

Original Article

The expression of IL-36 and the activation of p-p38 MAPK and NF- κ B p65 pathway in psoriasis vulgaris

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Abstract: Objective: To study the expression of IL-36 in psoriasis vulgaris and the activation changes of its downstream signal molecules, p-p38 and NF- κ B p65. Methods: Eighty patients with psoriasis vulgaris were selected as subjects of the study from Department of dermatology in our hospital from April 2015 to April 2017, and other thirty healthy subjects were selected as the control group. The skins of patients with psoriasis vulgaris and normal people were collected to detect the mRNA and protein levels of IL-36, p38 and NF- κ B p65 by RT-PCR method, immunohistochemical staining and the western blot, respectively. The correlation between the expression of IL-36 and the activation of p-p38 and NF- κ B p65 were evaluated using Pearson's correlation analysis. Results: The relative mRNA expression levels of IL-36 and NF- κ B p65 in the psoriasis vulgaris group were higher than those in the control group ($P=0.000$), but there was no statistical difference in mRNA expression level of p38 between the two groups ($P=0.146$). The results of immunohistochemical staining showed that staining intensities of IL-36, p-p38 and NF- κ B p65 in the psoriasis vulgaris group were significantly higher than those in the control group ($P=0.000$). The results of the western blot showed that the protein expression levels of IL-36, p-p38 and NF- κ B P65 were significantly higher than those in the control group ($P=0.000$). There were positive correlations among the expressions of IL-36, p-p38 and NF- κ B p65. Conclusion: There is high expression of IL-36 and increased activation of related p38 MAPK and NF- κ B signaling pathway in psoriasis vulgaris lesions.

Keywords: Psoriasis vulgaris, IL-36, p-p38, NF- κ B p65

Introduction

Psoriasis is a kind of inherited chronic dermatosis, which usually presents with inflammatory reactions and has great impacts on patients' skins and joints [1, 2]. Clinically the characteristic lesions of psoriasis can be seen, namely erythema covered with adherent scales. Currently, the knowledge of the pathogenesis of psoriasis has been scarce. Some scholars pointed that psoriasis was more likely to occur on the body whose immune system was disturbed [3-5]. Psoriasis has many characteristics in histology, such as hyperproliferation of Keratinocytes (KCs), inflammatory infiltration, vascular proliferation, etc. IL-36 in the body is mainly secreted by KCs. Some studies have proved the impacts of IL-36 on animal dermatitis, which has caught more and more attention, so has the signal pathway of IL-36 [6, 7]. This study observed the expression of IL-36 and the activation states of p38 MAPK and NF- κ B signaling

pathways in the lesions of patients with psoriasis vulgaris.

Materials and methods

Materials

Subjects of study: Eighty patients with psoriasis vulgaris were admitted to our department of dermatology from April 2015 to April 2017 and collected as the subjects of the study.

Inclusion criteria: Patients without any other dermatosis, autoimmune disease (such as dermatomyositis, rheumatoid arthritis, etc.), infectious disease or other basic diseases; patients with normal cerebrovascular and cardiovascular function and liver and kidney function; patients who didn't receive any systemic treatment or take any hormonal drugs or immunosuppressive agents in the past two months. And another 30 samples from healthy people

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Table 1. The primer sequences of IL-36, p38 and NF-κB p65

	Forward primer	Reverse primer
IL-36	5'-TCTACCTGGGCCTGAATGGA-3'	5'-AAAGGACTTCACAGGCTCGG-3'
P-38	5'-ATGCATAATGGCCGAGCTGT-3'	5'-GGTGTTCCTGTCAGACGCAT-3'
NF-κB p65	5'-GGGGACTACGACCTGAATGC-3'	5'-GATCTTGAGCTCGGCAGTGT-3'
β-actin	5'-ACAGAGCCTCGCCTTTGCCG-3'	5'-ACATGCCGGAGCCGTTGTCG-3'

were included as the control group at the same time, whose normal skin pieces were removed and reserved during the plastic surgeries in this hospital. The specimens were divided into two parts after sampling, one part was frozen and preserved in liquid nitrogen immediately, the other part was preserved in the freshly prepared 10% neutral buffered formalin liquid for the follow-up experiments. All the patients signed informed consents and this study was discussed and approved by the Ethics Committee of our hospital.

