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LARP7 alleviates psoriasis symptoms in mice by regulating the SIRT1/NF-κB signaling pathway

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transcriptional regulator (LARP7)

Abstract
Objective: To unravel the role of La ribonucleoprotein 7 (LARP7), a transcriptional regulator, in the progression of psoriasis and the underlying molecular mechanisms.
Methods: The psoriasis-like mice model was created by daily administering of imiquimod on shaved skin. The histological analysis and skin damage were evaluated in each group. The inflammation and oxidative stress response were assessed by enzyme-linked-immunosorbent serologic and immunoblot assays. The involvement of silent information regulator 1 (member of the Sirtuin family; SIRT1/nuclear factor kappa B (NF-κB) signaling pathway in LARP7-mediated psoriasis progression was also detected by immunoblot assay.
Results: LARP7 relieved psoriasis symptoms in the mice model. LARP7 inhibited the expression of inflammatory cytokines as well as chemokines in psoriasis-like skin tissues. Additionally, LARP7 suppressed oxidative stress in the psoriasis-like skin tissues of mice. LARP7 inhibited the activation of the SIRT1/NF-κB signaling pathway, and therefore affected the progression of psoriasis.
Conclusion: LARP7 relieved psoriasis symptoms in mice by regulating the SIRT1/NF-κB signaling pathway.

Introduction

Psoriasis is a complex, immune-mediated hyperproliferative disease featured by overgrowth of epidermal keratinocytes (KC), accompanied by abnormal terminal differentiation.[1] The exact pathogenesis of psoriasis is still unclear, but it is widely believed that abnormal crosstalk plays an important role in the pathogenesis of psoriasis.[2,3] An extensive network of growth factor circuits, such as epidermal growth factor, vascular endothelial growth factor, and keratinocyte growth factor, as well as cytokines, further mediates keratinocyte cell proliferation.[4] In addition, the critical role of

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reactive oxygen species (ROS) in the pathogenesis of psoriasis has been reported in patients.[5] The silent information regulator 1 (SIRT1)/nuclear factor kappa B (NF-κB) signaling pathway is also critical in psoriasis regulation. Activation of NF-κB in psoriasis leads to the production of several immune-related proteins, such as chemokines CCL-20, CXCL-1, and interleukin (IL)-36, which promote inflammatory responses.[6] SIRT1 is a member of the Sirtuin family. Its activation inhibits oxidative stress-related signaling pathways, down-regulates expression of inflammatory factors, and inhibits inflammation as well as excessive proliferation of keratinocytes.[7] To combat this disease, new and more targets are needed to be explored.

La ribonucleoprotein 7 (LARP7), a transcriptional regulator, is a La family RNA-binding protein with methyl phosphate endase.[8] LARP7 has been described to inhibit breast cancer progression and metastasis by inhibiting positive transcription elongation factor b (P-TEFb) activity.[9] Recent studies have shown that LARP7 attenuated aging and atherogenesis by inhibiting NF-κB transcriptional activity through SIRT1 activation.[10] In addition, knockdown of LARP7 aggravates neutrophil inflammation.[11] Whether LARP7 plays a role in psoriasis by regulating SIRT1/NF-κB signaling pathway requires further investigation.

In this study, we investigated the role of LARP7 in the progression of psoriasis. We also explored the underlying molecular mechanisms.

Materials and Methods

Animal procedures

Male BALB/c mice (aged 8 weeks; SLAC, Shanghai, China) were anesthetized and the hair on back and left ears was shaved. The mice were housed in an air-condition-regulated environment (20˚C and 40% humidity) under a 12-h light/dark cycle with ad libitum access to food and water. Anesthesia was induced by inhalation of 2.5% isoflurane, and maintained with 1% isoflurane. The animals were randomly divided into the following five groups, with eight mice in each group: control, imiquimod (IMQ), IMQ + adeno-associated virus vector (AAV), IMQ + AAV sh LARP7 (to deplete its expression), and IMQ + AAV LARP7 (n = 8). AAV was injected intraperitoneally as 1×10^{10} viral genome particles before construction of psoriasis model. In order to induce psoriasis, mice were administrated 62.5-mg IMQ consecutively on the shaved skin for 7 days.[12] On the seventh day, thickness of the left ear was measured 4 h after the administration of IMQ. Erythema and thickness were recorded based on the Psoriasis Area and Severity Index (PASI) score.[13] All the experiments were conducted 3 weeks post-AAV injection. The study was approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University.

