Biochemical and Biophysical Research Communications 422 (2012) 157–163

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The characteristics of genome-wide DNA methylation in naïve CD4+ T cells of patients with psoriasis or atopic dermatitis

Jihye Han [a,1], Sin-Gi Park [b,1], Jae-Bum Bae [c], JungKyoon Choi [c], Jae-Myun Lyu [c], Sung Hee Park [b], Hei Sung Kim [a], Young-Joon Kim [c], Sangsoo Kim [b,*], Tae-Yoon Kim [a,*]

[a] Department of Dermato-Immunology, College of Medicine, The Catholic University of Korea, 137-040 Seoul, South Korea
[b] Department of Bioinformatics & Life Sciences, Soongsil University, 511 Sangdo-dong, Dongjak-gu, Seoul, South Korea
[c] Department of Biochemistry, College of Life Science and Technology, Yonsei University, 134 Sinchon-dong, Seodaemun-gu, Seoul, South Korea

ARTICLE INFO

Article history:
Received 6 April 2012
Available online 30 April 2012

Keywords:
Psoriasis
Atopic dermatitis
DNA methylation
Naïve CD4+ T cells

ABSTRACT

Psoriasis and atopic dermatitis (AD) are skin diseases that are characterized by polarized CD4+ T cell responses. During the polarization of naïve CD4+ T cells, DNA methylation plays an important role in the regulation of gene transcription. In this study, we profiled the genome-wide DNA methylation status of naïve CD4+ T cells in patients with psoriasis or AD and healthy controls using a ChIP-seq method. Only psoriasis patient T cells, not those of AD patients, showed distinct hypomethylation (>4-fold) compared to healthy control T cells in twenty-six regions of the genome ranging in size from 10 to 70 kb. These regions were mostly pericentromeric on 10 different chromosomes and incidentally coincided with various strong epigenomic signals, such as histone modifications and transcription factor binding sites, that had been observed in the ENCODE project implying the potential epigenetic regulation in psoriasis development. The gene-centric analysis indicated that the promoter regions of 121 genes on the X chromosome had dramatically elevated methylation levels in psoriasis patient T-cells compared to those from healthy controls (>4-fold). Moreover, immune-related genes on the X chromosome had higher hypermethylation than other genes ($P = 0.046$). No such patterns were observed with AD patient T cells. These findings imply that methylation changes in naïve CD4+ T cells may affect CD4+ T cell polarization, especially in the pathogenesis of psoriasis.

1. Introduction

Psoriasis and atopic dermatitis (AD) are common inflammatory skin diseases associated with CD4+ T cell polarization [1,2]. CD4+ T cells arise from naïve CD4+ T cells. However, naïve CD4+ T cells in psoriasis mainly differentiate into T helper type 1 (Th1) cells in response to Th1 cytokines such as interferon (IFN)-γ [1]. In contrast, AD is predominantly a Th2-polarized disease involving Th2 cytokines such as interleukin (IL)-4 and IL-5 [2]. Immune responses related to T cell polarization are regulated by epigenetic changes [3,4]. These epigenetic changes may be involved in various skin diseases, including systemic lupus erythematosus and vitiligo [5–7]. However, the epigenetic changes of naïve CD4+ T cells during psoriasis and AD are not yet fully understood.

Epigenetic regulation of gene expression is a dynamic process in which DNA methylation is a primary mechanism [8]. In mammals, methylation occurs predominantly on CpG dinucleotides that are sparsely distributed within the genome. CpG methylation regulates and stabilizes chromatin structure and controls the recruitment of transcriptional machinery [9]. In general, regulation of CpG methylation is established and maintained by DNA methyltransferases [9]. Karen et al. demonstrated that the expression of Th2 cytokines, such as IL-4, was regulated by DNA methyltransferase-1 in CD4+ T cells [10]. Based on findings from a previous study, proper maintenance of DNA methylation may be critical for the polarization of CD4+ T cells and the development of diseases such as psoriasis and AD. Because psoriasis and AD are associated with improper immune responses, such as imbalanced CD4+ T cell populations, fine-tuning T cell heritage may be important for managing these two diseases [1,2]. With this hypothesis, we investigated whether DNA methylation in naïve CD4+ T cells is associated with the development of psoriasis and AD.

Abbreviations: AD, atopic dermatitis; dMES, differential methylation enrichment score; IFN, interferon; IL, interleukin; MES, methylation enrichment score; MIRA, Methylated-CpG island recovery assay; Th1, T helper type 1.

* Corresponding authors. Fax: +82 2 824 4383 (S. Kim), +82 2 3482 8261 (T.-Y. Kim).
E-mail addresses: sskimb@ssu.ac.kr (S. Kim), tykimder@catholic.ac.kr (T.-Y. Kim).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.04.128

Here, we describe the genome-wide profile of methylation changes in naïve CD4+ T cells from patients with psoriasis or AD as compared with those of healthy controls. First, we surveyed the global changes along the chromosomes irrespective of gene loci, and found many regions that underwent hypomethylation in patients with psoriasis, not AD. The gene-centric analysis comparing the methylation changes of both promoter and gene-body regions indicated that gene promoter regions on the X chromosome were dramatically hypermethylated in patients with psoriasis. No such patterns were observed with AD patient T cells. Our findings indicate that methylation changes in naïve CD4+ T cells may have important roles during CD4+ T cell polarization, especially in the pathogenesis of psoriasis. Further study of CD4+ T cell polarization based on changes in DNA methylation is necessary to understand the development of psoriasis and AD.