Main reagents: Mouse anti-human IL-36 polyclonal antibodies, mouse anti-human p-p38 monoclonal antibodies, mouse anti-human NF-κB p65 monoclonal antibodies and mouse anti-human β-actin polyclonal antibodies were purchased from Santa Cruz Biotechnology. DAB chromogen, citrate buffer powder, hematoxylin and eosin were purchased from Wuhan Boster biological technology Co. Ltd.; TRIGene kit, real time PCR kit and reverse transcription kit were purchased from U.S.A. Thermo Scientific Co. Ltd., the total cell protein extraction kit was purchased from Jiangsu Beyotime biotechnology Co. Ltd.

Methods

Real-time PCR detection: The total RNA of the samples in the psoriasis vulgaris group and the control group were extracted respectively according to instructions of TRIGene kit, and their concentrations and purity were detected by spectrophotometer and the ratio of A_{260}/A_{280} was controlled between 1.8 and 2.0. The synthesis of primer sequences was finished by Shanghai Jiran biotechnology Co. Ltd. (**Table 1**). The sample cDNA was synthesized according to instructions of reverse transcription kit. The RT-PCR reaction systems (10 μL) were constructed: 5 μL SYBR Green mix, 1 μL upstream and 1 μL downstream primers respectively, 2 μL ddH₂O and 1 μL cDNA. Reaction conditions: being kept at 95°C for 10 min, 95°C for 15 s,

then annealed for 1 min, and then heated at 95°C for 15 s, 60°C for 30 s, 95°C for 15 s, 40 cycles of every amplification, and finally elongated at 72°C for 7 min once. After PCR was finished, ABI7900

real-time fluorescent quantitative PCR system was adopted for real-time quantitative analysis, and the relative values of ΔCt was calculated. β-actin was used as internal reference of copy number in order to calibrate PCR template, and the relative expression level of gene was calculated using $2^{-\Delta\Delta Ct}$ method.

Immunohistochemical staining: Slices were rehydrated after dewaxing and washed with PBS solution. Ten percent of normal goat serum was used to block non-specific background at room temperature for 15 min. The primary antibodies were added and then were incubated at 4°C overnight. After the incubation, the slices were washed with PBS solution, the secondary antibodies were added and then were incubated at room temperature for 30 min and then washed with PBS solution. Next, the slices were incubated in streptavidin-peroxidase solution at room temperature for 30 min, washed with PBS solution, colorated by DAB, washed by running water, then counterstained by hematoxylin and mounted by neutral gums.

The western blot detection: According to the instructions of total cell protein extraction kit, the total proteins of samples in psoriasis vulgaris group and control group were respectively extracted, and the concentrations of extracted proteins were detected. And then all the proteins were preserved at -70°C. Gels with different concentrations were prepared and sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed. The gels' positions of two kind of proteins were confirmed according to marker band. The proteins were transferred to the membrane for 35 min and then they were blocked with 5% skimmed milk liquid at 37°C for 90 min. The primary antibodies (1:600) were added and then the whole system was incubated at 4°C overnight, then the band was washed and rocked with TBST3 times for 15 min each time in rocking bed. Secondary antibodies (1:400) were added, the whole system was incubated at 37°C for 1 h and then the band

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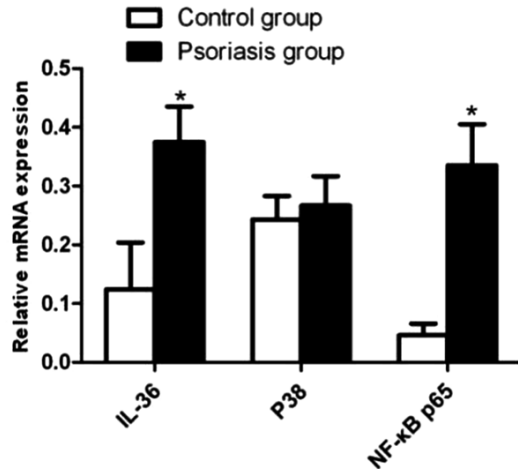


Figure 1. The relative mRNA expression levels of IL-36, p38, and NF- κ B p65 of the 80 patients with psoriasis vulgaris and 30 patients in the control group detected by qRT-PCR. Compared with the control group, * $P < 0.05$.

was washed and rocked with TBST3 times for 15 min each time in rocking bed. Enhanced chemiluminescence substrate was added to colorate at darkroom, then exposures and developing were performed. Finally, the images were analyzed and the values of absorbance were recorded using Image J Professional Image Analysis Software.

