

Evaluation of the effects of IL-22 on the proliferation and differentiation of keratinocytes *in vitro*

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Abstract. Psoriasis is one of the most common chronic inflammatory skin diseases, it is characterized by hyperproliferation of keratinocytes and infiltration of inflammatory cells. Several *in vitro* studies have reported that interleukin (IL)-22 is involved in excessive proliferation and abnormal differentiation of human keratinocytes. However, the association between IL-22 and CCAAT enhancer binding protein α (C/EBP α) in the pathogenesis of psoriasis remains unclear. Therefore, the present study aimed to investigate the association between IL-22 and C/EBP α , and the effects of IL-22 on the proliferation and differentiation of keratinocytes. Keratinocytes were treated with different concentrations of IL-22 (30, 60 and 90 ng/ml) and subsequently cells were collected at different time intervals. The expression levels of the key molecules of the mitogen-activated protein kinase (MAPK) signaling pathway were detected using western blot analysis. In addition, the effect of IL-22 on the proliferation rate of keratinocytes and the mRNA expression levels of C/EBP α were determined using a Cell Counting Kit-8 assay and reverse transcription-quantitative PCR, respectively. Furthermore, keratinocytes were transfected with C/EBP α small interfering (si)RNA or control using Lipofectamine[®] 2000. The results revealed that IL-22 significantly induced the proliferation of keratinocytes and the expression of phosphorylated (p)-JNK, p-ERK and p-p38 ($P < 0.05$). Additionally, IL-22 significantly inhibited the differentiation of keratinocytes, and the mRNA and protein expression of C/EBP α ($P < 0.05$). Furthermore, downregulation of C/EBP α increased the proliferation rate of keratinocytes and reduced the expression levels of cytokeratin 10 and involucrin. Therefore, these results suggested that the effect of IL-22 on the proliferation and differentiation

of keratinocytes may be mediated via the regulation of the MAPK signaling pathway and the expression of C/EBP α .

Introduction

Psoriasis is a common chronic inflammatory skin disease in the clinic, its typical pathological changes include hyperproliferation of keratinocytes and infiltration of inflammatory cells (1). Although it is commonly recognized as the result of interactions between multiple factors, such as genetic and environmental factors (2), the exact etiology of psoriasis remains unclear. It has been reported that a large number of inflammatory cells infiltrate the psoriatic lesions (3). T helper 17 (Th17) cells, a T cell subpopulation that secretes pro-inflammatory cytokines, such as interleukin (IL)-17 and IL-22, have been associated with the pathogenesis of psoriasis (4). Furthermore, *in vitro* studies have demonstrated that IL-22 induces excessive proliferation and abnormal differentiation of human keratinocytes (5-7).

Keratinocytes are an important type of immune cell. The excessive secretion of pro-inflammatory cytokines and chemokines, the aggregation of neutrophils and the formation of new blood vessels often occur in psoriasis (8). Therefore, a vicious circle is formed, resulting in excessive proliferation and abnormal differentiation of keratinocytes (2). In addition, the abnormal activation of signal transduction pathways, associated with immune responses, is considered to play a critical role in the pathogenesis of psoriasis (9). Previous studies have revealed that IL-22 activates the three main mitogen-activated protein kinase (MAPK) pathways, namely p38 kinase, mitogen-activated protein kinase (MEK)/ERK and JNK/stress-activated protein kinase (SAPK) (10,11). Furthermore, it has been reported that IL-22 upregulates the expression of STAT3 and inhibits the differentiation and promotes the proliferation of keratinocytes (12). However, the regulatory effects of IL-22 on the MAPK pathway in keratinocytes have not yet been fully investigated.

CCAAT enhancer binding protein α (C/EBP α), which is a member of the C/EBP transcription factor family, serves a key role in regulating the development, proliferation and differentiation of keratinocytes (13). Additionally, C/EBP α regulates the synthesis and metabolism of phospholipids in several types of cells, including keratinocytes, fat cells and bone marrow cells (14). Previous studies have confirmed that C/EBP α is expressed in the basal lamina of epidermis and its overexpression leads to keratinocyte cycle arrest (15).

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In the present study, primary cultured keratinocytes were used to evaluate the regulatory effects of IL-22 on the MAPK signaling pathway and to further investigate the role of IL-22 in the pathogenesis of psoriasis.

Materials and methods

Tissues. A total of 12 foreskin specimens were collected from young children (6-18 years old) from September to October 2011 who underwent circumcision in the Qilu Hospital of Shandong University (China). The size of the skin biopsies was 0.6x1.5 cm. The study was approved by the Ethics Committee of the Shandong University, China and all subjects provided written informed consent by their parents or guardians.

Cell culture. Foreskin samples were immediately cultured in Epilife-HKGS medium (M-EPI-500-CA; Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with penicillin-streptomycin and stored on ice. Foreskin tissue was removed under aseptic conditions, following which it was rinsed, soaked and shred, then digested with 0.25% pancreatin and incubated at 37°C for 5 min. Digestion was terminated using 10% FBS in 1640 medium, then the sample was centrifuged at 300 x g for 5 min at room temperature. A 100-mesh filter was used to collect the cell pellet from the suspension and then the cells were incubated at 37°C in a MCO-5AC incubator (Sanyo Electric Co., Ltd.) with 5% CO₂. Following incubation for 5 days and when cells reached 70-80% confluency, cells were passaged and immunocytochemistry was performed to detect cytokeratin (CK) expression on the cell slides.