Hematoxylin and eosin (H&E) staining

Skin samples in each group were fixed and embedded into paraffin and sliced as 6-μm sections. Then these sections were stained in H&E staining solution.

Immunoblot assay

Proteins were extracted with radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Beijing, China). The samples were collected and subsequently subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, the proteins were transferred onto polyvinylidene fluoride (PVDF) membranes, followed by blocking with 5% bovine serum albumin (BSA). Subsequently, membranes were incubated overnight at 4˚C with the following primary antibodies: LARP7 (1:1000, ab134746; Abcam, Cambridge, UK), COX-2 (1:1000, ab179800; Abcam), induced nitric oxide synthase (iNOS) (1:1000, ab178945; Abcam), p-IκBα (1:1000, ab157846, Abcam), IκBα (1:1000, ab237777; Abcam), p-p65 (1:1000, ab183559; Abcam), p65 (1:1000, ab32536; Abcam), sirtuins 1 (SIRT1, (1:1000, ab110304; Abcam), and beta-actin (1:5000, ab8226; Abcam). Subsequently, the membranes were incubated with specific secondary antibodies for 1 h. All blots were observed using the ECL kit and analyzed using the ImageJ 9.0 software (National Institutes of Health. MD, USA)

Enzyme-linked-immunosorbent serologic assay (ELISA)

The levels of CXCL-1, CCL-20, IL-1β, and IL-17 were assessed using the ELISA kit (Beyotime) following the manufacturer's guidelines. Samples were aspirated into wells and incubated with biotin-conjugated primary antibodies followed by avidin-conjugated horseradish peroxidase (HRP). Then the enzyme substrate was used for color reaction.

Detection of antioxidant activity

The levels of glutathione (GSH), r-glutamyl-cysteinyl-glycine, and malondialdehyde (MDA) were measured using the detection kits obtained from Nanjing Jiancheng Institute (Jiangsu, China) following the manufacturer's guidelines.

Statistical analysis

Data were analyzed using the GraphPad 8.0 software (GraphPad Software Inc., CA, USA). Error bars represent mean ± standard errors of mean (SEM); all experiments were repeated for three times. The unpaired Student's *t*-test was used to determine statistical significance between two groups. One-way ANOVA followed by Tukey's *post hoc* test was used for multiple comparisons. $P < 0.05$ indicated significant difference between two groups.

Results

LARP7 alleviated IMQ-induced psoriasis symptoms in the mice model

As shown by Figure 1A, the expression of LARP7 was reduced by IMQ stimulation. LARP7 expression was decreased by AAV-LARP7-shRNA insertion, and enhanced

