The Special DNA Methylation Status in the Promoter of IL-12B in Psoriatic Epidermis

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Background: It is unclear the methylation status of CpG islands within interleukin-12B (IL-12B) in psoriasis, although increasing evidence indicates that the abnormal DNA methylation is involved in the pathogenesis of psoriasis.

Objective: We investigated the methylation status of IL-12B gene in psoriatic epidermis.

Methods: The DNA specimens were obtained from the involved and uninvolved epidermis of 30 patients with plaque psoriasis and 28 healthy persons as the control group. Methylation of 26 CpG sites in the promoter within IL-12B gene was examined by bisulfite sequencing. The severity of disease was evaluated by psoriasis area and severity index (PASI).

Results: The mean methylation rate in the promoter of IL-12B in involved psoriatic epidermis, uninvolved psoriatic epidermis and healthy control is 0.0230 ± 0.0101, 0.0188 ± 0.0118 and 0.0105 ± 0.0114, respectively. The methylation rate of IL-12B in both involved psoriatic epidermis (p<0.001) and uninvolved psoriatic epidermis (p<0.05) is significantly higher than in healthy controls, respectively. The methylation rate of IL-12B in uninvolved psoriatic epidermis is lower than in involved psoriatic epidermis (p=0.031). The mean methylation rate in involved psoriatic epidermis was correlated to patient PASI scores (r=0.633, p<0.001).

Conclusions: Abnormal methylation in the promoter of IL-12B have been found in psoriatic epidermis, and is associated with severity of the disease, which suggests the promoter methylation of IL-12B may be involved to the pathogenesis of psoriasis.

Keywords: Psoriasis; Interleukin-12; DNA methylation

Introduction

Psoriasis is a chronic, immunologically-mediated, inflammatory disease of the skin and joints. As being public believed, epigenetic mechanisms are involved in psoriasis, especially DNA methylation. Some studies have shown significant increase of global DNA methylation level both in psoriatic PBMCs and psoriatic lesions compared to health controls, and a significant positive correlation was observed between the DNA methylation level and PASI scores in skin samples among the patients with psoriasis [1,2]. There even has been a study shows that a subset of hyper-methylated CpG sites in psoriatic lesions [2]. Many previous studies have shown that abnormal DNA hyper-methylation in promoters of some tumor suppressor genes is involved in pathogenesis of psoriasis, such as P16 and P14 [1,3], and other genes, for example, gene PDCD5, which encodes apoptosis-related protein and gene TIMP2, which encodes an inhibitor of matrix metalloproteinases (MMPs), have been found significantly increase in psoriatic lesions [4]. Then, how about the methylation status of CpG islands within the susceptibility genes in psoriasis? But no previous work can be referred to.

A growing number of SNPs in interleukin-12B (IL-12B) have been found being associated with the risk of psoriasis, exhibiting the linkage between the genetic polymorphisms in IL-12B and psoriasis [5]. Little of researches showed that the polymorphism in the promoter region of IL-12B is associated with IL-12 secretion [6]. As previous studies shown the pathophysiology of psoriasis is immunologically mediated by the infiltration of T cells in the skin and elaboration of inflammatory cytokines by immune cells, endothelial cells, and keratinocytes. There is growing evidence for the importance of the pro-inflammatory cytokines IL-12 and IL-23 in psoriasis. IL-12 promotes the development of Th1 populations, while IL-23 seems to be important in the survival and proliferation of Th17 cells [7]. IL-12 and IL-23 share the common subunit, the p40 subunit, which is encoded by IL-12B overexpressed in psoriatic lesions [8]. And the IL-23R encodes the protein forming a receptor for IL-23 together with the p1 subunit of IL-12 receptor (encoded by IL-12BRβ1) [9]. In order to explore the deeper association between these genes and psoriasis, and provide clues and directions for further studies in the pathogenesis of...
psoriasis, highly specific and sensitive techniques have been used to examine the density and sites of methylation in promoter in IL-12B genes.