Immunocytochemistry. Cells were rinsed three times with PBS and fixed at room temperature with 95% ethanol for ~1 h. Endogenous peroxidase was blocked with 3% H₂O₂ in PBS at room temperature for ~10 min and unspecific Ab binding was prevented with 5% goat serum (cat. no. ZLI-9021; OriGene Technologies, Inc.). Specific proteins were detected by immunohistochemistry using primary antibody anti-CK10 (1:200; cat. no. ab76318). Primary antibodies were added and samples were incubated in a humid chamber at 4°C overnight. Secondary antibodies (supplied with the DAB Immunochemistry kit; cat. no. PV-9000; OriGene Technologies, Inc) conjugated with peroxidase were used and the Ag-Ab reaction was visualized, according to the manufacturer's instructions. Images were captured using a light microscope (magnification, x200; cat. no. CX31; Olympus Corporation).

IL-22 stimulation. Normal human epidermal keratinocytes (NHEKs) at passage two were passaged and harvested using trypsin/EDTA. The culture medium (M-EPI-500-CA; Invitrogen; Thermo Fisher Scientific, Inc.) was added to prepare a single-cell suspension. When cells adhered to the wall and cell fusion reached 60%, IL-22 (cat. no. RC-212-33; Bio Basic, Inc.) was added to 6-well plates at concentrations of 30, 60 and 90 ng/ml, and subsequently total RNA and proteins were extracted from the cells for further analysis.

Cell transfection. Before transfection, 2x10⁵ cells were inoculated and cultivated in the incubator at 37°C with

5% CO₂ until the cell confluence reached 50%. NHEKs were transfected with 33 nM C/EBP α or control scramble siRNAs (cat. no. siNooooo1-1-10) (both Guangzhou RiboBio Co., Ltd.) with Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The sequence of C/EBP α siRNA was 5'-GGC AAAGAGAUUGACCUGG-3'. Subsequent experiments were performed 24 h after cell transfection.

Reverse transcription (RT)PCR analysis. Following stimulation of keratinocytes with IL-22 for 24 h at 37°C, total RNA was isolated using TRIzol (cat. no. 15596-026; Invitrogen; Thermo Fisher Scientific, Inc.) and its purity and concentration were measured for further analysis. Subsequently, total RNA was reverse transcribed into cDNA using a BioTeke Super RT kit (cat. no. PR6601; BioTeke Corporation), according to the manufacturer's instructions. The temperature protocol for reverse transcription was as follows: 30°C for 5 min, 42°C for 60 min, and 95°C for 5 min. The C/EBP α mRNA expression levels were detected using 2% agarose gel electrophoresis. A total of 10 μ l/lane PCR product was loaded with 2 μ l 6X loading buffer and separated at a constant voltage of 80 v. Bands were stained with 0.5 μ g/ml EB solution (cat. no. E1020; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 10 min. Bands were visualized using a Tanon-2500R gel image analysis system (Tanon Science and Technology Co., Ltd.). The primers used in the present study were synthesized by Sangon Biotech, Co., Ltd., and their sequences are presented in Table I.

Western blot assay. Following stimulation of NHEKs with IL-22 (cat. no. RC-212-33; Bio Basic, Inc.) at 37°C for 30, 60, 90 min and 48 h, cells were harvested, and total protein extracts were isolated using RIPA buffer (cat. no. P0013B; Beyotime Institute of Biotechnology). Protein concentration was determined using a BCA protein assay kit (cat. no. P0010S; Beyotime Institute of Biotechnology) and protein expression levels were determined using western blotting. A total of 40 μ g/lane total protein from each sample was loaded and separated via 10% SDS-PAGE, then transferred to a PVDF membrane. The membrane was blocked with blocking-buffer (cat. no. P0023B; Beyotime Institute of Biotechnology) for 1 h at room temperature, and then incubated with the primary antibody overnight at 4°C. The next day, the membrane was washed with TBST (0.1% Tween-20) and subsequently incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. The following primary antibodies were used: Anti-C/EBP α (1:1,000; cat. no. ab74404), anti-CK10 (1:1,000; cat. no. ab76318), anti-involucrin (1:2,000; cat. no. ab53112; all from Abcam), anti-phosphorylated (p)-JNK1/2/3 (1:2,000; cat. no. bs-1640R), anti-JNK1/2/3 (1:2,000; cat. no. bs-2592R), anti-p38 (1:2,000; cat. no. bs-0637R), anti-p-p38 (1:2,000; cat. no. bs-5476R), anti-ERK (1:2,000; cat. no. bs-0022R) and anti-p-ERK (1:2,000; cat. no. bs-1522R; all BIOSS) and anti- β -actin (1:1,000; cat. no. 4970s; Cell Signaling Technology, Inc.). The following secondary antibody was used: Goat anti-rabbit IgG H&L (HRP) (1:5,000; cat. no. ab6721; Abcam). Visualization was performed using a BeyoECL Moon kit (cat. no. PB0018FS; Beyotime Institute of Biotechnology). The protein bands were analyzed with ImageJ

Table I. Genes and primer sequences.

Gene	Primer sequence (5'-3')
C/EBP α	F: ACGTGGAGACGCAGCAGAA R: GTAGGCATTGGAGCGGTGA
β -actin	F: CACCAACTGGGACGACAT R: CAGAGGCGTAGAGGGACA

F, forward; R, reverse; C/EBP α , CCAAT enhancer binding protein α .

software (version Java1.8.0_112; imagej.nih.gov/ij/docs/index.html).

Cell viability assay. NHEKs were seeded into 96-well plates at a density of 1×10^4 /well and were then incubated at 37°C for 24 h. Subsequently, IL-22 (RC-142; Bio Basic, Inc.) was added to the wells at a final concentration of 30, 60 and 90 ng/ml. Finally, following stimulation for 12, 24, 48 and 72 h, 10 μ l Cell Counting Kit-8 (CCK-8) reagent (Beyotime Institute of Biotechnology) was added to each well and the cells were cultured at 37°C for an additional 2 h, according to the manufacturer's instructions. Optical density (OD) values were measured at 450 nm.