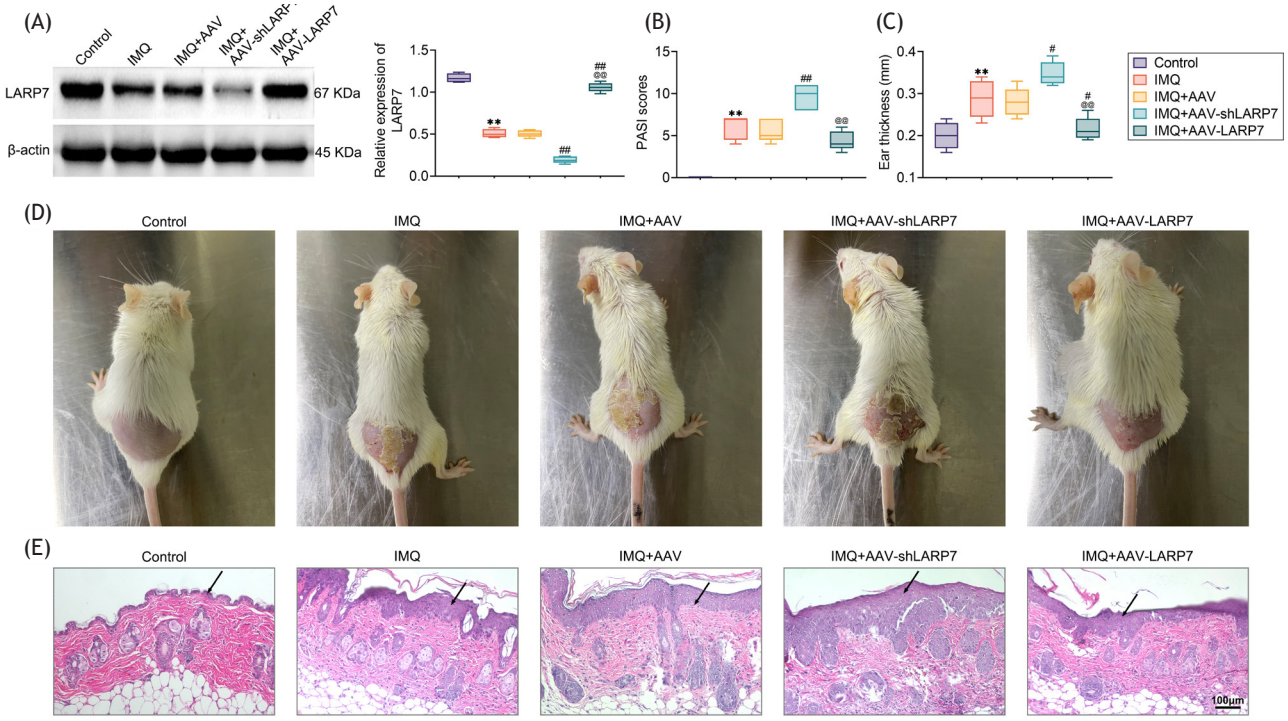

Figure 1 LARP7 alleviated IMQ-induced psoriasis symptom of mice. (A) The expression of LARP7 in the skin tissues of control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 mice. (B) and (C) The PASI scores and ear thickness in mice of control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 groups. (D) The representative images of skin damage in each group. (E) The histological exhibition in each group. Cell apoptosis in response to the indicated treatment was detected by flow cytometry. **P < 0.01 vs. control group; ##P < 0.01 vs. IMQ mice; @@P < 0.01 vs. IMQ + AAV.

by AAV-LARP7 infection. The PASI score and ear thickness were enhanced in IMQ-treated mice. LARP7 knockdown further increased the PASI score and ear thickness, which was reversed in LARP7-overexpressed mice (Figures 1B and C). LARP7 knockdown exaggerated epidermal hyperplasia, which was alleviated in LARP7-overexpressed mice (Figure 1D). Moreover, mice in the IMQ group demonstrated increased epidermal spinous hypertrophy, keratinosis, and a higher degree of epidermal thickening, and these symptoms were relieved after LARP7 overexpression (Figure 1E).

LARP7 inhibited the production of inflammatory cytokine and chemokine in psoriasis mice

The levels of IL-17, IL-1β, CCL-20, and CXCL-1 were enhanced in IMQ-stimulated mice. However, LARP7 overexpression significantly decreased the levels of IL-17, IL-1β, CCL-20, and CXCL-1 in psoriasis mice (Figure 2).

