev_201910_19311

94. Li Q, Cheng F, Zhou K, Fang L, Wu J, Xia Q, et al. Increased sensitivity to tnf-α promotes keloid fibroblast hyperproliferation by activating the nf-κb, jnk and P38 mapk pathways. *Exp Ther Med* (2021) 21(5):502. doi: 10.3892/etm.2021.9933

95. Khattab FM, Samir MA. Correlation between serum il 37 levels with keloid severity. *J Cosmet Dermatol* (2020) 19(9):2428–31. doi: 10.1111/jocd.13290

96. Zhao Y, Shi J, Lyu L. Critical role and potential therapeutic efficacy of interleukin-37 in the pathogenesis of keloid scarring. *J Cosmet Dermatol* (2020) 19 (7):1805–6. doi: 10.1111/jocd.13357

97. Liu X, Chen W, Zeng Q, Ma B, Li Z, Meng T, et al. Single-cell rna-sequencing reveals lineage-specific regulatory changes of fibroblasts and vascular endothelial cells in keloids. *J Invest Dermatol* (2022) 142(1):124–35.e11. doi: 10.1016/j.jid.2021.06.010

98. Shim J, Oh SJ, Yeo E, Park JH, Bae JH, Kim SH, et al. Integrated analysis of single-cell and spatial transcriptomics in keloids: Highlights on fibrovascular interactions in keloid pathogenesis. *J Invest Dermatol* (2022) 142(8):2128–39.e11. doi: 10.1016/j.jid.2022.01.017

99. Xie J, Chen L, Cao Y, Wu D, Xiong W, Zhang K, et al. Single-cell sequencing analysis and weighted co-expression network analysis based on public databases identified that tnc is a novel biomarker for keloid. *Front Immunol* (2021) 12:783907. doi: 10.3389/fimmu.2021.783907

100. Jones LR, Young W, Divine G, Datta I, Chen KM, Ozog D, et al. Genome-wide scan for methylation profiles in keloids. *Dis Markers* (2015) 2015:943176. doi: 10.1155/2015/943176

101. Xu H, Zhu Z, Hu J, Sun J, Wo Y, Wang X, et al. Downregulated cytotoxic Cd8 (+) t-cell identifies with the Nkg2a-soluble hla-e axis as a predictive biomarker and potential therapeutic target in keloids. *Cell Mol Immunol* (2022) 19(4):527–39. doi: 10.1038/s41423-021-00834-1

102. Eishi K, Bae SJ, Ogawa F, Hamasaki Y, Shimizu K, Katayama I. Silicone gel sheets relieve pain and pruritus with clinical improvement of keloid: Possible target of mast cells. *J Dermatolog Treat* (2003) 14(4):248–52. doi: 10.1080/09546630310016808

103. Shaker SA, Ayuob NN, Hajrah NH. Cell talk: A phenomenon observed in the keloid scar by immunohistochemical study. *Appl Immunohistochem Mol Morphol* (2011) 19(2):153–9. doi: 10.1097/PAI.0b013e3181efa2ef

104. Zhang Q, Kelly AP, Wang L, French SW, Tang X, Duong HS, et al. Green tea extract and (-)-Epigallocatechin-3-Gallate inhibit mast cell-stimulated type i collagen expression in keloid fibroblasts *Via* blocking pi-3k/Akt signaling pathways. *J Invest Dermatol* (2006) 126(12):2607–13. doi: 10.1038/sj.jid.5700472

105. Ammendola M, Leporini C, Marech I, Gadaleta CD, Scognamillo G, Sacco R, et al. Targeting mast cells tryptase in tumor microenvironment: A potential antiangiogenetic strategy. *BioMed Res Int* (2014) 2014:154702. doi: 10.1155/2014/154702

106. Dong X, Zhang C, Ma S, Wen H. Mast cell chymase in keloid induces profibrotic response *Via* transforming growth factor-β1/Smad activation in keloid fibroblasts. *Int J Clin Exp Pathol* (2014) 7(7):3596–607.

107. Wang R, Chen J, Zhang Z, Cen Y. Role of chymase in the local renin-angiotensin system in keloids: Inhibition of chymase may be an effective therapeutic approach to treat keloids. *Drug Des Devel Ther* (2015) 9:4979–88. doi: 10.2147/dddt.S87842

108. Shiota N, Kakizoe E, Shimoura K, Tanaka T, Okunishi H. Effect of mast cell chymase inhibitor on the development of scleroderma in tight-skin mice. *Br J Pharmacol* (2005) 145(4):424–31. doi: 10.1038/sj.bjp.0706209

109. Takai S, Jin D. Improvement of cardiovascular remodelling by chymase inhibitor. *Clin Exp Pharmacol Physiol* (2016) 43(4):387–93. doi: 10.1111/1440-1681.12549

110. Takai S, Jin D. Chymase as a possible therapeutic target for amelioration of non-alcoholic steatohepatitis. *Int J Mol Sci* (2020) 21(20):7543. doi: 10.3390/ijms21207543

111. Gallant-Behm CL, Hildebrand KA, Hart DA. The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red duroc pigs. *Wound Repair Regener* (2008) 16(2):226–33. doi: 10.1111/j.1524-475X.2008.00363.x

112. Mukhopadhyay A, Do DV, Ong CT, Khoo YT, Masilamani J, Chan SY, et al. The role of stem cell factor and c-kit in keloid pathogenesis: Do tyrosine kinase inhibitors have a potential therapeutic role? *Br J Dermatol* (2011) 164(2):372–86. doi: 10.1111/j.1365-2133.2010.10035.x

113. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* (2016) 44(3):450–62. doi: 10.1016/j.immuni.2016.02.015

114. Fukui S, Iwamoto N, Takatani A, Igawa T, Shimizu T, Umeda M, et al. M1 and M2 monocytes in rheumatoid arthritis: A contribution of imbalance of M1/M2 monocytes to osteoclastogenesis. *Front Immunol* (2017) 8:1958. doi: 10.3389/fimmu.2017.01958

115. Hesketh M, Sahin KB, West ZE, Murray RZ. Macrophage phenotypes regulate scar formation and chronic wound healing. *Int J Mol Sci* (2017) 18(7):1545. doi: 10.3390/ijms18071545

116. Clark RA, Kupper TS. Il-15 and dermal fibroblasts induce proliferation of natural regulatory t cells isolated from human skin. *Blood* (2006) 109(1):194–202. doi: 10.1182/blood-2006-02-002873

117. Chen Z, Zhou L, Won T, Gao Z, Wu X, Lu L. Characterization of Cd45ro+ memory t lymphocytes in keloid disease. *Br J Dermatol* (2018) 178(4):940–50. doi: 10.1111/bjd.16173

118. Turner JA, Stephen-Victor E, Wang S, Rivas MN, Abdel-Gadir A, Harb H, et al. Regulatory t cell-derived tgf-β1 controls multiple checkpoints governing allergy and autoimmunity. *Immunity* (2020) 53(6):1202–14.e6. doi: 10.1016/j.immuni.2020.10.002

119. Flavell RA, Sanjabi S, Wrzesinski SH, Licona-Limón P. The polarization of immune cells in the tumour environment by tgfbeta. *Nat Rev Immunol* (2010) 10 (8):554–67. doi: 10.1038/nri2808

120. Ganeshan K, Bryce PJ. Regulatory t cells enhance mast cell production of il-6 *Via* surface-bound tgf-β. *J Immunol* (2012) 188(2):594–603. doi: 10.4049/jimmunol.1102389

121. Fickenscher H, Hör S, Küpers H, Knappe A, Wittmann S, Sticht H. The interleukin-10 family of cytokines. *Trends Immunol* (2002) 23(2):89–96. doi: 10.1016/S1471-4906(01)02149-4

122. Hasegawa T, Nakao A, Sumiyoshi K, Tsuboi R, Ogawa H. Ifn-gamma fails to antagonize fibrotic effect of tgf-beta on keloid-derived dermal fibroblasts. *J Dermatol Sci* (2003) 32(1):19–24. doi: 10.1016/s0923-1811(03)00044-6

123. Villalta SA, Rosenthal W, Martinez L, Kaur A, Sparwasser T, Tidball JG, et al. Regulatory t cells suppress muscle inflammation and injury in muscular dystrophy. *Sci Transl Med* (2014) 6(258):258ra142. doi: 10.1126/scitranslmed.3009925

124. Ariotti S, Hogenbirk MA, Dijkgraaf FE, Visser LL, Hoekstra ME, Song JY, et al. T cell memory. skin-resident memory $Cd8^+$ t cells trigger a state of tissue-wide pathogen alert. *Science* (2014) 346(6205):101–5. doi: 10.1126/science.1254803

125. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, et al. Anti-Nkg2a mab is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both t and nk cells. *Cell* (2018) 175(7):1731–43.e13. doi: 10.1016/j.cell.2018.10.014

126. McMahon CW, Zajac AJ, Jamieson AM, Corral L, Hammer GE, Ahmed R, et al. Viral and bacterial infections induce expression of multiple nk cell receptors in responding Cd8(+) t cells. *J Immunol* (2002) 169(3):1444–52. doi: 10.4049/jimmunol.169.3.1444

127. Mingari MC, Ponte M, Bertone S, Schiavetti F, Vitale C, Bellomo R, et al. Hla class i-specific inhibitory receptors in human t lymphocytes: Interleukin 15-induced expression of Cd94/Nkg2a in superantigen- or alloantigen-activated Cd8+ t cells. *Proc Natl Acad Sci U.S.A.* (1998) 95(3):1172–7. doi: 10.1073/pnas.95.3.1172

128. Bertone S, Schiavetti F, Bellomo R, Vitale C, Ponte M, Moretta L, et al. Transforming growth factor-Beta-Induced expression of Cd94/Nkg2a inhibitory receptors in human t lymphocytes. *Eur J Immunol* (1999) 29(1):23–9. doi: 10.1002/(sici)1521-4141(199901)29:01<23::Aid-immu23>3.0.Co;2-y

129. Galot R, Le Tourneau C, Saada-Bouzid E, Daste A, Even C, Debruyne P, et al. A phase ii study of monalizumab in patients with Recurrent/Metastatic squamous cell carcinoma of the head and neck: The I1 cohort of the eortc-Hncg-1559 upstream trial. *Eur J Cancer* (2021) 158:17–26. doi: 10.1016/j.ejca.2021.09.003

130. Herbst RS, Majem M, Barlesi F, Carcereny E, Chu Q, Monnet I, et al. Coast: An open-label, phase ii, multidrug platform study of durvalumab alone or in combination with oleclumab or monalizumab in patients with unresectable, stage iii non-Small-Cell lung cancer. *J Clin Oncol* (2022) 40(29):Jco2200227. doi: 10.1200/jco.22.00227

131. Tinker AV, Hirte HW, Provencher D, Butler M, Ritter H, Tu D, et al. Dose-ranging and cohort-expansion study of monalizumab (Iph2201) in patients with advanced gynecologic malignancies: A trial of the canadian cancer trials group (Cctg): Ind221. *Clin Cancer Res* (2019) 25(20):6052–60. doi: 10.1158/1078-0432.Ccr-19-0298

132. Onodera M, Ueno M, Ito O, Suzuki S, Igawa HH, Sakamoto H. Factor xiiia-positive dermal dendritic cells in keloids and hypertrophic and mature scars. *Pathol Int* (2007) 57(6):337–42. doi: 10.1111/j.1440-1827.2007.02105.x

133. Altman DA, Nickoloff BJ, Fivenson DP. Differential expression of factor xiiia and Cd34 in cutaneous mesenchymal tumors. *J Cutan Pathol* (1993) 20(2):154–8. doi: 10.1111/j.1600-0560.1993.tb00233.x

134. Luo X, Chen J, Yang H, Hu X, Alphonse MP, Shen Y, et al. Dendritic cell immunoreceptor drives atopic dermatitis by modulating oxidized camkii-involved mast cell activation. *JCI Insight* (2022) 7(5):e152559. doi: 10.1172/jci.insight.152559

135. Yoon J, Leyva-Castillo JM, Wang G, Galand C, Oyoshi MK, Kumar L, et al. Il-23 induced in keratinocytes by endogenous Tlr4 ligands polarizes dendritic cells to drive il-22 responses to skin immunization. *J Exp Med* (2016) 213(10):2147–66. doi: 10.1084/jem.20150376

136. O'Reilly S, Ciechomska M, Cant R, van Laar JM. Interleukin-6 (Il-6) trans signaling drives a Stat3-dependent pathway that leads to hyperactive transforming growth factor-β (Tgf-β) signaling promoting Smad3 activation and fibrosis *Via* gremlin protein. *J Biol Chem* (2014) 289(14):9952–60. doi: 10.1074/jbc.M113.545822

137. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J Dermatol Sci* (2001) 27(2):140–6. doi: 10.1016/s0923-1811(01)00128-1

138. Jones SA. Directing transition from innate to acquired immunity: Defining a role for il-6. *J Immunol* (2005) 175(6):3463–8. doi: 10.4049/jimmunol.175.6.3463

139. Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, et al. Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. *Immunity* (2001) 14(6):705–14. doi: 10.1016/s1074-7613(01)00151-0

140. Fielding CA, Jones GW, McLoughlin RM, McLeod L, Hammond VJ, Uceda J, et al. Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity* (2014) 40(1):40–50. doi: 10.1016/j.immuni.2013.10.022

141. Johnson BZ, Stevenson AW, Prêle CM, Fear MW, Wood FM. The role of il-6 in skin fibrosis and cutaneous wound healing. *Biomedicines* (2020) 8(5):101. doi: 10.3390/biomedicines8050101

142. Tzavlaki K, Moustakas A. Tgf-β signaling. *Biomolecules* (2020) 10(3):487. doi: 10.3390/biom10030487

143. Wang H, Chen Z, Li XJ, Ma L, Tang YL. Anti-inflammatory cytokine tsg-6 inhibits hypertrophic scar formation in a rabbit ear model. *Eur J Pharmacol* (2015) 751:42–9. doi: 10.1016/j.ejphar.2015.01.040

144. Gieseck RL3rd, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol* (2018) 18(1):62–76. doi: 10.1038/nri.2017.90

145. Nguyen JK, Austin E, Huang A, Mamalis A, Jagdeo J. The il-4/Il-13 axis in skin fibrosis and scarring: Mechanistic concepts and therapeutic targets. *Arch Dermatol Res* (2020) 312(2):81–92. doi: 10.1007/s00403-019-01972-3

146. Salmon-Ehr V, Ramont L, Godeau G, Birembaut P, Guenounou M, Bernard P, et al. Implication of interleukin-4 in wound healing. *Lab Invest* (2000) 80(8):1337–43. doi: 10.1038/labinvest.3780141

147. Ong C, Wong C, Roberts CR, Teh HS, Jirik FR. Anti-Il-4 treatment prevents dermal collagen deposition in the tight-skin mouse model of scleroderma. *Eur J Immunol* (1998) 28(9):2619–29. doi: 10.1002/(sici)1521-4141(199809)28:09<2619::Aid-immu2619>3.0.Co;2-m

148. Luk K, Fakhoury J, Ozog D. Nonresponse and progression of diffuse keloids to dupilumab therapy. *J Drugs Dermatol* (2022) 21(2):197–9. doi: 10.36849/jdd.6252

149. Wong AJS, Song EJ. Dupilumab as an adjuvant treatment for keloid-associated symptoms. *JAAD Case Rep* (2021) 13:73–4. doi: 10.1016/j.jdcr.2021.04.034

150. Minutti CM, Knipper JA, Allen JE, Zaiss DM. Tissue-specific contribution of macrophages to wound healing. *Semin Cell Dev Biol* (2017) 61:3–11. doi: 10.1016/j.semcdb.2016.08.006

151. Van Dyken SJ, Locksley RM. Interleukin-4- and interleukin-13-Mediated alternatively activated macrophages: Roles in homeostasis and disease. *Annu Rev Immunol* (2013) 31:317–43. doi: 10.1146/annurev-immunol-032712-095906

152. Su S, Zhao Q, He C, Huang D, Liu J, Chen F, et al. Mir-142-5p and mir-130a-3p are regulated by il-4 and il-13 and control profibrogenic macrophage program. *Nat Commun* (2015) 6:8523. doi: 10.1038/ncomms9523

153. Crawford J, Nygard K, Gan BS, O'Gorman DB. Periostin induces fibroblast proliferation and myofibroblast persistence in hypertrophic scarring. *Exp Dermatol* (2015) 24(2):120–6. doi: 10.1111/exd.12601

154. Yamaguchi Y, Ono J, Masuoka M, Ohta S, Izuhara K, Ikezawa Z, et al. Serum periostin levels are correlated with progressive skin sclerosis in patients with systemic sclerosis. *Br J Dermatol* (2013) 168(4):717–25. doi: 10.1111/bjd.12117

155. Fuschiotti P, Medsger TAJr., Morel PA. Effector Cd8+ t cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. *Arthritis Rheum* (2009) 60(4):1119–28. doi: 10.1002/art.24432

156. Peng D, Fu M, Wang M, Wei Y, Wei X. Targeting tgf-β signal transduction for fibrosis and cancer therapy. *Mol Cancer* (2022) 21(1):104. doi: 10.1186/s12943-022-01569-x

157. Chen Z, Gao Z, Xia L, Wang X, Lu L, Wu X. Dysregulation of Dpp4-Cxcl12 balance by tgf-β1/Smad pathway promotes Cxcr4(+) inflammatory cell infiltration in keloid scars. *J Inflammation Res* (2021) 14:4169–80. doi: 10.2147/jir.S326385

158. Younai S, Nichter LS, Wellisz T, Reinisch J, Nimni ME, Tuan TL. Modulation of collagen synthesis by transforming growth factor-beta in keloid and hypertrophic scar fibroblasts. *Ann Plast Surg* (1994) 33(2):148–51. doi: 10.1097/00000637-199408000-00005

159. Babu M, Diegelmann R, Oliver N. Keloid fibroblasts exhibit an altered response to tgf-beta. *J Invest Dermatol* (1992) 99(5):650–5. doi: 10.1111/1523-1747.ep12668146

160. Chodon T, Sugihara T, Igawa HH, Funayama E, Furukawa H. Keloid-derived fibroblasts are refractory to fas-mediated apoptosis and neutralization of autocrine transforming growth factor-Beta1 can abrogate this resistance. *Am J Pathol* (2000) 157(5):1661–9. doi: 10.1016/s0002-9440(10)64803-1

161. Lee CH, Hong CH, Chen YT, Chen YC, Shen MR. Tgf-Beta1 increases cell rigidity by enhancing expression of smooth muscle actin: Keloid-derived fibroblasts as a model for cellular mechanics. *J Dermatol Sci* (2012) 67(3):173–80. doi: 10.1016/j.jdermsci.2012.06.004

162. Hasegawa T, Nakao A, Sumiyoshi K, Tsuchihashi H, Ogawa H. Sb-431542 inhibits tgf-Beta-Induced contraction of collagen gel by normal and keloid fibroblasts. *J Dermatol Sci* (2005) 39(1):33–8. doi: 10.1016/j.jdermsci.2005.01.013

163. Jiao H, Dong P, Yan L, Yang Z, Lv X, Li Q, et al. Tgf-β1 induces polypyrimidine tract-binding protein to alter fibroblasts proliferation and fibronectin deposition in keloid. *Sci Rep* (2016) 6:38033. doi: 10.1038/srep38033

164. Bran GM, Goessler UR, Schardt C, Hormann K, Riedel F, Sadick H. Effect of the abrogation of tgf-Beta1 by antisense oligonucleotides on the expression of tgf-Beta-Isoforms and their receptors i and ii in isolated fibroblasts from keloid scars. *Int J Mol Med* (2010) 25(6):915–21. doi: 10.3892/ijmm_00000422

165. Sadick H, Herberger A, Riedel K, Bran G, Goessler U, Hoermann K, et al. Tgf-Beta1 antisense therapy modulates expression of matrix metalloproteinases in keloid-derived fibroblasts. *Int J Mol Med* (2008) 22(1):55–60. doi: 10.3892/ijmm.22.1.55

166. Chin GS, Liu W, Peled Z, Lee TY, Steinbrech DS, Hsu M, et al. Differential expression of transforming growth factor-beta receptors i and ii and activation of smad 3 in keloid fibroblasts. *Plast Reconstr Surg* (2001) 108(2):423–9. doi: 10.1097/00006534-200108000-00022

167. Lee TY, Chin GS, Kim WJ, Chau D, Gittes GK, Longaker MT. Expression of transforming growth factor beta 1, 2, and 3 proteins in keloids. *Ann Plast Surg* (1999) 43(2):179–84.

168. Bock O, Yu H, Zitron S, Bayat A, Ferguson MW, Mrowietz U. Studies of transforming growth factors beta 1-3 and their receptors i and ii in fibroblast of keloids and hypertrophic scars. *Acta Derm Venereol* (2005) 85(3):216–20. doi: 10.1080/00015550410025453

169. Chu Y, Guo F, Li Y, Li X, Zhou T, Guo Y. A novel truncated tgf-beta receptor ii downregulates collagen synthesis and tgf-beta i secretion of keloid fibroblasts. *Connect Tissue Res* (2008) 49(2):92–8. doi: 10.1080/03008200801913924

170. Liu W, Cai Z, Wang D, Wu X, Cui L, Shang Q, et al. Blocking transforming growth factor-beta receptor signaling down-regulates transforming growth factor-Beta1 autoproduction in keloid fibroblasts. *Chin J Traumatol* (2002) 5(2):77–81.

171. Hahn JM, McFarland KL, Combs KA. Supp DM. partial epithelial-mesenchymal transition in keloid scars: Regulation of keloid keratinocyte gene expression by transforming growth factor-β1. *Burns Trauma* (2016) 4(1):30. doi: 10.1186/s41038-016-0055-7

172. Yan L, Cao R, Wang L, Liu Y, Pan B, Yin Y, et al. Epithelial-mesenchymal transition in keloid tissues and tgf-β1-Induced hair follicle outer root sheath keratinocytes. *Wound Repair Regener* (2015) 23(4):601–10. doi: 10.1111/wrr.12320

173. Xia W, Phan TT, Lim IJ, Longaker MT, Yang GP. Complex epithelial-mesenchymal interactions modulate transforming growth factor-beta expression in keloid-derived cells. *Wound Repair Regener* (2004) 12(5):546–56. doi: 10.1111/j.1067-1927.2004.012507.x

174. Wang XM, Liu XM, Wang Y, Chen ZY. Activating transcription factor 3 (Atf3) regulates cell growth, apoptosis, invasion and collagen synthesis in keloid fibroblast through transforming growth factor beta (Tgf-Beta)/Smad signaling pathway. *Bioengineered* (2021) 12(1):117–26. doi: 10.1080/21655979.2020.1860491

175. Nagar H, Kim S, Lee I, Kim S, Choi SJ, Piao S, et al. Downregulation of Cr6-interacting factor 1 suppresses keloid fibroblast growth *Via* the tgf-β/Smad signaling pathway. *Sci Rep* (2021) 11(1):500. doi: 10.1038/s41598-020-79785-y

176. Ma HL, Zhao XF, Chen GZ, Fang RH, Zhang FR. Silencing Nlrc5 inhibits extracellular matrix expression in keloid fibroblasts *Via* inhibition of transforming growth factor-β1/Smad signaling pathway. *BioMed Pharmacother* (2016) 83:1016–21. doi: 10.1016/j.biopha.2016.08.012

177. Jin SF, Wang YX, Xu N, Sun Q, Wang CC, Lv MZ, et al. High temperature requirement factor A1 (Htra1) regulates the activation of latent tgf-β1 in keloid fibroblasts. *Cell Mol Biol (Noisy-le-grand)* (2018) 64(1):107–10. doi: 10.14715/cmb/2018.64.2.19

178. Zhao YX, Ho CK, Xie Y, Chen YH, Li HZ, Zhang GY, et al. Calcimycin suppresses S100a4 expression and inhibits the stimulatory effect of transforming growth factor β1 on keloid fibroblasts. *Ann Plast Surg* (2018) 81(2):163–9. doi: 10.1097/sap.0000000000001502

179. Cui J, Jin S, Jin C, Jin Z. Syndecan-1 regulates extracellular matrix expression in keloid fibroblasts *Via* tgf-β1/Smad and mapk signaling pathways. *Life Sci* (2020) 254:117326. doi: 10.1016/j.lfs.2020.117326

180. Zhu HY, Bai WD, Li C, Zheng Z, Guan H, Liu JQ, et al. Knockdown of lncrna-atb suppresses autocrine secretion of tgf-β2 by targeting Znf217 *Via* mir-200c in keloid fibroblasts. *Sci Rep* (2016) 6:24728. doi: 10.1038/srep24728

181. Zhu HY, Bai WD, Li J, Tao K, Wang HT, Yang XK, et al. Peroxisome proliferator-activated receptor-Γ agonist troglitazone suppresses transforming growth factor-β1 signalling through mir-92b upregulation-inhibited axl expression in human keloid fibroblasts in vitro. *Am J Transl Res* (2016) 8(8):3460–70.

182. Yao X, Cui X, Wu X, Xu P, Zhu W, Chen X, et al. Tumor suppressive role of mir-1224-5p in keloid proliferation, apoptosis and invasion *Via* the tgf-β1/Smad3 signaling pathway. *Biochem Biophys Res Commun* (2018) 495(1):713–20. doi: 10.1016/j.bbrc.2017.10.070

183. Huang Y, Wang Y, Lin L, Wang P, Jiang L, Liu J, et al. Overexpression of mir-133a-3p inhibits fibrosis and proliferation of keloid fibroblasts by regulating Irf5 to inhibit the tgf-β/Smad2 pathway. *Mol Cell Probes* (2020) 52:101563. doi: 10.1016/j.mcp.2020.101563

184. Wu J, Fang L, Cen Y, Qing Y, Chen J, Li Z. Mir-21 regulates keloid formation by downregulating Smad7 *Via* the tgf-β/Smad signaling pathway. *J Burn Care Res* (2019) 40(6):809–17. doi: 10.1093/jbcr/irz089

185. Wu D, Zhou J, Tan M, Zhou Y. Linc01116 regulates proliferation, migration, and apoptosis of keloid fibroblasts by the tgf-β1/Smad3 signaling *Via* targeting mir-3141. *Anal Biochem* (2021) 627:114249. doi: 10.1016/j.ab.2021.114249

186. Huang H, Fu S, Liu D. Detection and analysis of the hedgehog signaling pathway-related long non-coding rna (Lncrna) expression profiles in keloid. *Med Sci Monit* (2018) 24:9032–44. doi: 10.12659/msm.911159

187. Guo L, Xu K, Yan H, Feng H, Chai L, Xu G. Expression profile of long noncoding rnas in human earlobe keloids: A microarray analysis. *BioMed Res Int* (2016) 2016:5893481. doi: 10.1155/2016/5893481

188. Liang X, Ma L, Long X, Wang X. Lncrna expression profiles and validation in keloid and normal skin tissue. *Int J Oncol* (2015) 47(5):1829–38. doi: 10.3892/ijo.2015.3177

189. Lin L, Wang Y, Liu W, Huang Y. Bambi inhibits skin fibrosis in keloid through suppressing tgf-β1-Induced hypernomic fibroblast cell proliferation and excessive accumulation of collagen i. *Int J Clin Exp Med* (2015) 8(8):13227–34.

190. Li Y, Liu H, Liang Y, Peng P, Ma X, Zhang X. Dkk3 regulates cell proliferation, apoptosis and collagen synthesis in keloid fibroblasts *Via* tgf-β1/Smad signaling pathway. *BioMed Pharmacother* (2017) 91:174–80. doi: 10.1016/j.biopha.2017.03.044

191. Zhou P, Shi L, Li Q, Lu D. Overexpression of Rack1 inhibits collagen synthesis in keloid fibroblasts *Via* inhibition of transforming growth factor-β1/Smad signaling pathway. *Int J Clin Exp Med* (2015) 8(9):15262–8.

192. Hu ZC, Shi F, Liu P, Zhang J, Guo D, Cao XL, et al. Tieg1 represses Smad7-mediated activation of tgf-β1/Smad signaling in keloid pathogenesis. *J Invest Dermatol* (2017) 137(5):1051–9. doi: 10.1016/j.jid.2016.12.019

193. Yan Q, Li B. Traf3ip2 regulated by Foxo4 affects fibroblast proliferation, migration, and extracellular matrix deposition in keloid through the tgf-β1/Smad pathway. *J Cosmet Dermatol* (2022) 21:5148–55. doi: 10.1111/jocd.15009

194. Cavalli G, Dinarello CA. Suppression of inflammation and acquired immunity by il-37. *Immunol Rev* (2018) 281(1):179–90. doi: 10.1111/imr.12605

195. Bulau AM, Nold MF, Li S, Nold-Petry CA, Fink M, Mansell A, et al. Role of caspase-1 in nuclear translocation of il-37, release of the cytokine, and il-37 inhibition of innate immune responses. *Proc Natl Acad Sci U.S.A.* (2014) 111(7):2650–5. doi: 10.1073/pnas.1324140111

196. Cavalli G, Tengesdal IW, Gresnigt M, Nemkov T, Arts RJW, Domínguez-Andrés J, et al. The anti-inflammatory cytokine interleukin-37 is an inhibitor of trained immunity. *Cell Rep* (2021) 35(1):108955. doi: 10.1016/j.celrep.2021.108955

197. Kim MS, Baek AR, Lee JH, Jang AS, Kim DJ, Chin SS, et al. Il-37 attenuates lung fibrosis by inducing autophagy and regulating tgf-β1 production in mice. *J Immunol* (2019) 203(8):2265–75. doi: 10.4049/jimmunol.1801515

198. Kuo YR, Wu WS, Wang FS. Flashlamp pulsed-dye laser suppressed tgf-Beta1 expression and proliferation in cultured keloid fibroblasts is mediated by mapk pathway. *Lasers Surg Med* (2007) 39(4):358–64. doi: 10.1002/lsm.20489

199. He S, Liu X, Yang Y, Huang W, Xu S, Yang S, et al. Mechanisms of transforming growth factor Beta(1)/Smad signalling mediated by mitogen-activated protein kinase pathways in keloid fibroblasts. *Br J Dermatol* (2010) 162(3):538–46. doi: 10.1111/j.1365-2133.2009.09511.x

200. Cui J, Li Z, Jin C, Jin Z. Knockdown of fibronectin extra domain b suppresses tgf-β1-Mediated cell proliferation and collagen deposition in keloid fibroblasts *Via* Akt/Erk signaling pathway. *Biochem Biophys Res Commun* (2020) 526(4):1131–7. doi: 10.1016/j.bbrc.2020.04.021

201. Zhang Q, Qian D, Tang DD, Liu J, Wang LY, Chen W, et al. Glabridin from glycyrrhiza glabra possesses a therapeutic role against keloid *Via* attenuating Pi3k/Akt and transforming growth factor-β1/Smad signaling pathways. *J Agric Food Chem* (2022) 70(35):10782–93. doi: 10.1021/acs.jafc.2c02045

202. Song R, Li G, Li S. Aspidin pb, a novel natural anti-fibrotic compound, inhibited fibrogenesis in tgf-β1-Stimulated keloid fibroblasts *Via* pi-3k/Akt and smad signaling pathways. *Chem Biol Interact* (2015) 238:66–73. doi: 10.1016/j.cbi.2015.06.005

203. Liang CJ, Yen YH, Hung LY, Wang SH, Pu CM, Chien HF, et al. Thalidomide inhibits fibronectin production in tgf-β1-Treated normal and keloid fibroblasts *Via* inhibition of the P38/Smad3 pathway. *Biochem Pharmacol* (2013) 85(11):1594–602. doi: 10.1016/j.bcp.2013.02.038

204. Xia W, Longaker MT, Yang GP. P38 map kinase mediates transforming growth factor-Beta2 transcription in human keloid fibroblasts. *Am J Physiol Regul Integr Comp Physiol* (2006) 290(3):R501–8. doi: 10.1152/ajpregu.00472.2005

205. Daian T, Ohtsuru A, Rogounovitch T, Ishihara H, Hirano A, Akiyama-Uchida Y, et al. Insulin-like growth factor-i enhances transforming growth factor-Beta-Induced extracellular matrix protein production through the P38/Activating transcription factor-2 signaling pathway in keloid fibroblasts. *J Invest Dermatol* (2003) 120(6):956–62. doi: 10.1046/j.1523-1747.2003.12143.x

206. Wang W, Qu M, Xu L, Wu X, Gao Z, Gu T, et al. Sorafenib exerts an anti-keloid activity by antagonizing tgf-β/Smad and Mapk/Erk signaling pathways. *J Mol Med (Berl)* (2016) 94(10):1181–94. doi: 10.1007/s00109-016-1430-3

207. Griffin MF, Borrelli MR, Garcia JT, Januszyk M, King M, Lerbs T, et al. Jun promotes hypertrophic skin scarring *Via* Cd36 in preclinical in vitro and in vivo models. *Sci Transl Med* (2021) 13(609):eabb3312. doi: 10.1126/scitranslmed.abb3312

208. Gao N, Lu L, Ma X, Liu Z, Yang S, Han G. Targeted inhibition of Yap/Taz alters the biological behaviours of keloid fibroblasts. *Exp Dermatol* (2022) 31(3):320–9. doi: 10.1111/exd.14466

209. Zhao MJ, Chen SY, Qu XY, Abdul-Fattah B, Lai T, Xie M, et al. Increased Cthrc1 activates normal fibroblasts and suppresses keloid fibroblasts by inhibiting tgf-β/Smad signal pathway and modulating yap subcellular location. *Curr Med Sci* (2018) 38(5):894–902. doi: 10.1007/s11596-018-1959-1

210. Hamburg-Shields E, DiNuoscio GJ, Mullin NK, Lafyatis R, Atit RP. Sustained β-catenin activity in dermal fibroblasts promotes fibrosis by up-regulating expression of extracellular matrix protein-coding genes. *J Pathol* (2015) 235(5):686–97. doi: 10.1002/path.4481

211. Sato M. Upregulation of the Wnt/Beta-catenin pathway induced by transforming growth factor-beta in hypertrophic scars and keloids. *Acta Derm Venereol* (2006) 86(4):300–7. doi: 10.2340/00015555-0101

212. Zanconato F, Cordenonsi M, Piccolo S. Yap and taz: A signalling hub of the tumour microenvironment. *Nat Rev Cancer* (2019) 19(8):454–64. doi: 10.1038/s41568-019-0168-y

213. Panciera T, Azzolin L, Cordenonsi M, Piccolo S. Mechanobiology of yap and taz in physiology and disease. *Nat Rev Mol Cell Biol* (2017) 18(12):758–70. doi: 10.1038/nrm.2017.87

214. Kiya K, Kubo T, Kawai K, Matsuzaki S, Maeda D, Fujiwara T, et al. Endothelial cell-derived endothelin-1 is involved in abnormal scar formation by dermal fibroblasts through Rhoa/Rho-kinase pathway. *Exp Dermatol* (2017) 26(8):705–12. doi: 10.1111/exd.13264

215. Mascharak S, desJardins-Park HE, Davitt MF, Griffin M, Borrelli MR, Moore AL, et al. Preventing engrailed-1 activation in fibroblasts yields wound regeneration without scarring. *Science* (2021) 372(6540):eaba2374. doi: 10.1126/science.aba2374

216. Clark RAF. To scar or not to scar. *N Engl J Med* (2021) 385(5):469–71. doi: 10.1056/NEJMcibr2107204

217. Lim CP, Phan TT, Lim IJ, Cao X. Stat3 contributes to keloid pathogenesis *Via* promoting collagen production, cell proliferation and migration. *Oncogene* (2006) 25 (39):5416–25. doi: 10.1038/sj.onc.1209531

218. Lim CP, Phan TT, Lim IJ, Cao X. Cytokine profiling and Stat3 phosphorylation in epithelial-mesenchymal interactions between keloid keratinocytes and fibroblasts. *J Invest Dermatol* (2009) 129(4):851–61. doi: 10.1038/jid.2008.337

219. Park G, Yoon BS, Moon JH, Kim B, Jun EK, Oh S, et al. Green tea polyphenol epigallocatechin-3-Gallate suppresses collagen production and proliferation in keloid fibroblasts *Via* inhibition of the Stat3-signaling pathway. *J Invest Dermatol* (2008) 128 (10):2429–41. doi: 10.1038/jid.2008.103

220. Lee YS, Liang YC, Wu P, Kulber DA, Tanabe K, Chuong CM, et al. Stat3 signalling pathway is implicated in keloid pathogenesis by preliminary transcriptome and open chromatin analyses. *Exp Dermatol* (2019) 28(4):480–4. doi: 10.1111/exd.13923

221. Vinaik R, Barayan D, Auger C, Abdullahi A, Jeschke MG. Regulation of glycolysis and the warburg effect in wound healing. *JCI Insight* (2020) 5(17):e138949. doi: 10.1172/jci.insight.138949

222. McCann KJ, Yadav M, Alishahedani ME, Freeman AF, Myles IA. Differential responses to folic acid in an established keloid fibroblast cell line are mediated by Jak1/2 and Stat3. *PloS One* (2021) 16(3):e0248011. doi: 10.1371/journal.pone.0248011

223. Hong YK, Wu CH, Lin YC, Huang YL, Hung KS, Pai TP, et al. Asc-J9 blocks cell proliferation and extracellular matrix production of keloid fibroblasts through inhibiting Stat3 signaling. *Int J Mol Sci* (2022) 23(10):5549. doi: 10.3390/ijms23105549

224. Zhou Y, Sun Y, Hou W, Ma L, Tao Y, Li D, et al. The Jak2/Stat3 pathway inhibitor, Ag490, suppresses the abnormal behavior of keloid fibroblasts. *In vitro. Int J Mol Med* (2020) 46(1):191–200. doi: 10.3892/ijmm.2020.4592

225. Moriana C, Moulinet T, Jaussaud R, Decker P. Jak inhibitors and systemic sclerosis: A systematic review of the literature. *Autoimmun Rev* (2022) 21(10):103168. doi: 10.1016/j.autrev.2022.103168

226. Kazemi T, Farah R, Hordinsky M. Oral janus kinase inhibitors may have a beneficial role in the treatment of keloidal scars: A case report. In press. (2020).

227. Wang Y, Yuan B, Qiao L, Yang H, Li X. Stat3 operates as a novel transcription factor that regulates Nedd4 in keloid. *Biochem Biophys Res Commun* (2019) 518 (4):638–43. doi: 10.1016/j.bbrc.2019.08.110

228. Farag AGA, Khaled HN, Hammam MA, Elshaib ME, Tayel NR, Hommos SEI, et al. Neuronal precursor cell expressed developmentally down regulated 4 (Nedd4) gene polymorphism contributes to keloid development in egyptian population. *Clin Cosmet Investig Dermatol* (2020) 13:649–56. doi: 10.2147/ccid.S253603

229. Zhao Y, Liu SL, Xie J, Ding MQ, Lu MZ, Zhang LF, et al. Nedd4 single nucleotide polymorphism Rs2271289 is associated with keloids in chinese han population. *Am J Transl Res* (2016) 8(2):544–55.

230. Ogawa R, Watanabe A, Than Naing B, Sasaki M, Fujita A, Akaishi S, et al. Associations between keloid severity and single-nucleotide polymorphisms: Importance of Rs8032158 as a biomarker of keloid severity. *J Invest Dermatol* (2014) 134(7):2041–3. doi: 10.1038/jid.2014.71

231. Chung S, Nakashima M, Zembutsu H, Nakamura Y. Possible involvement of Nedd4 in keloid formation; its critical role in fibroblast proliferation and collagen production. *Proc Jpn Acad Ser B Phys Biol Sci* (2011) 87(8):563–73. doi: 10.2183/pjab.87.563

232. Zhu G, Cai J, Zhang J, Zhao Y, Xu B. Abnormal nuclear factor (Nf)-kappab signal pathway and aspirin inhibits tumor necrosis factor alpha-induced nf-kappab activation in keloid fibroblasts. *Dermatol Surg* (2007) 33(6):697–708. doi: 10.1111/j.1524-4725.2007.33146.x

233. Niedermeyer J, Kriz M, Hilberg F, Garin-Chesa P, Bamberger U, Lenter MC, et al. Targeted disruption of mouse fibroblast activation protein. *Mol Cell Biol* (2000) 20 (3):1089–94. doi: 10.1128/mcb.20.3.1089-1094.2000

234. Fitzgerald AA, Weiner LM. The role of fibroblast activation protein in health and malignancy. *Cancer Metastasis Rev* (2020) 39(3):783–803. doi: 10.1007/s10555-020-09909-3

235. Dienus K, Bayat A, Gilmore BF, Seifert O. Increased expression of fibroblast activation protein-alpha in keloid fibroblasts: Implications for development of a novel treatment option. *Arch Dermatol Res* (2010) 302(10):725–31. doi: 10.1007/s00403-010-1084-x

236. Tran E, Chinnasamy D, Yu Z, Morgan RA, Lee CC, Restifo NP, et al. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J Exp Med* (2013) 210(6):1125–35. doi: 10.1084/jem.20130110

237. Roberts EW, Deonarine A, Jones JO, Denton AE, Feig C, Lyons SK, et al. Depletion of stromal cells expressing fibroblast activation protein-α from skeletal muscle and bone marrow results in cachexia and anemia. *J Exp Med* (2013) 210 (6):1137–51. doi: 10.1084/jem.20122344

238. Dorst DN, van Caam APM, Vitters EL, Walgreen B, Helsen MMA, Klein C, et al. Fibroblast activation protein targeted photodynamic therapy selectively kills activated skin fibroblasts from systemic sclerosis patients and prevents tissue contraction. *Int J Mol Sci* (2021) 22(23):12681. doi: 10.3390/ijms222312681

239. Mousavi MJ, Farhadi E, Vodjgani M, Karami J, Tahmasebi MN, Sharafat Vaziri A, et al. Role of fibroblast activation protein alpha in fibroblast-like synoviocytes of rheumatoid arthritis. *Iran J Allergy Asthma Immunol* (2021) 20(3):338–49. doi: 10.18502/ijaai.v20i3.6335

240. Schmidkonz C, Rauber S, Atzinger A, Agarwal R, Götz TI, Soare A, et al. Disentangling inflammatory from fibrotic disease activity by fibroblast activation protein imaging. *Ann Rheum Dis* (2020) 79(11):1485–91. doi: 10.1136/annrheumdis-2020-217408

241. Chen J, Zeng B, Yao H, Xu J. The effect of Tlr4/7 on the tgf-β-Induced smad signal transduction pathway in human keloid. *Burns* (2013) 39(3):465–72. doi: 10.1016/j.burns.2012.07.019

242. Bagabir RA, Syed F, Rautemaa R, McGrouther DA, Paus R, Bayat A. Upregulation of toll-like receptors (Tlrs) 6, 7, and 8 in keloid scars. *J Invest Dermatol* (2011) 131(10):2128–30. doi: 10.1038/jid.2011.163

243. Li J, Li Z, Wang S, Bi J, Huo R. Exosomes from human adipose-derived mesenchymal stem cells inhibit production of extracellular matrix in keloid fibroblasts *Via* downregulating transforming growth factor-β2 and notch-1 expression. *Bioengineered* (2022) 13(4):8515–25. doi: 10.1080/21655979.2022.2051838

244. Yuan R, Dai X, Li Y, Li C, Liu L. Exosomes from mir-29a-Modified adipose-derived mesenchymal stem cells reduce excessive scar formation by inhibiting tgf-β2/Smad3 signaling. *Mol Med Rep* (2021) 24(5):758. doi: 10.3892/mmr.2021.12398

245. Yang J, Li S, He L, Chen M. Adipose-derived stem cells inhibit dermal fibroblast growth and induce apoptosis in keloids through the arachidonic acid-derived cyclooxygenase-2/Prostaglandin E2 cascade by paracrine. *Burns Trauma* (2021) 9:tkab020. doi: 10.1093/burnst/tkab020

246. Liu J, Ren J, Su L, Cheng S, Zhou J, Ye X, et al. Human adipose tissue-derived stem cells inhibit the activity of keloid fibroblasts and fibrosis in a keloid model by paracrine signaling. *Burns* (2018) 44(2):370–85. doi: 10.1016/j.burns.2017.08.017

247. Sato C, Yamamoto Y, Funayama E, Furukawa H, Oyama A, Murao N, et al. Conditioned medium obtained from amnion-derived mesenchymal stem cell culture prevents activation of keloid fibroblasts. *Plast Reconstr Surg* (2018) 141(2):390–8. doi: 10.1097/prs.0000000000004068

248. Fang F, Huang RL, Zheng Y, Liu M, Huo R. Bone marrow derived mesenchymal stem cells inhibit the proliferative and profibrotic phenotype of hypertrophic scar fibroblasts and keloid fibroblasts through paracrine signaling. *J Dermatol Sci* (2016) 83(2):95–105. doi: 10.1016/j.jdermsci.2016.03.003

249. Arno AI, Amini-Nik S, Blit PH, Al-Shehab M, Belo C, Herer E, et al. Effect of human wharton's jelly mesenchymal stem cell paracrine signaling on keloid fibroblasts. *Stem Cells Transl Med* (2014) 3(3):299–307. doi: 10.5966/sctm.2013-0120

250. Taylor A, Budd DC, Shih B, Seifert O, Beaton A, Wright T, et al. Transforming growth factor beta gene signatures are spatially enriched in keloid tissue biopsies and ex vivo-cultured keloid fibroblasts. *Acta Derm Venereol* (2017) 97(1):10–6. doi: 10.2340/00015555-2462

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Deciphering the causal association and co-disease mechanisms between psoriasis and breast cancer

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Background: Prior research has indicated a link between psoriasis and the susceptibility to breast cancer (BC); however, a definitive causal relationship remains elusive. This study sought to elucidate the causal connection and shared underlying mechanisms between psoriasis and BC through bidirectional Mendelian randomization (MR) and bioinformatic approaches.

Methods: We employed a bidirectional MR approach to examine the potential causal connection between psoriasis and BC. Genetic data pertaining to psoriasis and BC were sourced from extensive published genome-wide association studies. The inverse -variance weighted or wald ratio served as the primary method for estimating causal effects. Sensitivity analysis of the MR results was applied with multiple methods. Leveraged datasets from the Gene Expression Omnibus and the Cancer Genome Atlas repositories to identify common differentially expressed genes, shedding light on the shared mechanisms underlying these two conditions.

Results: The MR analysis revealed that when considering psoriasis as an exposure factor, the incidences of BC (OR=1.027) and estrogen receptor negative (ER-) BC (OR=1.054) were higher than in the general population. When using Her2+ BC as an exposure factor, the risk of psoriasis was 0.822 times higher (OR=0.822) than in the general population. Sensitivity analysis indicated that the results were robust. Transcriptome analysis showed that CXCL13 and CCL20 were activated in both BC and psoriasis. Both diseases were also linked to neutrophil chemotaxis, the IL-17 pathway, and the chemokine pathway.

Conclusion: The results suggest that psoriasis may increase the risk of BC, especially ER- BC, while reverse MR suggests a decreased risk of psoriasis in Her2+ BC. Transcriptome analysis revealed a shared mechanism between psoriasis and BC.

KEYWORDS

psoriasis, breast cancer, causal effect, Mendelian randomization, co-disease mechanisms

1 Introduction

Breast cancer (BC) is one of the most prevalent malignant tumors affecting the female population (1). In the year 2020, there were approximately 2.3 million fresh BC cases reported worldwide, comprising 11.7% of the total cancer incidents (2). Psoriasis is a chronic immune-mediated disease (3), and the association between autoimmunity and cancer is well recognized (4). While the pathophysiology of psoriasis remains intricate, we have pinpointed a crucial role played by T cells. Furthermore, specific pathological processes, including the activation of the tumor necrosis factor (TNF)/interleukin (IL-)23/IL-17 cytokine axis, contribute significantly to the differentiation and activation of effector T cells, as well as their accumulation within affected tissues (5, 6). Epigenetic modifications play a significant role in the molecular pathogenesis of psoriasis, as they can modify gene expression without altering the underlying genomic sequence (7, 8). Changes in epigenetic characteristics have been evident across various autoimmune diseases (9). Despite previous research revealing an association between psoriasis and the risk of BC (10, 11), elucidating the precise role of psoriasis in the development of BC remains a formidable challenge. Further investigations are warranted to ascertain the existence of causal genetic molecular mechanisms linking these two conditions.

Genome-wide association studies (GWAS) have transformed the landscape of complex disease genetics by uncovering associations between genotypes and phenotypes through the examination of millions of genetic variations (12). GWAS is a comprehensive genomic analysis technique designed to uncover associations between common single nucleotide polymorphisms (SNPs) and various diseases or traits. It offers novel avenues for comprehending the pathogenesis of complex diseases. Mendelian Randomization (MR) analyses utilize genetic variants as instrumental variables (IVs), typically in the form of SNPs, to infer potential causality—that is, the link between an exposure factor and an outcome (13–15). Since SNPs are randomly allocated during conception and remain unaffected by confounding factors, MR analysis minimizes the influence of confounders and reverse causation. Consequently, MR analysis can provide more robust evidence compared to traditional observational studies in establishing causality (15, 16).

We undertook this European-based investigation to delve into the potential causal linkage between psoriasis and BC. This study enhances our comprehension of the involvement of psoriasis in the initiation and advancement of BC. Additionally, it lends support to the advancement of more efficient cancer surveillance initiatives for the early detection of cancer or precancerous lesions, thus mitigating the burden of care.

2 Methods

To investigate the causal relationship between psoriasis and BC, we employed a bidirectional two-sample MR approach. In this investigation, we employed psoriasis and BC as both exposure and outcome variables. To ensure the rigor of our study, we employed independent genetic variants as IVs, subject to meeting three critical criteria: (1) IVs were required to exhibit a robust association with the exposure; (2) no pleiotropic association of IVs with any known confounders; (3) IVs were assessed for their lack of association with outcome, except when related to exposure. We acquired genetic data for psoriasis and BC from distinct and non-overlapping GWAS datasets to ensure the independence of our analyses (Figure 1A). Furthermore, we systematically reviewed prior studies (Figure 1B) and conducted a transcriptome-based investigation into shared mechanisms (Figure 1C) between these two conditions. For this research, we adhered to the STROBE-MR statement for reporting MR studies (Supplementary Table S1) (17). Given that the datasets used in this study were available from public databases, additional ethical approval or informed consent were not required.

2.1 MR analysis

2.1.1 Data sources

Data regarding psoriasis were sourced from FinnGen (https://www.finngen.fi) and the European Bioinformatics Institute (EBI) (https://www.ebi.ac.uk/). These databases comprised 216,752 and 33,394 individuals of European descent, respectively. Importantly, our analysis did not identify any weak IVs, and all F-statistics > 10, indicating minimal bias due to weak instruments (Supplementary Table S2). The FinnGen's primary objective is to collect and scrutinize genomic and health data from 500,000 participants in the Finnish BioBank, with the aim of enhancing our comprehension of genomic influences on health (18, 19). EBI offers freely accessible bioinformatics resources to the scientific community, champions fundamental research, offers training opportunities, and disseminates state-of-the-art technologies (20). EBI is responsible for the management and upkeep of various extensive public bioinformatics databases, spanning diverse domains like genomics, proteomics, chemoinformatics, transcriptomics, and systems biology. Additionally, EBI provides a diverse array of tools to aid researchers in the analysis and sharing of information.

BC data were sourced from the Integrative Epidemiology Unit (IEU) database (IEU Open GWAS project) (https://gwas.mrcieu.ac.uk/) and the FinnGen database, both of which are associated with GWAS studies related to cancer. In reverse MR analyses, all F-statistics > 10, signifying minimal bias stemming from weak IVs (Supplementary Table S3). Within the IEU database, there were 89,677, 50,225, and 69,970 individuals of European descent included for BC, estrogen receptor-negative (ER-) BC, and ER-positive (ER+) BC data, respectively. As for the FinnGen database, it encompassed data from 102,359 and 103,530 European individuals for Her2- and Her2+ BC, respectively. The IEU database encompasses a wealth of information, comprising over 214.7 billion genetic associations drawn from 42,484 GWAS summary datasets (21).

Furthermore, to strengthen the credibility of the findings, we extracted data on BC and psoriasis from the UK Biobank (UKB) cohort as testing dataset. The UKB cohort is a prospective general population cohort that was assessed at baseline between 2006 and 2010 at 22 different assessment centers in the UK. A total of 502,628

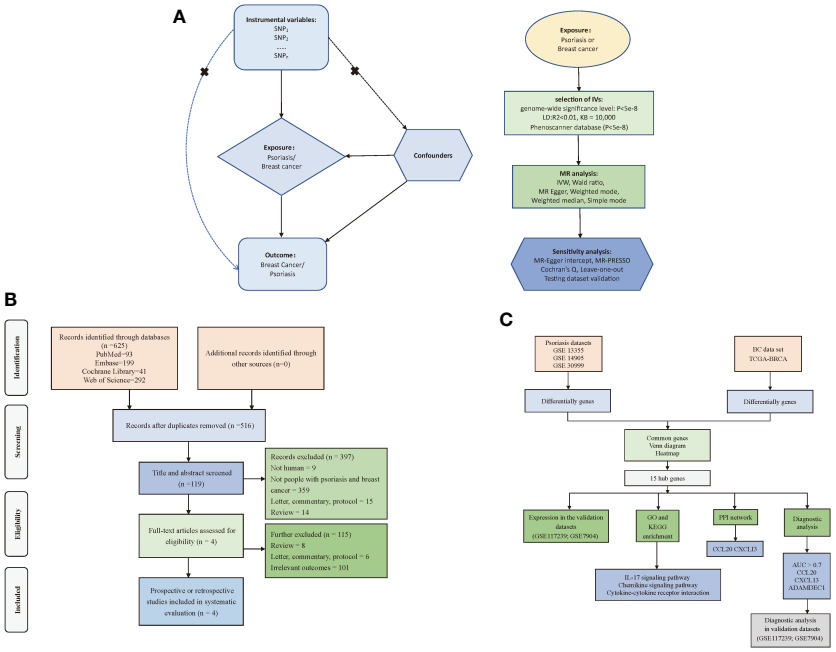

FIGURE 1
Flow of study design. **(A)** Principles and main processes of Mendelian randomization; **(B)** Literature screening process for systematic evaluation; **(C)** Transcriptome-based co-morbidity exploration process. MR, Mendelian randomization; BC, Breast cancer, IVs, Instrumental variables; SNP, Single nucleotide polymorphisms; IVW, Inverse-variance weighted; PPI, Protein-Protein Interaction; LD, Linkage disequilibrium; KB, Pairwise distance.

participants aged between 40 and 70 years were recruited to complete questionnaires covering topics such as lifestyle, sociodemographic characteristics, physical and mental health, and habitual food intake (22, 23). Summary data for the UKB cohort were available from the IEU database. Supplementary Table S4 displayed the characteristics of the psoriasis and BC datasets.

2.1.2 Instrumental variable selection

To ensure the robustness of our conclusions regarding the mutual risk of morbidity between psoriasis and BC, we implemented rigorous quality control measures to select the most appropriate IVs. Here are the key steps we followed: (1) we identified SNPs that displayed significant associations with exposure (psoriasis or BC) across the entire genome, employing a significance threshold of $P < 5 \times 10^{-8}$; (2) we applied a minor allele frequency (MAF) threshold of 0.01 for the specific variant of interest; (3) to minimize the potential for biased results, we carefully chose SNPs strongly associated with exposure (psoriasis or BC) that demonstrated no linkage disequilibrium (LD) ($R^2 < 0.01$, and clumping distance = 10,000 kb); (4) we excluded palindromic SNPs (e.g., those with A/T or G/C alleles) to prevent any distortions in chain orientation or allele coding. In order to ensure a sufficient number of SNPs, we also tried to infer positive strand alleles, using allele frequencies for palindromes; (5) we cross-referenced these alleles with the human genome reference sequence (build 37) and removed any SNPs that were unspecified or duplicated; (6) Following the PhenoScanner database (24), these SNPs as IVs were examined whether they might be strongly associated with the occurrence of BC (estrogen, reproductive factors, and so on) or psoriasis (mechanical stress, smoking, alcohol use, and so forth).

2.1.3 MR analysis and sensitivity analysis

We employed a two-sample MR study to explore the potential causal relationship between psoriasis and BC, encompassing both ER and Her2 receptor subtypes. When the number of SNPs was greater than or equal to two, we utilized the inverse-variance weighted (IVW) method as the primary analysis approach (25). In cases where only one SNP was available, we employed the Wald ratio method for analysis. Additionally, we applied supplementary methods, including weighted mode (26), MR-Egger (27), weighted median (28), and simple mode (29). In certain scenarios, the IVW method demonstrated slightly superior performance compared to the other methods (28). To reduce false positives in the findings, we corrected the results of all MR analyses for multiple testing using the method of false discovery rate (FDR). FDR < 0.05 indicated a significant causal effect, while FDR ≥ 0.05 and P < 0.05 denoted a suggestive causal effect.

To ensure the reliability of our findings, we conducted sensitivity analysis. Firstly, we employed MR-Pleiotropy Residual Sum and Outlier (PRESSO) (30) and examined the MR-Egger intercept to evaluate the potential presence of horizontal pleiotropy. The MR-PRESSO method allowed us to identify and exclude SNPs that might introduce bias. P ≥ 0.05 indicated the absence of horizontal pleiotropy, allowing us to include the remaining SNPs in the MR analysis. A deviation of the MR-Egger intercept from the origin signaled potential horizontal pleiotropy of the IVs (P < 0.05), while no evidence of horizontal pleiotropy was indicated when P ≥ 0.05. Secondly, as a supplementary sensitivity analysis, we employed the weighted median method to assess the robustness of the MR estimates. Thirdly, we calculated the F-statistic to gauge the potential bias stemming from weak

instrumental variables. F-statistic < 10 suggested a weak IV, which could introduce bias and therefore needed to be excluded (31). Additionally, we utilized Cochran's Q statistic to detect heterogeneity within the IVW models. If the Q-value exceeded the number of instruments minus one, it suggested the presence of heterogeneity and ineffective instruments. $P < 0.05$ indicated the potential existence of heterogeneity (32). Lastly, all results that appeared statistically significant were validated using the testing dataset to investigate the robustness of the MR analysis as well as to reduce possible potential false-negative errors after FDR correction.

All these analyses were conducted using the open-source statistical software R (version 4.3.0) along with the TwoSampleMR package (version 0.5.7).

2.1.4 Reverse-direction MR analysis

We explored the potential causal relationship between BC and psoriasis through reverse-direction MR analysis. In our analysis, when only one SNP was identified, we employed the wald ratio method for this single-SNP analysis. Given the limited availability of only one SNP for analysis, we were unable to conduct assessments for heterogeneity, pleiotropy, and sensitivity. Furthermore, when there were multiple SNPs characterized, we utilized five MR methods including IVW, weighted mode, MR-Egger, weighted median, and simple mode. The sensitivity analysis was conducted in the same way as the forward MR.

2.2 Systematic evaluation approach

We conducted a comprehensive search across four databases (Embase, PubMed, Cochrane Library, and Web of Science) to identify studies investigating the association between psoriasis and BC. Our search included keywords such as psoriasis, BC, and disease risk. The search period extended from the inception of the database to September 2023. Inclusion criteria were as follows: (1) prospective or retrospective studies, (2) confirmed diagnoses of both psoriasis and BC, (3) studies examining the risk of BC in psoriasis patients, (4) availability of complete primary data suitable for direct or indirect statistical analysis, and (5) publication of articles in the English language. Exclusion criteria encompassed: (1) diagnosis of malignancy prior to psoriasis diagnosis, (2) studies lacking sufficient published data for analysis, and (3) articles falling into categories such as meta-analyses, letters, commentaries, editorial materials, case reports, or expert opinions. Ultimately, four studies meeting these criteria were identified and subjected to systematic evaluation. The evaluation encompassed new BC cases, incidence rates per 100,000 person-years (PY), hazard ratios (HR), odds ratios (OR), and standardized incidence rates (SIR).

2.3 Transcriptome analysis

2.3.1 Data acquisition and processing

We obtained the datasets for psoriasis and BC from the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA). Differential expression analysis was conducted using the limma package. In this analysis, we set a log2Foldchange threshold of 0.5 for the three psoriasis datasets (GSE13355, GSE14905, and GSE30999), and a log2Foldchange threshold of 1.5 for the TCGA-BRCA dataset of BC. All adjusted $P < 0.05$ were considered statistically different. Differentially expressed genes (DEGs) were identified separately for each dataset, and the DEGs obtained were then taken to be intersected. The results of this intersection analysis were visualized using upset plots and Venn diagrams. To validate the findings, we further examined the DEGs in both the GEO and TCGA datasets. Supplementary Table S5 provided details on the characteristics of the transcriptome dataset.

2.3.2 Functional enrichment analysis

Gene ontology (GO) and The Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to further explore relevant biological pathways and higher genomic functions. We used the clusterProfiler and org.Hs.eg.db packages to enrich the KEGG pathway and evaluate the GO functionality of possible targets. The threshold was set to $P < 0.01$.

2.3.3 Protein-protein interaction network analysis

Potential protein interactions with DEGs were collected and integrated by using the STRING database (https://string-db.org/). The obtained genes were analyzed by PPI network analysis. Cytoscape was used for further subsequent analysis and visualization. The top 3 genes related signaling pathways were reconstructed through the CytoHubba and MCODE.

2.3.4 Receiver operator characteristic curve

ROC curves were performed by using the pROC package (v1.17.0.1) and plotted with the ggplot2 package. Area under the curve (AUC) more than 0.7 has the good diagnosis accuracy.

