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ORIGINAL RESEARCH

Proteomic Profiling and Clinical Insights: The Role of MMP9 in Differentiating Psoriasis Vulgaris from Generalized Pustular Psoriasis

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Background: Generalized pustular psoriasis (GPP) constitutes a rare, severe inflammatory disorder that differs from psoriasis vulgaris (PV). The IL-36 pathway has been identified as a key element in GPP pathogenesis.

Objective: To explore protein expression between PV and GPP, providing insights into potential mechanisms.

Methods: We performed proteomic analysis of tissue specimens from patients with PV and GPP to identify differentially expressed proteins. Comparative analysis of the proteomic data was performed and proteins with significant differences were further identified using immunofluorescence and Western blot techniques. Differential proteins were also explored by evaluating the efficacy of IL-36R inhibitors before and after GPP treatment, providing potential avenues for targeted therapeutic strategies.

Results: Tissue proteomic profiling showed that matrix metallopeptidase 9 (MMP9) increased significantly in the GPP as compared to PV. Immunofluorescence and Western blot analysis confirmed that MMP9 is higher expressed in GPP. And after therapy with IL-36 inhibitors showed that the level of MMP9 expression was markedly reduced.

Conclusion: MMP9 may be involved with the pathogenesis of GPP.

Keywords: generalized pustular psoriasis, psoriasis vulgaris, IL-36R inhibitors, matrix metallopeptidase 9, proteomics profiling

Introduction

Generalized pustular psoriasis (GPP) is a rare and severe variant of psoriasis that differs from the clinical presentation of psoriasis vulgaris (PV), also known as plaque psoriasis, in that it is characterized by widespread tiny pustules and generalized inflammation,[1] of which the global prevalence of GPP is approximately 0.27–4.6 per 10,000 people.[2] Patients with GPP have episodes of widespread eruption of macroscopically visible pustules and frequently experience comorbidities, significantly reduced quality of life, and high medication use.[3,4]

Although the underlying biological mechanisms of GPP have not been fully clarified, recent studies have reported that overexpression of the IL-36 pathway and overactivation of recruitment of neutrophils plays a key role in the pathogenesis of GPP.[5,6]

So far, the traditional systemic treatments for GPP such as retinoids, cyclosporine, and methotrexate remain highly controversial due to poor outcomes and side effects.[7] However, these are still used as the first-line non-biologic treatments for GPP in most countries, although with limited GPP-specific evidence, and no randomized controlled trial data available.[8,9]

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In recent years, biologic therapies including TNF-α inhibitors, IL-17 inhibitors, IL-23 inhibitors, and IL36 inhibitors, have been reported to be effective in the treatment of GPP.[10–15] Recently, only one GPP-specific treatment, the IL-36 receptor antagonist, spesolimab, has been approved by the Food and Drug Administration (FDA) and the European Commission for the treatment of GPP.[16,17] It was suggested that IL-36 inhibitors may reduce the acute inflammatory reaction of GPP by blocking the IL-36-associated pathway that involves the recruitment of neutrophils.[18]

In our study, we discovered that Matrix Metalloproteinase 9 (MMP9), a protein associated with neutrophil infiltration and migration, was overexpressed in GPP compared to PV and healthy controls (HC). MMP-9, a zinc-dependent endopeptidase from the MMP family, is primarily produced by neutrophils and is crucial for physiological processes such as growth, development, angiogenesis, and wound healing[19] Dysregulation of MMP9 is implicated in various diseases, playing a significant role in immune-mediated conditions, cardiovascular diseases, respiratory illnesses, and the progression of malignant tumors.[20]

However, the mechanism of the role of MMP9 in GPP is still unclear. Based on the application of spesolimab, a new treatment for GPP, this study intends to explore the relevance of MMP9 in GPP with clinical treatment.

Methods

Study Design and Patients

We enrolled three moderate-to-severe PV adults, and four GPP patients, who were admitted to the Department of Dermatology in the First Affiliated Hospital of Fujian Medical University. Participants have provided information on their medical history at baseline assessment through electronic signature and touchscreen questionnaires, interviews, and physical measurements. They also donated blood for future analysis and agreed to have health followed up through telephone and outpatient review for 2 weeks after discharge. Informed consent was obtained from all patients. The exclusion criterion for the GPP group is shown below:

a) History of allergy to IL-36R inhibitors.
b) Pregnant or breastfeeding women.
c) Severe infectious conditions.
d) History of central nervous systemic demyelinating diseases.
e) History of lymphoproliferative diseases.
f) Active and latent tuberculosis.
g) HIV carriers with a low CD4+ T cell count (<200/mL).
h) Active HBV /HCV infection.
i) In oral treatments (eg, methotrexate, cyclosporine, and acitretin), or other biologics.