2. Materials and methods

2.1. Subjects

Peripheral blood samples were obtained from 12 patients with plaque type psoriasis, 15 patients with atopic dermatitis and 10 healthy controls in each experiment. Patients with psoriasis or AD were restricted from taking systemic glucocorticoids, topical corticosteroids and immune-suppressants during at least 4 weeks before blood collection. Detailed information of the subjects included in the study is listed in Supplementary Table 1. For controls, healthy subjects who have no history of psoriasis, atopic dermatitis, allergic rhinitis and asthma were enrolled. Approval was obtained from the institutional review boards (IRB) of the Catholic University of Korea, College of Medicine and informed consent was provided according to the Declaration of Helsinki. Patients and healthy controls were subjected only to male because in female, epigenetic modification was reported to be dependent largely on hormonal regulation [11].

2.2. Isolation of naïve CD4+ T cells

To obtain naïve CD4+ T cells, peripheral blood mononuclear cells (PBMCs) from blood were isolated by Ficoll-paque™ PLUS (GE Healthcare bio-Science) density gradient centrifugational method and naïve CD4+ T cells were purified by naïve CD4+ T cells isolation kit II (Miltenyi Biotec). The purity of naïve CD4+ T cells was over 90% by flow cytometric analysis.

2.3. Genomic DNA isolation and affinity purification of methylated genomic DNA

For epigenetic analysis, genomic DNA of all the samples was isolated with DNA blood mini kit (QIAGEN). The individual DNAs were pooled and purified using 3 M NaOAc (pH 5.2) along with ethanol precipitation method. In each experiment set, genomic DNAs of 12 ~ 20 individual sample were pooled to reduce individual variation in further analysis.

First, for preparation of DNA for Solexa sequencing, glutathione *S*-transferase (GST)-tagged with methyl-CPG-binding domain (MBD)-2b and His-tagged MBD-3L1 proteins were prepared. Briefly, the purified genomic DNA was sonicated into 100–500 bp fragments and incubated with GST-MBD-2b protein, His-MBD-3L1 protein, and the JM110 bacterial RNA. MagneGST beads (Promega) were pre-blocked with the JM110 bacterial RNA to block nonspecific interaction between nucleic acid and MBD proteins, and incubated in the MIRA binding buffer (10 mM Tris–HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 3 mM MgCl2, 0.1% Triton X-100, 5% glycerol, 25 μg/ml bovine serum albumin) and washed with washing buffer (10 mM Tris–HCl, pH 7.5, 300 mM NaCl, 1 mM EDTA, 3 mM MgCl2, 0.1% Triton X-100) at 4 °C.

Second, for preparation of DNA for confirm the Solexa sequencing, DNA (2 μg) was dissolved in 50 μl of the appropriate buffer and 10 U of MseI (NEB), and incubated at 37 °C overnight. After purification of DNA, pull-down of methylated DNA fragments (restricted using MseI) was performed using the Methylcollector kit (Active Motif) according to the manufacturers' protocols.

All DNA was eluted and purified with QIAquick PCR Purification Kit (QIAGEN).

2.4. Preparation of affinity-purified methylated DNA for Solexa sequencing

The DNAs were ligated to a pair of Solexa adaptors for Illumina Genome Analyzer Sequencing. The ligation products were eluted from 175 to 225 bp fragments on an agarose gel after PCR amplification. Cluster generation and 36 cycles of sequencing were done according to the manufacturer's instructions. The sequence tags were mapped to the human genome with the University of California, Santa Cruz (UCSC) hg18 (http://genome.ucsc.edu/) assembly based on NCBI build 36.1 through the Solexa Analysis Pipeline (version 0.3.0). Those reads that were mapped to multiple locations were discarded and only those uniquely mapped were retained in the analysis. We obtained about 15 ~ 20 million Illumina sequencing reads at every 200 base pair (bp) interval, across the whole genome from MIRA-Seq. As a result, 34 bp reading sequences were obtained. The first and last nucleotides were excluded for exact sequence match.

2.5. Methylation Enrichment Score (MES) calculation and data analysis

The 34 bp reading sequence was extended toward the 3'end to cover DNA fragments bound by the MBD proteins. To visualize the data, the readout was converted to browser extensible data (BED) and wiggle (WIG) files compatible with the UCSC Genome Browser (http://genome.ucsc.edu). Tagged overlapping sequences were counted with 200 bp resolution. To find enriched genomic regions, numbers of mapped reads in window of 1 kb were compared with the total number of reads in the genome. MES was then calculated by the following formula:

$$\log_2\left(\frac{\text{target count/target interval}}{\text{genome count/genome interval}}\right)$$

The difference of MES between samples of patients and controls, dMES, is defined as:

$$\text{dMES} = \text{MES}_{\text{patient}} - \text{MES}_{\text{control}}$$

For initial survey of hypermethylated or hypomethylated regions, we typically used the criteria of dMES >+2.0 or dMES <−2.0, respectively, corresponding to more than 4-fold changes in methylation level. The genomic coordinates of promoters and genebodies were downloaded from the UCSC Genome Browser database build hg18. The each 10 control data in psoriasis and AD experiment was combined and analyzed in this analysis.