3 Results

3.1 MR analysis

3.1.1 Genetic instruments

Following a rigorous quality control process, we selected a total of 146 genetic variants (SNPs) associated with the exposure phenotype for the psoriasis validation analysis, with a significance threshold set at $P < 5 \times 10^{-8}$ (Supplementary Table S2). For the reverse MR analysis focused on BC, 87 genetic variants (SNPs) linked to the exposure phenotype were used (Supplementary Table S3). The specific count of selected IVs and the screening process were provided in Supplementary Tables S2, S3.

3.1.2 Causal relationship between psoriasis and BC

In our MR study, we observed that while the application of various analytical methods, including MR-Egger, weighted mode, simple mode, and weighted median, did not reveal a significant

correlation between psoriasis and BC (P > 0.05), the analysis employing the IVW approach indicated that psoriasis indeed represents a risk factor for BC development and there was a causal relationship between them. These findings established that psoriasis (OR=1.027, 95% CI: 1.003-1.050, P=0.021, FDR=0.054) is approximately 1.027 times more likely to predispose individuals to BC compared to the general population when considered as an exposure factor (Supplementary Table S6; Figures 2, 3). Meanwhile, the IVW results suggested that compared to the normal population, psoriasis was associated with a 1.056-fold increased risk of developing ER- BC (OR=1.056, 95% CI: 1.010-1.103, P=0.015, FDR=0.054), whereas there was no statistically significant difference in the risk of developing ER+ BC (P > 0.05; Supplementary Table S6; Figures 2; S1, S2). The results of the remaining four MR analysis methods were in general agreement with the IVW results. In addition, no causal relationship was observed between psoriasis and the Her2 receptor subtype of BC (Supplementary Figures S3, S4).

Remarkably, when we conducted a reverse MR analysis, an unexpected revelation emerged. It came to light that the Her2+ BC actually functions as a protective factor against psoriasis and there was a causal relationship between them. The wald ratio analysis demonstrated that employing the Her2+ BC subtype as an exposure factor (OR=0.822, 95% CI: 0.679-0.995, P=0.044, FDR=0.059) results in a 0.822-fold reduction in the risk of psoriasis development when compared to the general population (Supplementary Table S7; Supplementary Figure S5). When BC and its other subtypes (ER-, ER+, and Her2-) were investigated as exposures, we did not identify the causal relationship between these exposures and psoriasis risk (Supplementary Table S7; Supplementary Figures S5–S7).

3.1.3 Sensitivity analysis

To ensure the robustness of our MR causal effect estimates, we conducted multiple sensitivity analysis. Cochran's Q test indicated that all of our results were devoid of bias and showed no signs of heterogeneous associations (Supplementary Table S8). Additionally, a pleiotropy test employing both the MR-Egger intercept and MR-PRESSO methods yielded all P > 0.05, signifying an absence of pleiotropy (Supplementary Table S8). Furthermore, sensitivity analysis suggested that the results were no heterogeneity or pleiotropy when BC and ER subtype of BC were utilized as exposures for reverse MR (Supplementary Table S9). However, during the reverse MR analysis, only a single SNP was identified when utilizing the Her2 receptor subtype of BC as the exposure variable. Consequently, we were unable to perform heterogeneity test or pleiotropy test in this context (Supplementary Table S9).

Furthermore, we employed the leave-one-out method for sensitivity analysis. After individually excluding each SNP, our findings revealed that no single SNP exerted a significant influence on the overall causal estimates derived from all instrumental variables. This reaffirmed the reliability of the results obtained in our MR study (Supplementary Tables S10, S11).

Finally, to further confirm the causal relationship between psoriasis and breast cancer, we performed the MR analysis again for statistically significant exposures and outcomes using the testing dataset. In the testing dataset, the IVW results indicated that psoriasis remained a risk factor contributing to an elevated risk of BC development (OR=1.001, 95% CI: 1.000-1.003, P=0.032), whereas there was no causal association between Her2+ BC and psoriasis risk (OR=1.000, 95% CI: 0.999-1.001, P=0.907; Supplementary Figure S9). The results of the other four methods were generally consistent with the IVW results. However, due to the unavailability of the testing dataset for ER- BC, the causal relationship between psoriasis and ER- BC was unable to validate.

3.2 Systematic evaluation

Our study also included a comprehensive evaluation of pertinent literature through a systematic review. Among the studies examined, Boffetta P et al. (33) (SIR=1.27, 95% CI: 1.00-1.58) and Kimball AB et al. (34) (OR=1.32, 95% CI: 1.24-1.40)

Exposure	Outcome	Method	NSNP	OR	95% CI	P−value	FDR
Psoriasis (finn)	BC (ieu)	MR Egger	16	1.021	0.983−1.061	0.288	0.720
Psoriasis (finn)	BC (ieu)	Weighted median	16	1.019	0.989−1.050	0.220	0.550
Psoriasis (finn)	BC (ieu)	Inverse variance weighted	16	1.027	1.004−1.051	0.021	0.054
Psoriasis (finn)	BC (ieu)	Simple mode	16	1.019	0.952−1.090	0.599	0.654
Psoriasis (finn)	BC (ieu)	Weighted mode	16	1.017	0.987−1.048	0.282	0.704
Psoriasis (finn)	ER− BC (ieu)	MR Egger	16	1.102	1.027−1.183	0.017	0.087
Psoriasis (finn)	ER− BC (ieu)	Weighted median	16	1.083	1.024−1.045	0.005	0.025
Psoriasis (finn)	ER− BC (ieu)	Inverse variance weighted	16	1.056	1.010−1.103	0.015	0.054
Psoriasis (finn)	ER− BC (ieu)	Simple mode	16	1.073	0.973−1.183	0.180	0.450
Psoriasis (finn)	ER− BC (ieu)	Weighted mode	16	1.081	1.023−1.141	0.014	0.069
Psoriasis (finn)	ER+ BC (ieu)	MR Egger	16	0.991	0.939−1.046	0.748	0.817
Psoriasis (finn)	ER+ BC (ieu)	Weighted median	16	0.993	0.959−1.029	0.706	0.938
Psoriasis (finn)	ER+ BC (ieu)	Inverse variance weighted	16	1.009	0.977−1.043	0.579	0.703
Psoriasis (finn)	ER+ BC (ieu)	Simple mode	16	1.102	1.001−1.212	0.065	0.326
Psoriasis (finn)	ER+ BC (ieu)	Weighted mode	16	0.990	0.954−1.028	0.614	0.858
Psoriasis (ebi)	Her2− BC (finn)	MR Egger	49	1.002	0.986−1.018	0.817	0.817
Psoriasis (ebi)	Her2− BC (finn)	Weighted median	49	0.999	0.982−1.016	0.915	0.938
Psoriasis (ebi)	Her2− BC (finn)	Inverse variance weighted	49	1.002	0.990−1.015	0.703	0.703
Psoriasis (ebi)	Her2− BC (finn)	Simple mode	49	1.006	0.979−1.034	0.654	0.654
Psoriasis (ebi)	Her2− BC (finn)	Weighted mode	49	1.003	0.988−1.019	0.687	0.858
Psoriasis (ebi)	Her2+ BC (finn)	MR Egger	49	1.005	0.992−1.017	0.473	0.789
Psoriasis (ebi)	Her2+ BC (finn)	Weighted median	49	0.999	0.985−1.014	0.938	0.938
Psoriasis (ebi)	Her2+ BC (finn)	Inverse variance weighted	49	1.003	0.994−1.013	0.499	0.703
Psoriasis (ebi)	Her2+ BC (finn)	Simple mode	49	1.009	0.986−1.032	0.470	0.654
Psoriasis (ebi)	Her2+ BC (finn)	Weighted mode	49	1.000	0.987−1.013	0.947	0.947

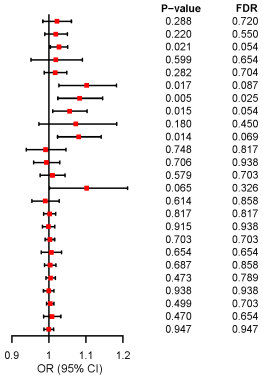

FIGURE 2
The forest plots for psoriasis on breast cancer risk. OR>1 indicates the positive correlation between psoriasis and a particular breast cancer. OR<1 indicates the Negative correlation between the two. The P < 0.05 indicated statistical significance. MR, Mendelian randomization; ER-, ER-negative; ER+, ER-positive; SNP, Single nucleotide polymorphism; OR, Odds ratio; 95%CI, 95% confidence interval; NSNP, Number of SNP; FDR, False discovery rate; BC, Breast cancer.

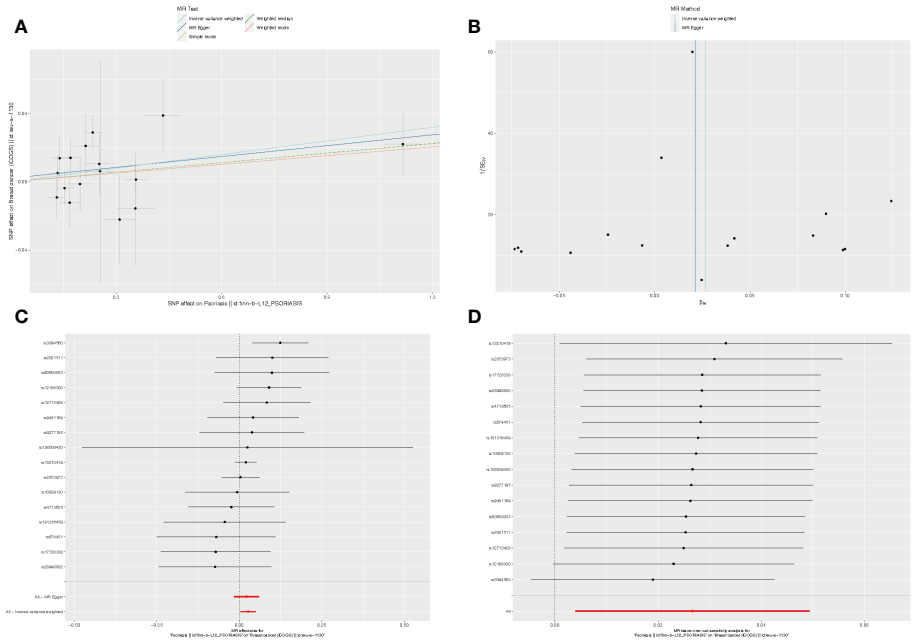

FIGURE 3
The causality of psoriasis on breast cancer risk in European populations. **(A)** Scatter plot. The slope of each line denotes the estimated effect of per mendelian randomization method. **(B)** Funnel plot. Vertical lines represent estimates with all SNPs. **(C)** Forest plot. The red points demonstrate the integrated estimates using all SNPs together, using IVW method. Horizontal lines represent 95% confidence intervals. **(D)** Leave-one-out analysis. Black points depict the IVW method was used to assess the causal effect, excluding single specific variant from the analysis. The red point denotes the inverse-variance weighted estimate using all SNPs. MR, Mendelian randomization; SNPs, Single nucleotide polymorphisms; IVW, Inverse-variance weighted.

indicated that psoriasis serves as a risk factor for BC. Conversely, studies conducted by Chiesa Fuxench ZC et al. (35).

(HR=1.04, 95% CI: 0.97-1.12) and Prizment AE et al. (36) (HR=1.00, 95% CI: 0.70-1.50) suggested that there was no significant difference in BC incidence between patients with psoriasis and the general population (Table 1).

3.3 Transcriptome-based exploration of co-morbid mechanisms

3.3.1 CXCL13 and CCL20 activation in psoriasis and BC

To provide further clarity regarding the association between psoriasis and BC, we selected three GEO datasets related to psoriasis and performed screening with a log2Foldchange threshold of 0.5. Subsequently, we conducted differential expression analysis using the limma package. In the GSE13355 dataset, we identified 199 up-regulated genes and 123 down-regulated genes (Figure 4A). In the GSE14905 dataset, we found 149 up-regulated genes and 39 down-regulated genes (Figure 4B). In the GSE30999 dataset, 266 up-regulated genes and 156 down-regulated genes were detected (Figure 4C). For BC, we identified 1843 up-regulated genes and 923 down-regulated genes using the TCGA database with a log2Foldchange of 1.5 (Figure 4D). By intersecting the differential genes identified in all four datasets, we identified 15 common genes (Figure 4E) that exhibited high expression in both BC and psoriasis, with the exception of INA, WIF1, and TIMP4. We validated the expression of these 15 genes in the psoriasis dataset GSE117239 and the BC dataset GSE7904. All of these genes were confirmed in both datasets, except for TNIP3, RHCG, INA, S100A9, and SERPINB4, which did not show statistically significant differences in BC (Figures 4F–H).

To gain deeper insights into the pathways influenced by these common genes, we conducted enrichment analyses using GO and KEGG. The results revealed significant enrichments in biological processes (BP) and molecular functions (MF) related to neutrophil chemotaxis and peptidase activation. In the KEGG analysis, pathways related to chemokines and IL-17 were prominently enriched. However, due to the limited number of genes, the cellular component (CC) category did not yield any meaningful pathway enrichments (Figures 5A–C). To further explore the interactions among these genes, we constructed a protein-protein interaction (PPI) network using the String database, which revealed 15 hub genes (Figure 5D). Employing CytoHubba and MCODE algorithms, we identified the top three hub genes as CCL20, CXCL13, and S100A9, along with another set of top hub genes: CCL20, CXCL13, and GZMB (Figures 5D–F).

3.3.2 Diagnostic efficacy and prognostic evaluation of 15 Hub Genes

We assessed the diagnostic efficacy of the 15 hub genes using the pROC package, considering an AUC greater than 0.7 as indicative of good diagnostic performance. The results demonstrated that

TABLE 1 Systematic evaluation of the risk of developing psoriasis in relation to breast cancer.

Study	Data Sources	Participants, No.			New breast cancer cases, No.		Incidence per 100 000 PY		Observation indicators
		Control	Psoriasis	Total sample size	Control	Psoriasis	Control	Psoriasis	Psoriasis vs Control
Chiesa Fuxench ZC	The Health Improvement Network (THIN)	937716	198366	1136082	6281	1118	180.87	177.84	HR: 1.04 (0.97-1.12)
Prizment AE	IWHS/Medicare data/ Lowa SEER cancer registry	402199	5477	407676	2037	29	510	530	HR: 1.00 (0.70–1.50)
Boffetta P	Swedish Cancer Register	84003	9773	93776	NA	78	NA	NA	SIR: 1.27 (1.00-1.58)
Kimball AB	Truven Health Analytics Marketscan Database	179066	179066	358132	NA	NA	NA	NA	OR: 1.32 (1.24-1.40)

OR, Odds ratio; HR, Hazard ratios; SIR, Standardized incidence rates; NA, Not applicable; PY, Person-years; No, Number.

CCL20, ADAMDEC1, and CXCL13 exhibited strong diagnostic performance in both BC and psoriasis. These findings were further validated in the psoriasis dataset GSE117239 and the BC dataset GSE7904 (Figures 6A, B).

4 Discussion

Psoriasis, characterized as an autoimmune disease (37, 38), is closely intertwined with autoimmune deficiencies, which contribute significantly to cancer development and progression. Epidemiological investigations have unveiled a robust link between psoriasis and cancer. For instance, an Italian population-based retrospective study conducted by Borghi A et al. revealed that individuals afflicted with plaque psoriasis exhibited an elevated cancer risk (SIR = 1.30, CI 95%: 0.9-1.8). Similarly, a study conducted by Calapai F et al. underscored an augmented cancer risk in psoriasis patients compared to the general population. The pathogenesis of psoriasis involves immune system activation, intricate interplay between T cells, dendritic cells, and cytokines, including IL-12, IL-17, and IL-23. The equilibrium between IL-12 and IL-23 assumes paramount importance in carcinogenesis, and disruptions in IL-12 and/or IL-23 signaling can potentiate tumor growth. Extensive genetic association studies have also pinpointed the correlation between single nucleotide polymorphisms in IL-23 and an escalated risk of malignancy (39). Furthermore, research conducted by Ahmed AR et al. unveiled the presence of autoantibodies to laminin-332 (LM-332Pg) across various autoimmune diseases, including psoriasis, with a concomitant heightened cancer risk. Nevertheless, the question of whether psoriasis is associated with an increased risk of developing BC remains elusive. Hence, we undertook this study with the objective of exploring the causal connection between psoriasis and BC, along with investigating the underlying mechanisms of these co-morbid conditions using transcriptomic approaches.

Our study chosen genetic variants from psoriasis GWAS that exhibited strong associations and comprehensive genetic data. With this study, we found that psoriasis may be a potential risk factor for BC and established a causal relationship between them. The implications of these findings could be significant for public health interventions aimed at mitigating the risk of cancer.

Our MR analysis results revealed a notable association between Psoriasis as an exposure factor and BC (OR=1.027, 95% CI: 1.003-1.050, P=0.021), indicating that individuals with Psoriasis are approximately 1.027 times more likely to develop BC compared to the general population. These results align with the observations made by Schairer C et al. (11), who similarly reported an elevated risk of BC among individuals with psoriasis (OR=1.16, 95% CI: 1.06-1.27). Although the results of MR analysis tended to lose statistical differences after correction (FDR=0.054), psoriasis was still found to be a risk factor for BC development in the MR-PRESSO analysis (P=0.035) and testing cohort (P=0.032), suggesting that our finding was robust and reliable. In our study, we also conducted a systematic evaluation of pertinent research through an extensive literature search. Among the four studies included, it was observed that two studies (33, 34) provided evidence supporting the notion that psoriasis could be a risk factor for BC. In contrast, the remaining two studies (35, 36) concluded that there was no substantial difference in the incidence of BC between individuals with psoriasis and the general population. While it is well-documented that many autoimmune diseases have a higher prevalence among women, possibly linked to hormonal factors (40), our study did not identify an association between psoriasis and the ER+ status in BC, despite the strong association between BC and hormone levels. A study conducted by Gadalla SM et al. arrived at a similar conclusion, indicating that the risk of BC in individuals with autoimmune diseases is independent of ER status. Interestingly, our study found that psoriasis led to the higher ER- BC risk (OR=1.056, 95% CI: 1.010-1.103, P=0.015), which at the same time might have contributed to the elevated overall BC risk. Moreover, our study also revealed that psoriasis was not causally linked to the risk of BC with different Her2 receptor status. Schairer

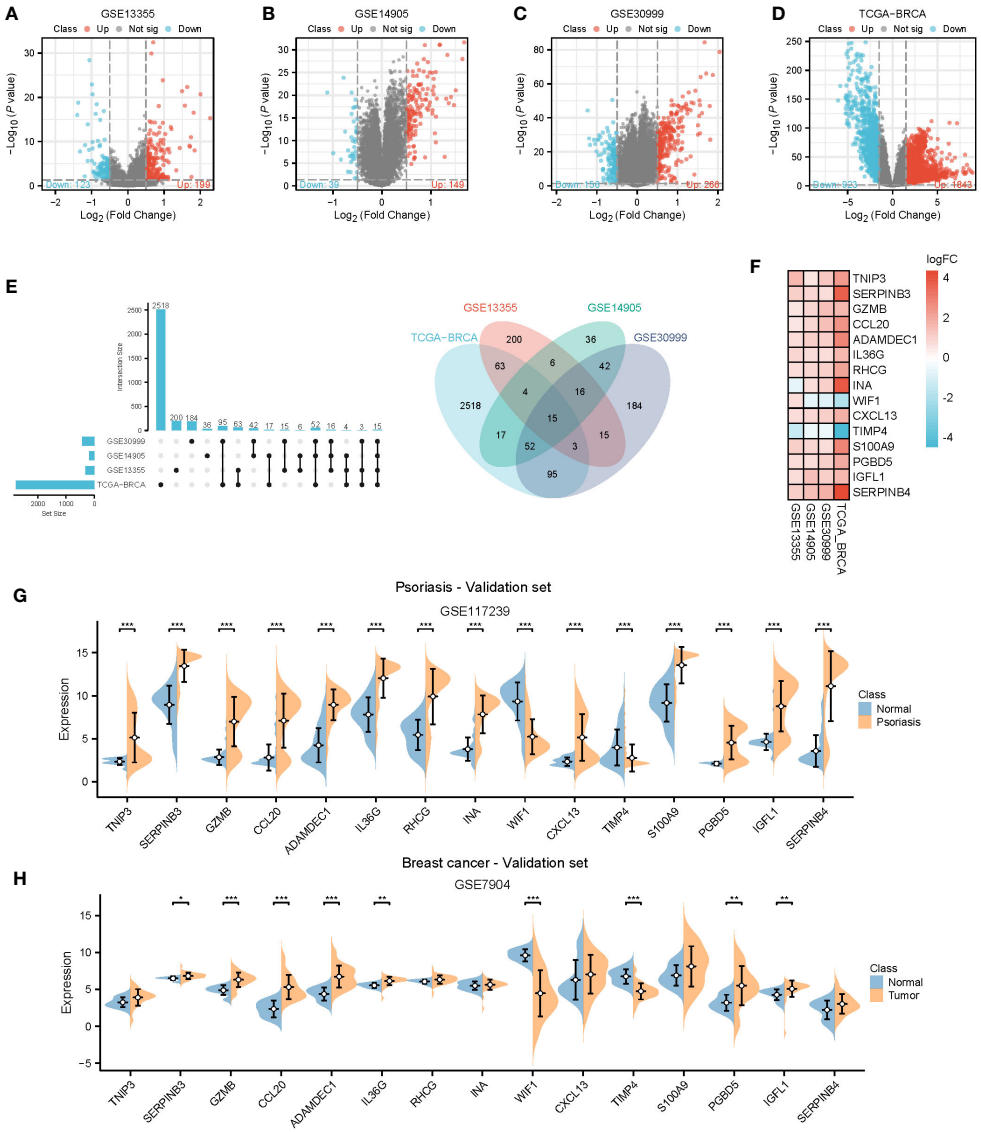

FIGURE 4
Identification of common genes. **(A–D)** Volcano plots for differentially genes (DEGs) expression. Red dots represent upregulated gene expression, whereas blue dots represent downregulated ones, and the grey dots stand for no significant difference. **(A–C)** Psoriasis dataset; **(D)** Breast cancer dataset. **(E, F)** Venn diagram and heatmap of genes common to both psoriasis and breast cancer. **(G, H)** Expression of 15 common genes in the validation datasets. TCGA, The Cancer Genome Atlas; DEGs, Differentially genes; *, Statistically significant. Wilcoxon test. *p<0.05; **p<0.01; ***p<0.001.

et al. demonstrated that psoriasis caused an elevated risk of progesterone receptor (PR)-positive BC, but not PR-negative BC (11). Since in the current BC cohort we only have access to a single ER status or Her2 status, it was not possible to identify BC subtypes with a diverse combination of PR or other receptors added. Therefore, the conclusions that psoriasis caused changes in the risk of single-receptor BC subtypes were not yet comprehensive. Importantly, the different BC subtypes combined from ER, PR, and Her2 receptors need to be further refined in larger populations so that more accurate findings can be drawn.

The forward MR has shown that psoriasis was the risk factor for the development of BC. Meanwhile, a Swedish population-based cohort study conducted by Yang H et al. (41) identified Her2+ BC as a risk factor for psoriasis. However, in a surprising twist, our inverse MR analysis revealed a protective association between Her2 + BC subtypes and psoriasis, marking a novel and unexpected discovery. Kim et al. have reported the development of new-onset psoriasis in a BC patient treated with trastuzumab (Her2 inhibitor) (42). Although the role of Her2 in the skin is unclear, some evidence pointed to an active role for Her2 in keratinocyte differentiation (43). Moreover, De potter et al. demonstrated that in a subpopulation of differentiated keratinocytes, some Her ligands activated a signaling pathway of Her2 heterodimers, but not of epidermal growth factor (EGFR) homodimers (43). Thus, certain Her2 inhibitors might induce alterations in normal epidermal differentiation and turnover regardless of EGFR. This to some

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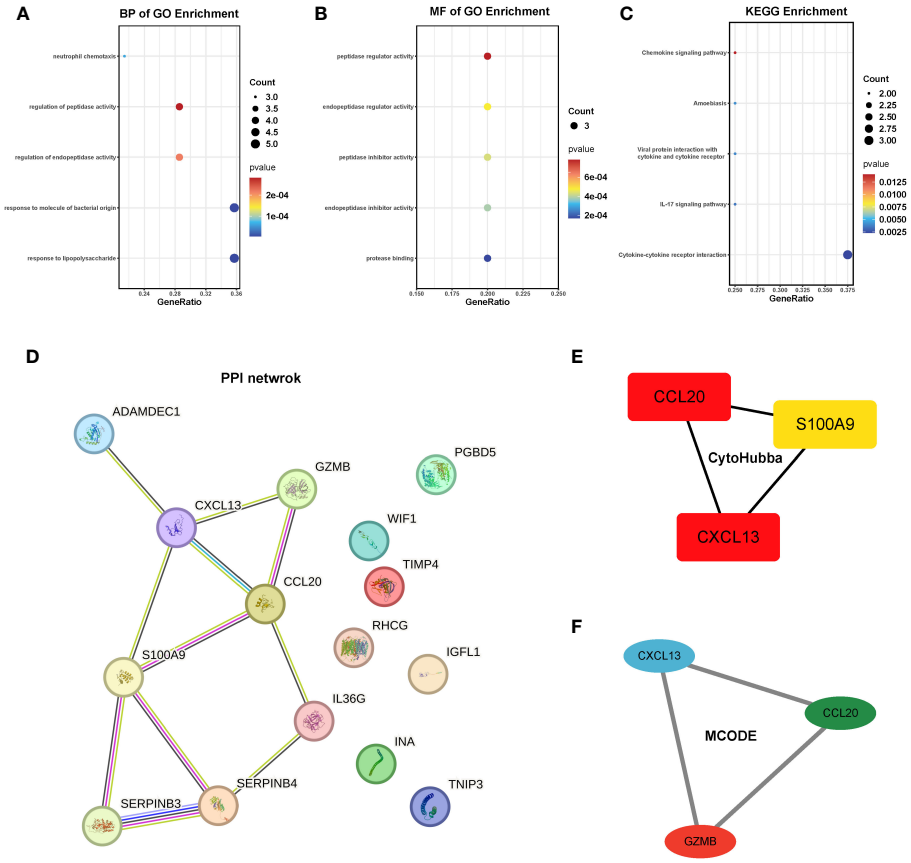

FIGURE 5
Enrichment analysis and PPI network of the 15 hub genes. **(A-C)** GO and KEGG pathway analysis. **(D)** PPI network. **(E, F)** The top 3 hub genes were identified by the CytoHubba and MCODE. GO, Gene ontology; KEGG, The Kyoto Encyclopedia of Genes and Genomes.

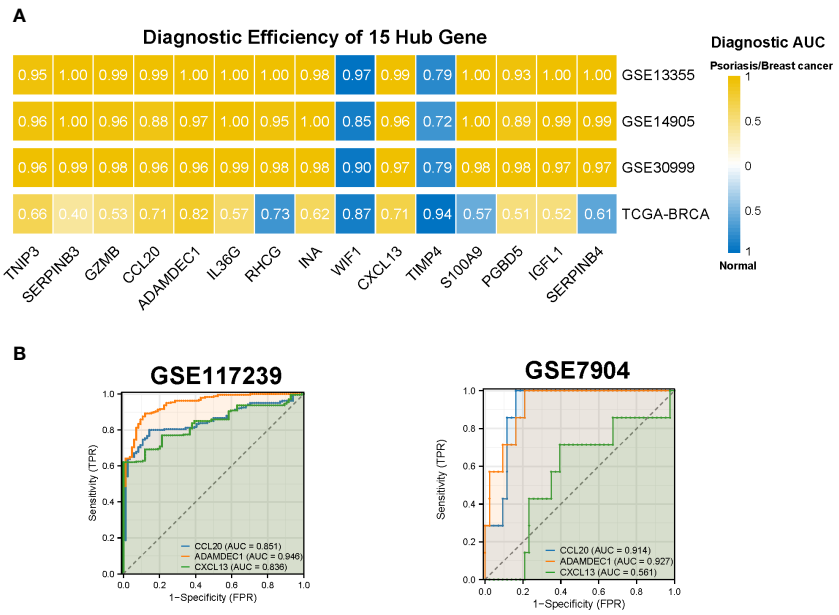

FIGURE 6
Diagnostic efficiency of the 15 hub genes. **(A)** Diagnostic AUC of 15 hub genes in psoriasis and breast cancer datasets. The AUC greater than 0.7 was considered to have high accuracy. **(B)** The AUC value of CCL20, ADAMDEC1, CXCL13 in the validation datasets. AUC, Area under curve; TCGA, The Cancer Genome Atlas.

extent implied that Her2+ BC might have the ability to reduce the risk of psoriasis. Nevertheless, the causal association between Her2+ BC and psoriasis was not validated once again in our testing dataset. Consequently, although there appeared to be a conflict with the findings of our forward MR, this conclusion needs to be viewed and analyzed with caution.

Our study delved into the shared mechanisms underlying these two conditions using transcriptome analysis. We discovered that CXCL13 and CCR20 activation was evident in both BC and psoriasis. Notably, these two genes, along with ADAMDEC1, demonstrated good diagnostic efficacy for both diseases. Further exploration through GO and KEGG enrichment analysis unveiled associations with neutrophil chemotaxis, the IL-17 pathway, and the chemokine pathway in both conditions. Of particular importance, IL-17A emerged as a central cytokine in the pathogenesis of psoriasis, driving the proliferation of epidermal keratinocytes (44). These keratinocytes, in turn, produce a multitude of antimicrobial peptides and chemokines, including CXCL1, CXCL2, CXCL8, and CCL20 (45). CCL20, in particular, plays a pivotal role in psoriasis by binding to its receptor CCR6. Moreover, CCL20 has been implicated in the progression of various cancers, such as liver, colon, breast, pancreatic, and gastric cancers (46). In summary, our transcriptome results offer insights into the potential interaction between psoriasis and BC.

Our study carries significant implications for pre-cancer screening and intervention efforts. While substantial progress has been achieved in elucidating genetic variations associated with human diseases, the majority of genetic risk factors remain enigmatic. Additional biological investigations are warranted to unveil the intricate interplay between psoriasis and BC.

Human behavior and the environments in which it manifests are intricate, shaped by the interplay between genetic factors and environmental influences (47–50). To mitigate confounding factors inherent in epidemiological investigations, we employed MR techniques. The SNPs utilized in our study exhibited robust associations with psoriasis and were subsequently cross-referenced with the BC database. The outcomes of our sensitivity analyses demonstrated statistical robustness, revealing the absence of pleiotropy or heterogeneity. Nonetheless, our investigation does have certain limitations. Firstly, it's important to acknowledge that the key assumptions of MR analysis come with inherent limitations. Despite our efforts to minimize confounding variables, we cannot completely rule out the presence of other confounders or potential multiplicative effects. Secondly, the use of aggregated data from GWAS introduces the possibility of results being influenced by variations in quality control and selection criteria across different studies. Thirdly, it's important to note that while MR can infer potential causal relationships, it does not provide insights into specific biological pathways. Fourth, our study primarily focused on European populations, which may limit its generalizability to other ethnic groups. Finally, the limited number of SNPs obtained in the analysis of Her2+ BC as a protective factor against psoriasis prevented us from conducting comprehensive heterogeneity, pleiotropy, and sensitivity analyses, potentially impacting the robustness of our conclusions. Furthermore, while our study did not identify an association between psoriasis and the status of ER and Her2 receptor in BC, it's important to acknowledge that the conclusions may be influenced by the limited sample size. We look forward to the availability of more SNP data in the future, which will provide opportunities for further validation and refinement of our conclusions.

5 Conclusion

Our study conducted a comprehensive evaluation of the relationship between psoriasis and BC, and we have reached the following conclusions. When psoriasis was considered as an exposure factor, it might pose a risk for the development of BC, especially ER- BC. In addition, when Her2+ BC considered as an exposure factor, it might exhibit a protective effect against psoriasis. A causal relationship appeared to exist between the above-mentioned findings. Transcriptome analysis further suggested a co-morbid mechanism for psoriasis and BC. In summary, this research offers novel insights into the genetic correlations and underlying mechanisms between psoriasis and BC.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

XL: Conceptualization, Data curation, Formal analysis, Software, Visualization, Writing – review & editing. LH: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. YY: Formal analysis, Project administration, Resources, Validation, Visualization, Writing – review & editing. YR: Conceptualization, Data curation, Methodology, Software, Writing – review & editing. XC: Formal analysis, Project administration, Supervision, Validation, Writing – review & editing. MG: Investigation, Methodology, Project administration, Software, Visualization, Writing – review & editing. JH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1304888/full#supplementary-material

SUPPLEMENTARY FIGURE S1
The causality of psoriasis on ER negative breast cancer risk in European populations.

SUPPLEMENTARY FIGURE S2
The causality of psoriasis on ER positive breast cancer risk in European populations.

SUPPLEMENTARY FIGURE S3
The causality of psoriasis on Her-2 negative breast cancer risk in European populations.

SUPPLEMENTARY FIGURE S4
The causality of psoriasis on Her-2 positive breast cancer risk in European populations.

SUPPLEMENTARY FIGURE S5
The forest plots for breast cancer on psoriasis risk.

SUPPLEMENTARY FIGURE S6
The causality of breast cancer on psoriasis risk in European populations.

SUPPLEMENTARY FIGURE S7
The causality of ER negative breast cancer on psoriasis risk in European populations.

SUPPLEMENTARY FIGURE S8
The causality of ER positive breast cancer on psoriasis risk in European populations.

SUPPLEMENTARY FIGURE S9
Validation of statistically significant MR analysis results using the testing dataset.

SUPPLEMENTARY TABLE S1
STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies.

SUPPLEMENTARY TABLE S2
IVs associated with psoriasis (exposure) and breast cancer (outcome).

SUPPLEMENTARY TABLE S3
IVs associated with breast cancer (exposure) and psoriasis (outcome).

SUPPLEMENTARY TABLE S4
Characterization of datasets for psoriasis and breast cancer.

SUPPLEMENTARY TABLE S5
Characterization of datasets for transcriptome.

SUPPLEMENTARY TABLE S6
MR estimates of the casual relationships between psoriasis (exposure) and breast cancer (outcome) risks.

SUPPLEMENTARY TABLE S7
MR estimates of the casual relationships between breast cancer (exposure) and psoriasis (outcome) risks.

SUPPLEMENTARY TABLE S8
Sensitivity results of MR analysis between psoriasis (exposure) and breast cancer (outcome)

SUPPLEMENTARY TABLE S9
Sensitivity results of MR analysis between breast cancer (exposure) and psoriasis (outcome).

SUPPLEMENTARY TABLE S10
Sensitivity results of MR analysis between psoriasis (exposure) and breast cancer (outcome) (leave-one-out).

SUPPLEMENTARY TABLE S11
Sensitivity results of MR analysis between breast cancer (exposure) and psoriasis (outcome) (leave-one-out).

References

1. Kong X, Song J, Gao P, Gao R, Zhang L, Fang Y, et al. Revolutionizing the battle against locally advanced breast cancer: A comprehensive insight into neoadjuvant radiotherapy. *Med Res Rev*. (2024) 44:606–31. doi: 10.1002/med.21998

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

3. Greb JE, Goldminz AM, Elder JT, Lebwohl MG, Gladman DD, Wu JJ, et al. Psoriasis. *Nat Rev Dis Primers*. (2016) 2:16082. doi: 10.1038/nrdp.2016.82

4. Giat E, Ehrenfeld M, Shoenfeld Y. Cancer and autoimmune diseases. *Autoimmun Rev*. (2017) 16:1049–57. doi: 10.1016/j.autrev.2017.07.022

5. Carvalho AL, Hedrich CM. The molecular pathophysiology of psoriatic arthritis-the complex interplay between genetic predisposition, epigenetics factors, and the microbiome. *Front Mol Biosci*. (2021) 8:662047. doi: 10.3389/fmolb.2021.662047

6. Hu P, Wang M, Gao H, Zheng A, Li J, Mu D, et al. The role of helper T cells in psoriasis. *Front Immunol*. (2021) 12:788940. doi: 10.3389/fimmu.2021.788940

7. Cuddapah S, Barski A, Zhao K. Epigenomics of T cell activation, differentiation, and memory. *Curr Opin Immunol*. (2010) 22:341–7. doi: 10.1016/j.coi.2010.02.007

8. Frias AB, Boi SK, Lan X, Youngblood B. Epigenetic regulation of T cell adaptive immunity. *Immunol Rev*. (2021) 300:9–21. doi: 10.1111/imr.12943

9. Ballestar E, Sawalha AH, Lu Q. Clinical value of DNA methylation markers in autoimmune rheumatic diseases. *Nat Rev Rheumatol*. (2020) 16:514–24. doi: 10.1038/s41584-020-0470-9

10. Wilton KM, Crowson CS, Matteson EL. Malignancy incidence in patients with psoriatic arthritis: a comparison cohort-based incidence study. *Clin Rheumatol*. (2016) 35:2603–7. doi: 10.1007/s10067-016-3396-5

11. Schairer C, Pfeiffer RM, Gadalla SM. Autoimmune diseases and breast cancer risk by tumor hormone-receptor status among elderly women. *Int J Cancer*. (2018) 142:1202–8. doi: 10.1002/ijc.31148

12. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. 10 years of GWAS discovery: biology, function, and translation. *Am J Hum Genet*. (2017) 101:5–22. doi: 10.1016/j.ajhg.2017.06.005

13. Sekula P, Del Greco MF, Pattaro C, Köttgen A. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol JASN*. (2016) 27:3253–65. doi: 10.1681/ASN.2016010098

14. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. (2003) 32:1–22. doi: 10.1093/ije/dyg070

15. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. (2014) 23:R89–98. doi: 10.1093/hmg/ddu328

16. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *Bmj*. (2018) 362:k601. doi: 10.1136/bmj.k601

17. Skrivankova VW, Richmond RC, Woolf BAR, Yarmolinsky J, Davies NM, Swanson SA, et al. Strengthening the reporting of observational studies in epidemiology using mendelian randomization: the STROBE-MR statement. *Jama*. (2021) 326:1614–21. doi: 10.1001/jama.2021.18236

18. Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. *Hum Mol Genet*. (1999) 8:1913–23. doi: 10.1093/hmg/8.10.1913

19. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner KM, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature*. (2023) 613:508–18. doi: 10.1038/s41586-022-05473-8

20. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res*. (2019) 47:D1005–d1012. doi: 10.1093/nar/gky1120

21. Lyon MS, Andrews SJ, Elsworth B, Gaunt TR, Hemani G, Marcora E. The variant call format provides efficient and robust storage of GWAS summary statistics. *Genome Biol*. (2021) 22:32. doi: 10.1186/s13059-020-02248-0

22. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PloS Med*. (2015) 12:e1001779. doi: 10.1371/journal.pmed.1001779

23. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of sociodemographic and health-related characteristics of UK biobank participants with those of the general population. *Am J Epidemiol*. (2017) 186:1026–34. doi: 10.1093/aje/kwx246

24. Kamat MA, Blackshaw JA, Young R, Surendran P, Burgess S, Danesh J, et al. PhenoScanner V2: an expanded tool for searching human genotype-phenotype associations. *Bioinf (Oxford England)*. (2019) 35:4851–3. doi: 10.1093/bioinformatics/btz469

25. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol*. (2015) 30:543–52. doi: 10.1007/s10654-015-0011-z

26. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol*. (2017) 46:1985–98. doi: 10.1093/ije/dyx102

27. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. (2015) 44:512–25. doi: 10.1093/ije/dyv080

28. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. (2016) 40:304–14. doi: 10.1002/gepi.21965

29. Verbanck M, Chen CY, Neale B, Do R. Publisher Correction: Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. (2018) 50:1196. doi: 10.1038/s41588-018-0164-2

30. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. (2018) 50:693–8. doi: 10.1038/s41588-018-0099-7

31. Burgess S, Thompson SG. Bias in causal estimates from Mendelian randomization studies with weak instruments. *Stat Med*. (2011) 30:1312–23. doi: 10.1002/sim.4197

32. Bowden J, Del Greco MF, Minelli C, Zhao Q, Lawlor DA, Sheehan NA, et al. Improving the accuracy of two-sample summary-data Mendelian randomization: moving beyond the NOME assumption. *Int J Epidemiol*. (2019) 48:728–42. doi: 10.1093/ije/dyy258

33. Boffetta P, Gridley G, Lindelöf B. Cancer risk in a population-based cohort of patients hospitalized for psoriasis in Sweden. *J Invest Dermatol*. (2001) 117:1531–7. doi: 10.1046/j.0022-202x.2001.01520.x

34. Kimball AB, Sundaram M, Cloutier M, Gauthier-Loiselle M, Gagnon-Sanschagrin P, Guérin A, et al. Increased prevalence of cancer in adult patients with psoriasis in the United States: A claims based analysis. *J Drugs Dermatol*. (2018) 17:180–6.

35. Chiesa Fuxench ZC, Shin DB, Ogdie Beatty A, Gelfand JM. The risk of cancer in patients with psoriasis: A population-based cohort study in the health improvement network. *JAMA Dermatol*. (2016) 152:282–90. doi: 10.1001/jamadermatol.2015.4847

36. Prizment AE, Alonso A, Folsom AR, Ahmed RL, Virnig BA, Warshaw EM, et al. Association between psoriasis and incident cancer: the Iowa's Women's Health Study. *Cancer Causes Control*. (2011) 22:1003–10. doi: 10.1007/s10552-011-9773-0

37. Natoli V, Charras A, Hofmann SR, Northey S, Russ S, Schulze F, et al. DNA methylation patterns in CD4(+) T-cells separate psoriasis patients from healthy controls, and skin psoriasis from psoriatic arthritis. *Front Immunol*. (2023) 14:1245876. doi: 10.3389/fimmu.2023.1245876

38. Sheikh A, Hazari SA, Molugulu N, Alshehri SA, Wahab S, Sahebkar A, et al. Hyaluronic acid engineered gallic acid embedded chitosan nanoparticle as an effective delivery system for treatment of psoriasis. *Environ Res*. (2023) 238:117086. doi: 10.1016/j.envres.2023.117086

39. Calapai F, Mannucci C, Cardia L, Currò M, Calapai G, Esposito E, et al. Suspected oncologic adverse reactions associated with interleukin-23 inhibitors in EudraVigilance: Comparative study and gender distribution. *Pharmacol Res Perspect*. (2023) 11:e01130. doi: 10.1002/prp2.1130

40. Quintero OL, Amador-Patarroyo MJ, Montoya-Ortiz G, Rojas-Villarraga A, Anaya JM. Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. *J Autoimmun*. (2012) 38:J109–119. doi: 10.1016/j.jaut.2011.10.003

41. Yang H, Brand JS, Li J, Ludvigsson JF, Ugalde-Morales E, Chiesa F, et al. Risk and predictors of psoriasis in patients with breast cancer: a Swedish population-based cohort study. *BMC Med*. (2017) 15:154. doi: 10.1186/s12916-017-0915-4

42. Kim DH, Jeong NJ, Im M, Lee Y, Seo YJ, Lee JH. Psoriasis induced by trastuzumab (herceptin®). *Ann Dermatol*. (2013) 25:229–31. doi: 10.5021/ad.2013.25.2.229

43. De Potter IY, Poumay Y, Squillace KA, Pittelkow MR. Human EGF receptor (HER) family and heregulin members are differentially expressed in epidermal keratinocytes and modulate differentiation. *Exp Cell Res*. (2001) 271:315–28. doi: 10.1006/excr.2001.5390

44. Liu S, Wu F, Wu Z, Li Y, Zhang S, Yu N. IL-17A synergistically enhances TLR3-mediated IL-36γ production by keratinocytes: A potential role in injury-amplified psoriatic inflammation. *Exp Dermatol*. (2019) 28:233–9. doi: 10.1111/exd.13871

45. Furue M, Furue K, Tsuji G, Nakahara T. Interleukin-17A and keratinocytes in psoriasis. *Int J Mol Sci*. (2020) 21(4):1275. doi: 10.3390/ijms21041275

46. Chen W, Qin Y, Liu S. CCL20 signaling in the tumor microenvironment. *Adv Exp Med Biol*. (2020) 1231:53–65. doi: 10.1007/978-3-030-36667-4_6

47. Meisel SF, Beeken RJ, van Jaarsveld CH, Wardle J. Genetic susceptibility testing and readiness to control weight: Results from a randomized controlled trial. *Obes (Silver Spring)*. (2015) 23:305–12. doi: 10.1002/oby.v23.2

48. Tucker-Drob EM, Bates TC. Large cross-national differences in gene × Socioeconomic status interaction on intelligence. *Psychol Sci*. (2016) 27:138–49. doi: 10.1177/0956797615612727

49. Agustí A, Melén E, DeMeo DL, Breyer-Kohansal R, Faner R. Pathogenesis of chronic obstructive pulmonary disease: understanding the contributions of gene-environment interactions across the lifespan. *Lancet Respir Med*. (2022) 10:512–24. doi: 10.1016/S2213-2600(21)00555-5

50. Meisel SF, Walker C, Wardle J. Psychological responses to genetic testing for weight gain: a vignette study. *Obes (Silver Spring)*. (2012) 20:540–6. doi: 10.1038/oby.2011.324

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Integrated bioinformatics combined with machine learning to analyze shared biomarkers and pathways in psoriasis and cervical squamous cell carcinoma

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Background: Psoriasis extends beyond its dermatological inflammatory manifestations, encompassing systemic inflammation. Existing studies have indicated a potential risk of cervical cancer among patients with psoriasis, suggesting a potential mechanism of co-morbidity. This study aims to explore the key genes, pathways, and immune cells that may link psoriasis and cervical squamous cell carcinoma (CESC).

Methods: The cervical squamous cell carcinoma dataset (GSE63514) was downloaded from the Gene Expression Omnibus (GEO). Two psoriasis-related datasets (GSE13355 and GSE14905) were merged into one comprehensive dataset after removing batch effects. Differentially expressed genes were identified using Limma and co-expression network analysis (WGCNA), and machine learning random forest algorithm (RF) was used to screen the hub genes. We analyzed relevant gene enrichment pathways using GO and KEGG, and immune cell infiltration in psoriasis and CESC samples using CIBERSORT. The miRNA-mRNA and TFs-mRNA regulatory networks were then constructed using Cytoscape, and the biomarkers for psoriasis and CESC were determined. Potential drug targets were obtained from the cMAP database, and biomarker expression levels in hela and psoriatic cell models were quantified by RT-qPCR.

Results: In this study, we identified 27 key genes associated with psoriasis and cervical squamous cell carcinoma. NCAPH, UHRF1, CDCA2, CENPN and MELK were identified as hub genes using the Random Forest machine learning algorithm. Chromosome mitotic region segregation, nucleotide binding and DNA methylation are the major enrichment pathways for common DEGs in the mitotic cell cycle. Then we analyzed immune cell infiltration in psoriasis and cervical squamous cell carcinoma samples using CIBERSORT. Meanwhile, we used the cMAP database to identify ten small molecule compounds that interact with the central gene as drug candidates for treatment. By analyzing miRNA-mRNA and TFs-mRNA regulatory networks, we identified three miRNAs and nine transcription factors closely associated with five key genes and validated their expression in external validation datasets and clinical samples. Finally, we

examined the diagnostic effects with ROC curves, and performed experimental validation in hela and psoriatic cell models.

Conclusions: We identified five biomarkers, *NCAPH, UHRF1, CDCA2, CENPN*, and *MELK*, which may play important roles in the common pathogenesis of psoriasis and cervical squamous cell carcinoma, furthermore predict potential therapeutic agents. These findings open up new perspectives for the diagnosis and treatment of psoriasis and squamous cell carcinoma of the cervix.

KEYWORDS

psoriasis, cervical squamous cell carcinoma (CESC), immune cell infiltration, machine learning, biomarkers

Introduction

Psoriasis is a chronic inflammatory and hyperproliferative skin condition, which is mediated by the immune system. The inflammatory features have been acknowledged with a deeper understanding of its biological properties (1–6). Several co-morbidities such as metabolic syndrome, tumors and inflammatory diseases can be induced by the cytokines involved in psoriasis (7–12). In addition, psoriasis patients receiving systemic and UV therapy are more likely to develop general and organ-specific cancers (13, 14).

Cervical cancer is a malignant tumor that arises in the cervix and vagina, with the second highest incidence rate among female tumors (15). Furthermore, it remains the second most common cause of cancer-related deaths among women in developing nations (16). The incidence of cervical cancer is on the rise, necessitating further exploration of new treatments for cervical squamous cell carcinoma (17, 18). The grave issue of patients with advanced cervical cancer experiencing poor prognosis and survival rates persists (19, 20). Previous studies have shown that the pathogenesis of cervical cancer is hypothesized to stem from multifactorial interactions between the host system, HPV(Human Papilloma Virus) infection, and diverse behavioral, environmental, or inherited variables (21).

Clinical data reveals that the majority of patients presenting with both cervical cancer and psoriasis exhibit advanced inoperable stages or postoperative recurrence. These cases are characterized by pathologically confirmed squamous cell carcinoma, a history of psoriasis, and a recurrent pattern of immunosuppressive therapy usage (22, 23). A traditional Chinese medicine known as Wolf Poison demonstrates dual efficacy—internally for treating cervical cancer and externally for addressing psoriasis. This dual therapeutic application suggests a potential common pathogenesis between cervical cancer and psoriasis (24, 25). In addition, both psoriasis and cervical squamous cell carcinoma show hyperproliferation of squamous epithelial cells and both have angiogenic mechanisms (26–29). Several studies have suggested that prolonged immunosuppression in individuals with psoriasis hampers immune responses, elevating their vulnerability to tumorigenesis, including CESC (30–32). However, the underlying mechanisms of this comorbidity remain unclear and warrant further investigation.

Thus, this study employs a systems biology approach to elucidate potential biomolecular mechanisms shared between psoriasis and CESC. Our findings aim to identify candidate biomarker signatures that could be common between psoriasis and cervical squamous cell carcinoma, contributing valuable insights to the field.

Materials and methods

Data processing

The research flowchart of this research is shown in Figure 1. Data Source GEO (http://www.ncbi.nlm.nih.gov/geo) is a public database containing a large number of high-throughput sequencing and microarray datasets submitted by research organizations around the world. The epithelial cell microarray dataset of cervical squamous cell carcinoma patients (GSE63514), including 24 normal 28 cervical squamous cell carcinoma epithelial cell specimens, was obtained through GEO. Two expression profiling datasets, GSE13355 and GSE14905, were downloaded from the GEO database for psoriasis and controls. The GSE13355 dataset consisted of total RNA extracted from puncture biopsies of 58 patients with psoriasis and 64 normal healthy controls, and the GSE14905 dataset consisted of skin biopsy specimens from 21 normal healthy donors and 56 from 28 patients with psoriasis skin biopsy samples. Batch correction integration, normalization, and gene ID transformation were performed on the 2 psoriasis datasets carried out using the R software package SVA (v4.2.1). RNAseq data for the STAR process of the TCGA-CESC project were downloaded and organized from The Cancer GenomeAtlas Program (TCGA) database (https://portal.gdc.cancer.gov) and extracted in TPM format. Table 1 presents detailed dataset information, including the microarray platform, sample groups, and numbers.

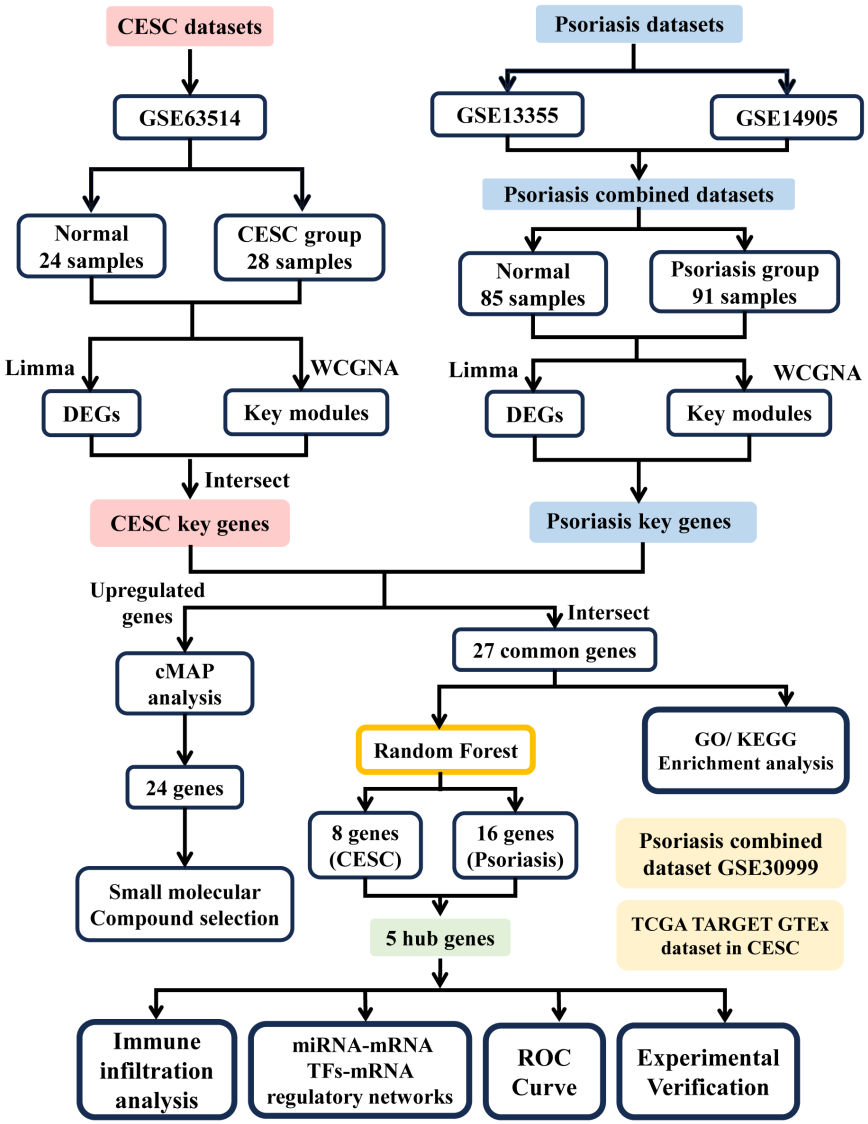

FIGURE 1
Flowchart of analytical steps in this study.

TABLE 1 Basic information of datasets used in the study.

Datasets	Type	Sample size		Platform
		Normal	Psoriasis	
GSE13355	RNA	64	58	GPL570
GSE14905	RNA	21	33	GPL570
GSE30999	RNA	85	170	GPL570
		Control	CESC	
GSE63514	RNA	24	28	GPL570
TCGA-CESC	RNA	3	306	

Identification of DEGs

Limma, a differential expression screening method based on generalized linear models, was utilized to obtain the differential genes between different comparator groups and the control group. We conducted the differential analysis using the R package limma (version 3.40.6) (33). We obtained the expression profiling dataset and performed multiple linear regression utilizing the lmFit function. We then utilized the eBays function to compute moderated t-statistics, moderated F-statistics, and log-odds of differential expression through empirical Bayes moderation of the standard errors towards a common value. Finally, we determined the significance of differences for each gene. Technical terms were explained upon first usage and the language used was neutral and objective.

Weighted gene co-expression network analysis

Using gene expression profiles, we calculated the MAD (Median Absolute Deviation) of each gene separately, eliminated the top 50% of genes with the smallest MAD, removed outlier genes and samples using the goodSamplesGenes method of the R package WGCNA, and further constructed scale-free co-expression networks using WGCNA. β is a soft-threshold parameter that can emphasize strong correlations between genes and penalize weak correlations. The neighbor-joining matrix was converted to a topological overlap matrix (TOM), which measures the network connectivity of a gene, defined as the sum of the neighbor-joining matrices of the gene and all other genes assigned to the network gene, and the corresponding dissimilarity (1-TOM) was calculated. To cluster genes with similar expression profiles into gene modules, we utilized average linkage hierarchical clustering based on the TOM similarity measure. It should be noted that the gray modules were classified as the set of genes unassigned to any module.

PPI network construction and module analysis

Search Tool for the Retrieval of Interacting Genes (STRING, http://string-db.org) (version 11.0) searches for relationships between proteins of interest, such as direct binding relationships, or coexisting upstream and downstream regulatory pathways, to construct PPI networks with complex regulatory relationships.

Functional enrichment analysis

Sangerbox (http://www.sangerbox.com/tool) was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. Gene Ontology (GO) analysis is a common technique utilized for conducting large-scale functional enrichment studies that encompass biological processes, molecular functions, and cellular components (34). Kyoto Encyclopedia of Genes and Genomes (KEGG) is a popular database for storing information pertaining to genomes, biological pathways, diseases, and pharmaceuticals (35). Adjusted P-value < 0.05 was considered significant.

Machine learning

Machine learning algorithms are used to screen the core genes for diagnosis. Using the Random Forest (RF) algorithm which integrates multiple trees for better accuracy through the idea of ensemble learning, we narrowed down the candidate biomarkers, which integrates multiple trees for better accuracy through the idea of ensemble learning. The genes with MeanDecreaseGini > 2 in the RF model were defined as the central genes.

Immune infiltration analysis

The CIBERSORT algorithm is utilized for evaluating the percentage of immune cells present in cells or tissues. The bar graphs show the proportion of each type of immune cell in various samples, and the "corrplot" R package is used to generate a heat map of the correlation between 22 immune cells. The vioplot was used to visualize the differences between the Psoriasis and normal immune cell groups.

Identification of transcription factors and miRNAs interact with key genes

Hub transcription factors (TFs) were identified using the JASPAR database, and the effect of binding of hub miRNAs to hub gene transcripts on protein expression was detected by miRNet (https://www.mirnet.ca/). We constructed topological networks of TFs genes and miRNA genes using Cytoscape software.

Isolation of human primary keratinocytes

Skin samples were obtained from the foreskin tissue of eight children, aged 6 to 12 years, at Northwest Women's and Children's Hospital in Xi'an, China. Prior to the procedure, the researchers obtained ethical permits and secured written informed consent from the parents or legal guardians of the participants. The researchers isolated primary keratinocytes using the standard two-step digestion method (36).

Cell culture

The HeLa cells were acquired from ATCC and grew in DMEM with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere with 5% CO2. PKC was cultured following prior procedures (37).

Establishment of the psoriatic cell model

PKCs were stimulated by M5 (TNF-a, IL-17A, IL-22, IL-1a, and oncostatin M) at a concentration of 10 ng/mL for a duration of 24 hours, as previously described (37).

qRT-PCR

RNA extraction and qRT-PCR procedures were conducted following the previously described method (38). Relevant mRNA levels were determined utilizing the $2^{\wedge(-\Delta\Delta Ct)}$ formula. The primers used in the study are summarized in Table 2.

Statistical analysis

All statistical analyses were conducted using R software version 4.2.2 and Sangerbox. To assess the statistical significance between normally distributed variables in the two groups of continuous variables, we employed the independent Student's t-test. Conversely, differences between non-normally distributed variables were determined using the Mann-Whitney U-test. The statistical significance between the two groups of categorical variables was analyzed using either the chi-square test or Fisher's exact test. Estimation of correlation coefficients between different genes was conducted through Pearson correlation analysis. All statistical tests conducted were two-sided and the level of statistical significance was set at a p-value of less than 0.05.

Results

WGCNA identifies key modules in psoriasis and cervical cancer

The investigators merged two psoriasis-related GEO datasets, GSE14905 and GSE13355. The data sets were merged and normalized to ensure uniformity for principal component analysis and to rectify batch effects. The final training dataset consisted of 91 patients and 85 matched controls, and the evaluation showed that the data preprocessing was valid and reliable. From the density plot, we can observe that the sample distributions of the individual datasets before removing the batch effect varied greatly, suggesting a batch effect, and after removing the batch effect the data distributions between the individual datasets converged, with similar means and variances (Figures 2A, B). Weighted gene co-expression network analysis was conducted utilizing the R package WGCNA, the genes with expression variance in the top 50% were used as the screening conditions, and the genes with less volatility were excluded, and the co-expression network was constructed for 20547 genes of psoriasis and 10,275 genes of cervical cancer.

Combining the analysis of scale independence and average connectivity, in the psoriasis samples, b=12 was chosen (Figures 2C, D) as the soft threshold. The minimum module size was set to 30 and 15 gene modules were obtained (Figure 2G). The results showed that the brown module had the highest correlation with psoriasis (correlation coefficient = 0.92, p= 2.2e-72, Figure 2I). Ultimately, 969 psoriasis-significantly correlated genes were identified in brown color module with high MM (> 0.8) and GS (> 0.1) values. In the cervical cancer samples, 8 was chosen as the optimal soft threshold to build a scale-free network (Figures 2E, F). Subsequently, cluster analysis was used to identify highly similar modules with the minimum module size set to 30, sensitivity set to 3, and modules with distances less than 0.25 were merged to obtain 18 gene modules (Figure 2H). The correlation between cervical cancer and gene modules (Figure 2J) showed that the green module had the highest correlation with cervical cancer (2270 genes, r=0.71, p=5.4e-9), and the green module was taken as the key module. The genes in the green module: MM>0.8 and GS>0.1 were selected as pivotal genes, and a total of 421 key genes significantly associated with cervical cancer were identified.