LARP7 ameliorated oxidative stress in psoriasis-like skin tissues

As shown in Figure 3A, the IMQ-stimulated mice had a higher levels of MDA and lower levels of GSH, which were partially inhibited by LARP7 overexpression. Moreover, the expressions of COX-2 and iNOS were enhanced by IMQ stimulation and inhibited by LARP7 overexpression (Figure 3B). Altogether, LARP7 ameliorated oxidative stress in psoriasis-like skin tissues.

LARP7 regulated SIRT1/NF-κB signaling pathway in psoriasis-like mice

Application of IMQ strongly promoted the activation of p-IκBα and p-p65, and suppressed SIRT1 expression (Figure 4). LARP7 overexpression led to an obvious decrease in p-IκBα and p-p65 expression and increase in SIRT1 level in mice with IMQ stimulation (Figure 4). Our data suggested that LARP7 regulated SIRT1/NF-κB signaling pathway in psoriasis-like mice.

Discussion

Psoriasis is a chronic inflammatory skin disease with a long course as well as relapse tendency.[14,15] Proper symptomatic treatment is required for treating this disease.[16] Since it is known as a chronic recurrent disease, several patients require long-term treatment.[17] The management mainly includes combinatory, alternate, sequential, and intermittent therapy.[18] The critical role of ROS in the pathogenesis of psoriasis is reported, with elevated levels of oxidative stress markers as well as decreased activity of antioxidant enzymes.[5,19] The SIRT1/NF-κB signaling pathway is critical in psoriasis regulation.[2] The present study revealed that LARP7 alleviated psoriasis symptoms and promoted its progression in mice.

ELISA revealed that LARP7 suppressed the production of inflammatory cytokines and chemokines in psoriasis-like skin tissues of mice. The study also confirmed that LARP7

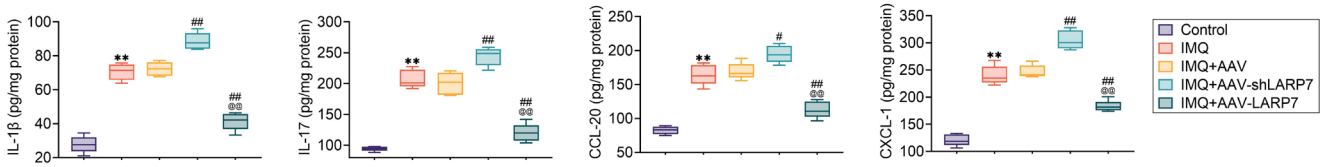

Figure 2 LARP7 inhibited inflammatory cytokine and chemokine in psoriasis mice. Inflammatory cytokines and chemokines in skin tissues of control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 mice. **P < 0.01 vs. control group; ##P < 0.01 vs. IMQ mice; @@P < 0.01 vs. IMQ + AAV.