Statistical analysis. All statistical analyses were performed using SPSS 18.0 software (SPSS, Inc.). All data are presented as the mean \pm SD from independent experiments performed in triplicate. The differences between 2 groups were analyzed using Student's t-test. In addition, when ≥ 3 groups were compared, the differences were analyzed using one-way ANOVA, followed by Tukey's post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of CK10 in cells assessed via immunocytochemistry. Brown staining in the cytoplasm demonstrated that keratinocytes were successfully isolated from foreskin samples (Fig. 1).

Expression levels of JNK, ERK and p38 in NHEKs following stimulation with IL-22. NHEKs were stimulated for 0, 30, 60 and 90 min with 60 ng/ml IL-22. Subsequently, total protein extracts were isolated and western blot analysis was performed to detect the expression levels of JNK, p-JNK, p38, p-p38, ERK and p-ERK. The results revealed that JNK phosphorylation was significantly increased after cell stimulation with IL-22 for 30 min and decreased at 60 min post-stimulation (Fig. 2). Accordingly, the expression levels of p-p38 and p-ERK were significantly increased at 30 min and reached the highest levels at 60 min post-stimulation. However, expression levels decreased following incubation for 90 min in the presence of IL-22.

IL-22 promotes the proliferation of keratinocytes. The effect of IL-22 on the proliferation of keratinocytes was assessed using a CCK-8 assay. Therefore, keratinocytes were stimulated

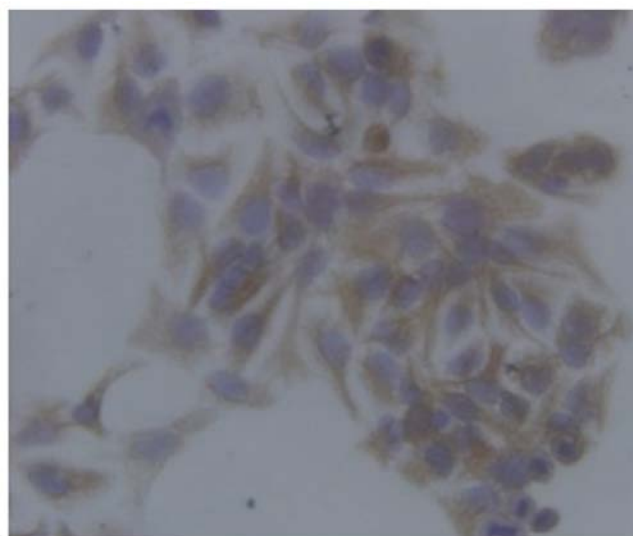


Figure 1. Cell slide immunocytochemistry. Expression levels of CK10 in the cytoplasm of keratinocytes. Brown, CK10; blue, nuclear staining. Magnification, x200).

for 12, 24, 48 and 72 h with 30, 60 and 90 ng/ml IL-22. The results demonstrated that IL-22 promoted the proliferation of keratinocytes in a dose- and time-dependent manner (Fig. 3).

IL-22 inhibits the differentiation of keratinocytes. Total protein extracts were isolated from keratinocytes treated with 60 ng/ml IL-22 for 48 h to detect the expression levels of keratinocyte differentiation markers, namely CK10 and involucrin. IL-22 treatment for 48 h significantly reduced the protein expression levels of CK10 and involucrin in keratinocytes compared with the control ($P < 0.05$; Fig. 4).

IL-22 decreases the expression of C/EBP α at the mRNA and protein level. Following stimulation of keratinocytes with 30, 60 and 90 ng/ml IL-22 for 48 h, C/EBP α mRNA and protein expression levels were assessed using RT-qPCR and western blot analysis, respectively. The results showed that mRNA and protein expression levels of C/EBP α were significantly down-regulated in the IL-22 group compared with that in the control group ($P < 0.05$) (Figs. 5 and 6). However, no statistically significant differences were observed among the different concentration groups ($P > 0.05$).

Downregulation of C/EBP α promotes proliferation of keratinocytes. NHEKs, were transfected with C/EBP α siRNAs or control using Lipofectamine 2000 to evaluate the effect of C/EBP α on cell proliferation. The CCK-8 proliferation assay demonstrated that C/EBP α siRNA promoted the proliferation of NHEKs at 24, 48 and 72 h (all $P < 0.05$) compared with that of the control siRNA and empty vector groups (Fig. 7). The results suggested that downregulation of C/EBP α promoted proliferation of keratinocytes.

Downregulation of C/EBP α inhibits the differentiation of keratinocytes. To investigate the effect of C/EBP α on the differentiation of keratinocytes, cells were transfected with C/EBP α siRNAs or control using Lipofectamine 2000.