Identification of differentially expressed genes and machine learning screening of key genes

By Limma analysis, 2066 differentially expressed genes (DEGs) between psoriasis patients and healthy controls were identified in the integrated dataset, of which 1134 genes were up-regulated and 932 genes were down-regulated. These DEGs were presented by volcano plot visualization (Figure 3A). In addition, the cervical squamous cell carcinoma dataset generated 6573 DEGs, including 2689 up-regulated genes and 1586 down-regulated genes (Figure 3B). The DEGs from the cervical squamous cell carcinoma and psoriasis samples were intersected with key genes taken from the WGCNA to obtain a total of 27 genes for subsequent analysis (Figure 3C). 27 genes were uploaded to the STRING database to construct a protein-protein interaction network (Figure 3D), then we analyzed the top 10 genes

TABLE 2 Basic information of datasets used in the study.

Gene	Forward (5′→3′)	Reverse (5′→3′)
CDCA2	TCTGATTCGTTTCATTGCTCGG	ACATTTCGATACAGTGCAGGG
CENPN	TGAACTGACAACAATCCTGAAGG	CTTGCACGCTTTTCCTCACAC
MELK	AACTCCAGCCTTATGCAGAAC	AACGATTTGGCGTAGTGAGTATT
NCAPH	GTCCTCGAAGACTTTCCTCAGA	TGAAATGTCAATACTCCTGCTGG
UHRF1	AGGTGGTCATGCTCAACTACA	CACGTTGGCGTAGAGTTCCC

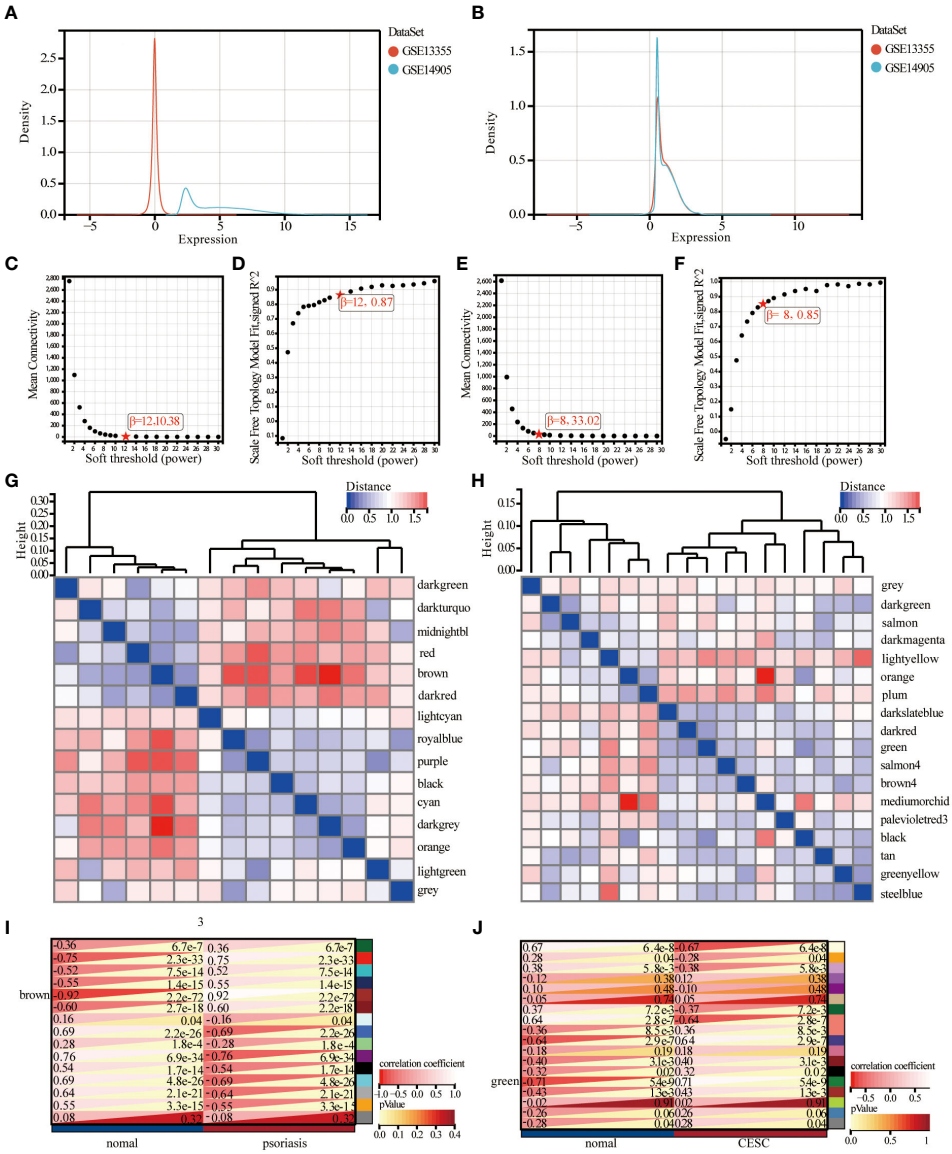

FIGURE 2
Identification and analysis of key module of psoriasis and cervical squamous cell carcinoma by WGCNA. **(A)** Principal component analysis of the two original Psoriasis datasets before batch effect correction. **(B)** Principal component analysis of the corrected Psoriasis dataset. **(C, D)** Scale independence and average connectivity plots of psoriasis. **(E, F)** Scale independence and average connectivity plots of cervical cancer. **(G, H)** Gene dendrogram and heatmap of the modular signature gene network. **(I, J)** Identification of weighted gene co-expression network modules associated with psoriasis and cervical cancer, and module characterized genes in relation to psoriasis and cervical cancer status.

by using the "degree" algorithm with the CytoHubba application in Cytoscape to identify the key genes, and the color of the nodes indicated the strength of the correlation (Figure 3E). The color of the nodes indicates the strength of the correlation. Random forest pairs were used for screening and finally 16 characterized genes were identified in psoriasis samples, including *MELK*, *AURKA*, *CENPN*, *CDCA5*, *KIF2C*, *NDC80*, *PRSS3P2*, *PRC1*, *DEPDC1B*, *FOXM1*, *UHRF1*, *WDR53*, *MCM10*, *BUB1B*, *NCAPH*, *CDCA2*(Figure 3F). Meanwhile, 10 cervical squamous cell carcinoma signature genes were also identified using RF algorithms, including *NCAPH, SMC4, CENPN, UHRF1, STIL, CDCA2, MELK, ZNF665* (Figure 3G). Next, the study found that these algorithms identified five overlapping genes (Figure 3H), namely *NCAPH, UHRF1, CENPN, CDCA2, MELK* which were used for sebsequent analysis (Table 3).

GO and KEGG enrichment analyses were performed to identify biological pathways and diseases associated with key genes

For biological processes in GO enrichment analysis, biological processes were highly enriched in mitotic cell cycle processes (Figure 4A, biological processes (BP)). And for the cellular components in GO, it involves intracellular non-membrane-bound

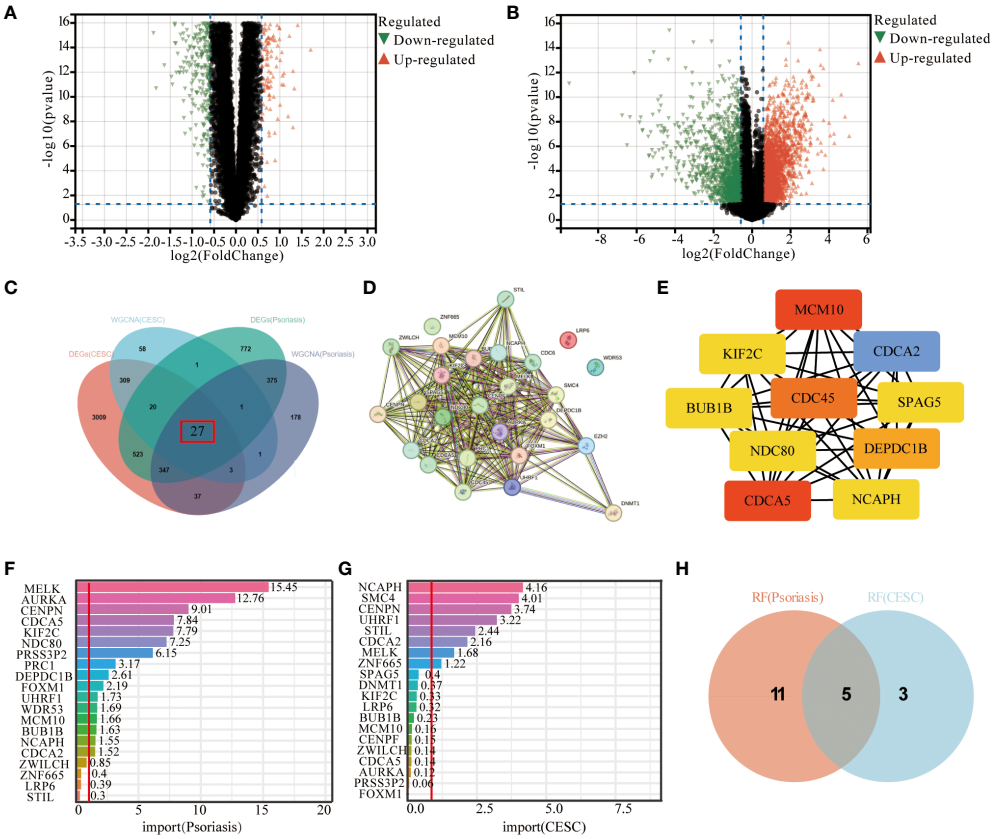

FIGURE 3
Screening of hub-genes by machine learning algorithm. **(A, B)** Volcano plot demonstrating an overview of the differential expression of all genes in CESC and Psoriasis. **(C)** DEGs in cervical cancer and psoriasis samples were intersected with key genes in WGCNA taken to obtain the Wayne plots of 27 genes. **(D)** PPI network of 27 genes. **(E)** Major PPI network analysis of the top 10 hub genes by CytoHubba software. **(F)** RF algorithm screened out 16 characterized genes in psoriasis samples. **(G)** The RF algorithm screened 8 characterized genes in cervical cancer samples. **(H)** Wayne diagram of 5 key genes identified. The threshold in the volcano plot was -log10 (adjusted P-value) > 2 and |log2 (fold change)| > 0.5; red dots indicate significant differential expressed genes. FDR was used for P value adjustment.

organelles, chromosomes, and mitotic regions (Figure 4B, cellular components (CC)). For the molecular functions enriched in GO, including nucleotide binding, phosphoribosylation, chromatin binding (Figure 4C, Molecular Functions (MF)). Based on the KEGG database further to decipher the biological pathways behind, the enriched molecular pathways included cell cycle, microRNAs in cancer, oocyte meiosis, breast cancer, gastric cancer, and mTOR signaling pathway (Figure 4D). These findings are in line with the results of GO enrichment analysis, providing further evidence of the association between cervical squamous cell carcinoma and psoriasis.

The CIBERSORT analysis tool calculated the proportions of 22 types of leukocyte subpopulations in psoriasis and CESC samples, respectively, including naïve B cells, memory B cells, plasma B cells, CD8 T cells, CD4 naive T cells, CD4 memory quiescent T cells, CD4 memory-activated T cells, follicular helper T cells, regulatory T cells (Tregs), γ δ T cells, resting natural killer (NK) cells, activated NK cells, monocytes, M0, M1 and M2 macrophages, resting and activated myeloid dendritic cells, and resting and activated mast cells. We also explore the relationship of key genes to immune infiltrating cells in both diseases and found that genes associated with psoriasis can also play a role in cervical squamous cell carcinoma. Stacked bar graphs of the two datasets show the percentage of 22 immune cells in each sample (Figure 5A). Analysis of the immune microenvironment in psoriasis patients revealed significant differences in the abundance of 20 immune cells. Analysis of the immune microenvironment in patients with cervical squamous cell carcinoma revealed notable variations in the abundance of seven immune cells. These differences were statistically significant (Figure 5B). In summary, patients with psoriasis and cervical squamous cell carcinoma have varying degrees of multiple immune cell infiltrations, and these immune cell infiltrations may be potential regulatory points for therapy. Then, the spearman correlation coefficient between hub genes and the infiltration level of the immune cell was calculated. As a result, resting mast cells and CD8T cells were negatively correlated with the expression of *NCAPH, UHRF1, CDCA2, CENPN* and *MELK* in patients with psoriasis and cervical squamous carcinoma, respectively (Figure 5C).

Identification of candidate small molecule compounds for the treatment of psoriasis and cervical squamous cell carcinoma

The intersection of DEGs genes upregulated in psoriasis and cervical squamous cell carcinoma was taken with hub genes in the

TABLE 3 Overview of the five hub genes.

Symbol	Description	Aspect	References
NCAPH	Non-SMC Condensin I Complex Subunit H	Interferes with plasmids and affects cell proliferation and migration	(39)
UHRF1	Ubiquitin Like With PHD And Ring Finger Domains 1	Required for G1/S phase transition; Regulation of DNA methylation, chromatin modification, cell proliferation and DNA repair	(40, 41)
CENPN	Centromere Protein N	Binds to filaments in S and G2 phases and recruits proteins	(42)
CDCA2	Cell Division Cycle Associated 2	Affects tumor cell proliferation and regulates the G0/G1 phase of the cell cycle	(43)
MELK	Maternal Embryonic Leucine Zipper Kinase	Induces inflammatory responses through secretion of pro-inflammatory factors Involved in mitosis, proliferation, apoptosis, differentiation and tumorigenesis	(44, 45)

WCGNA module, and 24 relevant pathogenic genes were obtained (Figure 6A). The screened 24 relevant pathogenic genes were imported into connectivity map (cMAP) database to predict small molecule compounds that could reverse the gene expression alterations in psoriasis-related pathogenesis and cervical squamous cell carcinoma. Phloretin, antimycin-a, palbociclib, purvalanol-a, aminopurvalanol-a, PD-102807, 7b-cis, pyrvinium-pamoate, angiogenesis-inhibitor, roscovitine were the top 10 compounds with the highest negative scores as potential drugs for therapy (Figure 6B). The targeting pathways and chemical structures of these 10 compounds are described in Figures 6C, D.

Validation of hub genes with GEO and TCGA databases and cellular experimental validation

To further confirm the accuracy of the comprehensive bioinformatics analysis described above, we first examined the expression patterns of the five hub genes in the patients of the two validation cohorts, and chose the psoriasis dataset, GSE63514 and the cervical squamous cell carcinoma dataset, TCGA-CESC, as the validation datasets. Multi-group box plots showed that the expression levels of *NCAPH, UHRF1, CDCA2, CENPN* and

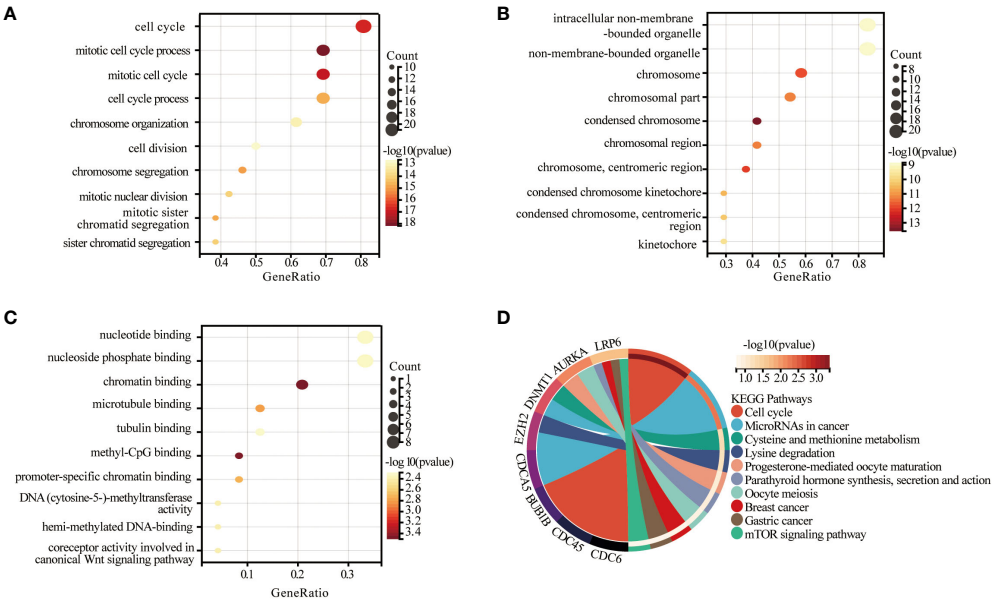

FIGURE 4
Significant gene module and enrichment analysis of the modular genes. **(A-C)** Results of GO analysis of 27 genes, biological process (BP), cellular component (CC) and molecular function (MF) of the genes. **(D)** Results of KEGG analysis of 27 genes.

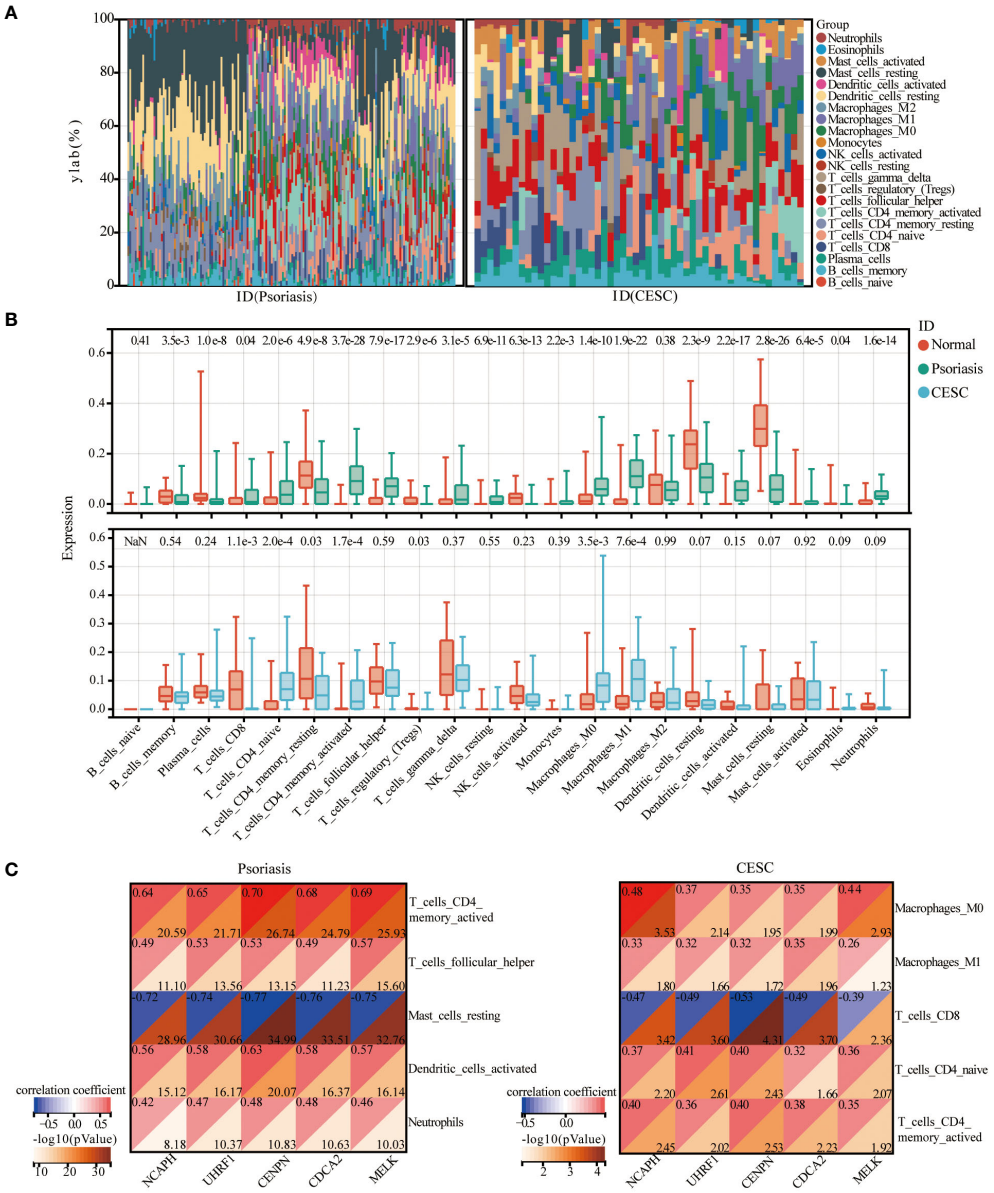

FIGURE 5
Immune cell infiltration analysis. **(A)** Heat map of the relative proportions of 22 types of infiltrating immune cells in patients with psoriasis and cervical cancer. **(B)** Violin plot of the abundance of each type of immune cell infiltration in the psoriasis and cervical cancer group. **(C)** Correlation graph representing the association of immune cells with five central genes.

MELK were significantly higher in psoriasis patients and cervical squamous cell carcinoma patients than in normal controls (Figures 7A, B). RT-qPCR results confirmed that the expression levels of *CENPN* and *MELK* mRNA levels were increased (Figure 7C), and that the expression of the five pivotal genes were consistently up-regulated in cervical cancer samples as compared to the control samples (Figure 7D).

Cohort validation of hub genes and enrichment analysis

We plotted ROC curves based on the five candidate genes to assess the diagnostic value of each gene. The calculated AUCs and 95% confidence intervals were as follows: *NCAPH* (AUC 0.92, CI 0.97–0.88), *UHRF1* (AUC 0.89, CI 0.94–0.84), *CDCA2* (AUC 0.96, CI 0.99–0.92), *CENPN* (AUC 0.94, CI 0.98–0.90) and *MELK* (AUC 0.96, CI 1.00–0.93). The findings indicated that the acquired genes had a significant diagnostic value in Psoriasis (Figure 8A). To investigate the potential functions of common central genes, we divided the samples from the psoriasis dataset into groups with high and low expressions based on median levels. We then identified DEGs between these groups and conducted GO/KEGG enrichment analysis. The significant enriched genes include "lysosomes, phagocytosis, SLE, pyrimidine metabolism, arachidonic acid metabolism, complement and coagulation cascades, and natural killer cell-mediated cytotoxicity (Figures 8B, C).

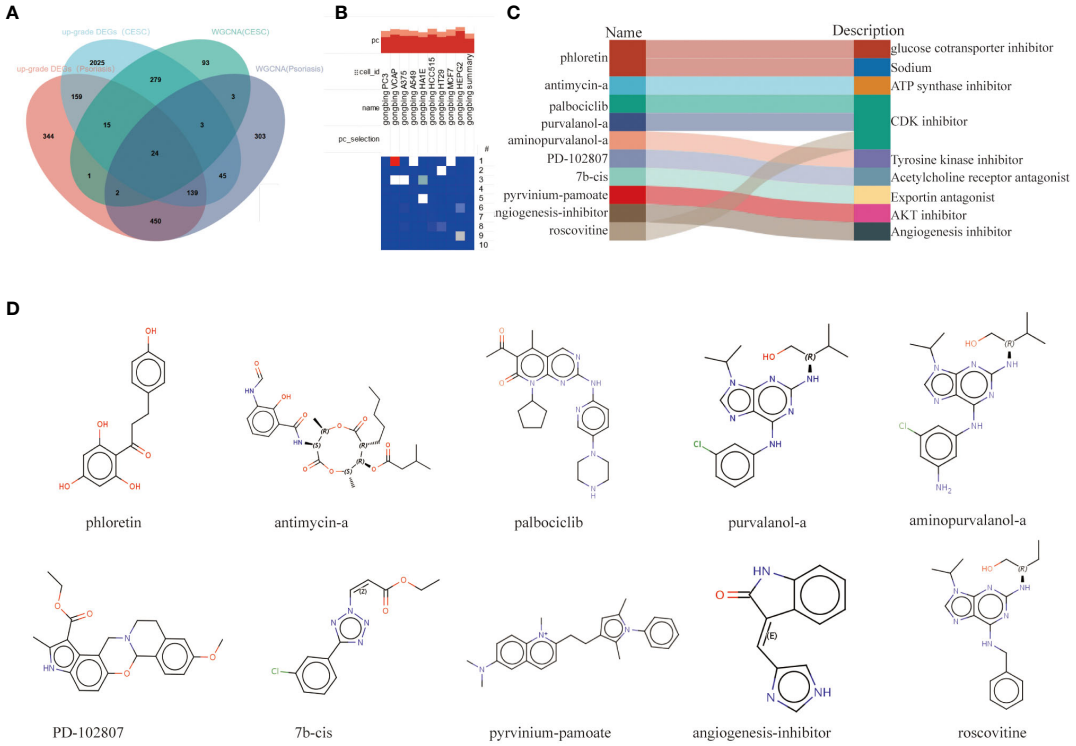

FIGURE 6
Screening of the potential small-molecular compounds for the treatment of psoriasis and CESC via cMAP analysis. **(A)** Intersection Wayne plots of DEGs genes up-regulated in psoriasis and cervical cancer with hub genes taken from the WCGNA module. **(B)** Heatmap of the top 10 compounds with the highest enrichment in 10 cell lines based on cMAP analysis. **(C)** Top 10 compounds information and targeting pathways. **(D)** Chemical structures of the 10 compounds.

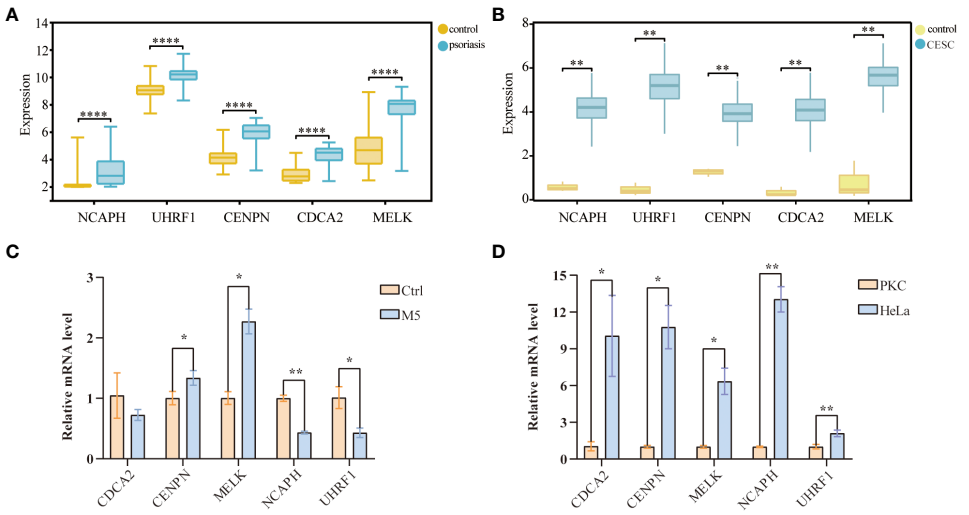

FIGURE 7
Validation of hub-genes in external datasets and experiments databases. **(A)** Validation of the center gene of cervical cancer in TCGA-CESC database. **(B)** Validation of center gene in the psoriasis dataset GSE35182. **(C)** RT-qPCR results of 5 key genes in psoriasis cell samples. **(D)** RT-qPCR results of 5 key genes in cervical cancer cell samples. (*p< 0.05, **p < 0.01, ***p < 0.001).

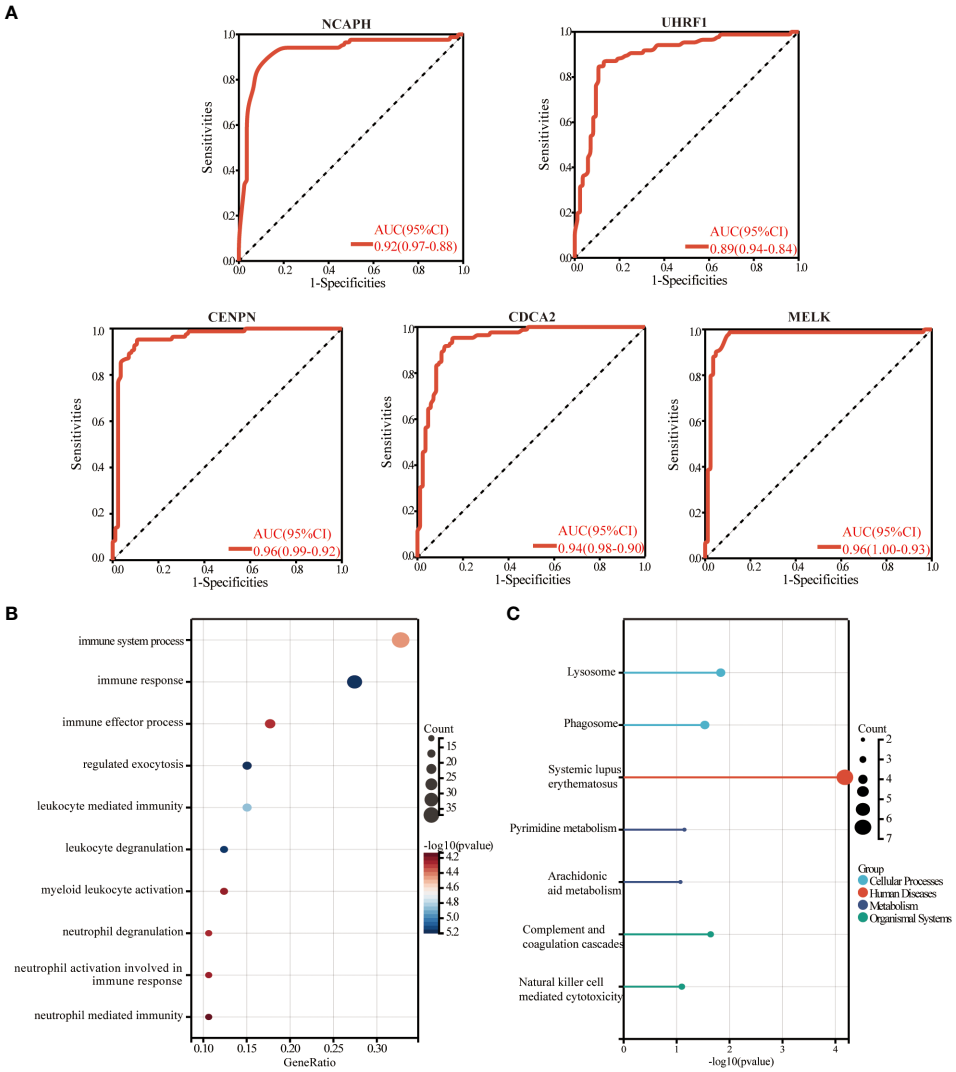

FIGURE 8
The diagnostic value evaluation in the validation cohort and enrichment analysis. **(A)** ROC plot of each key gene (*NCAPH, UHRF1, CDCA2, CENPN,* and *MELK*) based on the AUC. **(B)** The bubble plot demonstrates the results of GO enrichment analysis of hub gene-related differential genes in psoriasis. **(C)** The results of the KEGG enrichment analysis of hub gene-related differential genes in psoriasis are demonstrated by a lollipop plot. AUC, area under the curve.

The regulatory signatures analysis

We applied the miRNet database to screen the targeted miRNAs of NCA *NCAPH, UHRF1, CDCA2, CENPN* and *MELK*. As depicted in Figure 9A, the prediction identifies three miRNAs: hsa-miR-124–3p, hsa-mir-129–2-3p, and hsa-mir-147a. The Network analysis tool explored 9 transcription factors namely FOXC1, NFKB1, RELA, SREBF1, NRF1, GATA2, TFAP2A, USF1, USF2 (Figure 9B). The TFs and miRNAs related to three hub genes via network analysis were shown in Table 4.

Discussion

Cervical cancer is the fourth leading cause in cancer incidence and mortality among women, contributing to over 60,000 new cases and approximately 342,000 deaths across the world (60). In recent years, there has been a decline in the incidence of cervical cancer due to high-risk group screenings. Despite some progress, the 5-year survival rate for patients with advanced cervical cancer is only 16.7%. And early recognition and diagnosis of cervical cancer is one of the best measures to improve prognosis and reduce social burden (61).

Psoriasis, a chronic inflammatory skin disease, is increasingly recognized as a systemic inflammatory condition and can coexist with other diseases (62). The link between psoriasis and cancer is also gaining attention. In a cohort study, individuals who underwent treatment for severe psoriasis displayed a 41% greater likelihood of succumbing to malignant tumors than non-psoriasis attendees (63). A meta-analysis of 11 retrospective studies showed an increased risk of cancer in non-melanoma skin cancer (NMSC) (95% confidence interval [CI] 1.07–1.25) (64). A cohort study

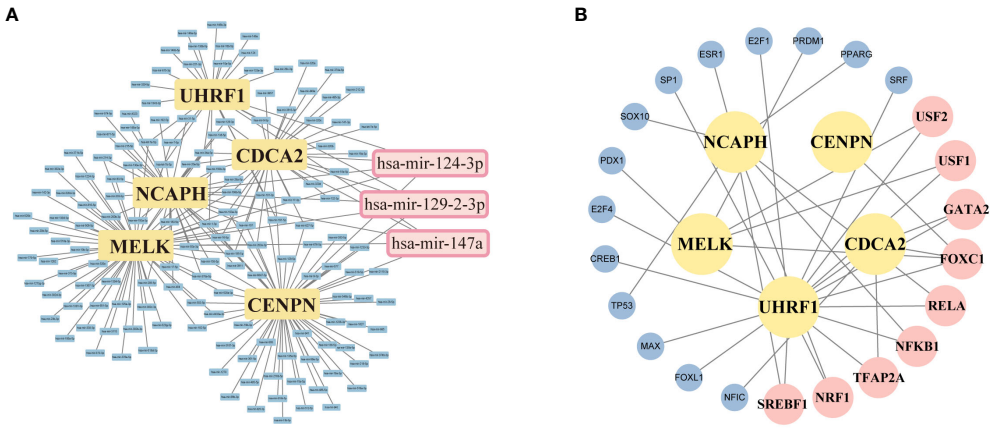

FIGURE 9
Screening of potential miRNAs and TF-mRNA network of 5 targeting hub-gene. **(A)** An Interaction network of five hub genes and potential miRNAs-targeted. **(B)** TF-mRNA network of 5 hub genes. The pink squares represent the top TFs associated with the hub genes.

TABLE 4 Top transcription factors and miRNA predicted from miRNA-mRNA, TFs-mRNA regulatory networks.

TFs/miRNAs	Description	Biological function	Reference
FOXC1	Forkhead	Regulation of cell proliferation, migration and invasion through PI3K/AKT signaling	(46)
NFKB1	nuclear factor kappa B subunit 1	Inhibition of cell proliferation, colony formation and migration in cervical cancer	(47)
RELA	v-rel avian reticuloendotheliosis viral oncogene homolog A	Control of NF-κB activity by autophosphorylation in inflammatory diseases and cancer。	(48)
SREBF1	sterol regulatory element binding transcription factor 1	Stimulates ubiquitination of SREBP1 and inhibits endoplasmic reticulum stress in CESC cells.	(49)
NRF1	nuclear respiratory factor 1	Leads to severe oxidative stress, genomic instability	(50)
GATA2	GATA binding protein 2b	A common regulatory elements in cervical cancer	(51)
TFAP2A	transcription factor AP-2 alpha	Promotes the growth of cervical tumors	(52, 53)
USF1/2	upstream transcription factor 1/2	Enhancement of cervical cancer cell malignancy by transcriptional activation of p65	(54, 55)
hsa-miR-124–3p	MicroRNA 124	Direct targeting of IGF2BP to inhibit cervical cancer growth and metastasis is considered to be an important marker and target for CC prognosis	(56)
hsa-mir-129–2-3p	MicroRNA 129	The methylation process of mir-129–2-3p increases cervical (pre)cancerous lesions.	(57)
hsa-mir-147a	MicroRNA 147a	Interacts with circ_0018289 binding and Linc00319 to promote cervical cancer progression.	(58, 59)

assessing cancer risk among psoriasis patients in the United Kingdom also found an increased risk of NMSC, lung cancer, and lymphoma, and this study also removed the effects of confounding factors such as smoking and alcohol consumption (65). Specifically cervical cancer, surveys have demonstrated that psoriasis patients taking biologics were more likely to be screened for cervical cancer than the general population without psoriasis (adjusted hazard ratio [HR] 1.09; 95% [CI] 1.02 - 1.16) (66). In addition, psoriasis lesions have been shown to contain HPV infection (67). Due to the immunomodulatory effects of medications used to treat psoriasis, which contribute to the development of cervical cancer, the ability of clearing HPV infection is impaired, leading to an increased risk of cervical tumors. This suggests that patients with psoriasis are at increased risk of developing HPV-associated cervical lesions; there

may be a co-morbid mechanism and risk association between the two, and our study provides new insights for clinicians to be aware of encouraging patients with psoriasis to follow a cervical tumor screening program (68).

Combining WGCNA, limma difference analysis and machine learning, we screened five key genes as markers of psoriasis and cervical cancer co-morbidities, including *NCAPH, UHRF1, CDCA2, CENPN* and *MELK*. *NCAPH* predominantly promotes sister chromatid entanglement, exacerbating chromosome segregation errors and cell division failure (69). Studies have confirmed that elevated levels of NCAPH expression are associated with an unfavorable prognosis and immune infiltration in several cancer types, including lung adenocarcinoma, breast cancer, and colorectal cancer (70). The expression of *NCAPH* in cervical cancer tissues was significantly higher than that in normal cervical tissues and was significantly correlated with the size, invasion and lymph node metastasis of cervical cancer tumor tissues, suggesting that *NCAPH* is a potential target for cervical cancer immunotherapy (71). *UHRF1* is a highly expressed epigenetic regulator within cancer cells that plays a significant role in double-strand break repair through homologous recombination. Overexpression of UHRF1 results in increased DNA methylation, promoting the further development, progression, and invasion of cancer (72, 73). Interestingly, human papillomavirus was found to induce cervical cancer through *UHRF1*-mediated promoter methylation, suggesting that treatment targeting *UHRF1* may inhibit cervical carcinogenesis through cell cycle arrest and apoptosis (74–77). The mitochondrial protein CENP-N regulates normal chromosome segregation by recognizing histone H3 in filamentous nucleosomes and promoting densification of filamentous chromatin (78, 79). In this study, *CENPN* expression was significantly elevated in both psoriasis and cervical cancer tissues compared to control samples, which could serve as a potential diagnostic indicator for identifying cervical cancer in psoriasis patients. In conclusion, our study suggests that these five central genes may play a key role in psoriasis and cervical cancer.

The pathophysiology of psoriasis involves abnormal activation of the autoimmune system, both intrinsic and acquired. This dysregulation is a key component of mechanisms that prevent and interfere with cancer (79). There exists a robust association between cancer and inflammation, with inflammation representing a paramount risk factor in the development of cancer, often accompanied by inflammation (80). We explored the mechanisms of immune dialog between psoriasis and cervical cancer. Our study demonstrated that cervical cancer tissues are heavily infiltrated with T lymphocytes and the ratio of CD4+ to CD8+ is reversed, and there is evidence that this phenomenon promotes an inflammatory response in patients with cervical cancer, leading to elevated levels of CRP(C-reactive protein) and HbA1c% (81). Interestingly, previous studies have shown that Th1 subpopulation T cells promote macrophage- and cytotoxic T cell-mediated immune responses through the release of interferon-γ (IFN-γ) and TNF-α, which are key factors in the pathogenesis of psoriasis (82). In addition, our immune infiltration analysis showed that macrophage type M1, which promotes the development of inflammation, was also heavily infiltrated in cervical cancer tissues. It has been shown that depletion of macrophages attenuates psoriatic inflammation and reduces the levels of Th1 cytokines, including IL-1α, IL-6, IL-23, and TNF-α, to normal levels (83–86). Psoriasis and cervical cancer show common properties and potential in terms of immune processes.

Although biologics have shown better efficacy in psoriasis, the side effects of biologics pose certain hazards. Therefore, there is an urgent need to explore potential drugs. Small molecule compounds have the advantages of high tissue permeability, adjustable half-life, and high oral bioavailability, resulting in better therapeutic efficacy. We linked causative genes associated with psoriasis and cervical cancer through cMAP analysis to identify potential therapeutic agents. roscovitine, palbociclib, and purvalanol-a are CDK (cell cycle protein-dependent kinase) inhibitors. The CDK inhibitors block the proliferation inhibition of malignant tumor cells through cell cycle progression (87). In some inflammation models, roscovitine demonstrates a reduction in leukocyte-mediated inflammation (88). Pravachol A, a CDK2 inhibitor, induces apoptosis in human neutrophils (89). Most solid tumor cells produce energy by relying heavily on aerobic glycolysis, and phloretin can effectively inhibit cancer progression by targeting the glycolytic pathway as a glucose cotransporter inhibitor (90). Antimycin A is a promising anticancer agent (90), which can target mitochondria, reduce human papillomavirus E6/E7 oncogene protein, inhibit proliferation, and induce apoptosis in cervical cancer cells (91). Aminopurinol A as a Tyrosine kinase inhibitor can restore the abnormal process of pre-mRNA splicing in cancer (92). The anticancer effects of Pyrviniu are mainly manifested in the inhibition of mitochondrial function as well as the renewal of cancer stem cells (93), and in particular, it significantly impedes cancer cell invasion via the Wnt/β-catenin signaling pathway (94). These drugs have promising potential in the treatment of psoriasis and cervical cancer.

We recognize the potential challenges faced by patients with comorbidities. For example, the use of biologics during treatment tends to suppress the activation of the body's immune system, which implies an increased potential risk of tumorigenesis. To further validate this concern in patients with psoriasis treated with biologics, we need to conduct additional clinical cohort studies. How psoriasis and cervical cancer talk through key genes under the systemic neuro-immune-endocrine network also needs further experimental exploration.

Conclusion

Based on bioinformatics analysis and machine learning, we systematically identified five related candidate genes (NCAPH, UHRF1, CDCA2, CENPN and MELK). This study will facilitate the exploration of molecular mechanisms, particularly with regard to the immune response and drug action. A comprehensive understanding of disease pathogenes is vital for mediating their interaction and prevent the risk of complications. The screened genes could be used for clinical diagnosis and treatment.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by Ethics Committee of Xi'an Jiaotong University. The studies were conducted in accordance with the local legislation and institutional requirements (No.2022-1012). The participants provided their written informed consent to participate in this study.

Author contributions

LL: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. PY: Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. RY: Data curation, Project administration, Software, Writing – original draft, Writing – review & editing. GZ: Funding acquisition, Resources, Visualization, Writing – review & editing. CW: Conceptualization, Investigation, Software, Writing – review & editing. YZ: Data curation, Funding acquisition, Methodology, Supervision, Writing – review & editing. SW: Funding acquisition, Validation, Visualization, Writing – review & editing. ML: Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Blauvelt A, Lebwohl M, Langley RG, Rowland K, Yang YW, Chan D, et al. Malignancy rates through 5 years of follow-up in patients with moderate-to-severe psoriasis treated with guselkumab: Pooled results from the VOYAGE 1 and VOYAGE 2 trials. *J Am Acad Dermatol.* (2023) 89:274–82. doi: 10.1016/j.jaad.2023.03.035

2. Wu JJ, Kavanaugh A, Lebwohl MG, Gniadecki R, Merola JF. Psoriasis and metabolic syndrome: implications for the management and treatment of psoriasis. *J Eur Acad Dermatol Venereol.* (2022) 36(6):797–806. doi: 10.1111/jdv.18044

3. Canli Ö, Nicolas AM, Gupta J, Finkelmeier F, Goncharova O, Pesic M, et al. Myeloid cell-derived reactive oxygen species induce epithelial mutagenesis. *Cancer Cell.* (2017) 32:797–806. doi: 10.1016/j.ccell.2017.11.004

4. Ceccarelli M, Venanzi Rullo E, Vaccaro M, Facciolà A, d'Aleo F, Paolucci IA, et al. HIV-associated psoriasis: Epidemiology, pathogenesis, and management. *Dermatol Ther.* (2019) 32:e12806. doi: 10.1111/dth.12806

5. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity.* (2019) 51:27–41. doi: 10.1016/j.immuni.2019.06.025

6. Hijnen D, Knol EF, Gent YY, Giovannone B, Beijn SJP, Kupper TS, et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-γ, IL-13, IL-17, and IL-22. *J Invest Dermatol.* (2013) 133:973–9. doi: 10.1038/jid.2012.456

7. Bu J, Ding R, Zhou L, Chen X, Shen E. Epidemiology of psoriasis and comorbid diseases: A narrative review. *Front Immunol.* (2022) 13:880201. doi: 10.3389/fimmu.2022.880201

8. Balda A, Wani I, Roohi TF, Suman, Krishna KL, Mehdi S, et al. Psoriasis and skin cancer - Is there a link? *Int Immunopharmacol.* (2023) 121:110464. doi: 10.1016/j.intimp.2023.110464

9. Rademaker M, Rubel DM, Agnew K, Andrews M, Armour KS, Baker C, et al. Psoriasis and cancer. An Australian/New Zealand narrative. *Australas J Dermatol.* (2019) 60:12–8. doi: 10.1111/ajd.12889

10. Loft ND, Vaengebjerg S, Skov L. Cancer risk in patients with psoriasis: should we be paying more attention? *Expert Rev Clin Immunol.* (2020) 16:479–92. doi: 10.1080/1744666X.2020.1754194

11. Vaengebjerg S, Skov L, Egeberg A, Loft ND. Prevalence, incidence, and risk of cancer in patients with psoriasis and psoriatic arthritis: A systematic review and meta-analysis. *JAMA Dermatol.* (2020) 156:421–9. doi: 10.1001/jamadermatol.2020.0024

12. Jung JM, Lee KH, Kim Y-J, Chang SE, Lee MW, Choi JH, et al. Assessment of overall and specific cancer risks in patients with Hidradenitis suppurativa. *JAMA Dermatol.* (2020) 156:844–53. doi: 10.1001/jamadermatol.2020.1422

13. Beyaert R, Beaugerie L, Van Assche G, Brochez L, Renauld J-C, Viguier M, et al. Cancer risk in immune-mediated inflammatory diseases (IMID). *Mol Cancer.* (2013) 12:98. doi: 10.1186/1476-4598-12-98

14. Naldi L. Malignancy concerns with psoriasis treatments using phototherapy, methotrexate, cyclosporin, and biologics: facts and controversies. *Clin Dermatol.* (2010) 28:88–92. doi: 10.1016/j.clindermatol.2009.03.003

15. Buskwofie A, David-West G, Clare CA. A review of cervical cancer: incidence and disparities. *J Natl Med Assoc.* (2020) 112:229–32. doi: 10.1016/j.jnma.2020.03.002

16. Martínez-Rodríguez F, Limones-González JE, Mendoza-Almanza B, Esparza-Ibarra EL, Gallegos-Flores PI, Ayala-Luján JL, et al. Understanding cervical cancer through proteomics. *Cells.* (2021) 10(8):1854. doi: 10.3390/cells10081854

17. Bogani G, Raspagliesi F, di Donato V, Brusadelli C, Guerrisi R, Pinelli C, et al. Spotlight on the role of human papillomavirus vaccines. *Gynecologic Oncol.* (2021) 160:346–50. doi: 10.1016/j.ygyno.2020.08.034

18. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA: Cancer J Clin.* (2023) 73:17–48. doi: 10.3322/caac.21763

19. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide

analysis.?*Lancet Global Health*. (2020) 8:e191–203. doi: 10.1016/S2214-109X(19)30482-6

20. Peng H, He X, Wang Q. Immune checkpoint blockades in gynecological cancers: A review of clinical trials. *Acta Obstet Gynecol Scand*. (2022) 101:941–51. doi: 10.1111/aogs.14412

21. Wu JJ, Kavanaugh A, Lebwohl MG, Gniadecki R, Merola JF. Psoriasis and metabolic syndrome: implications for the management and treatment of psoriasis. *J Eur Acad Dermatol Venereol*. (2022) 36(6):797–806. doi: 10.1111/jdv.18044

22. Moscicki AB, Flowers L, Huchko MJ, Long ME, MacLaughlin KL, Murphy J, et al. Guidelines for cervical cancer screening in immunosuppressed women without HIV infection. *J Low Genit Tract Dis*. (2019) 23:87–101. doi: 10.1097/LGT.0000000000000468

23. Gennigens C, Jerusalem G, Lapaille L, De Cuypere M, Streel S, Kridelka F, et al. Recurrent or primary metastatic cervical cancer: current and future treatments. *ESMO Open*. (2022) 7:100579. doi: 10.1016/j.esmoop.2022.100579

24. Zheng Y, Huang Q, Zhang Y, He X. The external use of Wolf poison in the treatment of skin diseases. *2023 National Conference on Dermatology and venereal Diseases*. (2023).

25. Wu M, Deng Y, Li S, Chen Y, Guo D. The immunoregulatory effects of traditional chinese medicine on psoriasis via its action on interleukin: advances and considerations. *Am J Chin Med*. (2018) 46(4):739–750.

26. Tison A, Quéré G, Misery L, Funck-Brentano E, Danlos FX, Routier E, et al. Safety and efficacy of immune checkpoint inhibitors in patients with cancer and preexisting autoimmune disease: A nationwide, multicenter cohort study. *Arthritis Rheumatol (Hoboken NJ)*. (2019) 71:2100–11. doi: 10.1002/art.41068

27. Wang CY, Wang CW, Chen CB, Chen WT, Chang YC, Hui RC, et al. Pharmacogenomics on the treatment response in patients with psoriasis: an updated review. *Int J Mol Sci*. (2023) 24(8):7329. doi: 10.3390/ijms24087329

28. Furue K, Ito T, Tsuji G, Kadono T, Furue M. Psoriasis and the TNF/IL23/IL17 axis. *Giornale italiano di dermatologia e venereologia: organo ufficiale Societa italiana di dermatologia e sifilografia*. (2019) 154:418–24. doi: 10.23736/S0392-0488.18.06202-8

29. Elbalshy AEM, El-Refaie AM, Akl EM. Expression of pigment epithelium-derived factor in psoriasis, verrucae, squamous cell carcinoma and normal skin: An immunohistochemical study. *Indian J Dermatol Venereol Leprol*. (2020) 86:469. doi: 10.4103/ijdvl.IJDVL_396_19

30. Kim HW, Kim EH, Lee M, Jung I, Ahn SS. Risk of cancer, tuberculosis and serious infections in patients with ankylosing spondylitis, psoriatic arthritis and psoriasis treated with IL-17 and TNF-α inhibitors: a nationwide nested case-control analysis. *Clin Exp Rheumatol*. (2023) 41:1491–9. doi: 10.55563/clinexprheumatol/qkiorp

31. Takeshita J, Grewal S, Langan SM, Mehta NN, Ogdie A, Van Voorhees AS, et al. Psoriasis and comorbid diseases: Implications for management. *J Am Acad Dermatol*. (2017) 76:393–403. doi: 10.1016/j.jaad.2016.07.065

32. Dudley AC, Griffioen AW. Pathological angiogenesis: mechanisms and therapeutic strategies. *Angiogenesis*. (2023) 26:313–47. doi: 10.1007/s10456-023-09876-7

33. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. (2015) 43:e47. doi: 10.1093/nar/gkv007

34. The Gene Ontology Consortium. Gene Ontology Consortium: going forward. *Nucleic Acids Res*. (2015) 43(D1):D1049–56. doi: 10.1093/nar/gku1179

35. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res*. (2017) 45:D353–d61. doi: 10.1093/nar/gkw1092

36. Ścieżyńska A, Nogowska A, Sikorska M, Konys J, Karpińska A, Komorowski M, et al. Isolation and culture of human primary keratinocytes-a methods review. *Exp Dermatol*. (2019) 28:107–12. doi: 10.1111/exd.13860

37. Liu M, Zhang G, Wang Z, Liu X, He K, Luo R, et al. FOXE1 contributes to the development of psoriasis by regulating WNT5A. *J Invest Dermatol*. (2023) 143(12):2366–2377.e7. doi: 10.1016/j.jid.2023.04.035

38. Jia J, Li C, Luo S, Liu-Smith F, Yang J, Wang X, et al. Yes-associated protein contributes to the development of human cutaneous squamous cell carcinoma via activation of RAS. *J Invest Dermatol*. (2016) 136:1267–77. doi: 10.1016/j.jid.2016.02.005

39. Liao L, Cheng H, Liu S. Non-SMC condensin I complex subunit H promotes the Malignant progression and cisplatin resistance of breast cancer MCF-7 cells. *Oncol letters*. (2022) 24:317. doi: 10.3892/ol

40. Irwin RE, Scullion C, Thursby SJ, Sun M, Thakur A, Hilman L, et al. The UHRF1 protein is a key regulator of retrotransposable elements and innate immune response to viral RNA in human cells. *Epigenetics*. (2023) 18:2216005. doi: 10.1080/15592294.2023.2216005

41. Mousli M, Hopfner R, Abbady AQ, Monté D, Jeanblanc M, Oudet P, et al. ICBP90 belongs to a new family of proteins with an expression that is deregulated in cancer cells. *Br J cancer*. (2003) 89:120–7. doi: 10.1038/sj.bjc.6601068

42. Prosée RF, Wenda JM, Steiner FA. Adaptations for centromere function in meiosis. *Essays Biochem*. (2020) 64:193–203. doi: 10.1042/EBC20190076

43. Cui XH, Peng QJ, Li RZ, Lyu XJ, Zhu CF, Qin XH. Cell division cycle associated 8: A novel diagnostic and prognostic biomarker for hepatocellular carcinoma. *J Cell Mol Med*. (2021) 25:11097–112. doi: 10.1111/jcmm.17032

44. Pitner MK, Taliaferro JM, Dalby KN, Bartholomeusz C. MELK: a potential novel therapeutic target for TNBC and other aggressive Malignancies. *Expert Opin Ther Targets*. (2017) 21:849–59. doi: 10.1080/14728222.2017.1363183

45. Tang B, Zhu J, Fang S, Wang Y, Vinothkumar R, Li M, et al. Pharmacological inhibition of MELK restricts ferroptosis and the inflammatory response in colitis and colitis-propelled carcinogenesis. *Free Radical Biol Med*. (2021) 172:312–29. doi: 10.1016/j.freeradbiomed.2021.06.012

46. Huang L, Huang Z, Fan Y, He L, Ye M, Shi K, et al. FOXC1 promotes proliferation and epithelial-mesenchymal transition in cervical carcinoma through the PI3K-AKT signal pathway. *Am J Trans Res*. (2017) 9:1297–306.

47. Sena MM, Trugilo KP, Okuyama NCM, Pereira ÉR, Cezar-Dos-Santos F, Ferreira RS, et al. The role of NFKB1/NFKBIA genetic variants in HPV infection: A cross-sectional cohort study. *Exp Mol Pathol*. (2022) 124:104716. doi: 10.1016/j.yexmp.2021.104716

48. Lu X, Yarbrough WG. Negative regulation of RelA phosphorylation: emerging players and their roles in cancer. *Cytokine Growth Factor Rev*. (2015) 26:7–13. doi: 10.1016/j.cytogfr.2014.09.003

49. Wu Y, Min L, Zhang P, Zhang L, Xu Y, Li D, et al. ORP5 promotes migration and invasion of cervical cancer cells by inhibiting endoplasmic reticulum stress. *Cell Stress Chaperones*. (2023) 28:395–407. doi: 10.1007/s12192-023-01357-6

50. Yuan J, Zhang S, Zhang Y. Nrf1 is paved as a new strategic avenue to prevent and treat cancer, neurodegenerative and other diseases. *Toxicol Appl Pharmacol*. (2018) 360:273–83. doi: 10.1016/j.taap.2018.09.037

51. Kori M, Gov E, Arga KY. Novel genomic biomarker candidates for cervical cancer as identified by differential co-expression network analysis. *Omics: J Integr Biol*. (2019) 23:261–73. doi: 10.1089/omi.2019.0025

52. Zhang P, Hou Q, Yue Q. MiR-204–5p/TFAP2A feedback loop positively regulates the proliferation, migration, invasion and EMT process in cervical cancer. *Cancer biomarkers: section A Dis markers*. (2020) 28:381–90. doi: 10.3233/CBM-191064

53. Yang J, Gao Y, Yao S, Wan S, Cai H. TFAP2A promotes cervical cancer via a positive feedback pathway with PD–L1. *Oncol Rep*. (2023) 49(6):114. doi: 10.3892/or

54. Wang W, Yao S, Jiang H, Dong J, Cui X, Tian X, et al. Upstream transcription factor 1 prompts Malignancies of cervical cancer primarily by transcriptionally activating p65 expression. *Exp Ther Med*. (2018) 16:4415–22. doi: 10.3892/etm

55. Chi TF, Khoder-Agha F, Mennerich D, Kellokumpu S, Miinalainen I, Kietzmann T, et al. Loss of USF2 promotes proliferation, migration and mitophagy in a redox-dependent manner. *Redox Biol*. (2020) 37:101750. doi: 10.1016/j.redox.2020.101750

56. Wang P, Zhang L, Zhang J, Xu G. MicroRNA-124–3p inhibits cell growth and metastasis in cervical cancer by targeting IGF2BP1. *Exp Ther Med*. (2018) 15:1385–93. doi: 10.3892/etm.2017.5528

57. Wilting SM, Miok V, Jaspers A, Boon D, Sørgård H, Lando M, et al. Aberrant methylation-mediated silencing of microRNAs contributes to HPV-induced anchorage independence. *Oncotarget*. (2016) 7:43805–19. doi: 10.18632/oncotarget.v7i28

58. Gao YL, Zhang MY, Xu B, Han LJ, Lan SF, Chen J, et al. Circular RNA expression profiles reveal that hsa_circ_0018289 is up-regulated in cervical cancer and promotes the tumorigenesis. *Oncotarget*. (2017) 8:86625–33. doi: 10.18632/oncotarget.v8i49

59. Ma Z, Cai Y, Zhang L, Tian C, Lyu L. LINC00319 promotes cervical cancer progression via targeting miR-147a/IGF1R pathway. *Cancer Biother Radiopharm*. (2020). doi: 10.1089/cbr.2020.3722

60. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin*. (2021) 71:209–49. doi: 10.3322/caac.21660

61. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. *Lancet*. (2019) 393:169–82. doi: 10.1016/S0140-6736(18)32470-X

62. Takeshita J, Grewal S, Langan SM, Mehta NN, Ogdie A, Van Voorhees AS, et al. Psoriasis and comorbid diseases: Epidemiology. *J Am Acad Dermatol*. (2017) 76:377–90. doi: 10.1016/j.jaad.2016.07.064

63. Abuabara K, Azfar RS, Shin DB, Neimann AL, Troxel AB, Gelfand JM. Cause-specific mortality in patients with severe psoriasis: a population-based cohort study in the U.K. *Br J Dermatol*. (2010) 163:586–92. doi: 10.1111/j.1365-2133.2010.09941.x

64. Pouplard C, Brenaut E, Horreau C, Barnetche T, Misery L, Richard MA, et al. Risk of cancer in psoriasis: a systematic review and meta-analysis of epidemiological studies. *J Eur Acad Dermatol Venereol*. (2013) 27 Suppl 3:36–46. doi: 10.1111/jdv.12165

65. Chiesa Fuxench ZC, Shin DB, Ogdie Beatty A, Gelfand JM. The risk of cancer in patients with psoriasis: A population-based cohort study in the health improvement network. *JAMA Dermatol*. (2016) 152:282–90. doi: 10.1001/jamadermatol.2015.4847

66. Barbieri JS, Wang S, Ogdie AR, Shin DB, Takeshita J. Age-appropriate cancer screening: A cohort study of adults with psoriasis prescribed biologics, adults in the general population, and adults with hypertension. *J Am Acad Dermatol*. (2021) 84:1602–9. doi: 10.1016/j.jaad.2020.10.045

67. Rust A, McGovern RM, Gostout BS, Persing DH, Pittelkow MR. Human papillomavirus in cutaneous squamous cell carcinoma and cervix of a patient with psoriasis and extensive ultraviolet radiation exposure. *J Am Acad Dermatol*. (2001) 44:681–6. doi: 10.1067/mjd.2001.112359

68. Boehncke WH, Schön MP. Psoriasis. *Lancet*. (2015) 386:983–94. doi: 10.1016/S0140-6736(14)61909-7

69. Kim JH, Youn Y, Hwang JH. NCAPH stabilizes GEN1 in chromatin to resolve ultra-fine DNA bridges and maintain chromosome stability. *Molecules Cells*. (2022) 45:792–805. doi: 10.14348/molcells.2022.0048

70. Liu Y, Ma X, Feng L, Lin Z, Zhou X. An integrative pan-cancer analysis reveals the carcinogenic effects of NCAPH in human cancer. *Math Biosci Eng: MBE*. (2023) 20:76–92. doi: 10.3934/mbe.2023005

71. Wang M, Qiao X, Cooper T, Pan W, Liu L, Hayball J, et al. HPV E7-mediated NCAPH ectopic expression regulates the carcinogenesis of cervical carcinoma via PI3K/AKT/SGK pathway. *Cell Death Disease*. (2020) 11:1049. doi: 10.1038/s41419-020-03244-9

72. Sidhu H, Capalash N. UHRF1: The key regulator of epigenetics and molecular target for cancer therapeutics. *Tumor Biol*. (2017) 39:1010428317692205. doi: 10.1177/1010428317692205

73. Xue B, Zhao J, Feng P, Xing J, Wu H, Li Y. Epigenetic mechanism and target therapy of UHRF1 protein complex in Malignancies. *OncoTargets Ther*. (2019) 12:549–59. doi: 10.2147/OTT

74. Kim MJ, Lee HJ, Choi MY, Kang SS, Kim YS, Shin JK, et al. UHRF1 induces methylation of the TXNIP promoter and down-regulates gene expression in cervical cancer. *Molecules Cells*. (2021) 44:146–59. doi: 10.14348/molcells.2021.0001

75. Sidhu H, Capalash N. Plumbagin downregulates UHRF1, p-Akt, MMP-2 and? suppresses survival, growth and migration of cervical cancer CaSki cells. *Toxicol vitro: an Int J published Assoc BIBRA*. (2023) 86:105512. doi: 10.1016/j.tiv.2022.105512

76. Qi X, Liu Y, Peng Y, Fu Y, Fu Y, Yin L, et al. UHRF1 promotes spindle assembly and chromosome congression by catalyzing EG5 polyubiquitination. *J Cell Biol*. (2023) 222(11):e202210093. doi: 10.1083/jcb.202210093

77. D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biol Int*. (2019) 43:582–92. doi: 10.1002/cbin.11137

78. Chittori S, Hong J, Saunders H, Feng H, Ghirlando R, Kelly AE, et al. Structural mechanisms of centromeric nucleosome recognition by the kinetochore protein CENP-N. *Sci (New York NY)*. (2018) 359:339–43. doi: 10.1126/science.aar2781

79. Zhou K, Gebala M, Woods D, Sundararajan K, Edwards G, Krzizike D, et al. CENP-N promotes the compaction of centromeric chromatin. *Nat Struct Mol Biol*. (2022) 29:403–13. doi: 10.1038/s41594-022-00758-y

80. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. (2010) 140:883–99. doi: 10.1016/j.cell.2010.01.025

81. Das D, Sarkar B, Mukhopadhyay S, Banerjee C, Biswas Mondal S. An altered ratio of CD4+ And CD8+ T lymphocytes in cervical cancer tissues and peripheral blood – A prognostic clue? *Asian Pac J Cancer Prev: APJCP*. (2018) 19:471–8. doi: 10.22034/APJCP.2018.19.2.471

82. Perera GK, Di Meglio P, Nestle FO. Psoriasis. *Annu Rev Pathol*. (2012) 7:385–422. doi: 10.1146/annurev-pathol-011811-132448

83. Clark RA, Kupper TS. Misbehaving macrophages in the pathogenesis of psoriasis. *J Clin Invest*. (2006) 116:2084–7. doi: 10.1172/JCI29441

84. Wang H, Peters T, Kess D, Sindrilaru A, Oreshkova T, Van Rooijen N, et al. Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J Clin Invest*. (2006) 116:2105–14. doi: 10.1172/JCI27180

85. Lorthois I, Asselineau D, Seyler N, Pouliot R. Contribution of *in vivo* and organotypic 3D models to understanding the role of macrophages and neutrophils in the pathogenesis of psoriasis. *Mediators Inflamm*. (2017) 2017:7215072. doi: 10.1155/2017/7215072

86. Cook PW, Pittelkow MR, Piepkorn M. Overexpression of amphiregulin in the epidermis of transgenic mice induces a psoriasis-like cutaneous phenotype. *J Invest Dermatol*. (1999) 113:860. doi: 10.1046/j.1523-1747.1999.00753.x

87. Cheng W, Yang Z, Wang S, Li Y, Wei H, Tian X, et al. Recent development of CDK inhibitors: An overview of CDK/inhibitor co-crystal structures. *Eur J Med Chem*. (2019) 164:615–39. doi: 10.1016/j.ejmech.2019.01.003

88. Le Roy L, Letondor A, Le Roux C, Amara A, Timsit S. Cellular and molecular mechanisms of R/S-roscovitine and CDKs related inhibition under both focal and global cerebral ischemia: A focus on neurovascular unit and immune cells. *Cells*. (2021) 10(1):104. doi: 10.3390/cells10010104

89. Phoomvuthisarn P, Cross A, Glennon-Alty L, Wright HL, Edwards SW. The CDK inhibitor purvalanol A induces neutrophil apoptosis and increases the turnover rate of Mcl-1: potential role of p38-MAPK in regulation of Mcl-1 turnover. *Clin Exp Immunol*. (2018) 192:171–80. doi: 10.1111/cei.13107

90. Abdel-Wahab AF, Mahmoud W, Al-Harizy RM. Targeting glucose metabolism to suppress cancer progression: prospective of anti-glycolytic cancer therapy. *Pharmacol Res*. (2019) 150:104511. doi: 10.1016/j.phrs.2019.104511

91. Zhang W, Che Q, Tan H, Qi X, Li D, Zhu T, et al. A novel antimycin analogue antimycin A2c, derived from marine Streptomyces sp., suppresses HeLa cells via disrupting mitochondrial function and depleting HPV oncoproteins E6/E7. *Life Sci*. (2023) 330:121998. doi: 10.1016/j.lfs.2023.121998

92. Shi Y, Park J, Lagisetti C, Zhou W, Sambucetti LC, Webb TR. A triple exon-skipping luciferase reporter assay identifies a new CLK inhibitor pharmacophore. *Bioorg Med Chem Lett*. (2017) 27:406–12. doi: 10.1016/j.bmcl.2016.12.056

93. Schultz CW, Nevler A. Pyrvinium pamoate: past, present, and future as an anti-cancer drug. *Biomedicines*. (2022) 10(12):3249. doi: 10.3390/biomedicines10123249

94. Karamian A, Nazarian H, Ziai SA, Zarnani AH, Salehpour S, Paktinat S, et al. Pyrvinium pamoate inhibits proliferation and invasion of human endometriotic stromal cells. *Hum Exp Toxicol*. (2020) 39:662–72. doi: 10.1177/0960327119896612

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MicroRNA-939 amplifies *Staphylococcus aureus*-induced matrix metalloproteinase expression in atopic dermatitis

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Background: Atopic dermatitis (AD) is a common chronic inflammatory skin diseases that seriously affects life quality of the patients. *Staphylococcus aureus* (*S. aureus*) colonization on the skin plays an important role in the pathogenesis of AD; however, the mechanism of how it modulates skin immunity to exacerbate AD remains unclear. MicroRNAs are short non-coding RNAs that act as post-transcriptional regulators of genes. They are involved in the pathogenesis of various inflammatory skin diseases.