Experimental results evaluation

The immunohistochemical slices were observed under optical microscope (400X). Five visual field were randomly chosen to be observed, and 100 cells in each field were counted respectively, then the percentages of positive cells were calculated as the average percentage of positive cells. In this study, cells were ranged according to the staining degrees in the field, namely no staining (-), weakly positive staining (+), positive staining (++), strongly positive staining (+++).

Statistical analysis

The statistical analysis in this study was applied with SPSS 17.0. The measurement data were evaluated using mean \pm standard deviation and the comparisons between two groups were performed by using t-test. The enumeration data were expressed by ratio or percentage and the comparisons between two groups were performed by using chi-square test. The correlation analysis was performed with Pearson's correlation analysis. The difference had statistical significance when $P < 0.05$.

Results

The comparison of general information between the two groups

In the psoriasis vulgaris group, 45 males and 35 females (aged 31-70 years old with the average age of 44.12 ± 6.74 years old) were enrolled and their courses of disease were from 15 days to 25 years (41 cases in the active stage and 39 cases in the resting stage). In the control group, there were 16 males and 14 females (aged 30-68 years old with the average age of 42.87 ± 5.92 years old). There was no significant difference between the two groups in terms of age, sex and other general baseline data, so that the two groups were comparable.

The results of real-time fluorescence quantitative PCR detection

The results of real-time fluorescent quantitative PCR showed that the mRNA expressions of IL-36, p38 MAPK and NF- κ B p65 could be detected in the psoriasis vulgaris group and the control group. Meanwhile, the mRNA expression levels of IL-36 and NF- κ B p65 in the psoriasis vulgaris group were significantly higher than those in the control group, and the differences were statistically significant ($P = 0.000$). There was no statistical difference in the mRNA expression levels of P38 MAPK compared with the control group ($P = 0.146$, **Figure 1**).

The results of immunohistochemical staining

The results of immunohistochemical staining showed that the expression levels of IL-36, p-p38 and NF- κ B p65 in the psoriasis vulgaris group were significantly higher than those in the control group, their differences were statistically significant (all $P = 0.000$). In the psoriasis vulgaris group, most of IL-36 was expressed in the cytoplasm, while p-p38 as well as NF- κ B p65 could be expressed in each layer of the skin with deep staining and strong positive expression. However, in the control group there was no expression or there were sporadic weakly expressions (**Figure 2**).

The results of the western blot

The results of the western blot depicted that the protein expressions of IL-36, p-p38 and NF- κ B p65 could be detected, but the protein expression levels of IL-36, p-p38 and NF- κ B p65 were relatively lower in the control group.