Biopsy and Plasma Samples

Lesional PV, GPP (pre- and post-IL-36R inhibitors) skin punch biopsies, and plasma samples were obtained. The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University. All patients agreed to participate in this study and signed the informed consent.

Clinical Data Collection

The clinical severity of GPP was evaluated using the Generalized Pustular Psoriasis Area and Severity Index (GPPASI) and the Generalized Pustular Psoriasis Physician Global Assessment (GPPGA) respectively. Patient demographic and clinical characteristics, including age, gender, weight, prior treatment, duration of disease, triggers of the disease, family history, and associated adverse effects during treatment were recorded. Photographs of all participants were obtained for assessment of disease severity during hospitalization.

Treatment

Patients in the GPP group were given spesolimab (Boehringer Ingelheim) 15mg/kg in intravenous infusion. All subjects were thoroughly counseled, and consent was obtained before commencing any therapy.

Proteomic Profiling of Tissue Proteins

The putative markers in the skin of PV and GPP patients were detected by LC-MS/MS. The MS experiments were conducted utilizing a nanoscale EASYnLC 1200UHPLC system (Thermo Fisher Scientific) coupled to an Orbitrap Fusion Lumos instrument equipped with a nanoelectrospray source (Thermo Fisher Scientific). The mobile phases consisted of 0.1% formic acid (v/v) in water for phase A and 0.1% formic acid in 80% acetonitrile (ACN) for phase B. Peptides, dissolved in 0.1% formic acid (FA) with 2% acetonitrile, were subjected to separation on a reversed-phase high-performance liquid chromatography (RP-HPLC) analytical column (75 μm×25 cm) packed with 2 μm C18 beads (Thermo Fisher Scientific). The separation employed a linear gradient from 9% to 28% ACN in 90 minutes, followed by a linear increase to 44% B in 20 minutes, at a flow rate of 300 nL/min. The Orbitrap Fusion Lumos operated in a data-dependent mode, alternating between full-scan MS and MS2 scans. The spray voltage was set at 2.2 kV, and the ion transfer capillary temperature was maintained at 300°C. Full-scan MS spectra (350–1800 m/z) were acquired with a resolution of 120,000, automatic gain control (AGC) set at 4×10^5, and a maximal injection time of 50 ms. Selected ions were subjected to sequential fragmentation in a 3 s cycle using high collision dissociation (HCD) with 30% normalized collision energy, specific isolated windows of 1.6 m/z, and a resolution of 15,000. For MS/MS, AGC was set at 5×10^4, and the maximal injection time was 40 ms. Dynamic exclusion was implemented with a 30-second window. Ions unassigned or possessing a charge of 1+ and >7+ were excluded from MS/MS analysis.

Mass Spectrometry Data Analysis

The raw data obtained underwent processing using Proteome Discoverer (PD, version 2.2), and subsequent MS/MS spectra were subjected to searches against the curated SwissProt human proteome database. All search parameters included a precursor mass tolerance of 20 ppm, a fragment mass tolerance of 0.02Da, and variable modifications such as oxidation (Met) (+15.9949 Da) and acetylation (protein N-terminus) (+42.0106Da), with carbamidomethylation (Cys) (+57.0215Da) specified as a fixed modification. Additionally, the search allowed for three trypsin missed cleavages. Peptides with a minimum length of six amino acids were exclusively considered in the analysis. The peptide and protein identifications underwent filtration by PD, implementing stringent criteria to control the false discovery rate (FDR) to be less than 0.05%. At least one unique peptide was required for protein identification.

Data Visualization and Analysis

The examination of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted through the utilization of the Database for Annotation, Visualization, and Integrated Discovery (DAVID version 6.8) (https://david.ncifcrf.gov/). The analysis encompassed the exploration of biological processes, molecular functions, and cellular components, and this information was subsequently subjected to further analysis using Panther software, accessible through the online platform (http://www.pantherdb.org/). Heatmaps and volcano plots were done through the OmicShare platform (https://www.omicshare.com/). Heatmaps utilize the pheatmap or heatmap.2 function in the R language to generate graphs displaying protein expression patterns in different samples based on protein ID and expression value data, while volcano plots were generated by comparing log2(FC) and -log10 (FDR values) to show differentially expressed proteins, where significantly differentially expressed proteins are highlighted in different colors.