Methods: In this study, we established miRNA expression profiles for keratinocytes stimulated with heat-killed *S. aureus* (HKSA). The expression of miR-939 in atopic dermatitis patients was analyzed by fluorescence in situ hybridization (FISH). miR-939 mimic was transfected to human primary keratinocyte to investigate its impact on the expression of matrix metalloproteinase genes (MMPs) *in vitro*. Subsequently, miR-939, along with Polyplus transfection reagent, was administered to MC903-induced atopic dermatitis skin to assess its function *in vivo*.

Results: MiR-939 was highly upregulated in HKSA-stimulated keratinocytes and AD lesions. *In vitro* studies revealed that miR-939 increased the expression of matrix metalloproteinase genes, including MMP1, MMP3, and MMP9, as well as the cell adhesion molecule ICAM1 in human primary keratinocytes. *In vivo* studies indicated that miR-939 increased the expression of matrix metalloproteinases to promote the colonization of *S. aureus* and exacerbated *S. aureus*-induced AD-like skin inflammation.

Conclusions: Our work reveals miR-939 is an important regulator of skin inflammation in AD that could be used as a potential therapeutic target for AD.

KEYWORDS

atopic dermatitis, microRNA, *Staphylococcus aureus*, keratinocyte, matrix metalloproteinase

Introduction

Atopic dermatitis (AD) is a common chronic, recurrent, inflammatory skin disease, usually associated with intense itching and an eczema-like rash. It affects approximately 20% of children and 10% of adults worldwide. The pathogenesis of AD is closely associated with host genetics, skin microbiota disorders, type 2 inflammatory response, and skin barrier disruption (1). Although the type 2 inflammatory response is a major driver in AD, *S. aureus* colonization is the primary reason for infection-induced AD relapse (2, 3). Greater than 90% of AD patients are positive for *S. aureus* colonization in skin lesions. Only 5% of the normal healthy population carries *S. aureus* on their skin, although this percentage may vary based on ethnicity and geographical location (4). Moreover, the density of *S. aureus* in the lesions and non-lesion areas of AD patients is strongly correlated with the severity of the disease (5). Recent studies have indicated that retention of *S. aureus* Agr virulence function during infancy is associated with *S. aureus* skin colonization and the development of AD (6). Various *S. aureus*-derived molecules and byproducts contribute to skin barrier disruption and inflammation, including superantigens, toxins, protein A, proteases, and phenol-soluble modulins (7, 8). In addition, *S. aureus* colonization promotes the development of AD-like skin inflammation in mice (6, 9, 10); however, the precise mechanism for this association with AD remains unclear.

Recent studies of non-coding RNAs have resulted in a greater understanding of the complexity of gene regulation. Of these, miRNAs are approximately 22 nucleotides in length and regulate many cellular processes by acting as post-transcriptional regulators via targeting mRNA degradation and/or translational repression (11). Dysregulation of miRNA expression plays an important role in the pathogenesis of AD. Dicer is a key enzyme in the maturation of miRNAs (12). The absence of Dicer exacerbates skin inflammation and is accompanied by elevated TSLP, which implicates miRNAs in AD pathogenesis (13). MiR-146, which is highly expressed in chronic AD lesions, inhibits the expression of various proinflammatory factors in keratinocytes and ameliorates skin inflammation in AD by targeting the nuclear factor kappa B signaling pathway (14). Furthermore, upregulation of miR-155 expression in patients with AD inhibits CTLA-4 expression and promotes T helper cell proliferation, which in turn, promotes chronic skin inflammation (15). In this study, we aimed to identify the roles of miRNAs in the interactions between *S. aureus* infection and AD.

Recent studies indicate that miR-939 is associated with various malignant tumor types. MiR-939 targets TIMP2 to promote cell proliferation and invasion, and affects the growth of gliomas and non-small cell lung cancer (16, 17). High miR-939 expression predicts a poor prognosis for some cancer patients. LINC00460 can regulate the expression of miR-939 to promote colorectal cancer metastasis (18). In patients with chronic heart failure, miR-939 levels in the serum are increased. TNF-α and iNOS are target genes for miR-939, thus it can regulate inflammatory cytokine-induced apoptosis in endothelial and cardiomyocytes (19); however, the role of miR-939 in AD pathogenesis has not been investigated.

In this study, we demonstrated that miR-939 expression is elevated in HKSA-stimulated keratinocytes and skin lesions of AD patients. MiR-939 increases the expression of matrix metalloproteinases, promotes the colonization of *S. aureus*, and exacerbates *S. aureus*-induced AD-like skin inflammation.

Results

MiR-939 is upregulated in heat-killed S. aureus-stimulated keratinocytes and atopic dermatitis

Skin colonization with *S. aureus* may be a crucial factor in AD pathogenesis. To identify the aberrantly expressed miRNAs in keratinocytes by *S. aureus* colonization, we established miRNA expression profiles for keratinocytes stimulated with heat-killed *S. aureus* (HKSA). The significance analysis of microarrays algorithm was used to analyze the miRNA profiling data. We identified 9 significantly differentially expressed miRNAs (fold change >1.5, p-value <0.05) between HKSA-stimulated keratinocytes and controls (Figure 1A). Interestingly, hsa-miR-939–5p was the top upregulated miRNA in HKSA-stimulated keratinocytes (Figure 1A). To validate our profiling data, we measured miR-939 expression in keratinocytes stimulated with different doses of HKSA by qRT-PCR. HKSA significantly induced miR-939 expression in keratinocytes in a dose-dependent manner (Figure 1B). To study the effects of other Staphylococcal strains on the expression of miR-939, we used heat killed *Staphylococcal epidermidis* (HKSE) and heat killed *Staphylococcal hominis* (HKSH) to treat keratinocytes. We found neither HKSE nor HKSH had any effect on miR-939 expression (Figure 1C). To examine the expression pattern of miR-939 in AD, RNA fluorescence *in situ* hybridization (FISH) was carried out with miR-939-specific probes on skin lesion sections obtained from 3 AD patients and 3 healthy individuals. Consistent with the upregulation of miR-939 in HKSA-stimulated keratinocytes, miR-939 expression was significantly increased in the epidermis of the AD samples (Figure 1D).

Pathogens secrete pathogen-associated molecular patterns to activate pattern recognition receptors on host cells to activate downstream signaling cascades (20). To determine which receptor in keratinocytes is required for *S. aureus*-induced miR-939 expression, we treated keratinocytes with different TLR ligands, including flagellin (TLR5 ligand), LPS (TLR4 ligand), poly(I: C)

Abbreviations: AD, atopic dermatitis; NS, normal skin; ND, not detect; siRNA, small-interfering RNA; miRNA, microRNA; TLR, Toll-like receptor; HKSA, heat-killed *Staphylococcus aureus*; HKSE, heat-killed Staphylococcus epidermidis; HKSH, heat-killed Staphylococcus hominis. SA, *Staphylococcus aureus*; PCA, principal component analysis; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, quantitative real-time polymerase chain reaction; NHEKs, normal human epidermal keratinocytes; FPKM, fragment per kilobase of transcript per million mapped reads; Agr, accessory gene regulatory; CFU, colony-forming units; DAPI, 4′,6-diamidino-2-phenylindole; TSB, tryptic soy broth; TSA, tryptic soy agar; FISH, florescence *in situ* hybridization; HE, hematoxylin and eosin; logFC, log2fold change; PBS, phosphate buffer saline.

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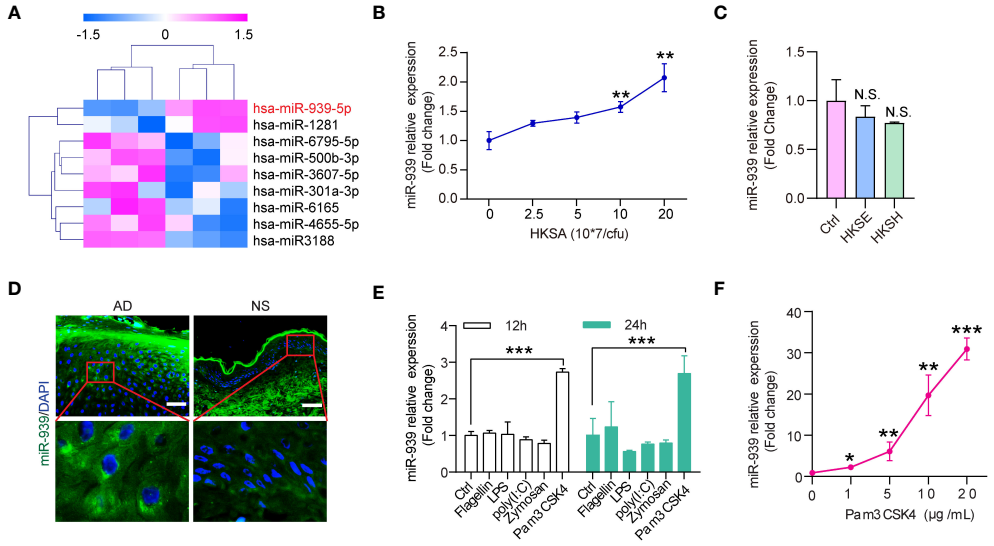

FIGURE 1
miR-939 is upregulated in heat-killed *S. aureus*-stimulated keratinocytes and atopic dermatitis. **(A)** Differentially expressed miRNA profiles in HKSA-stimulated keratinocytes compared with unstimulated keratinocytes. **(B)** qRT-PCR analysis of the expression of miR-939 in keratinocytes after treated with different concentrations of HKSA. **(C)** qRT-PCR analysis of the expression of miR-939 in keratinocytes after treated with $1x10^8$ CFU of HKSE and HKSH. **(D)** RNA FISH was performed in skin lesions from AD patients (AD) and normal skin from healthy donors (NS). 400x magnification, scale bars: 50 μm. Expression levels of miR-939 after stimulation of keratinocytes with various TLR ligands **(E)** and different doses of Pam3CSK4 **(F)**. The data are presented as mean ± SD. N.S., no significance. *P < 0.05, **P < 0.01, and ***P < 0.001 by One-way ANOVA test.

(TLR3 ligand), Zymosan (TLR2 ligand), and PamCSK4 (TLR2 ligand) (21, 22) (Figure 1E). Pam3CSK4 significantly induced miR-939 expression in a dose-dependent manner (Figure 1F). Taken together, these results likely indicate that *S. aureus* activates TLR2 to induce miR-939 expression in AD.

Transcriptome analysis reveals that miR-939 regulates the HKSA-induced immune response

To study the functional relevance of miR-939 expression under inflammatory conditions induced by HKSA in keratinocytes, we performed a global transcriptomic analysis in keratinocytes with miR-939 overexpression followed by HKSA stimulation. Principal Component Analysis (PCA) indicated that the clustering of samples was categorized into 4 distinct groups designated miRCtrl, miR-939, miRCtrl+HKSA, and miR-939+HKSA, indicating a clear difference in the transcriptome (Figure 2A). Subsequently, a two-by-two differential expression analysis of the four groups was performed to evaluate the differentially expressed genes (DEGs) regulated by miR-939 or HKSA in keratinocytes (Figures 2B, C; Supplementary Figures S1A, B). Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that cytokine-cytokine receptor interaction, TNF signaling pathway, and IL-17 signaling pathway were enriched in the HKSA-stimulated groups (Supplementary Figures S1C, D), which indicates the successful induction of an inflammatory response in keratinocytes by HKSA. Interestingly, these inflammation-related pathways were further enriched in upregulated genes by miR-939 overexpression; however, few enriched pathways were associated with the downregulated genes (Figures 2D–G). Therefore, we focused on 15 genes associated with inflammation-related signaling pathways upregulated by miR-939, most of which were upregulated by both miR-939 and HKSA (Figure 2H). These results indicate that miR-939 performs a regulatory role in amplifying the HKSA-induced inflammatory response in keratinocytes.

miR-939 amplifies the S. aureus-induced inflammatory response *in vitro*

To determine the potential interactions among 15 key genes, we constructed a functional protein association network using STRING network analyses (Figure 3A). Moreover, the plugin MCODE identified the four most important hub genes, including three matrix metalloproteinases (MMP1, MMP3, and MMP9) and one cell surface adhesion receptor gene, ICAM1 (Figure 3B), which regulates leukocyte recruitment from the circulation (23). To validate the results obtained from the above bioinformatics analysis, we treated keratinocytes with HKSA at different times. The qRT-PCR results confirmed that the expression of MMP1, MMP3, MMP9, and ICAM1 was significantly upregulated by HKSA in a time-dependent manner (Figures 3C–F). Moreover,

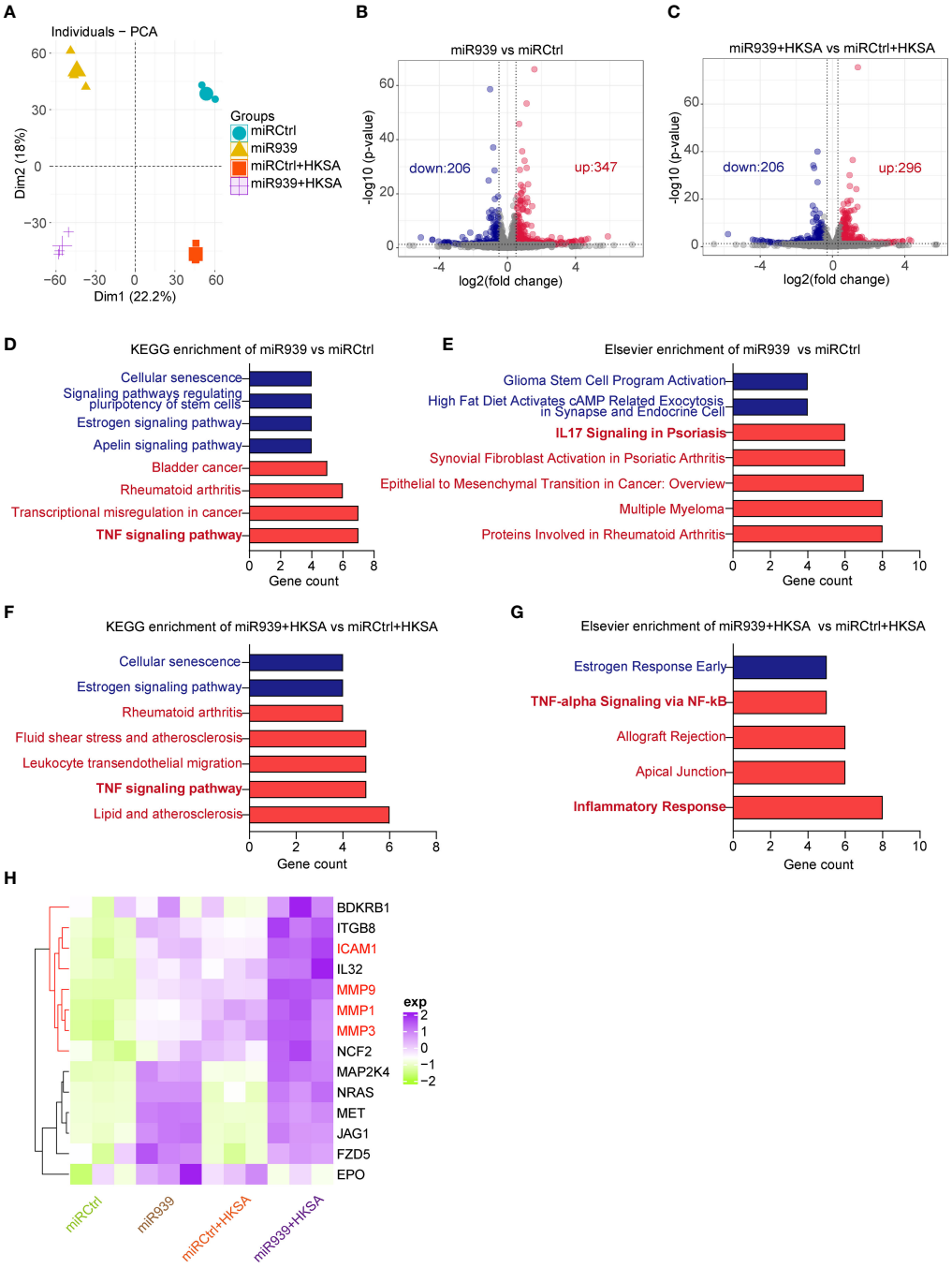

FIGURE 2
Transcriptome analysis shows that miR-939 regulates HKSA-induced immune response. Keratinocytes transfected with hsa-miR-939–5p mimic or the corresponding negative control, followed by treatment with HKSA or PBS for 8 h were subject to RNA sequencing. **(A)** PCA plot between miRCtrl, miR-939, miRCtrl+HKSA, and miR-939+HKSA. Differentially expressed genes in miR-939 compared with miRCtrl **(B)**, and in miR-939+HKSA compared with miRCtrl+HKSA **(C)** are shown in volcano maps. Top KEGG pathways **(D)** and Elsevier pathways **(E)** for the DEGs regulated by miR-939 as well as the top KEGG pathways **(F)** and Elsevier pathways **(G)** for the DEGs regulated by miR939+HKSA are shown in bar charts. **(H)** Heatmap of 15 top genes upregulated by miR-939.

overexpression of the miR-939 significantly amplified the expression of MMP1, MMP3, MMP9, and ICAM1 following an 8-hour treatment of keratinocytes with HKSA (Figures 3G–J). To confirm these results, we further performed immunostaining of MMP1 and MMP9 after miR-939 transfection followed by HKSA stimulation. We also found that miR-939 significantly upregulate HKSA-induced MMP1 and MMP9 protein expression (Figures 3K–M). To determine whether the HKSA-induced endogenous miR-939 can act as positive feedback in keratinocytes, we transfected the keratinocytes with miR-939 blocking oligonucleotides that

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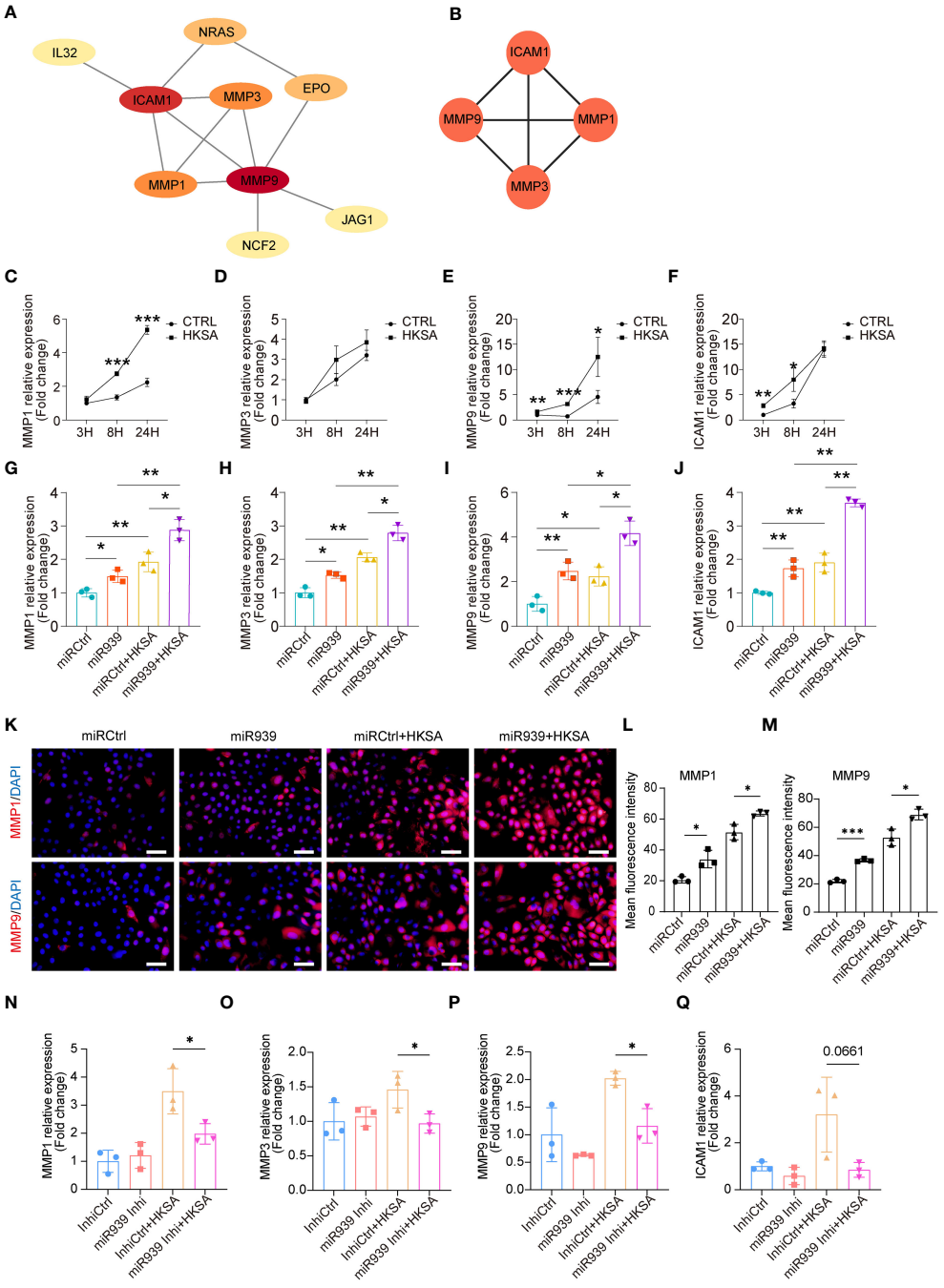

FIGURE 3
miR-939 amplifies the *S. aureus*-induced inflammatory response *in vitro*. **(A)** PPI network of 15 key genes. **(B)** The 4 most important hub genes were identified using the plugin MCODE of Cytoscape. **(C–F)** Expression levels of the four hub genes in keratinocytes after 3, 8, and 24 h of HKSA stimulation as measured by qRT-PCR. **(G–J)** qRT-PCR analysis of the four hub genes in keratinocytes transfected with miR939 or miRCtrl, followed by treatment with HKSA or PBS for 8 (h) **(K)** Immunofluorescence staining of MMP1 and MMP9 was performed on keratinocytes treated by miRCtrl, miR-939, miRCtrl&HKSA or miR-939&HKSA. The nucleus is stained with DAPI. 400x magnification, scale bars: 100 μm. **(L, M)** The mean fluorescence intensity of MMP1 and MMP9 of each field in different group is determined using ImageJ software in a blinded manner. The random 3 fields of each sample are scanned and then quantified. **(N–Q)** qRT-PCR analysis of the four hub genes in keratinocytes transfected with miR939 inhibitor or controls, followed by treatment with HKSA or PBS for 48 (h) The data are presented as mean ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001 by One-way ANOVA test.

hybridize to mature miRNAs (inhibitors) followed by HKSA stimulation. Inhibition of miR-939 is not able to change the expression of MMPs at basal level. This may be due to the low expression level of miR-939 in psychological conditions. However, miR-939 inhibitor significantly decreased HKSA-induced MMP1, MMP3 and MMP9 expression, demonstrating that HKSA-induced miR-939 expression acts as positive feedback in keratinocytes (Figures 3N–Q). Together, these data suggest that miR-939

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enhances the HKSA-induced inflammatory responses in human primary keratinocytes.

miR-939 amplifies the S. aureus-induced atopic dermatitis phenotype *in vivo*

We further explored the physiological relevance of miR-939 in human keratinocytes and AD mice. We mixed either the miR-939 agomir or the control agomir with *in vivo*-jetPEI transfection reagent and administered it through intradermal injection into the mice's skin followed by topical application of *S. aureus* (Figure 4A). Consistent with a previous study (9), *S. aureus* colonization was successfully established as an AD-like phenotype in mouse back skin (Figures 4B, C). The expression of AD-related cytokines, e.g. IL-13, TSLP and IL-6, were increased, while the antimicrobial peptide expression, e.g. mBD4, mBD14 and CAMP were deceased in *S. aureus* colonized skin (Supplementary Figure S2). Skin redness and epidermal thickness were significantly increased in the *S. aureus* colonization group (Figures 4B, D). Moreover, we also observed increased neutrophil infiltration in the skin dermis by *S. aureus* (Figures 4B, E). After injecting miR-939, *S. aureus* had a greater ability to colonize the skin of mice compared with the control mice (Figure 4F). This was evident by increased skin redness, epidermal thickness, and neutrophil infiltration in the miR-939+*S.aureus* group (Figures 4B–F). The increased *S. aureus* colonization and AD

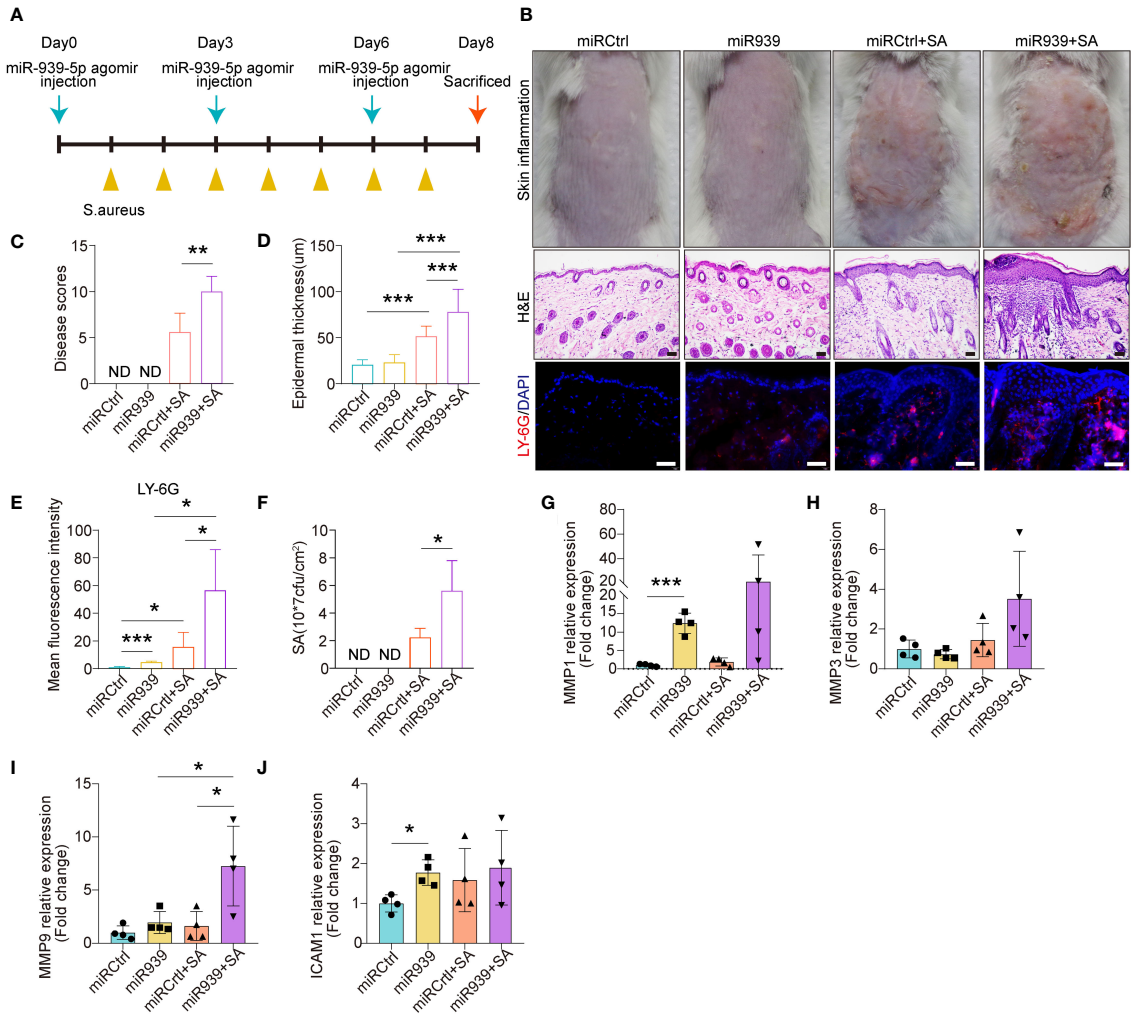

FIGURE 4
miR-939 amplifies *S. aureus*-induced atopic dermatitis phenotype *in vivo*. **(A)** Schematic of the animal study. **(B)** hsa-miR-939 agomir or corresponding negative control was mix with *in vivo*-jetPEI transfection reagent and intradermally injected to the mice back skin followed by colonization with *S. aureus* or treated with PBS. The above image is a representative photograph of a mouse skin lesion. The image in the middle is a corresponding H&E-stained section and below represents immunostaining for LY-6G. 200x magnification for H&E staining, 400x magnification for LY-6G immunostaining, scale bars: 100 μm. n = 4 mice per group. Skin disease scores **(C)**, epidermal thickness **(D)**, mean fluorescence intensity of LY-6G immunostaining **(E)** and the number of *S. aureus* colonized on the skin **(F)** were measured in each group. **(G–J)** qRT-PCR analysis of the expression levels of the four hub genes in each group. ND: not detected. The data are presented as mean ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001 by One-way ANOVA test.

phenotype may be attributed, in part, to upregulated MMP1 and MMP9 expression in the skin (Figures 4G–J). Together, these data demonstrate that miR-939 amplifies the *S. aureus*-induced AD phenotype *in vivo*.

Discussion

In this study, we established miRNA expression profiles in human primary keratinocytes after stimulation with heat-killed *S. aureus*. We focused on a highly upregulated miRNA by *S. aureus* stimulation, miR-939, and found that it was significantly upregulated in the epidermis of human AD lesions. We observed enhanced inflammatory responses in *S. aureus*-stimulated human primary keratinocytes. Overexpression of miR-939 increases the expression of matrix metalloproteinases to promote the colonization of *S. aureus* and exacerbate *S. aureus*-induced AD-like skin inflammation *in vitro* and *in vivo*. Moreover, inhibition of miR-939 decreased *S. aureus*-induced matrix metalloproteinases expression in keratinocytes. These results identified miR-939 as an important regulator in the immunopathogenesis of AD, which serves as a positive feedback loop after *S. aureus* stimulation and exacerbate matrix metalloproteinases expression.

MiR-939 exert diverse functions in different cells. Overexpression of miR-939 increased proliferation, migration, and invasion in glioma cell lines and increased miR-939–5p was associated with poor prognosis in glioma patients (16). The oncogenic role of miR-939 was confirmed in non–small cell lung cancer (NSCLC) indicating that miR–939 knockdown inhibits cell proliferation and invasion in NSCLC cell lines (17). MiR-939 also inhibited the expression of proinflammatory genes and decreased inflammation-induced apoptosis of endothelial cells (19). MiR-939 also exert specific roles in the regulation of innate immune response. McDonald et al. demonstrate that miR-939 inhibits the expression the proinflammatory cytokines in THP1 cells, e.g. IL-6, TNFa, NOS2 NOS2A and NFκB2 mRNAs (24). Hou et al. showed that MiR-939 abolished vascular integrity and repressed angiogenesis through directly targeting γ-catenin (25). In the present study, we demonstrated that miR-939 increased the expression of MMPs to enhance *S. aureus* colonization.

In contrast to coagulase-negative staphylococci (CoNS), such as *S. epidermidis* and *S. hominis*, *S. aureus* is characterized by a higher abundance of virulence factors. Our findings indicate that *S. aureus*, but not *S. epidermidis* or *S. hominis*, induces the expression of miR-939 which further enhances MMPs expression. Phenol-soluble modulins (PSMs), a family of amphipathic, α-helical peptides, serve as key virulence determinants, particularly in highly virulent *S. aureus* strains. PSMs, including PSMα, have been demonstrated to induce the expression of a wide range of pro-inflammatory chemokines and cytokines. Nakagawa et al. demonstrated that *S. aureus* produces PSMa, which induces keratinocyte damage and releases IL-1a and IL-36a, thereby triggering a downstream IL-17-dependent inflammatory response (26). The PSMs secreted by *S. aureus* may contribute to the observed synergistic effect in this study. Investigating the impact of PSMs on miR-939 and MMPs expression in human keratinocytes is a direction for future research.

Matrix metalloproteinases (MMPs) are a large family of calcium-dependent, zinc-containing endopeptidases, which are responsible for the degradation of the extracellular matrix (ECM) (27). A proteomics analysis of AD revealed that inflammatory markers, including matrix metalloproteinases (MMP1, MMP3, MMP9, and MMP12), Th2-type cytokines, and chemokines, are significantly upregulated in both lesion and non-lesion skin in patients with AD compared with healthy normal skin (28, 29). The upregulation of MMPs contribute to the breakdown of structural proteins in the skin, such as collagen and elastin, which are essential for maintaining the integrity of the skin barrier (30). Moreover, MMPs have been implicated in the modulation of sensory nerve function and itch sensation (31). Increased MMP activity in the skin may contribute to the neuroinflammatory processes underlying pruritus (itch) in AD. Disruption of the skin barrier allows allergens, irritants, and pathogens, such as *S. aureus* to penetrate the skin more easily, leading to inflammation and exacerbation of AD symptoms (32). In the present study, we demonstrated that miR-939 promotes *S. aureus*-induced MMP expression and colonization of *S. aureus* was increased in mice back skin after miR-939 administration. This phenomenon may be due to further damage of the skin barrier by MMPs.

MiRNAs normally bind to the 3' untranslated region (UTR) of messenger RNA (mRNA) to induce degradation and/or translational repression (33). However, multiple miRNAs positively regulate gene expression by interacting with the promoter to trigger the recruitment of transcription factors and RNA-Polymerase-II. For example, miRNA-551b interacts with RNA Pol II to recruit the TWIST1 transcription factor to the STAT3 promoter to activate STAT3 transcription (34). MiR-373 interacts with the E-cadherin promoter to induce gene expression by recruiting RNA Pol II (35). In the present study, we observed a similar gene activation phenomenon with miR-939 in HKSA-stimulated keratinocytes. MiR-939 may bind to gene promoters; however, the precise mechanism through which miR-939 promotes MMP expression requires further study.

In conclusion, we demonstrated that *S. aureus* increases miR-939 expression *in vitro* and *in vivo*. MiR-939 enhances the expression of matrix metalloproteinase genes to promote the colonization of *S. aureus* and exacerbates *S. aureus*-induced AD-like skin inflammation. The results suggest that decreasing miR-939 levels in the skin may be a strategy to control the immunopathogenesis of AD driven by *S. aureus* colonization and infection. Thus, miR-939 may be a promising candidate for AD treatment.

Methods

Human samples

Three AD and three normal healthy samples were obtained from biobank of Institute of Dermatology, Chinese Academy of

Medical Sciences, Jiangsu Biobank of Clinical Resources (BM2015004). All patients had signed informed consent for donating their samples. The study was approved by the Research Ethics Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (2023-KY-033).

Mouse model and *in vivo* studies

Seven-week-old male BALB/c mice were purchased from GemPharmatech (Nanjing, China) and acclimatized for one week. The mice were depilated on their backs and allowed to rest for one day to restore the skin barrier. The hsa-miR-939–5p agomir and corresponding negative control (GenePharma, Suzhou, China) were transfected into the mouse skin using *in vivo* jetPEI via intradermal injection, which is an *in vivo* transfection reagent (Polyplus), three times per week according to the manufacturer's protocol. During this period, the mice were externally colonized with *S. aureus* by placing ten million CFU of *S. aureus* onto sterile gauze, which was then immobilized on the dorsal skin of the mice with a transparent bio-occlusive dressing (Tegaderm; 3M). On the 8th day, the mice were photographed and euthanized. The skin was collected for various analyses, including qRT-PCR, H&E staining, immunofluorescence, and *S. aureus* enumeration. A disease score was determined by the cumulative scores of erythema, scale, edema and lichenosis in skin inflammation, with each being designated as none (0), mild (1), moderate (2), or severe (3). The animal studies were conducted under approved protocols by the Animal Use and Care Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (2023-DW-010).

S. aureus culture and enumeration

S. aureus (USA 300 LAC) was a gift from Professor Yuping Lai (East China Normal University). It was cultured with TSB (Solarbio, Beijing, China) in a shaker at 37°C and 220 rpm. The mixed *S. aureus* bacterial solution was diluted in a 1:10 concentration gradient. Different concentrations of bacterial solution as well as TSB were added to 96-well enzyme-labeled plates at 200 µl per well and the absorbance was read at 600 nm using a microplate reader (BioTek, Synergy™ H1). In addition, 10 µl of different concentrations of the bacterial solution was inoculated into the TSA (Solarbio, Beijing, China) plate and incubated at 37°C for 24 h to count CFUs. Finally, a standard growth curve of *S. aureus* was plotted based on the OD600 values and CFU counts for the different concentrations of *S. aureus*. To measure the concentration of *S. aureus*, one CFU was placed in 10 ml of a TSB in a shaker at 37°C and 220 rpm for 24 h. The next day, 10 µl of TSB was collected and incubated in 5 ml of fresh TSB for 3–4 h. The absorbance was measured and the concentration of *S. aureus* was calculated from the standard growth curve. To determine the number of *S. aureus* in colonized skin, skin of the same size was collected, homogenized in cold PBS, diluted, and placed onto TSA plates. The number of CFUs was determined after incubating at 37°C for 24 h.

qRT-PCR

Frozen mouse skin was homogenized with a Tissuelyser (JingXin, Shanghai, China). Total RNA was extracted from tissue homogenates and cells using RNAiso Plus (Takara). RNA quality and concentration were determined with a NanoDrop™ One/OneC (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized using PrimeScript™ RT Master Mix (Takara) based on the manufacturer's instructions. qRT-PCR was performed using TB Green® Premix Ex Taq™ II (Takara). For miRNA quantification, Bulge-loop miRNA qRT-PCR Primer Sets (one RT primer and a pair of qPCR primers for each set) specific for hsa-miR-939–5p were designed by RiboBio (Guangzhou, China). The expression of mRNA or miRNA was normalized to Gapdh or U6, respectively. The relative mRNA/miRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

FISH

FISH was done using a miRNA FISH Kit with FISH probes (GenePharma, Suzhou, China). Briefly, paraffin sections were deparaffinized, digested with proteinase K, denatured at 73°C, hybridized overnight at 37°C, washed, and stained for nuclei. All images were collected at a magnification of 400X using a fluorescence confocal microscope (Olympus, BX53, Tokyo, Japan). DAPI (Invitrogen, Thermo Fisher Scientific) and streptavidin Cy3 channels were used for signal detection.

Histology and immunofluorescence

Mouse skin tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. To calculate the average epidermal thickness of the mouse sections, we randomly selected five epidermal area on one image to measure the thickness by using ImageJ software. We totally analyzed four images from four mice. For immunofluorescence, fluorescence detection of neutrophil surface marker proteins in paraffin sections of mouse dorsal skin was done using an anti-Ly-6G/Ly-6C antibody (mouse monoclonal antibody; Thermo Fisher Scientific), followed by an anti-mouse AF488-conjugated secondary antibody (Invitrogen A32723). The fluorescent signal was observed using a fluorescence confocal microscope.

NHEK culture, transfection, and stimulation

NHEKs (Cat: FC0025, Lot 09213, Lifeline Cell Technology) were cultured in DermaLife Basal Medium (Lifeline Cell Technology) supplemented with DermaLife K lifefactor kit and 1× antibiotics [penicillin (100 U/ml), streptomycin (100 U/ml); Thermo Fisher Scientific] at 37°C and 5% CO_2. This donor is a 56-year-old Caucasian male. To study the effect of TLRs ligands on miR-939 expression, Flagellin (0.5ug/ml, InvivoGen), LPS (5ug/ml, InvivoGen), poly(I:C) (300ng/ml, InvivoGen), Zymosan (50ug/ml,

InvivoGen) and Pam3CSK4 (10ug/ml, InvivoGen) was used to treat keratinocyte for 24 hours. The expression of miR-939 was measured by qRT-PCR. Before stimulating keratinocytes with HKSA (Thermo Fisher Scientific) or TLR ligands (Thermo Fisher Scientific), 20 nmol/L of hsa-miR-939 mirVana miRNA mimic or mirVana miRNA mimic negative control #1 (Invitrogen, Thermo Fisher Scientific) was transfected into keratinocytes using Lipofectamine RNAiMAX (Thermo Fisher Scientific) when the confluence reached 50% to 60%. The final concentration of HKSA was 1 x 10^8 CFU/ml for RNA-sequencing and *in vitro* studies, whereas the final concentration of various TLR ligands was based on the manufacturer's instructions. NHEKs were only used for experiments between passages three and five.

Bioinformatic analysis

To determine the relationship between the samples, we performed PCA using the R packages "factoextra" and "FactoMineR", based on the normalized FPKM values for each sample. Raw Count values were used to perform a DEG analysis of the RNA-sequencing data using the R package "Deseq2". Briefly. the first step was to construct a DESeqDataSet object using the function DESeqDataSetFromMatrix. Then, the function DESeq was used for the difference analysis. To acquire DEGs regulated by HKSA or miR-939, genes with parameters of |logFC| > 0.5 and P-value <0.05 were considered significantly changed. The package "ggplot2" was used to generate a volcano map. We used an online tool (https://maayanlab.cloud/Enrichr/) for KEGG and Elsevier enrichment analysis of the DEGs, and the top five enriched pathways are shown as bar graphs. The heatmap was generated with DEGs from inflammation-related signaling pathways upregulated by miR-939 using the "ComplexHeatmap" package. Protein–protein interactions were determined using STRING (https://cn.string-db.org/) and visualized by Cytoscape (version 3.9.0). Four hub genes were identified using the plugin MCODE for Cytoscape.

RNA-sequencing

RNA-sequencing was done using HKSA-stimulated keratinocytes transfected with hsa-miR-939–5p mimic or the corresponding negative control. Briefly, total RNA was extracted and RNA quality was assessed. Then, mRNA was enriched using Oligo(dT), fragmented, and a cDNA Library was constructed using the NEB Next Ultra RNA Library Prep Kit for Illumina (NEB #7530, New England Biolabs, Ipswich, MA, USA). The resulting cDNA library was sequenced using an Illumina Novaseq6000 by Gene Denovo Biotechnology Co. (Guangzhou, China). The reads were subjected to quality control, sequence alignment, and gene abundance quantification by the FPKM and Count methods. The FPKM method eliminates the effects of varying gene lengths and sequencing data volume on gene expression calculations. Thus, the calculated gene expression may be used directly for subsequent bioinformatics analyses.

Quantification and statistical analysis

Statistical analysis was performed with GraphPad Prism version 8.0 (GraphPad). All data were presented as means ± SEM. Statistical significance between groups was determined using either a two-tailed Student's t-test or ANOVA analysis, by using GraphPad Prism 8 (GraphPad software Inc, California, USA). P values <0.05 were considered statistically significant.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: (https://www.ncbi.nlm.nih.gov/geo/, GSE249837).

Ethics statement

The studies involving humans were approved by the Research Ethics Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by the Animal Use and Care Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JW: Software, Visualization, Methodology, Formal analysis, Data curation, Writing – original draft. YH: Data curation, Formal analysis, Methodology, Validation, Visualization, Software, Writing – original draft. XW: Writing – review & editing, Visualization, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. DL: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1354154/full#supplementary-material

References

1. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet*. (2020) 396:345–60. doi: 10.1016/S0140-6736(20)31286-1

2. Kim J, Kim BE, Ahn K, Leung DYM. Interactions between atopic dermatitis and *staphylococcus aureus* infection: clinical implications. *Allergy Asthma Immunol Res*. (2019) 11:593–603. doi: 10.4168/aair.2019.11.5.593

3. Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, et al. The microbiome in patients with atopic dermatitis. *J Allergy Clin Immunol*. (2019) 143:26–35. doi: 10.1016/j.jaci.2018.11.015

4. Matsui K, Nishikawa A, Suto H, Tsuboi R, Ogawa H. Comparative study of *Staphylococcus aureus* isolated from lesional and non-lesional skin of atopic dermatitis patients. *Microbiol Immunol*. (2000) 44:945–7. doi: 10.1111/j.1348-0421.2000.tb02587.x

5. Tauber M, Balica S, Hsu CY, Jean-Decoster C, Lauze C, Redoules D, et al. *Staphylococcus aureus* density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. *J Allergy Clin Immunol*. (2016) 137:1272–4.e3. doi: 10.1016/j.jaci.2015.07.052

6. Nakamura Y, Takahashi H, Takaya A, Inoue Y, Katayama Y, Kusuya Y, et al. Staphylococcus Agr virulence is critical for epidermal colonization and associates with atopic dermatitis development. *Sci Transl Med*. (2020) 12. doi: 10.1126/scitranslmed.aay4068

7. Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Muñoz-Planillo R, Hasegawa M, et al. Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. *Nature*. (2013) 503:397–401. doi: 10.1038/nature12655

8. Geoghegan JA, Irvine AD, Foster TJ. *Staphylococcus aureus* and atopic dermatitis: A complex and evolving relationship. *Trends Microbiol*. (2018) 26:484–97. doi: 10.1016/j.tim.2017.11.008

9. Williams MR, Costa SK, Zaramela LS, Khalil S, Todd DA, Winter HL, et al. Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis. *Sci Transl Med*. (2019) 11. doi: 10.1126/scitranslmed.aat8329

10. Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, et al. Dysbiosis and *staphylococcus aureus* colonization drives inflammation in atopic dermatitis. *Immunity*. (2015) 42:756–66. doi: 10.1016/j.immuni.2015.03.014

11. Pu M, Chen J, Tao Z, Miao L, Qi X, Wang Y, et al. Regulatory network of miRNA on its target: coordination between transcriptional and post-transcriptional regulation of gene expression. *Cell Mol Life Sci*. (2019) 76:441–51. doi: 10.1007/s00018-018-2940-7

12. Vergani-Junior CA, Tonon-da-Silva G, Inan MD, Mori MA. DICER: structure, function, and regulation. *Biophys Rev*. (2021) 13:1081–90. doi: 10.1007/s12551-021-00902-w

13. Hener P, Friedmann L, Metzger D, Chambon P, Li M. Aggravated TSLP-induced atopic dermatitis in mice lacking Dicer in adult skin keratinocytes. *J Invest Dermatol*. (2011) 131:2324–7. doi: 10.1038/jid.2011.208

14. Rebane A, Runnel T, Aab A, Maslovskaja J, Rückert B, Zimmermann M, et al. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. *J Allergy Clin Immunol*. (2014) 134:836–47.e11. doi: 10.1016/j.jaci.2014.05.022

15. Sonkoly E, Janson P, Majuri ML, Savinko T, Fyhrquist N, Eidsmo L, et al. MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4. *J Allergy Clin Immunol*. (2010) 126:581–9.e1–20. doi: 10.1016/j.jaci.2010.05.045

16. Zheng H, Zhao H, Ye G. Overexpression of microRNA-939–5p contributes to cell proliferation and associates poor prognosis in glioma. *Neuromolecular Med*. (2021) 23:531–9. doi: 10.1007/s12017-021-08655-1

17. Chen A, Liu S, Lu X, Wei L, Chen Y. Inhibition of microRNA–939 suppresses the development of human non–small cell lung cancer via the upregulation of tissue inhibitor of metalloproteinases 2. *Mol Med Rep*. (2018) 18:4831–8. doi: 10.3892/mmr

18. Zhang Y, Liu X, Li Q, Zhang Y. lncRNA LINC00460 promoted colorectal cancer cells metastasis via miR-939–5p sponging. *Cancer Manag Res*. (2019) 11:1779–89. doi: 10.2147/CMAR

19. Chen C, Zong M, Lu Y, Guo Y, Lv H, Xie L, et al. Differentially expressed lnc-NOS2P3-miR-939–5p axis in chronic heart failure inhibits myocardial and endothelial cells apoptosis via iNOS/TNFα pathway. *J Cell Mol Med*. (2020) 24:11381–96. doi: 10.1111/jcmm.15740

20. Amarante-Mendes GP, Adjemian S, Branco LM, Zanetti LC, Weinlich R, Bortoluci KR. Pattern recognition receptors and the host cell death molecular machinery. *Front Immunol*. (2018) 9:2379. doi: 10.3389/fimmu.2018.02379

21. Radakovics K, Battin C, Leitner J, Geiselhart S, Paster W, Stöckl J, et al. A highly sensitive cell-based TLR reporter platform for the specific detection of bacterial TLR ligands. *Front Immunol*. (2021) 12:817604. doi: 10.3389/fimmu.2021.817604

22. Matsumoto M, Seya T. TLR3: interferon induction by double-stranded RNA including poly(I:C). *Adv Drug Delivery Rev*. (2008) 60:805–12. doi: 10.1016/j.addr.2007.11.005

23. Bui TM, Wiesolek HL, Sumagin R. ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*. (2020) 108:787–99. doi: 10.1002/JLB.2MR0220-549R

24. McDonald MK, Ramanathan S, Touati A, Zhou Y, Thanawala RU, Alexander GM, et al. Regulation of proinflammatory genes by the circulating microRNA hsa-miR-939. *Sci Rep*. (2016) 6:30976. doi: 10.1038/srep30976

25. Hou S, Fang M, Zhu Q, Liu Y, Liu L, Li X. MicroRNA-939 governs vascular integrity and angiogenesis through targeting γ-catenin in endothelial cells. *Biochem Biophys Res Commun*. (2017) 484:27–33. doi: 10.1016/j.bbrc.2017.01.085

26. Nakagawa S, Matsumoto M, Katayama Y, Oguma R, Wakabayashi S, Nygaard T, et al. *Staphylococcus aureus* virulent PSMα Peptides induce keratinocyte alarmin release to orchestrate IL-17-dependent skin inflammation. *Cell Host Microbe*. (2017) 22:667–77.e5. doi: 10.1016/j.chom.2017.10.008

27. Verma RP, Hansch C. Matrix metalloproteinases (MMPs): chemical-biological functions and (Q)SARs. *Bioorg Med Chem*. (2007) 15:2223–68. doi: 10.1016/j.bmc.2007.01.011

28. Del Duca E, Renert-Yuval Y, Pavel AB, Mikhaylov D, Wu J, Lefferdink R, et al. Proteomic characterization of atopic dermatitis blood from infancy to adulthood. *J Am Acad Dermatol*. (2023) 88:1083–93. doi: 10.1016/j.jaad.2022.12.050

29. Pavel AB, Zhou L, Diaz A, Ungar B, Dan J, He H, et al. The proteomic skin profile of moderate-to-severe atopic dermatitis patients shows an inflammatory signature. *J Am Acad Dermatol*. (2020) 82:690–9. doi: 10.1016/j.jaad.2019.10.039

30. Kuźmiński A, Przybyszewski M, Graczyk M, Bartuzi Z. The role of extracellular matrix metalloproteinases and their inhibitors in allergic diseases. *Adv Dermatol Allergology/ Postępy Dermatologii i Alergologii*. (2012) 29:384–9. doi: 10.5114/pdia.2012.31493

31. Marek-Jozefowicz L, Nedoszytko B, Grochocka M, Czajkowski R, Cubała WJ, Slominski AT. Molecular mechanisms of neurogenic inflammation of the skin. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms24055001

32. Namrata K, Bai B-X. Role of tight junctions and their protein expression in atopic dermatitis. *Int J Dermatol Venereology*. (2021) 4:40–4. doi: 10.1097/JD9.0000000000000114

33. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol (Lausanne)*. (2018) 9:402. doi: 10.3389/fendo.2018.00402

34. Chaluvally-Raghavan P, Jeong KJ, Pradeep S, Silva AM, Yu S, Liu W, et al. Direct upregulation of STAT3 by microRNA-551b-3p deregulates growth and metastasis of ovarian cancer. *Cell Rep*. (2016) 15:1493–504. doi: 10.1016/j.celrep.2016.04.034

35. Place RF, Li LC, Pookot D, Noonan EJ, Dahiya R. MicroRNA-373 induces expression of genes with complementary promoter sequences. *Proc Natl Acad Sci U S A*. (2008) 105:1608–13. doi: 10.1073/pnas.0707594105

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Granzyme K mediates IL-23-dependent inflammation and keratinocyte proliferation in psoriasis

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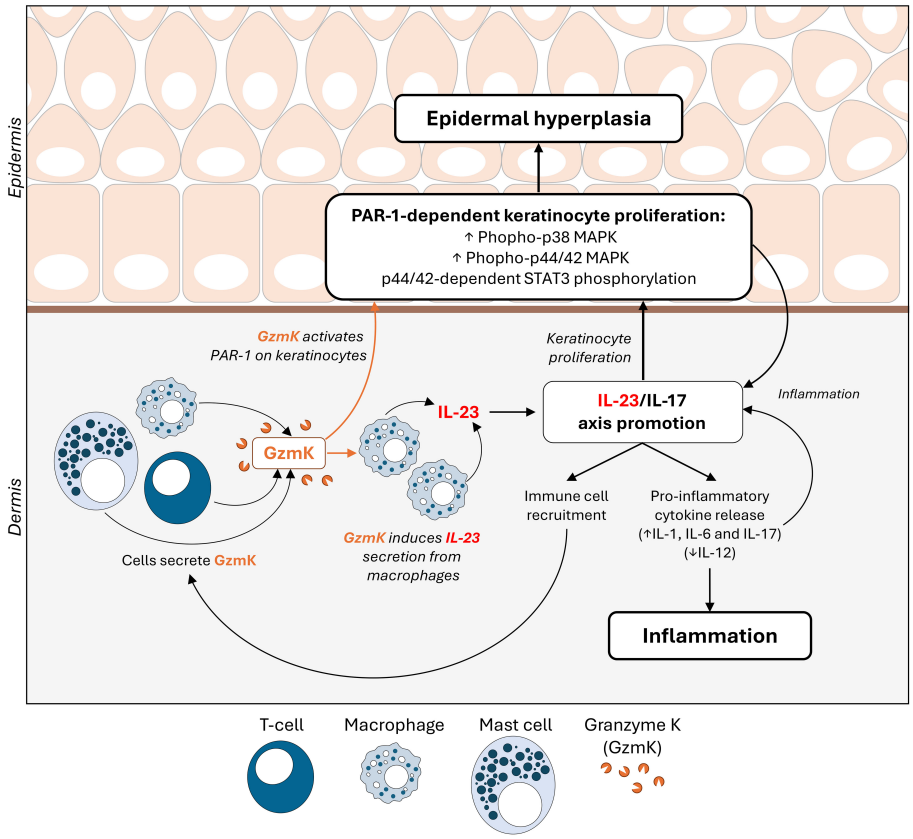
Psoriasis is an inflammatory disease with systemic manifestations that most commonly presents as itchy, erythematous, scaly plaques on extensor surfaces. Activation of the IL-23/IL-17 pro-inflammatory signaling pathway is a hallmark of psoriasis and its inhibition is key to clinical management. Granzyme K (GzmK) is an immune cell-secreted serine protease elevated in inflammatory and proliferative skin conditions. In the present study, human psoriasis lesions exhibited elevated GzmK levels compared to non-lesional psoriasis and healthy control skin. In an established murine model of imiquimod (IMQ)-induced psoriasis, genetic loss of GzmK significantly reduced disease severity, as determined by delayed plaque formation, decreased erythema and desquamation, reduced epidermal thickness, and inflammatory infiltrate. Molecular characterization *in vitro* revealed that GzmK contributed to macrophage secretion of IL-23 as well as PAR-1-dependent keratinocyte proliferation. These findings demonstrate that GzmK enhances IL-23-driven inflammation as well as keratinocyte proliferation to exacerbate psoriasis severity.

