



Allergologia et immunopathologia

Sociedad Española de Inmunología Clínica,
Alergología y Asma Pediátrica

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ORIGINAL ARTICLE

OPEN ACCESS

Knockdown of ANXA3 regulates NF- κ B/STAT3 pathway to alleviate inflammation and hyperproliferation in psoriasis models

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Received 6 November 2024; Accepted 10 January 2025

Available online 1 March 2025

KEYWORDS

Annexin A3 (ANXA3);
inflammation;
NF- κ B/STAT3
pathway;
psoriasis;
skin

Abstract

Psoriasis is an immune-mediated inflammatory skin disorder and its pathological mechanism remains incompletely understood. Detailed exploration of this mechanism is crucial to identify key regulatory molecules influencing its progression. In previous studies, Annexin A3 (ANXA3), a calcium-dependent phospholipid-binding protein from the annexin family, has been linked to psoriasis progression. However, its specific effects on the disease remain unclear. This study aimed to investigate the role of ANXA3 in psoriasis progression. For this purpose, we employed an imiquimod (IMQ)-induced mouse model and *in-vitro* experiments to uncover the underlying cellular mechanisms. A mixture of five inflammatory factors (TNF- α , IL-1 α , IL-17A, IL-22, and statin M) was used to stimulate HaCaT cells, mimicking the psoriasis microenvironment. Our findings demonstrate that ANXA3 is highly expressed in psoriatic skin, and its knockdown alleviates skin lesions in IMQ-induced mice. Further analysis revealed that ANXA3 knockdown reduces skin tissue hyperplasia and decreases the expression of inflammatory factors in IMQ mice. Mechanistically, ANXA3 knockdown inhibits the NF- κ B/STAT3 pathway in skin tissue. Additionally, ANXA3 knockdown inhibits inflammation and hyperproliferation in HaCaT cells. Collectively, these results indicate that ANXA3 alleviates psoriasis progression both *in-vivo* and *in-vitro* by inhibiting the NF- κ B/STAT3 pathway.

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<https://doi.org/10.15586/aei.v53i2.1260>

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Introduction

Psoriasis is an immune-mediated inflammatory skin disease that affects approximately 2% of the global population.^{1,2} It is typically characterized by red, squamous, and thickened patches of skin lesions caused by excessive proliferation of keratinocytes and infiltration of immune cells.³ Although the precise pathogenesis of psoriasis remains unclear, growing evidence highlights the critical role of keratinocytes, immune cells, and their interactions in disease development.⁴ To improve the therapeutic outcomes, further investigation into the pathological mechanisms of psoriasis is essential with a focus on identifying the key regulatory molecules that influence its progression.⁵

Annexin A3 (ANXA3), also known as lipocortin 3 or placental anticoagulant protein 3 (PAP-III), is a class of calcium-dependent phosphatide-binding proteins that belongs to the annexin family.⁶ ANXA3 has been implicated in various malignancies, including lung cancer, where it exhibits a stimulative effect on tumor progression.⁷ Additionally, ANXA3 has regulatory effects on proliferation, cell migration, apoptosis, cytoskeletal regulation, and immune regulation. Its ability to promote hypoxia-induced inflammatory factor expression highlights its broad physiological and pathological functions in human diseases.^{8,9} ANXA3 overexpression has been observed in the serum and plasma of sepsis patients.¹⁰ Reducing ANXA3 levels in sepsis patients alleviates lung injury, as evidenced by decreased pulmonary edema, reduced inflammatory cell infiltration, and inhibited apoptosis.¹⁰ Moreover, ANXA3 overexpression has been linked to increased phosphorylation of NF- κ B subunit P65 further underscoring its role in inflammation.¹¹ Despite these findings, the role of ANXA3 in psoriasis progression and the underlying mechanisms remain largely unexplored.

Nuclear factor κ B (NF- κ B) plays a pivotal role as an inflammatory mediator in the pathogenesis of psoriasis. Elevated NF- κ B expression has been consistently observed in psoriatic lesions.¹² The alteration of NF- κ B signal transduction disrupts the balance of apoptotic signals, resulting in the upregulation of cyclin and survivin, which inhibit apoptosis.¹³ Furthermore, NF- κ B promotes the production of proinflammatory cytokines such as IL-17 and TNF- α , amplifying the downstream inflammatory response and contributing to disease progression.¹⁴

This study was carried out with the objective to comprehensively investigate the role of ANXA3 in the progression of psoriasis and to elucidate the underlying molecular mechanisms. By utilizing both *in-vivo* and *in-vitro* models, the research explored the influence of ANXA3 on psoriasis progression through regulation of the NF- κ B/STAT3 signaling pathway.

In this study, the analyses of the psoriatic expression profile revealed the high expression of ANXA3 in psoriatic lesions and further revealed its influence on psoriatic progression and its mechanism. We found that knocking down ANXA3 regulates the NF- κ B/STAT3 pathway to reduce psoriasis inflammation and hyperproliferation *in vivo* and *in vitro*. Therefore, ANXA3 is considered a potential therapeutic target for psoriasis.

Materials and Methods

GEO analysis

The microarray data was downloaded from the GEO database (GSE161683) and analyzed using R software (version 4.2.2). Differential expression analysis was performed using the “limma” package, and the results were visualized using heatmaps and volcano plots.

Animal experiments and ethics

Male BALB/c mice (6 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experiments were conducted in compliance with ethical guidelines and were approved by Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University (Approval No. 2021DZGKJDWLS-00115). The mice were housed in a controlled environment with a 12-hour light/dark cycle, and standard laboratory chow and water *ad libitum*. To ensure animal welfare, all procedures were performed under institutional guidelines. The mice were anesthetized using isoflurane (Sigma-Aldrich, USA) for the experiments.

