

Silencing of miR-155 suppresses inflammatory responses in psoriasis through inflammasome NLRP3 regulation

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Abstract. Psoriasis is a dermatosis with the major clinical symptoms of scale, erythema and itching, and it has a long disease course. In addition, it is easily recurrent and refractory, greatly affecting the physical and mental health of patients. In the present study, it was hypothesized that the function of miR-155 increases psoriasis-induced inflammation and that its expression may be dependent on inflammasome activation. miR-155 expression was examined by gene chip array and quantitative polymerase chain reaction analysis. miR-155 expression levels were significantly increased in an *in vivo* model of psoriasis compared with normal tissues, as was the expression of NLR family pyrin domain containing 3 (NLRP3). *In vitro*, using keratinocyte-induced HaCaT cells as a model for psoriasis, silencing of miR-155 was confirmed to significantly decrease inflammation and NLRP3/caspase-1 signaling. Activation of toll-like receptor 4 (TLR4) enhanced the miR-155-induced inflammatory response in the *in vitro* keratinocyte model. Treatment with a TLR4 inhibitor or an NLRP3 inhibitor reversed the miR-155-mediated inflammation in the same cell system. The present study demonstrated that miR-155 silencing suppressed psoriasis-associated inflammatory responses through inflammasome NLRP3 regulation.

Introduction

The etiology of psoriasis remains unclear. Modern research has demonstrated that it is related to various factors, including heredity, infection, immunity, spirit, endocrine, trauma, diet and metabolic disorder (1). At present, it is suggested that immune mediation accounts for the major pathogenesis (2). Its clinical manifestation consists of reddish

and scaly skin damage, with an initial reddish rash (2). The epidermis is covered by layers of silvery scales, with dry, flaking and crusting skin (2). In addition, several skin symptoms are contiguous. Some may be itching, suppurant and blood-stained. Psoriasis can be classified into Plaque, erythrodermic, pustular, and arthritic types (3). Blood-heat syndrome in traditional Chinese medicine is dominated by papule and maculopapule, where new rash occurs continuously, basal skin is bright red in color, and punctuate hemorrhage can be observed after scraping the scales (4). In addition, it is accompanied with various degrees of itching, which equals to the progressive psoriasis in western medicine. Prospective treatment and future drug therapies have been proposed under the guidance of gradually updated pathogenesis concept (5). Furthermore, more attention has been paid to the complicated pathogenesis inducing the pathological changes of the disease (4). Treatment may not be limited to the traditional antiproliferative, antimetabolic and anti-inflammatory measures based on the cellular morphological changes (1).

MicroRNAs (miRNAs) are derived from the non-coding region of the genome (6). They are a group of evolutionarily conserved single-strand RNAs with length of ~21-24 nucleotides (7). A miRNA can target the mRNA of multiple genes. In addition, the mRNA of a single gene can be simultaneously regulated by multiple miRNAs, suggesting a great diversity and complexity of miRNAs biological activities (7). Research focusing on the regulation of miRNAs in immune responses, as well as their regulatory mechanisms, are currently a hot topic in immunology. The involvement of miRNAs in regulating inflammation, natural immune responses and antiviral immune responses has been reported in numerous studies (7,8).

Previous research has indicated that NLR family pyrin domain containing 3 (NLRP3) can be stimulated by various pathogenic microorganisms (9). In addition, it can be stimulated by multiple pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and environmental factors (9). These mainly include ATP, hyaluronic acid, β -amyloid protein, extracellular glucose, monosodium urate crystal, shoe stone, asbestos, ultraviolet ray, picric acid and dinitrofluorobenzene (10). Additionally, NLRP3 expression has been demonstrated to be increased in multiple diseases, including myocardial infarction, Alzheimer

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disease, type 2 diabetes and its complications, gouty arthritis, lung disease and dermatitis (11). Toll-like receptor 4 (TLR4) signaling regulates miR-155 to promote liver fibrosis and alcohol-induced steatohepatitis (12). In the present study, it was hypothesized that the function of miR-155 may increase psoriasis-induced inflammation and that its expression may be dependent on inflammasome activation.

Materials and methods

Imiquimod-induced skin-inflammation model. Female BALB/c mice (6 week, 20–22 g, n=12) were purchased from the Center of Laboratory Animal Science of Guangdong (Guangdong, China), and housed at 22–23°C, 55–60% humidity, 12 h light/dark cycle, and freely access to food and water. The mice (n=12) were randomly assigned into two groups: Normal (n=6) and psoriasis model (n=6). In the psoriasis model group, mice were treated on regions of shaved skin with 62.5 mg of 5% imiquimod cream (Aldara; 3M Pharmaceuticals, Maplewood, MN, USA) at 8:00 pm for 7 days. The present study was approved by the Medical Ethics Committee of Guangzhou Institute of Dermatology (Guangzhou, China).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay and microarray data analysis. Total RNA from skin samples and cells was isolated using TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Total RNA was reverse transcribed using 200 ng total RNA with the RevertAid Reverse Transcription kit (Thermo Fisher Scientific, Inc.). qPCR was conducted using a Roche LightCycler 480 PCR machine (Roche Diagnostics, Basel, Switzerland) with the SYBR-Green miScript PCR kit (Qiagen, Inc., Valencia, CA, USA). The reaction conditions were: 95°C for 10 min, followed by 40 cycles of 95°C for 30 sec and 60°C for 30 sec. Primer sequences were as follows: miR-155, forward 5'-TTA ATGCTAATCGTGTAGGGG-3' and reverse 5'-CGAATT CTAGAGCTCGAGGCAGG-3'; U6, forward 5'-CTCGCT TCGGCACA-3' and reverse 5'-CGAATTCTAGAGCTC GAGGCAGG-3'. mRNA expression was calculated using the formula $2^{-\Delta\Delta C_q}$ (12).

A total of 500 ng total RNA was used for microarray data analysis (8x15 K G4471A-021828 platform) Agilent Technologies, Inc., Santa Clara, CA, USA) (13). Array data was acquired using the Agilent Feature Extraction software (Agilent Technologies, Inc.) according to the manufacturer's protocols. The GeneSpring GX v12.1 software package (Agilent Technologies, Inc.) was used for quantile normalization and subsequent data processing.

