SPRR2B knockdown inhibits proliferation and inflammatory response in M5-treated human HaCaT keratinocytes

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ABSTRACT

Psoriasis is a chronic inflammatory skin disease characterized by keratinocyte hyperproliferation and excessive immune activation, for which curative therapies remain limited. This study aimed to investigate the role of small proline-rich protein 2B (SPRR2B) in psoriasis pathogenesis. In this study, gene Expression Omnibus (GEO) dataset GSE13355 was analyzed using R Language. Cell proliferation, gene and protein expression were measured with CCK8, RT-qPCR and immunofluorescence staining, respectively. Bioinformatics analysis of the GSE13355 dataset revealed that SPRR2B was significantly overexpressed in psoriatic lesions and was positively correlated with immune cell infiltration and multiple inflammatory pathways. In an M5-induced HaCaT keratinocyte psoriatic model, SPRR2B knockdown markedly suppressed cell proliferation and promoted apoptosis via regulation of the MDM2/p53/CDKN1A axis. Furthermore, SPRR2B knockdown reduced the secretion of IL-6, IFN-γ, TNF-α, and IL-1β, and inhibited JAK1 phosphorylation. These findings demonstrated that SPRR2B promotes keratinocyte proliferation and inflammatory response in psoriasis, suggesting it may serve as a promising diagnostic biomarker and therapeutic target for the disease.

Key words: psoriasis; SPRR2B; HaCaT; JAK1; MDM2; p53.

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Introduction

Psoriasis is a common chronic inflammatory skin disease, primarily characterized by erythema, scales, and pruritus affecting 2-3% of the population worldwide.[1] The pathology involves uncontrolled keratinocyte growth and disrupted differentiation, along with lymphocytic infiltration extending into both epidermal and dermal layers.[2] In addition, it is not merely a skin disease but also a systemic immune-mediated disease that is closely associated with metabolic syndrome and other conditions.[2] The IL-23/Th17 axis is widely considered to be the core driver of psoriasis pathogenesis.[3]

Currently, psoriasis treatment strategies are divided into two major categories: «conventional therapy» and «biologics/targeted therapy.» Conventional therapy includes glucocorticoids, retinoids, methotrexate, cyclosporine, and acitretin. Targeted therapy includes TNF-α inhibitors (etanercept and infliximab), IL-17 inhibitor secukinumab and IL-23 inhibitor guselkumab.[4] However, current treatment approaches focus on symptom control rather than cure. Once medications are discontinued, most patients experience recurrence to varying degree.

The Small Proline-Rich Protein (SPRR) family is a multi-gene family that encodes a set of small proteins rich in proline. These proteins are mainly involved in epidermal differentiation, barrier function formation, and cellular stress response.[5] The human SPRR gene family contains four main categories: SPRR1, SPRR2, SPRR3, and SPRR4. Among them, SPRR2B is an important epidermal differentiation structural protein that plays a key role in maintaining epithelial tissue integrity and barrier function.[6] Its expression is regulated by estrogen and other factors, and it is associated with various skin and respiratory diseases, making it an important target protein in skin biology and disease research.[7]

JAK (Janus Kinase) signaling family is an important intracellular non-receptor tyrosine kinase family that plays a core role in cytokine signal transduction.[8] Meanwhile, the JAK pathway plays a key role in the immune pathogenesis of psoriasis; its inhibitors regulate inflammatory responses and improve clinical symptoms by inhibiting the JAK-STAT signaling pathway.[9] In this study, we analyzed skin samples from healthy controls and lesional skin samples from patients with psoriasis. We found that SPRR2B was abnormally overexpressed in the lesional skin of patients with psoriasis. Furthermore, an *in vitro* psoriasis cell model was used to further investigate the role of SPRR2B and the JAK signaling pathway in the occurrence and development of psoriasis.

Methods and Materials

Bioinformatics analysis

Microarray dataset GSE13355 from the Gene Expression Omnibus (GEO) database was included, comprising a total of 122 skin tissue samples, consisting of 64 healthy control skin samples and 58 psoriasis lesional skin samples, for screening and analysis of differentially expressed genes. The limma package (version 3.62.2) in R was used to analyze the fold change of gene expression between groups; *p*-values were corrected for multiple comparisons using the false discovery rate (FDR). Differentially expressed genes (DEGs) were screened based on the criteria of |Log2 fold change (Log2 FC)| >1 and FDR <0.05. The cluster Profiler package (version 4.14.6) in R was used to perform Gene Ontology (GO) enrichment analysis, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, with *p*-value <0.05 used to screen significantly enriched pathways.

The pROC package (version 1.18.5) in R was used for Receiver Operating Characteristic (ROC) analysis (prognosis-related analysis) to evaluate the area under the ROC curve (AUC). Additionally, the CIBERSORT software was used to calculate the relative proportions of 22 immune cell types in each sample.[10] Using a deconvolution algorithm, 547 barcode genes from the reference profile were used to characterize the composition of infiltrating immune cells. The sum of all estimated immune cell type proportions in each sample equals 1.

Cell culture and M5 cytokines stimulation

Human HaCaT keratinocytes were purchased from Meisen Cell (Hangzhou, China). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, Waltham, MA, USA) containing 10% Fetal Bovine Serum (FBS, Gibco) and 1% penicillin-streptomycin (Hyclong, Logan, UT, USA) and cultured at 37°C with 5% CO_2. A psoriasis-like keratinocyte model was established by adding an M5 cytokine cocktail (IL-17A, IL-22, oncostatin M, IL-1α, and TNF-α, each at a final concentration of 2.5 ng/ml) to the medium of HaCaT keratinocytes.

