IL-36 Cytokine Expression and Its Relationship with p38 MAPK and NF-κB Pathways in Psoriasis Vulgaris Skin Lesions

Qi HE (贺 岯), Hong-xiang CHEN (陈宏翔), Wen LI (李 家), Yan WU (吴 雯), Shan-juan CHEN (陈善娟), Qing YUE (岳 青), Min XIAO (肖 敏), Jia-wen LI (李家文)

Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

© Huazhong University of Science and Technology and Springer-Verlag Berlin Heidelberg 2013

Summary: This study examined the correlation of the expression of interleukin-36 (IL-36), a novel member of interleukin-1 (IL-1) family, with p38 mitogen-activated protein kinase (p38 MAPK) and nuclear factor-kappa B (NF-xB) pathways in psoriasis vulgaris skin lesions. The expression levels of IL-36α, IL-36β, IL-36γ, phosphorylated p38 MAPK, and NF-xBp65 were detected in the skin tissues of 38 psoriasis patients and 17 healthy control subjects by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blotting. The cytokine expression levels were compared between the psoriasis group and the control group. A correlation analysis between cytokine proteins was performed in the psoriasis group. Results showed that the expression levels of IL-36α, IL-36β, IL-36γ, phosphorylated p38 MAPK, and NF-xBp65 in the psoriasis group were significantly higher than those in the control group (P<0.001). In the psoriasis group, the IL-36 cytokine expression was positively correlated with phosphorylated p38 MAPK and NF-xBp65 expression (P<0.05). A significant positive correlation was also found between the phosphorylated p38 MAPK and NF-xBp65 expression (P<0.01). It was concluded that the increased IL-36 expression is correlated with p38 MAPK and NF-xB pathways in psoriasis vulgaris skin lesions. All the three factors may be jointly involved in the pathogenesis and local inflammatory response of psoriasis.

Key words: interleukin-36; p38 mitogen-activated protein kinase; nuclear factor-kappa B; psoriasis vulgaris

Psoriasis is a common chronic inflammatory skin disease with genetic predisposition. Approximately 2% to 3% of the world’s population suffers from this disease, particularly in America and Canada[1, 2]. Although the pathogenesis of psoriasis has been extensively examined, it remains largely elusive because of its complex etiology and relationship with various factors (such as genetic, immune, and environmental factors, etc.).

Several studies demonstrated that two signaling systems, namely, the mitogen-activated protein kinase (MAPK) and the nuclear factor-kappa B (NF-xB), are implicated in the immune response of many chronic inflammatory diseases, including psoriasis[3-6]. These signaling systems can affect and regulate the expression of various chemokines, inflammation mediators, and adhesion molecules and their release, which play important roles in maintaining inflammation[3-6].

Recently, interleukin-36 (IL-36), a novel member of the interleukin-1 (IL-1) family was found to be involved in the pathogenesis of psoriasis[7]. The members of IL-36 family, including IL-36 receptor antagonist (IL-36Ra), IL-36α, IL-36β, and IL-36γ (previously designated as IL-1F5, IL-1F6, IL-1F8, and IL-1F9) can form a novel and relatively independent signaling system in Jurkat cells cultured in vitro. These members can also induce the activation of MAPK and NF-xB pathways, which are involved in the subsequent immune responses[8]. Therefore, IL-36α, IL-36β, and IL-36γ expression as well as expression of MAPK and NF-xB family members, including p38 MAPK, phosphorylated p38 MAPK and NF-xBp65, were examined in skin lesions of patients with psoriasis vulgaris in this study to determine the correlation of IL-36 expression with p38 MAPK and NF-xB pathways in psoriasis vulgaris.

1 MATERIALS AND METHODS

1.1 Subjects of Study
Two groups were established in this study: psoriasis group and control group. Skin lesions from the psoriasis group were obtained from 38 patients (aged 21 to 64 years, mean age 43 years; 22 males, 16 females) diagnosed as having psoriasis vulgaris in the Department of
Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (China) from 2011 to 2012. All of the patients didn’t receive any systemic treatment prior to the study and skin-directed therapy during the two weeks before biopsy. Normal skin was obtained from 17 subjects (aged 16 to 35 years, mean age 22; 17 males) who had no skin disease or systemic disease, and were circumcised in the Department of Urology in 2012. Each skin sample was divided into two parts after biopsy: one sample was fixed in 10% formalin and another was frozen immediately and stored at –80 °C for Western blotting and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). This study was approved by the Ethics Review Committee of Tongji Medical College, Huazhong University of Science and Technology (China). Informed consents were obtained from all participants.