Cell culture and cell transfection. HaCaT cells were cultured at 37°C/5% CO₂ in MEM (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and antibiotics (Sigma-Aldrich; Merck KGaA). miR-155, forward 5'-GATCCCTGTTAATGCTAATCGTGATAG GGGTTTTTGCCTCCAAGTACTGCTCCTACATATTAGCA TTAACAGA-3', reverse 5'-AGCTTCTGTTAATGCTAA TATGTAGGAGTCAGTTGGAGGCCAAAACCCCTATCA CGATTAGCATTAACAGG-3'; miR-155 inhibitor, forward 5'-ACCCCUAUCACGAUUAAGCAUUA-3', reverse 5'-ACG

UGACACGUUCGGAGAATT-3'; negative control, forward 5'-CCCCCCCCCCCCCCCCCCCC-3' and reverse 5'-CCC CCCCCCCCCCCCCCCCCC-3'. miR-155 mimic, miR-155 inhibitor and negative control oligos were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). miR-155 mimic (100 ng), miR-155 inhibitor (100 ng) and negative control (100 ng) were transfected into cells using Lipofectamine 2000 (Thermo Fisher Scientific, Inc.). Following transfection for 6 h, the medium was removed and fresh medium was added, containing 0.1 ng/ml human interleukin (IL)-4 (Sigma-Aldrich; Merck KGaA), 1 ng/ml human tumor necrosis factor (TNF)- α (Sigma-Aldrich; Merck KGaA) and 10 ng/ml human interferon (IFN)- γ (Sigma-Aldrich; Merck KGaA) for 24 h. Cells were then cultured as keratinocyte-induced HaCaT cells, an *in vitro* model for psoriasis, as previously reported in the literature (14). Cells were induced with 100 ng/ml LPS (Beyotime Institute of Biotechnology, Jiangsu, China) for 24 h. Cells were treated with NLRP3 inhibitor, MCC950 (2 nM; MedChemExpress, Shanghai, China) for 8 h.

ELISA. Following culture of the *in vitro* model of psoriasis for 8 h, the culture supernatant was centrifuged at 2,000 x g for 10 min at 4°C and TNF- α (H052), IL-18 (H015), IL-6 (H007) and IL-1 β (H002) levels were quantified with a ELISA kits (Nanjing Jiancheng Biology Engineering Institute, Nanjing, China). Absorbency was detected at 450 nm using an AIA 600II Automated Enzyme Immunoassay Analyzer (Tosoh Corporation, Tokyo, Japan).

Western blot analysis. Following culture of the *in vitro* model of psoriasis for 8 h, cells were lysed with RIPA buffer (Beyotime Institute of Biotechnology) and protein concentration was quantified using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). A total of 50 μ g proteins were separated using 8–12% SDS-PAGE gels and blotted onto a polyvinylidene difluoride membrane (Life Technologies, Grand Island, NY, USA). The membrane was blocked with 5% non-fat dried milk for 1 h at 37°C and then probed with antibodies against TLR4 (1:1,000; sc-10741; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), NF- κ B (1:1,000; sc-109, Santa Cruz Biotechnology, Inc.), NLRP3 (1:1,000; sc-66846, Santa Cruz Biotechnology), caspase-1 (1:1,000; sc-514, Santa Cruz Biotechnology, Inc.) and GAPDH (1:5,000; sc-25778, Santa Cruz Biotechnology, Inc.) overnight at 4°C. Following three washes with PBST, the PVDF membrane was incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:5,000; 7074, Cell Signaling Technology, Inc., Danvers, MA, USA) for 1 h at 37°C. Signals were developed using an enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc.) and protein expression was quantified using Image-Pro Plus software version 6.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analysis. Data are expressed as the mean \pm standard deviation from three independent repeats. Statistical analyses were performed by one-way analysis of variance following by a Tukey post-hoc test using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

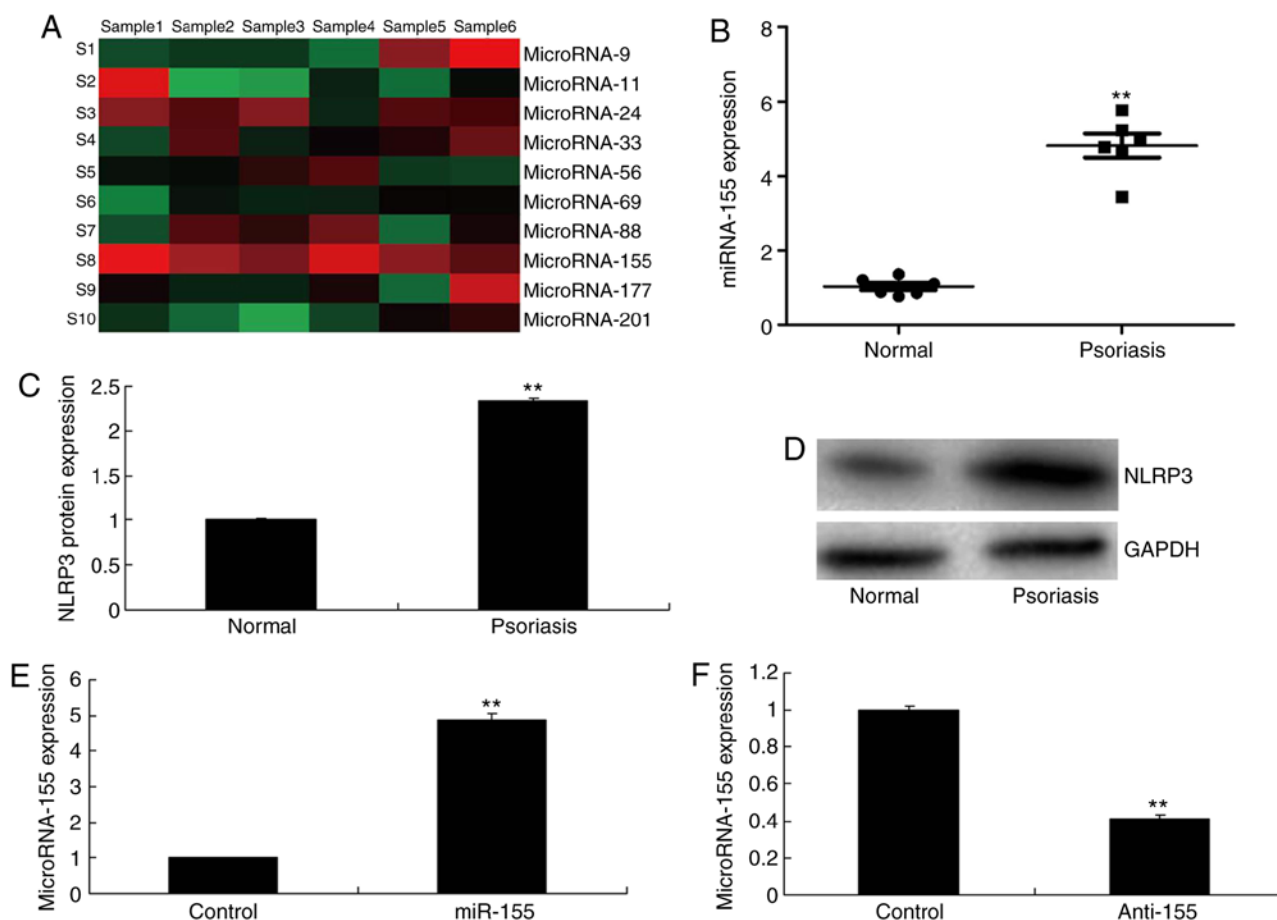


Figure 1. miR-155 expression in psoriasis. (A) Gene chip analysis for miRNA expression and (B) quantitative polymerase chain reaction analysis for miR-155 expression in psoriasis and normal mouse skin tissues (n=6 per group). (C) Quantification and (D) representative images from western blot analysis of NLRP3 protein expression levels in psoriasis and normal mouse skin tissues. (E) Confirmation of successful upregulation and (F) silencing of miR-155 by transfection of a mimic or an inhibitor, respectively, in HaCaT cells. **P<0.01 compared with control. NLRP3, NLR family pyrin domain containing 3.