2.6. Methylation-specific PCR of promoter regions

To confirm Solexa sequencing, methylated DNA fragments by Methylcollector kit (Active Motif) were amplified using with HotStarTaq Polymerase (Qiagen) and included an initial incubation at 95 °C for 10 min, followed by 37 cycles of 95 °C for 1 min, 64 °C for 1 min and 72 °C for 1 min, followed by one cycle of 72 °C for

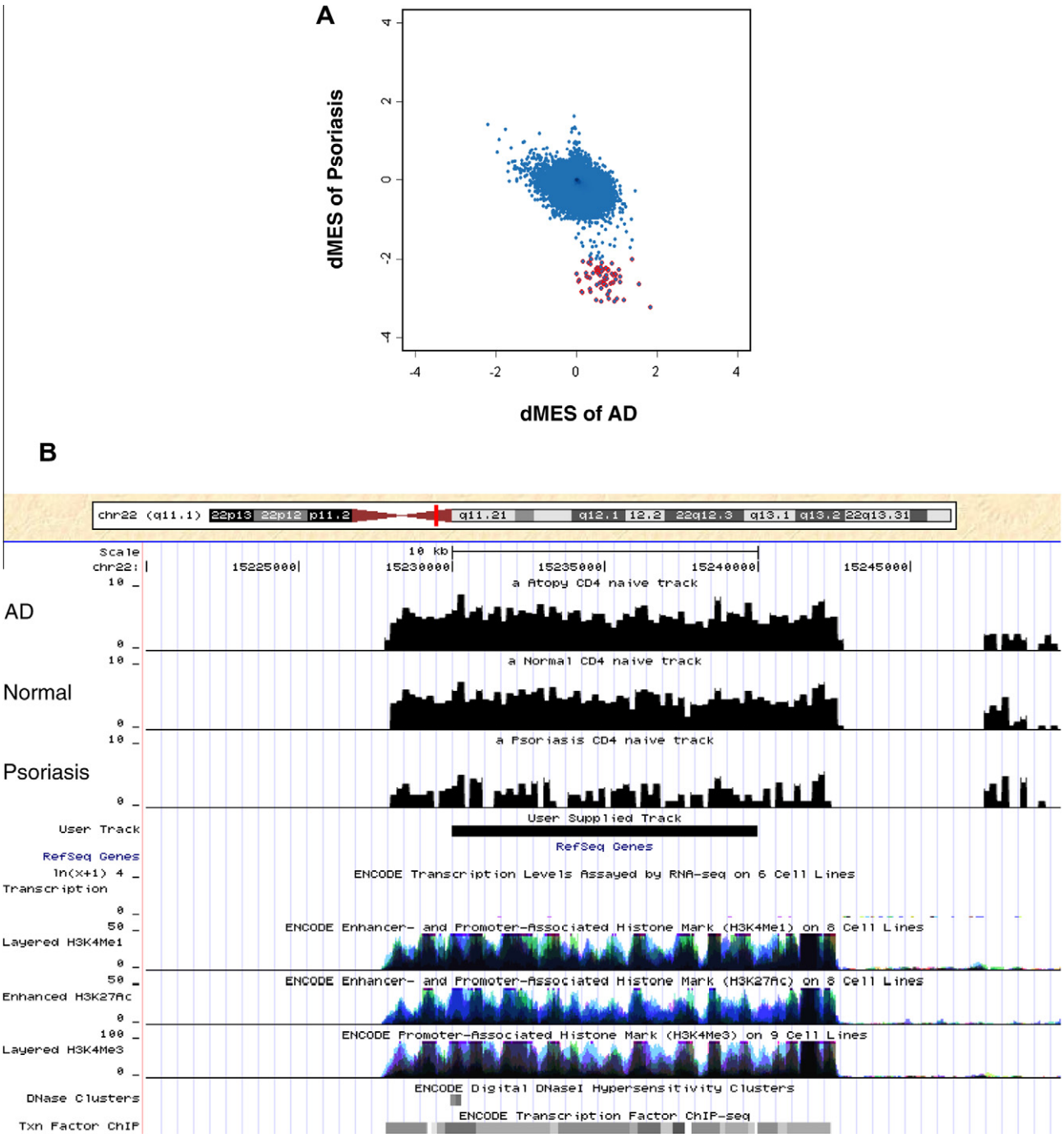

Fig. 1. Genome-wide methylation changes in 10-kb bins along each chromosome in naïve CD4+ T cells from psoriasis and AD patients and healthy controls. (A) Comparison of DNA methylation changes with dot plotting of averaged dMESs in 10-kb intervals along each chromosome from psoriasis and AD patients. dMESs of less than −2 in patients with psoriasis were presented by red circles. (B) A representative region shown by red circles in (a) using UCSC Genome Browser (ENCODE Regulation Super-track with hg18). The tracks for AD, normal, and psoriasis values, transformed by $\log_2$, are layered above the ENCODE data. The region detected by our analysis is given in 'User Supplied Track'.