1.2 Reagents

Goat anti-IL-36α and goat anti-IL-36β polyclonal antibodies (R&D Systems, USA), rabbit anti-IL-36γ polyclonal antibody (Gene Tex, USA), mouse anti-phospho-p38 MAPK monoclonal antibody (Cell Signaling Technology, USA), mouse anti-NF-κBp65 monoclonal antibody (Santa Cruz, USA) and mouse anti-β-actin monoclonal antibody (Santa Cruz, USA) were used as primary antibodies for Western Blotting and immunohistochemistry.

1.3 RNA Extraction and qRT-PCR

Total RNA was isolated from tissues using TRIzol reagent (Ambion, USA). Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The sequences of primer pairs used in the study were as follows: 5'-TCTACCTGGGCGTCAATGGA-3' (forward IL-36α), 5'-AAAGGACTTCAACAGGCTCGG-3' (reverse IL-36α); 5'-TTCTCATCCATGCGGACAA-3' (forward IL-36β), 5'-GATGTTGGAACTTCTCCTCCC-3' (reverse IL-36β); 5'-TGTAGGCAACAAGGCATCTG-3' (forward IL-36γ), 5'-TAAACGGCAGAAGCTCTGAA-3' (reverse IL-36γ); 5'-AGCATATAATGGCCGACGCTGT-3' (forward p38 MAPK), 5'-GGGACTCAGACCTGATGC-3' (reverse p38 MAPK); 5'-GGGACTCAGACCTGATGC-3' (forward NF-κBp65), 5'-GATCTTGAGCTCGGACGT-3' (reverse NF-κBp65); 5'-ACAGACCTGCGCTTTGCCG-3' (forward β-actin), and 5'-ACATGCCC-GGACCGCTTGTCG-3' (reverse β-actin). Real-time PCR was performed using the FastStart Universal SYBR Green Master [Rox] (Rothe, Germany). Real-time quantities were performed using the ABI7900 Detection System software (ABI, USA) and the amplification parameters consisted of 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each sample was tested thrice within the same run. Ct method was used to calculate the relative gene expression changes that were normalized against the reference gene β-actin.

1.4 Western Blotting

Total proteins were extracted from tissues, purified, applied onto 10% sodium dodecyl sulphate polyacrylamide gel for electrophoresis, and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% non-fat dry milk for 45 min and subsequently incubated with a primary antibody (IL-36α, IL-36β, IL-36γ, phospho-p38 MAPK, NF-κBp65, or β-actin) overnight at 4°C and a secondary antibody for 60 min at room temperature. The bands were detected using chemiluminescence and analyzed by AlphaEaseFC 4.0 (AlphaInnotech, USA). The relative protein expression was normalized against the internal control β-actin. Each sample was analyzed at least thrice.

1.5 Immunohistochemistry (S-P Method)

All of the 10% formalin-fixed samples were paraffin-embedded and cut into sections. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The sections were then incubated with primary antibodies (IL-36α, IL-36β, IL-36γ, phospho-p38 MAPK, or NF-κBp65) overnight at 4°C, followed by secondary antibodies for 60 min at room temperature. The slides were developed with newly prepared 3,3′-diaminobenzidine and counterstained with hematoxylin. For the negative control setup, all of the procedures were the same except that tris-buffered saline was used as the primary antibody.

Cells that were stained brown were considered to be positive cells. Staining and distribution of positive cells within each layer of the epidermis were evaluated under the microscope. On each section, five visual fields were randomly selected at 400× magnification. The percentage of positive epidermal cells in each field was calculated. The average percentage was used as the positive cell proportion.

1.6 Statistical Analysis

SPSS 18.0 software package was used for statistical analysis. Data were expressed as mean±SE. Student’s t-test was used to compare the difference between the two groups. Pearson’s Bivariate correlation analysis was adopted for the correlation analysis. P value<0.05 was considered to be statistically significant.

2 RESULTS

2.1 qRT-PCR and Western Blotting Results

qRT-PCR results showed that IL-36α, IL-36β, IL-36γ and NF-κBp65 were weakly expressed at transcriptional level or even found absent in normal skin samples. However, they were significantly increased in the psoriasis vulgaris samples (P<0.001 for all). There was no significant difference in the total p38 MAPK mRNA expression between the two groups (P>0.05; fig. 1A). Western Blotting also revealed that IL-36α, IL-36β, IL-36γ, phospho-p38 MAPK and NF-κBp65 proteins were intensely expressed in psoriasis vulgaris samples compared with those in normal controls (fig. 1B, fig. 2) (P<0.001 for all).

2.2 Immunohistochemical Staining Results

IL-36α, IL-36β and IL-36γ were expressed in both skin lesions and normal epidermis. Moreover, it was