Results

miR-155 expression in psoriasis. The expression of miR-155 in psoriasis was first examined by using gene chip and qPCR analyses. In the *in vivo* model of psoriasis, miR-155 expression levels were significantly increased in psoriasis tissues compared with normal tissues (Fig. 1A and B). Notably, the protein expression levels of NLRP3 were also significantly induced in the *in vivo* psoriasis model group, compared with the control group (Fig. 1C and D). Based on these initial experiments, it was hypothesized that miR-155 may promote psoriasis by modifying gene expression. Then, miR-155 mimics increased the expression of miR-155, and anti-miR-155 mimics decreased the expression of miR-155 *in vitro* model (Fig. 1E and F).

Effects of miR-155 on the inflammatory response of lipopolysaccharide (LPS)-induced HaCaT cells. Next, LPS was used to induce HaCaT cells and a mimic was used to overexpress miR-155. The results demonstrated that the protein expression levels of TLR4, nuclear factor (NF)- κ B, NLRP3 and caspase-1 were lower in the miR-155 overexpression alone group compared with the LPS group (Fig. 2). The protein expression of TLR4 and NF- κ B in the miR-155 overexpression and LPS groups was similar to those of the LPS alone group (Fig. 2A,

B, and E). However, the protein expression of NLRP3 and caspase-1 in the miR-155 overexpression and LPS group was higher compared with the LPS alone group (Fig. 2C-E). In addition, the levels of secreted TNF- α , IL-18, IL-6 and IL-1 β in the culture supernatant of the miR-155 overexpression alone group were lower compared with the LPS group (Fig. 3). However, the levels of secreted TNF- α , IL-18, IL-6 and IL-1 β levels in the miR-155 overexpression and LPS group were higher than those of the LPS alone group (Fig. 3). These results suggested that miR-155 regulated the inflammasome NLRP3 activation, but did not affect the TLR4/NF- κ B signaling pathway.

Effects of miR-155 expression on inflammation. Then, the *in vitro* psoriasis model of keratinocyte-induced HaCaT cells was used to either upregulate or silence miR-155 expression by transfecting a mimic or an inhibitor, respectively (Fig. 1E, F). Overexpression of miR-155 significantly increased TNF- α , IL-18, IL-6 and IL-1 β levels (Fig. 4A-D). By contrast, silencing of miR-155 significantly decreased TNF- α , IL-18, IL-6 and IL-1 β levels in keratinocyte-induced HaCaT cells (Fig. 4E-H).

miR-155 regulates the NLRP3/caspase-1 signaling pathway. To examine the potential mechanism by which miR-155 may mediate inflammation in psoriasis, the expression levels of proteins in the NLRP3/caspase-1 pathway were measured. As

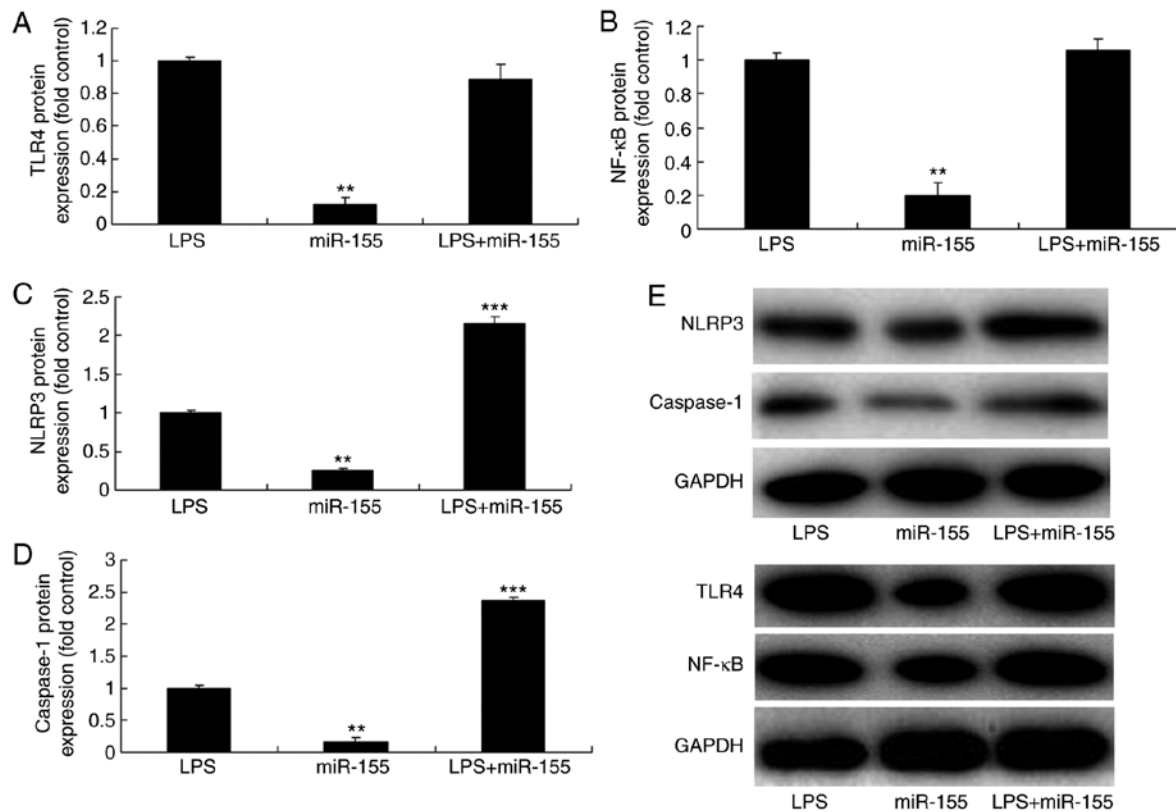


Figure 2. Effects of miR-155 on inflammasome NLRP3 activation in LPS-induced HaCaT cells. HaCaT cells were induced with LPS (100 ng/ml for 6 h) and/or transfected with miR-155 mimic. Protein expression levels of (A) TLR4, (B) NF-κB, (C) NLRP3 and (D) caspase-1 were analyzed by western blotting (representative blots are presented in panel E). ** $P < 0.01$ compared with LPS, *** $P < 0.01$ compared with miR-155. NLRP3, NLR family pyrin domain containing 3; LPS, lipopolysaccharide; TLR4, toll-like receptor 4; NF-κB, nuclear factor-κB.