SPRR2B siRNA transfection

HaCaT keratinocytes were plated in cell plates and incubated for 24 hours. After cells achieved 60-80% confluency, treatment with SPRR2B siRNA1 (5'-AGCUUAUCAUGGAUUCAUAdT dT-3'), siRNA2 (5'-CCAGCCAAAGUAUCCACCGdTdT-3') or negative control (NC; 5'-AGCUGGAAUAUUAUUCUCAdTdT-3') using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) for 24 h at 37°C.

Cell viability assay

Cell viability was measured with CCK-8 assay. HaCaT keratinocytes were plated in 96-well plates and cultured for 24 h. Cells at 50-60% confluence were treated with SPRR2B siRNA or/and M5 cytokines for 24 h. Then, 10 µl of CCK-8 solution was added to each well and incubated at 37°C with 5% CO_2 for 2 h. Absorbance value at 450 nm was measured using Multiskan Spectrum (Thermo Fisher Scientific) and was directly proportional to the number of living cells.

RT-qPCR assay

Total RNA was extracted employing Trizol reagent (Ambion, Waltham, MA, USA), and cDNA was generated through reverse transcription with HiScript Reverse Transcriptase (RNase H) (VAZYME). Quantitative PCR analysis was executed utilizing SYBR Green Master Mix (VAZYME) for gene expression profiling. Gene expression data were normalized to GAPDH and analyzed by the 2-ΔΔCt method. Primer sequences used in this study are summarized in Table 1.

Cell proliferation analysis

HaCaT keratinocytes were plated in 6-well plates and incubated for 24 hours. After cells achieved 60-80% confluency, treatment with SPRR2B siRNA and/or M5 cytokines was performed for 24 h. To assess HaCaT cell proliferation, a Ki67 kit (Beyotime, Shanghai, China) was employed. For staining, HaCaT cells were fixed with 4% paraformaldehyde for 2 h at room temperature. Subsequent staining with Ki67 solution was carried out at 37°C for 1 h in the dark, followed by DAPI incubation for 15 min at room temperature. Finally, fluorescence microscopy (Nikon Co., Tokyo,

Japan) was used to quantify Ki-positive cells from three randomly selected fields.

Western blotting analysis

HaCaT keratinocytes were seeded in 6-well plates and cultured for 24 h. Upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. Subsequently, cells were harvested and lysed on ice for 20 min in cell lysis buffer (Beyotime). Protein concentration in the supernatants was determined using a BCA protein assay kit (Pierce, Thermo Fisher Scientific). Approximately 10 μg of total protein per lane was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred onto

Table 1. Sequences of primers used in this study..

Name	Primer	Sequence (5'-3')
Homo GAPDH	Forward	TCAAGAAGGTGGTGAAGCAGG
	Reverse	TCAAAGGTGGAGGAGTGGGT
Homo SPRR2B	Forward	CATGTCCACCCCCGAAGTG
	Reverse	TCGGTGGATACTTTGGCTGG
Homo CK10	Forward	TCTTTGGAGGGGGCAGTTTC
	Reverse	TCCAGAGCCCGAACTTTGTC
Homo CK1	Forward	GGCTAAAGGCTGCAACAAAG
	Reverse	TCCTGAAAAGAATGCGGAAT
Homo CXCL1	Forward	TCCAAAGTGTGAACGTGAAGTC
	Reverse	TTGGATTTGTCACTGTTCAGCA