Materials and Methods

Study subject

30 patients were enrolled in the study. Their characteristics are summarized in Table 1. All of them were referred to the Institute of Dermatology, Chinese Academy of Medical Sciences.

| Case | Gender | Age (years) | Length of disease (Years) | PASI | Family history | Original site |
|------|--------|-------------|---------------------------|------|----------------|---------------|
| 1    | M      | 32          | 1                         | 21   | -              | Trunk         |
| 2    | M      | 46          | 20                        | 32   | +              | Trunk         |
| 3    | F      | 45          | 10                        | 16.2 | -              | Trunk         |
| 4    | F      | 28          | 8                         | 8.9  | -              | Trunk         |
| 5    | M      | 65          | 8                         | 43.6 | -              | Trunk         |
| 6    | M      | 30          | 6                         | 12   | -              | Trunk         |
| 7    | F      | 30          | 14                        | 18.6 | +              | Trunk         |
| 8    | F      | 48          | 1                         | 5.1  | -              | Trunk         |
| 9    | M      | 31          | 1                         | 5.6  | -              | Trunk         |
| 10   | M      | 21          | 3                         | 10.6 | -              | Trunk         |
| 11   | F      | 39          | 8                         | 20.6 | -              | Trunk         |
| 12   | M      | 36          | 10                        | 20.2 | +              | Trunk         |
| 13   | F      | 33          | 20                        | 9.2  | -              | Trunk         |
| 14   | M      | 18          | 1                         | 16.8 | -              | Trunk         |
| 15   | F      | 38          | 20                        | 5.6  | -              | Trunk         |
| 16   | M      | 53          | 1                         | 18.2 | -              | Trunk         |
| 17   | M      | 48          | 18                        | 8.9  | -              | Trunk         |
| 18   | M      | 33          | 8                         | 8.2  | +              | Trunk         |
| 19   | F      | 43          | 1                         | 9.6  | -              | Trunk         |
| 20   | F      | 45          | 1                         | 35.8 | -              | Trunk         |
| 21   | M      | 20          | 5                         | 6    | +              | Trunk         |
| 22   | F      | 29          | 1                         | 9.2  | -              | Trunk         |
| 23   | M      | 48          | 20                        | 26.5 | -              | Trunk         |
| 24   | F      | 32          | 8                         | 6    | -              | Trunk         |
| 25   | M      | 48          | 3                         | 4.2  | -              | Trunk         |
| 26   | F      | 58          | 16                        | 28   | -              | Trunk         |
| 27   | M      | 64          | 1                         | 20   | -              | Trunk         |
| 28   | F      | 34          | 16                        | 16   | -              | Trunk         |
| 29   | M      | 17          | 3                         | 46.2 | -              | Trunk         |
| 30   | M      | 47          | 2                         | 9.2  | +              | Trunk         |

Table 1: Clinical details of 30 patients with psoriasis.
Evaluation of disease severity was made using the Psoriasis Area and Severity Index (PASI) scoring system. The score ranged from 4.2 to 46.2. None of the patients received phototherapy or systemic therapy for 4 weeks prior to entry into this study. Pregnant patients were excluded from participating in the study. 28 healthy controls were defined as persons who did not have self-reported psoriasis history and clinically did not have skin lesions. All the enrolled volunteers signed an informed consent document approved by the ethical committee. Skin specimens were obtained by 8-mm punch biopsy under aseptic conditions with local anaesthesia. The specimens were frozen and stored at -80 until processed.

Epidermal isolation, DNA extraction

Epidermis was separated from skin specimens by treatment with 0.25% dispase (Roche, Indianapolis, IN, U.S.A.) for about 8 hours, and samples were homogenized in sterile 5-mL tissue grinders (placed on ice). For DNA isolation, into each tube was placed 500 lL of reaction solution containing 10% sodium dodecyl sulphate, 10 mol.L⁻¹ Tris–HCl and 0.1 mol.L⁻¹ ethylenediamine tetraacetic acid (EDTA), followed by gentle mixing and incubation for 1 h at 37, then 3IL proteinase K (20 mg.mL⁻¹) was added, followed by overnight incubation at 55. After phenolization for 10 min, tubes were centrifuged for 20 min at 4. Then the supernatants were placed in 1.5 mL reaction tubes, phenolization was repeated, followed by centrifugation for 20 min at 4, and DNA was precipitated from the supernatant by addition of 2–2.5 volumes ethanol. After centrifugation, the pellet was washed with 70% ethanol, air dried, and redissolved in 100 mL doubledistilled water.