Immunofluorescence Staining

Formalin-fixed paraffin-embedded human skin samples (3 hC, 3 PV, and 3 GPP) were sectioned in 4 μm. After deparaffinization and dehydration, antigen retrieval was performed for the sections in Tris-EDTA buffer at 100 °C for 20 min. The sections were incubated for 1h at 37 °C with primary antibodies. The following primary antibodies were used: anti-MMP9 (Abcam, ab283575, 1:300), CD15 Monoclonal Antibody (Q89) (Immunoway, YM3105, 1:200). After

washing three times with PBST, these sections were incubated in dark for 1 h at room temperature with secondary antibodies including DyLight 488, goat anti-mouse (Abkine, ATUMA1901, 1:500), Goat Anti Rabbit IgG (H&L) - Alexa Fluor 594 (Immunoway, RS3611, 1:500). Finally, the nuclei were made visible by staining with Antifade Mounting Medium with DAPI (Beyotime, P0131-25mL) at room temperature for 5 min. Images were captured with an inverted fluorescence microscope (Leica, Model DMi8 manual) using the same exposure parameters. Images were analyzed with OPLENIC software (Ver.1.920).

Western Blot Analysis

As per the manufacturer's guidelines, the quantification of plasma protein concentrations was conducted employing a Bicinchoninic Acid (BCA) Protein Assay Kit sourced from Meilunbio, China. Bovine Serum Albumin (BSA) served as the standard for calibration. Subsequently, Western Blotting was employed to assess MMP9 antibodies (Abcam, ab283575, 1:1000), with an anti-rabbit secondary antibody (Cell Signaling, #7074, 1:8000) utilized in the process. For the Western Blotting procedure, a protein lysate of 10μg was loaded onto either 10% or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, the selection depending on the molecular weight of the target proteins. Following electrophoresis, the proteins were transferred to a methanol-activated polyvinylidene difluoride (PVDF) membrane (Merck Millipore, USA). The membrane was immersed in a blocking buffer containing 5% skim milk powder in 1x tris-buffered saline with Tween 20 (TBST) for one hour at room temperature. Subsequently, it was incubated overnight with the primary antibody at 4°C. Following primary antibody incubation, the membrane underwent three washes with TBST to remove excess antibodies, followed by another incubation with the secondary antibody for one hour at room temperature. The membrane was then exposed to an enhanced chemiluminescence (ECL) substrate (Merck Millipore, USA) for five minutes at room temperature, and the resulting chemiluminescent signals were captured using the Chemiscope 3300 mini (Clinx, China). This systematic procedure ensured the precise evaluation and detection of the target proteins.

Statistics

The graphical representation of data and statistical analyses were conducted using GraphPad Prism (version 9.0; GraphPad Software) for figure plotting and SPSS (version 27.0; IBM) for statistical data analysis, respectively. Continuous variables are expressed as the mean±standard deviations and medians (interquartile ranges [IQRs]). Statistical comparisons between the two groups were performed utilizing the independent-samples *t*-test (SPSS) for mean values and the Mann–Whitney *U*-test (SPSS) for medians. In scenarios involving three or more groups, the ANOVA test was employed. Statistical significance was established at a P value of less than 0.05, denoting meaningful differences between groups in the analyzed parameters.

Results

Tissue Proteomics Profiling of PV and GPP

This study included two groups of clinical patients, PV and GPP, respectively. The study protocol and disposition in this study are shown in Figure 1. To investigate the differences in proteins and signaling pathways between PV and GPP, a proteomics study was conducted using nano-liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) on tissue samples from PV and GPP patients. To determine the differentially expressed proteins (DEPs), we set two filter criteria: the multiplicity of differences was adjusted to a range greater than 1.5 and the significance threshold was adjusted to a range less than 0.05. According to our analysis, 160 DEPs passed the filter criteria (82 rose, 78 fell) (Supplemental Figure 1A and B). The heat map of hierarchical clustering indicated the candidates for differentially expressed proteins in the PV and GPP lesional skin (Figure 2). The Gene Ontology (GO) analysis was performed to evaluate the candidates for differentially expressed proteins (Supplemental Figure 1C) to obtain a comprehensive image of the proteins in lesional skin from PV and GPP patients. Moreover, these candidates DEPs in PV and GPP have been described as a connectivity protein-protein interaction (PPI) network relating to several Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways, including pathways in neutrophil extracellular trap formation, viral carcinogenesis, phagosome, complement and coagulation cascades, leukocyte transendothelial migration and