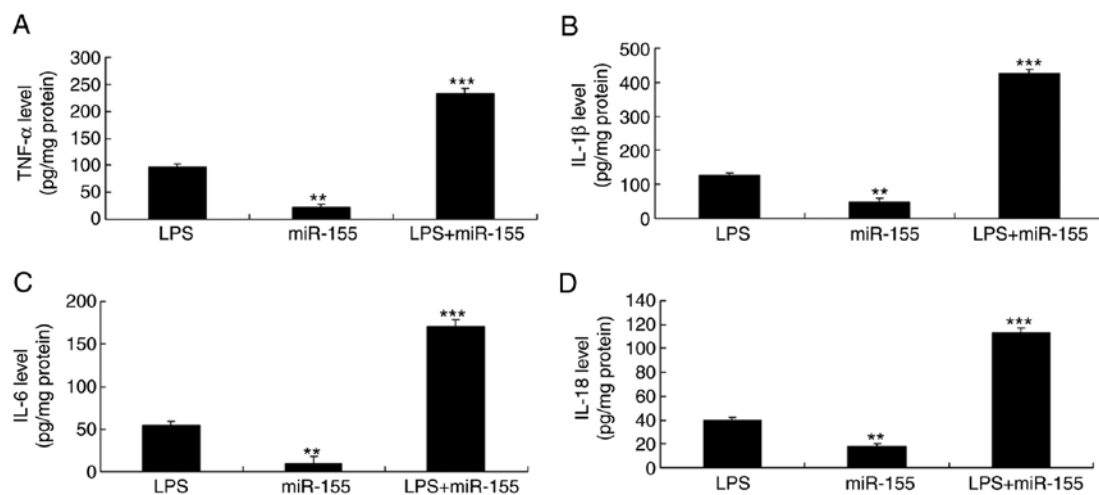


Figure 3. Effects of miR-155 on inflammation in LPS-induced HaCaT cells. HaCaT cells were induced with LPS (100 ng/ml for 6 h) and/or transfected with miR-155 mimic. Secreted levels of (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) IL-18 were measured by ELISA. ** $P < 0.01$ compared with LPS, *** $P < 0.01$ compared with miR-155. LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin.

illustrated in Fig. 5, overexpression of miR-155 significantly enhanced NLRP3 and caspase-1 protein expression, while miR-155 silencing significantly suppressed NLRP3 and caspase-1 protein expression in keratinocyte-induced HaCaT cells.

Activation of TLR4 by LPS stimulation enhances miR-155-mediated inflammation in keratinocyte-induced

HaCaT cells. Bala *et al* (15) have demonstrated that TLR4 signaling regulates miR-155 to promote liver fibrosis and alcohol-induced steatohepatitis. To directly examine the upstream pathways of miR-155 in psoriasis-related inflammation, the TLR4/NF-κB signaling pathway was analyzed. NLRP3 and caspase-1 protein expression levels were significantly increased by LPS stimulation and miR-155 overexpression, compared with the miR-155 overexpression

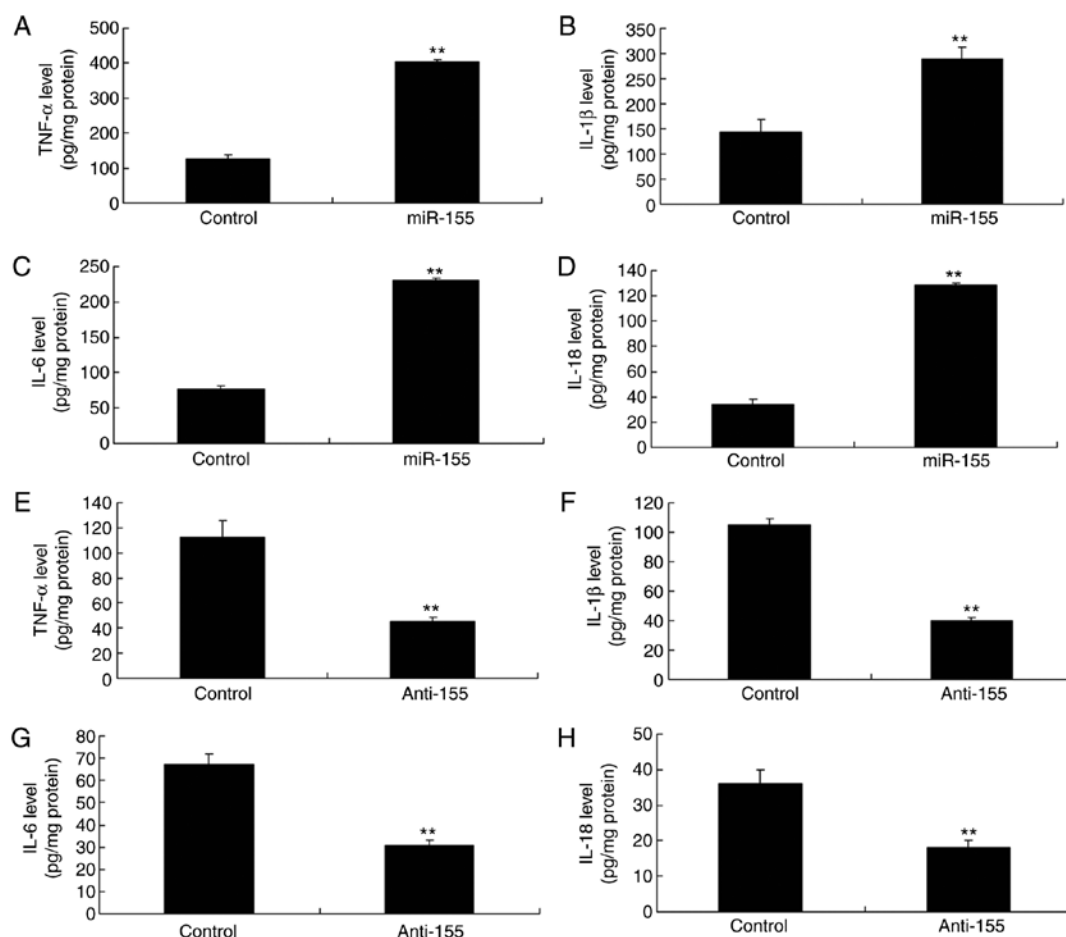


Figure 4. Effects of miR-155 on the inflammatory response in a psoriasis *in vitro* model. Psoriasis-induced cells were transfected with either (A-D) a miR-155 mimic or (E-H) a miR-155 inhibitor and levels of secreted TNF- α , IL-1 β , IL-6 and IL-18 were measured by ELISA. **P<0.01 compared with control. TNF, tumor necrosis factor; IL, interleukin; miR-155, miR-155 mimic; anti-155, miR-155 inhibitor.