KEYWORDS

granzymes, granzyme K, psoriasis, IL-23, PAR-1, inflammation, proliferation, skin



GRAPHICAL ABSTRACT

1 Introduction

Psoriasis is a chronic inflammatory disease that affects over 125 million people worldwide (1). Psoriasis vulgaris (plaque psoriasis) is the most common clinical variant, observed in 85-90% of psoriasis cases, and usually manifests as erythematous plaques covered with silvery scales on the extensor surfaces, trunk, and scalp (2). Persistent pruritus, pain, and visibility of plaques characteristic of the disease considerably impair quality of life (3, 4).

While biologics targeting the IL-23/IL-17 pathway have demonstrated clinical efficacy, their broad use is limited by costs, side effects related to immunomodulation, and disease recurrence following treatment discontinuation (5, 6). As such, an impetus to develop more affordable and efficacious therapeutics for psoriasis remains (7, 8). To address this need, a clearer understanding of the molecular mechanisms underlying pathogenesis is required.

Psoriasis pathogenesis is driven by elevated inflammation and keratinocyte proliferation. These processes are dependent on IL-23 and JAK/STAT signaling (9–12). During active disease, IL-23, produced by myeloid cells, activates and maintains the pathogenic phenotypes of Th17, Tc17, and possibly Treg cells, leading to the release of various pro-inflammatory cytokines, most notably IL-17 (13–15). This cytokine cascade fuels inflammation, perpetuating vasodilation and immune cell infiltration (9). Concurrently, STAT3 hyperactivation in keratinocytes drives increased cellular proliferation and impaired differentiation, leading to epidermal hyperplasia which presents clinically as skin plaques (9–12). Provided the central roles of IL-23 and STAT3 in psoriasis pathogenesis, exploring novel mediators within this context is imperative to advance our understanding of disease mechanisms and yield new opportunities for therapeutic development.

The immune cell-secreted serine protease, Granzyme K (GzmK), conventionally known for its role in targeted cell death, has emerged as a key player in inflammation (16). Elevated GzmK levels are consistently observed in biospecimens from individuals with diverse inflammatory conditions compared with healthy controls, including acute lung inflammation (bronchoalveolar lavage fluid) (17), endotoxemia (blood) (18), sepsis (blood) (19), viral infection (blood) (20), thermal injury (skin) (21), atopic dermatitis (skin) (22), and inflammaging (various tissues) (23). Corresponding to its presence in biofluids, extracellular roles for GzmK have been forwarded. Notably, GzmK has been shown to induce the release of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1/CCL2)) (21, 24–26) and to promote cellular proliferation through activation of protease activated receptor-1 (PAR-1) (21, 24, 26, 27). Further, PAR-1-induced keratinocyte proliferation may contribute to psoriasis (28–31). Taken together, we hypothesized that GzmK contributes to inflammation and/or keratinocyte proliferation in psoriasis.

2 Results

2.1 GzmK is elevated in human psoriasis lesions and predominantly localized in the infiltrating immune cells of the papillary dermis

To evaluate GzmK levels in human psoriasis tissues, GzmK expression was assessed using microarray data available through the Gene Expression Omnibus (GEO, NCBI) database (32). According to dataset GDS4602 (33–35), GzmK mRNA expression is elevated in lesional psoriasis skin compared to both healthy control and non-lesional psoriasis skin (Figure 1A). Similar results were obtained using another dataset, GSD4600 (36, 37) (Supplementary Figure 1A). To further assess the presence of GzmK in psoriatic skin, a database query for GzmK was performed on skin mRNA microarray datasets publicly available on SEEK (Princeton University) (38). According to this search, the majority of prioritized datasets were related to psoriasis, underscoring the significance of GzmK to psoriasis pathology (Supplementary Table 1). Importantly, dataset enrichment analysis revealed 'psoriasis' as the disease category with the highest number of relevant datasets retrieved, further emphasizing its significance in the context of GzmK (Supplementary Table 2).

To examine GzmK at the protein level and its distribution in human psoriasis tissue, formalin-fixed paraffin-embedded sections of healthy control skin and lesional psoriasis skin were evaluated. In agreement with the GzmK mRNA expression data, all psoriasis skin samples exhibited a significant increase in GzmK protein levels compared with healthy control skin (Figures 1B, C). This increased GzmK detection occurred in all psoriasis samples despite reported differences in clinical appearance, sex, and age of individuals (patient data listed in Supplementary Table 3). Within psoriasis lesions, GzmK-positivity was also observed in the extracellular milieu surrounding the GzmK-positive cells compared with healthy controls (Supplementary Figure 1B), indicating potential extracellular secretion.

In all stained psoriasis lesions, GzmK-positive cells were primarily located within the inflammatory cell infiltrate of the papillary dermis and adjacent to small blood vessels (Figure 1B). In healthy skin, GzmK was detected almost exclusively in mast cells (Toluidine Blue O (TBO)$^+$ and tryptase$^+$), as previously reported (22). GzmK-positive mast cells were observed in psoriatic skin as well (Supplementary Figures 1C–E); however, there was a decrease in the percentage of GzmK-positive mast cells. Serial immunostaining and co-immunofluorescence of human psoriasis lesions further revealed a population of CD68$^+$ cells (monocytes/macrophages) positive for GzmK (Figure 1D, Supplementary Figure 2A). We also detected a minor population of CD3$^+$ cells (both CD4$^+$ and CD8$^+$ T cells) and Neutrophil Elastase (NE)$^+$ cells (neutrophils) positive for GzmK (Supplementary Figures 2A, 3A). No GzmK was detected in CD56$^+$ (natural killer cells), CD1a$^+$ (Langerhans cells), nor CD11c$^+$ cells (dermal dendritic cells) despite each of these cell populations being elevated in human psoriasis lesions (Supplementary Figures 2A, 3A). Further, human THP-1 monocytes and macrophages were confirmed as a source of GzmK *in vitro* (Figure 1E), corresponding with previous observations (21).

2.2 GzmK depletion attenuates disease severity in a murine model of IMQ-induced psoriasis-like skin inflammation

To investigate whether GzmK exerts a pathologic role in psoriasis, *GZMK* knockout (GzmK KO) mice were utilized. In comparative sequence analysis, human and mouse GzmK exhibited a 71.82% amino-acid sequence identity and full conservation of the catalytic triad (His, Asp, Ser) (Supplementary Figure 4A), underscoring the relevance of the mouse model for investigating the mechanistic role of GzmK in the context of psoriasis. Using the established murine model of imiquimod (IMQ)-induced psoriasis-like skin inflammation (39), a psoriasis-like phenotype was induced in both wild-type (WT) and GzmK KO mice (Figure 2A). WT and GzmK KO mice showed no visible signs of distress, and their body weights remained stable throughout the study (Supplementary Figure 4B).

Clinically recognizable lesions developed earlier in IMQ-treated WT mice (day 5) compared to GzmK KO mice (day 6) (Figure 2B). Erythema and desquamation severity were reduced in GzmK KO mice, as determined using the modified psoriasis area and severity index (PASI) scoring system (scoring outline listed in Supplementary Table 4) (40). Both sets of individual scores were significantly reduced in GzmK KO mice at days 6 and/or 7 (Figure 2C). Accordingly, GzmK KO mice exhibited a reduced cumulative severity score (defined as combined erythema and desquamation severity scores) for the duration of the study and a significant decrease in cumulative severity score at day 7 compared to WT mice (Figure 2C). Other phenotypes observed in GzmK KO mice included reduced inflammatory infiltrate in the upper dermis and the absence of parakeratosis and vascular dilation in the papillary dermis compared to WT mice (Figure 2D, Supplementary Table 5). Altogether, these results indicate that GzmK deficiency attenuates disease severity in the murine model of IMQ-induced psoriasis.

2.3 GzmK depletion reduces IMQ-induced inflammatory cell infiltrate

Compared with IMQ-treated WT mice, GzmK KO mice displayed an attenuated inflammatory response, characterized by a decreased leukocyte infiltration (Figures 3A, B). T cells (CD3$^+$) were most abundant in both IMQ-treated WT and GzmK KO mice compared to other cell types, similar to human psoriatic tissue (41). However, there was a decrease in the number of CD3$^+$ per area in IMQ-treated lesions of GzmK KO mice compared to WT mice (Supplementary Figures 5A, B). Additionally, a decrease in microvascular density (CD31$^+$) was observed in IMQ-treated GzmK KO mice compared to WT mice (Supplementary Figures 5C, D). These findings highlight the potential importance of GzmK in modulating inflammatory responses during disease development.

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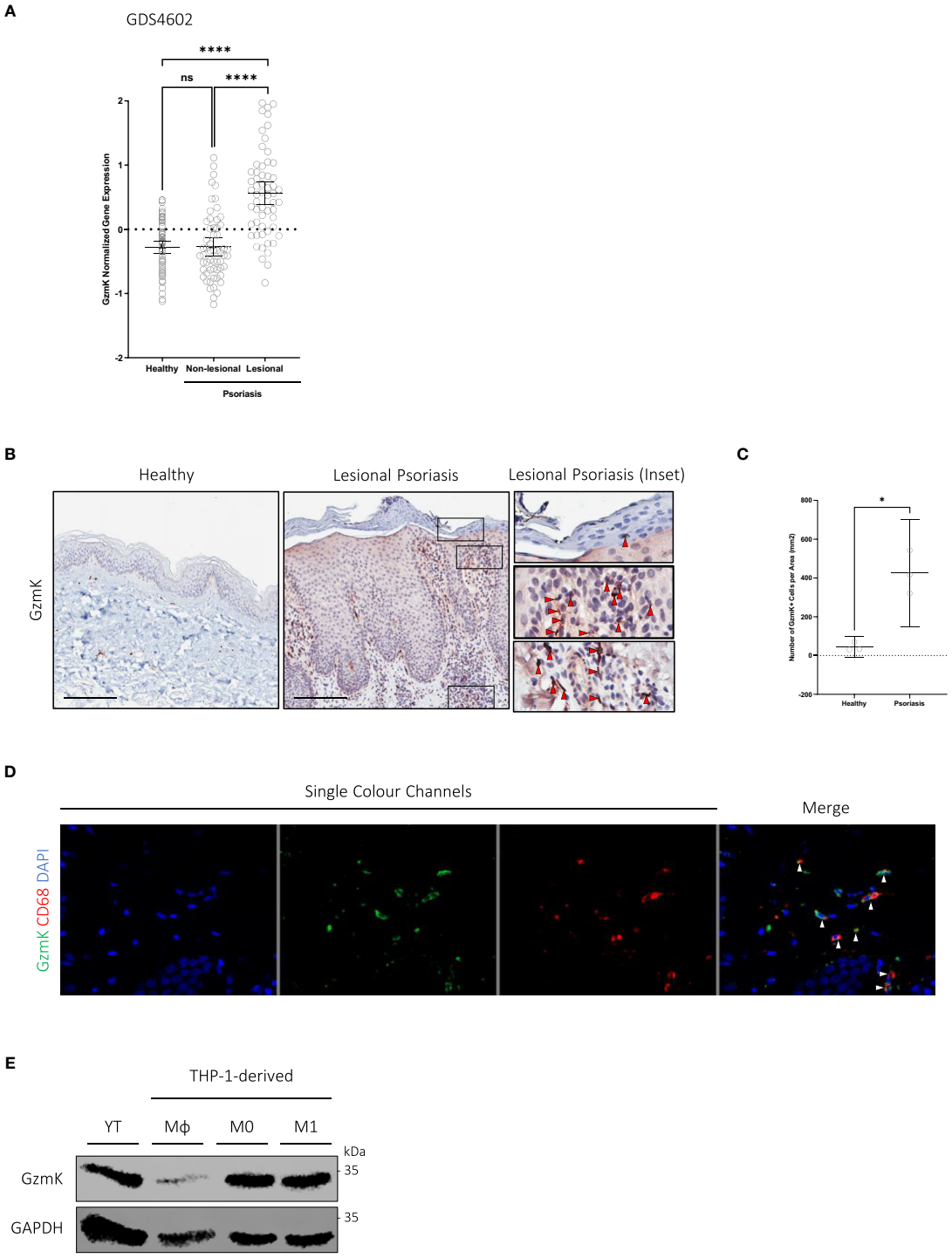

FIGURE 1
GzmK is abundant in human psoriasis lesions and predominantly localized in the infiltrating immune cells of the papillary dermis **(A)** GzmK normalized gene expression in the skin of healthy control subjects and psoriasis patients (both non-lesional and lesional). **(B)** GzmK immunostaining in the skin of healthy control subjects and psoriasis patients. The inset shows high magnification of regions of interest. Red arrows indicate $GzmK^+$ cells. **(C)** Quantification of GzmK-positive cells presented as number of GzmK-positive cells per area (mm^2) in the skin of healthy control subjects and psoriasis patients. **(D)** Two-colour immunofluorescence of GzmK and CD68 in psoriasis-affected human skin. 'Merge' showcases overlap of all colour channels. White arrows indicate co-staining cells ($GzmK^+/CD68^+$). **(E)** Western blot immunodetection of GzmK in NK cells (YT) and THP-1-derived monocytes (Mϕ), M0 and M1 macrophages. n ≥ 58 per group **(B–E)**. Data were analyzed by Kruskal-Wallis test with Dunn's *post-hoc* test for multiple comparisons and presented as mean with 95% CI **(A)**, or Welch's t-test and presented as mean with 95% CI **(C)**. In all plots, *P≤0.05, ****P≤0.0001. Scale bars represent 200 µm **(B)**, with representative images shown.

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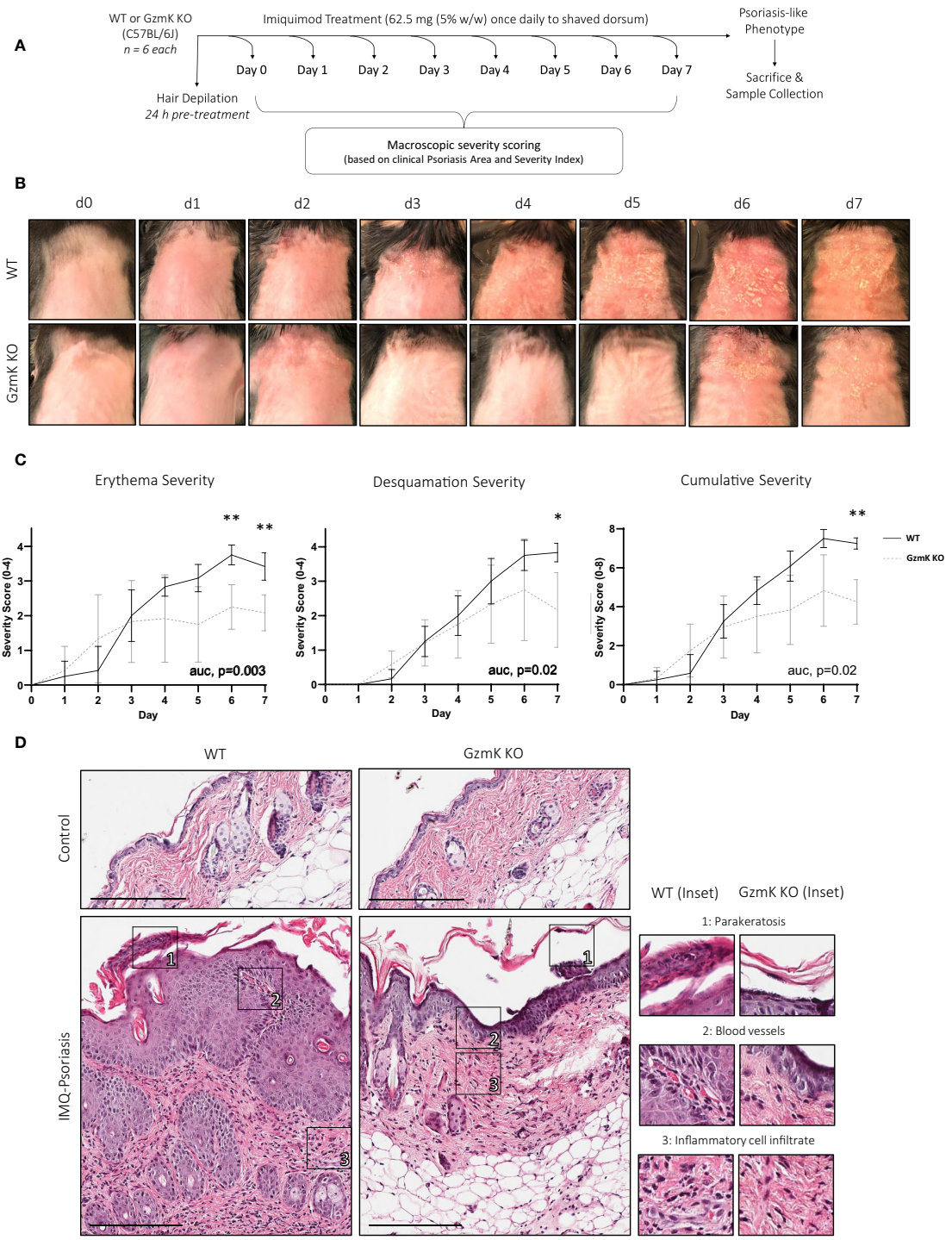

FIGURE 2
GzmK depletion attenuates disease severity in a murine model of IMQ-induced psoriasis-like skin inflammation. **(A)** Experimental protocol involving daily topical application of IMQ for 7 days to induce a psoriasis-like phenotype. **(B)** Images of IMQ-treated WT and GzmK KO mice from day 0 to day 7. **(C)** Daily changes in skin severity (erythema and desquamation) in IMQ-treated WT and GzmK KO mice. Cumulative skin severity is represented as cumulative erythema and scaling scores. **(D)** Hematoxylin and Eosin staining of paraffin sections from the dorsal skin of both IMQ-treated WT and GzmK KO mice. The inset shows high magnification of regions of interest. n ≥ 6 per group **(A-D)**. Data were analyzed by multiple unpaired t-tests with Welch correction and Holm-*Šídák post-hoc* test for multiple comparisons and presented as multivariable linear regression with three degrees of interaction **(C)**. In all plots, area under curve (AUC) p-values are as written and adjusted p-values are shown *P≤0.05, **P≤0.01. Scale bars represent 200 μm **(D)**, with representative images shown.

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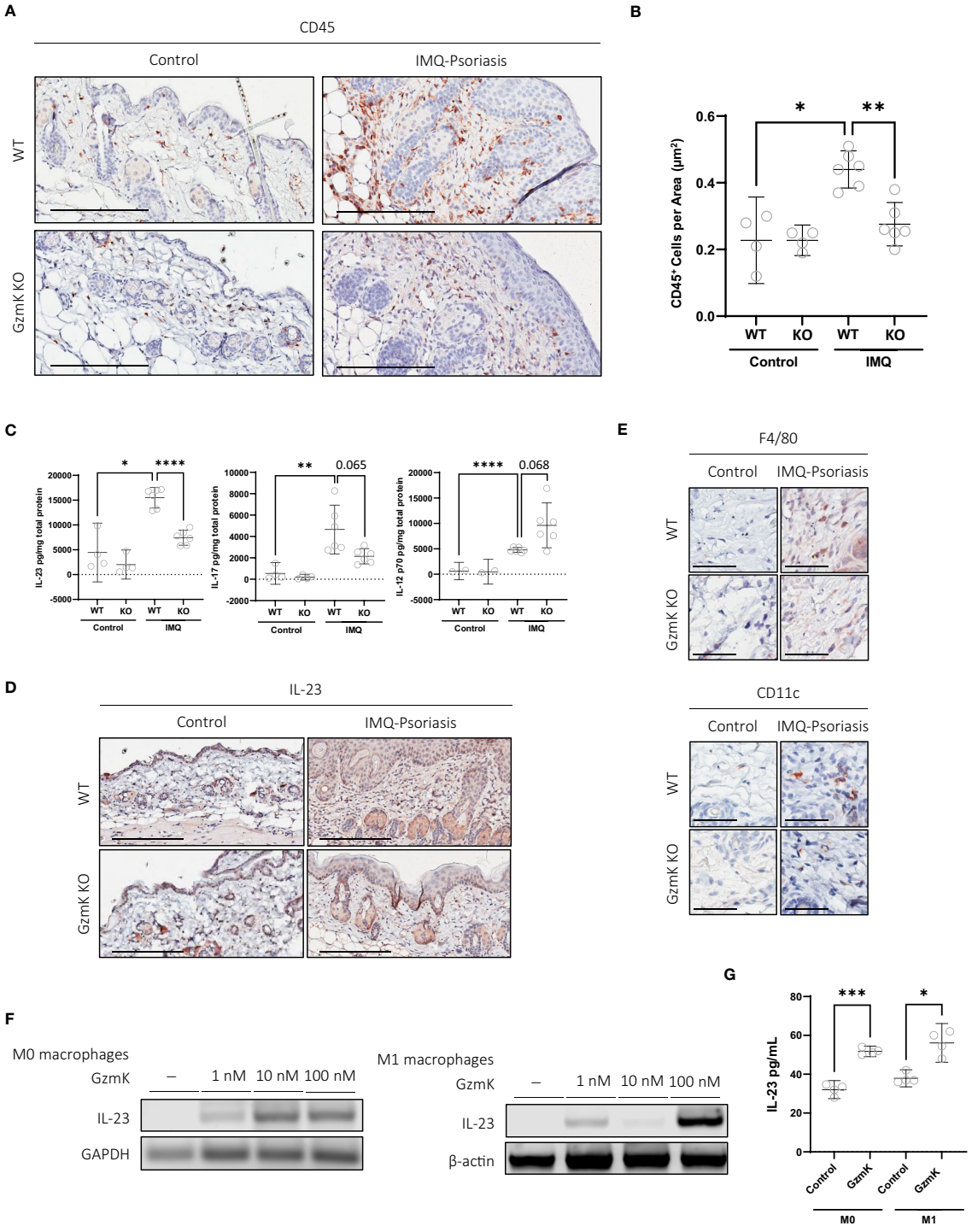

FIGURE 3
GzmK depletion reduces IMQ-induced inflammatory cell infiltrate, GzmK induces IL-23 secretion from macrophages. **(A)** CD45 immunohistochemistry of dorsal skin in untreated and IMQ-treated WT and GzmK KO mice. **(B)** Data presented as number of CD45-positive cells per area (um^2). **(C)** Quantification of IL-12, IL-17 and IL-23 in WT and GzmK KO tissue homogenates by ELISA. Data presented as pg cytokine per mg total protein. **(D)** IL-23A immunohistochemistry of dorsal skin in untreated and IMQ-treated WT and GzmK KO mice. **(E)** F480 (monocytes/macrophages) and CD11c (dendritic cells) immunohistochemistry of dorsal skin in untreated and IMQ-treated WT and GzmK KO mice skin. **(F)** IL-23 gene expression analyzed by RT-PCR in THP-1 macrophages ± GzmK exposure. β-actin and GAPDH were used as loading controls. **(G)** IL-23 protein secretion analyzed by ELISA of culture medium from THP-1 macrophages ± GzmK exposure. Data presented as pg cytokine per ml. n ≥ 3 per group **(A-G)**. Data were analyzed by Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 *post-hoc* test for multiple comparisons and presented as mean with 95% CI **(B, C)**, or Welch's t-test and Mann-Whitney test and presented as mean with 95% CI **(G)**. In all plots, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$. Scale bars represent 200 µm **(A, D)** and 50 µm **(E)**, with representative images shown.

2.4 GzmK induces IL-23 secretion from macrophages

In IMQ-treated GzmK KO mice, IL-17 and IL-23 tissue levels were reduced compared to IMQ-treated WT mice, whereas IL-12p70 levels were increased in GzmK KO mice (Figure 3C). To examine the lesional tissue distribution of IL-23, immunohistochemistry was performed. IL-23A staining of murine tissues identified that the majority of IL-23$^+$ cells were located in the dermis within the vicinity of blood vessels and exhibited a morphology characteristic of mononuclear cells (Figure 3D). In contrast to GzmK KO mice, infiltration of macrophages (F4/80$^+$) (CD11c$^+$), two major sources of IL-23 in psoriasis (42), were observed in IMQ-treated WT mice (Figure 3E). The role of GzmK in inducing IL-23 release from macrophages was therefore investigated. THP-1-derived M0 and M1 macrophages were exposed to increasing concentrations of active recombinant human GzmK. GzmK increased IL-23 mRNA expression in a dose-dependent manner and promoted IL-23 secretion from both M0 and M1 macrophages (Figures 3F, G). These results support a pro-inflammatory function for GzmK in psoriasis through the stimulation of IL-23 production.

2.5 GzmK depletion attenuates IMQ-induced epidermal hyperplasia

The contribution of GzmK to epidermal hyperplasia, a hallmark of psoriasis, was assessed in the murine model of IMQ-induced psoriasis-like skin inflammation. IMQ-treated GzmK KO mice exhibited a reduction in epidermal thickness compared with WT mice at day 7 (Figures 4A, B). In IMQ-treated GzmK KO mice and untreated controls, the proliferation marker

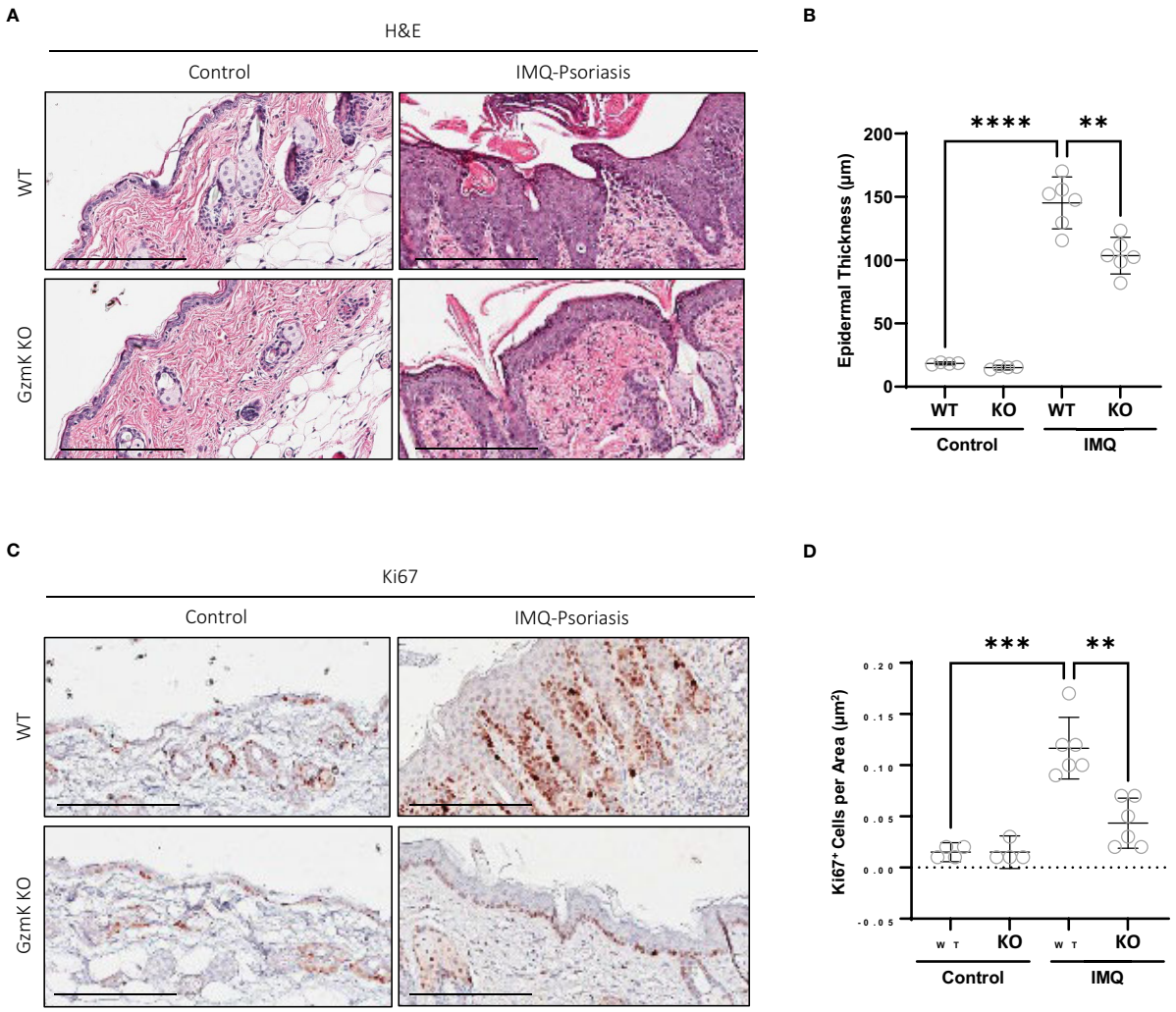

FIGURE 4
GzmK depletion attenuates IMQ-induced epidermal proliferation. **(A)** Hematoxylin and Eosin staining of dorsal skin in untreated and IMQ-treated WT and GzmK KO mice comparing epidermal thickness. **(B)** Data presented as mean epidermal thickness (µm). **(C)** Ki67 immunohistochemistry of dorsal skin in untreated and IMQ-treated WT and GzmK KO mice. **(D)** Data presented as number of Ki67-positive cells per area (µm^2). n ≥ 6 per group **(A-D)**. Data were analyzed by Brown-Forsythe and Welch ANOVA tests with Dunnett's T3 *post-hoc* test for multiple comparisons and presented as mean with 95% CI **(B, D)**. In all plots, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$. Scale bars represent 200 µm **(A, C)**, with representative images shown.

Ki67 was restricted to basal keratinocytes, whereas in IMQ-treated WT mice, a large number of suprabasal keratinocytes also exhibited positive staining for this marker (Figure 4C). Comparatively, GzmK KO mice exhibited a decrease in the mean number of $Ki67^+$ cells per μm^2 compared with WT mice (Figure 4D). In IMQ-treated GzmK KO mice, a similar decrease in proliferating cell nuclear antigen (PCNA) was observed compared to IMQ-treated WT (Supplementary Figure 6A). Further, when both primary (NHEKs) and immortalized (HaCaTs) keratinocytes were cultured in the presence of GzmK, increased levels of PCNA were observed (Supplementary Figure 6B). Consequently, GzmK may contribute to psoriasis epidermal hyperplasia through the stimulation of keratinocyte proliferation.

2.6 GzmK stimulates human keratinocyte proliferation via the PAR-1/MAPK/STAT3 pathway

As documented previously, both normal human epidermal keratinocytes (NHEKs) and keratinocyte cell lines (HaCaTs) express PAR-1 (43, 44). GzmK is known to activate PAR-1 through cleavage of its tethered ligand, initiating receptor activation and cellular responses from various cell types (21, 24, 26). We examined whether extracellular GzmK was inducing proliferation through the cleavage and subsequent activation of PAR-1. To address this, we used 2 siRNA sequences designed against human *F2R* (PAR-1). Both siRNAs were transfected at a final concentration of 40 nM in HaCaTs, which achieved an 87% (siRNA #1) and 100% (siRNA #2) reduction at 72 h post-transfection and 100% (siRNA #1 and #2) at 120 hours post-transfection (Figures 5A, B). Those HaCaTs transfected with PAR-1 siRNA demonstrated a marked reduction of GzmK-mediated proliferation as visualized by Ki67 immunocytochemistry (Figure 5C), supporting our hypothesis that GzmK contributes to keratinocyte proliferation through a PAR-1 dependent extracellular mechanism.

As activation of the MAPK signaling pathway has been previously linked to keratinocyte proliferation (45–47), we next assessed their potential downstream activation by PAR-1 in keratinocytes. Western blot analysis revealed a robust phosphorylation of p38 (MAPK14) and p44/42 (ERK1/2), but not p46/54 (SAPK/JNK) MAPK, in keratinocytes stimulated by GzmK for up to 3 hours (Supplementary Figures 7A–C).

We next examined the involvement of PAR-1 in GzmK-dependent MAPK signaling. When keratinocytes were transfected with *F2R* (PAR-1) siRNA as above, GzmK-mediated phosphorylation of both p38 and p44/42 MAPK was inhibited (Figures 6A, B). Taken together, the findings suggest that GzmK activation of the PAR-1/MAPK pathway contributes to the induction of keratinocyte proliferation.

To further understand the downstream signaling pathways involved in GzmK/PAR-1/MAPK-mediated keratinocyte proliferation, STAT3 phosphorylation was assessed. Upon GzmK stimulation of human keratinocytes, we observed a marked increase in STAT3 phosphorylation at Ser727 and to a lesser extent at Tyr705, both peaking at 15 mins and maintaining elevated levels up to 3 hours-post stimulation (Supplementary Figure 7A). This finding suggests a sustained activation of STAT3 signaling in response to GzmK.

We subsequently examined whether the observed GzmK-mediated STAT3 signaling was PAR-1 and/or MAPK pathway-dependent. Keratinocytes transfected with *F2R* (PAR-1) siRNA showed inhibited GzmK-mediated phosphorylation of STAT3 at both Ser727 and Tyr705 (Figure 6B). To determine whether this effect occurs downstream of MAPK, keratinocytes were pre-treated with U0126, a p44/42 MAPK inhibitor. Following this treatment, the phosphorylation of STAT3 Ser727 was notably abrogated (Figure 6C), indicating that STAT3 phosphorylation occurs downstream of PAR-1-mediated p44/42 activation.

3 Discussion

The mechanisms by which psoriasis is regulated are poorly understood. Currently, mechanisms underlying IL-23-driven inflammation and STAT3-driven keratinocyte proliferation are areas of high therapeutic interest and active investigation.

Of relevance to the present study, a role for serine proteases and their endogenous inhibitors (Kallikreins-Related Peptidases (48, 49), Marapsin (50), Vaspin (51), SerpinB7 (52), Leukocyte Elastase (53)) in psoriasis have been investigated. When compared with other classes of proteases, serine proteases are among the most likely to mediate psoriasis pathology since a majority are released to the extracellular milieu indiscriminately (54). The emerging pro-inflammatory effects of the serine protease, GzmK, initially prompted us to explore its potential role in psoriasis pathogenesis. Further, chromosome 5q, which contains the GzmK gene in humans, includes a susceptibility region for psoriasis (55) and numerous other autoimmune and inflammatory conditions including Crohn's disease (56), rheumatoid arthritis (57), asthma (58), and atopic dermatitis (59).

In the current study, GzmK levels were significantly increased in skin lesions from human psoriasis patients, predominantly within the dermal inflammatory cell infiltrate. Macrophages were identified as the largest population of GzmK-positive cells present exclusively within psoriasis lesions. It is known that infiltrating macrophages are recruited in inflammatory conditions and play an important role in psoriasis development, particularly during the initiation stages (60). Further, M1 polarization in psoriasis is associated with increased disease severity (61). Hence, GzmK produced and secreted by inflammatory macrophages may contribute to early psoriasis development. This is supported by a previous study in acute thermal injury, where GzmK was reported to be expressed and secreted by inflammatory macrophages (21). Monocytes/macrophages are also the dominant immune population in the synovial fluid of psoriatic arthritis, an inflammatory spondyloarthritis that affects up to 30% of people with psoriasis (62). Although the proportion of GzmK-positive mast cells in psoriatic lesions does not significantly differ from that in healthy skin, this does not rule out a pathogenic role in psoriasis. Notably, approximately 30% of dermal mast cells in psoriatic lesions lack the endogenous inhibitor for GzmK, bikunin (63). The prevalence of bikunin-negative mast cells in healthy controls is unclear; however, in both atopic dermatitis and chronic eczema, almost all tryptase-positive mast cells also express bikunin (63).

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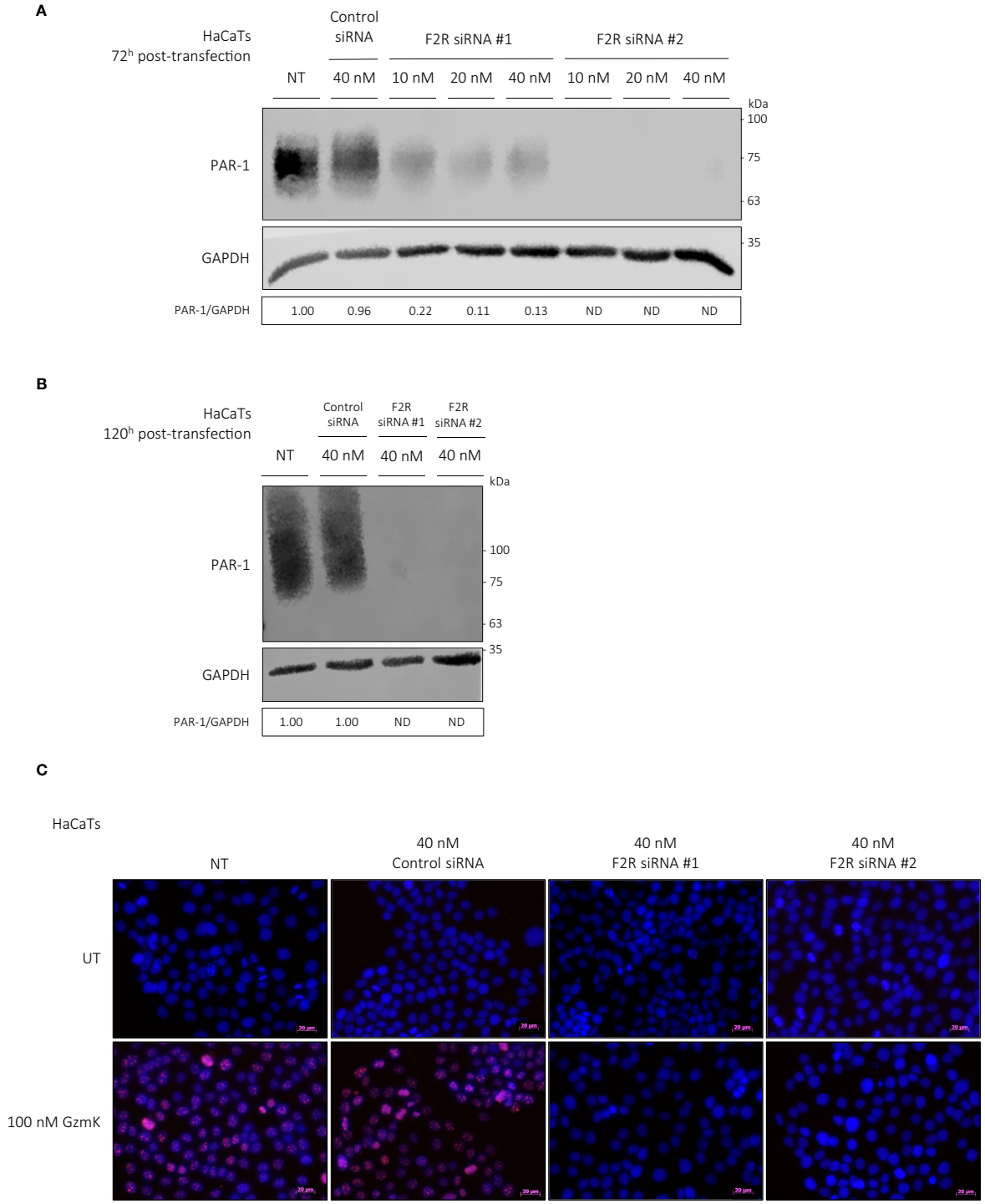

FIGURE 5
GzmK stimulates human keratinocyte proliferation via PAR-1. **(A)** Western blot immunodetection of PAR-1 and GAPDH of HaCaTs transfected with or without *F2R* (PAR-1) or Control siRNA (10 nM or 20 nM or 40 nM, 72 h). **(B)** Western blot immunodetection of PAR-1 and GAPDH of HaCaTs transfected with or without *F2R* (PAR-1) or Control siRNA (40 nM, 120 h). **(C)** HaCaTs transfected with or without *F2R* (PAR-1) or Control siRNA (40 nM, 120 h) and stimulated with or without GzmK (100 nM, 48 h). Proliferation was detected by Ki67 immunocytochemistry, Ki67 = red, Hoechst nuclear stain = blue. n ≥ 3 per group **(C)**. Scale bars represent 20 μm **(C)**, with representative images shown.

This suggests that the lack of bikunin expression in dermal mast cells may be unique to psoriasis. Despite the presence of GzmK-positive mast cells in both healthy and psoriatic skin, their potential contribution to the development of psoriasis warrants further investigation. The present study suggests that there may be multiple cell sources that contribute to elevated GzmK that is observed in psoriasis lesions. It is important to note that the pathogenic impact of GzmK observed in the current study is specifically attributed to the proteolytic activity of extracellular GzmK regardless of the independent cellular source(s).

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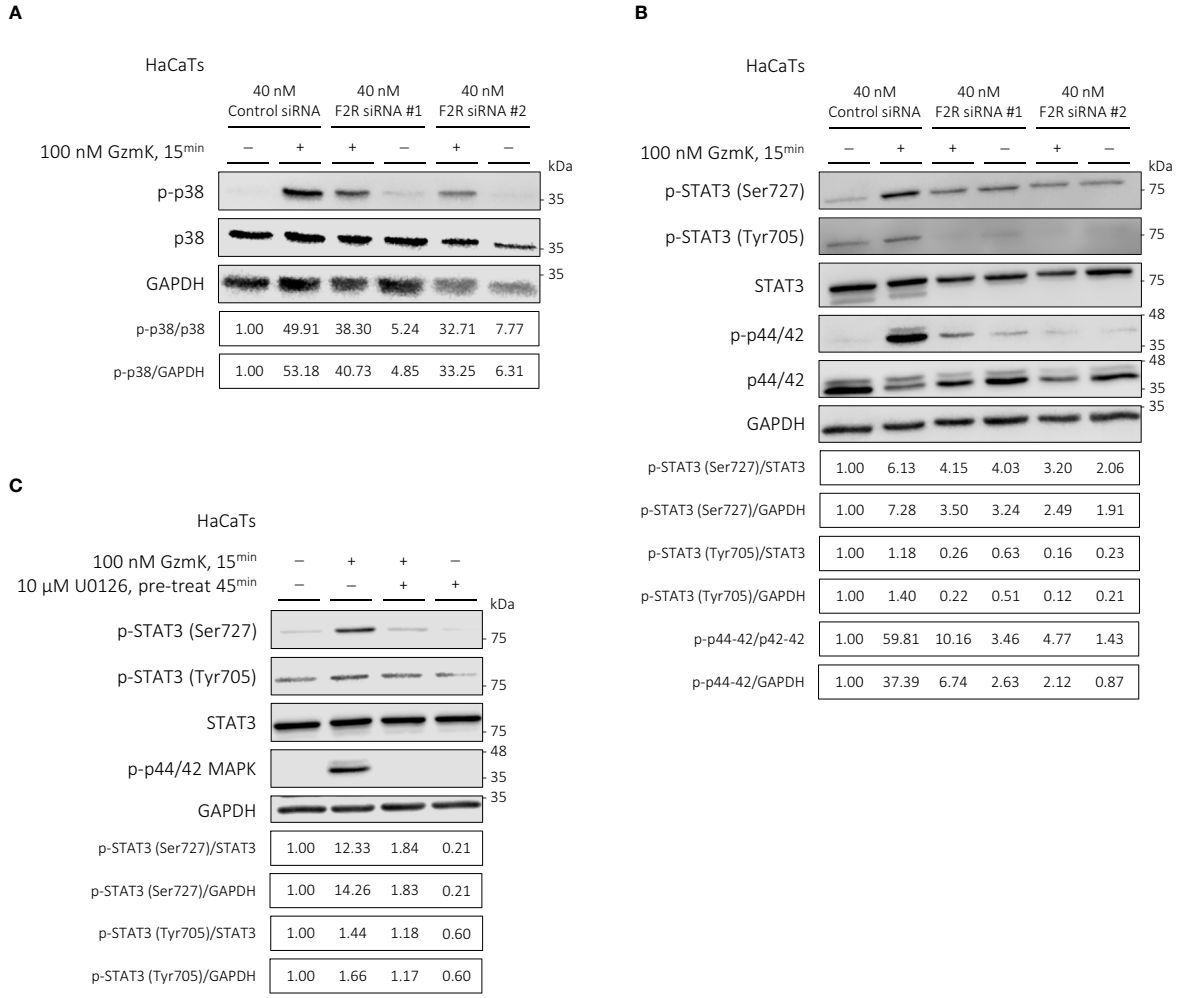

FIGURE 6
GzmK activation of PAR-1 phosphorylates p38 and p44/42 MAPK in human keratinocytes; GzmK induces STAT3 (Ser727) phosphorylation in a PAR-1/p44/42 MAPK-dependent manner in human keratinocytes. **(A)** Western blot immunodetection of p-p38 MAPK, total p38 MAPK, and GAPDH of HaCaTs transfected with or without *F2R* (PAR-1) or Control siRNA and stimulated with or without GzmK (100 nM, 15 min). **(B)** Western blot immunodetection of p-STAT3 (Ser727), p-STAT3 (Tyr705), total STAT3, p-p44/42 MAPK, total p44/42 MAPK, and GAPDH of HaCaTs transfected with or without *F2R* (PAR-1) or Control siRNA and stimulated with or without GzmK (100 nM, 15 min). **(C)** Western blot immunodetection of p-STAT3 (Ser727), p-STAT3 (Tyr705), total STAT3, p-p44/42 MAPK, and GAPDH of HaCaTs pre-treated with U0126 (10 µM, 45 min) followed by stimulation with or without GzmK (100 nM, 15 min).

The functional role of GzmK in psoriasis was studied in WT and GzmK KO mice using the established IMQ model of psoriasis-like skin inflammation (39). The IMQ-induced psoriasis model is amongst the most widely used mouse models of human psoriasis. It recapitulates several histological and immunological features of human psoriasis including an IL-23 and/or IL-17 dependent inflammatory response (39, 64–67). Accordingly, IMQ-treated WT mice presented with multiple features reminiscent of the phenotype observed in human psoriasis lesions including increased epidermal thickness/hyperplasia, perivascular inflammation (predominantly characterized by the presence of $CD3^+$ T cells), vascularization, and secretion of IL-23/IL-17 axis cytokines. GzmK contributed to disease severity in the murine model of IMQ-induced psoriasis, as reflected in earlier clinical disease with more severe erythema and desquamation in WT mice compared to GzmK KO mice. Histologically, GzmK primarily contributed to disease severity by inciting lymphocytic infiltration and epidermal hyperplasia.

Of particular relevance to clinically approved biologics, GzmK depletion in GzmK KO mice reduced the levels of both IL-17 and IL-23 in IMQ-induced psoriasis-like lesional skin. The decreased inflammatory reaction associated with the lower production of these cytokines provides insights with respect to the milder disease phenotype observed in GzmK KO mice compared to WT mice. These findings suggest that GzmK contributes to a pro-inflammatory response that favours the Th17 lineage and is upstream of IL-23/IL-17. In support, GzmK induced macrophage production and secretion of IL-23. IL-23 is a heterodimer composed of two subunits: p19 and p40, which it shares with IL-12. For this reason, previous therapies targeting IL-23p40 have also targeted IL-12. However, IL-12 is important for the activation of Th1 cells and recent research has revealed that IL-12 may serve a protective role

in psoriasis (68, 69). Interestingly, IL-12 levels were heightened in GzmK KO mice lesional skin compared to WT mice, which may also contribute to the milder phenotype observed. Thus, by inhibiting GzmK, it would appear we are able to inhibit IL-23 and not IL-12, bringing several advantages over current therapies. Further, there is strong evidence to support the role of IL-17 in the protection of epithelial barrier function in the gut mucosa and airways; as such, systemic inhibition of IL-17 presents potential risk of candida infection and is associated with infection and disease worsening in these tissues (70–73). Although IL-23 is upstream of IL-17, studies suggest that blocking IL-23 does not impair IL-17 production by innate non-T-cell lymphocytes in the gut (74), thereby preserving intestinal integrity (72, 75). Provided the role of GzmK in selectively inducing IL-23p19, it could be a potential therapeutic target for psoriasis treatment.

Further, IL-23 binds to the IL-23 receptor (IL-23R) which, at least in Th17 cells, is induced by stimulation with IL-1β, IL-6 and transforming growth factor (TGF)-β. Previously, we have demonstrated that GzmK can induce macrophages and resident skin cells to secrete IL-1β and IL-6 (21), emphasizing its potential support of IL-23/IL-17 pathogenic mechanisms. Together, the overproduction of pro-inflammatory cytokines, IL-1β, IL-6, IL-17, and IL-23, associated with elevated GzmK would be expected to elicit a severe inflammatory imbalance thereby driving keratinocyte proliferation and psoriatic plaque development.

In addition, this phenotype is exacerbated by the ability of GzmK to directly induce keratinocyte proliferation and epidermal hyperplasia. Previous research has demonstrated that GzmK can induce PAR-1-mediated proliferation in human fetal lung fibroblasts (24). Extending these findings, our study reveals that GzmK induces PAR-1-mediated proliferation in keratinocytes. Furthermore, we observed that GzmK concurrently activates p38 and p44/42 MAPK pathways, with the latter specifically leading to the phosphorylation of STAT3 (predominantly at Ser727). In addition to STAT3, the significance of PAR-1 and MAPK signaling in psoriasis is well-established with respect to keratinocyte proliferation (45–47). Within psoriasis lesions, PAR-1, p38, and p44/42 MAPK are consistently active and present throughout the epidermis (48, 76). Additionally, the modulation of STAT3 transcriptional activity in keratinocytes by MAPK signaling has been observed (77). Provided the interconnected nature of these signaling events, our findings underscore the therapeutic potential of targeting GzmK, not only as a means to modulate IL-23-driven inflammation, but also to regulate STAT3-driven keratinocyte proliferation through the PAR-1/MAPK pathway, offering a novel approach to psoriasis management. A schematic overview depicting the role of GzmK in psoriasis can be seen in the Graphical Abstract.

At present, pharmacologic agents targeting GzmK activity do not exist. However, there is a natural physiological inhibitor of human and mouse GzmK found in plasma, known as Inter-alpha inhibitor protein (IaIP). IaIP acts as an extracellular inhibitor, mediated by the second Kunitz-type domain of its bikunin subunit (78). In a survey of over 2,007 genes expressed in epithelial tissues, Itoh et al. identified 7 genes that show high expression levels only in non-lesional/uninvolved psoriasis skin compared to matched lesional psoriasis skin, which includes *AMBP*, the precursor for alpha-1-microglobulin and bikunin (79). In this case, free GzmK may be accumulating and show upregulated activity in lesional psoriasis skin.

Although several treatment interventions for psoriasis exist, barriers to effective care include cost of advanced biologics, cumulative toxicity, and potential for adverse events (5, 6, 80). As GzmK is a protease and produced locally in affected tissues, the present study offers an alternative approach that could mitigate expensive biological approaches and associated long-term off-target effects.

4 Materials and methods

4.1 Human database queries

Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) was used to retrieve raw scRNA-seq gene expression profiles from healthy control subjects, non-lesional and lesional psoriasis patients (accession numbers: GSE13355, GSE30999). The GSE13355 dataset (GDS4602) was generated using human skin punch biopsies obtained from 58 psoriasis patients that sampled both lesional and non-lesional skin and 64 healthy control subjects (33–35), while the GSE30999 dataset (GDS4600) included lesional skin collected from 85 psoriasis patients along with matched biopsies of non-lesional psoriasis skin (36, 37). The resulting datasets were queried for *GZMK* normalized gene expression using the GEO Profiles database. The expression values in the table are after adjustment of RMA expression values (on the log scale) to account for batch and sex effects as appropriate.

SEEK (http://seek.princeton.edu/) is a search engine used to identify/query gene(s) co-expression with other genes or datasets. After defining the search range to (non-cancer) skin-related datasets, available datasets were ordered by their relevance to/enrichment for *GZMK* (see Supplementary Tables 1, 2).

4.2 Human samples

Paraffin-embedded psoriasis skin was obtained retroactively from patients diagnosed with psoriasis ($N > 3$). Psoriasis patients had not received any treatments prior to biopsy (additional patient data is available in Supplementary Table 3). Paraffin-embedded healthy control skin was obtained from subjects undergoing elective abdominoplasty ($N \geq 3$).

4.3 Mice

All animal ethics and procedures were approved and performed in accordance with the guidelines for animal experimentation as per the University of British Columbia (UBC) Animal Care Committee. C57BL/6 mice (listed as WT in text) were purchased from Jackson Laboratories (Bar Harbor, ME). GzmK knockout mice (C57BL/6 background) (listed as GzmK KO in text) were kindly gifted from

Dr. Phillip Bird (Department of Biochemistry and Molecular Biology, Monash University, Australia) and are routinely backcrossed with C57BL/6 mice. Both WT and GzmK KO mice were bred and housed at the International Collaboration on Repair Discoveries (ICORD) vivarium. Six female mice (aged 6-8 weeks) were used per genotype.

4.4 IMQ-induced psoriasis model

The IMQ-induced mouse model of psoriasis was performed as previously described (39). Daily topical administration of IMQ on shaved dorsal skin induced erythematous, scaly lesions resembling human psoriasis. On day 8 of the experiment, mice were euthanized, and tissue collected for analysis.

4.5 Immunostaining & histology

Five-micrometer-thick, vertical sections were prepared from all formalin-fixed, paraffin-embedded skin samples and processed for staining using Hematoxylin and Eosin for morphological analysis, Toluidine Blue O (TBO) for mast cells, or specific antibodies for immunohistology as listed in Supplementary Table 6. All histological and histochemical stains were imaged with the Aperio CS2 slide scanner (Leica Biosystems). The resulting images were prepared using the Aperio ImageScope Viewer (Leica Biosystems) or QuPath (University of Edinburgh) (81).

The density of positive cells and/or intensity of staining was determined using the formula: (number of positive cells or total intensity of strong positive staining)/(total skin area examined), which was measured using the Aperio ImageScope Viewer (Leica Biosystems) or by evaluating H-Score or Positive Cell Detection using QuPath (University of Edinburgh). Epidermal thickness was determined using the formula: (tissue epidermal area)/(tissue length), which was measured using the Aperio ImageScope Viewer (Leica Biosystems). Areas of skin layers (dermis and subcutaneous fat), hair follicles and glands were carefully excluded as appropriate.

4.6 Mice scoring of disease severity (modified psoriasis area and severity index)

Evaluations of disease severity were performed daily using a modified version of the Psoriasis Area and Severity Index as previously described (Supplementary Table 4) (40). IMQ-induced psoriasis-like skin severity was graded independently by two dermatology researchers. The two scores were averaged.

4.7 Tissue sampling

Biopsies from the dorsal region were harvested and placed in formalin for subsequent paraffin-embedding or snap-frozen in liquid nitrogen.

4.8 Primary and immortalized cells

Human keratinocytes (HaCaTs) were cultured in DMEM (Sigma-Aldrich) containing 10% (volume/volume) fetal bovine serum and 1% (volume/volume) penicillin/streptomycin. Normal human keratinocytes (NHEKs) were cultured in Keratinocyte Basal Medium 2 with supplements (Promo Cell) and 1% (volume/volume) penicillin/streptomycin. Human THP-1 monocytes (ATCC® TIB202™) were cultured and differentiated into M0 macrophages, and polarized into M1 macrophages, as previously described (82). Cellular phenotypes of cells transformed from THP-1 monocytes were validated using flow cytometry (Supplementary Figure S8A). All cells were cultured in serum-free media conditions for 24 hours before and during each experiment. Where pertinent, cells were treated with rhGzmK protein (BonOpus, non-commercial supply).

4.9 siRNA transfection

HaCaT cells were seeded into 6-well plates at a density of 0.25 x10^6 cells/well approximately 24 h before transfection. Two siRNA targeting the *F2R* gene (PAR-1) and a negative control siRNA with no complementary target sequences were used (Qiagen). Cells were transfected with 10-40 nM siRNA using HiPerFect Transfection Reagent (Qiagen), as per supplier instructions. The efficiency of gene knockdown was evaluated by Western blot after incubation in normal cell culture conditions for 72 and 120 h.

4.10 Statistics

Coefficient of Variation (CV) was equal to an average of 1%. CV ≤ 5% was defined a-priori as the acceptable limit. Data was analyzed using Graphpad Software for Windows. Data was analyzed for normal (Gaussian) distribution using the Shapiro-Wilk test (alpha=0.05). Statistical significance was determined by t-tests or ANOVA with *post-hoc* test for multiple comparisons, as appropriate (particulars of statistical tests specified in figure legends). $^*P \leq 0.05$ was defined as statistically significant.

4.11 Ethics statement

This study was carried out in accordance with the recommendations of institutional guidelines of the University of British Columbia. All human studies were approved by the University of British Columbia Human Research Ethics Committee. All animal experiments were approved by the Animal Care Committee.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by University of British Columbia Clinical Research Ethics Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because the samples used were excess tissue slides that were generated for diagnostic purposes and all samples were fully de-identified. The animal study was approved by University of British Columbia Animal Care Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KR: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. AA: Investigation, Methodology, Writing – review & editing. CT: Methodology, Writing – review & editing. LN: Methodology, Writing – review & editing. SH: Methodology, Writing – review & editing. MP: Investigation, Writing – review & editing. RC: Investigation, Writing – review & editing. HZ: Resources, Writing – review & editing. KJ: Funding acquisition, Writing – review & editing. AB: Investigation, Writing – review & editing. RC: Resources, Writing – review & editing. DG: Funding acquisition, Resources, Writing – review & editing.

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Conflict of interest

DG is a co-founder and serves as a Chief Scientific Officer and consultant for viDA Therapeutics, Inc. However, no viDA products or reagents were used in this study.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

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Supplementary material

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References

1. Griffiths CEM, van der Walt JM, Ashcroft DM, Flohr C, Naldi L, Nijsten T, et al. The global state of psoriasis disease epidemiology: a workshop report. *Br J Dermatol.* (2017) 177:e4–7. doi: 10.1111/bjd.15610

2. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med.* (2009) 361(5):496–509. doi: 10.1056/NEJMra0804595

3. MacDonald A, Burden AD. Psoriasis: advances in pathophysiology and management. *Postgrad Med J.* (2007) 83:690–7. doi: 10.1136/pgmj.2007.061473

4. Sarkar R, Chugh S, Bansal S. General measures and quality of life issues in psoriasis. *Indian Dermatol Online J.* (2016) 7:481–8. doi: 10.4103/2229-5178.193908

5. Al-Janabi A, Yiu ZZN. Biologics in psoriasis: updated perspectives on long-term safety and risk management. *Psoriasis Targets Ther.* (2022) 12:1–14. doi: 10.2147/PTT.S328575

6. Kamata M, Tada Y. Efficacy and safety of biologics for psoriasis and psoriatic arthritis and their impact on comorbidities: A literature review. *Int J Mol Sci.* (2020) 21:1690. doi: 10.3390/ijms21051690

7. Strober BE, van der Walt JM, Armstrong AW, Bourcier M, Carvalho AVE, Chouela E, et al. Clinical goals and barriers to effective psoriasis care. *Dermatol Ther.* (2018) 9:5–18. doi: 10.1007/s13555-018-0279-5

8. World Health Organization. *Global Report on Psoriasis*. Geneva, Switzerland: World Health Organization (2016). Available at: https://apps.who.int/iris/handle/10665/204417.

9. Zhou X, Chen Y, Cui L, Shi Y, Guo C. Advances in the pathogenesis of psoriasis: from keratinocyte perspective. *Cell Death Dis.* (2022) 13:1–13. doi: 10.1038/s41419-022-04523-3

10. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci.* (2019) 20:1475. doi: 10.3390/ijms20061475

11. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. *Lancet Lond Engl.* (2021) 397:1301–15. doi: 10.1016/S0140-6736(20)32549-6

12. Calautti E, Avalle L, Poli V. Psoriasis: A STAT3-centric view. *Int J Mol Sci.* (2018) 19:171. doi: 10.3390/ijms19010171

13. Fotiadou C, Lazaridou E, Sotiriou E, Ioannides D. Targeting IL-23 in psoriasis: current perspectives. *Psoriasis Auckl NZ.* (2018) 8:1–5. doi: 10.2147/PTT

14. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol.* (2009) 10:314–24. doi: 10.1038/ni.1698

15. Meglio PD, Nestle FO. The role of IL-23 in the immunopathogenesis of psoriasis. *F1000 Biol Rep.* (2010) 2:40. doi: 10.3410/B

16. Richardson KC, Jung K, Pardo J, Turner CT, Granville DJ. Non-cytotoxic roles of granzymes in health and disease. *Physiol Bethesda.* (2022) 37:323–48. doi: 10.1152/physiol.00011.2022

17. Bratke K, Klug A, Julius P, Kuepper M, Lommatzsch M, Sparmann G, et al. Granzyme K: a novel mediator in acute airway inflammation. *Thorax.* (2008) 63:1006–11. doi: 10.1136/thx.2007.091215

18. Wensink AC, Wiewel MA, Jongeneel LH, Boes M, van der Poll T, Hack CE, et al. Granzyme M and K release in human experimental endotoxemia. *Immunobiology.* (2016) 221:773–7. doi: 10.1016/j.imbio.2016.02.006

19. Rucevic M, Fast LD, Jay GD, Trespalcios FM, Sucov A, Siryaporn E, et al. Altered levels and molecular forms of granzyme K in plasma from septic patients. *Shock*. (2007) 27:488–93. doi: 10.1097/01.shk.0000246905.24895.e5

20. Bade B, Lohrmann J, Brinke At, Wolbink AM, Wolbink GJ, Berge IJMt, et al. Detection of soluble human granzyme K in vitro and in *vivo*. *Eur J Immunol*. (2005) 35:2940–8. doi: 10.1002/eji.200526249

21. Turner CT, Zeglinski MR, Richardson KC, Zhao H, Shen Y, Papp A, et al. Granzyme K expressed by classically activated macrophages contributes to inflammation and impaired remodeling. *J Invest Dermatol*. (2019) 139:930–9. doi: 10.1016/j.jid.2018.09.031

22. Turner CT, Zeglinski MR, Boivin W, Zhao H, Pawluk MA, Richardson KC, et al. Granzyme K contributes to endothelial microvascular damage and leakage during skin inflammation. *Br J Dermatol*. (2022) 189(3):279–91. doi: 10.1093/bjd/ljac017

23. Mogilenko DA, Shpynov O, Andhey PS, Arthur L, Swain A, Esaulova E, et al. Comprehensive profiling of an aging immune system reveals clonal GZMK+ CD8+ T cells as conserved hallmark of inflammaging. *Immunity*. (2021) 54:99–115.e12. doi: 10.1016/j.immuni.2020.11.005

24. Cooper DM, Pechkovsky DV, Hackett TL, Knight DA, Granville DJ. Granzyme K activates protease-activated receptor-1. *PloS One*. (2011) 6:e21484. doi: 10.1371/journal.pone.0021484

25. Joeckel LT, Wallich R, Martin P, Sanchez-Martinez D, Weber FC, Martin SF, et al. Mouse granzyme K has pro-inflammatory potential. *Cell Death Differ*. (2011) 18:1112–9. doi: 10.1038/cdd.2011.5

26. Sharma M, Merkulova Y, Raithatha S, Parkinson LG, Shen Y, Cooper D, et al. Extracellular granzyme K mediates endothelial activation through the cleavage of protease-activated receptor-1. *FEBS J*. (2016) 283:1734–47. doi: 10.1111/febs.13699

27. Kaiserman D, Zhao P, Rowe CL, Leong A, Barlow N, Joeckel LT, et al. Granzyme K initiates IL-6 and IL-8 release from epithelial cells by activating protease-activated receptor 2. *PloS One*. (2022) 17:e0270584. doi: 10.1371/journal.pone.0270584

28. Derian CK. Differential regulation of human keratinocyte growth and differentiation by a novel family of protease-activated receptors. *Cell Growth Differ*. (1997) 8:743–9.