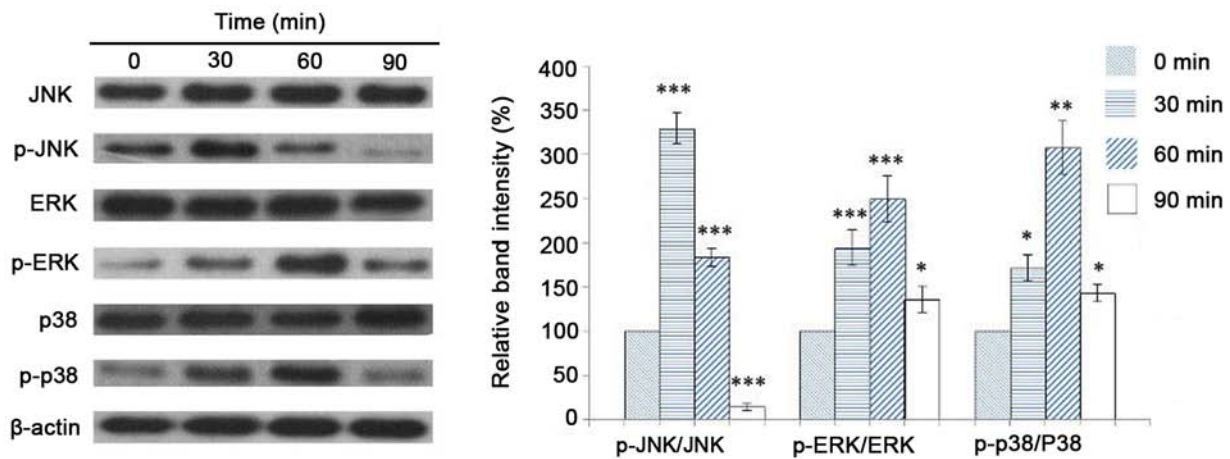


Figure 2. Expression levels of JNK, ERK and p38 in IL-22-treated (60 ng/ml) keratinocytes. β -actin served as an internal control. The intensity of the immunoreactive bands was evaluated using ImageJ. Experiments were performed independently three times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. 0 min. IL-22, interleukin 22; p-, phosphorylated.

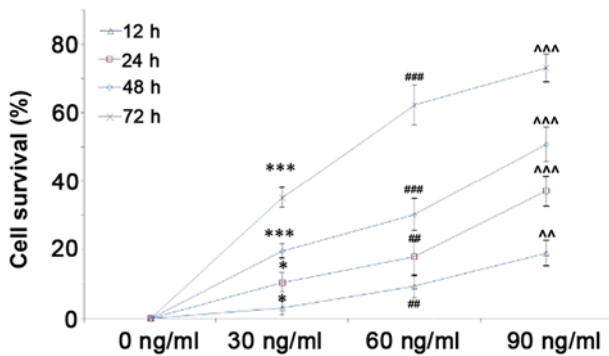


Figure 3. Effect of IL-22 on proliferation of NHEKs. NHEKs were treated with IL-22 for 12, 24, 48 and 72 h and then proliferation assays were performed using a CCK-8 assay. The results are presented as the mean \pm standard deviation. The significance indicators are represented as 30 ng/ml vs. 0 ng/ml, 60 ng/ml vs. 30 ng/ml and 90 ng/ml vs. 60 ng/ml at each time point. The differences were analyzed using one-way ANOVA followed by Tukey's post hoc test. * $P < 0.05$, *** $P < 0.001$ vs. 0 ng/ml; ## $P < 0.01$, ### $P < 0.001$ vs. 30 ng/ml; ^^ $P < 0.01$, ^^^ $P < 0.001$ vs. 60 ng/ml. IL-22, interleukin 22; NHEKs, normal human epidermal keratinocytes.

Western blot analysis revealed that involucrin and CK10 expression levels were significantly decreased in the C/EBP α siRNA group compared with those in the control siRNA and non-transfected groups (Fig. 8). The aforementioned results indicated that C/EBP α downregulation inhibited the differentiation of keratinocytes.

Discussion

Psoriasis vulgaris, one of the most common chronic inflammatory diseases in the clinic, is considered to occur as a consequence of genetic and environmental factors, leading to the induction of keratinocyte proliferation via immune-mediated signaling pathways (1,2).

A previous study has demonstrated that in psoriasis vulgaris the proliferation rate and the number of generated keratinocytes is increased by 2 and 28 times, respectively, compared with normal epidermis (16). However, the exact underlying mechanism of the increased keratinocyte proliferation rate

in psoriasis remains unknown. The abnormal differentiation of keratinocytes is one of the most important features of psoriatic lesions, resulting from the abnormal expression of several keratins (17). In addition, it has been reported that transglutaminase 1 kinase, involucrin, peptidase inhibitor 3, ATP binding cassette subfamily C member 8 and CK6/CK16 proteins are upregulated in psoriatic lesions (18). Furthermore, keratin K1/K10, a characteristic protein of the terminal differentiation stage of keratinocytes, is significantly reduced in the psoriatic lesions compared with the normal skin (19). Therefore, in the present study, CK10 and involucrin were selected as differentiation markers to investigate the effect of IL-22 on keratinocyte differentiation. The results revealed that IL-22 significantly decreased the expression of CK10 and involucrin in keratinocytes. In addition, IL-22 significantly promoted keratinocyte proliferation and differentiation in a time- and dose-dependent manner.

Th17 cells, a subpopulation of CD4⁺ T lymphocytes that differs from Th1, Th2 and regulatory T cells, mainly secrete the cytokines IL-17 and IL-22 (20). Previous studies have confirmed that IL-22 is one of the key pro-inflammatory cytokines in inflammatory diseases (21,22). IL-22 has been found in IL-9-stimulated BW5147 cells (mouse T lymphoma cells) and exerted its biological effects by interacting with its receptor (23). An *in vitro* study demonstrated that IL-22 promoted the excessive proliferation and abnormal differentiation of human keratinocytes (5). Furthermore, Ma *et al* (24) established a psoriasis mouse model by transferring CD4⁺CD45RB^{hi} T cells from BALB/cBy to C.B-17/Prkdc^{scid/scid} mice and revealed that subcutaneous injection of IL-22 antibodies prevented the progression of psoriasis. They also reported that the levels of Th17-related cytokines, namely IL-17A, IL-17F, IL-22 and IL-23p19, were significantly reduced (24). These findings suggest that IL-22 serves an important role in the pathogenesis of psoriasis by promoting the secretion ability of Th17 cells, which in turn acts on keratinocytes. Therefore, IL-22 may be considered the link between the immune system and keratinocytes.