10 min. The promoter of a gene was defined as the region of genomic DNA encompassing 1000 bp upstream and 600 bp downstream of the transcription start site. The DNA sequences were downloaded from the UCSC Genome Browser (version hg18). The primer sets for each gene were as follows: EMD: 5′-CCTCGCGTTGATGACGTT-3′, 5′-GGTGGTCAGCTCGGTATCC-3′, 342 bp; HDAC6: 5′-CGCTTCCTTGCTTGCTCT-3′, 5′-GTGCTGCGCCTACGTAAAA-3′, 181 bp; IKBKG: 5′-AGACGAGGGGGCGTGTAG-3′, 5′-CTTCCGGTGTCATAGCTGTGG-3′, 216 bp; ZIC3: 5′-AGTTCCCCGCAGCCACTC -3′, 5′-GCGAGTCCCAGCCTCTTC-3′, 318 bp; SLITRK4: 5′-TGCCGAGCGCTAGCAAT-3′, 5′-TCATGCCGCTGAGGACC-3′, 251 bp; CXorf40A: 5′-TTGCAGCCTGGACACTAGC-3′, 5′-CCAGCCCTCAGGTACCGT-3′, 281 bp; SH3KBP1: 5′-GCGAGACTCCTCGAGACCG-3′, 5′-AGGGCCACCTGCCATCA-3′, 188 bp; OTUD5: 5′-AACCCCGGGAGCTTGAC-3′, 5′-AGGCCCGCTACCAGGAC-3′, 271 bp; NDUFA1: 5′-CGAGATTCTCCCCGGACT-3′, 5′-CGCAGGCTGACGGTCTC-3′, 175 bp; WNK3: 5′-AGCAGACGCACGCGTACT-3′, 5′-TAGTTGGCTGGCCACTCG-3′, 175 bp; MSL3: 5′-CTGTGCTTCGAGCCTGACC-3′, 5′-CAAACGGTAGCCCCGGTAA-3′, 324 bp; CNKSR2: 5′-ACTTCGCGCGCCTCTGTT-3′, 5′-AAGCCCCGAGCACGACTAG-3′, 291 bp; MAMLD1: 5′-GCTCACCAGCTCGTTTTCC-3′, 5′-ACTCAGTGGAGGGT-

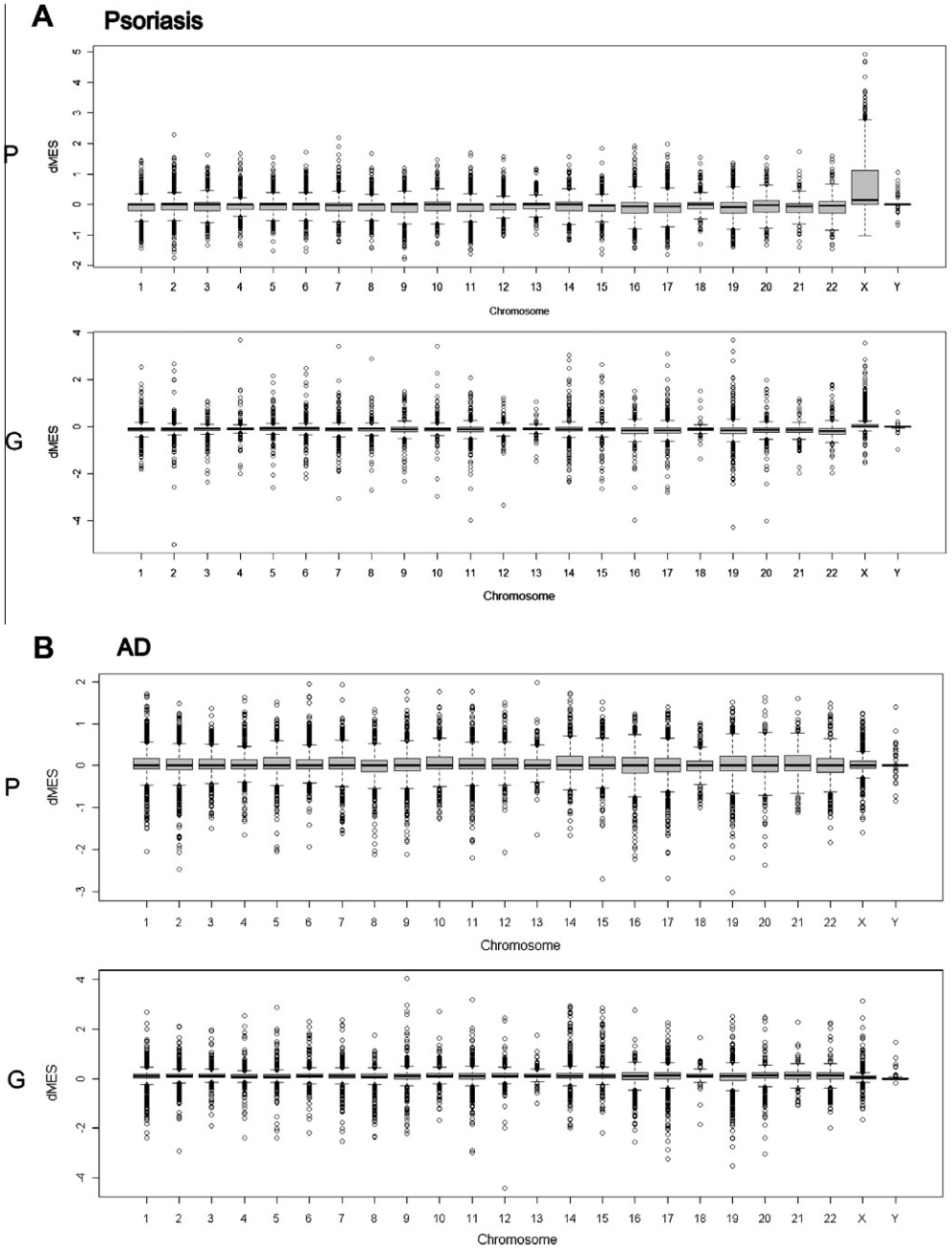

Fig. 2. DNA methylation changes in promoter and gene body regions summarized by chromosome in naïve CD4+ T cells from patients with psoriasis or AD. Distribution of dMESs for each promoter and gene body site is presented as box plots. A promoter was defined as the region of genomic DNA from −1000 to 600 bp relative to the transcription start site. P: Promoter, G: Gene body.