29. Wang H, Ubl JJ, Stricker R, Reiser G. Thrombin (PAR-1)-induced proliferation in astrocytes via MAPK involves multiple signaling pathways. *Am J Physiol-Cell Physiol*. (2002) 283:C1351–64. doi: 10.1152/ajpcell.00001.2002

30. Sabri A, Short J, Guo J, Steinberg SF. Protease-activated receptor-1–mediated DNA synthesis in cardiac fibroblast is via epidermal growth factor receptor transactivation. *Circ Res*. (2002) 91:532–9. doi: 10.1161/01.RES.0000035242.96310.45

31. Kiseleva EV, Sidorova MV, Gorbacheva LR, Strukova SM. Peptide-agonist of protease-activated receptor (PAR1) stimulates keratinocyte proliferation and epithelial layer wound healing similarly to activated protein C. *Biochem Mosc Suppl Ser B BioMed Chem*. (2015) 9:199–204. doi: 10.1134/S1990750815020092

32. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res*. (2013) 41:D991–5. doi: 10.1093/nar/gks1193

33. Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet*. (2009) 41:199–204. doi: 10.1038/ng.311

34. Swindell WR, Johnston A, Carbajal S, Han G, Wohn C, Lu J, et al. Genome-wide expression profiling of five mouse models identifies similarities and differences with human psoriasis. *PloS One*. (2011) 6:e18266. doi: 10.1371/journal.pone.0018266

35. Ding J, Gudjonsson JE, Liang L, Stuart PE, Li Y, Chen W, et al. Gene expression in skin and lymphoblastoid cells: Refined statistical method reveals extensive overlap in cis-eQTL signals. *Am J Hum Genet*. (2010) 87:779–89. doi: 10.1016/j.ajhg.2010.10.024

36. Suárez-Fariñas M, Li K, Fuentes-Duculan J, Hayden K, Brodmerkel C, Krueger JG. Expanding the psoriasis disease profile: interrogation of the skin and serum of patients with moderate-to-severe psoriasis. *J Invest Dermatol*. (2012) 132:2552–64. doi: 10.1038/jid.2012.184

37. Correa da Rosa J, Kim J, Tian S, Tomalin LE, Krueger JG, Suárez-Fariñas M. Shrinking the psoriasis assessment gap: early gene-expression profiling accurately predicts response to long-term treatment. *J Invest Dermatol*. (2017) 137:305–12. doi: 10.1016/j.jid.2016.09.015

38. Zhu Q, Wong AK, Krishnan A, Aure MR, Tadych A, Zhang R, et al. Targeted exploration and analysis of large cross-platform human transcriptomic compendia. *Nat Methods*. (2015) 12:211–4. doi: 10.1038/nmeth.3249

39. Fits Lvd, Mourits S, Voerman JSA, Kant M, Boon L, Laman JD, et al. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J Immunol*. (2009) 182:5836–45. doi: 10.4049/jimmunol.0802999

40. Kjær TN, Thorsen K, Jessen N, Stenderup K, Pedersen SB. Resveratrol ameliorates imiquimod-induced psoriasis-like skin inflammation in mice. *PloS One*. (2015) 10(5):e0126599. doi: 10.1371/journal.pone.0126599

41. Boehncke WH, Schön MP. Psoriasis. *Lancet*. (2015) 386:983–94. doi: 10.1016/S0140-6736(14)61909-7

42. Tato CM, Cua DJ. Reconciling id, ego, and superego within interleukin-23. *Immunol Rev*. (2008) 226:103–11. doi: 10.1111/j.1600-065X.2008.00715.x

43. Santulli RJ, Derian CK, Darrow AL, Tomko KA, Eckardt AJ, Seiberg M, et al. Evidence for the presence of a protease-activated receptor distinct from the thrombin receptor in human keratinocytes. *Proc Natl Acad Sci U S A*. (1995) 92:9151–5. doi: 10.1073/pnas.92.20.9151

44. Algermissen B, Sitzmann J, Nürnberg W, Laubscher JC, Henz BM, Bauer F. Distribution and potential biologic function of the thrombin receptor PAR-1 on human keratinocytes. *Arch Dermatol Res*. (2000) 292:488–95. doi: 10.1007/s004030000168

45. Sakurai K, Dainichi T, Garcet S, Tsuchiya S, Yamamoto Y, Kitoh A, et al. Cutaneous p38 mitogen-activated protein kinase activation triggers psoriatic dermatitis. *J Allergy Clin Immunol*. (2019) 144:1036–49. doi: 10.1016/j.jaci.2019.06.019

46. Huang X, Yu P, Liu M, Deng Y, Dong Y, Liu Q, et al. ERK inhibitor JSI287 alleviates imiquimod-induced mice skin lesions by ERK/IL-17 signaling pathway. *Int Immunopharmacol*. (2019) 66:236–41. doi: 10.1016/j.intimp.2018.11.031

47. Yang J, Sun L, Han J, Zheng W, Peng W. DUSP1/MKP-1 regulates proliferation and apoptosis in keratinocytes through the ERK/Elk-1/Egr-1 signaling pathway. *Life Sci*. (2019) 223:47–53. doi: 10.1016/j.lfs.2019.03.018

48. Billi AC, Ludwig JE, Fritz Y, Rozic R, Swindell WR, Tsoi LC, et al. KLK6 expression in skin induces PAR1-mediated psoriasiform dermatitis and inflammatory joint disease. *J Clin Invest*. (2020) 130:3151–7. doi: 10.1172/JCI133159

49. Zani MB, Sant'Ana AM, Tognato RC, Chagas JR, Puzer L. Human tissue kallikreins-related peptidases are targets for the treatment of skin desquamation diseases. *Front Med*. (2022) 8. doi: 10.3389/fmed.2021.777619

50. Li W, Danilenko DM, Bunting S, Ganesan R, Sa S, Ferrando R, et al. The serine protease marapsin is expressed in stratified squamous epithelia and is up-regulated in the hyperproliferative epidermis of psoriasis and regenerating wounds. *J Biol Chem*. (2009) 284:218–28. doi: 10.1074/jbc.M806267200

51. Sayed KS, Said ER, Mohammed FN, Algorhary M, Rashed LA. Down-regulation of tissue levels of serine protease inhibitor (vaspin) in psoriasis vulgaris patients: a possible mechanism of narrowband ultraviolet B radiation. *Arch Dermatol Res*. (2019) 311:637–41. doi: 10.1007/s00403-019-01947-4

52. Zheng H, Gu L, Zhao F, Zhang C, Wang Z, Zhou H, et al. SerpinB7 deficiency contributes to development of psoriasis via calcium-mediated keratinocyte differentiation dysfunction. *Cell Death Dis*. (2022) 13:1–13. doi: 10.1038/s41419-022-05045-8

53. Wiedow O, Wiese F, Christophers E. Lesional elastase activity in psoriasis. Diagnostic and prognostic significance. *Arch Dermatol Res*. (1995) 287:632–5. doi: 10.1007/BF00371734

54. Vergnolle N. Protease inhibition as new therapeutic strategy for GI diseases. *Gut*. (2016) 65:1215–24. doi: 10.1136/gutjnl-2015-309147

55. Friberg C, Björck K, Nilsson S, Inerot A, Wahlström J, Samuelsson L. Analysis of Chromosome 5q31–32 and Psoriasis: Confirmation of a Susceptibility Locus but no Association with SNPs within SLC22A4 and SLC22A5. *J Invest Dermatol*. (2006) 126:998–1002. doi: 10.1038/sj.jid.5700194

56. Li Y, Chang M, Schrodi SJ, Callis-Duffin KP, Matsunami N, Civello D, et al. The 5q31 variants associated with psoriasis and Crohn's disease are distinct. *Hum Mol Genet*. (2008) 17:2978–85. doi: 10.1093/hmg/ddn196

57. Tokuhiro S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, et al. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet*. (2003) 35:341–8. doi: 10.1038/ng1267

58. Palmer LJ, Daniels SE, Rye PJ, Gibson NA, Tay GK, Cookson WO, et al. Linkage of chromosome 5q and 11q gene markers to asthma-associated quantitative traits in Australian children. *Am J Respir Crit Care Med*. (1998) 158:1825–30. doi: 10.1164/ajrccm.158.6.9804037

59. Wu H, Ke X, Huang W, Shi W, Yao S, Duan YY, et al. Multitissue integrative analysis identifies susceptibility genes for atopic dermatitis. *J Invest Dermatol*. (2023) 143(4):602–11.e14. doi: 10.1016/j.jid.2022.09.006

60. Kamata M, Tada Y. Dendritic cells and macrophages in the pathogenesis of psoriasis. *Front Immunol*. (2022) 13:941071. doi: 10.3389/fimmu.2022.941071

61. Lin SH, Chuang HY, Ho JC, Lee CH, Hsiao CC. Treatment with TNF-α inhibitor rectifies M1 macrophage polarization from blood CD14+ monocytes in patients with psoriasis independent of STAT1 and IRF-1 activation. *J Dermatol Sci*. (2018) 91:276–84. doi: 10.1016/j.jdermsci.2018.05.009

62. FitzGerald O, Ogdie A, Chandran V, Coates LC, Kavanaugh A, Tillett W, et al. Psoriatic arthritis. *Nat Rev Dis Primer*. (2021) 7:59. doi: 10.1038/s41572-021-00293-y

63. Ashenagar MS, Sugihara K, Maeda A, Isogai R, Takahashi M, Aisu K, et al. The presence of tryptase-positive and bikunin-negative mast cells in psoriatic skin lesions. *Arch Dermatol Res*. (2007) 298:421–6. doi: 10.1007/s00403-006-0704-y

64. Chamcheu JC, Chaves-Rodriquez MI, Adhami VM, Siddiqui IA, Wood GS, Longley BJ, et al. Upregulation of PI3K/AKT/mTOR, FABP5 and PPARβ/δ in human psoriasis and imiquimod-induced murine psoriasiform dermatitis model. *Acta Derm Venereol*. (2016) 96:854–6. doi: 10.2340/00015555-2359

65. Grine L, Dejager L, Libert C, Vandenbroucke RE. Dual inhibition of TNFR1 and IFNAR1 in imiquimod-induced psoriasiform skin inflammation in mice. *J Immunol Baltim Md 1950*. (2015) 194:5094–102. doi: 10.4049/jimmunol.1403015

66. Ha HL, Wang H, Pisitkun P, Kim JC, Tassi I, Tang W, et al. IL-17 drives psoriatic inflammation via distinct, target cell-specific mechanisms. *Proc Natl Acad Sci*. (2014) 111:E3422–31. doi: 10.1073/pnas.1400513111

67. Vinter H, Kragballe K, Steiniche T, Gaestel M, Iversen L, Johansen C. Tumour necrosis factor-α plays a significant role in the Aldara-induced skin inflammation in mice. *Br J Dermatol*. (2016) 174:1011–21. doi: 10.1111/bjd.14320

68. Crowley JJ, Warren RB, Cather JC. Safety of selective IL-23p19 inhibitors for the treatment of psoriasis. *J Eur Acad Dermatol Venereol*. (2019) 33:1676–84. doi: 10.1111/jdv.15653

69. Kulig P, Musiol S, Freiberger SN, Schreiner B, Gyülveszi G, Russo G, et al. IL-12 protects from psoriasiform skin inflammation. *Nat Commun*. (2016) 7:13466. doi: 10.1038/ncomms13466

70. Blauvelt A, Chiricozzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. *Clin Rev Allergy Immunol*. (2018) 55:379–90. doi: 10.1007/s12016-018-8702-3

71. Gurczynski SJ, Moore BB. IL-17 in the lung: the good, the bad, and the ugly. *Am J Physiol - Lung Cell Mol Physiol*. (2018) 314:L6–16. doi: 10.1152/ajplung.00344.2017

72. Lee JS, Tato CM, Joyce-Shaikh B, Gulan F, Cayatte C, Chen Y, et al. IL-23-independent IL-17 production regulates intestinal epithelial permeability. *Immunity*. (2015) 43:727–38. doi: 10.1016/j.immuni.2015.09.003

73. Torres T. Selective interleukin-23 p19 inhibition: another game changer in psoriasis? Focus on risankizumab. *Drugs*. (2017) 77:1493–503. doi: 10.1007/s40265-017-0794-1

74. Whibley N, Gaffen SL. Gut-busters: IL-17 ain't afraid of no IL-23. *Immunity*. (2015) 43:620–2. doi: 10.1016/j.immuni.2015.10.001

75. Maxwell JR, Zhang Y, Brown WA, Smith CL, Byrne FR, Fiorino M, et al. Differential roles for interleukin-23 and interleukin-17 in intestinal immunoregulation. *Immunity*. (2015) 43:739–50. doi: 10.1016/j.immuni.2015.08.019

76. Johansen C, Kragballe K, Westergaard M, Henningsen J, Kristiansen K, Iversen L. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br J Dermatol*. (2005) 152:37–42. doi: 10.1111/bjd.2005.152.issue-1

77. Andrés RM, Hald A, Johansen C, Kragballe K, Iversen L. Studies of Jak/STAT3 expression and signalling in psoriasis identifies STAT3-Ser727 phosphorylation as a modulator of transcriptional activity. *Exp Dermatol*. (2013) 22:323–8. doi: 10.1111/exd.12128

78. Wilharm E, Tschopp J, Jenne DE. Biological activities of granzyme K are conserved in the mouse and account for residual Z-Lys-SBzl activity in granzyme A-deficient mice. *FEBS Lett*. (1999) 459:139–42. doi: 10.1016/S0014-5793(99)01200-4

79. Itoh K, Kawasaki S, Kawamoto S, Seishima M, Chiba H, Michibata H, et al. Identification of differentially expressed genes in psoriasis using expression profiling approaches. *Exp Dermatol*. (2005) 14:667–74. doi: 10.1111/j.0906-6705.2005.00338.x

80. Yasir M, Goyal A, Sonthalia S. *Corticosteroid Adverse Effects*. Treasure Island (FL: StatPearls Publishing (2022). Available at: http://www.ncbi.nlm.nih.gov/books/NBK531462/.

81. Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, et al. QuPath: Open source software for digital pathology image analysis. *Sci Rep*. (2017) 7:16878. doi: 10.1038/s41598-017-17204-5

82. Genin M, Clement F, Fattaccioli A, Raes M, Michiels C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. *BMC Cancer*. (2015) 15:577. doi: 10.1186/s12885-015-1546-9

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Therapeutic strategies focusing on immune dysregulation and neuroinflammation in rosacea

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Rosacea is a complex inflammatory condition characterized by papulopustular lesions and erythema on the central face for which there is no cure. The development of rosacea is influenced by both external triggers and genetics, but the common pathophysiology is overactivation of the immune system. Here, we review the current data on proinflammatory cytokines and dysregulation of the neurovascular system as targetable components of rosacea. Amelioration of cutaneous and gastrointestinal dysbiosis and other external factors impacts the immune state and has been observed to improve rosacea. While multiple treatments exist, many patients do not achieve their goals for rosacea control and highlights an unmet need for dermatologic care. Current interventions encompass topical/oral drugs, light devices, and avoidance of triggers management. Additional understanding of the underlying pathogenesis may help us develop novel targeted therapeutic strategies to improve rosacea.

KEYWORDS

rosacea, immune dysregulation, neuroinflammation, microbiota, therapeutics

1 Introduction

Rosacea is a chronic, relapsing, inflammatory skin disease characterized by symptoms such as burning, stinging, erythema, and papulopustular lesions on the central face (1). To date, rosacea is considered a multifactorial disease, but our current understand has not led to a cure, nor sufficient control of the disease in many cases. Both immune dysregulation and neuroinflammation have been implicated in the development of rosacea. Here, we review the current literature on these components (2–4) which may lead to novel targets and pathways for future clinical trials.

For instance, rosacea can worsen due to various external trigger factors of the immune system, such as ultraviolet (UV) radiation and *Demodex* mites (5, 6). Neuroinflammation is

also significant in rosacea, triggered by factors like alcohol, spicy foods, and temperature changes, with activation of calcium channels and vasodilation manifest as skin redness, a common symptom of the condition (3).

2 Pathophysiology

The precise pathophysiology of rosacea continues to be investigated. So far, rosacea is known to be a multifactorial, chronic, inflammatory skin disease (7, 8). The contributing pathogenic mechanisms of rosacea can be categorized into four major categories which can interact with each other: external triggers (e.g., exposure to UV radiation and *Demodex* mites), genetic predisposition, immune dysregulation, and neurovascular dysregulation (2) (Figure 1).

Rosacea may be exacerbated by external factors such as ultraviolet (UV) radiation, alcohol, spicy food, extreme temperatures, and *Demodex* mites and other microbes (1). Exposure to UV radiation and *Demodex* mites activates Toll-like receptor 2 (TLR2), leading to the activation and subsequent release of cathelicidin—an antimicrobial peptide integral to the pathogenesis of rosacea (5, 6). Kallikrein 5 (KLK5) converts cathelicidin into its active form, LL-37, which has been implicated in inflammation, angiogenesis, and telangiectasis in rosacea (9).

Neuroinflammation is a major component of rosacea (4), though it is unclear if the initial trigger is immunologic, leading to stimulation of nerves that mediate itch and burning or vice versa. Some data exists that the nerves can be activated first by common triggers leading to blood vessel dilation. For instance, triggers such as alcohol and spicy food consumption and temperature changes can activate calcium channels, specifically transient receptor potential vanilloid (TRPV)1/transient receptor potential ankyrin (TRPA)1, thereby inducing the release of neurotransmitters and the dilatation of microvessels (3). These changes manifest as skin erythema, a characteristic clinical sign of rosacea.

The following sections shed more light on these mechanisms of inflammation and the components of the immune system involved in rosacea (Figure 1).

2.1 External triggers of inflammation and the immune system

Exposure to UV radiation and external pathogens activates TLR2 (5, 10), leading to the subsequent activation and release of KLK5 (11). In addition, inflammatory cells such as neutrophils and macrophages release matrix metalloproteinases (MMPs), which convert pro-KLK5 into KLK5 (12). KLK5 is a serine protease that cleaves cathelicidin, an antimicrobial peptide secreted by epidermal keratinocytes, into the human antimicrobial peptide LL-37, an immune-stimulating factor (9). This process, referred to as the cathelicidin activation pathway, plays a crucial role in the inflammatory response in rosacea (9). Given that LL-37 plays a pivotal role in rosacea-related inflammation, this pathway is frequently referenced in the subsequent discussion on immune dysregulation.

Regarding the triggers of rosacea, researchers have extensively focused on the cutaneous microbiome (13). Rosacea may be associated with certain skin microorganisms such as *Demodex folliculorum*, *Staphylococcus epidermidis*, *Bacillus oleronius* (a

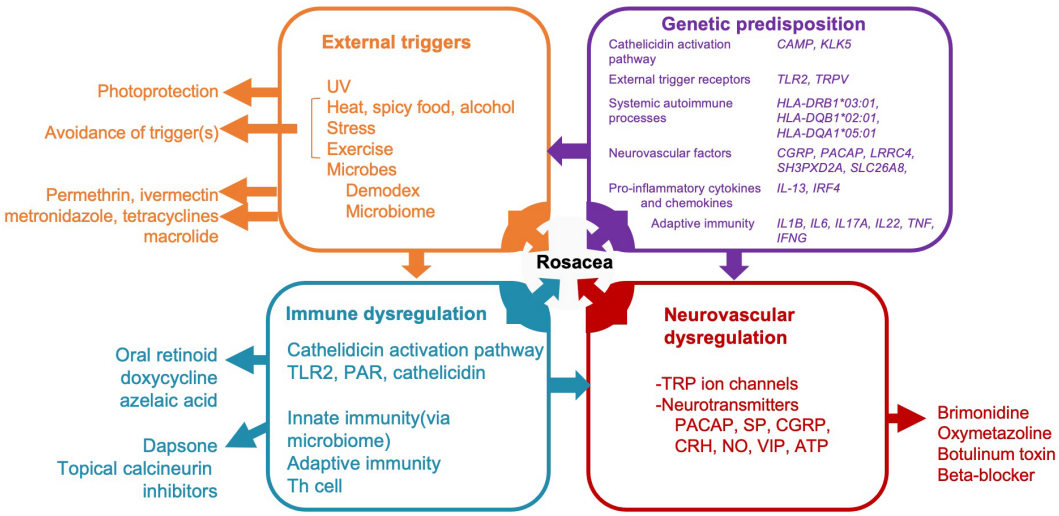

FIGURE 1
A diagram illustrating potential pathogenic mechanisms of rosacea categorized into four major categories and interaction with immune system. Rosacea, a multifactorial condition, involves external triggers (orange box), genetic predisposition (purple box), immune dysregulation (blue box), and neurovascular dysregulation (red box). Treatment modalities correspond to these mechanisms, as shown outside of the boxes in the diagram. Only drugs aligned with Swiss S1 and National Rosacea Society guidelines are indicated. UV, ultraviolet; CAMP, cathelicidin antimicrobial peptide; KLK-5, Kallikrein 5; TLR2, toll-like receptor 2; TRP-, transient receptor potential channel; CGRP, calcitonin gene related peptide; PACAP, pituitary adenylate cyclase-activating peptide; IL-, interleukin; IRF4, interferon regulatory factor-4; TNF, tumor necrosis factor; IFNG, Interferon gamma; PAR, protease-activated receptor; SP, substance P; CRH, corticotropin-releasing hormone; NO, Nitric oxide; VIP, vasoactive intestinal polypeptide; ATP, adenosine triphosphate.

bacterium carried by *Demodex* mites), *Bartonella quintana*, and *Chlamydia pneumoniae* (8). However, studies examining the relationship between these microorganisms and rosacea have reported inconsistent results. Among these microorganisms, *Demodex* has garnered the most attention and has the largest evidence for its role in rosacea. *Demodex*, with its chitin exoskeleton, can increase TLR2 levels and induce a proinflammatory response (5). An *in vitro* study revealed that *Demodex* antigens can activate the human inflammasome (5). Furthermore, *Demodex*-associated microorganisms, such as *B. oleronius*, may induce the differentiation of immune cells and promote the secretion of various substances, including cathelicidin, MMP-9, tumor necrosis factor (TNF), and interleukin (IL)-8 (14). The current literature strongly supports the role of *Demodex* as a trigger of rosacea.

2.2 Genetic predisposition including immune associated genes

Recent epidemiological studies, family and twin studies, genetic association studies, and *in vitro* analyses have increasingly suggested the involvement of a genetic component in the development of rosacea (6, 15–20). By including both identical and fraternal twins, a twin study differentiated genetic susceptibility from the role of environmental factors in rosacea development; the results suggested that genetics contribute to 46% of individuals' predisposition to rosacea (19).

The genes mentioned in the literature can be broadly classified into the following major categories:

1. Cathelicidin activation pathway: Overexpression of genes such as cathelicidin antimicrobial peptides (6), *KLK5*, and *LL-37* (21) has been validated in molecular and functional studies using human keratinocytes and skin explants of rosacea compared to healthy skin controls.
2. External triggers: RNA sequencing revealed alteration of gene expression levels such as *TLR2* and *TRPV* in samples from rosacea patients compared to non-lesional skins or healthy skin controls (22–25).
3. Systemic autoimmune processes: A genome wide association study (GWAS) of Caucasian rosacea patients without any autoimmune disease found single nucleotide polymorphism (SNP) in genes such as *HLA-DRB1*03:01*, *HLA-DQB1*02:01*, and *HLA-DQA1*05:01*, which are associated with MHC class II and antigen presentation (26).
4. Neurovascular factors: The roles of neurotransmitters such as calcitonin gene-related peptide (*CGRP*) and pituitary adenylate cyclase-activating peptide (*PACAP*) in rosacea were supported by a case-control observational study and a retrospective study (27–29). Moreover, a whole-genome sequencing in 3 large rosacea families and whole exome sequencing in 49 additional validation families from Han population which revealed rare, single deleterious variants of *LRRC4*, *SH3PXD2A*, and *SLC26A8*, which are genes for neural synaptic processes and cell adhesion, in large families with rosacea. Subsequent *in vitro* and mouse studies have revealed that these genes induced the production of vasoactive neuropeptides, thereby leading to rosacea-like skin inflammation (4).
5. Proinflammatory cytokines and chemokines: Another GWAS study of Caucasian rosacea patients found SNP in genes such as interleukin-13 (*IL-13*) and interferon regulatory factor-4 (*IRF4*) (30).
6. Altered adaptive immunity: Genes related to the Th1/Th17 pathway, specifically IL1B, IL6, IL17A, and IL22 are upregulated in rosacea patients compared to healthy skin controls (23, 24, 31).

2.3 Immune dysregulation

Data on immune dysregulation in rosacea primarily involves disturbances in the innate immune system (Figure 2). Rosacea-affected skin tissues exhibit substantially higher concentrations of antimicrobial peptides, particularly cathelicidin and LL-37, than do normal skin tissues (2, 9). In addition, rosacea upregulates the expression level of TLR2, thereby increasing the secretion of KLK5 from keratinocytes. This promotes the cathelicidin activation pathway, resulting in increased LL-37 production (2, 5). These molecules play crucial roles in regulating the innate immune system in patients with rosacea. Innate immunity–related inflammatory cells such as mast cells play important roles in rosacea. While there is limited research specifically on antihistamines or mast cell stabilizers in rosacea, some studies suggest they may have a beneficial effect in reducing inflammation and symptoms (6). The proportion of mast cells is markedly increased in affected tissues. LL-37 can induce mast cells to secrete cathelicidin, which, in turn, increases the level of LL-37 (32). LL-37 can also activate neutrophils and macrophages, leading to the release of various cytokines and MMPs (33). MMPs can convert pro-KLK5 into KLK5, further increasing the level of LL-37 in affected tissues (12). These findings indicate that the innate immune system of patients with rosacea is trapped in a cycle of activation and inflammation under the effects of various triggers.

Vitamin D plays a major role in both the innate and adaptive immune systems. In particular, this vitamin regulates the expression of cathelicidin in keratinocytes (34). Increased vitamin D levels and altered *VDR* single-nucleotide polymorphisms are strongly associated with rosacea (35). It is not known whether vitamin D supplementation or toxicity leads to facial redness.

Rosacea is a chronic inflammatory skin condition. The role of the antioxidative system in the regulation of oxidative stress (e.g., during inflammation) has been implicated in the pathogenesis of rosacea (36). A study revealed marked differences between patients with rosacea and individuals without this condition in the expression levels of glutathione S-transferase polymorphisms, indicating that these polymorphisms are associated with an increased risk of rosacea (37).

In addition to the innate immune system, the adaptive immune system is affected by rosacea. Abundant Th1/Th17 cells have been

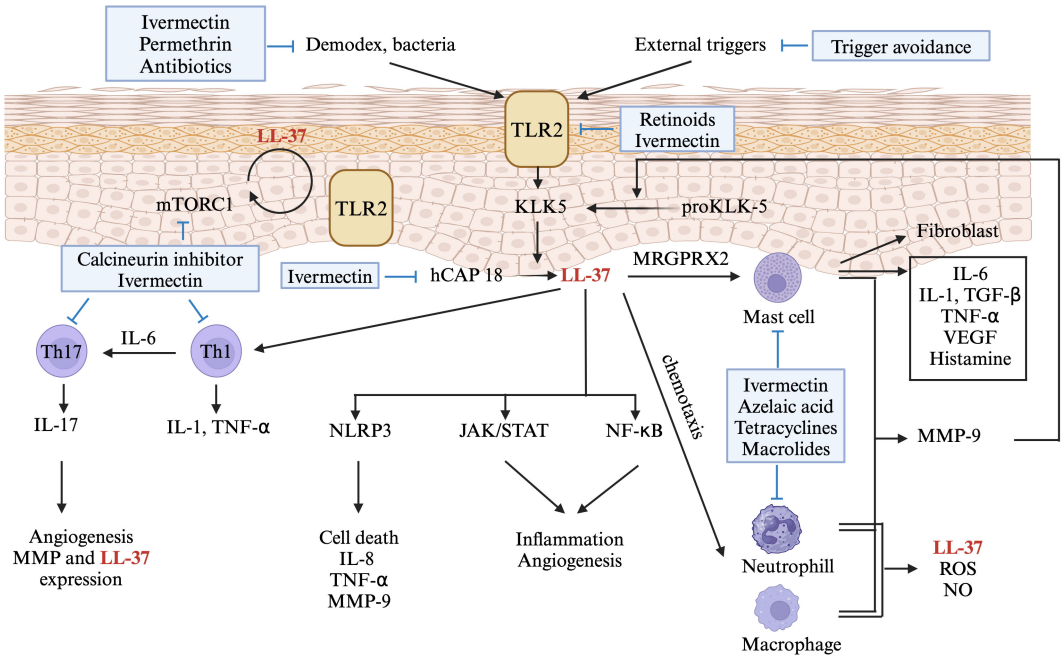

FIGURE 2
A diagram showing the role of immune dysregulation in the pathogenesis of rosacea. Environmental factors activate toll-like receptor (TLR)2 on keratinocytes, leading to kallikrein (KLK) 5 expression in rosacea. KLK5 cleaves human cathelicidin (hCAP), producing LL-37 (also known as hCAP18), triggering pathways like NLR family pyrin domain containing (NLRP)3 inflammasome, Janus protein tyrosine kinase/Signal Transducers and Activators of Transcription (JAK/STAT), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB). LL-37 stimulates mast cells via Mas-related G-protein coupled receptor member (MRGPR) X2, inducing the production of inflammatory cytokines and matrix metalloproteinase (MMP)9. MMP-9 further enhances KLK5 production or releases LL-37, creating a positive feedback loop. LL-37 also stimulates Th-1 and Th-17 cells, with IL-17 contributing to angiogenesis. Production of hCAP is regulated by LL-37-induced mammalian target of rapamycin (mTORC) 1 signaling, again creating a positive feedback loop. Immune-targeted therapies for rosacea are shown in blue boxes. Only drugs aligned with Swiss S1 and National Rosacea Society guidelines are indicated. This figure was created with BioRender.com and obtained authorization on February 11, 2024. TLR2, toll-like receptor 2; KLK-5, Kallikrein 5; hCAP, human cathelicidin; mTOR, mammalian target of rapamycin; IL-, interleukin; TGF-β, Transforming growth factor beta; TNF-α, tumor necrosis factor; VEGF, vascular endothelial growth factor; MRGPRX2, Mas-related G-protein coupled receptor member X2; Th-, T helper cell; NLRP3, NLR family pyrin domain containing 3; JAK/STAT, Janus protein tyrosine kinase/Signal Transducers and Activators of Transcription; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; MMP, matrix metalloproteinase; ROS, reactive oxygen species; NO, Nitric oxide.

observed in affected tissues (31). Th17 cells secrete interleukin (IL)-17, which induces angiogenesis through vascular endothelial growth factor and affects the expression of LL-37 in keratinocytes (38).

2.4 Neurovascular dysregulation and inflammation

Transient receptor potential (TRP) channels, including TRPV1, TRPV4, and TRPA1, play key roles in neurovascular dysregulation (Figure 3). These TRPs are expressed not only in nerve endings but also in the endothelium and keratinocytes (27). External factors such as cold, heat, alcohol, spicy foods, and chemicals can theoretically activate these TRPs, leading to the release of vasoactive neurotransmitters, such as PACAP, CGRP, vasoactive intestinal peptide, and substance P (39). These neurotransmitters result in vasodilation and stimulate downstream inflammatory cells such as mast cells, macrophages, and neutrophils, thereby inducing inflammatory response (3, 27). Neuropeptides released from sensory neurons affect vascular endothelial cells, causing vasodilation and increasing capillary permeability (27, 40). In addition, these neuropeptides activate cutaneous mast cells through MRGPRX2, leading to the degranulation of these cells and the production of chemokines and cytokines (27, 40), thereby exacerbating rosacea.

Several studies suggest that adenosine 5'-triphosphate (ATP), a purinergic nucleotide and neurotransmitter, also participates in rosacea pathogenesis by regulating vascular tone and immune responses (41). In human microvascular endothelial cells (HMEC-1), an ATP analogue enhances the secretion of pro-inflammatory cytokines such as IL-6 and IL-8, promotes the release of chemokines like CCL-2, CCL-5, CCL-21, and CXCL1, and increases the expression of adhesion molecules (41). These actions indicate that ATP may exacerbate inflammation and vasodilation, which are key components in rosacea.

Rosacea patients exhibit heightened responses to heat or stress, leading to increased sweating and cutaneous vasodilation (42). This involves sympathetic nerve excitation, affecting vascular smooth muscle contraction in superficial peripheral vessels through multiple alpha-receptors (43). Additionally, rosacea may feature dilated blood and lymphatic vessels, contributing to erythema and edema (2). Patients with neurogenic rosacea, which resembles small fiber neuropathy, often present with gastrointestinal symptoms and dysautonomia (44). Both conditions share similar manifestations,

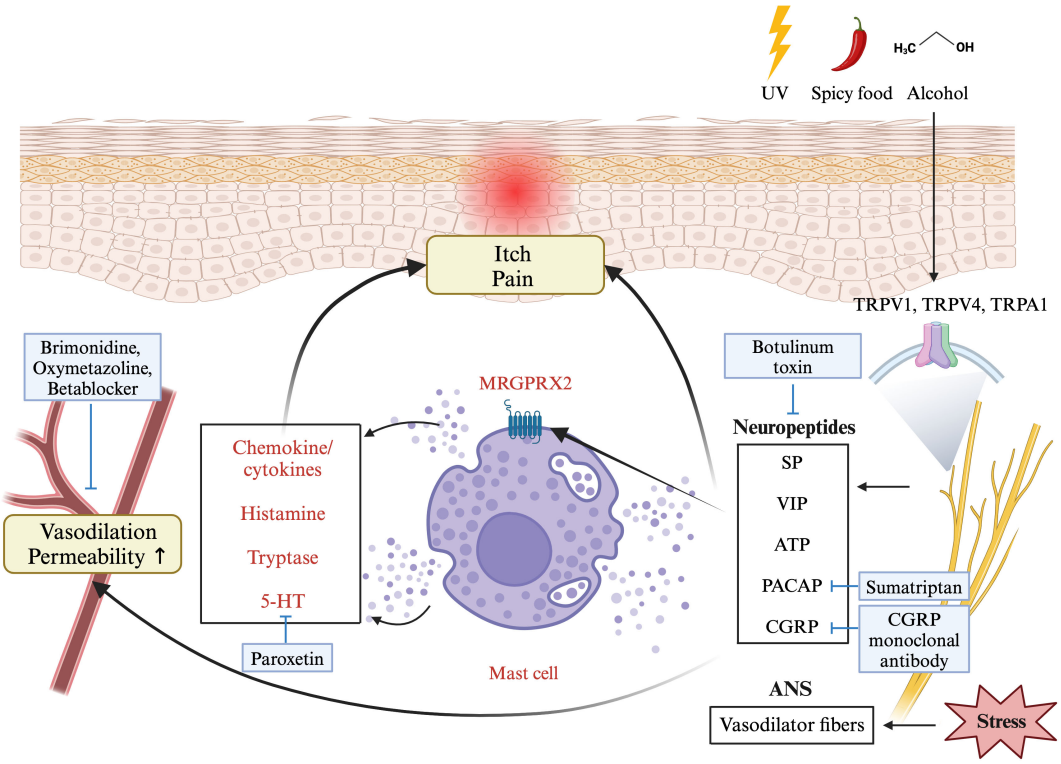

FIGURE 3
A diagram showing the pathophysiology of rosacea involving neurovascular dysregulation. External factors can activate sensory neurons via transient receptor potential cation channel (TRPV) receptors, leading to neuropeptide release. This activates mast cells through Mas-related G-protein coupled receptor member (MRGPR) X2, causing degranulation of histamine, serotonin, and chemokines, resulting in vasodilation, pain, and itch. Stress causes the autonomic nervous system to overstimulate vasodilator fibers, affecting the layer of smooth muscle enveloping the blood vessels, ultimately resulting in vasodilation. The figure includes medications recommended by Swiss S1 and National Rosacea Society guidelines (blue boxes) associated with this mechanism. This figure was created with BioRender.com and obtained authorization on July 03, 2024. UV, ultraviolet; TRP-, transient receptor potential channel; SP, substance P; VIP, vasoactive intestinal polypeptide; ATP, adenosine triphosphate; PACAP, pituitary adenylate cyclase-activating peptide; CGRP, calcitonin gene related peptide; ANS, autonomic nervous system.

complications, and treatments, indicating common autonomic nervous system dysregulation (44).

The aforementioned hypothesis remains to be confirmed through studies on the associations of these vasoactive neurotransmitters with rosacea. An *in vitro* study revealed that mast cells require prior upregulation of TRPV4 for effective LL-37-mediated stimulation and subsequent complete degranulation (45). This indicates a possible crosstalk between neurovascular dysregulation and immune dysregulation—two major pathogenic mechanisms underlying rosacea (32).

3 Current therapeutic strategies and clinical trials

Currently, treatment options for rosacea include topical and oral drugs, light devices, and appropriate skincare and lifestyle management (46).

This section provides an overview of various treatment strategies for rosacea particularly focused on modulating the immune system either directly or indirectly. To highlight key therapeutic targets, the strategies were categorized on the basis of their mechanisms of action. Although a single medication may affect multiple mechanisms, we focused on the primary target of each strategy. The following sections present commonly recommended treatment options with an overview of emerging treatment approaches based on The Oxford 2011 Levels of Evidence (47) (Supplementary Tables 1-4). Medications that have been approved by the US Food and Drug Administration (FDA) are marked with an asterisk (*).

Laser, light therapies, and surgery are essential for treating rosacea-associated telangiectasia resistant to standard therapies. Treatments such as potassium titanyl phosphate (KTP) laser, pulsed dye laser (PDL), neodymium-doped yttrium aluminum garnet (Nd : YAG) laser, and intense pulsed light (IPL) therapies address telangiectasis, erythema, and flushing (48). However, their impact on immune dysregulation and neuroinflammation lacks evidence and is beyond this article's scope.

3.1 Immune-targeted therapy

Immune dysregulation in rosacea involves innate immune disturbances, including TLR2 activation, KLK5 release, and

cathelicidin cleavage into LL-37 (9). These processes, triggered by UV radiation and pathogens, are crucial in rosacea inflammation (5, 6). Adaptive immunity, especially Th1/Th17 cells, is also affected in rosacea (31), indicating a complex immune response. Supplementary Table 2 lists selected therapeutics targeting immune dysregulation for rosacea. FDA-approved anti-inflammatory agents for rosacea include oral tetracyclines (49), topical minocycline (50), and azelaic acid (51, 52). Off-label, oral isotretinoin (53, 54) and topical calcineurin inhibitors (55, 56) are widely used. Studies have explored other potential anti-inflammatory agents, such as secukinumab, targeting IL-17, due to data indicating Th17 cells' role in rosacea.

3.1.1 Oral tetracyclines as anti-inflammatory agents

Oral doxycycline (40 mg/day; 30 mg immediate release and 10 mg delayed release) is a well-established, FDA-approved treatment option for rosacea (57). Its benefits have been validated by a phase III randomized controlled trial (49). Submicrobial doses of doxycycline can effectively alleviate inflammation by suppressing the production of proinflammatory cytokines, reactive oxygen species, nitric oxide synthetase, and MMPs, while also avoiding antibiotic resistance or dysbiosis (58, 59). Furthermore, doxycycline can inhibit the cathelicidin activation pathway and immune cell recruitment (59). These anti-inflammatory effects of doxycycline have led to its recommendation by treatment guidelines (46, 60). While tetracyclines are commonly used in rosacea and acne for their anti-inflammatory effects, their antimicrobial properties likely alter the composition of the skin microbiome. A systematic review found that these treatments mostly decrease *Cutibacterium acnes* and increase the alpha diversity of the cutaneous microbiota (61).

Other tetracyclines also demonstrate significant anti-inflammatory effects, including the inhibition of chemotaxis, granuloma formation, and proteases (62). Therefore, various tetracycline drugs, formulations, and dosages are commonly used off-label in rosacea treatment (62). These alternatives include tetracycline (250–1000 mg/day), doxycycline (100–200 mg/day), and minocycline (100–200 mg/day). These agents are effective against PPR (59, 63, 64).

Other forms of oral tetracyclines have recently shown efficacy in treating rosacea. DFD-29, a minocycline extended-release oral capsule (40 mg), exhibited a significantly higher level of efficacy than did placebo and doxycycline (40 mg) in the treatment of PPR with the plasma level of drug was maintained below its antimicrobial threshold (65).

A new generation tetracycline, sarecycline, has proven effective and safe in the treatment of PPR. Compared with control, sarecycline significantly improved patients' IGA scores, reduced inflammatory lesion counts, and alleviated rosacea's secondary symptoms such as burning sensation, without causing any adverse effects (66). While not extensively examined in humans, sarecycline has demonstrated anti-inflammatory activity comparable to doxycycline and minocycline in a rat paw edema model (67).

Notably, the mechanism of action of tetracyclines in rosacea primarily involves modulating immune dysregulation, not neurovascular dysregulation. Therefore, when using tetracyclines in patients with ETR, significant improvement is generally not expected (46). Tetracycline may inhibit the proinflammatory process induced by the ATP analogue in vascular endothelial cells *in vitro* (41, 68). While there may be a slight effect on persistent erythema, the level of evidence supporting this is not strong (46).

3.1.2 Topical azelaic acid

Azelaic acid gel (15%) is a well-established, FDA-approved topical agent. According to standard guidelines and multiple randomized controlled, double-blinded multicenter trials, azelaic acid is beneficial against mild to moderate inflammatory papules or pustules (46, 57, 60, 69, 70). However, it exhibits a low level of efficacy against erythema, primarily in patients with the papulopustular phenotype (51, 52). Azelaic acid works by suppressing KLK5 and cathelicidin expression and activating peroxisome proliferator-activated receptor γ, thus reducing the degrees of inflammatory responses and the levels of proinflammatory factors such as IL-1, IL-6, and TNF-α (71, 72). Additionally, this medication can suppress the UV-induced activation of nuclear factor (NF)-κB p65 subunit (73).

3.1.3 Topical tetracyclines

Minocycline foam 1.5% (FMX103), which was approved by the US FDA in 2020, was found to be effective and safe against moderate to severe papulopustular rosacea (PPR) in two 12-week, randomized, double-blinded, vehicle-control phase III trials (50). Another topical formulation is minocycline gel, whose 1% and 3% concentrations were tested in a 12-week, randomized, double-blinded, vehicle-controlled phase IIb trial; the results revealed that both concentrations, particularly the 3% concentration, significantly reduced inflammatory lesion counts and improved the Investigator's Global Assessment (IGA) scores (74). As mentioned above, oral minocycline act as anti-inflammatory agents. While topical minocycline shows promise in delivering anti-inflammatory effects with reduced risk of systemic adverse effects, further evaluation is needed.

3.1.4 Oral isotretinoin

Isotretinoin is an off-label treatment option for rosacea. It is indicated for moderate to severe PPR (46, 57, 60). Isotretinoin is typically administered at a low daily dose (0.25–0.3 mg/kg) over a 4-month period; this is followed by a gradual dose reduction (54). Mechanistically, isotretinoin works by downregulating TLR2 expression in keratinocytes (53, 54). By inhibiting the activity of the sebaceous gland and reducing the production of sebum, isotretinoin can delay the progression of inflamed phyma when used during the prefibrotic phase of PPR (54, 75). Although the anti-inflammatory effect of isotretinoin might theoretically benefit erythema by inhibiting neuro- inflammation, its efficacy in treating erythema in rosacea patients shows inconsistent results (76). Some studies indicate that low-dose (0.25 mg/kg/day) isotretinoin is not

effective in treating erythema and telangiectasia (77), while others suggest that an intermediate dose (20 mg/day) effectively improves erythema within four weeks (78).

3.1.5 Oral macrolides

Macrolide drugs, such as erythromycin, clarithromycin, and azithromycin, are secondary agents used in the systemic treatment of rosacea. For azithromycin, the dosage is 500 mg three times weekly for 4 weeks, followed by 250 mg three times weekly for additional weeks. For clarithromycin, the dosage is 250 mg twice daily for 4 weeks, followed by 250 mg once daily for the next 4 weeks. They are particularly useful for patients with contraindications to tetracycline, such as those who are refractory or pregnant (57, 79). The therapeutic effects of macrolides extend beyond their antimicrobial properties to include immunomodulatory effects. For example, azithromycin can modulate transcription factors such as NF-κB, reduce the release of inflammatory cytokines, impede the migration of neutrophils, inhibit the activation of neutrophils and eosinophils, and suppress the release of reactive oxygen species (57, 80). However, despite the low level of evidence for the efficacy of azithromycin in treating rosacea, its gastrointestinal adverse effects are increasingly becoming a concern (81, 82).

3.1.6 Hydroxychloroquine

Hydroxychloroquine effectively suppresses the activation of mast cells by inhibiting Ca^{2+}-activated K+ channels, leading to a reduction in local Ca^{2+} influx (83). This suppression results in the inhibition of inflammatory factor release, chemotaxis, and degranulation, ultimately reducing chemokine production and neutrophil and monocyte recruitment (83).

Patients who received hydroxychloroquine for 8 weeks exhibited improvements in their rosacea phenotypes along with improved IGA and Clinician Erythema Assessment (CEA) scores, indicating a tendency toward symptomatic relief (83). In a separate multicenter, randomized, double-blind, double-placebo pilot study, participants received either oral hydroxychloroquine (200 mg twice daily) or doxycycline (100 mg once daily) along with their respective placebos for 8 weeks; in this study, hydroxychloroquine was found to be noninferior to doxycycline in terms of rosacea-specific quality-of-life scores (84).

3.1.7 Secukinumab

IL-17, a proinflammatory cytokine produced by Th17 cells, has been implicated in rosacea-related inflammation (31, 85). Th17 activation contributes to the upregulation of LL-37 expression, which, in turn, promotes the production of cathelicidin (85). The synergistic actions of LL-37 and IL-17 result in the release of C-X-C motif chemokine ligand 8 and IL-6, facilitating neutrophil chemotaxis and Th17 differentiation, respectively (85). This Th1/Th17 polarized inflammation, often underrecognized, is common across all subtypes of rosacea (31). In an exploratory study having an open-label, rater-blinded design, secukinumab, an IL-17A inhibitor, significantly reduced papule counts and improved global severity and median RosaQOL scores after 16 weeks of systemic therapy (86). However, more comprehensive and definitive evidence from high-quality randomized controlled trials is needed to fully understand the efficacy of secukinumab in the management of rosacea.

3.1.8 Janus kinase inhibitors

The JAK-STAT signaling pathway plays a pivotal role in the proinflammatory processes within immune cells. In the pursuit of effective rosacea treatments, oral JAK inhibitors like tofacitinib and abrocitinib have been explored (87, 88). A compelling case series involving 21 patients with erythematotelangiectatic and papulopustular rosacea demonstrated promising results, with 71.4% experiencing significant regression of facial erythema (IGA ≤ 1) and a mean change of -2.24 in the IGA score (87). Additionally, a separate case series highlighted the efficacy of abrocitinib in improving steroid-induced rosacea (88). These findings underscore the substantial potential of JAK inhibitors for broader utilization in the management of rosacea.

3.1.9 Topical calcineurin inhibitors

Pimecrolimus cream proves to be an effective treatment option for those with mild to moderate inflammatory rosacea (55). Pimecrolimus exhibits anti-inflammatory activity against PPR by inhibiting the activation of T cells, suppressing the production of inflammatory cytokines, and preventing the release of cytokines from T and mast cells through the inhibition of calcineurin phosphatase (89). The calcineurin inhibitor tacrolimus can treat erythema on rosacea patients (90). However, a higher level of evidence is available for 1% pimecrolimus than for tacrolimus (55, 56, 60). Prescribing these drugs should be done cautiously due to the potential risk of causing rosacea-like eruptions (91). The potential adverse events may be attributable to their immunosuppressive effects, which results in the overgrowth of microorganisms such as *D. folliculorum* (91). Therefore, pimecrolimus and tacrolimus may be regarded as dual-edged swords in the treatment of rosacea (91).

3.1.10 Mammalian target of rapamycin inhibitor

Mammalian target of rapamycin complex 1 (mTORC1) can regulate cathelicidin through a positive feedback loop (92). In this loop, LL-37 activates mTORC1 signaling by binding to TLR2, thereby upregulating the expression of cathelicidin in keratinocytes (92). Excess LL-37 may induce NF-κB activation through mTORC1 signaling, leading to the production of disease-specific cytokines and chemokines (92). Overexpression of TLR7 in keratinocytes stimulates the mTORC1 pathway through NF-κB signaling (93). In a pilot study involving 18 women with rosacea, the patients were randomized to receive either a placebo (n = 8) or 0.4% topical rapamycin (sirolimus) ointment (n = 10) for 4 weeks (92). After the intervention, the level of clinical improvement was significantly higher in the rapamycin group than in the placebo group. Topical treatment with rapamycin significantly reduced the patients' CEA and IGA scores (92).

3.1.11 Artemisinin

Artemisinin, a widely recognized antimalarial drug, effectively suppressed the infiltration of CD4+ T cells in a mouse model of LL-37-induced rosacea (94, 95). This drug also suppressed the LL-37-induced invasion of neutrophils and macrophages, thereby mitigating innate immune responses against rosacea (95). In HaCaT cells, artemisinin suppressed the release of proinflammatory factors, potentially through the NF-κB pathway (95). An open-label trial involving 130 patients with rosacea who received artemether emulsion, one of the most extensively studied lipid-based derivatives of artemisinin, revealed markedly reduced papule and pustule scores as early as 4 weeks into treatment; notably, the erythema scores of patients treated with artemether emulsion were similar to those of patients treated with standard metronidazole for 4 weeks (96).

3.1.12 ACU-D1

The 26S proteasome, a protein complex responsible for NF-κB degradation, regulates the activation of NF-κB. Therefore, inhibiting the 26S proteasome can result in the inhibition of NF-κB; this offers a novel target for rosacea treatment. A two-arm, vehicle-controlled trial evaluated the efficacy of a novel proteasome inhibitor, topical ACU-D1—pentaerythritol tetrakis (3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propionate). The trial included 27 patients in the active arm and 12 patients in the vehicle arm, all presenting with moderate to severe PPR. ACU-D1 was found to be safe and well-tolerated by the patients; it effectively reduced inflammatory lesions and erythema in the patients. Although this trial lacked the statistical power to determine between-arm significance, the evaluation of both efficacy and safety yielded favorable results (97).

3.1.13 Dapsone

Dapsone exerts its anti-inflammatory effects by inhibiting the production of ROS, mitigating the effects of eosinophil peroxidase on mast cells, and suppressing inflammatory responses mediated by neutrophils (98). A case report indicated sustained remission of granulomatous rosacea after the systemic administration of dapsone (dose: 100 mg/day) (99). A double-blind, randomized clinical trial involving 56 adults with PPR demonstrated that 5% dapsone gel exhibited efficacy similar to that of 0.75% metronidazole gel in improving IGA scores and reducing the lesion counts (100). The first to study dapsone use on ETR, reported that 5% dapsone gel used alone for 12 weeks showed significant improvement in the Investigators Global Assessment score, burning sensation, pruritus, edema, and erythema (101).

3.1.14 ε-Aminocaproic acid

ε-Aminocaproic acid can effectively inhibit trypsin-like proteases, including KLK5, which regulates the cleavage of the cathelicidin precursor protein into LL-37 (102). In a randomized, double-blind, placebo-controlled study involving 11 patients with PPR, no significant differences in the IGA or CEA scores were noted between the treatment and placebo groups at any time point. Notably, in the treatment group, these scores were significantly improved in week 12 compared with baseline (102).

3.1.15 Tranexamic acid

Tranexamic acid (TXA), an antifibrinolytic drug used to treat bleeding conditions, functions by inhibiting the conversion of plasminogen to plasmin, thus suppressing plasmin-induced angiogenesis (103). This suppression is achieved through the reduction of $CD31^+$ microvessel counts and the downregulation of vascular endothelial growth factor expression (104). In the context of skin inflammation, TXA inhibits the production of TLR2, proinflammatory cytokines (IL-6 and TNF-α), and chemokines in keratinocytes primed by LL-37 (104). In an unblinded study, TXA solution–infused wet dressing and microneedling with TXA solution ameliorated the symptoms of erythematotelangiectatic rosacea (103). In a retrospective study investigating the effects of intradermal microinjections of TXA on erythematotelangiectatic rosacea, the observed improvements in symptoms persisted for 3 months (105). In a randomized, vehicle-controlled, split-face study, topical TXA enhanced the barrier function of the epidermal layer and alleviated the clinical signs of rosacea (106). These effects are attributed to the inhibition of protease activated receptor 2 activation, which reduces the calcium influx (106). For oral TXA, one study divided patients into two groups. Seventy patients were treated with doxycycline therapy plus oral TXA or doxycycline therapy alone for 8 weeks. The results showed that the TXA group had better outcomes compared to doxycycline (107).

3.2 Targeted neurovascular therapy for inflammation

Neurovascular dysregulation in rosacea may be linked to immune dysregulation (3, 27). Activation of TRP channels and release of vasoactive neurotransmitters trigger vasodilation, inflammation, and mast cell degranulation, contributing to the symptoms (40). Supplementary Table 3 lists selected therapeutics targeting neurovascular dysregulation for rosacea. FDA-approved treatments for inflammation include topical brimonidine and oxymetazoline (46), acting as vasoconstrictors. Off-label, oral beta blockers are widely used (108). Pilot studies on agents like paroxetine (109), sumatriptan (110), and monoclonal antibodies against CGRP (28) show promise, suggesting a novel treatment approach targeting vasoactive neurotransmitters in rosacea.

3.2.1 Brimonidine

Among drugs targeting neurovascular dysfunction in rosacea, two have received FDA approval (46). The first one is brimonidine topical gel (0.33%), which is a vasoconstrictive alpha-2 adrenergic receptor agonist acting on the microvascular smooth muscles of the facial skin (46). Brimonidine specifically addresses facial erythema instead of papules and pustules (60). Clinical trials ranging in duration from 1 month to 12 months have consistently

demonstrated that topical brimonidine is both safe and effective as a maintenance therapy for rosacea (111, 112). A short-term multicenter, randomized study demonstrated its efficacy in improving moderate rosacea (at least a 1-grade improvement) (113). However, brimonidine is not effective in managing telangiectasia (57, 60). Brimonidine had significant risk of developing adverse effects include local erythema, pruritus, burning sensation, and exacerbated flushing symptoms (57, 60, 114).

3.2.2 Oxymetazoline

Oxymetazoline is the second FDA-approved vasoconstrictor for the topical management of persistent erythema in patients with rosacea (46). As a sympathomimetic agent, oxymetazoline acts as a selective alpha1A adrenoreceptor agonist, exhibiting vasoconstrictive and anti-inflammatory properties (57). Multiple phase III clinical trials have reported that 1% oxymetazoline hydrochloride cream, administered for either 29 days or 52 weeks, was well-tolerated, safe, and effective in treating persistent facial erythema in patients with rosacea (115–117). Only a small proportion of patients experienced adverse effects such as apparent worsening of facial erythema, rebound effects, and occasional paradoxical erythema (115–117). A meta-analysis of adverse effects found that using oxymetazoline, compared to the vehicle, had a significantly higher risk of application-site dermatitis (RR = 8.91, 95% CI: 1.76–45.23), while other adverse effects did not show significant statistical differences (118). These findings highlight the differences between oxymetazoline and topical brimonidine in terms of safety and efficacy (114).

3.2.3 β-blocker

The potential mechanism of action of non-selective beta-blockers lies in the inhibition of beta-2 receptors on cutaneous blood vessels, reducing vasodilation (108). Additionally, systemic administration exhibits a reduction in sympathetic tone, thereby alleviating patient anxiety (119). A systematic review indicates that carvedilol and propranolol are effective for rosacea patients with persistent facial erythema and flushing (108). For carvedilol, the dosage ranges from 6.25 to 37.5 mg daily, which can be divided into doses such as 6.25 mg twice daily. For propranolol, the dosage is typically 30-40 mg daily. In a randomized controlled trial, carvedilol demonstrated superior efficacy compared to brimonidine in addressing telangiectasia and facial erythema (120). Moreover, carvedilol significantly improved patients' depression or anxiety status (120). While beta-blockers offer alternative therapeutic options for rosacea, it is essential to be cautious about common side effects such as hypotension, bradycardia, bronchospasm, and dizziness (121).

3.2.4 Paroxetine

Treatment with paroxetine, a potent selective serotonin (5-HT) reuptake inhibitor, led to improvements in CEA scores, flushing, and burning sensation in a multicentered, randomized, double-blinded, and placebo-controlled trial involving patients with rosacea (109). Paroxetine modulates 5-HT uptake, and dysfunctional 5-HT regulation may result in abnormal blood vessel dilation and constriction (2, 122, 123). Mast cells and platelets were also proved to release 5-HT in several inflammatory skin diseases (124, 125). An association has been reported between rosacea and a single-nucleotide polymorphism of the 5-HT2A receptor (126).

3.2.5 Sumatriptan

PACAP is a potent vasodilator that directly affects smooth muscle cells in arterioles (127). A study reported elevated levels of PACAP expression in rosacea-affected tissues, suggesting its potential relevance in TRPV-mediated edema (29). Another study utilizing both clinical and experimental models confirmed that PACAP38 induces sustained flushing and facial edema (110). In a double-blind, randomized, placebo-controlled, crossover trial, sumatriptan alleviated these symptoms (110). Sumatriptan acts as a 5-HT1B/1D receptor agonist, inhibiting mast cell degranulation and reducing PACAP levels (128, 129). A case report suggested that oral sumatriptan (50 mg) successfully treated a patient with severe and painful rosacea, markedly reducing burning sensation, swelling, redness, and pain (130).

3.2.6 Monoclonal antibodies against CGRP

Erenumab, galcanezumab, fremanezumab, and eptinezumab are four FDA-approved monoclonal antibodies that target CGRP. These antibodies are primarily used for preventing migraine (131, 132). Another novel oral CGRP inhibitor in the pipeline for chronic migraine is atogepant (133). CGRP is involved in the pathophysiology of migraine through nociceptive processes in the trigeminovascular system (134). Notably, CGRP serves as a vasoactive neurotransmitter in rosacea, causing vasodilation and inducing inflammatory response (3, 135). In 2020, an exploratory trial was initiated to investigate the efficacy and tolerability of erenumab in the preventive treatment of persistent redness and flushing associated with rosacea; this trial was anticipated to conclude in 2021 (136). Furthermore, an exploratory comparative case series involving 13 patients explored the benefits of CGRP monoclonal antibodies for patients with both rosacea and migraine (28). The results revealed marked posttreatment improvements in the patients' severity scores (28). However, two patients experienced dermatitis at the injection site (28). Although most patients in this study experienced improvements in rosacea, large-scale randomized clinical trials are still needed to confirm the efficacy and safety of CGRP monoclonal antibodies.

3.2.7 Botulinum toxin

Botulinum toxin (BTX) improves neurovascular dysfunction in rosacea by blocking the release of neurotransmitters, such as acetylcholine, calcitonin gene-related peptide, vasoactive intestinal peptide, substance P, and glutamate, or by reducing non-noxious stimulation (135, 137, 138). This action inhibits the skin's vasodilator system (stimulated by peripheral autonomic nerves), thereby suppressing rosacea-associated neuroinflammation and vasodilation (4, 139). BTX inhibits the activation of TRPV1 by disrupting the lipid raft activity associated with TRPV1 through structural and functional interactions with it (140). Moreover, BTX directly blocks the degranulation of mast cells by cleaving SNARE

protein in these cells (141). In a randomized, controlled, split-face study involving 22 patients, one side of the cheek was treated with a combination of BTX and broadband light. Compared with the pretreatment condition and control groups, the experimental group exhibited reduced global flushing symptom scores, erythema index scores, transepidermal water loss, and sebum secretion (142). The effects of using BTX typically appear within 1-2 weeks and can last for 3-6 months. The most common side effect was localized pain, while the rarer and more concerning side effect was paralysis of motor muscles, with a pooled incidence of 4.3% (95% CI: 1.8 - 7.8%). Most cases of paralysis resolved on their own (143).