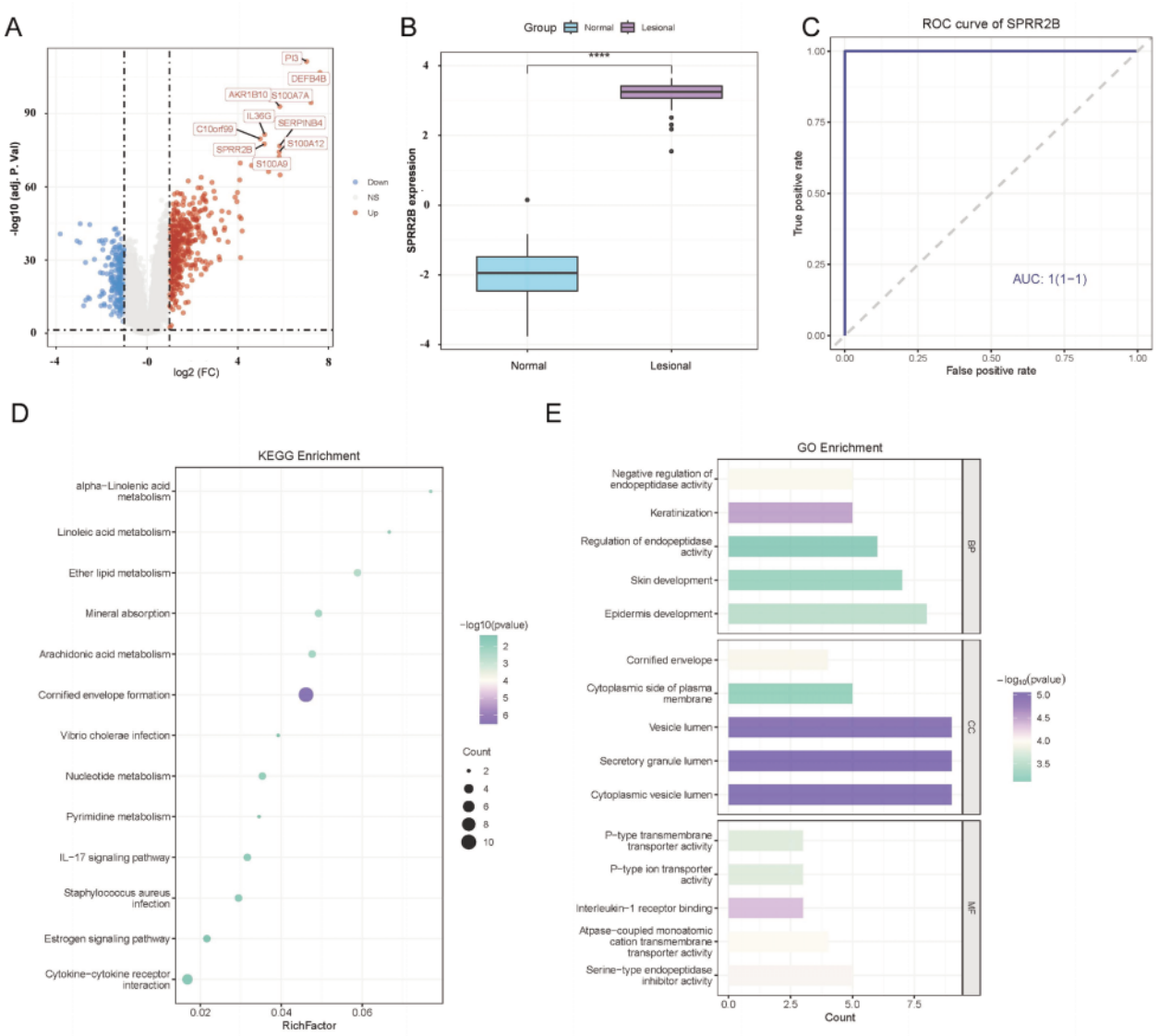

Figure 1. SPRR2B was abnormally overexpressed in the lesional skin of patients with psoriasis. (**A**) Microarray dataset GSE13355 comprising a total of 122 skin tissue samples, consisting of 64 healthy control skin samples and 58 psoriasis lesional skin samples; the limma package (version 3.62.2) in R was used to analyze the fold change of gene expression between groups. (**B**) The expression of SPRR2B in this dataset was analyzed. (**C**) The pROC package (version 1.18.5) in R was used for ROC analysis (prognosis-related analysis) to evaluate the area under the ROC curve (AUC). (**D,E**) The cluster Profiler package (version 4.14.6) in R was used to perform Gene Ontology (GO) enrichment analysis, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, with $p<0.05$ used to screen significantly enriched pathways.

polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk at room temperature for 2 h. Following blocking, the membranes were incubated with primary antibodies against Bax, p-Bcl-2, cleaved caspase 3, and β-actin (all at 1:1000 dilution) overnight at 4°C. After washing with TBS-T buffer three times, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 2 h. Subsequently, the membranes were incubated with a chemiluminescence substrate (Pierce, Thermo Fisher Scientific). Protein signals were visualized using the ChemiDoc™ XRS + System (Bio-Rad Laboratories, Hercules, CA, USA), and band intensities were quantified with ImageJ software. β-actin was used as the loading control.

Immunofluorescence staining

HaCaT keratinocytes were seeded in 24-well plates and cultured for 24 h. Upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. After blocking with BSA, cells were incubated overnight at 4°C with primary antibodies specific for SPRR2B, p53, CDKN1A, MDM2 or p-JAK1. Subsequently, cells were treated with fluorescently labeled secondary antibodies at room temperature for immunostaining. Nuclei were counterstained using DAPI-containing mounting medium to facilitate visualization. Final image acquisition was performed using a fluorescence microscope (Nikon Co.).

TUNEL staining

HaCaT keratinocytes were seeded in 24-well plates and cultured for 24 h. Upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. Cell apoptosis was assessed using the One Step TUNEL Apoptosis Assay Kit (Beyotime). In brief, cells were fixed with 4% paraformaldehyde for 30 min at room-temperature, followed incubated with 0.3% Triton X-100 for 5 min at room-temperature. Subsequently, a TUNEL reagent staining was conducted according to the manufacturer's protocol. Nuclei were counterstained with DAPI, and images were captured using a fluorescence microscope. TUNEL-positive cells from three randomly selected fields were quantified.

Statistical analysis

Statistical comparisons between groups were analyzed by Student's t-test or one-way ANOVA followed by Tukey's test using GraphPad Prism software. Results are expressed as mean ±SD. A p-value <0.05 was taken as the threshold for statistical significance.