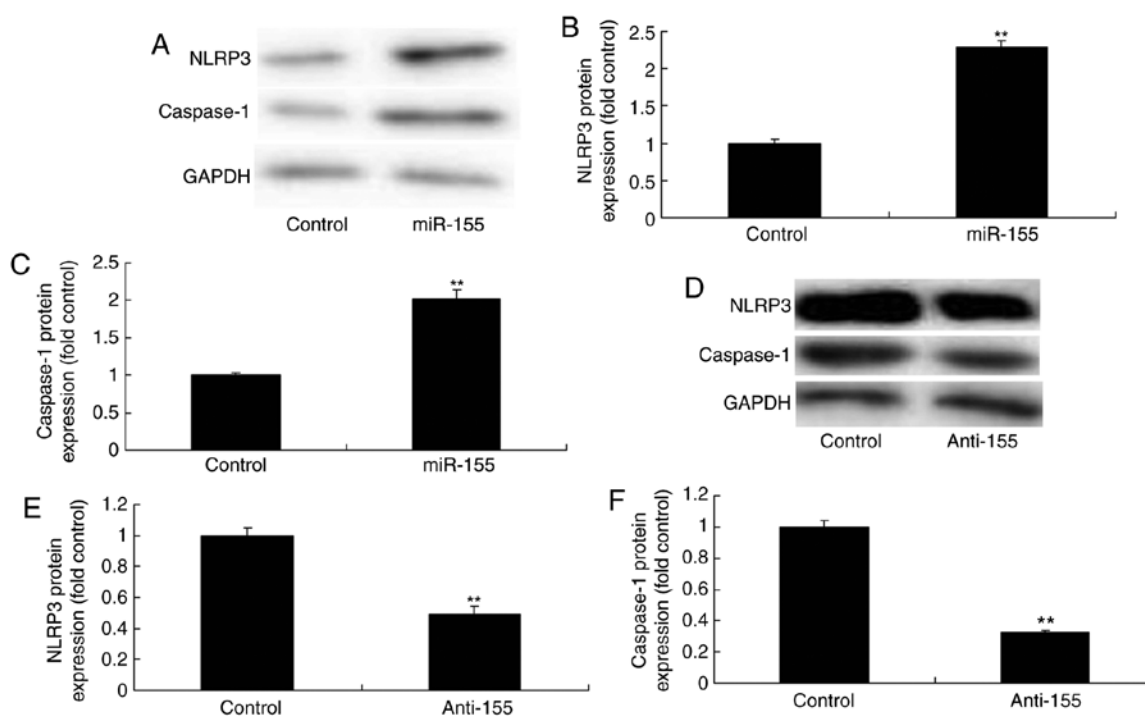


Figure 5. Effects of miR-155 on NLRP3/caspase-1 signaling pathway in a psoriasis *in vitro* model. Psoriasis-induced cells were transfected with either (A-C) a miR-155 mimic or (D-F) a miR-155 inhibitor, and NLRP3 and caspase-1 protein expression levels were analyzed by western blotting. **P<0.01 compared with control. NLRP3, NLR family pyrin domain containing 3; miR-155, miR-155 mimic; anti-155, miR-155 inhibitor.

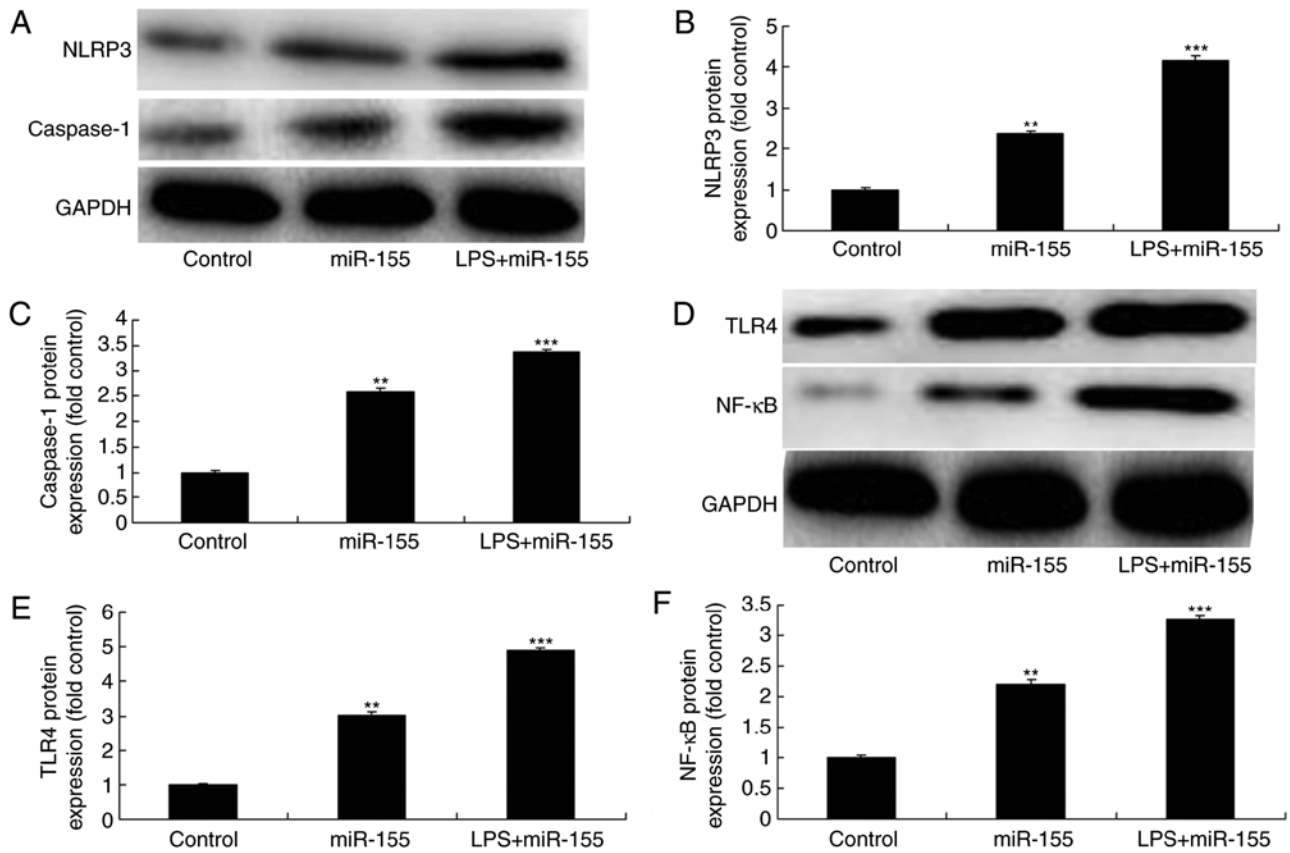


Figure 6. Activation of TLR4 enhances the function of miR-155 in the *in vitro* keratinocyte psoriasis model. Psoriasis-induced cells were induced with LPS and/or transfected with miR-155 mimic. Representative blots and quantification of western blot analysis for (A-C) NLRP3 and caspase-1, and (D-F) TLR4 and NF-κB protein expression levels. ** $P < 0.01$ compared with control group; *** $P < 0.01$ compared with miR-155-overexpression alone group. TLR4, toll-like receptor 4; LPS, lipopolysaccharide; NLRP3, NLR family pyrin domain containing 3; NF-κB, nuclear factor-κB.