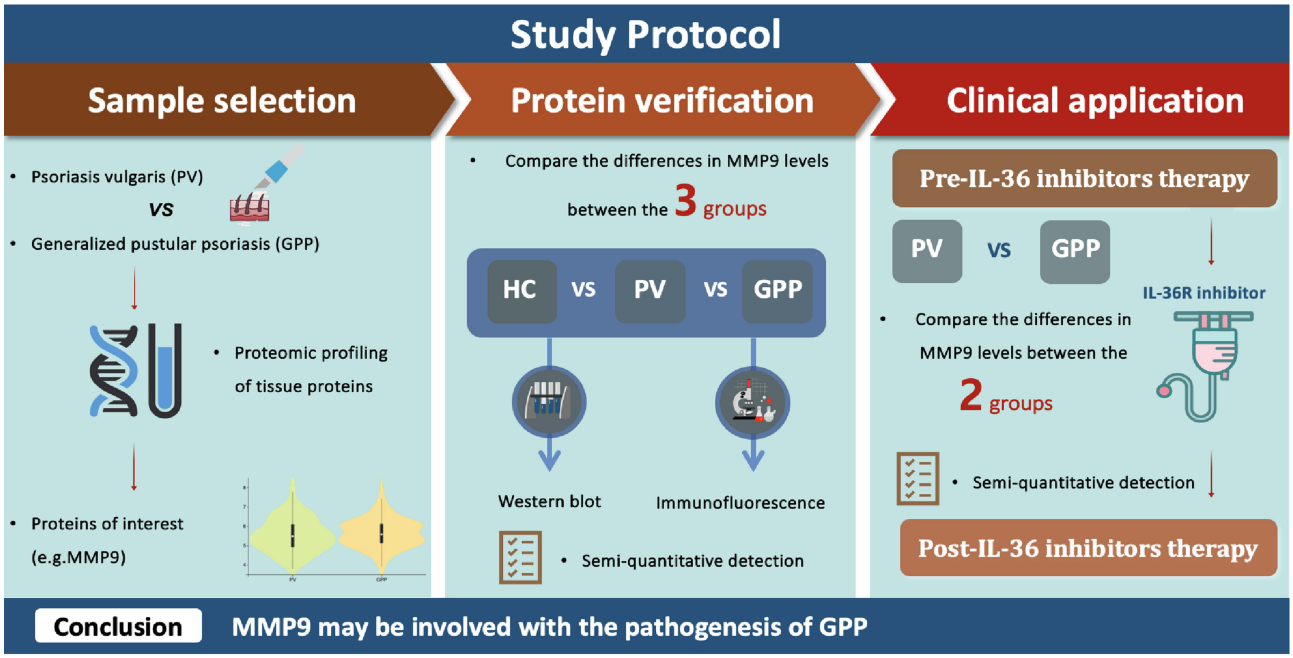

Figure 1 Study protocol. Two groups of clinical patients were enrolled in this study.

NF-kappa B (NF- kB) signaling pathway, etc. (top 15 KEGG pathways are shown in Supplemental Figure 1D). Among those DEPs, one protein of particular interest was MMP9, which belongs to the zinc-metalloproteinases family.

MMP9 Is Higher Expressed in GPP Than in PV

Tissue proteomics results showed that MMP9, which was one of the most significantly elevated plasma proteins in our study, was reported as a protein involved in extracellular matrix degradation and neutrophil migration. To investigate the relationship between MMP9 with PV and GPP, we performed further validation and statistical analysis. The Immunofluorescence results showed that MMP9 is expressed in the neutrophils of patients, especially at the pustule site of the GPP (Supplemental Figure 2). The results of Western blot analysis further validated that MMP9 levels in GPP were notably higher than both PV ($P<0.05$) and HC ($P<0.0001$) (Figure 3, Supplemental 3A). Therefore, MMP9 may play a pivotal role in modulating the development of GPP.

MMP9 Expression Level Was Positively Correlated with the Severity of GPP

To further investigate the correlation between MMP9 expression and the severity of GPP, we analyzed four patients with GPP pre- and post-use of IL-36 inhibitors.