Results

SPRR2B was abnormally overexpressed in the lesional skin of patients with psoriasis

We firstly analyzed skin samples from healthy controls and lesional skin samples from patients with psoriasis using the

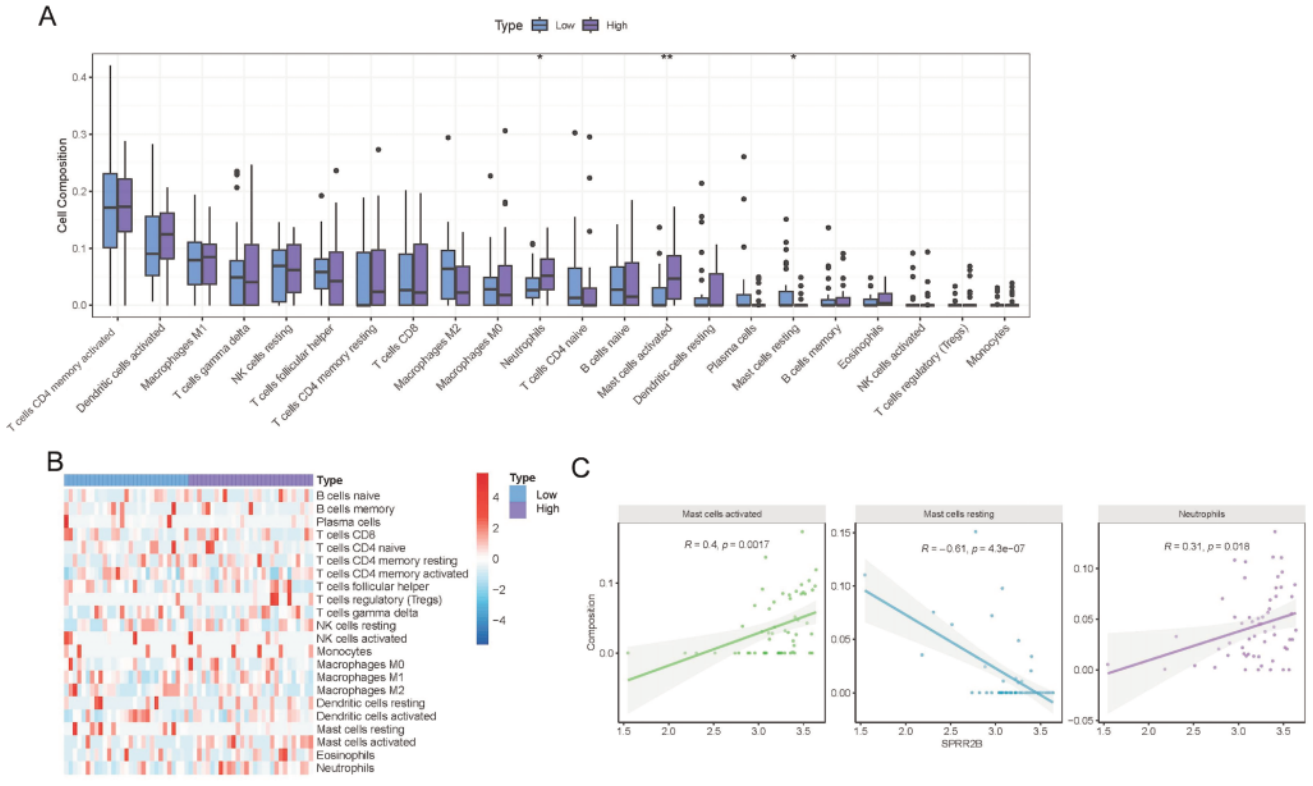

Figure 2. SPRR2B demonstrated a significant association with immune cell infiltration in the skin lesions of psoriasis patients. (**A**) Using a deconvolution algorithm, 547 barcode genes from the reference profile were used to characterize the composition of infiltrating immune cells. The sum of all estimated immune cell type proportions in each sample equals 1. (**B,C**) The CIBERSORT software was used to calculate the relative proportions of 22 immune cell types in each sample.

GSE13355 dataset. Compared with the normal group, there were 248 down-regulated genes and 452 up-regulated genes in the disease group (Figure 1A). Based on gene expression profiles and diagnostic accuracy for the disease, combined with literature review.[11,12] SPRR2B was selected as the follow-up research gene (Figure 1 B,C). Subsequently, the disease group was divided into high-expression group and low-expression group. The differentially expressed genes between the two groups were analyzed and pathway enrichment was performed. The results showed that SPRR2B was significantly associated with 13 KEGG pathways (Figure 1D), 157 biological processes (BP), 27 cellular components (CC), 55 molecular functions (MF); and significantly associated with 25 Hallmark pathways (Figure 1E). Therefore, we hypothesized that SPRR2B could play a role, and this self-amplifying positive feedback loop may significantly contribute to psoriasis pathogenesis.

SPRR2B demonstrated a significant association with immune cell infiltration in the skin lesions of psoriasis patients

By utilizing CIBERSORT algorithm, we compared immune cell infiltration profiles between high and low SPRR2B expression groups. Compared with the low expression group, the proportions of neutrophils and activated mast cells were significantly elevated in the high expression group, whereas the proportion of resting mast cells was significantly reduced (Figure 2 A,B). Furthermore, SPRR2B expression levels showed a significant positive correlation with neutrophil and activated mast cell proportions, and a significant negative correlation with the proportion of resting mast cells (Figure 2C). These results suggested SPRR2B has a significant association with immune cell infiltration in the skin lesions of psoriasis patients.

SPRR2B knockdown reversed M5-induced HaCaT keratinocytes proliferation

In order to further explore the role of SPRR2B in the progression of psoriasis, an *in vitro* psoriatic model was applied. We first knocked down the expression of SPRR2B in HaCaT cells using SPRR2B-specific siRNAs (Figure 3A). M5 treatment significantly upregulated the levels of psoriasis markers CK10, CK1, and CXCL1 in HaCaT cells, while these effects were reversed by SPRR2B-specific siRNA2 (Figure 3B). In addition, significant upregulation of SPRR2B was observed in M5-treated HaCaT cells, while this effect was reversed by SPRR2B-specific siRNA2 (Figure 3C). Ki67 staining assay demonstrated that M5-induced HaCaT keratinocyte proliferation was notably inhibited by SPRR2B-specific siRNA2 (Figure 3D). Furthermore, SPRR2B-specific siRNA2 was able to induce obvious apoptosis in M5-treated HaCaT cells. All these data suggest that SPRR2B knockdown was able to inhibit M5-induced HaCaT keratinocyte proliferation by inducing apoptosis.