3.3 Targeted antimicrobial therapy

Research on the cutaneous microbiome in rosacea focuses on *Demodex folliculorum*, *Staphylococcus epidermidis*, *Bacillus oleronius*, *Bartonella quintana*, and *Chlamydia pneumoniae* (5, 8, 13, 14). Demodex, with its chitin exoskeleton, triggers inflammation by increasing TLR2 levels (5, 8), and therefore becomes the major target of antimicrobial therapy for rosacea. Supplementary Table 4 lists therapeutics targeting microbiota for rosacea. FDA-approved antimicrobials include topical metronidazole (82, 144), ivermectin (145), and microencapsulated benzoyl peroxide (146). Off-label, oral metronidazole (147), oral ivermectin (148), topical sulfur preparation and topical permethrin (148) target Demodex as well. Ongoing studies investigate topical omiganan (149) and oral rifaximin (150). Despite optimism for treatments like bacterial transfer, probiotics, or prebiotics to balance the microbiome, the lack of large-scale trials hampers confirming their efficacy (151).

3.3.1 Metronidazole: oral and topical uses

Because of its dual efficacy as an antibiotic and antiparasitic agent, FDA-approved topical metronidazole is widely used for the treatment of rosacea across the globe and indirectly reduces inflammation by putative reduction of *Demodex* colonization. *Demodex* mites are implicated in the pathogenesis of rosacea, and the antiparasitic effect of metronidazole plays a key role in this context (57). Metronidazole's efficacy in rosacea treatment is also attributed to its anti-inflammatory, immunosuppressive, and antioxidative characteristics, involving the reduction of reactive oxygen species production from neutrophils and inhibition of IL-17 (152–154). The efficacy of topical metronidazole is supported by clinical trials, including randomized, placebo-controlled double-blind studies, as highlighted in a Cochrane review (82, 144). Clinical guidelines recommend topical metronidazole for the treatment of PPR (46, 57, 60). The efficacy of oral metronidazole was evaluated in a double-blind trial, where a combination therapy comprising metronidazole (200 mg twice a day) and 1% hydrocortisone cream was administered for 6 weeks; this therapy reduced the severity of rosacea in 10 out of 14 patients (147).

3.3.2 Ivermectin: oral and topical uses

The US FDA have approved 1% ivermectin cream for the treatment of PPR (155). Clinical guidelines recommend topical ivermectin as a first-line treatment for rosacea, citing high-level evidence for its efficacy in addressing papules and pustules—the key features of rosacea (46, 60). A 2016 network meta-analysis identified ivermectin 1% cream to be the most effective treatment option for inflammatory papules and pustules (145). Regarding the Demodex decrease rate, both topical and systemic ivermectin are superior to other anti-Demodex therapies, achieving nearly a 100% decrease rate (156). Its therapeutic mechanism involves effectively reducing *Demodex* infestation through neurotoxic activity (157). Owing to its anti-inflammatory properties, ivermectin reduces the levels of IL-1b and TNF-α while increasing the level of IL-10. Additionally, ivermectin reduces the expression of proinflammatory genes such as IL-8, LL-37, human β-defensin 3, and TNF-α (158). At the protein level, significant reductions were observed in the levels of LL-37 and IL-8 (158).

3.3.3 Sulfur preparation

The US FDA-approved topical treatment also included sulfur preparations in various formulation such as creams, gels and cleansers. The most common formulation is sodium sulfacetamide, 10% (46). Sulfur preparations have shown positive effects on both erythematotelangiectatic rosacea (ETR) and papulopustular rosacea (PPR), similar to metronidazole (159). This effect may be due to the significant reduction in Demodex count with the sulfur-sodium combination (160). However, sulfur has a more noticeable irritating effect compared to metronidazole, permethrin, lindane, and crotamiton (161).

3.3.4 Microencapsulated benzoyl peroxide cream

Benzoyl peroxide, a potent oxidizing agent, is commonly used in acne treatment (155) and indirectly reduces inflammation through putative antibacterial effects. In 2022, the US FDA approved encapsulated cream benzoyl peroxide (E-BPO) 5% for patients with rosacea (155). The advantage of this silica-based encapsulated formulation is that it facilitates a gradual release of benzoyl peroxide into the skin. This approach enhances efficacy and prevents skin irritation associated with traditional bolus formulations of benzoyl peroxide, thereby improving tolerability (155, 162). Although E-BPO's mechanism of action in the treatment of rosacea remains hypothetical, it primarily centers around the antibacterial effect of this drug (136, 163). In two 12-week, identical, parallel, randomized, double-blind phase III trials, a high proportion of E-BPO-treated patients achieved a clear/almost clear status in the IGA and a reduced lesion count (146). However, a few adverse events were noted; these included pain, erythema, pruritus, and edema (146). Furthermore, a 52-week, single-arm phase III trial investigating the long-term use of E-BPO indicated that prolonged E-BPO use is a safe and well-tolerated therapeutic approach (164).

3.3.5 Topical permethrin

The role of *Demodex* mites in the development of rosacea is well-established and its anti-inflammatory effect is indirect though putative reduction of mites that may stimulate the immune system. Researchers have explored permethrin, an antiparasitic belonging to

the pyrethrin group, as a treatment choice for rosacea (154). The results indicated a notable decrease in the density of *Demodex* mites, indicating permethrin as a primary treatment choice for *Demodex* infestation (165). In a 12-week, double-blind study involving 20 patients with PPR, the application of 5% permethrin cream resulted in a marked reduction in the density of *Demodex* mites and an improvement in the symptoms of rosacea compared with the effects of a cream base (140). However, the use of permethrin for the treatment of rosacea is currently considered off-label.

3.3.6 Omiganan topical gel

Omiganan is an anti-microbial peptide tested against rosacea. In randomized, double-blind, vehicle-controlled, parallel-group, multicenter phase III trial involving patients with severe PPR, topical application of 1.6% omiganan gel significantly outperformed the vehicle in reducing mean inflammatory lesion counts and improving IGA scores in week 12 compared with baseline (149). The underlying mechanism of action involves the rapid bactericidal and fungicidal effects of omiganan against a wide range of pathogens (166).

3.3.7 Rifaximin

Rifaximin, a non-absorbed, gut-active antibiotic with broad-spectrum efficacy, is used to treat small intestinal bacterial overgrowth (SIBO) (155). Studies have indicated a higher prevalence of SIBO among patients with rosacea than in the normal population (167, 168). Administering rifaximin to these patients not only mitigate SIBO but also ameliorate rosacea symptoms. The underlying mechanism suggests that SIBO disrupts immunity, triggering rosacea by increasing the levels of TNF-α or other cytokines, reducing the level of IL-17, and stimulating Th1-mediated immune response (150). In a prospective trial involving 113 patients, a 10-day treatment rifaximin (1200 mg/day) significantly reduced the number of cutaneous lesions compared with the findings in the control group (150). Nonetheless, further research is needed to confirm the efficacy of rifaximin. In 2022, a phase IIa, multicenter, double-blind, placebo-controlled, randomized clinical trial (NCT 05150587) investigated the efficacy and safety of extended-release rifaximin against moderate to severe rosacea (169). The research results are yet to be published.

4 Promising therapeutic strategies in preclinical study with anti-inflammatory activity

Several therapeutic strategies for rosacea show promise in preclinical studies (Supplementary Table 5). Recent years have seen a growing interest in the potential of herbal medicines against rosacea. Compounds such as osthole, celastrol, *Coptis chinensis* Franch, and paeoniflorin have demonstrated efficacy in animal or cellular models by inhibiting inflammatory pathways, thereby alleviating rosacea symptoms (170–172). In an animal model of LL-37-induced rosacea-like lesions, the use of erythroid differentiation regulator 1 mitigated erythema, inflammatory cell infiltration, and microvessel density (173). The same model was used for benvitimod—an aryl hydrocarbon receptor agonist; upon stimulation, benvitimod inhibited TLR2-induced inflammatory responses (174). Furthermore, this agonist significantly ameliorated the rosacea-like symptoms (174).

Among antidiabetic medications, both metformin and pioglitazone have gained prominence. Pioglitazone, which is delivered using a new nan-oemulsion formulation, enhances the drug–skin contact and dermal retention while exhibiting anti-inflammatory activity (175). Metformin is effective in improving rosacea-like lesions, reducing LL-37- and TNF-α-induced reactive oxygen production, and mitigating mitogen-activated protein kinase/NF-κB signal activation in keratinocytes (176).

Melatonin, whose therapeutic potential was demonstrated through network pharmacology analysis, reduces the levels of inflammatory cytokines (177). Other compounds such as thalidomide and aspirin, known for their roles in slowing down innate immunity, reducing inflammatory cytokines, and suppressing NF-κB activation, may be effective in treating rosacea (178, 179).

5 Conclusion/discussion

The diagnosis of rosacea primarily relies on patients' symptoms and phenotypes, as outlined by the 2018 National Rosacea Society Expert Committee guidelines (46). Given the multifactorial nature of rosacea pathophysiology and the diverse triggers and mechanisms involved, treatment approaches must be personalized, taking into account predominant symptoms, phenotypes, pathogenic mechanisms, and trigger factors. Factors such as drug efficacy, tolerability, and previous treatment history should also be considered.

In addressing inflammatory lesions such as papules and pustules, treatment selection should align with underlying pathogenic mechanisms such as immune dysregulation or the presence of *Demodex* mites (46, 60, 180). Recommended topical treatments include ivermectin, azelaic acid, metronidazole, or microencapsulated benzoyl peroxide cream (69, 146, 157, 181). For severe cases, FDA-approved minocycline foam 1.5% or systemic doxycycline may be appropriate (46, 50). A network meta-analysis showed that, compared to low-dose doxycycline 40 mg, topical ivermectin, and metronidazole 0.75%, oral minocycline 100 mg had the best effect on papulopustules with a low incidence of side effects (81). There are also many off-label treatment options; for refractory papulopustules, oral isotretinoin is an option (60, 182). Oral macrolides can serve as secondary agents, particularly for patients with contraindications to tetracyclines, such as those who are refractory or pregnant (79). However, azithromycin has a high risk of adverse effects (RR, 8.9, 95% CI: 1.3-83), such as gastrointestinal issues (81). Topical pimecrolimus is also mentioned in the Swiss guidelines as an anti-inflammatory agent, but care must be taken due to its immunosuppressive effect, which may cause Demodex overgrowth and pose a potential risk of rosacea-like eruptions (91). Several treatments are still under investigation. As we gain a better understanding of rosacea's pathophysiological pathways and develop new precision

medicines, targets like the pro-inflammatory cytokine IL-17, activation of NF-κB, and the JAK-STAT signaling pathway are becoming therapeutic goals. Drugs such as secukinumab, novel proteasome inhibitors, and Janus kinase inhibitors are aimed at these targets and have shown promising results in novel research (86, 87, 97).

For persistent erythema or flushing, these symptoms are highly associated with neurovascular dysregulation (3). Therefore, drug mechanisms focus on promoting vasoconstriction and inhibiting various vasoactive neuropeptides. FDA-approved medications include brimonidine and oxymetazoline (46). Off-label use of propranolol and carvedilol has also been shown to help control persistent erythema (120). Additionally, many new drugs targeting neurogenic inflammation are under investigation, such as paroxetine, sumatriptan, monoclonal antibodies against CGRP, and botulinum toxin (28, 109, 110, 135). These medications aim to reduce neuropeptide-induced vascular dysfunction. However, the side effects and cost-effectiveness of these new drugs need to be considered, and their efficacy still requires validation through more extensive clinical trials.

Many therapeutic approaches coming down the pipeline address the central role of the immune and neurovascular systems in rosacea. Controlling the immune and neurovascular dysregulations will likely lead to increased improvement in rosacea signs and symptoms, and hopefully, with less side effects. Although some of the cited studies were conducted using animal models or at the cellular level, these references were included to illustrate the breadth of ongoing research and the potential for promising new treatment options for rosacea. However, these findings need validation and rigorous evidence to support their efficacy in humans. Future large-scale clinical trials will be essential to provide such evidence and confirm the effectiveness of these treatments in clinical practice. Understanding the triggers and concomitant medications may assist the clinician in selecting the appropriate treatment regimen for particular patients to maximize chances or rosacea symptom control. Research into correlation between genotypes and phenotypes in rosacea patients may also one day lead to a personalized approach to manage rosacea.

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Publisher's note

Supplementary material

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References

1. van Zuuren EJ. Rosacea. *N Engl J Med*. (2017) 377:1754–64. doi: 10.1056/NEJMcp1506630

2. Steinhoff M, Schauber J, Leyden JJ. New insights into rosacea pathophysiology: A review of recent findings. *J Am Acad Dermatol*. (2013) 69:S15–26. doi: 10.1016/j.jaad.2013.04.045

3. Marek-Jozefowicz L, Nedoszytko B, Grochocka M, Żmijewski MA, Czajkowski R, Cubała WJ, et al. Molecular mechanisms of neurogenic inflammation of the skin. *Int J Mol Sci*. (2023) 24(5):5001. doi: 10.3390/ijms24055001

4. Deng Z, Chen M, Zhao Z, Xiao W, Liu T, Peng Q, et al. Whole genome sequencing identifies genetic variants associated with neurogenic inflammation in rosacea. *Nat Commun*. (2023) 14:3958. doi: 10.1038/s41467-023-39761-2

5. Lacey N, Russell-Hallinan A, Zouboulis CC, Powell FC. Demodex mites modulate sebocyte immune reaction: possible role in the pathogenesis of rosacea. *Br J Dermatol*. (2018) 179:420–30. doi: 10.1111/bjd.16540

6. Melnik BC. Rosacea: the blessing of the celts - an approach to pathogenesis through translational research. *Acta Derm Venereol*. (2016) 96:147–56. doi: 10.2340/00015555-2220

7. Awosika O, Oussedik E. Genetic predisposition to rosacea. *Dermatol Clin*. (2018) 36:87–92. doi: 10.1016/j.det.2017.11.002

8. Zhu W, Hamblin MR, Wen X. Role of the skin microbiota and intestinal microbiome in rosacea. *Front Microbiol*. (2023) 14:1108661. doi: 10.3389/fmicb.2023.1108661

9. Yamasaki K, Gallo RL. Rosacea as a disease of cathelicidins and skin innate immunity. *J Investig Dermatol Symp Proc*. (2011) 15:12–5. doi: 10.1038/jidsymp.2011.4

10. Ahn CS, Huang WW. Rosacea pathogenesis. *Dermatol Clin*. (2018) 36:81–6. doi: 10.1016/j.det.2017.11.001

11. Yamasaki K, Kanada K, Macleod DT, Borkowski AW, Morizane S, Nakatsuji T, et al. Tlr2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J Invest Dermatol*. (2011) 131:688–97. doi: 10.1038/jid.2010.351

12. Two AM, Del Rosso JQ. Kallikrein 5-mediated inflammation in rosacea: clinically relevant correlations with acute and chronic manifestations in rosacea and how individual treatments may provide therapeutic benefit. *J Clin Aesthet Dermatol*. (2014) 7:20–5.

13. Zaidi AK, Spaunhurst K, Sprockett D, Thomason Y, Mann MW, Fu P, et al. Characterization of the facial microbiome in twins discordant for rosacea. *Exp Dermatol*. (2018) 27:295–8. doi: 10.1111/exd.13491

14. Mylonas A, Hawerkamp HC, Wang Y, Chen J, Messina F, Demaria O, et al. Type I ifns link skin-associated dysbiotic commensal bacteria to pathogenic inflammation and angiogenesis in rosacea. *JCI Insight*. (2023) 8(4):e151846. doi: 10.1172/jci.insight.151846

15. Abram K, Silm H, Maaroos HI, Oona M. Risk factors associated with rosacea. *J Eur Acad Dermatol Venereol*. (2010) 24:565–71. doi: 10.1111/j.1468-3083.2009.03472.x

16. Sobolewska B, Angermair E, Deuter C, Doycheva D, Kuemmerle-Deschner J, Zierhut M. Nlrp3 A439v mutation in a large family with cryopyrin-associated periodic syndrome: description of ophthalmologic symptoms in correlation with other organ symptoms. *J Rheumatol*. (2016) 43:1101–6. doi: 10.3899/jrheum.150681

17. Ee HL, Tan HH, Ng SK. Autosomal dominant familial chronic mucocutaneous candidiasis associated with acne rosacea. *Ann Acad Med Singap*. (2005) 34:571–4.

18. Second J, Korganow AS, Jannier S, Puel A, Lipsker D. Rosacea and demodicidosis associated with gain-of-function mutation in stat1. *J Eur Acad Dermatol Venereol*. (2017) 31:e542–e4. doi: 10.1111/jdv.14413

19. Aldrich N, Gerstenblith M, Fu P, Tuttle MS, Varma P, Gotow E, et al. Genetic vs environmental factors that correlate with rosacea: A cohort-based survey of twins. *JAMA Dermatol*. (2015) 151:1213–9. doi: 10.1001/jamadermatol.2015.2230

20. Palleschi GM, Torchia D. Rosacea in a monozygotic twin. *Australas J Dermatol*. (2007) 48:132–3. doi: 10.1111/j.1440-0960.2007.00353.x

21. Thibaut de Ménonville S, Rosignoli C, Soares E, Roquet M, Bertino B, Chappuis JP, et al. Topical treatment of rosacea with ivermectin inhibits gene expression of cathelicidin innate immune mediators, ll-37 and klk5, in reconstructed and ex vivo skin models. *Dermatol Ther (Heidelb)*. (2017) 7:213–25. doi: 10.1007/s13555-017-0176-3

22. Sun Y, Chen LH, Lu YS, Chu HT, Wu Y, Gao XH, et al. Identification of novel candidate genes in rosacea by bioinformatic methods. *Cytokine*. (2021) 141:155444. doi: 10.1016/j.cyto.2021.155444

23. Harden JL, Shih YH, Xu J, Li R, Rajendran D, Hofland H, et al. Paired transcriptomic and proteomic analysis implicates il-1beta in the pathogenesis of papulopustular rosacea explants. *J Invest Dermatol*. (2021) 141:800–9. doi: 10.1016/j.jid.2020.08.013

24. Shih YH, Xu J, Kumar A, Li R, Chang ALS. Alterations of immune and keratinization gene expression in papulopustular rosacea by whole transcriptome analysis. *J Invest Dermatol*. (2020) 140:1100–3 e4. doi: 10.1016/j.jid.2019.09.021

25. Sulk M, Seeliger S, Aubert J, Schwab VD, Cevikbas F, Rivier M, et al. Distribution and expression of non-neuronal transient receptor potential (Trpv) ion channels in rosacea. *J Invest Dermatol*. (2012) 132:1253–62. doi: 10.1038/jid.2011.424

26. Chang ALS, Raber I, Xu J, Li R, Spitale R, Chen J, et al. Assessment of the genetic basis of rosacea by genome-wide association study. *J Invest Dermatol*. (2015) 135:1548–55. doi: 10.1038/jid.2015.53

27. Helfrich YR, Maier LE, Cui Y, Fisher GJ, Chubb H, Fligiel S, et al. Clinical, histologic, and molecular analysis of differences between erythematotelangiectatic rosacea and telangiectatic photoaging. *JAMA Dermatol*. (2015) 151:825–36. doi: 10.1001/jamadermatol.2014.4728

28. Sia T, Webb T, Li S, Moskatel LS, Chang ALS. An exploratory comparative case series of calcitonin gene-related peptide monoclonal antibodies in patients with migraine with rosacea. *Br J Dermatol*. (2023) 189:776–8. doi: 10.1093/bjd/ljad277

29. Seeliger S, Buddenkotte J, Schmidt-Choudhury A, Rosignoli C, Shpacovitch V, von Arnim U, et al. Pituitary adenylate cyclase activating polypeptide: an important vascular regulator in human skin in vivo. *Am J Pathol*. (2010) 177:2563–75. doi: 10.2353/ajpath.2010.090941

30. Aponte JL, Chiano MN, Yerges-Armstrong LM, Hinds DA, Tian C, Gupta A, et al. Assessment of rosacea symptom severity by genome-wide association study and expression analysis highlights immuno-inflammatory and skin pigmentation genes. *Hum Mol Genet*. (2018) 27:2762–72. doi: 10.1093/hmg/ddy184

31. Buhl T, Sulk M, Nowak P, Buddenkotte J, McDonald I, Aubert J, et al. Molecular and morphological characterization of inflammatory infiltrate in rosacea reveals activation of Th1/Th17 pathways. *J Invest Dermatol*. (2015) 135:2198–208. doi: 10.1038/jid.2015.141

32. Wang L, Wang YJ, Hao D, Wen X, Du D, He G, et al. The theranostics role of mast cells in the pathophysiology of rosacea. *Front Med (Lausanne)*. (2019) 6:324. doi: 10.3389/fmed.2019.00324

33. Margalit A, Kowalczyk MJ, Żaba R, Kavanagh K. The role of altered cutaneous immune responses in the induction and persistence of rosacea. *J Dermatol Sci*. (2016) 82:3–8. doi: 10.1016/j.jdermsci.2015.12.006

34. DeLuca HF. verview of general physiologic features and functions of vitamin D. *Am J Clin Nutr*. (2004) 80:1689s–96s. doi: 10.1093/ajcn/80.6.1689S

35. Akdogan N, Alli N, Incel Uysal P, Candar T. Role of serum 25-hydroxyvitamin D levels and vitamin D receptor gene polymorphisms in patients with rosacea: A case-control study. *Clin Exp Dermatol*. (2019) 44:397–403. doi: 10.1111/ced.13769

36. Baz K, Cimen MY, Kokturk A, Aslan G, Ikizoglu G, Demirseren DD, et al. Plasma reactive oxygen species activity and antioxidant potential levels in rosacea patients: correlation with seropositivity to helicobacter pylori. *Int J Dermatol*. (2004) 43:494–7. doi: 10.1111/j.1365-4632.2004.02137.x

37. Yazici AC, Tamer L, Ikizoglu G, Kaya TI, Api H, Yildirim H, et al. Gstm1 and gstt1 null genotypes as possible heritable factors of rosacea. *Photodermatol Photoimmunol Photomed*. (2006) 22:208–10. doi: 10.1111/j.1600-0781.2006.00220.x

38. Hayran Y, Şen O, Fırat Oğuz E, Yücel Ç, Eren F, Külcü Çakmak S, et al. Serum il-17 levels in patients with rosacea. *J Cosmet Dermatol*. (2022) 21:1147–53. doi: 10.1111/jocd.14169

39. Choi JE, Di Nardo A. Skin neurogenic inflammation. *Semin Immunopathol*. (2018) 40:249–59. doi: 10.1007/s00281-018-0675-z

40. Roy S, Chompunud Na Ayudhya C, Thapaliya M, Deepak V, Ali H. Multifaceted mrgprx2: new insight into the role of mast cells in health and disease. *J Allergy Clin Immunol*. (2021) 148:293–308. doi: 10.1016/j.jaci.2021.03.049

41. Seiffert K, Ding W, Wagner JA, Granstein RD. Atpgammas enhances the production of inflammatory mediators by a human dermal endothelial cell line *via* purinergic receptor signaling. *J Invest Dermatol*. (2006) 126:1017–27. doi: 10.1038/sj.jid.5700135

42. Metzler-Wilson K, Toma K, Sammons DL, Mann S, Jurovcik AJ, Demidova O, et al. Augmented supraorbital skin sympathetic nerve activity responses to symptom trigger events in rosacea patients. *J Neurophysiol*. (2015) 114:1530–7. doi: 10.1152/jn.00458.2015

43. Del Rosso JQ. Management of facial erythema of rosacea: what is the role of topical α-adrenergic receptor agonist therapy? *J Am Acad Dermatol*. (2013) 69:S44–56. doi: 10.1016/j.jaad.2013.06.009

44. Li M, Tao M, Zhang Y, Pan R, Gu D, Xu Y. Neurogenic rosacea could be a small fiber neuropathy. *Front Pain Res (Lausanne)*. (2023) 4:1122134. doi: 10.3389/fpain.2023.1122134

45. Mascarenhas NL, Wang Z, Chang YL, Di Nardo A. Trpv4 mediates mast cell activation in cathelicidin-induced rosacea inflammation. *J Invest Dermatol*. (2017) 137:972–5. doi: 10.1016/j.jid.2016.10.046

46. Thiboutot D, Anderson R, Cook-Bolden F, Draelos Z, Gallo RL, Granstein RD, et al. Standard management options for rosacea: the 2019 update by the national rosacea society expert committee. *J Am Acad Dermatol*. (2020) 82:1501–10. doi: 10.1016/j.jaad.2020.01.077

47. Group OLoEW. The Oxford 2011 Levels of Evidence. In: *Oxford Centre for Evidence-Based Medicine*. Oxford, United Kingdom: University of Oxford (2011). Available at: http://www.cebm.net/indexaspx?o=5653.

48. Hofmann MA, Lehmann P. Physical modalities for the treatment of rosacea. *J Dtsch Dermatol Ges*. (2016) 14:38–43. doi: 10.1111/ddg.13144

49. Del Rosso JQ, Webster GF, Jackson M, Rendon M, Rich P, Torok H, et al. Two randomized phase iii clinical trials evaluating anti-inflammatory dose doxycycline (40-mg doxycycline, usp capsules) administered once daily for treatment of rosacea. *J Am Acad Dermatol*. (2007) 56:791–802. doi: 10.1016/j.jaad.2006.11.021

50. Gold LS, Del Rosso JQ, Kircik L, Bhatia ND, Hooper D, Nahm WK, et al. Minocycline 1.5% Foam for the topical treatment of moderate to severe papulopustular rosacea: results of 2 phase 3, randomized, clinical trials. *J Am Acad Dermatol*. (2020) 82:1166–73. doi: 10.1016/j.jaad.2020.01.043

51. Bjerke R, Fyrand O, Graupe K. Double-blind comparison of azelaic acid 20% Cream and its vehicle in treatment of papulo-pustular rosacea. *Acta Derm Venereol*. (1999) 79:456–9. doi: 10.1080/000155599750009906

52. Thiboutot D, Thieroff-Ekerdt R, Graupe K. Efficacy and safety of azelaic acid (15%) gel as a new treatment for papulopustular rosacea: results from two vehicle-controlled, randomized phase iii studies. *J Am Acad Dermatol*. (2003) 48:836–45. doi: 10.1067/mjd.2003.308

53. Dispenza MC, Wolpert EB, Gilliland KL, Dai JP, Cong Z, Nelson AM, et al. Systemic isotretinoin therapy normalizes exaggerated Tlr-2-mediated innate immune responses in acne patients. *J Invest Dermatol*. (2012) 132:2198–205. doi: 10.1038/jid.2012.111

54. Bagatin E, Costa CS, Rocha M, Picosse FR, Kamamoto CSL, Pirmez R, et al. Consensus on the use of oral isotretinoin in dermatology - Brazilian society of dermatology. *Bras Dermatol*. (2020) 95 Suppl 1:19–38. doi: 10.1016/j.abd.2020.09.001

55. Kim MB, Kim GW, Park HJ, Kim HS, Chin HW, Kim SH, et al. Pimecrolimus 1% Cream for the treatment of rosacea. *J Dermatol*. (2011) 38:1135–9. doi: 10.1111/j.1346-8138.2011.01223.x

56. Karabulut AA, Izol Serel B, Eksioglu HM. A randomized, single-blind, placebo-controlled, split-face study with pimecrolimus cream 1% for papulopustular rosacea. *J Eur Acad Dermatol Venereol*. (2008) 22:729–34. doi: 10.1111/j.1468-3083.2008.02589.x

57. Clanner-Engelshofen BM, Bernhard D, Dargatz S, Flaig MJ, Gieler U, Kinberger M, et al. S2k guideline: rosacea. *J Dtsch Dermatol Ges*. (2022) 20:1147–65. doi: 10.1111/ddg.14849

58. Kanada KN, Nakatsuji T, Gallo RL. Doxycycline indirectly inhibits proteolytic activation of tryptic kallikrein-related peptidases and activation of cathelicidin. *J Invest Dermatol*. (2012) 132:1435–42. doi: 10.1038/jid.2012.14

59. Del Rosso JQ, Webster G, Weiss JS, Bhatia ND, Gold LS, Kircik L. Nonantibiotic properties of tetracyclines in rosacea and their clinical implications. *J Clin Aesthet Dermatol*. (2021) 14:14–21.

60. Anzengruber F, Czernielewski J, Conrad C, Feldmeyer L, Yawalkar N, Häusermann P, et al. Swiss S1 guideline for the treatment of rosacea. *J Eur Acad Dermatol Venereol*. (2017) 31:1775–91. doi: 10.1111/jdv.14349

61. Lam M, Hu A, Fleming P, Lynde CW. The impact of acne treatment on skin bacterial microbiota: A systematic review. *J Cutan Med Surg*. (2022) 26:93–7. doi: 10.1177/12034754211037994

62. Sapadin AN, Fleischmajer R. Tetracyclines: nonantibiotic properties and their clinical implications. *J Am Acad Dermatol*. (2006) 54:258–65. doi: 10.1016/j.jaad.2005.10.004

63. van der Linden MMD, van Ratingen AR, van Rappard DC, Nieuwenburg SA, Spuls PI. Domino, Doxycycline 40 mg Vs. Minocycline 100 mg in the Treatment of Rosacea: A Randomized, Single-Blinded, Noninferiority Trial, Comparing Efficacy and Safety. *Br J Dermatol*. (2017) 176:1465–74. doi: 10.1111/bjd.15155

64. Baldwin HE. Systemic therapy for rosacea. *Skin Ther Lett*. (2007) 12:1–5.

65. Tsianakas A, Pieber T, Baldwin H, Feichtner F, Alikunju S, Gautam A, et al. Minocycline extended-release comparison with doxycycline for the treatment of rosacea: A randomized, head-to-head, clinical trial. *J Clin Aesthet Dermatol*. (2021) 14:16–23.

66. Rosso JQ, Draelos ZD, Effron C, Kircik LH. Oral sarecycline for treatment of papulopustular rosacea: results of a pilot study of effectiveness and safety. *J Drugs Dermatol*. (2021) 20:426–31. doi: 10.36849/JDD.2021.5923

67. Bunick CG, Keri J, Tanaka SK, Furey N, Damiani G, Johnson JL, et al. Antibacterial mechanisms and efficacy of sarecycline in animal models of infection and inflammation. *Antibiotics (Basel)*. (2021) 10(4):439. doi: 10.3390/antibiotics10040439

68. Bender A, Zapolanski T, Watkins S, Khosraviani A, Seiffert K, Ding W, et al. Tetracycline suppresses atp gamma S-induced Cxcl8 and Cxcl1 production by the human dermal microvascular endothelial cell-1 (Hmec-1) cell line and primary human dermal microvascular endothelial cells. *Exp Dermatol*. (2008) 17:752–60. doi: 10.1111/j.1600-0625.2008.00716.x

69. Draelos ZD, Elewski BE, Harper JC, Sand M, Staedtler G, Nkulikiyinka R, et al. A phase 3 randomized, double-blind, vehicle-controlled trial of azelaic acid foam 15% in the treatment of papulopustular rosacea. *Cutis*. (2015) 96:54–61.

70. Draelos ZD, Elewski B, Staedtler G, Havlickova B. Azelaic acid foam 15% in the treatment of papulopustular rosacea: A randomized, double-blind, vehicle-controlled study. *Cutis*. (2013) 92:306–17.

71. Coda AB, Hata T, Miller J, Audish D, Kotol P, Two A, et al. Cathelicidin, kallikrein 5, and serine protease activity is inhibited during treatment of rosacea with azelaic acid 15% Gel. *J Am Acad Dermatol*. (2013) 69:570–7. doi: 10.1016/j.jaad.2013.05.019

72. Searle T, Ali FR, Al-Niaimi F. The versatility of azelaic acid in dermatology. *J Dermatolog Treat*. (2022) 33:722–32. doi: 10.1080/09546634.2020.1800579

73. MastroFrancesco A, Ottaviani M, Aspite N, Cardinali G, Izzo E, Graupe K, et al. Azelaic acid modulates the inflammatory response in normal human keratinocytes through ppargamma activation. *Exp Dermatol*. (2010) 19:813–20. doi: 10.1111/j.1600-0625.2010.01107.x

74. Webster G, Draelos ZD, Graber E, Lee MS, Dhawan S, Salman M, et al. A multicentre, randomized, double-masked, parallel group, vehicle-controlled phase iib study to evaluate the safety and efficacy of 1% and 3% Topical minocycline gel in patients with papulopustular rosacea. *Br J Dermatol*. (2020) 183:471–9. doi: 10.1111/bjd.18857

75. Schaller M, Almeida LM, Bewley A, Cribier B, Dlova NC, Kautz G, et al. Rosacea treatment update: recommendations from the global rosacea consensus (Rosco) panel. *Br J Dermatol*. (2017) 176:465–71. doi: 10.1111/bjd.15173

76. Gollnick H, Blume-Peytavi U, Szabó EL, Meyer KG, Hauptmann P, Popp G, et al. Systemic isotretinoin in the treatment of rosacea - doxycycline- and placebo-controlled, randomized clinical study. *J Dtsch Dermatol Ges*. (2010) 8:505–15. doi: 10.1111/j.1610-0387.2010.07345.x

77. Sbidian E, Vicaut É, Chidiack H, Anselin E, Cribier B, Dréno B, et al. A randomized-controlled trial of oral low-dose isotretinoin for difficult-to-treat papulopustular rosacea. *J Invest Dermatol*. (2016) 136:1124–9. doi: 10.1016/j.jid.2016.01.025

78. Uslu M, Şavk E, Karaman G, Şendur N. Rosacea treatment with intermediate-dose isotretinoin: follow-up with erythema and sebum measurements. *Acta Derm Venereol*. (2012) 92:73–7. doi: 10.2340/00015555-1204

79. Bakar O, Demirçay Z, Gürbüz O. Therapeutic potential of azithromycin in rosacea. *Int J Dermatol*. (2004) 43:151–4. doi: 10.1111/j.1365-4632.2004.01958.x

80. Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacol Ther*. (2014) 143:225–45. doi: 10.1016/j.pharmthera.2014.03.003

81. Xiao W, Chen M, Wang B, Huang Y, Zhao Z, Deng Z, et al. Efficacy and safety of antibiotic agents in the treatment of rosacea: A systemic network meta-analysis. *Front Pharmacol*. (2023) 14:1169916. doi: 10.3389/fphar.2023.1169916

82. van Zuuren EJ, Fedorowicz Z, Carter B, van der Linden MM, Charland L. Interventions for rosacea. *Cochrane Database Syst Rev*. (2015) 2015:Cd003262. doi: 10.1002/14651858.CD003262.pub5

83. Li J, Yuan X, Tang Y, Wang B, Deng Z, Huang Y, et al. Hydroxychloroquine is a novel therapeutic approach for rosacea. *Int Immunopharmacol*. (2020) 79:106178. doi: 10.1016/j.intimp.2019.106178

84. Wang B, Yuan X, Huang X, Tang Y, Zhao Z, Yang B, et al. Efficacy and safety of hydroxychloroquine for treatment of patients with rosacea: A multicenter, randomized, double-blind, double-dummy, pilot study. *J Am Acad Dermatol*. (2021) 84:543–5. doi: 10.1016/j.jaad.2020.05.050

85. Amir Ali A, Vender R, Vender R. The role of Il-17 in papulopustular rosacea and future directions. *J Cutan Med Surg*. (2019) 23:635–41. doi: 10.1177/1203475419867611

86. Kumar AM, Chiou AS, Shih YH, Li S, Chang ALS. An exploratory, open-label, investigator-initiated study of interleukin-17 blockade in patients with moderate-to-severe papulopustular rosacea. *Br J Dermatol*. (2020) 183:942–3. doi: 10.1111/bjd.19172

87. Sun YH, Man XY, Xuan XY, Huang CZ, Shen Y, Lao LM. Tofacitinib for the treatment of erythematotelangiectatic and papulopustular rosacea: A retrospective case series. *Dermatol Ther*. (2022) 35:e15848. doi: 10.1111/dth.15848

88. Xu B, Xu Z, Ye S, Sun H, Zhao B, Wu N, et al. Jak1 inhibitor abrocitinib for the treatment of steroid-induced rosacea: case series. *Front Med (Lausanne)*. (2023) 10:1239869. doi: 10.3389/fmed.2023.1239869

89. Weissenbacher S, Merkl J, Hildebrandt B, Wollenberg A, Braeutigam M, Ring J, et al. Pimecrolimus cream 1% for papulopustular rosacea: A randomized vehicle-controlled double-blind trial. *Br J Dermatol*. (2007) 156:728–32. doi: 10.1111/j.1365-2133.2006.07669.x

90. Bamford JT, Elliott BA, Haller IV. Tacrolimus effect on rosacea. *J Am Acad Dermatol*. (2004) 50:107–8. doi: 10.1016/s0190-9622(03)02157-1

91. Zhang H, Yang L, Wang Y, Zhang D, Tang K, Fang R, et al. Topical calcineurin inhibitors as a double-edged sword in rosacea: A systematic review. *J Cosmet Dermatol*. (2022) 21:1695–704. doi: 10.1111/jocd.14315

92. Deng Z, Chen M, Liu Y, Xu S, Ouyang Y, Shi W, et al. A positive feedback loop between mtorc1 and cathelicidin promotes skin inflammation in rosacea. *EMBO Mol Med*. (2021) 13:e13560. doi: 10.15252/emmm.202013560

93. Huang Y, Liu D, Chen M, Xu S, Peng Q, Zhu Y, et al. Tlr7 promotes skin inflammation *via* activating Nfκb-mtorc1 axis in rosacea. *PeerJ*. (2023) 11:e15976. doi: 10.7717/peerj.15976

94. Efferth T, Oesch F. The immunosuppressive activity of artemisinin-type drugs towards inflammatory and autoimmune diseases. *Med Res Rev*. (2021) 41:3023–61. doi: 10.1002/med.21842

95. Yuan X, Li J, Li Y, Deng Z, Zhou L, Long J, et al. Artemisinin, a potential option to inhibit inflammation and angiogenesis in rosacea. *BioMed Pharmacother*. (2019) 117:109181. doi: 10.1016/j.biopha.2019.109181

96. Wang GJ, Gao XY, Wu Y, He HQ, Yu Y, Qin HH, et al. Evaluation of the efficacy and tolerance of artemether emulsion for the treatment of papulopustular rosacea: A randomized pilot study. *J Dermatolog Treat*. (2019) 30:809–12. doi: 10.1080/09546634.2019.1610549

97. Jackson JM, Coulon R, Arbiser JL. Evaluation of a first-in-class proteasome inhibitor in patients with moderate to severe rosacea. *J Drugs Dermatol*. (2021) 20:660–4. doi: 10.36849/jdd.2021.5925

98. Khalilzadeh M, Shayan M, Jourian S, Rahimi M, Sheibani M, Dehpour AR. A comprehensive insight into the anti-inflammatory properties of dapsone. *Naunyn Schmiedebergs Arch Pharmacol*. (2022) 395:1509–23. doi: 10.1007/s00210-022-02297-1

99. Merlo G, Cozzani E, Russo R, Parodi A. Dapsone for unresponsive granulomatous rosacea. *Am J Ther*. (2020) 27:e304–e6. doi: 10.1097/MJT.0000000000000903

100. Faghihi G, Khosravani P, Nilforoushzadeh MA, Hosseini SM, Assaf F, Zeinali N, et al. Dapsone gel in the treatment of papulopustular rosacea: A double-blind randomized clinical trial. *J Drugs Dermatol*. (2015) 14:602–6.

101. Gökşin Ş, İmren IG, Kaçar N. Efficacy of topical dapsone 5% Gel for the treatment of erythematotelangiectatic rosacea: new treatment option with old drug. *Dermatol Pract Concept*. (2024) 14(1):e2024034. doi: 10.5826/dpc.1401a34

102. Two AM, Hata TR, Nakatsuji T, Coda AB, Kotol PF, Wu W, et al. Reduction in serine protease activity correlates with improved rosacea severity in a small, randomized pilot study of a topical serine protease inhibitor. *J Invest Dermatol*. (2014) 134:1143–5. doi: 10.1038/jid.2013.472

103. Bageorgou F, Vasalou V, Tzanetakou V, Kontochristopoulos G. The new therapeutic choice of tranexamic acid solution in treatment of erythematotelangiectatic rosacea. *J Cosmet Dermatol*. (2019) 18:563–7. doi: 10.1111/jocd.12724

104. Li Y, Xie H, Deng Z, Wang B, Tang Y, Zhao Z, et al. Tranexamic acid ameliorates rosacea symptoms through regulating immune response and angiogenesis. *Int Immunopharmacol*. (2019) 67:326–34. doi: 10.1016/j.intimp.2018.12.031

105. Daadaa N, Litaiem N, Karray M, Bacha T, Jones M, Belajouza Noueiri C, et al. Intradermal tranexamic acid microinjections: A novel treatment option for erythematotelangiectatic rosacea. *J Cosmet Dermatol*. (2021) 20:3324–9. doi: 10.1111/jocd.14209

106. Zhong S, Sun N, Liu H, Niu Y, Chen C, Wu Y. Topical tranexamic acid improves the permeability barrier in rosacea. *Dermatologica Sin*. (2015) 33:112–7. doi: 10.1016/j.dsi.2015.04.012

107. Xu Z, Yu B, Xu B, Ye S, Qing Y, Zhao B, et al. Oral tranexamic acid treats papulopustular rosacea by improving the skin barrier. *J Cosmet Dermatol*. (2024). doi: 10.1111/jocd.16339

108. Logger JGM, Olydam JI, Driessen RJB. Use of beta-blockers for rosacea-associated facial erythema and flushing: A systematic review and update on

proposed mode of action. *J Am Acad Dermatol*. (2020) 83:1088–97. doi: 10.1016/j.jaad.2020.04.129

109. Wang B, Huang Y, Tang Y, Zhao Z, Shi W, Jian D, et al. Paroxetine is an effective treatment for refractory erythema of rosacea: primary results from the prospective rosacea refractory erythema randomized clinical trial. *J Am Acad Dermatol*. (2023) 88:1300–7. doi: 10.1016/j.jaad.2023.01.044

110. Wienholtz NKF, Christensen CE, Coskun H, Zhang DG, Ghanizada H, Egeberg A, et al. Infusion of pituitary adenylate cyclase-activating polypeptide-38 in patients with rosacea induces flushing and facial edema that can be attenuated by sumatriptan. *J Invest Dermatol*. (2021) 141:1687–98. doi: 10.1016/j.jid.2021.02.002

111. Moore A, Kempers S, Murakawa G, Weiss J, Tauscher A, Swinyer L, et al. Long-term safety and efficacy of once-daily topical brimonidine tartrate gel 0.5% for the treatment of moderate to severe facial erythema of rosacea: results of a 1-year open-label study. *J Drugs Dermatol*. (2014) 13:56–61.

112. Fowler J Jr., Jackson M, Moore A, Jarratt M, Jones T, Meadows K, et al. Efficacy and safety of once-daily topical brimonidine tartrate gel 0.5% for the treatment of moderate to severe facial erythema of rosacea: results of two randomized, double-blind, and vehicle-controlled pivotal studies. *J Drugs Dermatol*. (2013) 12:650–6.

113. Layton AM, Schaller M, Homey B, Hofmann MA, Bewley AP, Lehmann P, et al. Brimonidine gel 0.33% Rapidly improves patient-reported outcomes by controlling facial erythema of rosacea: A randomized, double-blind, vehicle-controlled study. *J Eur Acad Dermatol Venereol*. (2015) 29:2405–10. doi: 10.1111/jdv.13305

114. Del Rosso JQ, Tanghetti E. Topical oxymetazoline hydrochloride cream 1% for the treatment of persistent facial erythema of rosacea in adults: A comprehensive review of current evidence. *J Clin Aesthet Dermatol*. (2021) 14:32–7.

115. Draelos ZD, Gold MH, Weiss RA, Baumann L, Grekin SK, Robinson DM, et al. Efficacy and safety of oxymetazoline cream 1.0% for treatment of persistent facial erythema associated with rosacea: findings from the 52-week open label reveal trial. *J Am Acad Dermatol*. (2018) 78:1156–63. doi: 10.1016/j.jaad.2018.01.027

116. Baumann L, Goldberg DJ, Stein Gold L, Tanghetti EA, Lain E, Kaufman J, et al. Pivotal trial of the efficacy and safety of oxymetazoline cream 1.0% for the treatment of persistent facial erythema associated with rosacea: findings from the second reveal trial. *J Drugs Dermatol*. (2018) 17:290–8.

117. Kircik LH, DuBois J, Draelos ZD, Werschler P, Grande K, Cook-Bolden FE, et al. Pivotal trial of the efficacy and safety of oxymetazoline cream 1.0% for the treatment of persistent facial erythema associated with rosacea: findings from the first reveal trial. *J Drugs Dermatol*. (2018) 17:97–105.

118. Liu F, Zhou Q, Wang H, Fu H, Li Y, Tao M, et al. Efficacy and safety of oxymetazoline for the treatment of rosacea: A meta-analysis. *J Cosmet Dermatol*. (2023) 22:2408–19. doi: 10.1111/jocd.15747

119. Craige H, Cohen JB. Symptomatic treatment of idiopathic and rosacea-associated cutaneous flushing with propranolol. *J Am Acad Dermatol*. (2005) 53:881–4. doi: 10.1016/j.jaad.2005.07.021

120. Li J, Tang JY, Fu J, Zhang MW, Wan M, Chen DW, et al. Carvedilol ameliorates persistent erythema of erythematotelangiectatic rosacea by regulating the status of anxiety/depression. *J Dermatol*. (2022) 49:1139–47. doi: 10.1111/1346-8138.16525

121. Chen L, Tsai TF. The role of β-blockers in dermatological treatment: A review. *J Eur Acad Dermatol Venereol*. (2018) 32:363–71. doi: 10.1111/jdv.14566

122. Stearns V, Beebe KL, Iyengar M, Dube E. Paroxetine controlled release in the treatment of menopausal hot flashes: A randomized controlled trial. *Jama*. (2003) 289:2827–34. doi: 10.1001/jama.289.21.2827

123. MacLean MR. Pulmonary hypertension, anorexigens and 5-ht: pharmacological synergism in action? *Trends Pharmacol Sci*. (1999) 20:490–5. doi: 10.1016/S0165-6147(99)01389-9

124. Oliveira MC, Pelegrini-da-Silva A, Parada CA, Tambeli CH. 5-ht acts on nociceptive primary afferents through an indirect mechanism to induce hyperalgesia in the subcutaneous tissue. *Neuroscience*. (2007) 145:708–14. doi: 10.1016/j.neuroscience.2006.12.021

125. Nordlind K, Thorslund K, Lonne-Rahm S, Mohabbati S, Berki T, Morales M, et al. Expression of serotonergic receptors in psoriatic skin. *Arch Dermatol Res*. (2006) 298:99–106. doi: 10.1007/s00403-006-0652-6

126. Wang Z, Xie H, Gong Y, Ouyang Y, Deng F, Tang Y, et al. Relationship between rosacea and sleep. *J Dermatol*. (2020) 47:592–600. doi: 10.1111/1346-8138.15339

127. Dalsgaard T, Hannibal J, Fahrenkrug J, Larsen CR, Ottesen B. Vip and pacap display different vasodilatory effects in rabbit coronary and cerebral arteries. *Regul Pept*. (2003) 110:179–88. doi: 10.1016/S0167-0115(02)00205-7

128. Zagami AS, Edvinsson L, Goadsby PJ. Pituitary adenylate cyclase activating polypeptide and migraine. *Ann Clin Transl Neurol*. (2014) 1:1036–40. doi: 10.1002/acn3.113

129. Levy D, Jakubowski M, Burstein R. Disruption of communication between peripheral and central trigeminovascular neurons mediates the antimigraine action of 5ht 1b/1d receptor agonists. *Proc Natl Acad Sci U.S.A*. (2004) 101:4274–9. doi: 10.1073/pnas.0306147101

130. Wienholtz N, Ashina M, Thyssen JP, Egeberg A. Subtype-specific off-label treatment of rosacea. *Case Rep Dermatol*. (2021) 13:121–8. doi: 10.1159/000511984

131. Bagherzadeh-Fard M, Amin Yazdanifar M, Sadeghalvad M, Rezaei N. Erenumab efficacy in migraine headache prophylaxis: A systematic review. *Int Immunopharmacol*. (2023) 117:109366. doi: 10.1016/j.intimp.2022.109366

132. Shah T, Bedrin K, Tinsley A. Calcitonin gene relating peptide inhibitors in combination for migraine treatment: A mini-review. *Front Pain Res (Lausanne)*. (2023) 4:1130239. doi: 10.3389/fpain.2023.1130239

133. Pozo-Rosich P, Ailani J, Ashina M, Goadsby PJ, Lipton RB, Reuter U, et al. Atogepant for the preventive treatment of chronic migraine (Progress): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. (2023) 402:775–85. doi: 10.1016/S0140-6736(23)01049-8

134. Ho TW, Edvinsson L, Goadsby PJ. Cgrp and its receptors provide new insights into migraine pathophysiology. *Nat Rev Neurol*. (2010) 6:573–82. doi: 10.1038/nrneurol.2010.127

135. Scala J, Vojvodic A, Vojvodic P, Vlaskovic-Jovicevic T, Peric-Hajzler Z, Matovic D, et al. Botulin toxin use in rosacea and facial flushing treatment. *Open Access Maced J Med Sci*. (2019) 7:2985–7. doi: 10.3889/oamjms.2019.784

136. Okamoto K, Kanayama S, Ikeda F, Fujikawa K, Fujiwara S, Nozawa N, et al. Broad spectrum in vitro microbicidal activity of benzoyl peroxide against microorganisms related to cutaneous diseases. *J Dermatol*. (2021) 48:551–5. doi: 10.1111/1346-8138.15739

137. Patil S, Willett O, Thompkins T, Hermann R, Ramanathan S, Cornett EM, et al. Botulinum toxin: pharmacology and therapeutic roles in pain states. *Curr Pain Headache Rep*. (2016) 20:15. doi: 10.1007/s11916-016-0545-0

138. Meng J, Wang J, Lawrence G, Dolly JO. Synaptobrevin I mediates exocytosis of cgrp from sensory neurons and inhibition by botulinum toxins reflects their anti-nociceptive potential. *J Cell Sci*. (2007) 120:2864–74. doi: 10.1242/jcs.012211

139. Bloom BS, Payongayong L, Mourin A, Goldberg DJ. Impact of intradermal abobotulinumtoxina on facial erythema of rosacea. *Dermatol Surg*. (2015) 41:S9–16. doi: 10.1097/dss.0000000000000277

140. Raoufinejad K, Mansouri P, Rajabi M, Naraghi Z, Jebraeili R. Efficacy and safety of permethrin 5% Topical gel vs. Placebo for rosacea: A double-blind randomized controlled clinical trial. *J Eur Acad Dermatol Venereol*. (2016) 30:2105–17. doi: 10.1111/jdv.13801

141. Choi JE, Werbel T, Wang Z, Wu CC, Yaksh TL, Di Nardo A. Botulinum toxin blocks mast cells and prevents rosacea like inflammation. *J Dermatol Sci*. (2019) 93:58–64. doi: 10.1016/j.jdermsci.2018.12.004

142. Tong Y, Luo W, Gao Y, Liu L, Tang Q, Wa Q. A randomized, controlled, split-face study of botulinum toxin and broadband light for the treatment of erythematotelangiectatic rosacea. *Dermatol Ther*. (2022) 35:e15395. doi: 10.1111/dth.15395

143. Alsaati AA, Alsaadoun D, Kinkar LI, Alkhamis RS, Ahmed WA, Almathami AH. The efficacy and safety of botulinum toxin a for the treatment of rosacea: A systematic review. *Cureus*. (2023) 15:e51304. doi: 10.7759/cureus.51304

144. Breneman DL, Stewart D, Hevia O, Hino PD, Drake LA. A double-blind, multicenter clinical trial comparing efficacy of once-daily metronidazole 1 percent cream to vehicle in patients with rosacea. *Cutis*. (1998) 61:44–7.

145. Siddiqui K, Stein Gold L, Gill J. The efficacy, safety, and tolerability of ivermectin compared with current topical treatments for the inflammatory lesions of rosacea: A network meta-analysis. *Springerplus*. (2016) 5:1151. doi: 10.1186/s40064-016-2819-8

146. Bhatia ND, Werschler WP, Baldwin H, Sugarman J, Green LJ, Levy-Hacham O, et al. Efficacy and safety of microencapsulated benzoyl peroxide cream, 5%, in rosacea: results from two phase iii, randomized, vehicle-controlled trials. *J Clin Aesthet Dermatol*. (2023) 16:34–40.

147. Pye RJ, Burton JL. Treatment of rosacea by metronidazole. *Lancet*. (1976) 1:1211–2. doi: 10.1016/s0140-6736(76)92160-7

148. Forstinger C, Kittler H, Binder M. Treatment of rosacea-like demodicidosis with oral ivermectin and topical permethrin cream. *J Am Acad Dermatol*. (1999) 41:775–7. doi: 10.1016/s0190-9622(99)70022-8

149. Grada A, Van Doorn M, Lain E, Furst K, Feiss G. Lb1092 topical omiganan for severe papulopustular rosacea: A randomized, vehicle-controlled, double-blind, multicenter study. *J Invest Dermatol*. (2019) 139:B13. doi: 10.1016/j.jid.2019.06.057

150. Parodi A, Paolino S, Greco A, Drago F, Mansi C, Rebora A, et al. Small intestinal bacterial overgrowth in rosacea: clinical effectiveness of its eradication. *Clin Gastroenterol Hepatol*. (2008) 6:759–64. doi: 10.1016/j.cgh.2008.02.054

151. Knackstedt R, Knackstedt T, Gatherwright J. The role of topical probiotics in skin conditions: A systematic review of animal and human studies and implications for future therapies. *Exp Dermatol*. (2020) 29:15–21. doi: 10.1111/exd.14032

152. Sharma A, Kroumpouzos G, Kassir M, Galadari H, Goren A, Grabbe S, et al. Rosacea management: A comprehensive review. *J Cosmet Dermatol*. (2022) 21:1895–904. doi: 10.1111/jocd.14816

153. Akamatsu H, Oguchi M, Nishijima S, Asada Y, Takahashi M, Ushijima T, et al. The inhibition of free radical generation by human neutrophils through the synergistic effects of metronidazole with palmitoleic acid: A possible mechanism of action of metronidazole in rosacea and acne. *Arch Dermatol Res*. (1990) 282:449–54. doi: 10.1007/bf00402621

154. Koçak M, Yağli S, Vahapoğlu G, Ekşioğlu M. Permethrin 5% Cream versus metronidazole 0.75% Gel for the treatment of papulopustular rosacea. A randomized double-blind placebo-controlled study. *Dermatology*. (2002) 205:265–70. doi: 10.1159/000065849

155. Choe J, Barbieri JS. Emerging medical therapies in rosacea: A narrative review. *Dermatol Ther (Heidelb)*. (2023) 13:2933–49. doi: 10.1007/s13555-023-01048-1

156. Li J, Wei E, Reisinger A, French LE, Clanner-Engelshofen BM, Reinholz M. Comparison of different anti-demodex strategies: A systematic review and meta-analysis. *Dermatology*. (2023) 239:12–31. doi: 10.1159/000526296

157. Trave I, Micalizzi C, Cozzani E, Gasparini G, Parodi A. Papulopustular rosacea treated with ivermectin 1% Cream: remission of the demodex mite infestation over time and evaluation of clinical relapses. *Dermatol Pract Concept*. (2022) 12:e2022201. doi: 10.5826/dpc.1204a201

158. Schaller M, Gonser L, Belge K, Braunsdorf C, Nordin R, Scheu A, et al. Dual anti-inflammatory and anti-parasitic action of topical ivermectin 1% in papulopustular rosacea. *J Eur Acad Dermatol Venereol*. (2017) 31:1907–11. doi: 10.1111/jdv.14437

159. Nobeyama Y, Aihara Y, Asahina A. Real-world evidence for the treatment of rosacea with sulfur or metronidazole preparation in Japanese patients. *JMA J*. (2023) 6:448–54. doi: 10.31662/jmaj.2023-0100

160. Sarac G. A comparison of the efficacy and tolerability of topical agents used in facial demodex treatment. *J Cosmet Dermatol*. (2019) 18:1784–7. doi: 10.1111/jocd.12986

161. Forton F, Seys B, Marchal JL, Song AM. Demodex folliculorum and topical treatment: acaricidal action evaluated by standardized skin surface biopsy. *Br J Dermatol*. (1998) 138:461–6. doi: 10.1046/j.1365-2133.1998.02125.x

162. Leyden JJ. Randomized, phase 2, dose-ranging study in the treatment of rosacea with encapsulated benzoyl peroxide gel. *J Drugs Dermatol*. (2014) 13:685–8.

163. van Zuuren EJ, Arents BWM, van der Linden MMD, Vermeulen S, Fedorowicz Z, Tan J. Rosacea: new concepts in classification and treatment. *Am J Clin Dermatol*. (2021) 22:457–65. doi: 10.1007/s40257-021-00595-7

164. Werschler WP, Sugarman J, Bhatia N, Baldwin H, Green LJ, Nov O, et al. Long-term efficacy and safety of microencapsulated benzoyl peroxide cream, 5%, in rosacea: results from an extension of two phase iii, vehicle-controlled trials. *J Clin Aesthet Dermatol*. (2023) 16:27–33.

165. Jacob S, VanDaele MA, Brown JN. Treatment of demodex-associated inflammatory skin conditions: A systematic review. *Dermatol Ther*. (2019) 32:e13103. doi: 10.1111/dth.13103

166. Rubinchik E, Dugourd D, Algara T, Pasetka C, Friedland HD. Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models. *Int J Antimicrob Agents*. (2009) 34:457–61. doi: 10.1016/j.ijantimicag.2009.05.003

167. Drago F, De Col E, Agnoletti AF, Schiavetti I, Savarino V, Rebora A, et al. The role of small intestinal bacterial overgrowth in rosacea: A 3-year follow-up. *J Am Acad Dermatol*. (2016) 75:e113–e5. doi: 10.1016/j.jaad.2016.01.059

168. Weinstock LB, Steinhoff M. Rosacea and small intestinal bacterial overgrowth: prevalence and response to rifaximin. *J Am Acad Dermatol*. (2013) 68:875–6. doi: 10.1016/j.jaad.2012.11.038

169. Alfasigma SpA, bioRasi LLC. *Safety, Efficacy and Pharmacokinetics of Rifaximin in Patients with Moderate-to-Severe Papulopustular Rosacea*. ClinicalTrials.gov identifier: NCT05150587. (2022). Accessed July 16, 2024. https://clinicaltrials.gov/study/NCT05150587.