A previous study has confirmed that abnormal signal transduction pathways play a critical role in the pathogenesis

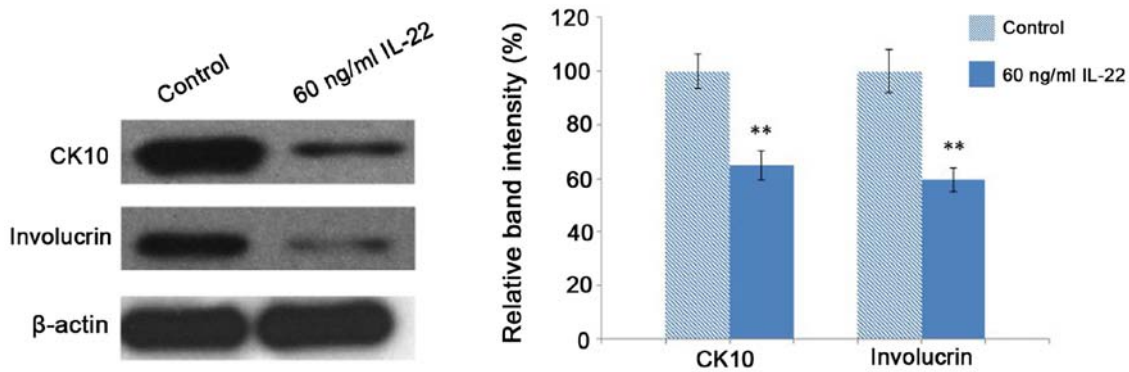


Figure 4. IL-22 significantly decreases the expression of CK10 and involucrin. Following stimulation for 48 h, the protein expression levels of CK10 and involucrin were determined in the control and IL-22-treated (60 ng/ml) groups using western blotting. β -actin served as an internal control. The intensity of the immunoreactive bands was measured using ImageJ. Experiments were performed independently three times. ** $P < 0.01$ vs. control. IL-22, interleukin 22; CK10, cytokeratin 10.

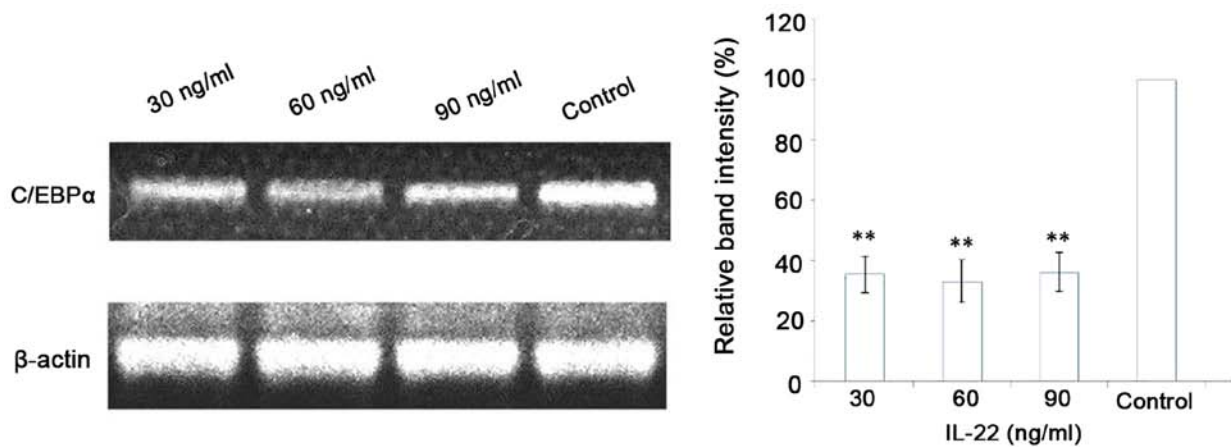


Figure 5. Effect of different concentrations of IL-22 on C/EBP α mRNA expression. The results are presented as the mean \pm SD of three independent experiments. ** $P < 0.01$ vs. control. IL-22, interleukin 22; C/EBP α , CCAAT enhancer binding protein α .

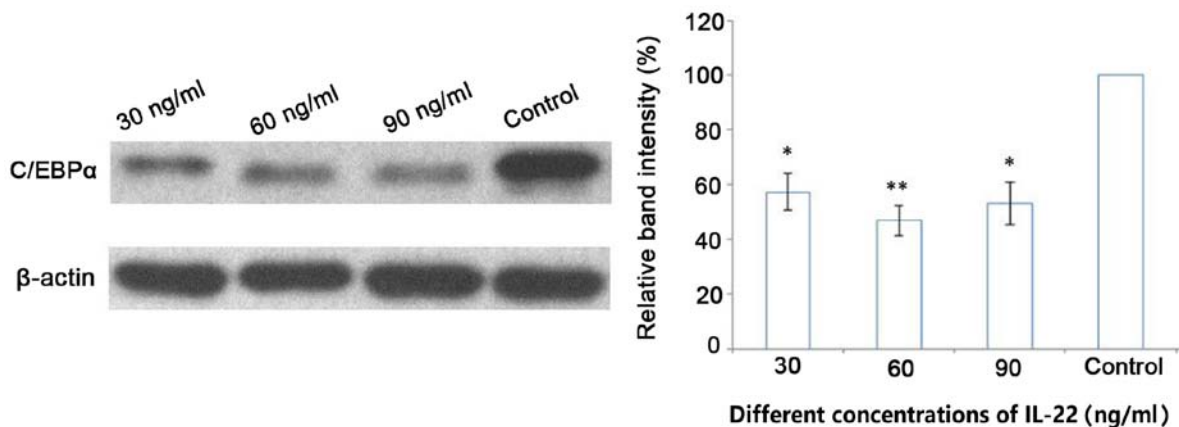


Figure 6. Effect of different concentrations of IL-22 on C/EBP α protein expression. Following stimulation for 48 h, the protein expression levels of C/EBP α at different concentrations of IL-22 were determined using western blot analysis. β -actin served as an internal control. The intensity of the immunoreactive bands was measured using ImageJ. Experiments were performed independently three times. * $P < 0.05$, ** $P < 0.01$ vs. control. IL-22, interleukin 22; C/EBP α , CCAAT enhancer binding protein α .