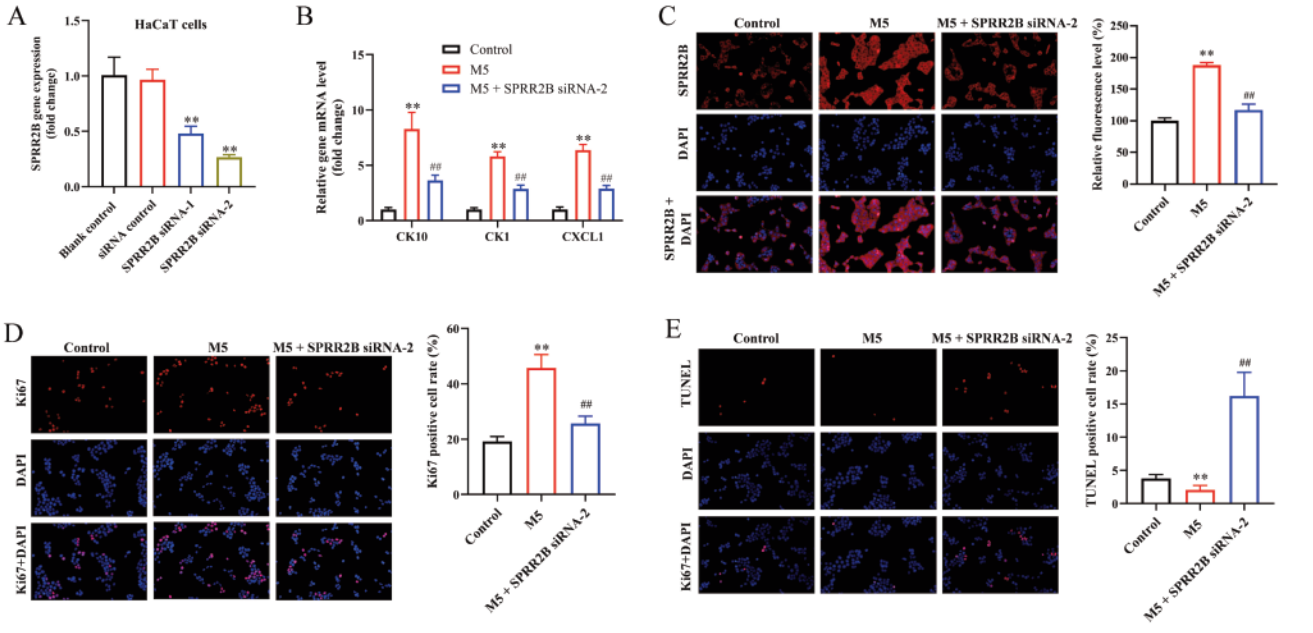

Figure 3. SPRR2B knockdown reversed M5-induced HaCaT keratinocytes proliferation. (**A**) HaCaT keratinocytes were treated with SPRR2B siRNA1, SPRR2B siRNA2 or SPRR2B siRNA NC for 24 h; the gene level of SPRR2B was detected with RT-qPCR. HaCaT keratinocytes were seeded in cell plates and cultured for 24 h; upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. (**B**) The gene level of markers CK10, CK1, and CXCL1 in HaCaT cells was measured with RT-qPCR. (**C**) The protein level of SPRR2B was detected with immunofluorescence staining. (**D**) The cell proliferation was detected with Ki67 staining. (**E**) The cell apoptosis was measured using TUNEL staining assay. $^{**}p<0.01$ compared with control group; $^{\#\#}p<0.01$ compared with M5 group; n=3.

SPRR2B knockdown reversed M5-induced apoptosis of HaCaT keratinocytes

Since SPRR2B was reported to be closely associated with the MDM2/p53 pathway,[13] Western blot and IF assays were performed for further investigation. Western blot results demonstrated that SPRR2B knockdown significantly increased pro-apoptotic protein levels, including Bax and cleaved caspase-3, while decreasing the anti-apoptotic protein Bcl-2 expression in M5-treated HaCaT cells (Figure 4A). Additionally, M5 inhibited p53 and CDKN1A expression, but these effects were reversed by SPRR2B knockdown (Figure 4 B,). Consistently, M5 promoted MDM2 expression, which was also reversed by SPRR2B knockdown (Figure 4D). Collectively, these results indicate that SPRR2B knockdown reverses M5-induced apoptosis in HaCaT keratinocytes by regulating the MDM2/p53 pathway.

SPRR2B knockdown suppressed M5-induced cytokine upregulation in HaCaT keratinocyte

To further investigate the effects of SPRR2B knockdown on the production of inflammatory cytokines in M5-treated HaCaT keratinocytes, ELISA assays were conducted to assess cytokine levels. Treatment with M5 significantly increased the levels of IL-6, IFN-γ, TNF-α, and IL-1β in the supernatant of HaCaT cells (Figure 5 A-D); notably, these up-regulatory effects were attenuated following SPRR2B knockdown. In addition, M5-induced p-JAK upregulation in HaCaT cells were revered by SPRR2B knockdown (Figure 5E). All the data shown above indicate that SPRR2B knockdown suppressed M5-induced cytokine upregulation in HaCaT keratinocytes and suggest a close involvement with JAK pathway signaling.