Demographic and Clinical Characteristics

The baseline features of all subjects are demonstrated in Table 1. The mean age of the patients with GPP was 8.25±4.03 years, with a mean weight of 35.00±18.29 kg. The median score of the Generalized Pustular Psoriasis Area and Severity Index (GPPASI) in the patients was 16.50±3.03. The mean score of the Generalized Pustular Psoriasis Physician Global Assessment (GPPGA) was 2.50±0.58. The mean Children Dermatology Life Quality Index (CDLQI) score was 24.00±1.41.

Assessment of Clinical Outcomes and Safety in the GPP Group

The primary outcome parameter was the changes in GPPASI at day 15 (Table 1). At day 15, all GPP patients achieved GPPASI 90. The mean score of GPPASI was 16.50±3.02 at baseline vs 0.48±0.15 at day 15. The pustules subsided rapidly within 1 day, erythema and scale reduced gradually in two weeks (Supplemental Figure 4) with the mean score of

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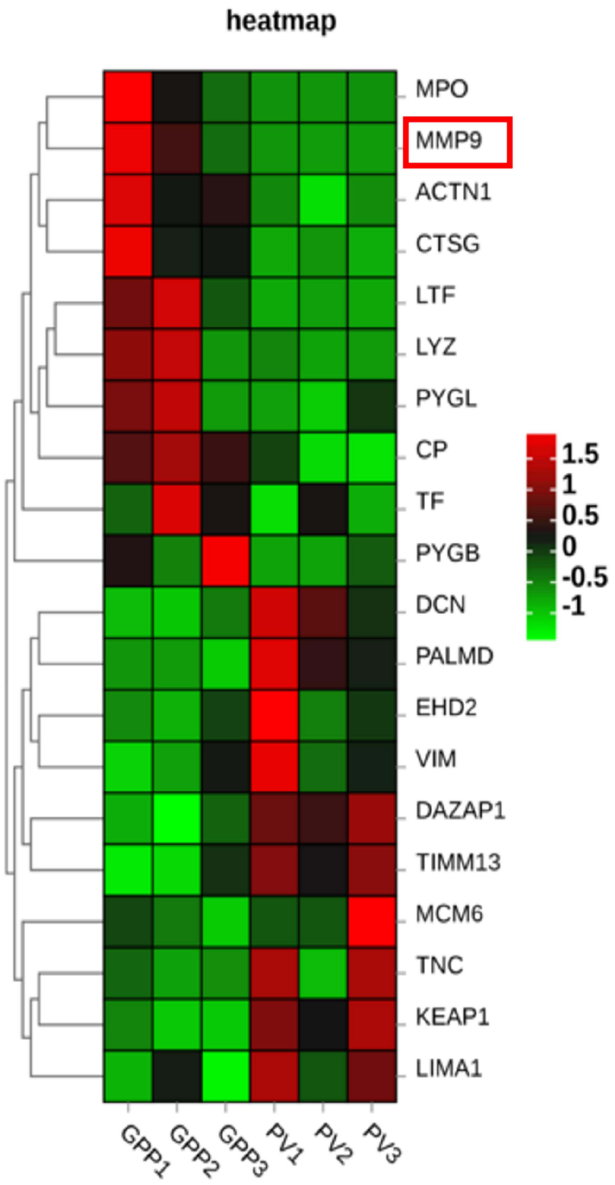

Figure 2 Tissue proteomics profiling of PV and GPP patients. The heat map of hierarchical clustering indicated the candidates for differentially expressed proteins in the PV and GPP tissue. Up/downregulated proteins are indicated by red/green color, respectively.

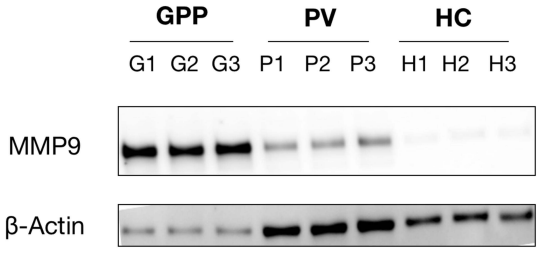

Figure 3 Protein levels of MMP9 were increased in GPP compared with PV and HC by Western blot analysis. HC, PV, and GPP patients.
Abbreviation: HC, healthy control; PV, psoriasis vulgaris; GPP, generalized pustular psoriasis.