Bisulfite Sequencing

To investigate the methylation status of the IL-12B promoter, we first mapped the corresponding CpG island containing 26 CpG dinucleotides in IL-12B by CpGplot (EBI Tools, EMBOSS CpGPlot; http://www.ebi.ac.uk/emboss/cpgplot). Subsequently, one microgram of genomic DNA was used to convert unmethylated CpGs using the EpiTectH Bisulfite kit (Qiagen, CA) following the manufacturer’s instructions. Finally, the prepared DNA was suspended in 10 µL TE to test immediately, or stored at -20. The following primer sets for IL-12B were designed using MethPrimer. Forward 5′-AGTAGGATTGAGGAAATGATT-3′, reverse 5′-CAAACAACCCACACTCAAACAC-3′, spanning part of the promoter of IL-12B.

The reaction was setup in a total volume of 25 µL containing 1 µL sample DNA, 0.2 mM dNTPs, 0.5 mM primers, 2.5 mM MgCl₂, 16 µL buffer II, and 1.25 unit HotTaq DNA polymerase (Takara, JP). PCR amplification was carried out with the following profile: 94 for 9 minutes to activate the polymerase; 44 cycles at 94 for 30 seconds, 55 for 30 seconds, and 68 for 45 seconds; and a 7-minute terminal extension at 68. The PCR-generated DNA fragments were cloned into plasmids using a TOPO TA Cloning kit according to the protocol recommended by the manufacturer (Invitrogen, Carlsbad, CA). Clones were screened by colony PCR followed by electrophoreses on the agarose gel to examine presence of target inserts. Plasmid DNAs from each clone were purified using the Qiaquick kit (Qiagen, CA). Individual clones were sequenced on an ABI PRISM 3730 automated sequencer. Sequence electrophorograms were aligned using Sequence Navigator software (Applied Biosystems), which shows cytosine if the original cytosine is methylated or thymine if the original cytosine is unmethylated. We selected 5-10 clones per sample for sequencing.

Statistical analysis

The SPSS 13.0 (SPSS, Chicago, IL, U.S.A.) was used for statistical analysis. The t-test was used to compare differences for paired and non-paired observations. Adjusted odds ratios (ORs) were calculated by fitting logistic regression models with adjustment for the relation between mean methylation rate and PASI score. The level of significance was P<0.05.

Results

Bisulfite sequencing was performed on samples from 30 patients, including both involved psoriatic epidermis and uninvolved psoriatic epidermis, and 28 healthy controls to validate the observed differences in DNA methylation of IL-12B. 93.33% (28/30) of samples from involved psoriatic epidermis and 86.67% (26/30) of ones from uninvolved psoriatic epidermis were found methylated in IL-12B. The mean methylation rate in the promoter of IL-12B in involved psoriatic epidermis, unmethylated psoriatic epidermis and healthy controls is 0.0230±0.0101, 0.0188±0.0118 and 0.0105±0.0114, respectively. Compared to healthy controls, there was significantly hyper-methylated in both involved psoriatic epidermis (t=4.426, p<0.001) and unmethylated psoriatic epidermis (t=2.695, p=0.009). The methylation rate of IL-12B in involved psoriatic epidermis is higher than that in unmethylated psoriatic epidermis (t=2.271, p=0.031). (Figure 1A) Correlation analyses showed patient PASI score was correlated to the mean DNA methylation levels in involved psoriatic epidermis (r=0.633, p<0.01) (Figure 2). In methylated clones, about 1-6 of 26 CpG dinucleotides in the promoter region of the HLA-C genome was methylated (Figure 3).
Figure 1A&1B: (A) Mean methylation rate in the promoter of IL-12B from psoriatic epidermis (n=30), including involved psoriatic epidermis (IP) and uninvolved psoriatic epidermis (UP), and healthy control epidermis (HC) (n=28). Compared to healthy controls, there was significantly hyper-methylated in both involved psoriatic epidermis (p<0.001) and uninvolved psoriatic epidermis (p=0.009). The methylation rate of IL-12B in uninvolved psoriatic epidermis is lower than that in involved psoriatic epidermis (p=0.031). (B) Methylation status in the promoter of IL-12B from psoriatic epidermis (n=30), including involved epidermis and uninvolved epidermis, and healthy control epidermis (n=28).