AAV-mediated knockdown of ANXA3 was achieved by injecting AAV vectors carrying shRNA specific to ANXA3 into the tail vein of mice. Each mouse received 1×10^{12} viral genome copies (vg) of the AAV vector suspended in 100 μ L of PBS. The efficiency of ANXA3 knockdown was confirmed by Western blot analysis conducted 2 weeks postinjection.

Following the procedures, the mice were carefully monitored during recovery and humanely euthanized at the designated endpoints. Erythema and scaling scores were calculated based on the standard scoring system. Erythema was assessed on a scale of 0-4 based on the intensity of redness, while scaling was scored from 0-4 based on the extent and thickness of scales. To ensure accuracy and minimize bias, these scores were evaluated independently by two blinded researchers, and the average score for each mouse was calculated.

Imiquimod

Imiquimod (IMQ) is a TLR7 agonist that induces an immune response by activating innate immunity and is commonly used in experimental models to mimic inflammatory conditions such as psoriasis. In this study, a 5% concentration of IMQ (Sigma-Aldrich, USA; Catalog #I7402) was topically applied to the shaved dorsal skin of mice three times per week for 2 weeks to induce localized inflammatory responses.

Reagents and antibodies

Reagents for the experiments were primarily purchased from Beyotime Biotechnology. Key reagents included imiquimod cream (62.5 mg, 5%, Beijing Tong Ren Tang, China)

for inducing psoriasis-like skin inflammation in mice. Other test kits used in the study such as IL-6 ELISA Kit (PI326, Beyotime, China), IL-1 β ELISA Kit (PI305, Beyotime, China), TNF- α ELISA Kit (PT518, Beyotime, China), IL-23 ELISA Kit (PI303, Beyotime, China), IL-17 ELISA Kit (PI317, Beyotime, China), and IL-22 ELISA Kit (PI315, Beyotime, China) were also obtained from Beyotime.

The antibodies used in this study were primarily obtained from Abcam and applied at specific concentrations for immunoblotting and immunohistochemistry. These included anti-ANXA3 (ab180889, 1:1000, Abcam, USA), anti-p65 (ab16502, 1:1000, Abcam, USA), anti-p-p65 (ab86299, 1:1000, Abcam, USA), anti-STAT3 (ab68153, 1:1000, Abcam, USA), and anti-p-STAT3 (ab76315, 1:1000, Abcam, USA). β -actin (AF7018, 1:5000, Beyotime, China) was used as the loading control.

Psoriasis-like mouse model

To induce psoriasis-like inflammation, imiquimod cream was topically applied to the shaved dorsal skin of mice for 6 consecutive days. The mice were divided into four groups (control, IMQ, IMQ+sh-NC, and IMQ+sh-ANXA3) to assess the effects of ANXA3 knockdown on skin lesions.

Cell culture and treatment

HaCaT cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and maintained at 37°C in a humidified atmosphere of 5% CO₂. To create a psoriatic-like environment, HaCaT cells were stimulated with a mixture of inflammatory cytokines, including TNF- α , IL-1 α , IL-17A and IL-22 (each at 10 ng/mL, PeproTech, USA), for 24 h. Following stimulation, the cells were transfected with shRNA targeting ANXA3 or a non-targeting control using Lipofectamine 3000 (L3000008, Invitrogen, USA). Cell proliferation was assessed using EDU staining, performed following the manufacturer's protocol using the Click-iT EDU Imaging Kit (Invitrogen, USA; Catalog #C10337).

Immunohistochemistry and immunofluorescence

Tissue sections or cells were deparaffinized, rehydrated, and subjected to antigen retrieval. The samples were then blocked with 5% BSA and incubated overnight at 4°C with primary antibodies. After washing, secondary antibodies were applied, and DAPI staining was performed to visualize nuclei. For immunofluorescence, sections were incubated with anti-ANXA3 (ab180889, 1:500, Abcam, USA) and anti-Ki-67 (ab15580, 1:500, Abcam, USA). Images were captured using a Zeiss LSM 880 confocal microscope (Zeiss, Germany).

Western blotting

Proteins were extracted from tissues and cells, quantified, and separated by SDS-PAGE. The separated proteins

were transferred onto PVDF membranes and blocked with 5% nonfat milk to prevent nonspecific binding. The membranes were then incubated overnight with primary antibodies. After washing, membranes were incubated with HRP-conjugated secondary antibodies. Protein bands were visualized using an ECL kit (P0018, Beyotime, China) and quantified using ImageJ software (NIH, USA).

Quantitative PCR (qPCR)

Total RNA was extracted using TRIzol reagent (15596018, Invitrogen, USA), and cDNA was transcribed using a reverse transcription kit (D2639, Beyotime, China). SYBR Green Master Mix (Q111-02, Vazyme, China) was used for qPCR analysis on a QuantStudio 5 Real-Time PCR system (Thermo Fisher Scientific, USA) to determine relative ANXA3 expression levels. GAPDH was used as an internal control, and the relative gene expression was calculated using the 2^{- $\Delta\Delta$ Ct} method.

Enzyme-linked immunosorbent assay (ELISA)

Skin tissue homogenates were prepared and analyzed using ELISA to measure the levels of IL-6, IL-1 β , TNF- α , IL-23, IL-17, and IL-22, following manufacturer's instructions (Beyotime, China). The absorbance was measured at 450 nm using a Multiskan MK3 microplate reader (Thermo Fisher Scientific, USA).

Statistical analysis

Data was presented as mean \pm standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, and a P-value < 0.05 was considered statistically significant. All the analyses were conducted using GraphPad Prism 8 (GraphPad Software, USA).