Discussion

Psoriasis is a chronic immune-mediated inflammatory skin disorder driven by aberrant keratinocyte hyperproliferation, impaired differentiation, and excessive inflammatory activation, with the IL-23/Th17 axis and JAK-STAT signaling as core pathogenic pathways.[1] In the present study, we identified that SPRR2B was significantly overexpressed in psoriatic lesions through bioinformatics analysis of the GSE13355 dataset, and its expression was closely correlated with multiple inflammation-related signaling pathways. Functional experiments further demonstrated that knockdown of SPRR2B suppressed M5-induced proliferation and inflammatory response in HaCaT keratinocytes, at least partially *via* regulating the MDM2/p53 apoptotic axis and inhibiting JAK signaling activation. These findings suggest that SPRR2B acts as a pro-inflammatory and pro-proliferative regulator in psoriasis, representing a potential diagnostic marker and therapeutic target.

SPRR2B belongs to the small proline-rich protein (SPRR) family, which is characterized by high proline content and plays critical roles in epidermal differentiation, barrier function, and cellular stress responses.[6] SPRR2B is primarily expressed in epithelial tissues, including skin, where it participates in maintaining tissue integrity and protection against environmental stressors.[14] Previous studies have reported aberrant expression of SPRR family members, including SPRR2B, in skin barrier disorders such as atopic dermatitis and in inflammatory skin conditions.[13] Consistent with these findings, our bioinformatics analysis revealed that SPRR2B was markedly upregulated in psoriatic lesions and demonstrated high diagnostic potential, suggesting that SPRR2B may serve as a candidate molecular biomarker, warranting further validation in clinical studies. Furthermore, The novel contributions

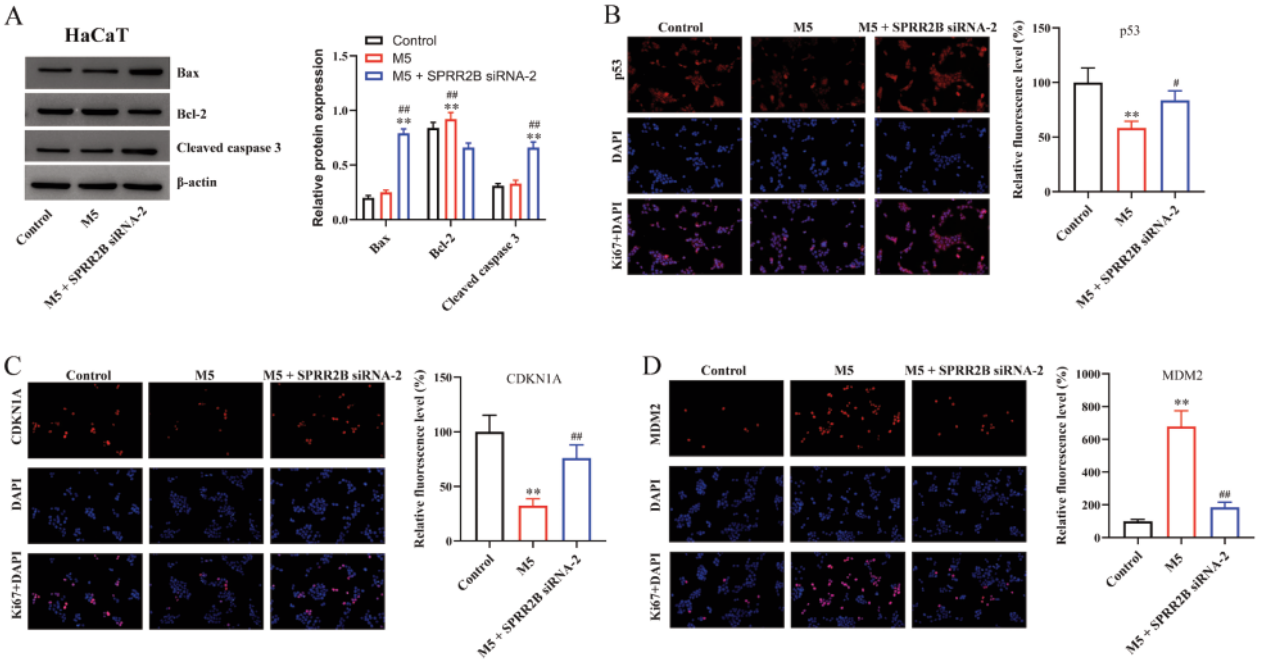

Figure 4. SPRR2B knockdown reversed M5-induced apoptosis of HaCaT keratinocytes. HaCaT keratinocytes were seeded in cell plates and cultured for 24 h. Upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. (**A**) Bax, Bcl-2 and cleaved caspase 3 protein expressions were measured with Western blot. (**B-D**) p53, CDKN1A and cleaved caspase 3 protein expressions were measured with immunofluorescence. $^{**}p<0.01$ compared with control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with M5 group; n=3.

of this study include our discovery that elevated SPRR2B expression correlated with increased infiltration of neutrophils and activated mast cells, immune cell populations known to be critical effectors in psoriatic inflammation.[15] Secondly, we first found SPRR2B may contribute to psoriasis pathogenesis by modulating local immune cell infiltration and amplifying inflammatory cascades, thereby expanding our understanding of SPRR2B's role in immune-mediated skin diseases.