of psoriasis (9). It has also been demonstrated that three main pathways, namely MEK-ERK, JNK/SAP and p38 kinase, are activated by IL-22 (25). Furthermore, IL-22 upregulates the expression of STAT3, inhibits keratinocyte differentiation,

promotes keratinocyte proliferation and induces the formation of psoriatic lesions (12). Additionally, the activity of the MAPK signaling pathway is significantly increased in psoriatic lesions (26). Notably, in psoriasis, p-ERK is upregulated in

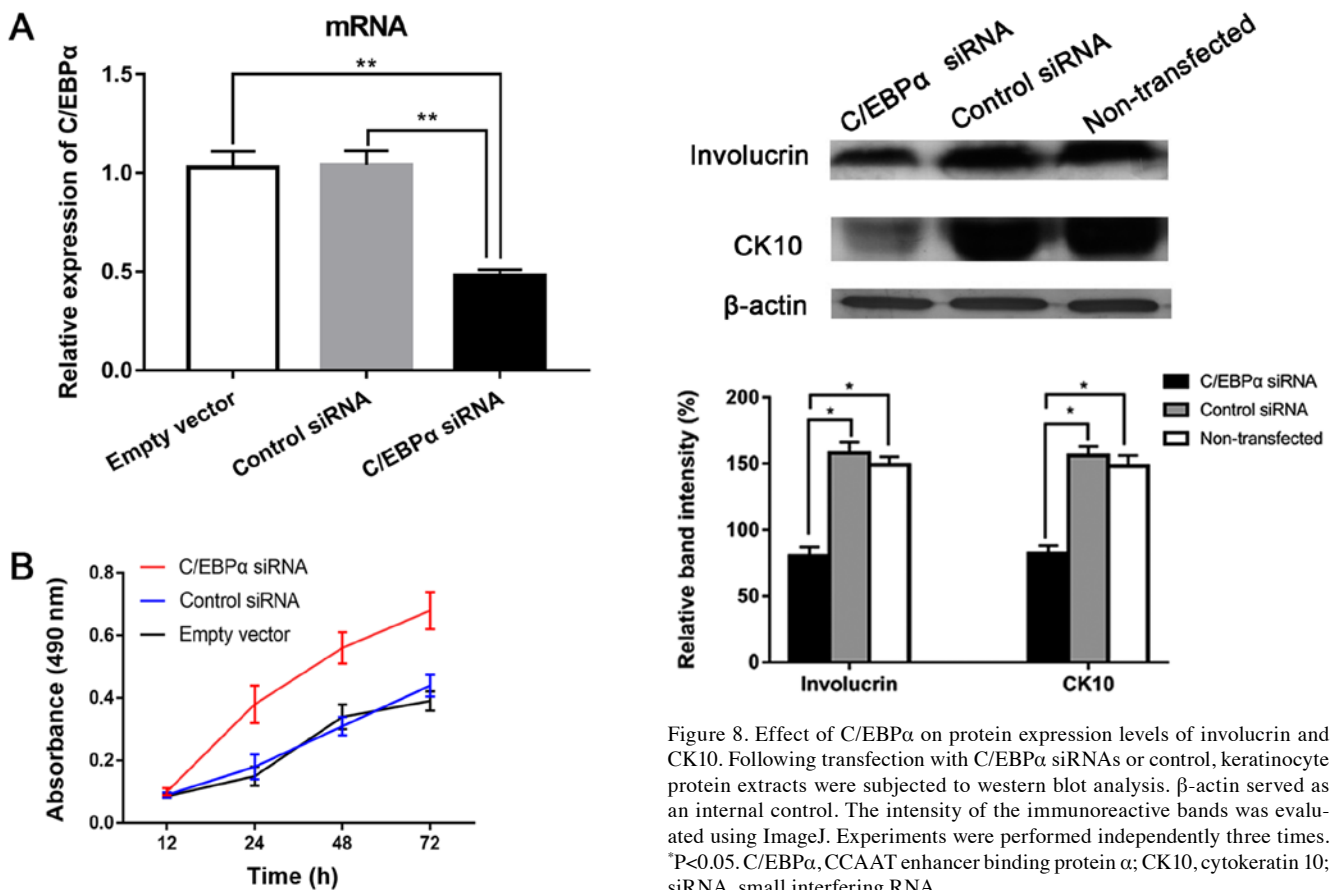


Figure 7. Effect of C/EBP α on keratinocyte proliferation. (A) Transfection with C/EBP α siRNAs, control siRNA or empty vector, the expression of C/EBP α mRNA was detected using RT-qPCR 24 h later. (B) Following transfection with C/EBP α siRNAs or control, the proliferation rate of keratinocytes was evaluated at 12, 24, 48 and 72 h using a CCK-8 assay. The optical density values at 490 nm of each point were calculated from three independent experiments. The results are presented as the mean \pm SD. ** $P < 0.01$. C/EBP α , CCAAT enhancer binding protein α ; siRNA, small interfering RNA.

the base layer and stratum spinosum cell nucleus, while in the normal skin p-ERK is only expressed in the basal layer (27). JNK and p38 kinase are expressed in the granular layer of the normal skin and in both the granular layer and stratum spinosum of the psoriatic lesions (28). The results of the present study revealed that primary cultured keratinocytes treated with 60 ng/ml IL-22 for 30 min upregulated the expression of p-ERK, p-JNK and p-p38, indicating that IL-22 was involved in the activation of the MAPK pathway in keratinocytes.