Results

High expression of ANXA3 in psoriatic skin tissues

To investigate the expression of ANXA3 in psoriasis, we analyzed gene expression profiles from two datasets. Analysis of the GSE161683 dataset, using a volcano plot (Figure 1A), identified differentially expressed genes between psoriatic and healthy skin tissues. Notably, ANXA3 was among the genes significantly upregulated in psoriatic skin. Consistent with the GSE161683 dataset, analysis of the GSE166388 dataset, using a volcano plot (Figure 1B), also identified ANXA3 as a gene significantly upregulated in psoriatic skin. The Venn diagram displays the overlap of upregulated genes identified in both datasets, with ANXA3 being a most common gene (Figure 1C). Box plots for the GSE161683 (Figure 1D) and GSE166388 (Figure 1E) datasets further confirm higher ANXA3 expression in psoriatic tissues compared to healthy tissues. Additionally, qPCR results show increased relative mRNA expression of ANXA3 in psoriatic

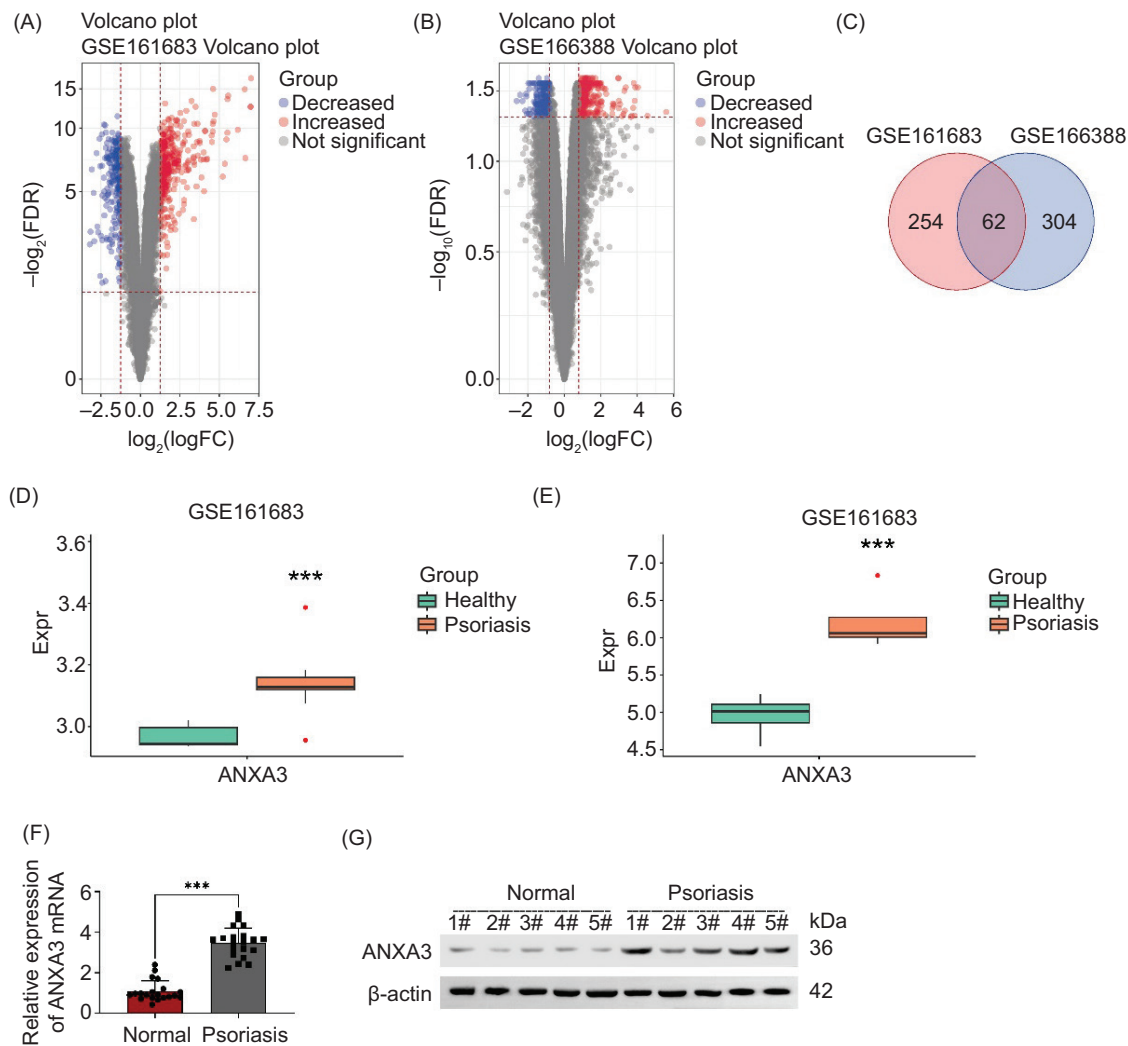


Figure 1 High expression of ANXA3 in psoriatic skin tissues. (A) Volcano plot of differentially expressed genes in psoriatic versus healthy skin tissues from the GSE161683 dataset. Red dots represent genes with increased expression, while blue dots indicate genes with decreased expression in psoriatic skin; (B) Volcano plot of differentially expressed genes in psoriatic versus healthy skin tissues from the GSE166388 dataset. Red dots represent genes with increased expression, while blue dots indicate genes with decreased expression in psoriatic skin; (C) Venn diagram of upregulated genes from the GSE161683 and GSE166388 datasets, showing the overlap of genes between the two datasets; (D) Box plot of ANXA3 expression in the GSE161683 dataset, comparing psoriatic and healthy skin tissues; (E) Box plot of ANXA3 expression in the GSE166388 dataset, comparing psoriatic and healthy skin tissues; (F) Relative mRNA expression of ANXA3 in psoriatic vs. normal skin tissues from collected patient samples; (G) Western blot analysis of ANXA3 protein expression in normal and psoriatic skin tissue samples, with β -actin as the loading control. *** indicates $P < 0.001$, representing significant differences. Exp, expression; ANXA3, Annexin A3.

skin samples (Figure 1F), and western blot analysis further confirms elevated ANXA3 protein levels in psoriatic tissues compared to controls (Figure 1G). These findings establish that ANXA3 is highly expressed in psoriatic skin tissues, suggesting a potential role in psoriasis pathology.