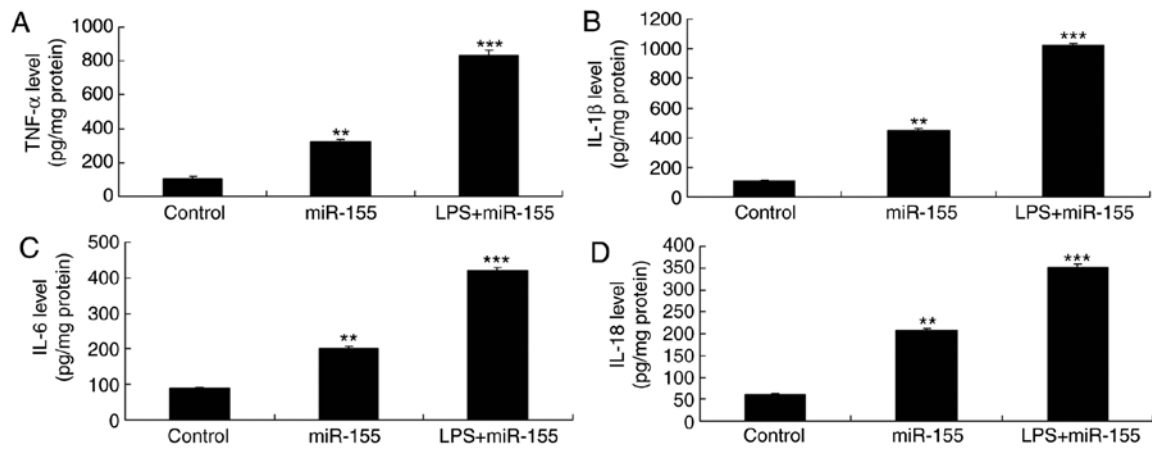


Figure 7. Activation of TLR4 enhances the function of miR-155 on the inflammatory response of the *in vitro* keratinocyte psoriasis model. Psoriasis-induced cells were induced with LPS and/or transfected with miR-155 mimic. The levels of secreted (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) IL-18 were measured by ELISA. ** $P < 0.01$ compared with control group; *** $P < 0.01$ compared with miR-155-overexpression alone group. TLR4, toll-like receptor 4; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin.

alone group (Fig. 6A-C). In addition, TLR4 and NF-κB protein expression levels were significantly induced by LPS stimulation and miR-155 overexpression, compared with the miR-155 overexpression alone group (Fig. 6D-F). Furthermore, LPS stimulation, and subsequently activation of TLR4, enhanced the miR-155-mediated increase in the secreted levels of TNF-α, IL-18, IL-6 and IL-1β in miR-155-overexpressing

keratinocyte-induced HaCaT cells, compared with the miR-155 overexpression alone group (Fig. 7).

Inhibition of TLR4 reverses the miR-155-mediated inflammation in keratinocyte-induced HaCaT cells. The TLR4 inhibitor, TAK-242, was added in the cells at 2 nM for 8 h to inhibit TLR4 activation. The results demonstrated that TLR4

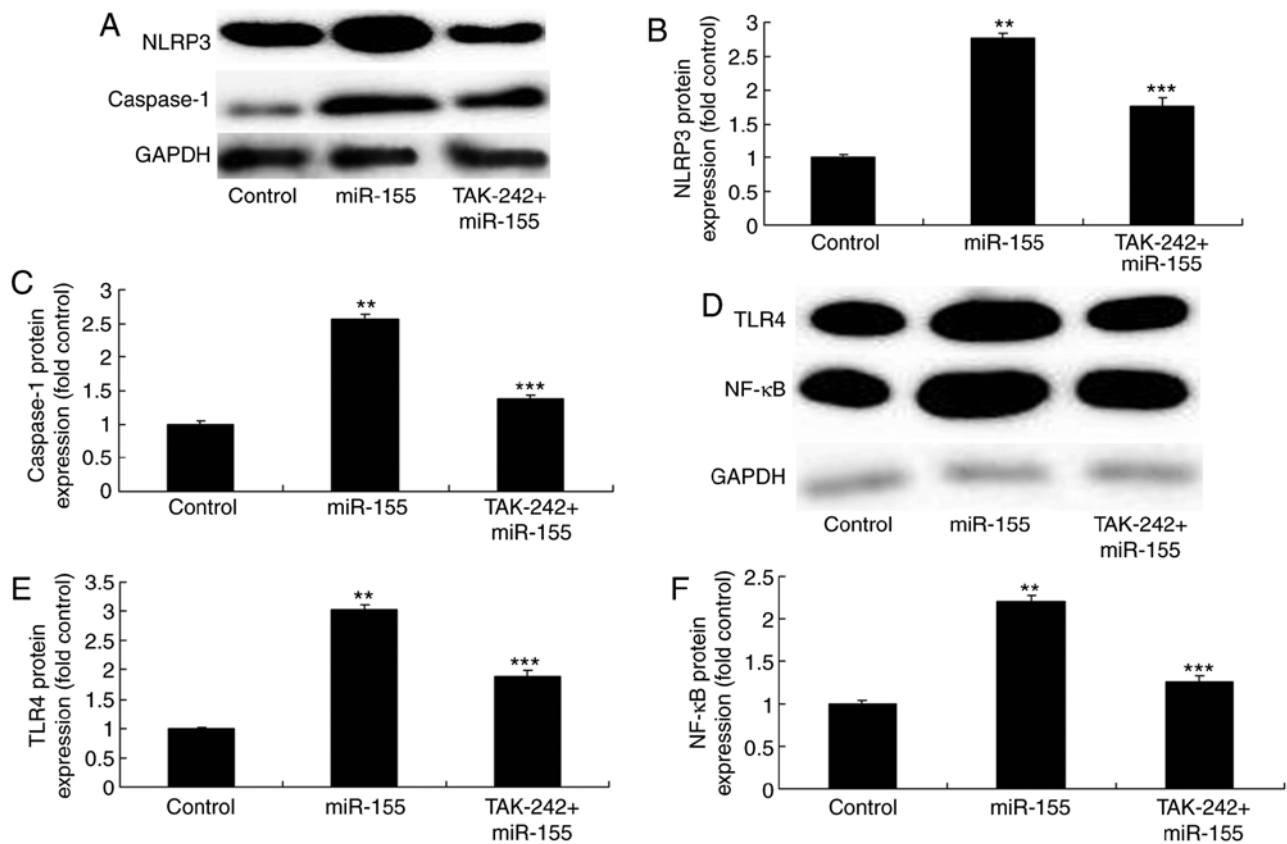


Figure 8. Inhibition of TLR4 reverses the function of miR-155 in the *in vitro* keratinocyte psoriasis model. Psoriasis-induced cells were treated with TAK-242, a TLR4 inhibitor, and/or transfected with miR-155 mimic. Representative blots and quantification of western blot analysis for (A-C) NLRP3 and caspase-1, and (D-F) TLR4 and NF-κB protein expression levels. ** $P < 0.01$ compared with control group; *** $P < 0.01$ compared with miR-155-overexpression alone group. TLR4, toll-like receptor 4; NLRP3, NLR family pyrin domain containing 3; NF-κB, nuclear factor-κB.