GGTCTGT-3′, 202 bp; SOX3: 5′-TTCACAGTCTGCCTGGGCTCTA-3′, 5′-AACGGGGGCTCGGTAATGAT-3′, 151 bp; GPC4: 5′-GACGCGTTCC-CACCTTT-3′, 5′-AAACCCCGAGGTCACCA-3′, 277 bp; SCML1: 5′-AG-CGCGCGAGTCTCTGAAA-3′, 5′-GTTGATCGGCCAATCCGC-3′, 242 bp. PCR products were separated on 1.5% agarose gels and visualized by ETBR staining.

2.7. Bioinformatic analysis and gene selection

DAVID Functional Annotation Clustering tool [12] (http://david.abcc.ncifcrf.gov) was used for functional annotation of the selected gene list. The Gene Ontology information was downloaded from its web server (http://www.geneontology.org). Statistical analysis was done with the R statistical package (http://www.r-project.org).

2.8. Data access

The raw Solexa sequencing reads and MES scores along the genomic coordinates have been deposited in the NCBI Gene Expression Omnibus (GEO) (http://www.ncbi.nih.gov/geo) under accession number GSE35582.

3. Results

3.1. Global changes in DNA methylation in naïve CD4+ T cells from patients with psoriasis or AD

To determine whether naïve CD4+ T cells from patients with psoriasis or AD have different DNA methylation patterns than

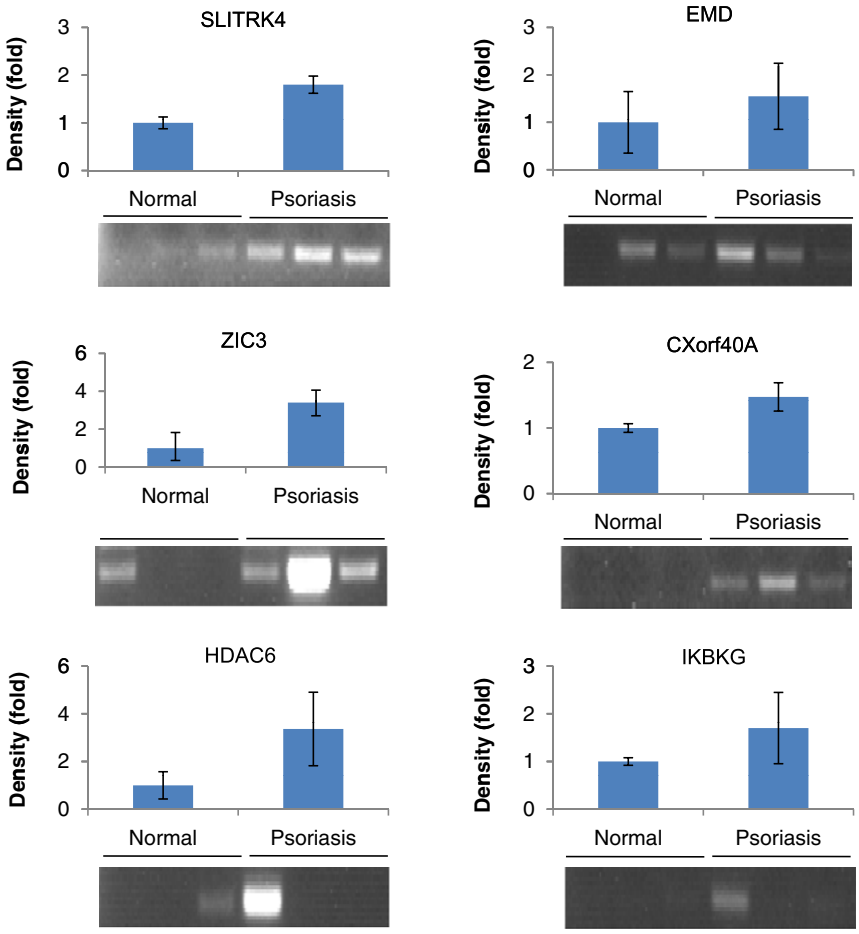

Fig. 3. Identification of the status of hypermethylated genes in patients with psoriasis. The DNA in naïve CD4+ T cells of three patients with psoriasis and three healthy controls were used for experiment. Densitometric analysis of PCR data (integrated density) was performed using Multigauge v3.0 software (Fujifilm).

those from healthy controls, we performed comprehensive CpG methylation profiling of whole genomes. Genomic DNA samples isolated from naïve CD4+ T cells were separately pooled from patients with psoriasis or AD, and from healthy controls. To measure the enrichment of local methylation signals, methylation enrichment scores (MESs) were calculated (see Section 2 for definition). Sequence reads and MESs followed Poisson distributions, but differential MES (dMES) signal distributions followed a normal distribution [13]. dMESs were calculated by subtracting the MESs of healthy controls from those of the two patient groups (see Section 2 for definition).