C/EBP α , a member of the C/EBP family, was first isolated from rat liver cell nuclei by McKnight in 1987. C/EBP α is a heat-stable transcription factor that interacts with the CCAAT motif (29). Previous studies have shown that C/EBP α is involved in the regulation of keratinocyte differentiation and its expression is gradually elevated in stratum spinosum, granular layer and stratum corneum keratinocytes in a space- and time-dependent manner (13,15), which indicated that C/EBP α was expressed in basal layer keratinocytes, and is upregulated as keratinocytes exit the basal layer, enter the spinous and granular layers and undergo terminal differentiation. Maytin and Habener (30) found that CK10 was primarily expressed in BALB/MK keratinocytes following stimulation with 0.12 mM Ca²⁺ for 24 h and its expression was decreased

Figure 8. Effect of C/EBP α on protein expression levels of involucrin and CK10. Following transfection with C/EBP α siRNAs or control, keratinocyte protein extracts were subjected to western blot analysis. β -actin served as an internal control. The intensity of the immunoreactive bands was evaluated using ImageJ. Experiments were performed independently three times. * $P < 0.05$. C/EBP α , CCAAT enhancer binding protein α ; CK10, cytokeratin 10; siRNA, small interfering RNA.

after 48 h. However, the expression levels of C/EBP α were increased by 5 times (31). In the terminal differentiation stage, the markers of spinous and granular layer, K1/K10, are induced by C/EBP α binding to DNA (13). The expression levels of C/EBP α and CK10 over time (C/EBP α at first, CK10 later) and at different sites (C/EBP α in the basal layer and CK10 in the spinous layer) suggests that these molecules may be involved in the differentiation of keratinocytes. C/EBP α is considered an important negative regulator of cell proliferation and its anti-tumor effects have been reported in numerous types of skin tumors (31). For example, Loomis *et al* (32) demonstrated that epidermal-specific C/EBP α knockout mice were highly susceptible to skin squamous carcinoma. However, the underlying mechanisms of C/EBP α in regulating cell proliferation remains unclear. It has been considered that C/EBP α interacts with several cell cycle proteins in its dimer form, it can interact directly with CDK2 and CDK4 enzymes to prevent cyclin binding thus regulating the cell cycle (33). In addition, C/EBP α regulates, stabilizes and activates the cell cycle inhibitor p21 or directly inhibits CDK2-, CDK4- and E2F-induced transcription. These findings indicate that C/EBP α may block the cell cycle process and inhibit cell proliferation (34). In the present study the expression levels of C/EBP α were significantly decreased in keratinocytes following treatment with IL-22, suggesting a potential role in the pathogenesis of psoriasis vulgaris. Additionally, the MAPK signaling pathway may act as a key molecule in the interaction between IL-22 and C/EBP α . Therefore, elevated IL-22 expression levels in psoriasis vulgaris may activate the MAPK

signaling pathway, which in turn may mediate the inactivation of C/EBP α . However, the mechanism of cell signal transduction is complex. A signal transducer may not only participate in the cell signal transduction of one pathway, and functional molecules in one signal transduction pathway may affect and regulate other pathways. The present study primarily investigated the relationship between IL-22 and the MAPK signaling pathway. Moreover, it was found that the effect of IL-22 on the proliferation and differentiation of keratinocytes may be mediated via the regulation of the MAPK signaling pathway. The result of this study cannot exclude whether there are other signaling pathways that play a role in the regulatory mechanism of IL-22. This will be further studied in the future.

In conclusion, the present study demonstrated that IL-22 promoted proliferation and inhibited differentiation of keratinocytes. The C/EBP α expression levels were significantly decreased in IL-22-treated keratinocytes. Furthermore, downregulation of C/EBP α promoted keratinocyte proliferation and decreased the expression of CK10 and involucrin. Therefore, IL-22 may contribute to the abnormal proliferation and differentiation of keratinocytes via downregulating the expression of C/EBP α . Finally, the results suggest that the IL-22/MAPK/C/EBP α axis may be involved in the pathogenesis of psoriasis.

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Availability of data and materials

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

Authors' contributions

HZ and LZ designed the present study and performed the experiments, and should be regarded as corresponding and first author, respectively. WM analyzed and interpreted patients' data. JY contributed to the clinical diagnosis and histological examination during the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Qilu Hospital of Shandong University, China and was in accordance with The Declaration of Helsinki (1964) and its later amendments. All patients provided written informed consent for publication of the results.

Patient consent for publication

All patients provided written informed consent.

Competing interests

The authors declare that they have no competing interests.