The M5 cytokine cocktail is widely used to establish an *in vitro* psoriasiform keratinocyte model that recapitulates key pathological features, including keratinocyte hyperproliferation and proinflammatory cytokine production.[16] In this study, M5 stimulation significantly upregulated SPRR2B expression in HaCaT cells, concomitant with enhanced proliferation, elevated psoriasis-related markers (CK10, CK1, CXCL1), and suppressed apoptosis. Notably, SPRR2B knockdown reversed these M5-induced effects *via* inhibiting proliferation and promoting apoptosis of HaCaT cells. Mechanistically, we confirmed that SPRR2B knockdown upregulated pro-apoptotic proteins Bax and cleaved caspase-3, downregulated anti-apoptotic Bcl-2, and restored the expression of p53 and CDKN1A (p21) while reducing MDM2 levels. The MDM2/p53/CDKN1A signaling pathway is a critical cell growth inhibitory pathway, in which MDM2 acts as an important negative feedback regulator of the p53 protein by binding to p53 and promoting its ubiquitin-mediated degradation to suppress p53 activity. When cells are subjected to stress stimuli such as DNA damage, p53 is activated and stabilized, subsequently inducing the expression of downstream target gene CDKN1A, leading to cell cycle arrest or apoptosis.[17] These data are consistent with previous reports that SPRR2B promotes cell proliferation by accelerating p53 degradation *via* SPRR2B.[13] Our results therefore clarify that SPRR2B drives keratinocyte hyperproliferation in psoriasis by inhibiting p53-dependent apoptosis through the MDM2/p53/ CDKN1A axis. Inflammation serves as a core driver of psoriasis pathogenesis, with sustained cytokine release contributing to keratinocyte dysfunction and amplifying immune cell infiltration.[18,19] Our results showed that M5 significantly upregulated IL-6, IFN-γ, TNF-α, and IL-1β in HaCaT cells, and this upregulation was markedly attenuated by SPRR2B knockdown. Furthermore, SPRR2B silencing suppressed M5-induced JAK1 phosphorylation. The JAK signaling pathway functions as a critical mediator of multiple cytokines in psoriasis pathogenesis, and JAK inhibitors have demonstrated clinical efficacy in reducing psoriatic inflammation.[9] Our findings demonstrate that SPRR2B promotes inflammatory responses in keratinocytes through JAK signaling activation, establishing a positive feedback loop that maintains psoriatic inflammation. Taken together, these results indicate that SPRR2B serves as a critical regulator that connects keratinocyte proliferation, apoptosis, and inflammatory signaling in psoriasis. This study is subject to several limitations. First, the conclusions are based solely on *in vitro* cell experiments and bioinformatics analyses; future studies employing *in vivo* animal models and clinical sample validation are necessary to further confirm the role of SPRR2B. Second, the precise molecular mechanism underlying SPRR2B-mediated regulation of JAK phosphorylation