To evaluate genome-wide changes in DNA methylation in naïve CD4+ T cells from patients with psoriasis or AD, we averaged dMESs in 10-kb bins along each chromosome (Fig. 1). While most bins had dMESs ranging from −2 to +2 in both diseases, a set of 68 bins was distinctively clustered with dMESs that were markedly lower in psoriasis patients (dMES <−2, Red dots), but not in AD patients (Fig. 1A). By concatenating the contiguous bins, 26 unique regions ranging in size from 10 to 70 kb were cataloged (Supplementary Table 2). All but one of these regions were pericentromeric on 10 different chromosomes. Pericentromeric regions contain repetitive elements [14]; therefore, mapping short reads in these regions should be done with caution. We discarded all reads that were mapped ambiguously, and retained only the uniquely-mapped reads. The same analytical pipeline was applied to the three different samples, and only the sample from psoriasis patients showed distinct patterns. To understand the epigenomic significance, we visually examined the data from the ENCODE project [15] using the UCSC Genome Browser [16] (ENCODE Regulation Super-track with hg18). Interestingly, the uniquely-mapped regions coincided with strong histone modification signals, such as H3K4Me1, H3K27Ac, and H3K4Me3, as well as transcription factor binding sites in various types of cell lines (Fig. 1B and Supplementary Fig. 1).

Histone modifications in psoriasis patients have been reported only recently [17], and further study will be necessary to determine whether the DNA methylation changes are accompanied by changes in histone modification in these regions. This differential DNA methylation in pericentromeric regions is not new; similar differences have been identified between germ cells and somatic cells [18]. Hypomethylation in some pericentromeric regions of naïve CD4+ T cells may be a hallmark of psoriasis, not AD. It is not yet clear what roles the epigenetic changes in these regions play in T cell development. Nevertheless, our data for the first time illustrate the importance of such changes in the development of immune-mediated skin diseases.

3.2. DNA methylation changes of promoter and gene body regions in naïve CD4+ T cells from patients with psoriasis or AD

DNA methylation changes in promoters and gene bodies have been reported in cancers and immune diseases [6,19–21]. We examined whether similar changes could be observed in naïve naïve CD4+ T cells from patients with psoriasis or AD. In this study, a promoter was defined as the region of genomic DNA from −1000 to 600 bp relative to the transcription start site. We calculated

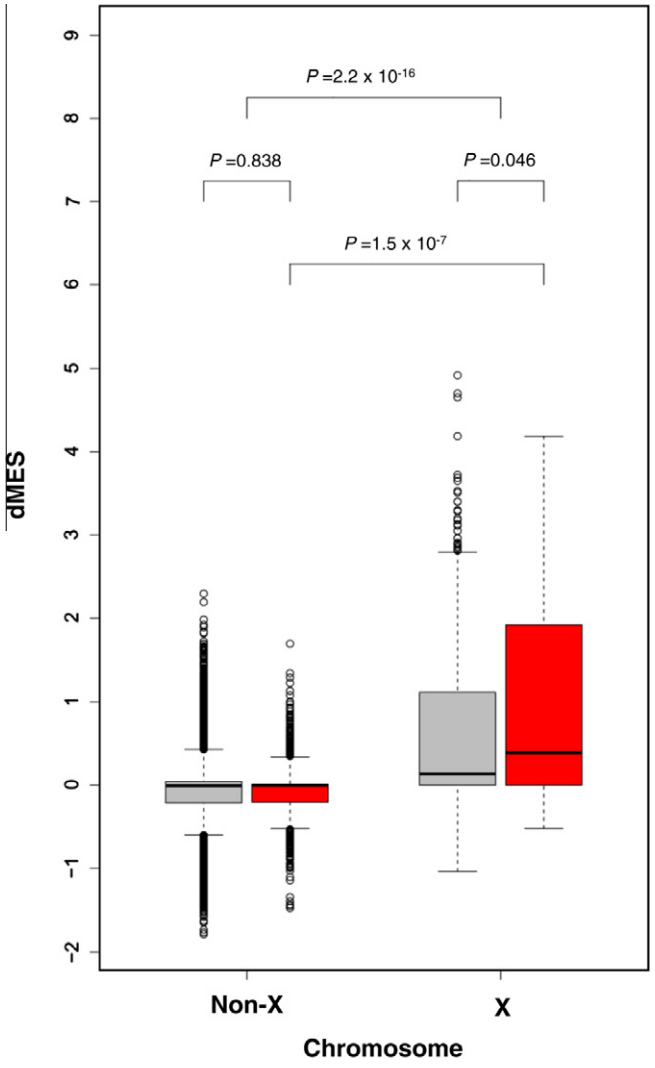

Fig. 4. Methylation patterns of immune-related genes on the non-X and X chromosomes, respectively, in psoriasis. Grey box show dMES of all gene promoters. Red box show dMES of immune-related gene promoters sorting by Gene Ontology (GO) immune system process term (GO: 0002376).

dMESs in gene promoters and bodies for both psoriasis and AD patients relative to healthy controls, and summarized them by chromosome (Fig. 2). Only the gene promoters on the X chromosome of psoriasis patients revealed dramatically-increased methylation levels (Fig. 2A) ($P < 2.2 \times 10^{-16}$). There were 124 genes having dMES greater than 2 (4-fold) in patients with psoriasis as compared to healthy controls (Supplementary Table 3). All of these promoters harbored a CpG island [16], and 121 of them were located on the X chromosome. To confirm this high throughput ChIP-seq result, we performed the methyl-specific PCR analysis for 16 genes that were randomly selected from the 121 X chromosome genes (SLITRK4, EMD, ZIC3, CXorf40A, HDAC6, IKBKG, SH3KBP1, OTUD5, NDUFA1, WNK3, MSL3, CNKSR2, MAMLD1, SOX3, GPC4, and SCML1). The individual methylation status was profiled in both three psoriasis patients and three controls that were randomly chosen for each gene. The promoters of the first 11 genes showed hypermethylated patterns in psoriasis patients. Six of them are shown in Fig. 3. Most genes were hypermethylated in all three patients with psoriasis, while some genes such as HDAC6 and IKBKG had hypermethylated promoter in the only one patient.