ANXA3 knockdown suppresses inflammation and hyperproliferation in HaCaT cells

We next evaluated the effect of ANXA3 knockdown on inflammation and hyperproliferation in HaCaT cells

exposed to a psoriatic-like environment. Western blot analysis and Immunostaining showed successful knockdown of ANXA3 in HaCaT cells confirming reduced ANXA3 protein levels (Figures 2A and 2B). EDU staining indicates a decrease in the percentage of EDU-positive cells in the ANXA3 knockdown group, reflecting reduced proliferation (Figure 2C). ELISA assay also revealed a decrease in the levels of inflammatory cytokines such as, IL-6, IL-1 β , TNF- α , IL-23, IL-17, and IL-22 in those cells (Figure 2D). Additionally, western blots of proteins involved in NF- κ B pathway show a reduced phosphorylation of p65 (Figure 2E), while the proteins involved in STAT3 pathway show a decrease in

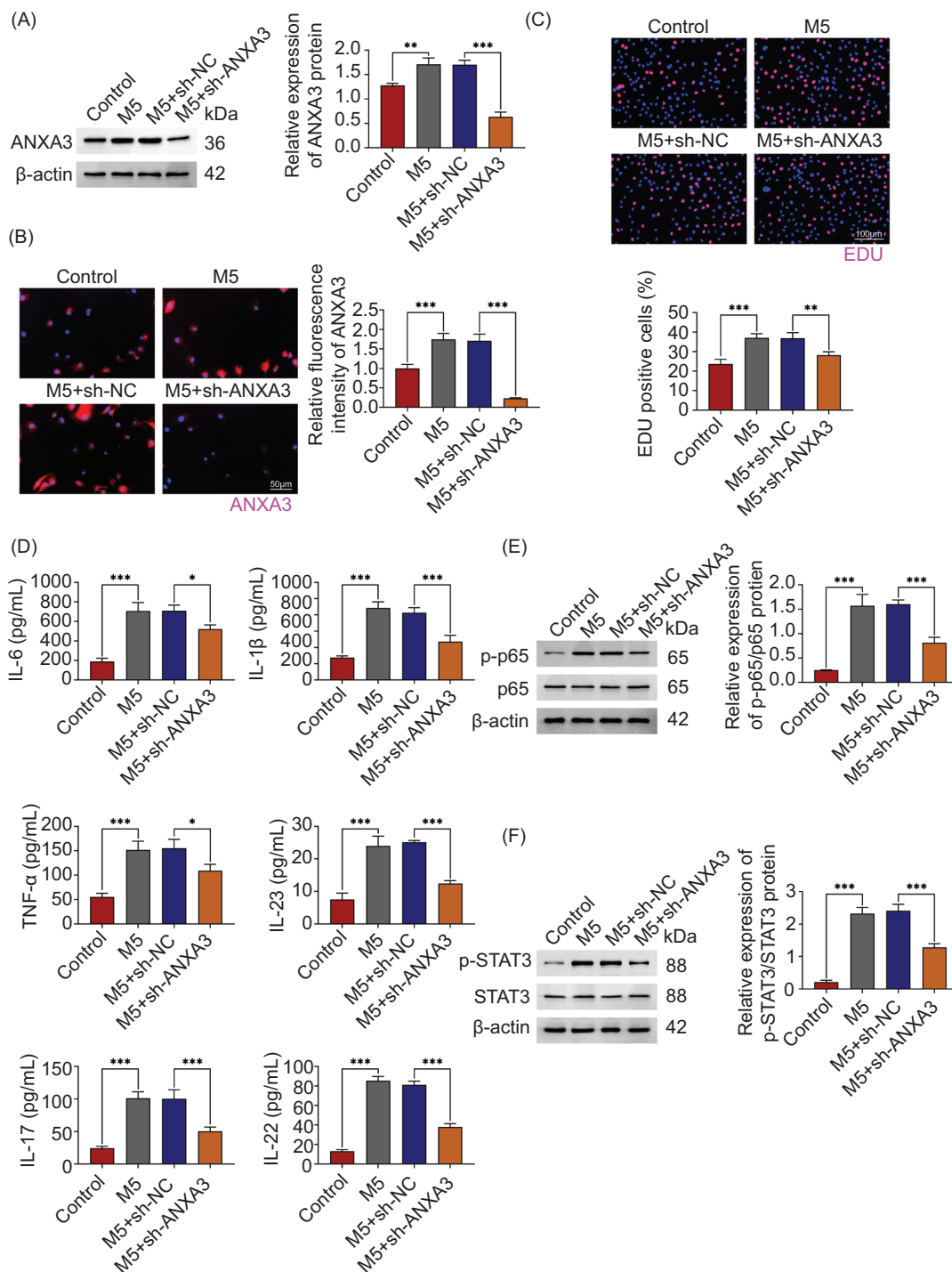


Figure 2 ANXA3 knockdown suppresses inflammation and hyperproliferation in HaCaT cells. (A) Western blot analysis of ANXA3 protein levels in HaCaT cells under different treatments, with quantification of relative ANXA3 expression; (B) Immunostaining analysis of ANXA3 protein levels in HaCaT cells under different treatments, with quantification of relative ANXA3 expression. Scale bar, 100 μ m; (C) Representative images of EDU staining to assess cell proliferation in HaCaT cells across different groups, with quantification of EDU-positive cells. Scale bar, 100 μ m; (D) ELISA results showing the levels of inflammatory cytokines (IL-6, IL-1 β , TNF- α , IL-23, IL-17, and IL-22) in HaCaT cells under various treatment conditions; (E) Western blot analysis of p-p65 and p65 proteins in HaCaT cells across different groups, with quantification of relative p-p65 expression; (F) Western blot analysis of p-STAT3 and STAT3 proteins in HaCaT cells under different treatment conditions, with quantification of relative p-STAT3 expression. ** indicates $P < 0.01$, *** indicates $P < 0.001$. EDU, 5-ethynyl-2'-deoxyuridine; ANXA3, Annexin A3; IL, interleukin; TNF- α , tumor necrosis factor- α ; STAT3, signal transducer and activator of transcription 3.

phosphorylation of STAT3 in the ANXA3 knockdown group (Figure 2F). These results indicate that ANXA3 knockdown effectively suppresses inflammation and hyperproliferation in HaCaT cells, suggesting a regulatory role for ANXA3 in psoriasis-related cellular responses.

ANXA3 knockdown alleviates skin lesions in the IMQ-induced mouse models

To assess the impact of ANXA3 knockdown on psoriasis-like lesions *in-vivo*, we employed the IMQ-induced psoriasis model in mice. Representative images demonstrate visible improvements in skin lesions in ANXA3 knockdown mice compared to control groups (Figure 3A). Histological analysis using H&E staining further showed reduced epidermal thickening and inflammatory cell infiltration in the ANXA3 knockdown group (Figure 3B). Additionally, quantification of erythema and scaling scores showed a significant decrease in these parameters in ANXA3 knockdown group (Figures 3C and 3D). Collectively, these findings indicate that ANXA3 knockdown alleviates the severity of psoriatic lesions in the IMQ-induced mouse model, highlighting its potential as a therapeutic target for psoriasis treatment.

ANXA3 knockdown reduces hyperproliferation and inflammatory cytokine expression in IMQ-treated mouse skin tissues

We further investigated the effects of ANXA3 knockdown on hyperproliferation and cytokine expression in the skin tissues of IMQ-treated mice. Immunofluorescence staining for Ki-67 revealed a decreased proliferation in the ANXA3 knockdown group, as indicated by a reduction in Ki-67-positive cells (Figure 4A). ELISA analysis of skin tissue homogenates also showed significantly reduced levels of proinflammatory cytokines IL-6, IL-1 β , TNF- α , IL-23, IL-17, and IL-22 in the ANXA3 knockdown group (Figure 4B). These results demonstrate that ANXA3 knockdown effectively reduces both hyperproliferation and inflammation in psoriatic skin tissues, supporting its role in modulating psoriatic pathology.

ANXA3 knockdown inhibits the NF- κ B/STAT3 pathway in psoriatic skin tissues

To explore the mechanism by which ANXA3 affects psoriasis progression, we examined the NF- κ B and STAT3