170. Callahan BN, Kammala AK, Syed M, Yang C, Occhiuto CJ, Nellutla R, et al. Osthole, a natural plant derivative inhibits mrgprx2 induced mast cell responses. *Front Immunol*. (2020) 11:703. doi: 10.3389/fimmu.2020.00703

171. Zeng Q, Yang J, Yan G, Zhang L, Wang P, Zhang H, et al. Celastrol inhibits ll37-induced rosacea by inhibiting Ca(2+)/camkii-mtor-nf-κb activation. *BioMed Pharmacother*. (2022) 153:113292. doi: 10.1016/j.biopha.2022.113292

172. Roh KB, Ryu DH, Cho E, Weon JB, Park D, Kweon DH, et al. Coptis chinensis franch directly inhibits proteolytic activation of kallikrein 5 and cathelicidin associated with rosacea in epidermal keratinocytes. *Molecules*. (2020) 25(23):5556. doi: 10.3390/molecules25235556

173. Kim M, Kim KE, Jung HY, Jo H, Jeong SW, Lee J, et al. Recombinant erythroid differentiation regulator 1 inhibits both inflammation and angiogenesis in a mouse model of rosacea. *Exp Dermatol*. (2015) 24:680–5. doi: 10.1111/exd.12745

174. Sun Y, Chen L, Wang H, Zhu P, Jiang S, Qi R, et al. Activation of aryl hydrocarbon receptor ameliorates rosacea-like eruptions in mice and suppresses the tlr signaling pathway in ll-37-induced hacat cells. *Toxicol Appl Pharmacol*. (2022) 451:116189. doi: 10.1016/j.taap.2022.116189

175. Espinoza LC, Silva-Abreu M, Calpena AC, Rodríguez-Lagunas MJ, Fábrega MJ, Garduño-Ramírez ML, et al. Nanoemulsion strategy of pioglitazone for the treatment of skin inflammatory diseases. *Nanomedicine*. (2019) 19:115–25. doi: 10.1016/j.nano.2019.03.017

176. Li Y, Yang L, Wang Y, Deng Z, Xu S, Xie H, et al. Exploring metformin as a candidate drug for rosacea through network pharmacology and experimental validation. *Pharmacol Res*. (2021) 174:105971. doi: 10.1016/j.phrs.2021.105971

177. Zhang H, Zhang Y, Li Y, Wang Y, Yan S, Xu S, et al. Bioinformatics and network pharmacology identify the therapeutic role and potential mechanism of melatonin in ad and rosacea. *Front Immunol*. (2021) 12:756550. doi: 10.3389/fimmu.2021.756550

178. Deng Z, Xu S, Peng Q, Sha K, Xiao W, Liu T, et al. Aspirin alleviates skin inflammation and angiogenesis in rosacea. *Int Immunopharmacol*. (2021) 95:107558. doi: 10.1016/j.intimp.2021.107558

179. Chen M, Xie H, Chen Z, Xu S, Wang B, Peng Q, et al. Thalidomide ameliorates rosacea-like skin inflammation and suppresses Nf-κb activation in keratinocytes. *BioMed Pharmacother*. (2019) 116:109011. doi: 10.1016/j.biopha.2019.109011

180. Lazaridou E, Giannopoulou C, Fotiadou C, Vakirlis E, Trigoni A, Ioannides D. The potential role of microorganisms in the development of rosacea. *J Dtsch Dermatol Ges*. (2011) 9:21–5. doi: 10.1111/j.1610-0387.2010.07513.x

181. Taieb A, Ortonne JP, Ruzicka T, Roszkiewicz J, Berth-Jones J, Peirone MH, et al. Superiority of ivermectin 1% Cream over metronidazole 0·75% Cream in treating inflammatory lesions of rosacea: A randomized, investigator-blinded trial. *Br J Dermatol*. (2015) 172:1103–10. doi: 10.1111/bjd.13408

182. Assiri A, Hobani AH, AlKaabi HA, Mojiri ME, Daghriri SA, Suwaid OA, et al. Efficacy of low-dose isotretinoin in the treatment of rosacea: A systematic review and meta-analysis. *Cureus*. (2024) 16:e57085. doi: 10.7759/cureus.57085

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NETosis of psoriasis: a critical step in amplifying the inflammatory response

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NETosis, a regulated form of neutrophil death, is crucial for host defense against pathogens. However, the release of neutrophil extracellular traps (NETs) during NETosis can have detrimental effects on surrounding tissues and contribute to the pro-inflammatory response, in addition to their role in controlling microbes. Although it is well-established that the IL-23-Th17 axis plays a key role in the pathogenesis of psoriasis, emerging evidence suggests that psoriasis, as an autoinflammatory disease, is also associated with NETosis. The purpose of this review is to provide a comprehensive understanding of the mechanisms underlying NETosis in psoriasis. It will cover topics such as the formation of NETs, immune cells involved in NETosis, and potential biomarkers as prognostic/predicting factors in psoriasis. By analyzing the intricate relationship between NETosis and psoriasis, this review also aims to identify novel possibilities targeting NETosis for the treatment of psoriasis.

KEYWORDS

neutrophils, NEtosis, psoriasis, immune, immune disease

1 Introduction

Psoriasis is a chronic immune-mediated disease that not only affects skin but is also associated with conditions such as arthritis, diabetes mellitus, metabolic syndrome, vascular complications (including stroke and ischemic heart disease), and depression (1). It is widely recognized that Th1 and Th17 and their associated cytokines, including IL-17A, IL-12, and IL-23, play a key role in the pathogenesis of psoriasis. Inhibitors targeting these cytokines have proven effective in achieving clinical remission by rapidly clearing skin lesions. However, some patients do not respond to these inhibitors or experience disease recurrence during treatment, suggesting the involvement of other immune cells in the pathogenesis of psoriasis.

Dysfunctional immune system involvement has been well-documented in typical psoriatic dermatoses, making it a focal point for investigating the pathogenesis of the disease (2). Neutrophils have been observed to be highly abundant in psoriatic lesions, particularly in the epidermis, where they accumulate in Munro micro-abscesses in the

stratum corneum and spongy pustules of Kogoj in the spinous layer (3). Some researchers believe that neutrophils, as the most abundant of the innate immune cells, play a crucial role in the development and progression of psoriasis (4). Although the precise role of neutrophils in the development and progression of psoriasis remains unknown, a wealth of clinical data supports their relevance (5–7). For instance, the treatment drug for psoriasis, *dimethyl fumarate* has been shown to reduce neutrophil levels, thereby mitigating the immune system's impact on the body. Additionally, *secukizumab*, a drug that significantly reduces epidermal neutrophil levels, has demonstrated efficacy in treating moderate-to-severe psoriasis (8). These findings suggest that neutrophils are involved in the pathogenesis of psoriasis and represent a potential target for therapeutic intervention.

Activated neutrophils employ a mechanism known as NETosis to capture and eliminate pathogens by releasing neutrophil extracellular traps (NETs) into the cell. The formation of NETs is accompanied by a unique form of neutrophil death, distinct from apoptosis and necrosis, known as NETosis (9). These NETs have been identified in peripheral tissues, such as the skin and kidneys, of individuals with autoimmune small vessel vasculitis, SLE, and rheumatoid arthritis (10–12).

In this narrative review, we extensively examine published articles focusing on the formation of NETs and their key components, as well as the potential role of NETosis in psoriasis. We also explore new treatments for psoriasis.

2 Overview of NETosis

2.1 NET formation and NET components in psoriasis

NETs, which are composed of decondensed chromatin forming a reticulated DNA structure with pores of approximately 200 nm, are surrounded by nuclear proteins (13). These proteins can be classified into three categories: histones, granule proteins, and cytoplasmic proteins. Granule proteins mainly include neutrophil elastase and myeloperoxidase. Cytoplasmic proteins include representatives such as S100 calcium-binding proteins A8, A9, and A12, as well as actin and α-actin (14–16).

The formation of NETs can involve two main mechanisms, namely vital NETs and suicidal NETs (17). Suicidal NETosis is the release of DNA networks by neutrophils through apoptosis, in which the nucleus is discharged into the surrounding environment (18). When neutrophils perceive a stimulus, the stimulus directly activates the protein kinase C (PKC) and Raf-MEK-ERK-MAP kinase pathways. Next, the activation of MAP kinase will initiate the formation of the NADPH oxidase complex (19, 20), leading to the rapid generation of reactive oxygen species (ROS) (21). Neutrophil elastase (NE) and myeloperoxidase (MPO) contribute to the enhancement of nuclear membrane permeability and the promotion of chromatin formation (22), as well as in the nucleus, where NE and MPO can facilitate the digestion of histones H2b and H4 through synergistic effects (23, 24). At the same time, ROS may increase Ca^{2+} in the cytoplasm by disrupting the endoplasmic reticulum or mitochondrial membrane, thereby activating peptide-based arginine deaminase 4 (PAD4) (25). Then, PAD4 modifies histone H3 by converting arginine to citrulline, leading to chromatin depolymerization (26). During nuclear rupture, citrulline histones (26, 27) and nuclear DNA (28)are released together. The released DNA is further decorated by granular (NE and MPO) (29) and cytosolic proteins (Calpain) (30). The increase of intracellular ROS can also activate receptor interacting protein kinase 3 (RIPK3) and mixed lineage kinase domain like protein (MLKL), promoting membrane rupture (31, 32).

Vital NETosis is formed by neutrophils passing through the cell membrane and releasing DNA and proteins into the surrounding environment to form NETs (33). At first, PMA stimulates neutrophils, leading to rapid activation of intracellular NADPH oxidase and ultimately increasing ROS (19, 34). When DNA dissociated from chromatin leaves the cytoplasm through vesicles, it can be modified by granular proteins (NE, MPO, and PR3) (29, 34). Subsequently, neutrophils maintain activity and exert further functions (35), using the increased intracellular ROS to mobilize the cytoskeleton to transport particles and mitochondria (36), and using ATP to transport particles to the outside of neutrophils through actin (37).

2.2 NET components in psoriasis

There is a higher likelihood of NET formation in neutrophils in individuals with psoriasis than in healthy individuals (38). Furthermore, it has been observed that there may be alternations in NETs during the onset of the disease. A recent study suggests that NETs in individuals with psoriasis exhibit an increased presence of proteins, including inflammatory mediators and antimicrobial proteins such as histone, myeloperoxidase, neutrophil elastase LL37, and RNA-LL37 (39). These proteins are believed to play a role in the inflammatory process that contributes to the development of skin lesions.

3 NETosis is associated with the amplification of psoriasis inflammation

NETosis, a process in which neutrophils release NETs, plays a significant role in amplifying inflammation in psoriasis. During NETosis, neutrophils produce LL37, which can bind to the P2X7 receptor on monocytes and promote RNA uptake. This RNA is then directed to intracellular compartments, triggering the activation of endosomal toll-like receptors and subsequent secretion of IL-1β, leading to inflammatory vesicle activation (40).. Additionally, LL37 promotes RNA uptake by neutrophils and facilitates its transportation to intracellular compartments, resulting in TLR induction, cytokine release, and IL-8 production and CD62L shedding upon stimulation of the neutrophils (41, 42). The released IL-8 can restimulate neutrophils (43), recruiting more neutrophils to the lesion site (2). In a study conducted by Franziska et al., it was demonstrated that NETs contain RNA, and the RNA-LL37 complex has the ability to induce the release of new NETs by neutrophils, creating a repetitive cycle of immune activation that further amplifies psoriatic inflammation (42).

Furthermore, the tyrosine phosphatase SHP2 has been found to be highly correlated with neutrophils and the development of psoriasis. Experimental results from Ding Y et al. suggested that SHP2 promotes the production of NETs and increases the expression of inflammatory cytokines associated with psoriasis through the ERK5 pathway. SHP2 predominantly increased in macrophages and acts as an IL-10 inhibitor to exacerbate psoriasis progression. It is worth noting that the inhibition of SHP2 significantly improves psoriasis-like skin inflammation in mice (44–48). In addition, molecular complexes containing the adapter molecule Act1 and SHP2 mediate autonomous IL-17R signaling, thereby accelerating and maintaining inflammation (49). Consequently, SHP2 exacerbates the progression of psoriasis, making it a potential therapeutic target for the treatment of psoriasis (50).

4 Immune cells and inflammatory factors associated with NETosis in psoriasis

4.1 Neutrophils

Psoriasis, as an inflammatory skin disease, is characterized by the infiltration of neutrophils. In response to inflammatory signals, circulating neutrophils are recruited to an inflammatory site and become activated. These activated neutrophils produce and release large amounts of ROS as part of their antimicrobial activity. Two key enzymes involved in the respiratory burst and subsequent ROS production are NADPH oxidase (NOX2) (51) and MPO (52). Research has shown that neutrophils from psoriasis patients have higher MPO and NOX2 activity, leading to increased ROS release compared with neutrophils from healthy individuals (53, 54). An imbalance in ROS production, either through overproduction or insufficient clearance of ROS, can result in oxidative-stress-related dysfunctions.

In patients with psoriasis, neutrophils are pre-activated and form NETs within psoriatic lesions. These NETs are increased in blood samples and correlate with the severity of psoriasis. NETs create a highly immunogenic environment and are involved in the initial and maintenance phases of psoriasis. They are enriched in RNA, particularly LL37. When RNA binds to LL37 and subsequently stimulates neutrophils, this can lead to the release of IL-8 and a moderate shedding of CD62L (41, 42). The data suggest that the involvement of neutrophils and their activation in psoriasis highlight their significant role in the pathogenesis of the disease.

4.2 Keratinocytes

One of the central features of psoriasis is the dysregulated crosstalk between keratinocytes and immune cells. Activated keratinocytes in psoriatic skin release pro-inflammation, IL-1, TNF, and IL-6. These cytokines not only amplify the inflammatory response but also induce the production of chemokines, which attract immune cells to the sites of inflammation. These cytokines activate dendritic cells, which in turn produce the cytokines IL-12 and IL-23, leading to the differentiation of TH1 and TH17 cells.

As shown in Figure 1, in the context of NETosis, keratinocytes have been shown to be with NETs and their components. Studies have demonstrated that keratinocytes can internalize NETs and take up antimicrobial peptides, such as LL37 and human beta-defensin 2 (HBD2), present in these structures (55).

NETs, including psoriatic NETs, have been shown to induce HBD-2 mRNA production in epidermal keratin-forming cells, thereby promoting the expression of HBD-2 (56). Moreover, it is well-established that HBD-2 is primarily expressed in keratinocytes (57–59). In line with previous studies, Gambichler et al. reported elevated levels of HBD-2 in psoriatic skin compared with healthy controls (60). The induction of HBD-2 expression in keratinocytes by NETs suggests a potential mechanism through which neutrophils and keratinocytes contribute to the inflammatory cascade observed in psoriatic skin.

Similarly, Kanda et al. shed light on the association between LL37 levels and psoriasis. Their findings revealed significantly higher levels of LL-37 in the sera of patients with psoriasis than in normal subjects. Interestingly, the researchers also observed a correlation between serum LL-37 levels and HBD-2 levels in patients (61). HBD-2 is believed to contribute to psoriasis development by acting on neutrophils.

Taken together, the interaction between keratinocytes and neutrophils, mediated by factors such as HBD-2 and LL37, plays a role in promoting inflammation and NETosis in psoriasis. Further studies of HBD-2 may provide valuable insights into the pathogenesis of psoriasis and potential therapeutic targets.

4.3 Mast cells

Mast cells (MCs) play a crucial role in immune modulation through the release of various pro- and anti-inflammatory mediators (62). The role of mast cells in psoriasis has been extensively studied, with investigations indicating a significant increase in the number of mast cells at psoriatic lesions compared to healthy individuals (63). In the context of psoriasis, MCs contribute to the inflammatory state by producing caspase-1 and chymotrypsin. These enzymes play a crucial role in activating immature IL-1β into mature active IL-1β (64). The production of IL-1β by MCs further amplifies inflammation and leads to an increase in the number of infiltrating neutrophils in response to protease release (65).

Neutrophils release LL37-RNA through the NETosis process. This LL37-RNA can be recognized by MCs triggering their activation and the subsequent production of IL33. IL33 then binds to ST2R on the surface of macrophages, stimulating the production of IL-36 by macrophages.

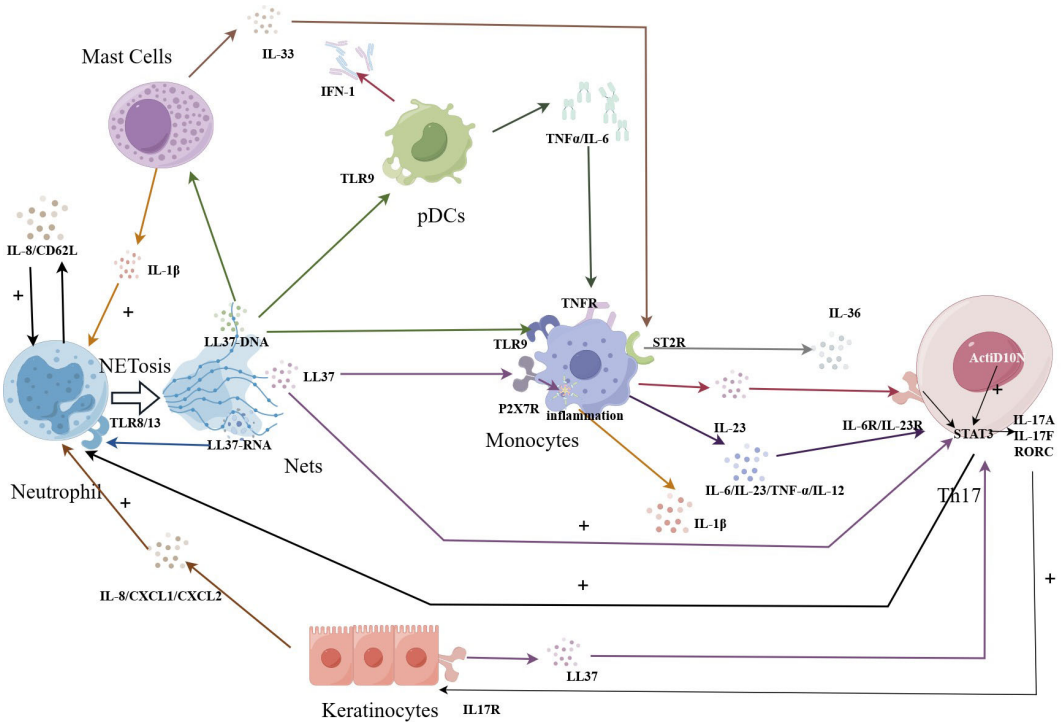

FIGURE 1
NETosis amplifies immune effects in psoriasis. The LL37-DNA causes Th17 cells to secrete cytokines, which promote keratin-forming cells to secrete LL37, which can in turn act on Th17 cells to amplify the immune effect. Beyond that, the LL37-DNA complex produced by NETosis cell can also stimulate the TLR9 receptor of PCDs, and the released TNFα binds to the receptor on monocytes. The further released IL23 can be recognized by Th17 cells. IL-17α produced by Th17 cells reactivates neutrophils, amplifying the immune and inflammatory effects.

4.4 DCs

Dendritic cells (DCs) are specialized antigen-presenting cells that play a crucial role in the immune system. They act as a bridge between innate and adaptive immunity by capturing, processing, and presenting antigens to T cells. They are a heterogeneous population of cells comprising different subpopulations, including plasmacytoid DCs (pDCs), classical/myeloid DCs (cDCs/mDCs), and monocyte-derived dendritic cells (moDCs) (66, 67). cDCs are a major subset of DCs specialized in presenting antigens to CD4+ helper T cells. Through this antigen presentation, effector T cells including Th2 and Th17 cells are activated (68). pDCs mainly produce a large amount of IFN-α and IFN-β, which can also directly activate T cells through stimulation (69). moDC can effectively express TNF- α and inducible nitric oxide synthase (iNOS), and iNOS-mediated NO production inhibits T cell proliferation (70).

In psoriasis, studies have shown that the number and activity of pDCs are increased in areas of skin lesions. These pDCs are responsible for producing interferon in psoriatic plaques (71). Interferon production by pDCs in psoriasis may trigger an immune response that exacerbates symptoms. The overproduction of interferon can lead to the abnormal proliferation of skin cells and inflammation, ultimately resulting in the formation of typical psoriatic lesions. Recent studies have also found that the overexpressed antimicrobial peptide LL37 in the skin of patients with psoriasis can form a new complex with self -DNA. These complexes can trigger TLR9 in dendritic cells, specifically pDCs (72, 73). When neutrophils capture pathogens and release NETs, the DNA-LL37 complexes within these NETs can be captured by dendritic cells, particularly pDCs. Dendritic cells then process these antigens and present them to T cells, initiating a specific immune response. This immune response involves the production of TNF-α and IL-6 (41, 73). These data indicate that the interaction between DCs and NETs is involved in the pathogenesis and progression of psoriasis.

When grouping DCs in the blood of psoriasis patients, it can be found that not only the skin but also the mDCs in the blood have Th1 polarization and Th1/Th17 recruitment abilities (74). This discovery provides a possible blood testing target for the diagnosis of psoriasis. In addition to directly stimulating Th1 polarization, mDCs can also co-culture with inflammatory polymorphonuclear leukocytes (PMNs) to form NETs, from which they can absorb antigens. This process potentially allows for antigen processing and presentation, indirectly stimulating Th1 polarization. Reducing mDCs can block the occurrence of NETosis. In a mouse model, mDCs activated by NETs can induce antineutrophil cytoplasmic antibody (ANCA) and autoimmune responses (75). When psoriasis patients experience renal organ damage, ANCA positivity may occur. mDCs may work together with NETs in this situation, exacerbating the progression of the disease.

4.5 Monocytes/macrophages

Monocytes and macrophages are key components of the immune system and play key roles in immune defense, surveillance, and self-

stabilization. They are capable of phagocytosing and eliminating intracellular parasites, foreign bacteria, and mutated tumor cells, as well as their own senescent and abnormal cells. Macrophages can differentiate into different cell subpopulations, including two representative subpopulations, M1 and M2, based on the stimulation of different stimuli and the production of different cytokines.

Studies on mouse models of psoriasis have demonstrated a strong correlation between macrophages and the severity of psoriasis (76, 77). Pathological sections of skin lesions from psoriasis patients have also shown the presence of aggregated macrophages (78). When staining the skin lesions of psoriasis patients, it can be observed that $CD68^+iNOS^+$M1 increase and $CD68^+CD163^+$M2 decrease (79). Increased M1 polarization in psoriasis patients is associated with increased disease severity (80). In addition, the number of $CD68^+$ (81) and $CD163^+$ macrophages expressing TNF-a in the dermis (82) also increased in human skin with psoriasis lesions (82). Activation of the NLRP3 inflammasome by macrophages can also be involved in psoriasis (83). Research has shown that NLRP3 may be a promising therapeutic target for the treatment of psoriasis (84). The method of inhibiting NLRP3 inflammasome activation can alleviate psoriasis inflammation (85).

In this context, when dying neutrophils release LL37 through NETosis, the P2X7 receptor on monocytes is activated. This activation triggers the release of inflammatory vesicles and the production of IL-1β (32, 33). Similarly, when LL37-DNA complexes are released, the TLR9 receptor on monocytes is activated, leading to the release of IL-6, IL-12, IL-23, and TNF-α (20). These cytokines, including those produced by pDCs, can further stimulate monocytes/macrophages to produce IL-23. The accumulation of IL-23 at the site of a skin lesion can lead to the production of additional cytokines by macrophages, including IL-17A, IL-22, and IFN-γ, in addition to TNF-α (86).

4.6 Th17 cells

It is widely recognized that IL-23 plays a crucial role in maintaining the activation of Th17 cells (87). IL-23 promotes the production of IL-17A by Th17 cells, which in turn leads to the recruitment and activation of neutrophils (88). This cytokine cascade contributes to the inflammatory response observed in psoriasis. In *in vitro* experiments, the percentage of CD3+CD4+IL-17+ (Th17) cells among T cells is significantly higher in the presence of NETs compared to the control group without NETs. Act1 is a key mediator for IL-17 signal transduction (89). In the presence of NETs, the downstream key factor Act1D10N of the psoriasis susceptibility gene *TRAF3IP2* mutation is enhanced, further inducing the production of Th17 cells (90). In summary, these results indicate that NETs are of great significance in the immunogenetic study of neutrophil-induced human Th17 cells and psoriasis.

Studies have indicated that NETs are abundant in environments rich in myeloid cells and memory T cells. This suggests that NETS play a role in inducing the formation of other immune cells (91, 92). Experimental findings by Evans et al. support this notion, demonstrating a link between NETs and Th17 responses in psoriasis patients. The researchers further explored this interaction, showing that NETs can induce the differentiation of memory CD4 T cells into LL37-specific Th17 cells (93). These memory T cells not only secrete IL-17A but also express IL-17F and RORC, which stimulates keratinocytes to secrete LL37 (90). Subsequently, IL-6/IL-23 secreted by monocytes induces LL37-specific Th17 cells to migrate to the epidermis, where they recognize the LL37 expressed by keratinocytes (94). This mechanism creates an immune amplification effect. Th17 cells identify the synthesis of LL37 as a T-cell antigen, and their responses are further fueled by a synergistic interaction of IL-1, IL-6, and IL-23 (34, 35).

5 NET markers as prognostic/predicting factors in psoriasis

MicroRNAs (miRNAs) are small non-coding RNAs with important roles in post-transcriptional gene expression. Deregulation of miRNAs and the corresponding target gene expression have been shown to be involved in psoriasis (95). Pathogenesis MiRNA-155 (96, 97), 210 (98), and 20b (99) are significantly increased in psoriasis lesions. Among them, the expression of miRNA-155 is increased in diseased psoriasis skin compared with normal skin (100). The pathological miRNA-210 is positively correlated with the Psoriasis Area and Severity Index (PASI) and body surface area (BSA) affected by psoriasis (99). MiRNA pathway enrichment and target gene network analysis were performed on the serum of psoriasis patients, and researchers found a high correlation between miR-214–3p, miR-7–5p, miR-761, miR-665, and miR-1207–5p (101). The above results indicate an important relationship between this miRNA and disease activity, and may encourage further studies to explore the possibility of using this miRNA as one of the markers of psoriasis severity.

Studies have shown that circulating MPO/DNA or NE/DNA conjugates, as well as plasma circulating citrullinated histone H3 (H3Cit) levels, have a stronger specificity for NET formation than evaluating microRNAs alone (102). A study was conducted on the sera of 50 adult patients with chronic plaque psoriasis and 25 healthy controls, and it was found that there was a significant difference in serum myeloperoxidase levels between the two groups (103). MPO-DNA complex level is also an important detection method. It has been reported that the MPO-DNA complex level in serum was significantly increased in patients with PsA/PsO compared with healthy controls. The level of MPO-DNA was also positively associated with the Disease Activity in Psoriatic Arthritis score (DAPAS) and its components (104). Vascular endothelial cells play an important role in maintaining the vascular barrier and controlling blood flow. Additionally, they can target immune cells to specific areas of vascular damage, infection, or foreign objects (105). According to one report, H3Cit can directly cause inflammatory damage by disrupting the microvascular endothelial barrier (106). However, currently, there are no clinical data to prove the association between H3Cit and psoriasis. Whether H3Cit can become a diagnostic marker in the blood of psoriasis patients deserves further research.

Some data suggest that circulating NETs may play a role in predicting the severity of psoriasis. However, owing to the lack of specific antibodies for NETs and specific and standardized testing methods for NETs at present, NET substitutes are usually used. These detectable alternatives include circulating cell-free DNA (cfDNA), or

circulating NET-associated proteins such as NE or MPO, and/or levels of circulating histone H3 (H3Cit) or other NET-associated proteins (107). Therefore, further research and technological improvements are needed to better define the prognosis and/or predictive ability of NETs at different stages of psoriasis.

6 The potential of drugs targeting NETosis in psoriasis

Exploring potential drugs targeting NETosis holds great promise for individuals with psoriasis, considering the involvement of NETosis in the condition. NETs inhibitors can target various stages of NETosis to inhibit the generation of NETs. For example, researchers have focused on the stress response protein REDD1, which is closely related to NETosis. Key mediators, such as endothelin-1 (ET-1) and hypoxia inducible factor-1α (HIF-1α), drive the generation of NETs through REDD1. Inhibitors like *bosentan* and *L-ascorbic acid* can respectively inhibit ET-1 and HIF-1α, thereby inhibiting NETosis in neutrophils (108).

Another crucial enzyme involved in NET formation is a protein arginine deiminase, PAD4, which catalyzes the conversion of arginine to citrulline and mediates NET formation. Drugs targeting PAD4, such as *JBI-589* (109), have shown efficacy in rheumatoid arthritis mouse models. Additionally, drugs like *dipyridamole* (110) and *cannabidiol* (111) can inhibit NETosis and have potential in the treatment of psoriasis.

Neutrophil elastase inhibitor *sivelepristal sodium* (112) and myeloperoxidase inhibitor *PF-1355* (113) have demonstrated effectiveness in inhibiting NET formation, making them potential treatments for acute respiratory distress syndrome (ARDS) or systemic inflammatory response syndrome (SIRS) with acute lung injury (ALI) (114). *Metformin* commonly used as a first-line drug for the treatment of type 2 diabetes, has also shown potential in downregulating the generation of NETs and reducing the release of NET DNA in the mouse model of systemic lupus erythematosus (SLE) (115). However, there is a need for relevant clinical and *in vivo* experiments to determine their effectiveness in psoriasis.

Clearing NETs and preventing their accumulation in the body is another approach worth considering. *Deoxyribonuclease I* (*DNase I*) (116) has been shown to effectively clear NETs in experiments. Although it can promote inflammation resolution and reduce the accumulation of ROS, it has the disadvantage of a short action time and limited range of action. In 2021, Xin's team developed a new nanocarrier that can release DNase I in response to MMP-9, effectively degrading the structure of NETs (117). This carrier successfully addresses the drawbacks of DNase I drugs. *Tofacitinib* (118), another type of NET scavenger, can simultaneously regulate the formation and degradation of NETs. A clinical trial conducted in SLE patients showed that *Tofacitinib* can reduce low-density granulocytes and circulating NETs, indicating its potential for treating psoriasis (119). Figure 2 provides a brief summary of these possible NETosis drugs.

In summary, drug development targeting NETosis and the interactions between NETs and various immune cells holds great

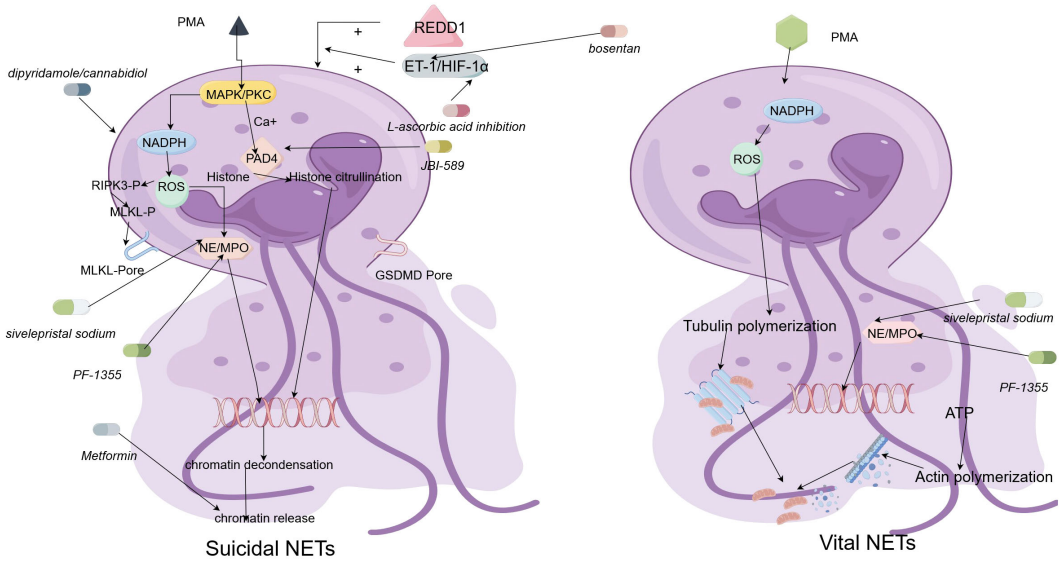

FIGURE 2
The regulatory mechanisms underlying the formation of vital neutrophil extracellular traps (Vital NETs) and suicidal neutrophil extracellular traps (Suicidal NETs), as well as a schematic diagram of the mechanism of action of NETosis drugs. The characteristics of suicidal NETosis are the production of ROS and the rupture of neutrophils. Neutrophils are stimulated and activated, inducing the phosphorylation of NOX complexes and release of ROS, a process dependent on high Ca2+concentrations. Subsequently, PAD4 is activated and causes NE and MPO to be transported from neutrophilic granules to the nucleus. NE and MPO binding to PAD4 leads to histone citrullination and chromatin deconcentration. After the nuclear membrane ruptures, the desorbed chromatin mixes with granular proteins and enters the cytoplasm. Finally, the cytoplasmic membrane leaks, and the modified chromatin is released from neutrophils, forming NETs. The formation of Vital NET can occur without NOX complexes and ROS. The formation of Vital NET is initiated by stimulation, which activates PAD4 and transports NE and MPO to the nucleus, promoting chromatin deconcentration. Decondensed chromatin decorated with granular proteins and histones is enveloped in vesicles germinating from the nucleus. Subsequently, these vesicles are expelled from intact neutrophils and form NETs near the neutrophils. Under this method, neutrophils remain intact and can further phagocytose. NETosis-related drugs can exert effects on the occurrence process of these two types of NETosis.

promise. However, it is important to consider that NETosis also serves as a mechanism to trap and kill bacteria and other pathogens. In individuals with psoriasis, skin inflammation is associated with bacterial infection, and NETosis may be a means through which neutrophils fight infection. Furthermore, the adverse reactions and success rates of new drugs are worth further discussion.

7 Conclusion

Understanding the role of NETosis in the pathogenesis of psoriasis could provide insights into potential therapeutic strategies. NETosis, psoriasis, and the immune response are interconnected and closely related. Recent advancements in understanding NETosis have the potential to improve our comprehension of the complex process of psoriasis pathogenesis. This interaction provides new insights into the molecular mechanisms underlying the disease. The regulation of NETosis is rapidly emerging as a promising therapeutic target for psoriasis. Studying the exact mechanisms of NETosis in psoriasis is of significant importance for developing novel therapeutic approaches.

Author contributions

JZ: Data curation, Writing – original draft. YF: Investigation, Writing – review & editing. DS: Writing – review & editing, Funding acquisition.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Griffiths CEM, Armstrong AW, Gudjonsson JE, Barker JNWN. Psoriasis. *Lancet*. (2021) 397:1301–15. doi: 10.1016/S0140-6736(20)32549-6

2. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. *Annu Rev Immunol*. (2014) 32:227–55. doi: 10.1146/annurev-immunol-032713-120225

3. van de Kerkhof. PC, Chang A. Migration of polymorphonuclear leukocytes in psoriasis. *Skin Pharmacol*. (1989) 2:138–54. doi: 10.1159/000210811

4. Terui T, Ozawa. M, Tagami H. Role of neutrophils in induction of acute inflammation in T cell mediated immune dermatosis psoriasis a neutrophil-associated inflammation boosting loop. *Exp Dermatol*. (2000) 9:1–10. doi: 10.1034/j.1600-0625.2000.009001001.x

5. Herlin T, Borregaard N, Kragballe. K. On the mechanism of enhanced monocyte and neutrophil cytotoxicity in severe psoriasis. *J Invest Dermatol*. (1982) 79:2104–108. doi: 10.1111/1523-1747.ep12500035

6. Reich K, Papp KA, Matheson RT, Tu JH, Bissonnette R, Bourcier M, et al. Evidence that a neutrophil–keratinocyte crosstalk is an early target of IL-17A inhibition in psoriasis. *Exp Dermatol*. (2015) 24:529–35. doi: 10.1111/exd.12710

7. Yamanaka K, Umezawa Y, Yamagiwa A, Saeki H, Kondo M, Gabazza EC, et al. Biologic therapy improves psoriasis by decreasing the activity of monocytes and neutrophils. *J Dermatol*. (2014) 41:679–85. doi: 10.1111/1346-8138.12560

8. Krueger JG, Wharton KA Jr, Schlitt T, Suprun M, Torene RI, Jiang X, et al. IL-17A inhibition by secukinumab induces early clinical, histopathologic, and molecular resolution of psoriasis. *J Allergy Clin Immunol*. (2019) 144:750–63. doi: 10.1016/j.jaci.2019.04.029

9. de Bont CM, Boelens WC, Pruijn GJM. NETosis, complement, and coagulation: a triangular relationship. *Cell Mol Immunol*. (2018) 16:19–27. doi: 10.1038/s41423-018-0024-0

10. Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med*. (2009) 15:623–5. doi: 10.1038/nm.1959

11. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci Transl Med*. (2011) 3:73ra20. doi: 10.1126/scitranslmed.3001201

12. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Sci Transl Me*. (2013) 5:178ra40. doi: 10.1126/scitranslmed.3005580

13. Pires RH, Felix SB, Delcea M. The architecture of neutrophil extracellular traps investigated by atomic force microscopy. *Nanoscale*. (2016) 8:14193–202. doi: 10.1039/C6NR03416K

14. Chapman EA, Lyon M, Simpson D, Mason D, Beynon RJ, Moots RJ, et al. Caught in a trap? Proteomic analysis of neutrophil extracellular traps in rheumatoid arthritis and systemic lupus erythematosus. *Front Immunol*. (2019) 10:423. doi: 10.3389/fimmu.2019.00423

15. Petretto A, Bruschi M, Pratesi F, Croia C, Candiano G, Ghiggeri G, et al. Neutrophil extracellular traps (NET) induced by different stimuli: A comparative proteomic analysis. *PloS One*. (2019) 14:e0218946. doi: 10.1371/journal.pone.0218946

16. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. *PloS Pathog*. (2009) 5:e1000639. doi: 10.1371/journal.ppat.1000639

17. Hoffmann JH, Enk AH. Neutrophil extracellular traps in dermatology: Caught in the NET. *J Dermatol Sci*. (2016) 84:3–10. doi: 10.1016/j.jdermsci.2016.07.001

18. Tanaka K, Shimura T, Toiyama Y, Okugawa Y, Kusunoki M. Visualization of ex vivo Neutrophil Extracellular Traps by Fluorescence Microscopy. *Bio-Protocol*. (2015) 5. doi: 10.21769/BioProtoc.1550

19. Parker H, Dragunow M, Hampton MB, Kettle AJ, Winterbourn CC. Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus. *J Leukoc Biol*. (2012) 92:841–9. doi: 10.1189/jlb.1211601

20. Pieterse E, Rother N, Yanginlar C, Gerretsen J, Boeltz S, Munoz LE, et al. Cleaved N-terminal histone tails distinguish between NADPH oxidase (NOX)-dependent and NOX-independent pathways of neutrophil extracellular trap formation. *Ann Rheum Dis*. (2018) 77:1790–8. doi: 10.1136/annrheumdis-2018-213223

21. Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nat Chem Biol*. (2011) 7:75–7. doi: 10.1038/nchembio.496

22. Thiam HR, Wong SL, Qiu R, Kittisopikul M, Vahabikashi A, Goldman AE, et al. NETosis proceeds by cytoskeleton and endomembrane disassembly and PAD4-mediated chromatin decondensation and nuclear envelope rupture. *Proc Natl Acad Sci U.S.A*. (2020) 117:7326–37. doi: 10.1073/pnas.1909546117

23. Metzler KD, Goosmann C, Lubojemska A, Zychlinsky A, Papayannopoulos V. A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. *Cell Rep*. (2014) 8:883–96. doi: 10.1016/j.celrep.2014.06.044

24. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *J Cell Biol*. (2010) 191:677–91. doi: 10.1083/jcb.201006052

25. Remijsen Q, Kuijpers TW, Wirawan E, Lippens S, Vandenabeele P, Vanden Berghe T. Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death Differ*. (2011) 18:581–8. doi: 10.1038/cdd.2011.1

26. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *J Exp Med*. (2010) 207:1853–62. doi: 10.1084/jem.20100239

27. Neeli I, Khan SN, Radic M. Histone deimination as a response to inflammatory stimuli in neutrophils. *J Immunol*. (2008) 180:1895–902. doi: 10.4049/jimmunol.180.3.1895

28. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, et al. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *J Cell Biol*. (2009) 184:205–13. doi: 10.1083/jcb.200806072

29. Brinkmann. V, Reichard. U, Goosmann. C. Neutrophil extracellular traps kill bacteria. *Science*. (2004) 303:1532–5. doi: 10.1126/science.1092385

30. Gosswein S, Lindemann A, Mahajan A, Maueröder C, Martini E, Patankar J, et al. Citrullination licenses calpain to decondense nuclei in neutrophil extracellular trap formation. *Front Immunol*. (2019) 10:2481. doi: 10.3389/fimmu.2019.02481

31. Schreiber A, Rousselle A, Becker JU, von Massenhausen A, Linkermann A, Kettritz R. Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis. *Proc Natl Acad Sci USA*. (2017) 114:E9618–25. doi: 10.1073/pnas.1708247114

32. Desai J, Kumar SV, Mulay SR, Konrad L, Romoli S, Schauer C, et al. PMA and crystal-induced neutrophil extracellular trap formation involves RIPK1-RIPK3-MLKL signaling. *Eur J Immunol*. (2016) 46:223–9. doi: 10.1002/eji.201545605

33. Yipp BG, Kubes P. NETosis: how vital is it? *Blood*. (2013) 122:2784–94. doi: 10.1182/blood-2013-04-457671

34. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ*. (2009) 16:1438–44. doi: 10.1038/cdd.2009.96

35. Cristinziano L, Modestino L, Loffredo S, Varricchi G, Braile M, Ferrara AL, et al. Anaplastic thyroid cancer cells induce the release of mitochondrial extracellular DNA traps by viable neutrophils. *J Immunol*. (2020) 204:1362–72. doi: 10.4049/jimmunol.1900543

36. Stojkov D, Amini P, Oberson K, Sokollik C, Duppenthaler A, Simon H-U, et al. ROS and glutathionylation balance cytoskeletal dynamics in neutrophil extracellular trap formation. *J Cell Biol*. (2017) 216:4073–90. doi: 10.1083/jcb.201611168

37. Amini P, Stojkov D, Felser A, Jackson CB, Courage C, Schaller A, et al. Neutrophil extracellular trap formation requires OPA1-dependent glycolytic ATP production. *Nat Commun*. (2018) 9:2958. doi: 10.1038/s41467-018-05387-y

38. Meng X, Guo R, Fan C, Li Y, Liu X, Chen X, et al. RIPK1 downregulation enhances neutrophil extracellular traps in psoriasis. *Postepy Dermatol Alergol*. (2022) 39:72–80. doi: 10.5114/ada.2022.113803

39. Guo J, Tu J, Hu Y, Song G, Yin Z. Cathepsin G cleaves and activates IL-36gamma and promotes the inflammation of psoriasis. *Drug Des Devel Ther*. (2019) 13:581–8. doi: 10.2147/DDDT

40. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. *J Immunol*. (2013) 190:1217–26. doi: 10.4049/jimmunol.1202388

41. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med*. (2011) 3:73ra19. doi: 10.1126/scitranslmed.3001180

42. Herster F, Bittner Z, Archer NK, Dickhöfer S, Eisel D, Eigenbrod T, et al. Neutrophil extracellular trap-associated RNA and LL37 enable self-amplifying inflammation in psoriasis. *Nat Commun*. (2020) 11:105. doi: 10.1038/s41467-019-13756-4

43. Fujishima S, Hoffman AR, Vu T, Kim KJ, Zheng H, Daniel D, et al. Regulation of neutrophil interleukin 8 gene expression and protein secretion by LPS, TNF-alpha, and IL-1 beta. *J Cell Physiol*. (1993) 154:478–85. doi: 10.1002/jcp.1041540305

44. Wang J, Mizui M, Zeng L-F, Bronson R, Finnell M, Terhorst C, et al. Inhibition of SHP2 ameliorates the pathogenesis of systemic lupus erythematosus. *J Clin Invest*. (2016) 126:2077–92. doi: 10.1172/JCI87037

45. Sun Z, Liu Q, Lv Z, Li J, Xu X, Sun H, et al. Targeting macrophagic SHP2 for ameliorating osteoarthritis via TLR signaling. *Acta Pharm Sin B*. (2022) 12:3073–84. doi: 10.1016/j.apsb.2022.02.010

46. Xiao P, Zhang H, Zhang Y, Zheng M, Liu R, Zhao Y, et al. Phosphatase Shp2 exacerbates intestinal inflammation by disrupting macrophage responsiveness to interleukin-10. *J Exp Med*. (2019) 216:337–49. doi: 10.1084/jem.20181198

47. Liu Q, Qu J, Zhao M, Xu Q, Sun Y. Targeting SHP2 as a promising strategy for cancer immunotherapy. *Pharmacol Res*. (2020) 152:104595. doi: 10.1016/j.phrs.2019.104595

48. Paccoud R, Saint-Laurent C, Piccolo E, Tajan M, Dortignac A, Pereira O, et al. SHP2 drives inflammation-triggered insulin resistance by reshaping tissue macrophage populations ". *Sci Transl Med*. (2021) 13:eabe2587. doi: 10.1126/scitranslmed.abe2587

49. Luo Q, Liu Y, Shi K, Shen X, Yang Y, Liang X, et al. An autonomous activation of interleukin-17 receptor signaling sustains inflammation and promotes disease progression. *Immunity*. (2023) 56:2006–2020.e6. doi: 10.1016/j.immuni.2023.06.012

50. Ding Y, Ouyang Z, Zhang C, Zhu Y, Xu Q, Sun H, et al. Tyrosine phosphatase SHP2 exacerbates psoriasis-like skin inflammation in mice via ERK5-dependent NETosis. *MedComm (2020)*. (2022) 3:e120. doi: 10.1002/mco2.120

51. Belambri SA, Rolas L, Raad H, Hurtado-Nedelec M, Dang PMC, El-Benna J. NADPH oxidase activation in neutrophils: Role of the phosphorylation of its subunits. *Eur J Clin Invest*. (2018) 48:e12951. doi: 10.1111/eci.12951

52. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil function: from mechanisms to disease. *Annu Rev Immunol*. (2012) 30:459–89. doi: 10.1146/annurev-immunol-020711-074942

53. Bloomfield FJ, MM Y. Enhanced release of inflammatory mediators from lithium-stimulated neutrophils in psoriasis. *Br J Dermatol*. (1983) 109:9–13. doi: 10.1111/j.1365-2133.1983.tb03985.x

54. Bloomfield FJ, MM Y. Enhanced chemiluminescence production by phagocytosing neutrophils in psoriasis. *Inflammation*. (1988) 12:153–9. doi: 10.1007/BF00916398

55. Albanesi C, Madonna S, Gisondi P, Girolomoni G. The interplay between keratinocytes and immune cells in the pathogenesis of psoriasis. *Front Immunol*. (2018) 9:1549. doi: 10.3389/fimmu.2018.01549

56. Hu SC, Yu HS, Yen FL, Lin CL, Chen GS, Lan CC. Neutrophil extracellular trap formation is increased in psoriasis and induces human beta-defensin-2 production in epidermal keratinocytes. *Sci Rep*. (2016) 6:311129. doi: 10.1038/srep31119

57. Ryan LK, Dai J, Yin Z, Megjugorac N, Uhlhorn V, Yim S, et al. Modulation of human beta-defensin-1 (hBD-1) in plasmacytoid dendritic cells (PDC), monocytes, and epithelial cells by influenza virus, Herpes simplex virus, and Sendai virus and its possible role in innate immunity. *J Leukoc Biol*. (2011) 90:343–56. doi: 10.1189/jlb.0209079

58. Niyonsaba F, Ushio H, Nakano N, Ng W, Sayama K, Hashimoto K, et al. Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol*. (2007) 127:594–604. doi: 10.1038/sj.jid.5700599

59. Peng G, Tsukamoto S, Ikutama R, Nguyen HLT, Umehara Y, Trujillo-Paez JV, et al. Human beta-defensin-3 attenuates atopic dermatitis-like inflammation through autophagy activation and the aryl hydrocarbon receptor signaling pathway. *J Clin Invest*. (2022) 132:e156501. doi: 10.1172/JCI156501

60. Gambichler T, Skrygan M, Tomi NS, Othlinghaus N, Brockmeyer NH, Altmeyer P, et al. Differential mRNA expression of antimicrobial peptides and proteins in atopic dermatitis as compared to psoriasis vulgaris and healthy skin. *Int Arch Allergy Immunol*. (2008) 147:17–24. doi: 10.1159/000128582

61. Frasca L, Lande. R. Role of defensins and cathelicidin LL37 in auto-immune and auto-inflammatory diseases.pdf. *Curr Pharm Biotechnol*. (2012) 13:1882–97. doi: 10.2174/138920112802273155

62. Dinarello CA. Introduction to the interleukin-1 family of cytokines and receptors: Drivers of innate inflammation and acquired immunity. *Immunol Rev*. (2017) 281:5–7. doi: 10.1111/imr.12624

63. Kritas SK, Saggini A, V G. Impact of mast cells on the skin. *Int J Immunopathol Pharmacol*. (2013) 26:855–9. doi: 10.1177/039463201302600403

64. Franza L, Carusi V, Altamura S, Caraffa A, Gallenga CE, Kritas SK, et al. Interrelationship between inflammatory cytokines (IL-1, IL-6, IL-33, IL-37) and acquired immunity. *J Biol Regul Homeost Agents*. (2019) 33:1321–6. doi: 10.23812/Editorial

65. Filippo K, Dudeck A, Hasenberg M, Nye E, Rooijen van N, Hartmann K, et al. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood*. (2013) 121:4930–7. doi: 10.1182/blood-2013-02-486217

66. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, Buck MD, et al. Type 1 interferons induce changes in core metabolism that are critical for immune function. *Immunity*. (2016) 44:1325–36. doi: 10.1016/j.immuni.2016.06.006

67. Feng J, Pucella JN, Jang G, Alcántara-Hernández M, Upadhaya S, Adams NM, et al. Clonal lineage tracing reveals shared origin of conventional and plasmacytoid dendritic cells. *Immunity*. (2022) 55:405–422.e11. doi: 10.1016/j.immuni.2022.01.016

68. Macri C, Pang ES, Patton T, O'Keeffe M. Dendritic cell subsets. *Semin Cell Dev Biol*. (2018) 84:11–21. doi: 10.1016/j.semcdb.2017.12.009

69. Villani AC, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science*. (2017) 356:eaah4573. doi: 10.1126/science.aah4573

70. Rubio MT, Means TK, Chakraverty R, Shaffer J, Fudaba Y, Chittenden M, et al. Maturation of human monocyte-derived dendritic cells (MoDCs) in the presence of prostaglandin E2 optimizes CD4 and CD8 T cell-mediated responses to protein antigens: role of PGE2 in chemokine and cytokine expression by MoDCs. *Int Immunol*. (2005) 17:1561–72. doi: 10.1093/intimm/dxh335

71. Vyas NS, Charifa A, Desman GT, McNiff JM. Distinguishing pustular psoriasis and acute generalized exanthematous pustulosis on the basis of plasmacytoid dendritic cells and MxA protein. *J Cutaneous Pathol*. (2019) 46:317–26. doi: 10.1111/cup.13430

72. Zasloff. M. Antimicrobial peptides of multicellular organisms. *Nature*. (2002) 415:389–95. doi: 10.1038/415389a

73. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang Y-H, Homey B, et al. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature*. (2007) 449:564–9. doi: 10.1038/nature06116

74. Khasawneh A, Baráth S, Medgyesi B, Béke G, Dajnoki Z, Gáspár K, et al. Myeloid but not plasmacytoid blood DCs possess Th1 polarizing and Th1/Th17 recruiting capacity in psoriasis. *Immunol Lett*. (2017) 189:109–13. doi: 10.1016/j.imlet.2017.04.005

75. Sangaletti S, Tripodo C, Chiodoni C, Guarnotta C, Cappetti B, Casalini P, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. *Blood*. (2012) 120:3007–18. doi: 10.1182/blood-2012-03-416156

76. Clark RA. Misbehaving macrophages in the pathogenesis of psoriasis. *J Clin Invest*. (2006) 116:2084–7. doi: 10.1172/JCI29441

77. Wang H. Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J Clin Invest*. (2006) 116:2105–14. doi: 10.1172/JCI27180

78. Leite Dantas R, Masemann D, Schied T, Bergmeier V, Vogl T, Loser K, et al. Macrophage-mediated psoriasis can be suppressed by regulatory T lymphocytes. *J Pathol*. (2016) 240:366–77. doi: 10.1002/path.4786

79. Kim HJ, Jang J, Lee EH, Jung S, Roh JY, Jung Y. Decreased expression of response gene to complement 32 in psoriasis and its association with reduced M2 macrophage polarization. *J Dermatol*. (2019) 46:166–8. doi: 10.1111/1346-8138.14733

80. Lin SH, Chuang HY, Ho JC, Lee CH, Hsiao CC. Treatment with TNF-alpha inhibitor rectifies M1 macrophage polarization from blood CD14+ monocytes in patients with psoriasis independent of STAT1 and IRF-1 activation. *J Dermatol Sci*. (2018) 91:276–84. doi: 10.1016/j.jdermsci.2018.05.009

81. Marble DJ, Gordon KB, Nickoloff BJ. Targeting TNFalpha rapidly reduces density of dendritic cells and macrophages in psoriatic plaques with restoration of epidermal keratinocyte differentiation. *J Dermatol Sci*. (2007) 48:87–101. doi: 10.1016/j.jdermsci.2007.06.006

82. Fuentes-Duculan J, Suárez-Fariñas M, Zaba LC, Nograles KE, Pierson KC, Mitsui H, et al. A subpopulation of CD163-positive macrophages is classically activated in psoriasis. *J Invest Dermatol*. (2010) 130:2412–22. doi: 10.1038/jid.2010.165

83. Gaire BP, Lee CH, Kim W, Sapkota A, Lee DY, Choi JW. Lysophosphatidic acid receptor 5 contributes to imiquimod-induced psoriasis-like lesions through NLRP3 inflammasome activation in macrophages. *Cells*. (2020) 9:1753. doi: 10.3390/cells9081753

84. Zhang C, Tang B, Zheng X, Luo Q, Bi Y, Deng H, et al. Analysis of the potential pyroptosis mechanism in psoriasis and experimental validation of NLRP3 in vitro and in *vivo*. *Int Immunopharmacol*. (2023) 124:110811. doi: 10.1016/j.intimp.2023.110811

85. Chen X, Chen Y, Ou Y, Min W, Liang S, Hua L, et al. Bortezomib inhibits NLRP3 inflammasome activation and NF-kappaB pathway to reduce psoriatic inflammation. *Biochem Pharmacol*. (2022) 206:115326. doi: 10.1016/j.bcp.2022.115326

86. Das A, Sinha M, Datta S, Abas M, Chaffee S, Sen CK, et al. Monocyte and macrophage plasticity in tissue repair and regeneration. *Am J Pathol*. (2015) 185:2596–606. doi: 10.1016/j.ajpath.2015.06.001

87. Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, et al. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet*. (2009) 41:199–204. doi: 10.1038/ng.311

88. Stritesky GL, Yeh N, Kaplan. MH. IL-23 promotes maintenance but not commitment to the Th17 lineage. *J Immunol*. (2008) 181:5948–55. doi: 10.4049/jimmunol.181.9.5948

89. Wu L, Zepp J, Li X. Function of Act1 in IL-17 family signaling and autoimmunity. *Adv Exp Med Biol*. (2012) 946:223–35. doi: 10.1007/978-1-4614-0106-3_13

90. Lambert S, Hambro CA, Johnston A, Stuart PE, Tsoi LC, Nair RP, et al. Neutrophil extracellular traps induce human Th17 cells: effect of psoriasis-associated TRAF3IP2 genotype. *J Invest Dermatol*. (2019) 139:1245–53. doi: 10.1016/j.jid.2018.11.021

91. Kim J, Krueger JG. The immunopathogenesis of psoriasis. *Dermatol Clin*. (2015) 33:13–23. doi: 10.1016/j.det.2014.09.002

92. Clark RA. Resident memory T cells in human health and disease. *Sci Transl Med*. (2015) 7:269rv1. doi: 10.1126/scitranslmed.3010641

93. Hobbs RP, Smith SH, Getsios S. Act1: A psoriasis susceptibility gene playing its part in keratinocytes. *J Invest Dermatol*. (2017) 137:1410–2. doi: 10.1016/j.jid.2017.01.023

94. Brembilla NC, Senra L, Boehncke WH. The IL-17 family of cytokines in psoriasis: IL-17A and beyond. *Front Immunol*. (2018) 9:1682. doi: 10.3389/fimmu.2018.01682

95. Masalha M, Sidi Y, Avni D. The contribution of feedback loops between miRNAs, cytokines and growth factors to the pathogenesis of psoriasis. *Exp Dermatol*. (2018) 27:603–10. doi: 10.1111/exd.13520

96. Zibert JR, Lovendorf MB, Litman T, Olsen J, Kaczkowski B, Skov L. MicroRNAs and potential target interactions in psoriasis. *J Dermatol Sci*. (2010) 58:177–85. doi: 10.1016/j.jdermsci.2010.03.004

97. Xia J, Zhang W. MicroRNAs in normal and psoriatic skin. *Physiol Genomics*. (2014) 46:113–22. doi: 10.1152/physiolgenomics.00157.2013

98. Zhao M, Wang LT, Liang GP, Zhang P, Deng XJ, Tang Q, et al. Up-regulation of microRNA-210 induces immune dysfunction via targeting FOXP3 in CD4(+) T cells of psoriasis vulgaris. *Clin Immunol*. (2014) 150:22–30. doi: 10.1016/j.clim.2013.10.009

99. El-Komy M, Amin I, El-Hawary MS, Saadi D, Shaker O. Upregulation of the miRNA-155, miRNA-210, and miRNA-20b in psoriasis patients and their relation to IL-17. *Int J Immunopathol Pharmacol*. (2020) 34:2058738420933742. doi: 10.1177/2058738420933742

100. Hou RX, Liu RF, Zhao XC, Jia YR, An P, Hao ZP, et al. Increased miR-155-5p expression in dermal mesenchymal stem cells of psoriatic patients: comparing the microRNA expression profile by microarray. *Genet Mol Res*. (2016) 15:0.4238/gmr.15038631. doi: 10.4238/gmr.15038631

101. Xiao S, Liu X, Wang X, Lv H, Zhao J, Guo X, et al. Plasma microRNA expression profiles in psoriasis. *J Immunol Res*. (2020) 2020:1–12. doi: 10.1155/2020/1561278

102. Yoo DG, Floyd M, Winn M, Moskowitz SM, Rada B. NET formation induced by Pseudomonas aeruginosa cystic fibrosis isolates measured as release of myeloperoxidase-DNA and neutrophil elastase-DNA complexes. *Immunol Lett*. (2014) 160:186–94. doi: 10.1016/j.imlet.2014.03.003

103. Dilek N, Dilek AR, Taskin Y, Erkinuresin T, Yalcin O, Saral Y. Contribution of myeloperoxidase and inducible nitric oxide synthase to pathogenesis of psoriasis. *Postepy Dermatol Alergol*. (2016) 33:435–9. doi: 10.5114/ada.2016.63882

104. Li B, Li G, Yang X, Song Z, Wang Y, Zhang Z. NETosis in psoriatic arthritis: serum MPO-DNA complex level correlates with its disease activity. *Front Immunol*. (2022) 13:911347. doi: 10.3389/fimmu.2022.911347

105. Krüger G, Blocki, Franke, Jung. Vascular endothelial cell biology: an update. *Int J Mol Sci*. (2019) 20:4411. doi: 10.3390/ijms20184411

106. Meegan JE, Yang. X, Beard R Jr. Citrullinated histone 3 causes endothelial barrier dysfunction. *Biochem Biophys Res Commun*. (2018) 503:1498–502. doi: 10.1016/j.bbrc.2018.07.069

107. Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, et al. DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. *Nature*. (2020) 583:133–8. doi: 10.1038/s41586-020-2394-6

108. Frangou E, Chrysanthopoulou A, Mitsios A. REDD1 autophagy pathway promotes thromboinflammation and fibrosis in human systemic lupus erythematosus (SLE) through NETs decorated with tissue factor (TF) and interleukin-17A (IL-17A).". *Ann Rheum Dis*. (2019) 78:238–48. doi: 10.1136/annrheumdis-2018-213181

109. Deng H, Lin C, Garcia-Gerique L, Fu S, Cruz Z, Bonner EE, et al. A novel selective inhibitor JBI-589 targets PAD4-mediated neutrophil migration to suppress tumor progression. *Cancer Res*. (2022) 82:3561–72. doi: 10.1158/0008-5472.CAN-21-4045

110. Ali RA, Gandhi AA, Meng H, Yalavarthi S, Vreede AP, Estes SK, et al. Adenosine receptor agonism protects against NETosis and thrombosis in antiphospholipid syndrome. *Nat Commun*. (2019) 10:1916. doi: 10.1038/s41467-019-09801-x

111. Wojcik P, Garley M, Wronski A, Jablonska E, Skrzydlewska E. Cannabidiol modifies the formation of NETs in neutrophils of psoriatic patients. *Int J Mol Sci*. (2020) 21:6795. doi: 10.3390/ijms21186795

112. Gando S, Kameue T, Matsuda N, Hayakawa M, Hoshino H, Kato H. Serial changes in neutrophil-endothelial activation markers during the course of sepsis associated with disseminated intravascular coagulation. *Thromb Res*. (2005) 116:91–100. doi: 10.1016/j.thromres.2004.09.022

113. Wang CY, Lin T-T, Hu L, Xu C-J, Hu F, Wan L, et al. Neutrophil extracellular traps as a unique target in the treatment of chemotherapy-induced peripheral neuropathy. *EBioMedicine*. (2023) 90:104499. doi: 10.1016/j.ebiom.2023.104499

114. Hayakawa M, Katabami K, Wada T, Sugano M, Hoshino H, Sawamura A, et al. Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. *Shock*. (2010) 33:14–8. doi: 10.1097/SHK.0b013e3181aa95c4

115. Chen XC, Wu D, Wu HL, Li HY, Yang C, Su HY, et al. Metformin improves renal injury of MRL/lpr lupus-prone mice via the AMPK/STAT3 pathway. *Lupus Sci Med*. (2022) 9:e000611. doi: 10.1136/lupus-2021-000611

116. Zhang J, Dai Y, Wei C, Zhao X, Zhou Q, Xie L. DNase I improves corneal epithelial and nerve regeneration in diabetic mice. *J Cell Mol Med*. (2020) 24:4547–56. doi: 10.1111/jcmm.15112

117. Yin H, Lu H, Xiong Y, Ye L, Teng C, Cao X, et al. Tumor-associated neutrophil extracellular traps regulating nanocarrier-enhanced inhibition of Malignant tumor growth and distant metastasis. *ACS Appl Mater Interfaces*. (2021) 13:59683–94. doi: 10.1021/acsami.1c18660

118. Hasni SA, Gupta S, Davis M, Poncio E, Temesgen-Oyelakin Y, Carlucci PM, et al. Phase 1 double-blind randomized safety trial of the Janus kinase inhibitor tofacitinib in systemic lupus erythematosus. *Nat Commun*. (2021) 12:3391. doi: 10.1038/s41467-021-23361-z

119. Berekmeri A, Mahmood F, Wittmann M, Helliwell P. Tofacitinib for the treatment of psoriasis and psoriatic arthritis. *Expert Rev Clin Immunol*. (2018) 14:719–30. doi: 10.1080/1744666X.2018.1512404

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