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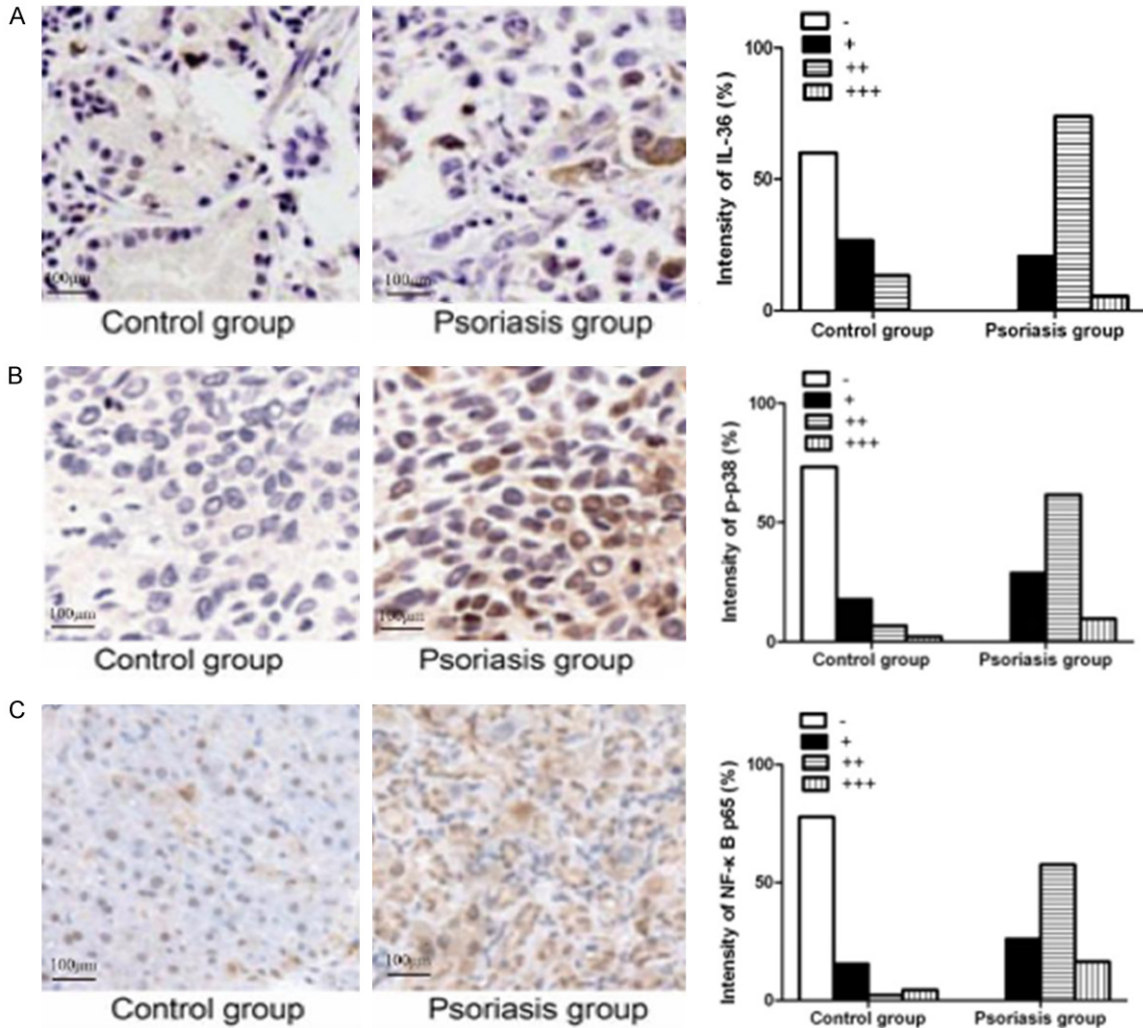


Figure 2. The intensities of IL-36, p-p38, and NF-κB p65 of the 80 patients with psoriasis vulgaris and 30 patients in the control group detected by immunohistochemical staining. A. The expression of IL-36; B. The expression of p-p38; C. The expression of NF-κB p65.

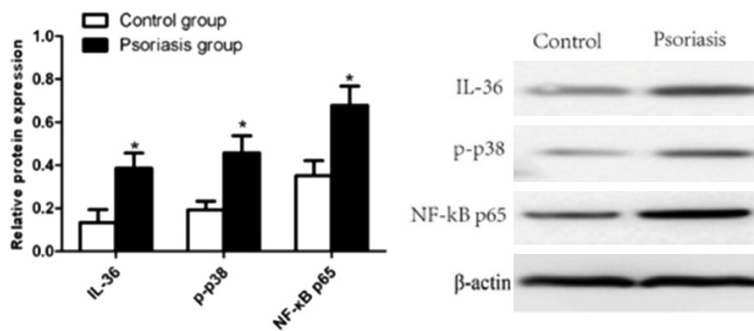


Figure 3. The protein expression levels of IL-36, p-p38, and NF-κB p65 of the 80 patients with psoriasis vulgaris and 30 patients in the control group detected by the western blot. Compared with the control group, *P=0.000.

proteins in the psoriasis vulgaris group was significantly higher than those in the control group, and all the differences were statistically significant (P=0.000, **Figure 3**).

Pearson's correlation analysis was applied to analyze the protein expression levels of IL-36, p-p38 and NF-κB p65 in the psoriasis vulgaris group. And the results showed that there were significant positive correlations between IL-36 and p-p38 as well as between

The statistical analysis revealed that the expression level of IL-36, p-p38 and NF-κB p65

IL-36 and NF-κB p65 ($r_1=0.354$, P=0.002, $r_2=0.386$, P=0.001), and there was significant

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Table 2. Correlation analysis between different factors

Index	Correlation coefficient (r)	P
IL-36 and p-p38	0.354	0.002
IL-36 and NF- κ B p65	0.386	0.001
p-p38 and NF- κ B p65	0.568	0.003

positive correlation between p-p38 and NF- κ B p65 ($r=0.568$, $P=0.003$). See **Table 2**.