References

- Schön MP and Boehncke WH: Psoriasis. *N Engl J Med* 352: 1899-1912, 2005.
- Lowes MA, Bowcock AM and Krueger JG: Pathogenesis and therapy of psoriasis. *Nature* 445: 866-873, 2007.
- Bos JD, de Rie MA, Teunissen MB and Piskin G: Psoriasis: Dysregulation of innate immunity. *Br J Dermatol* 152: 1098-1107, 2005.
- Di Cesare A, Di Meglio P and Nestle FO: The IL-23/Th17 axis in the immunopathogenesis of psoriasis. *J Invest Dermatol* 129: 1339-1350, 2009.
- Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC and Morel F: IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. *J Immunol* 174: 3695-3702, 2005.
- Ekman AK, Bivik Eding C, Rundquist I and Enerbäck C: IL-17 and IL-22 promote keratinocyte stemness in the germinative compartment in psoriasis. *J Invest Dermatol* 139: 1564-1573.e8, 2019.
- Jiang M, Ma W, Gao Y, Jia K, Zhang Y, Liu H and Sun Q: IL-22-induced miR-122-5p promotes keratinocyte proliferation by targeting Sprouty2. *Exp Dermatol* 26: 368-374, 2017.
- Boehncke WH: Etiology and pathogenesis of psoriasis. *Rheum Dis Clin North Am* 41: 665-675, 2015.
- Nestle FO, Kaplan DH and Barker J: Psoriasis. *N Engl J Med* 361: 496-509, 2009.
- Lejeune D, Dumoutier L, Constantinescu S, Kruijer W, Schuringa JJ and Renauld JC: Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. *J Biol Chem* 277: 33676-33682, 2002.
- Yu J, Xiao Z, Zhao R, Lu C and Zhang Y: Paeoniflorin suppressed IL-22 via p38 MAPK pathway and exerts anti-psoriatic effect. *Life Sci* 180: 17-22, 2017.
- Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, Vom Baur E, Witte K, Warszawska K, Philipp S, *et al*: IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. *J Mol Med (Berl)* 87: 523-536, 2009.
- Lopez RG, Garcia-Silva S, Moore SJ, Bereshchenko O, Martinez-Cruz AB, Ermakova O, Kurz E, Paramio JM and Nerlov C: C/EBP α and beta couple interfollicular keratinocyte proliferation arrest to commitment and terminal differentiation. *Nat Cell Biol* 11: 1181-1190, 2009.
- Zhang ZC, Liu Y, Li SF, Guo L, Zhao Y, Qian SW, Wen B, Tang QQ and Li X: Suv39h1 mediates AP-2 α -dependent inhibition of C/EBP α expression during adipogenesis. *Mol Cell Biol* 34: 2330-2338, 2014.
- Thompson EA, Zhu S, Hall JR, House JS, Ranjan R, Burr JA, He YY, Owens DM and Smart RC: C/EBP α expression is downregulated in human nonmelanoma skin cancers and inactivation of C/EBP α confers susceptibility to UVB-induced skin squamous cell carcinomas. *J Invest Dermatol* 131: 1339-1346, 2011.
- Weinstein GD, McCullough JL and Ross PA: Cell kinetic basis for pathophysiology of psoriasis. *J Invest Dermatol* 85: 579-583, 1985.
- Bernard BA, Asselineau D, Schaffar-Deshayes L and Darmon MY: Abnormal sequence of expression of differentiation markers in psoriatic epidermis: Inversion of two steps in the differentiation program? *J Invest Dermatol* 90: 801-805, 1988.
- Duvic M, Asano AT, Hager C and Mays S: The pathogenesis of psoriasis and the mechanism of action of tazarotene. *J Am Acad Dermatol* 39: S129-S133, 1998.
- Bernernd F, Magnaldo T and Darmon M: Delayed onset of epidermal differentiation in psoriasis. *J Invest Dermatol* 98: 902-910, 1992.
- Sallusto F and Lanzavecchia A: Heterogeneity of CD4+ memory T cells: Functional modules for tailored immunity. *Eur J Immunol* 39: 2076-2082, 2009.
- Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfarani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, *et al*: Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* 119: 3573-3585, 2009.

22. Zenewicz LA and Flavell RA: IL-22 and inflammation: Leukin' through a glass onion. *Eur J Immunol* 38: 3265-3268, 2008.
23. Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD and Gurney AL: Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R. *J Biol Chem* 275: 31335-31339, 2000.
24. Ma HL, Liang S, Li J, Napierata L, Brown T, Benoit S, Senices M, Gill D, Dunussi-Joannopoulos K, Collins M, *et al*: IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J Clin Invest* 118: 597-607, 2008.
25. Weber GF, Gaertner FC, Erl W, Janssen KP, Blechert B, Holzmann B, Weighardt H and Essler M: IL-22-mediated tumor growth reduction correlates with inhibition of ERK1/2 and AKT phosphorylation and induction of cell cycle arrest in the G2-M phase. *J Immunol* 177: 8266-8272, 2006.
26. Johansen C, Kragballe K, Westergaard M, Henningsen J, Kristiansen K and Iversen L: The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br J Dermatol* 152: 37-42, 2005.
27. Zhang X, Yang D, Ma S and Liu H: Up-regulation of activities of mitogen-activated protein kinase in psoriatic lesions. *J Dermatol Sci* 37: 118-119, 2005.
28. Funding AT, Johansen C, Kragballe K and Iversen L: Mitogen- and stress-activated protein kinase 2 and cyclic AMP response element binding protein are activated in lesional psoriatic epidermis. *J Invest Dermatol* 127: 2012-2019, 2007.
29. Johnson PF and McKnight SL: Eukaryotic transcriptional regulatory proteins. *Annu Rev Biochem* 58: 799-839, 1989.
30. Maytin EV and Habener JF: Transcription factors C/EBP alpha, C/EBP beta, and CHOP (Gadd153) expressed during the differentiation program of keratinocytes in vitro and in vivo. *J Invest Dermatol* 110: 238-246, 1998.
31. Oh HS and Smart RC: Expression of CCAAT/enhancer binding proteins (C/EBP) is associated with squamous differentiation in epidermis and isolated primary keratinocytes and is altered in skin neoplasms. *J Invest Dermatol* 110: 939-945, 1998.
32. Loomis KD, Zhu S, Yoon K, Johnson PF and Smart RC: Genetic ablation of CCAAT/enhancer binding protein alpha in epidermis reveals its role in suppression of epithelial tumorigenesis. *Cancer Res* 67: 6768-6776, 2007.
33. McKnight SL: McBindall-a better name for CCAAT/enhancer binding proteins? *Cell* 107: 259-261, 2001.
34. Wang H, Iakova P, Wilde M, Welm A, Goode T, Roesler WJ and Timchenko NA: C/EBPalpha arrests cell proliferation through direct inhibition of Cdk2 and Cdk4. *Mol Cell* 8: 817-828, 2001.



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