The functional enrichment analyses using DAVID [12] did not show any significant Gene Ontology connections among these 121 genes (data not shown). As the promoters of these genes may be under epigenetic regulation, we looked for enriched transcription-factor binding sites using DAVID. The binding sites of CDPCR3, GATA3, BRN2, and other transcription factors were identified as marginally enriched ($p \leqslant 10^{-3}$ after Benjamini-Hochberg correction). Although these results demonstrate the intriguing differences between psoriasis and AD in that many genes on the X chromosome are hypermethylated in their promoters only in psoriasis patients, not in AD patients, further studies may be needed to understand their functional implication in the pathogenesis of these diseases.

3.3. DNA methylation changes in the promoter regions of immune-related genes

It is well known that X chromosome encodes many immune-related genes and plays an important role in the pathogenesis of autoimmune diseases [22]. We wondered whether those immune-related genes on the X chromosome displayed DNA methylation patterns different from those of the non-immune genes on the same chromosome. Since many genes on the X chromosome showed distinctively hypermethylated patterns in psoriasis, those immune-related genes may also follow the similar pattern. This is *aka* 'hitchhiking' effect in that immune-related genes are not particularly hypermethylated but follow the general hypermethylation of the genes on the X chromosome. The 'hitchhiking' effect does not preclude the role of immune-related genes on the X chromosome in the pathogenesis of autoimmune diseases. On the other hand, if those immune-related genes show even higher hypermethylated pattern than other genes on the X chromosome, it would strongly argue that the immune-related genes on the X chromosome may indeed play important roles in psoriasis development. This argument may also be corroborated by comparing the dMES patterns of immune-related genes on the X chromosome to the immune-related genes in all other chromosomes. We cataloged 1787 immune-related genes from the Gene Ontology server ("immune system" process term (GO: 0002376)) [23]. As shown in Fig. 4, the hypermethylation of those immune-related genes on the X chromosome was distinctively higher than that of the non-immune genes in the same chromosome (Fig. 4 right panel, $p = 0.046$). Such a hypermethylation pattern was not observed in other chromosomes (Fig. 4 left panel, $p = 0.84$). Moreover, compared to the immune-related genes in other chromosomes, those on the X chromosome were remarkably hypermethylated ($p = 1.5 \times 10^{-7}$). This result imply that the associated methylation changes on the X chromosome can partly account for the developments in naïve CD4+ T cells of patients with psoriasis.

4. Discussion

We have performed the genome-wide analysis of DNA methylation in naïve CD4+ T cells from patients with psoriasis and AD. Compare to previous study [24,25], our results provide detailed methylation analysis of psoriasis and AD. We found that the pattern of DNA methylation has increased dramatically in the promoter region of genes on the X chromosome in patients with psoriasis. The X chromosome encodes many immune-related genes [22]. The immune-related genes on the X chromosome showed higher hypermethylation pattern than other genes. According to recently studies, the X chromosome-associated anomalies such as X chromosome inactivation can contribute to disorders in self recognition and eventually to autoimmunity [22]. In addition, DNA methylation is involved in the initiation of X chromosome

inactivation as well as in the stable maintenance of the silenced state [26]. These studies suggest that DNA methylation may affect the expression of gene on the X chromosome or T cell development in psoriasis.

Previous studies on psoriasis and AD have not considered the presence of epigenomic changes in naïve CD4+ T cells. Our findings of epigenomic changes in these T cells imply that naïve CD4+ T cells may be predisposed to inducing either psoriasis or AD prior to encountering antigens. This may be due to differential environmental exposure. Psoriasis and AD are affected by environmental exposure, which may result in changes in DNA methylation. Thus, further epigenomic examination of naïve CD4+ T cells is needed to understand T cell polarization in psoriasis and AD, as well as in other T cell-mediated diseases. The information from this study of changes in global DNA methylation patterns, as well as in promoters and gene bodies of naïve CD4+ T cells provides new insights into the mechanism of T cell polarization and the development of psoriasis and AD.

Acknowledgments

This work was supported by grants from the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (No. 20110027837 & 20100021811) and the Next-Generation BioGreen 21 Program (No. PJ007991), Rural Development Administration, Korea.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.128.