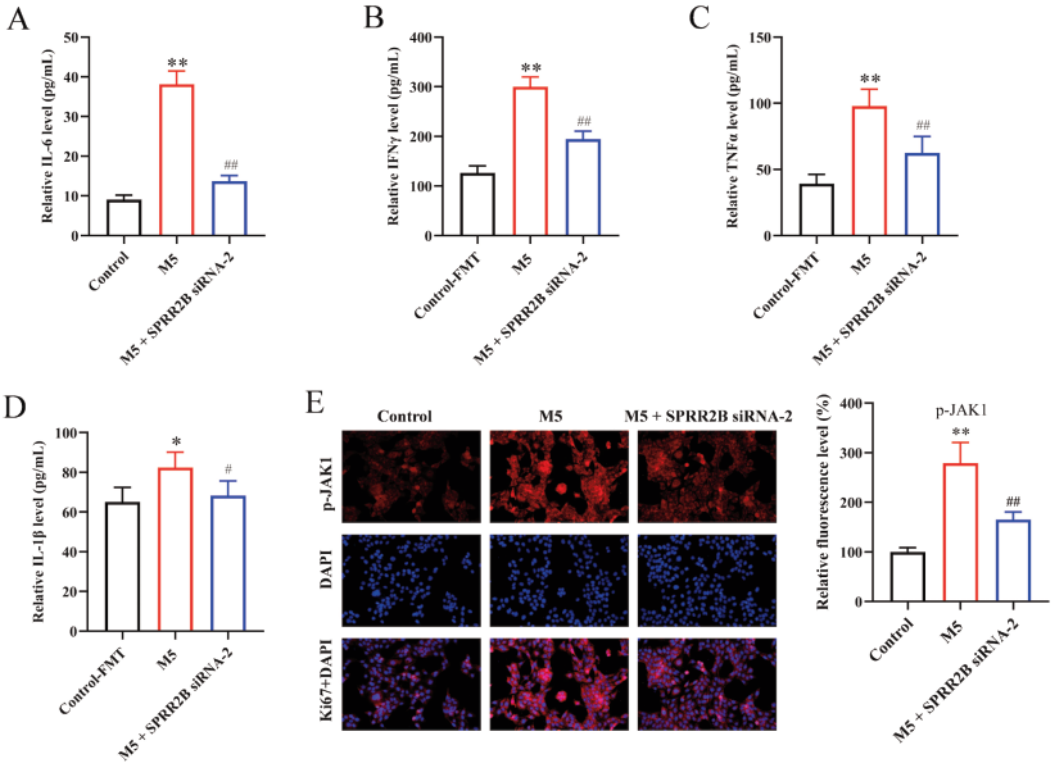

Figure 5. SPRR2B knockdown suppressed M5-induced cytokine upregulation in HaCaT keratinocyte. HaCaT keratinocytes were seeded in cell plates and cultured for 24 h. Upon reaching 60-80% confluence, the cells were treated with SPRR2B siRNA and/or M5 cytokines for an additional 24 h. (**A-D**) The the levels of IL-6, IFN-γ, TNF-α, and IL-1β in the supernatant of cells were measured with ELISA kits, respectively. (**E**) p-JAK1 protein expressions were measured with immunofluorescence. $^{**}p<0.01$ compared with control group; $^{\#}p<0.05$, $^{\#\#}p<0.01$ compared with M5 group; n=3.

remains to be fully elucidated, and the nature of SPRR2B's interaction with JAK kinases or upstream receptors requires further investigation. Third, upstream regulatory factors of SPRR2B in psoriasis pathogenesis, including cytokines, transcription factors, and epigenetic modifications, remain unexplored and warrant future investigation. Thus, validating the role of SPRR2B using an *in vivo* psoriasis mouse model and clinical patient samples will be the focus of future research. In conclusion, SPRR2B knockdown inhibits M5-induced HaCaT keratinocyte proliferation and inflammatory response by regulating the MDM2/p53 apoptotic pathway and suppressing JAK signaling activation. These results highlight SPRR2B as a promising therapeutic target for psoriasis and provide new insights into the molecular mechanism underlying keratinocyte dysfunction in psoriasis.

References

1. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med 2009;361:496-509.
2. Boehncke WH, Schön MP. Psoriasis. Lancet 2015;386:983-94.
3. Furue K, Ito T, Tsuji G, Kadono T, Furue M. Psoriasis and the TNF/IL23/IL17 axis. G Ital Dermatol Venereol 2019;154:418-24.
4. Armstrong AW, Read C. Pathophysiology, clinical presentation, and treatment of psoriasis: a review. JAMA 2020;323:1945-60.
5. Gibbs S, Fijneman R, Wiegant J, van Kessel AG, van De Putte P, Backendorf C. Molecular characterization and evolution of the SPRR family of keratinocyte differentiation markers encoding small proline-rich proteins. Genomics 1993;16:630-7.
6. Burke RM, Lighthouse JK, Quijada P, Dirkx RA Jr, Rosenberg A, Moravec CS, et al. Small proline-rich protein 2B drives stress-dependent p53 degradation and fibroblast proliferation in heart failure. Proc Natl Acad Sci U S A 2018;115:E3436-E45.
7. Kim J, Deshar B, Hwang M, Shrestha C, Ju E, Bin BH, et al. Quantum molecular resonance ameliorates atopic dermatitis through suppression of IL36G and SPRR2B. BMB Rep 2025;58:209-16.
8. Xue C, Yao Q, Gu X, Shi Q, Yuan X, Chu Q, et al. Evolving cognition of the JAK-STAT signaling pathway: autoimmune disorders and cancer. Signal Transduct Target Ther 2023;8:204.
9. Sugumaran D, Yong ACH, Stanslas J. Advances in psoriasis research: from pathogenesis to therapeutics. Life Sci 2024;355:122991.
10. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015;12:453-7.
11. Dull K, Lénárt K, Dajnoki Z, Póliska S, Uchiyama E, Hendrik Z, et al. Barrier function-related genes and proteins have an altered expression in acne-involved skin. J Eur Acad Dermatol Venereol 2023;37:1415-25.
12. Barygina V, Becatti M, Prignano F, Lotti T, Taddei N, Fiorillo C. Fibroblasts to keratinocytes redox signaling: the possible role of ROS in psoriatic plaque formation. Antioxidants (Basel) 2019;8:11.
13. Yao L, Yan J, Cheng F, Gan L, Huang Y, Zheng L, et al. Small proline-rich protein 2B facilitates gastric adenocarcinoma proliferation via MDM2-p53/p21 signaling pathway. Onco Targets Ther 2021;14:1453-63.
14. Dębińska A, Danielewicz H, Boznański A, Matusiak Ł, Szepietowski JC. A small proline-rich protein (SPRR) gene variant contributes to atopic eczema and eczema-associated asthma susceptibility. Postepy Dermatol Alergol 2022;39:965-71.
15. Gong X, Wang W. Profiles of innate immune cell infiltration and related core genes in psoriasis. BioMed Res Int 2021;2021:6656622.
16. Giraulo C, De Palma G, Plaitano P, Esposito R, Morretta E, Monti MC, et al. Oncostatin M upregulates CD73 via the MAPK pathway in keratinocytes to promote an adenosine-dependent anti-inflammatory response in psoriasis. Front Immunol 2026;17:1698290.
17. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004;303:844-8.
18. Ma R, Cui L, Cai J, Yang N, Wang Y, Chen Q, et al. Association between systemic immune inflammation index, systemic inflammation response index and adult psoriasis: evidence from NHANES. Front Immunol 2024;15:1323174.
19. Svedbom A, Mallbris L, González-Cantero Á, Playford M, Wu C, Mehta NN, et al. Skin inflammation, systemic inflammation, and cardiovascular disease in psoriasis. JAMA Dermatol 2025;161:81-6.

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