Table 1 Characteristics of Three GPP Patients Treated with IL-36R Inhibitors

Patient	Gender	Age, years	Weight (kg)	Prior Treatment	Duration of Disease (year)	Triggers	Course of GPP(day)	Treatment	Response	Family History	Side Effect	GPPASI	GPPGA	CDLQI
1	M	6	24	Intravenous glucocorticoid + acitretin	3	N	1	Spesolimab 360mg,ivtt	Good	N	N	Baseline:17.1 day 1: 16.7 day 7: 13.8 day 15: 0.4	Baseline: 3 day 1: 2 day 7: 1 day 15: 0	Baseline: 23 day 1: 21 day 7: 8 day 15: 1
2	M	10	56	Cyclospori + acitretin	3	N	2	Spesolimab 840mg,ivtt	Good	N	N	Baseline:16.8 day 1: 15.5 day 7: 7.8 day 15: 0.7	Baseline: 2 day 1: 2 day 7: 1 day 15: 0	Baseline: 24 day 1: 23 day 7: 9 day 15: 2
3	F	13	44	Intravenous glucocorticoid + acitretin	10	Chinese medical	10	Spesolimab 660mg,ivtt	Good	N	N	Baseline:12.4 day 1: 8.8 day 7: 5.4 day 15: 0.4	Baseline: 2 day 1: 2 day 7: 1 day 15: 0	Baseline: 23 day 1: 21 day 7: 7 day 15: 2
4	M	4	16	Intravenous glucocorticoid	3	N	8	Spesolimab 240mg,ivtt	Good	N	N	Baseline:19.7 day 1: 11.7 day 7: 4.6 day 15: 0.4	Baseline: 3 day 1: 2 day 7: 1 day 15: 0	Baseline:26 day 1:20 day 7:8 day 15: 1

Abbreviations: GPP, generalized pustular psoriasis; GPPASI, Generalized Pustular Psoriasis Area and Severity Index; GPPGA, Generalized Pustular Psoriasis Physician Global Assessment; CDLQI, Children's Dermatology Life Quality Index; N, None.

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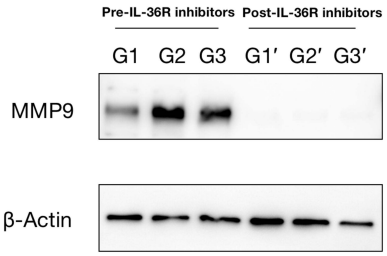

Figure 4 Protein levels of MMP9 were decreased after IL-36R inhibitors therapy.

GPPGA and CDLQI at baseline and day 15 was 2.50±0.58 vs 0.00±0.00and 24.00±1.41vs.1.50±0.58, respectively. During follow-up, no unexpected safety signals were observed.

MMP9 Levels Decreased After IL-36R Inhibitors treatment

Based on the effectiveness of IL-36R inhibitors treatment, we next performed immunofluorescence and Western blot experiments. The results showed that MMP9 expression was robustly reduced in tissue from GPP patients treated with IL-36R inhibitors ($P<0.0001$) (Figure 4, Supplemental Figures 3B and 5).

Discussion

GPP is a relatively uncommon, yet severe, and inflammatory disease characterized by the abrupt emergence of small, superficial, sterile pustules on an erythematous base. These pustules may coalesce to form larger pus-filled regions, accompanied by systemic symptoms.[7] PV represents a chronic, recurrent disease, typically manifesting as well-defined, salmon-pink plaques enveloped by silvery scales.[21] GPP and PV are distinct in terms of distribution on the body, and histopathologic and clinical appearance.[22]

To further explore the difference between PV and GPP, we performed tissue proteomic analysis, and among the variant proteins, MMP9 stood out for its clinical significance. MMP9 is a zinc-dependent endopeptidase that participates in a variety of physiological and biochemical processes and is probably involved in the pathogenesis of PV through the Erk-1/2 and p38 MAPK pathways.[23] As for GPP, which is distinguished from PV by generalized pustules, the amount of neutrophil aggregation in its pathological manifestations is more significant than that of PV.[22] So we hypothesized that MMP9, as a protein closely related to neutrophil chemotaxis, also has a role in GPP.