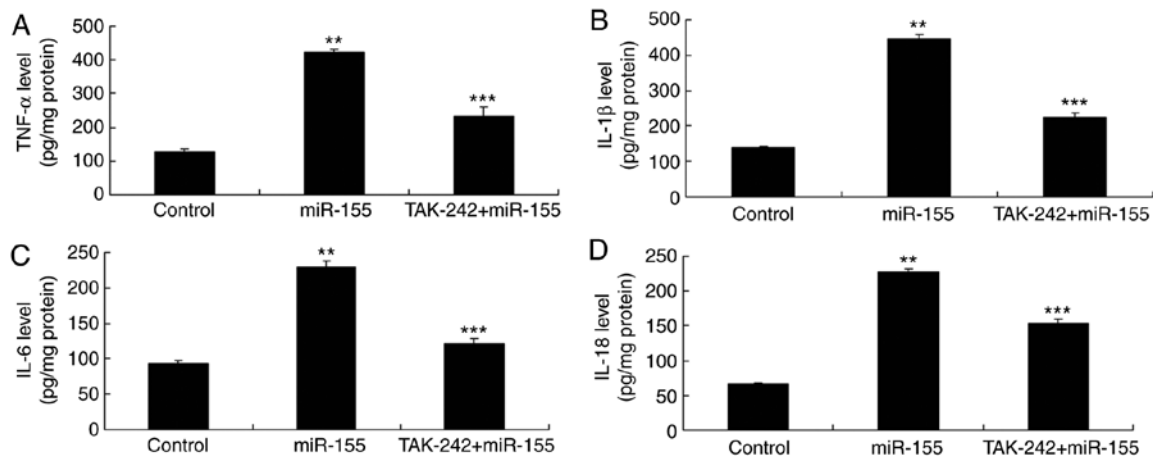


Figure 9. Inhibition of TLR4 reverses the function of miR-155 on the inflammatory response of the *in vitro* keratinocyte psoriasis model. Psoriasis-induced cells were treated with TAK-242, a TLR4 inhibitor, and/or transfected with miR-155 mimic. The levels of secreted (A) TNF-α, (B) IL-1β, (C) IL-6 and (D) IL-18 were measured by ELISA. ** $P < 0.01$ compared with control group; *** $P < 0.01$ compared with miR-155-overexpression alone group. TLR4, toll-like receptor 4; TNF, tumor necrosis factor; IL, interleukin.

inhibition suppressed the NLRP3 and caspase-1 protein expression levels in miR-155 overexpressing keratinocyte-induced HaCaT cells, compared with the miR-155 overexpression alone group (Fig. 8A-C). Similarly, inhibition of TLR4 reduced the miR-155-mediated upregulation of TLR4 and NF-κB, compared with the miR-155 overexpression alone group (Fig. 8D-F). In addition, inhibition of TLR4 reversed the miR-155-mediated

increase in the secreted levels of TNF-α, IL-18, IL-6 and IL-1β in keratinocyte-induced HaCaT cells, compared with the miR-155 overexpression alone group (Fig. 9).

Inhibition of NLRP3 reverses the miR-155-mediated inflammation in vitro. Finally, the hypothesis that miR-155-induced regulation of inflammation-related genes may occur through

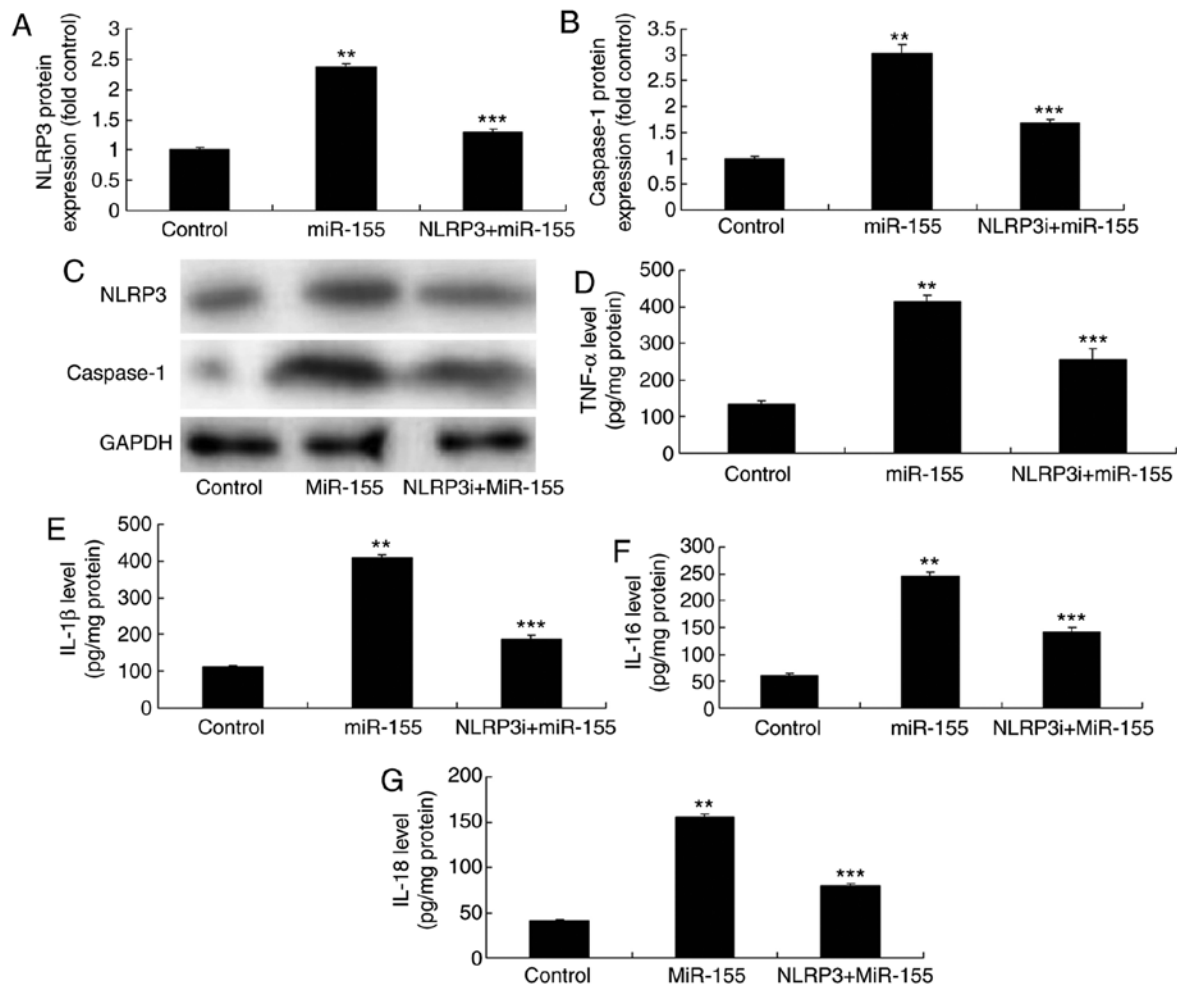


Figure 10. Inhibition of NLRP3 reverses the miR-155-induced inflammation in the *in vitro* keratinocyte psoriasis model. Psoriasis-induced cells were treated with a NLRP3 inhibitor, and/or transfected with miR-155 mimic. (A-C) Protein expression levels of NLRP3 and caspase-1 were measured by western blotting. Levels of secreted (D) TNF- α , (E) IL-1 β , (F) IL-6 and (G) IL-18 were measured by ELISA. **P<0.01 compared with control group; ***P<0.01 compared with miR-155-overexpression alone group. NLRP3, NLR family pyrin domain containing 3; TNF, tumor necrosis factor; IL, interleukin.