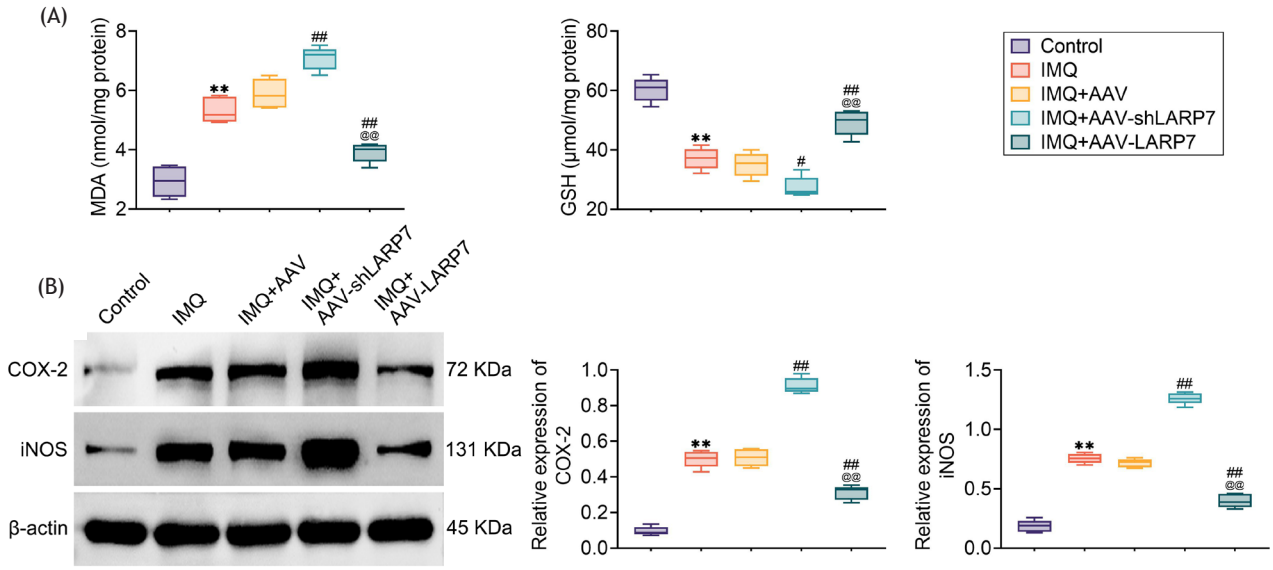

Figure 3 LARP7 ameliorated oxidative stress in psoriasis-like skin tissue of mice. (A) Levels of MDA and GSH in the skin tissues of control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 mice. (B) Immunoblot assay showed the levels of COX-2 and iNOS in control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 mice. **P < 0.01 vs. control group; ##P < 0.01 vs. IMQ mice; @@P < 0.01 vs. IMQ + AAV.

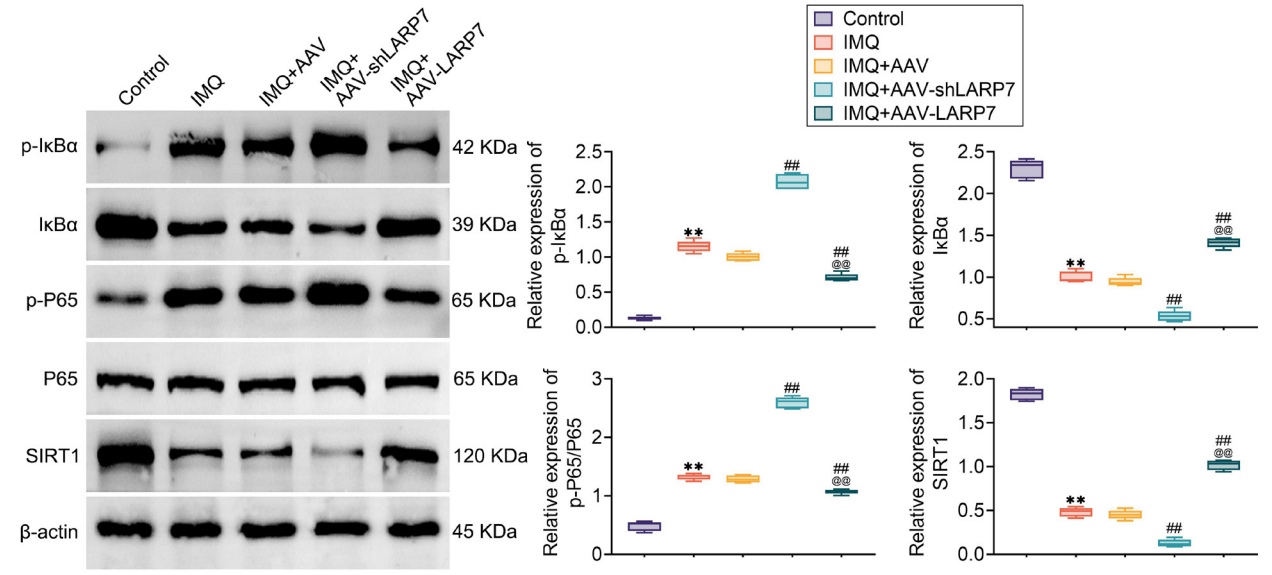

Figure 4 LARP7 regulated SIRT1/NF-κB signaling pathway in psoriasis-like mice. Immunoblot assays depicted the expression of p-IκBα, p-p65, and SIRT1 in the skin tissues of control, IMQ, IMQ + AAV, IMQ + AAV sh LARP7, and IMQ + AAV LARP7 mice. **P < 0.01 vs. control group; ##P < 0.01 vs. IMQ mice; @@P < 0.01 vs. IMQ + AAV.