We performed a preliminary validation by immunofluorescence staining and Western blot and found that the expression level of MMP9 was gradually increased in HC, PV, and GPP groups, and the difference between GPP and PV groups was significant. To investigate the role of MMP9 in GPP in more depth, first, we conducted an observational prospective study to evaluate the efficacy of spesolimab, IL-36R inhibitors, in three patients with GPP. After spesolimab treatment, patients with GPP showed remarkable improvement in their lesions with a favorable safety outcome. Spesolimab is the currently only FDA-approved biologic for the treatment of GPP, and it inhibits the over-activated IL-36 pathway in GPP by targeting IL-36R and attenuating IL-36 inflammatory signals.[6,16] Subsequently, we obtained blood and tissue specimens from the above GPP patients after treatment with spesolimab and again underwent immunofluorescence and Western blot analysis validation to determine the changes in the expression level of MMP9 before and after this treatment in the GPP patients. The results demonstrated, as we expected, that MMP9 levels decreased notably after treatment with spesolimab, suggesting a possible involvement of MMP9 in the development and prognosis of GPP.

The IL-36 pathway is critical in the pathogenesis of GPP.[6] Keratinocytes (KC) are the major source of IL-36 in the skin.IL-36 expression can be induced by pro-inflammatory cytokines such as IL-1, TNF, and IL-17A. When exposed to neutrophil-derived proteases, IL-36 is enzymatically cleaved into a truncated form and has more than 500-fold biological activity. Also, this truncated IL-36 can act on KCs via IL-36R to induce more IL-36 expression and amplify the circuit. Meanwhile, IL-36 signaling activates further downstream pro-inflammatory NF-κB and MAPK pathways by binding to IL-36R, which results in the secretion of pro-inflammatory factors and cytokines by KCs, leading to the activation of neutrophils, T cells, and dendritic cells.[24] Previous studies have shown that strong expression of IL-17A was observed in GPP patients, but at significantly lower

levels than in PV patients.[24] The IL-17/IL-36 axis acts as a bridge between innate and adaptive immunity. Since the IL-36 pathway is intertwined with the TNF-α/IL-23/IL-17/IL-22 axis, an inflammatory positive feedback loop is generated. At the same time, Th17 cells may not be the only source of IL-17 in the GPP, and neutrophils can also produce additional IL-17. Thus, the difference between the PV and the GPP is that the latter involves hyperactivation of the IL-36 axis, leading to intense neutrophil chemotaxis and inflammatory responses.[8]

MMP9 is involved in the regulation of immune cell regeneration and function, and n-terminal processing of IL-8 by MMP9 was shown to enhance the inflammatory activity of neutrophils by enhancing their chemotaxis.[25] Similarly, MMP9 increased neutrophil recruitment in a mouse model of peritonitis through N-terminal processing of CXCL5.[26] In addition, MMP9 is elevated in inflammatory skin diseases such as PV, hidradenitis suppurativa, autoimmune pemphigus, rosacea, and other inflammatory skin diseases with neutrophil involvement.[27–31] IL-36γ stimulates the ability of various cell types to express neutrophil chemokines, making it a key regulator of neutrophil responses. It was shown that IL-36γ regulates MMP9 expression in oral epithelial cells, and mechanistically, MMP9 expression was shown to be induced in an IRAK1- and NF-κB-dependent manner. In addition, NF-κB signaling may allow their expression to be independently regulated by IL-36γ.[32,33] Hence, the regulation of MMP9 by the IL-36 pathway may be another important mechanism controlling neutrophil responses in the GPP.

In our study, we found that MMP9 can act as a biomarker for GPP differently from PV and is associated with a decrease in expression after improvement, a finding that facilitates our further understanding of the pathogenesis of GPP. We will focus on MMP9 in our subsequent studies to explore its specific mechanism of promoting neutrophil aggregation, pustule formation and related inflammatory responses in GPP. Due to the small sample size, we aim to continue to collect more clinical samples to better evaluate the role of MMP9 in GPP and to provide further evidence for disease monitoring and development of therapeutic targets for this refractory and recurrent disease.

Our study has some limitations. Given the small sample size and short follow-up period of our study, there is a need for higher quality, paired cohort studies in the future to confirm our findings.

In conclusion, our study identified the contribution of MMP9 in GPP and hinted that MMP9 may be involved in the IL-36 pathway, suggesting that it may work in the pathogenesis of GPP.

Patient Consent

Consent for the publication of recognizable patient photographs or other identifiable material was obtained by the authors and included at the time of article submission to the journal stating that all patients gave consent with the understanding that this information may be publicly available.

IRB Approval Status

This study was approved by The Ethics Committee of the First Affiliated Hospital of Fujian Medical University (IRB [2022]007).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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