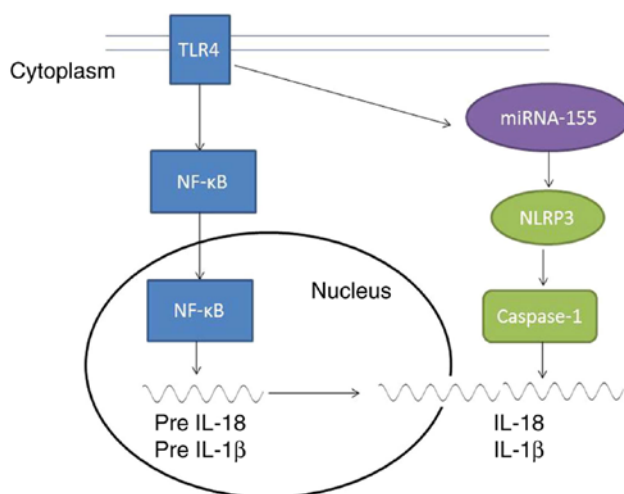


Figure 11. Schematic of the miR-155 function on the inflammatory responses in psoriasis through inflammasome NLRP3 activation.

NLRP3 was examined. The NLRP3 inhibitor, MCC950 (2 nM for 8 h), was added to the cells, and protein expression was analyzed by western blotting. Addition of the NLRP3 inhibitor

suppressed NLRP3 and caspase-1 protein expression levels in the *in vitro* psoriasis model, compared with the miR-155 overexpression alone group (Fig. 10A-C). Additionally, the miR-155 overexpression-induced TNF- α , IL-18, IL-6 and IL-1 β levels were significantly reduced in the *in vitro* keratinocyte model following NLRP3 inhibition, compared with the miR-155 overexpression alone group (Fig. 10D-G).

Discussion

Neurodermatitis, which is part of the cancer category in traditional Chinese medicine, is referred to as psoriasis. It is characterized by its refractoriness. Psoriasis has gained names such as cowhide carcinoma and incised wound, according to different skin lesion morphologies (5,16). Psoriasis is the most common chronic, recurrent and inflammatory dermatosis characterized by scaly erythema (2). It is the most common refractory disease in skin, with a morbidity of ~0.1-3% (3). In China, the morbidity in the north is higher compared with the south, in urban areas is higher compared with rural areas, and in males is higher compared with females. According to incomplete statistics, the morbidity of psoriasis in China is over 2 million (16). However, the urban population in China

is surging in recent years, and China has witnessed an annual new population of 20 million (5). Epidemiological statistics suggest that ~0.1 million psoriasis patients have increased in China every year. The present results clearly demonstrate that miR-155 and NLRP3 expression were significantly increased in psoriasis *in vivo* and *in vitro* models compared with control groups.

miRNAs are extensively distributed in nematode, fruit fly and multiple human tissues and cells (6). It is a type of single-strand non-coding RNA with a length of ~21-25 nucleotides (6). miRNAs can bind at the 3'-untranslated region of mRNA, and negatively regulate the expression of related proteins (6). Numerous previous studies have indicated that miRNAs are closely related to inflammation (6,15). They predominantly target important proteins in the NLRP3 inflammation-associated pathway, thus promoting or inhibiting the inflammatory response (17). The present study demonstrated that overexpression of miR-155 significantly increased TNF- α , IL-18, IL-6 and IL-1 β levels in keratinocyte-induced HaCaT cells, through the NLRP3 inflammasome. Obora *et al* (18) described that inflammation-induced miR-155 inhibits self-renewal of neural stem cells. However, it remains to be elucidated how miR-155 regulates NLRP3 in psoriasis.

The NLRP3 inflammasome is constituted by NLRP3, the apoptosis-associated speck-like protein containing CARD (ASC), and the cysteinyl aspartate specific proteinase-1, caspase-1 (19). NLRP3 binds to ASC once it is activated (11), and then it can recruit pro-caspase-1 and induce its self-cleavage to produce the activated caspase-1 (19). The activated caspase-1 can cleave the pro-IL-1 β into active IL-1 β , thus activating the downstream inflammatory response (20). The present results demonstrated that TLR4 induced the miR-155/NLRP3 inflammasome in keratinocyte-induced HaCaT cells. Bala *et al* (15) reported that TLR4 signaling regulates miR-155 to promote liver fibrosis and alcohol-induced steatohepatitis. Taken together, these data suggest that TLR4 signaling regulates miR-155-induced inflammation through NLRP3 inflammasome in psoriasis.

The NLRP3 inflammasome-activated molecular mechanism is extremely complicated, and it can be classified into three major pathways (21). In the first pathway, extracellular ATP stimulates the opening of the P2X7-dependent ion channel (21). This can promote K⁺ outflow and the formation of the pannexin-1 membrane channel. Thus, the extracellular NLRP3 agonist can enter the cell and directly promote the aggregation and activation of the NLRP3 inflammasome (20). NLRP3 inflammasome enhances the production of effector molecules, such as caspase-1 and IL-1p. NF- κ B in macrophages can upregulate NLRP3 expression (20). Intracellular inflammatory factors, such as IL-1 β , can also be released out of the cell, thus inducing inflammatory response (22). In the present study, addition of an NLRP3 inhibitor reversed the miR-155 mediated increased inflammation in keratinocyte-induced HaCaT cells. Artlett *et al* (23) reported that miR-155 required NLRP3 inflammasome-mediated collagen synthesis during fibrosis.

The present data imply that TLR4/miR-155 increased inflammatory responses in psoriasis through inflammasome NLRP3 activation. TLR4/NF- κ B signaling induced inflammation and miR-155/NLRP3/caspase-1 promoted the production of IL-18 and IL-1 β in psoriasis (Fig. 11). Further studies

targeting miR-155 may provide new prospects in discovering the causative factors involved in psoriasis and reveal novel strategies for psoriasis treatment.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

XZ designed the experiment; QL, JZ, WL, L L, XZ, XT, WL, LZ performed the experiment; XZ and QL analyzed the data; XZ wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Guangzhou Institute of Dermatology (Guangzhou, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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