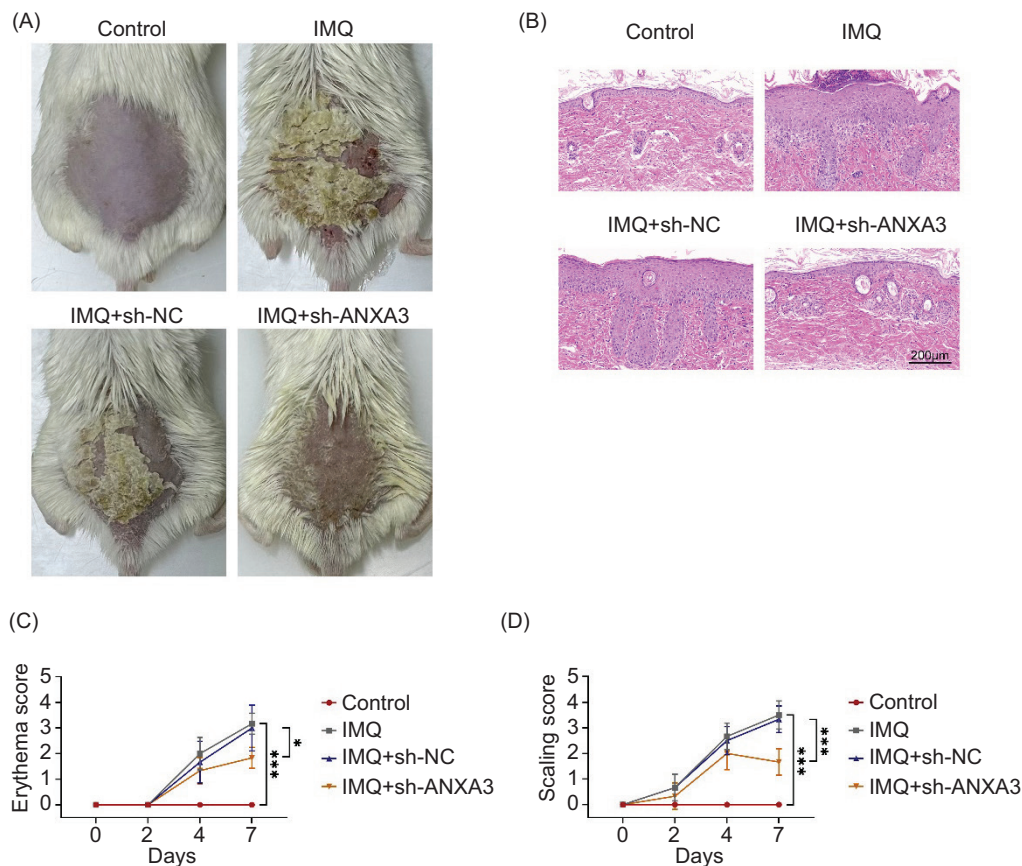


Figure 3 ANXA3 knockdown alleviates skin lesions in the IMQ-induced mouse model of psoriasis. (A) Representative images of mouse back skin showing the effects of ANXA3 knockdown on IMQ-induced skin lesions across different treatment groups; (B) H&E staining of skin tissue sections from each group, highlighting histopathological changes after ANXA3 knockdown. Scale bar, 200 μ m; (C) Quantification of erythema scores for each treatment group; (D) Measurement of scaling scores in skin sections from different groups, demonstrating the effects of ANXA3 knockdown. * indicates $P < 0.05$, *** indicates $P < 0.001$. IMQ, imiquimod; H&E, hematoxylin and eosin; ANXA3, Annexin A3.

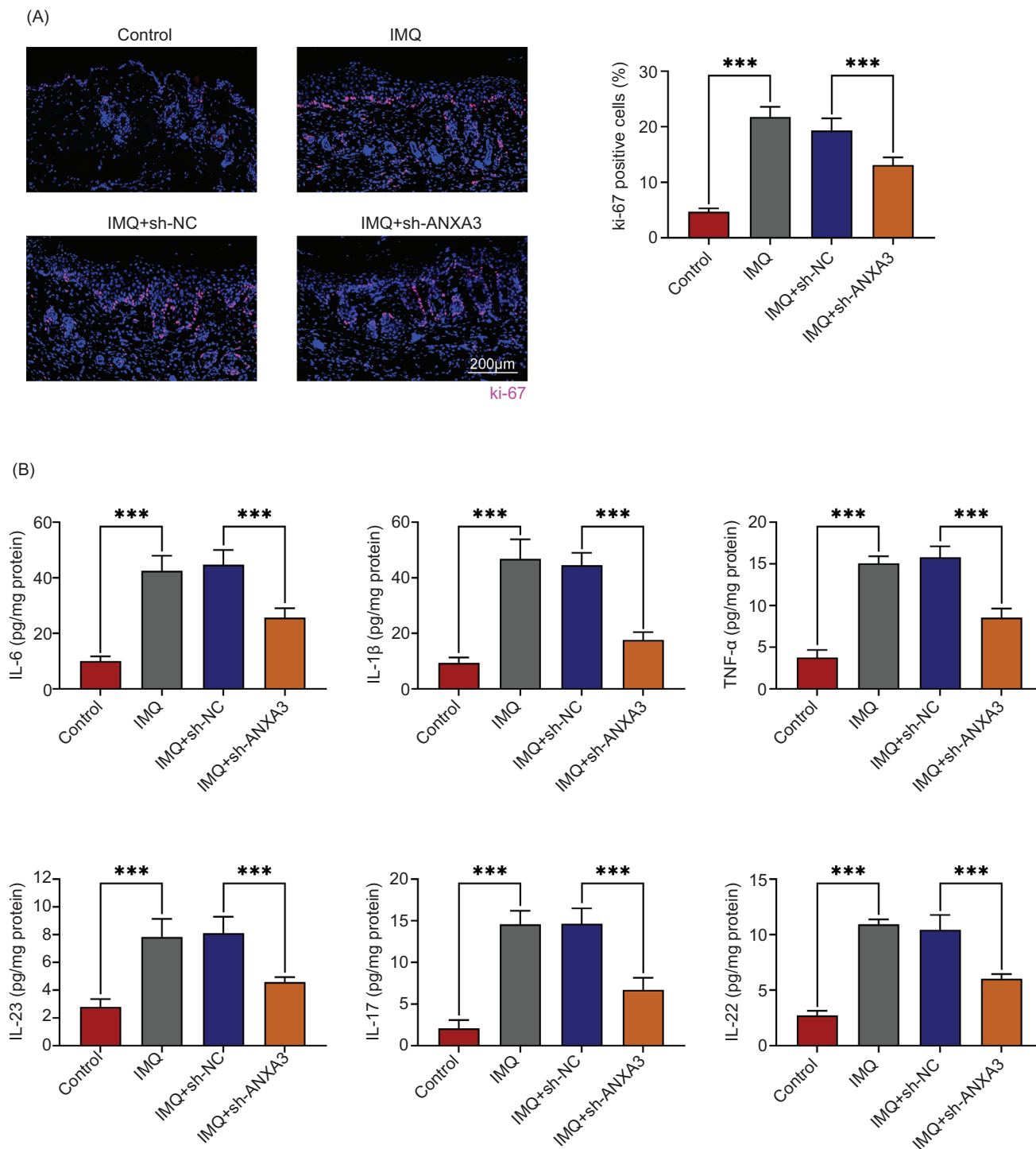


Figure 4 ANXA3 knockdown reduces hyperproliferation and inflammatory cytokine expression in IMQ-treated mouse skin tissues. (A) Immunofluorescence staining for Ki-67 in skin sections from different treatment groups, indicating cell proliferation levels. Scale bar, 200 μ m; (B) ELISA results show the concentrations of inflammatory cytokines (IL-6, IL-1 β , TNF- α , IL-23, IL-17, and IL-22) in skin tissues from each group. *** indicates $P < 0.001$, representing significant differences. IMQ, imiquimod; ANXA3, Annexin A3; IL, interleukin; TNF- α , tumor necrosis factor-alpha.

pathways in psoriatic skin tissues. Western blot analysis showed a reduced phosphorylation of p65, a key NF- κ B subunit, in the ANXA3 knockdown group, with quantification indicating a decreased p-p65/p65 ratio (Figure 5A). Similarly, western blot analysis of STAT3 pathway showed a reduction in p-STAT3 levels in the ANXA3 knockdown

group, with quantification indicating a decreased p-STAT3/STAT3 ratio (Figure 5B). These results suggest that ANXA3 knockdown inhibits the activation of both NF- κ B and STAT3 pathways, providing a mechanistic explanation for its anti-inflammatory and antiproliferative effects in psoriatic skin.