Figure 2: Correlation between individual PASI scores and the mean DNA methylation levels in involved psoriatic epidermis (r=0.633, p<0.001).

At 11 of 26 CpG sites (No. 1, 8, 14, 15, 18, 21-26), the hyper-methylation was more pronounced in involved psoriatic epidermis compared to uninvolved psoriatic epidermis, but methylation level reverses at 10 of 26 CpG sites (No. 3-7, 9, 11-13, 20), and the other five CpG sites show no methylation in both in involved and uninvolved psoriatic epidermis. Interestingly, at four continuous clustered CpG sites (No. 23-26), IL-12B was hyper-methylated in healthy controls compared to psoriatic epidermis. There was no methylation at five continuous clustered CpG sites (No. 9-13) in involved psoriatic epidermis and in healthy controls. And no methylation was found at the other five continuous clustered CpG sites (No. 15-19) in uninvolved psoriatic epidermis and in healthy controls. At five CpG sites (No. 2, 10, 16, 17, 19), no methylation was detected in involved psoriatic epidermis, uninvolved psoriatic epidermis and healthy controls (Table 2 and Figure 1B).

Table 2: Methylation rates of 26 CpG sites in a 405 bp region of IL-12B gene (-1166– -762) in involved psoriatic epidermis, uninvolved psoriatic epidermis and healthy controls.

| Groups          | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   | 21   | 22   | 23   | 24   | 25   | 26   | Mean |
|-----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Involved epidermis | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.02 |
| Uninvolved epidermis | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.63 |
| Controls        | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.0  | 0.63 |
Abnormal immune response leading to excessive keratinocyte proliferation and global epidermal thickening plays a crucial role in psoriasis, especially the responses in which Th1 and Th17 cells and their inflammatory cytokines involved [10]. There is growing evidence showing that IL-12 and IL-23 are associated with psoriasis because their important functions in promoting the development of Th1 and Th17 cells respectively [11]. IL-12 promotes the development of Th1 populations producing IFN-γ, TNF-α and IL-2, while IL-23 seems to be important in the survival and proliferation of Th17 cells, producing IL-17, IL-22, TNF-α and IL-18 [7]. IL-12 are up-regulated in involved psoriatic skin compared to uninvolved psoriatic skin [12]. The IL-12B encodes the p40 subunit which is shared by IL-12 and IL-23, while the IL-23R encodes a subunit forming the receptor of IL-23. A list of researches have found lots of SNPs associated with the risk of psoriasis, such as the IL-12B haplotype rs3212227 and rs6887695 [5] and the IL-23R haplotype rs1209026, rs7530511, and rs2201841 [13]. It also has been proved that the polymorphism in the promoter region of IL-12B is associated with the IL-12 secretion [6]. And recent biologic therapies to psoriasis such as Ustekinumab targeting the p40 subunit, thus inhibiting both IL-12 and IL-23 receptor-mediated signalling have shown efficacy [14]. These all confirm the crucial roles of IL-12B in pathology of psoriasis.

As widely known, epigenetic mechanisms play a role in psoriasis. Some studies have shown significant increase of global DNA methylation level both in psoriatic PBMCs and psoriatic lesions [2]. It even has found a significant positive correlation between the DNA methylation level and PASI scores among psoriasis patients in skin samples, but not in PBMCs, which may indicate the skin samples are more meaningful to research of DNA methylation in psoriasis [1]. We examined the methylation status of CpG islands within a set of susceptibility genes in the epidermis of psoriasis patients, and the first genes being chosen are IL-12B.

In our study, there was significantly hyper-methylated within IL-12B in psoriatic lesions compared to healthy controls, which is consistent with the previous studies which show global hyper-methylation in psoriatic lesions. But previous studies didn’t detect uninvolved psoriatic skin. Interestingly, our study found the methylation rate of IL-12B in involved psoriatic epidermis is higher than that in uninvolved psoriatic epidermis. In involved psoriatic epidermis, DNA hypermethylation of IL-12B was correlated with patient PASI scores. The special methylation pattern may be related to the pathogenesis of psoriasis, which is worth further study.