References

[1] F.O. Nestle, D.H. Kaplan, J. Barker, Psoriasis, N. Engl. J. Med. 361 (2009) 496–509.
[2] T. Bieber, Atopic dermatitis, N. Engl. J. Med. 358 (2008) 1483–1494.
[3] K.M. Ansel, D.U. Lee, A. Rao, An epigenetic view of helper T cell differentiation, Nat. Immunol. 4 (2003) 616–623.
[4] P.P. Lee, D.R. Fitzpatrick, C. Beard, H.K. Jessup, S. Lehar, K.W. Makar, M. Pérez-Melgosa, M.T. Sweetser, M.S. Schlissel, S. Nguyen, S.R. Cherry, J.H. Tsai, S.M. Tucker, W.M. Weaver, A. Kelso, R. Jaenisch, C.B. Wilson, A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival, Immunity 15 (2001) 763–774.
[5] E. Balada, J. Ordi-Ros, S. Serrano-Acedo, L. Martinez-Lostao, M. Rosa-Leyva, M. Vilardell-Tarrés, Transcript levels of DNA methyltransferases DNMT1, DNMT3A and DNMT3B in CD4+ T cells from patients with systemic lupus erythematosus, Immunology 124 (2008) 339–347.
[6] W. Lei, Y. Luo, W. Lei, Y. Luo, K. Yan, S. Zhao, Y. Li, X. Qiu, Y. Zhou, H. Long, M. Zhao, Y. Liang, Y. Su, Q. Lu, Abnormal DNA methylation in CD4+ T cells from patients with systemic lupus erythematosus, systemic sclerosis, and dermatomyositis, Scand. J. Rheumatol. 38 (2009) 369–374.
[7] M. Zhao, F. Gao, X. Wu, J. Tang, Q. Lu, Abnormal DNA methylation in peripheral blood mononuclear cells from patients with vitiligo, Br. J. Dermatol. 163 (2010) 736–742.
[8] R. Jaenisch, A. Bird, Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals, Nat. Genet. 33 (2003) 245–254.
[9] R. Singal, G.D. Ginder, DNA methylation, Blood 93 (1999) 4059–4070.
[10] K.W. Makar, C.B. Wilson, DNA methylation is a nonredundant repressor of the Th2 effector program, J. Immunol. 173 (2004) 4402–4406.
[11] J.M. Schwarz, B.M. Nugent, M.M. McCarthy, Developmental and hormone-induced epigenetic changes to estrogen and progesterone receptor genes in brain are dynamic across the life span, Endocrinology 151 (2010) 4871–4881.
[12] W. Huang da, B.T. Sherman, R.A. Lempicki, Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources, Nat. Protoc. 4 (2009) 44–57.
[13] S. Anders, W. Huber, Differential expression analysis for sequence count data, Genome Biol. 11 (2010) R106.
[14] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, et al., Initial sequencing and analysis of the human genome, Nature 409 (2001) 860–921.
[15] E. Birney, J.A. Stamatoyannopoulos, A. Dutta, R. Guigó, T.R. Gingeras, E.H. Margulies, et al., Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project, Nature 447 (2007) 799–816.
[16] W.J. Kent, C.W. Sugnet, T.S. Furey, K.M. Roskin, T.H. Pringle, A.M. Zahler, D. Haussler, The human genome browser at UCSC, Genome Res. 12 (2002) 996–1006.
[17] P. Zhang, Y. Su, M. Zhao, W. Huang, Q. Lu, Abnormal histone modifications in PBMCs from patients with psoriasis vulgaris, Eur. J. Dermatol. 21 (2011) 552–557.
[18] K. Yamagata, T. Yamazaki, H. Miki, N. Ogonuki, K. Inoue, A. Ogura, T. Baba, Centromeric DNA hypomethylation as an epigenetic signature discriminates between germ and somatic cell lineages, Dev. Biol. 312 (2007) 419–426.
[19] P.A. Jones, S.B. Baylin, The fundamental role of epigenetic events in cancer, Nat. Rev. Genet. 3 (2002) 415–428.
[20] Y. Koga, M. Pelizzola, E. Cheng, M. Krauthammer, M. Sznol, S. Ariyan, D. Narayan, A.M. Molinaro, R. Halaban, S.M. Weissman, Genome-wide screen of promoter methylation identifies novel markers in melanoma, Genome Res. 19 (2009) 1462–1470.
[21] N.H. Kwon, J.S. Kim, J.Y. Lee, M.J. Oh, D.C. Choi, DNA methylation and the expression of IL-4 and IFN-gamma promoter genes in patients with bronchial asthma, J. Clin. Immunol. 28 (2008) 139–146.
[22] C. Libert, L. Dejager, I. Pinheiro, The X chromosome in immune functions: when a chromosome makes the difference, Nat Rev Immunol. 10 (2010) 594–604.
[23] A.D. Diehl, J.A. Lee, R.H. Scheuermann, J.A. Blake, Ontology development for biological systems: immunology, Bioinformatics. 23 (2007) 913–915.
[24] P. Zhang, Y. Su, H. Chen, M. Zhao, Q. Lu, Abnormal DNA methylation in skin lesions and PBMCs of patients with psoriasis vulgaris, J Dermatol Sci. 60 (2010) 40–42.
[25] T. Nakamura, I. Sekigawa, H. Ogasawara, K. Mitsuishi, K. Hira, S. Ikeda, H. Ogawa, Expression of DNMT-1 in patients with atopic dermatitis, Arch Dermatol Res. 298 (2006) 253–256.
[26] D.C. Kaslow, B.R. Migeon, DNA methylation stabilizes X chromosome inactivation in eutherians but not in marsupials: evidence for multistep maintenance of mammalian X dosage compensation, Proc. Natl. Acad. Sci. USA 84 (1987) 6210–6214.