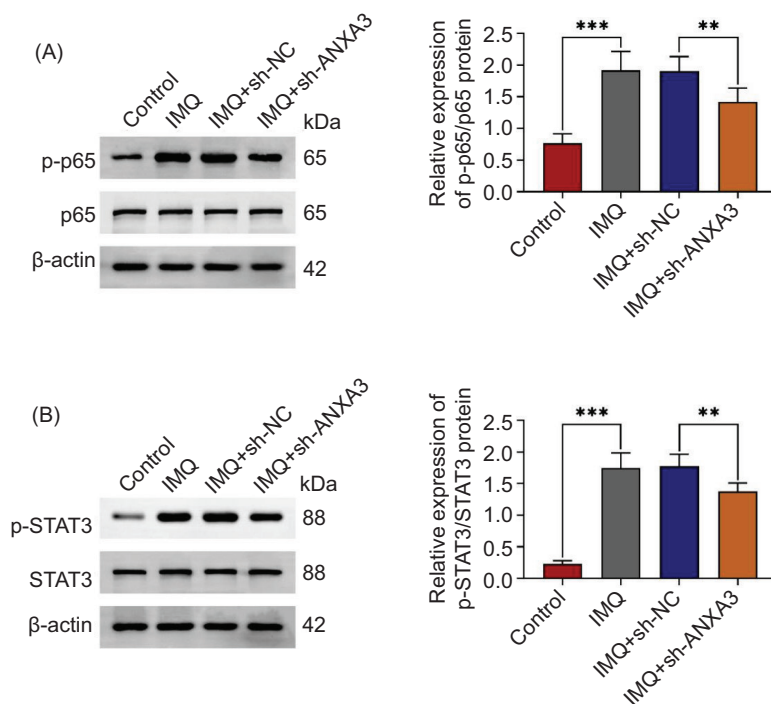


Figure 5 Knockdown of ANXA3 inhibits the NF- κ B/STAT3 pathway in psoriatic skin tissues. (A) Western blot analysis of phosphorylated p65 (p-p65) and total p65 protein levels in skin tissues from different treatment groups, with quantification of the relative p-p65/p65 expression; (B) Western blot analysis of phosphorylated STAT3 (p-STAT3) and total STAT3 protein levels in skin tissues from each group, with quantification of the relative p-STAT3/STAT3 expression. ** indicates $P < 0.01$, *** indicates $P < 0.001$, representing significant differences. IMQ, imiquimod; ANXA3, Annexin A3; STAT3, signal transducer and activator of transcription 3; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells.

Discussion

Psoriasis is an immune-mediated inflammatory skin disorder characterized by red, scaly, and thickened lesions resulting from excessive keratinocyte proliferation and immune cell infiltration.^{15,16} The role of inflammatory responses in psoriasis is well-documented. Studies have indicated that proinflammatory cytokines such as TNF- α , IL-1 α , IL-17A, and IL-22 are markedly elevated in psoriatic lesions and contribute to the chronic inflammatory state observed in patients.¹⁹ Despite significant research, its pathogenesis remains incompletely understood.^{17,18} This study sought to address the gap by investigating Annexin A3 (ANXA3), a protein with known regulatory functions in inflammatory and malignant conditions, in the context of psoriasis. We found that ANXA3 is significantly upregulated in psoriatic skin tissues, suggesting its potential involvement in psoriasis progression. By examining the effects of ANXA3 knockdown on psoriasis-related inflammation and hyperproliferation, we provide evidence that supports its potential as a promising target for alleviating psoriatic pathology. Additionally, our findings emphasize the therapeutic implications of modulating ANXA3 expression to control inflammation.

The use of both *in-vivo* and *in-vitro* models is crucial for advancing psoriasis research. In this study, we employed the IMQ-induced psoriasis-like skin inflammation in mice as an *in-vivo* model and, HaCaT cells stimulated by a mixture of proinflammatory cytokines (TNF- α , IL-1 α , IL-17A,

IL-22, and statin M) as an *in-vitro* psoriatic model. These models are valuable for simulating the psoriatic microenvironment and evaluating potential therapeutic targets. In the IMQ-induced *in-vivo* model, we observed that ANXA3 knockdown significantly alleviated psoriasis-like symptoms, including skin erythema, scaling, and thickening, thereby reducing overall lesion severity. Similarly, in the HaCaT cell model, ANXA3 knockdown reduced cellular hyperproliferation and inflammatory cytokine production. These results underscore the utility of our models for investigating ANXA3's role in psoriasis and confirm that targeting ANXA3 in these settings yields beneficial effects. This also validates the relevance of these models for studying the molecular mechanisms of psoriasis and testing potential therapeutic strategies.