As we know, the methylation of CpG sites within promoters may have effects on transcriptional levels, where methylation levels demonstrate an inverse correlation with expression level [15]. The previous studies show IL-12 was up-regulated in involved psoriatic skin compared to uninvolved psoriatic skin [12]. We didn’t examine the level of IL-12 mRNA in same samples as it is difficult to obtain enough epidermal specimens from the patients with psoriasis. It is just our hypotheses. If possible, the next work is to investigate the relationship between methylation status within IL-12B and their expression.

It is important to realize although many CpG islands are located at gene promoters, CpG islands also exist within gene bodies. Indeed, less than 3% of CpG islands in the promoters were found to methylated [4]. The current conclusion shows that increased gene body methylation also correlates with increased transcription genome-wide [2,5,7,8], though the precise mechanism is still unclear. Maybe it is more reasonable to explore CpG islands within the whole gene rather than only in the promoter. Basing on the information above, it is necessary to make sure the condition of DNA methylation in other CpG islands of the IL-12B in psoriasis. The methylation status in the CpG islands of IL-12B and other susceptibility genes may be expected to be a potential predictor of the psoriasis state and be useful to look for new treatments.

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References

1. Zhang P, Su Y, Chen H, Zhao M, Lu Q (2010) Abnormal dna methylation in skin lesions and pbmc of patients with psoriasis vulgaris. J Dermatol Sci 60: 40-42.
2. Roberson ED, Liu Y, Ryan C, Joyce CE, Duan S, et al. (2012) A subset of methylated cpg sites differentiate psoriatic from normal skin. J Invest Dermatol 132: 583-592.
3. Chen M, Chen ZQ, Cui PG, Yao X, Li YM, et al. (2008) The methylation pattern of p16ink4a gene promoter in psoriatic epidermis and its clinical significance. Br J Dermatol 158: 987-993.
4. Zhang P, Zhao M, Liang G, Yin G, Huang D, et al. (2013) Whole-genome dna methylation in skin lesions from patients with psoriasis vulgaris. J Autoimmun 41: 17-24.
5. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, et al. (2007) A large-scale genetic association study confirms il-12b and leads to the identification of il23r as psoriasis-risk genes. Am J Hum Genet 80: 273-290.
6. Muller-Berghaus J, Kern K, Paschen A, Nguyen XD, Kluter H, et al. (2004) Deficient il-12p70 secretion by dendritic cells based on IL-12b promoter genotype. Genes Immun 5: 431-434.
7. Trinchieri G (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3: 133-146.
8. Piskin G, Sylva-Steenland RM, Bos JD, Teunissen MB (2006) In vitro and in situ expression of il-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. J Immunol 176: 1908-1915.
9. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, et al. (2002) A receptor for the heterodimeric cytokine IL-23 is composed of IL-12beta1 and a novel cytokine receptor subunit, IL-23r. J Immunol 168: 5699-5708.

10. Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, et al. (2008) Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol 128: 1207-1211.

11. Lyakh L, Trinchieri G, Provezza L, Carra G, Gerosa F (2008) Regulation of interleukin-12/interleukin-23 production and the Th17 response in humans. Immunol Rev 226: 112-131.

12. Yawalkar N, Tscharner GG, Hunger RE, Hassan AS (2009) Increased expression of IL-12p70 and IL-23 by multiple dendritic cell and macrophage subsets in plaque psoriasis. J Dermatol Sci 54: 99-105.

13. Zhu KJ, Zhu CY, Shi G, Fan YM (2012) Association of IL23R polymorphisms with psoriasis and psoriatic arthritis: a meta-analysis. Inflamm Res 61: 1149-1154.

14. Quatresooz P, Hermans-Le T, Pierard GE, Humbert P, Delvenne P, et al. (2012) Ustekinumab in psoriasis immunopathology with emphasis on the Th17-IL23 axis: a primer. J Biomed Biotechnol 147413.

15. Bell JT, Pat AA, Pickrell JK, Gaffney DJ, Pique-Regi R, et al. (2011) DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. Genome Biol 12: 405.