Discussion

Psoriasis vulgaris is an immune-mediated disease with a high incidence, which is hard to cure and easy to recur. It can seriously affect the patients' physical and mental health and quality of life. The pathogenesis of psoriasis vulgaris has been unclear at present and its etiology is complex. However, most of the researches suggested that psoriasis was a kind of dysfunction of T lymphocytes, and the interleukin, interferon, tumor necrosis factor, etc., released by T lymphocytes involved in the process of the genesis and development of psoriasis [8, 9]. Among them, the interleukin is the important cytokine that can activate the secretion of T cells, which has been discovered in recent years. As a member of the interleukin-1 family, it can participate in the activation of dendritic cells and T helper cell, antigen presentation, the stimulation of synthesis of proinflammatory cytokine as well as the occurring process of inflammation. The immune activation and regulation functions of IL-36 play a momentous role in autoimmune diseases. Some studies have suggested that the expression of IL-36 α could be detected in the inner layer of bursa of patients with inflammatory arthritis, and the expression levels of IL-36 α in rheumatoid arthritis and psoriatic arthritis were significantly higher than that in osteoarthritis [10]. In the study of mouse models of arthritis, IL-36 was found to be interrelated to the severity of arthritis [11]. The skin lesions of IL-36 α^+ and IL-36Ra $^{+/-}$ transgenic mouse models were very close to the skin lesions of human psoriasis, and the skin lesions of mice were obviously reduced after being treated with the human psoriasis drugs [12]. At present, the relationship between IL-36 and psoriasis needs to be studied further. The results of this study showed that the mRNA and protein expression levels of IL-36 in psoriasis vulgaris lesions were

significantly higher than those in the control group, and the difference was statistically significant. This is basically consistent with the foreign research reports [13-15].

IL-36 involves in the pathophysiological process of chronic inflammation and autoimmune diseases mainly through combining with the corresponding receptors and then activating p38 MAPK and NF- κ B signal transduction pathway [16]. The signaling pathway of p38 MAPK and NF- κ B can induce the expression of proinflammatory genes, apply chemotaxis on the aggregation of inflammatory cells as well as inflammatory mediators to inflammatory sites, and then promote an inflammatory reaction, which is one of the important intracellular signal transduction mechanisms [17]. The p38 MAPK after phosphorylation can be transferred into the nucleus and involve in cell proliferation and differentiation as well as the activation of T cells and so on through the transduction of relative signals [18]. The NF- κ B usually expressed as a p50/p60 heterodimer. As the basic transcription factor of some chronic inflammatory diseases, it can be transferred into the nucleus after activation and then induce the expression of genes that NF- κ B manages [19]. More and more studies showed that NF- κ B pathway and MAPK pathway involved in the process of inflammatory reactions [20]. Psoriasis is a kind of immune related inflammatory dermatosis. Therefore, it is believed that the NF- κ B pathway and MAPK pathway may involve in the process of psoriasis vulgaris occurrence and its development.

In this study, the results of immunohistochemical staining showed that NF- κ B p65 and p-p38 were strong positive expression in psoriasis vulgaris lesions, while there was almost no expression in the control group. The results of real-time PCR and western blot showed that in terms of the expression level of mRNA, the expression level of NF- κ B p65 in the psoriasis vulgaris group was significantly higher than that in the control group, while there was no significant difference in the expression level of p38 mRNA between the two groups; in terms of the expression level of protein, the expression level of p-p38 protein in the psoriasis vulgaris group was significantly higher than that in the control group and this suggested that p-p38 MAPK was expressed abundantly in patients with psoriasis vulgaris, which was consistent with the

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results of immunohistochemical staining and indicated that large-amount phosphorylation of p38 MAPK in psoriasis vulgaris lesions might be related to the local inflammations in psoriasis vulgaris lesions. The activated p38 MAPK could up-regulate the expression of NF- κ B p65 and other transcription factors.

The conclusion of correlation analysis is that there is a significant positive correlation between the protein expressions of NF- κ B p65 and p-p38. In psoriasis vulgaris lesions, there are significant positive correlations between the expression of IL-36 and NF- κ B p65, as well as between the expression of IL-36 and p-p38, so it is believed that the high expression of IL-36 and the increased activation of related p38 MAPK and NF- κ B signaling pathway may be one of the pathogeneses of psoriasis vulgaris.

In summary, this study found that there was high expression of IL-36 in the skin of patients with psoriasis and the activation of relative p38 MAPK and NF- κ B signaling pathway, which is supposed to involve in the pathogenesis of psoriasis vulgaris. However, this study also had some limitations, such as the small sample size. What's more, further studies were needed to confirm these conclusions.

Disclosure of conflict of interest

None.

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