inhibited oxidative stress in psoriasis-like skin tissues. Therefore, it was hypothesized that LARP7 could alleviate psoriasis symptoms via inhibiting inflammatory response and oxidative stress.

The current treatment modalities are not effective due to their impact on patients' quality of life. At present, it is believed that abnormal activation of immune cells, mainly T cells, active proliferation of local keratinocytes, dysdifferentiation, and abnormal immune activity are important factors in the pathogenesis of psoriasis. Expression of inflammatory cytokines as well as chemokines in the skin is closely related to oxidative stress and development of psoriasis. Dendritic cells, immune T cells, macrophages, keratinocytes, etc. are involved in the inflammatory cascade, leading to the secretion of cytokines, including IL-17A, IL-23, IL-22, TNF-α, etc., which are involved in the mechanism of psoriasis.[20] In addition, mitogen-activated protein kinase (MAPK) signal induces inflammation in psoriasis. Oxidative stress also causes skin inflammation and induces the production of a large number of inflammatory cytokines, leading to the cutin cell proliferation and persistent inflammation, thus promoting psoriasis.[9] Different studies have confirmed that various proteins regulate the progression of psoriasis by regulating the expression of inflammatory factors or affecting oxidative stress.[8] However, the precise mechanism needs further study.

Interestingly, knockdown of LARP7 increases neutrophil inflammation, which was confirmed in the study conducted by Imbert-Bouteille et al.[11] T cell-mediated immunity plays an important role in the development and persistence of psoriasis. Chronic inflammation may develop during T cell and cutin cell interactions, namely T cell-mediated inflammatory continuous loop, while the aggregation of neutrophils can lead to acute inflammation in skin lesions or layer angles. Therefore, our data confirmed LARP7 mediated inflammatory response and oxidative stress via regulating SIRT1/NF-κB signaling pathway, and thus affecting the progression of psoriasis.

In this study, we found that LARP7 could mediate SIRT1/NF-κB signaling pathway. In fact, this pathway widely affected inflammatory response and oxidative stress. The specific role of SIRT1/NF-κB signaling pathway in psoriasis was unraveled. As a protein deacetylase, SIRT1 plays a primary regulatory role in genetics, metabolism, and anti-inflammatory and differentiation promotion. The expression of SIRT1 is decreased in psoriasis, suggesting that SIRT1 plays an important role in the development of psoriasis[21] through regulating NF-κB pathway, signal transducer and activator of transcription (STAT)-3 pathway, MAPK pathway, etc.[22] Different studies also indicated that SIRT1/NF-κB signaling pathway was involved in the pathology of psoriasis, and we therefore hypothesized that LARP7 mediated inflammatory response and oxidative stress via regulating SIRT1/NF-κB signaling pathway, and thus affecting the progression of psoriasis.[8,9,23]

Conclusion

LARP7 relieved psoriasis symptoms in mice. LARP7 inhibited the expression of inflammatory cytokines and chemokines and suppressed oxidative stress in psoriasis-like skin tissues of mice. LARP7 blocked the progression of psoriasis by regulating the SIRT1/NF-κB signaling pathway.

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Conflict of Interest

The authors stated that there were no conflicts of interest to disclose.

Data Availability

The authors declared that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Na Li and Yulei Liu designed and carried out the study. Both authors supervised data collection, analyzed and interpreted the data, and prepared the manuscript. Both authors reviewed, read, and approved the final draft of the manuscript for publication.

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