Annexin A3 is a calcium-dependent phospholipid-binding protein known to regulate various cellular processes such as cell proliferation, migration, apoptosis, cytoskeletal dynamics, and immune response.^{20,21} Emerging researches has highlighted ANXA3's involvement in inflammatory diseases and malignancies, where it modulates the expression of hypoxia-induced inflammatory factors and influences inflammation-associated signaling pathways.^{22,23} Psoriasis, characterized by inflammation and hyperproliferation, provides a relevant context for investigating ANXA3's role. Our findings demonstrate upregulated ANXA3 expression in psoriasis, suggesting its potential contribution to disease pathogenesis. Furthermore, the study

demonstrated that ANXA3 knockdown significantly reduced inflammatory cytokine levels and inhibited hyperproliferation in both *in-vitro* and *in-vivo* psoriasis models. These results collectively indicate that ANXA3 may play a crucial role in potentiating the inflammatory state and excessive cell growth observed in psoriasis. Therefore, targeting ANXA3 could represent a novel therapeutic strategy for this condition, providing valuable insights into its underlying pathophysiology.

Keratinocyte hyperproliferation is a hallmark of psoriasis, driving the formation of thickened, scaly lesions that define the disease's clinical presentation.²⁴ Inflammatory mediators further exacerbate this hyperproliferation in psoriatic lesions, amplifying the abnormal proliferation cycle in keratinocytes.²⁵ Targeting this proliferation is essential for managing psoriasis symptoms and preventing disease progression.²⁵ Our study shows that ANXA3 knockdown effectively reduces keratinocyte hyperproliferation, as evidenced by decreased expression of proliferation markers and inflammatory cytokines both in *in-vivo* and *in-vitro* models. These findings strongly support the potential of ANXA3 inhibition as a strategy to curb the hyperproliferative and inflammatory processes in psoriasis, offering a novel approach for reducing disease severity and improving patient outcomes.

The NF- κ B and STAT3 signaling pathways are key regulators of inflammation and have been implicated in various inflammatory and autoimmune diseases, including psoriasis.²⁶ Aberrant activation of these pathways is consistently observed in psoriatic lesions, where they promote the transcription of proinflammatory cytokines, sustaining a feedback loop that perpetuates inflammation and cellular proliferation.³ By driving the expression of cytokines such as IL-6, IL-8, IL-17, and TNF- α , these pathways contribute to the psoriatic phenotype and are thus attractive targets for therapeutic intervention.²⁵ Our study reveals that ANXA3 knockdown suppresses NF- κ B and STAT3 activation, indicating that ANXA3 may modulate psoriasis progression through its effects on these pathways. These findings align with our observations of reduced inflammation and hyperproliferation in ANXA3-depleted models, supporting the relevance of NF- κ B and STAT3 as mechanisms through which ANXA3 influences psoriatic pathology.

Mechanistically, our study demonstrated that ANXA3 knockdown specifically inhibits NF- κ B and STAT3 pathway activation, as shown by reduced phosphorylation levels of p65 and STAT3.²⁷ This provides insight into ANXA3's role in facilitating NF- κ B/STAT3 signaling in psoriatic skin, suggesting that ANXA3 could act upstream to regulate these pathways, thereby exacerbating the inflammatory response. By inhibiting NF- κ B and STAT3, ANXA3 knockdown attenuates inflammatory mediator production and keratinocyte hyperproliferation, supporting a role for ANXA3 as a modulator of inflammatory signaling in psoriasis. These findings open new avenues for investigating ANXA3-related pathways in psoriasis and highlight the potential for targeting ANXA3 to effectively disrupt the proinflammatory cascade that drives psoriasis progression.

The involvement of NF- κ B and STAT3 in psoriasis and other inflammatory diseases underscores their potential as therapeutic targets.²⁶ The dual inhibition of NF- κ B and STAT3 as observed in ANXA3 knockdown reinforces

the therapeutic relevance of these pathways in psoriasis management.²⁶ Our data indicate that modulating these pathways through ANXA3 depletion significantly impacts both inflammation and hyperproliferation. Thus, targeting ANXA3 or related pathways could yield promising results in psoriasis treatment. By implicating ANXA3 in the modulation of the NF- κ B/STAT3 pathway, this study positions ANXA3 as a key player in inflammation-driven diseases, particularly in the context of psoriasis.

This study has limitations, including the potential challenges in translating these findings to human patients. While our *in-vivo* and *in-vitro* models provide valuable insights into ANXA3's function in psoriasis, further studies are required to assess the long-term efficacy and safety of targeting ANXA3 in clinical settings. Additionally, comprehensive investigations are necessary to fully delineate the interactions between ANXA3 and other inflammatory pathways involved in psoriasis. This deeper understanding may reveal novel therapeutic targets or refine ANXA3-based interventions for more effective disease management.

In conclusion, our findings demonstrate that ANXA3 plays a crucial role in driving psoriasis progression by modulating the NF- κ B/STAT3 signaling pathway. We observed that ANXA3 knockdown effectively alleviates both psoriatic inflammation and hyperproliferation, suggesting that inhibiting ANXA3 may represent a promising novel therapeutic strategy for this disease. By elucidating the molecular mechanisms underlying ANXA3's role in psoriasis, this study contributes significantly to our understanding of psoriasis pathogenesis and offers promising directions for future therapeutic developments targeting chronic inflammatory skin diseases.

Acknowledgments

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics Approval

Ethical approval was obtained from the Ethics Committee of Jinling Hospital, Affiliated Hospital of Medical School, Nanjing University (Approval No. 2021DZGKJDWLS-00115).

Authors Contribution

Jin Li and Fang Ren designed the study, completed the experiment and supervised the data collection, Hongshan Yuan analyzed and interpreted the data. Wenliang Yan prepared the manuscript for publication. All authors read and approved the manuscript for publication.

Conflicts of Interest

The authors state that there are no conflicts of interest to disclose.

Funding

None.

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