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ORIGINAL PAPER

DNA methylation analysis of CD4+ T cells in patients with psoriasis

Geon Tae Park · Jihye Han · Sin-Gi Park · Sangsoo Kim · Tae-Yoon Kim

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Abstract Psoriasis is a chronic inflammatory skin disease that is characterized by aberrant cross-talk between keratinocytes and immune cells such as CD4+ T cells, resulting in keratinocyte hyperproliferation in the epidermis. DNA methylation, one of several epigenetic mechanisms, plays an important role in gene expression without changing the DNA sequence. Several studies have suggested the involvement of epigenetic regulation in skin lesions from patients with psoriasis. In this study, we investigated the genome-wide DNA methylation status of CD4+ T cells in patients with psoriasis compared with healthy subjects using methylated DNA immunoprecipitation sequencing (MeDIP-Seq). The results of MeDIP-Seq showed that the global methylation values of CD4+ T cells are higher in patients with psoriasis than in healthy controls, particularly in the promoter regions. Among the most hypermethylated genes in the promoter regions, we selected the genes whose expression is significantly reduced in the CD4+ T cells of psoriasis patients. Studies using the methylation inhibitor 5-azacytidine in vitro methylation assays have shown that the differential expression levels were associated with the methylation status of each gene. Bisulfite sequencing of the transcription start region of phosphatidic acid phosphatase type 2 domain containing 3 (*PPAPDC3*), one of the selected genes, showed hypermethylation in the CD4+ T cells of psoriasis patients. These results suggested that the methylation status, which is identified by MeDIP-Seq of the genes, was correlated with the mRNA expression level of the genes. Collectively, the DNA methylation status in CD4+ T cells might be associated with the pathogenesis of psoriasis.

Keywords Psoriasis · CD4+ T cells · DNA methylation · MeDIP-Seq

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G. T. Park · J. Han · T.-Y. Kim (✉)
Department of Dermatology, College of Medicine, The Catholic University of Korea, Seoul 137-040, South Korea
e-mail: tykimder@catholic.ac.kr

S.-G. Park · S. Kim
Department of Bioinformatics and Life Sciences, Soogsil University, Seoul 511, South Korea

Introduction

Psoriasis is a chronic inflammatory skin disease associated with aberrant interactions between keratinocytes and the immune system [13, 17, 26]. Psoriasis is primarily characterized by hyperproliferation and abnormal differentiation of keratinocytes, and the overactive immune responses are also one of the pathologic causes of psoriasis [10]. The main mechanisms of psoriasis development remain poorly understood, but many results have suggested that both genetic and environmental issues may be involved in the development of psoriasis [12, 22]. Environmental factors associated with the onset of psoriasis include various infections, psychological stress, injuries, prescription drug use such as non-steroidal anti-inflammatory agents, and excessive alcohol consumption [7, 9].

DNA methylation is one of several epigenetic mechanisms of gene expression during developmental and cellular processes such as X-chromosome inactivation and parental imprinting [20]. In mammals, DNA methylation occurs predominantly at the 5′-carbon position of cytosine residues within CpG pairs that are sparsely distributed in the genome [2]. Generally, CpG methylation, particularly when occurring within the promoters of genes, can lead to gene

silencing, a feature found in several human cancers. For CD4+ T cells, much has been reported about the involvement of epigenetic regulation in differentiation and lineage commitment [25]. Because the pathological causes of skin inflammatory diseases such as psoriasis are deeply related to improper immune responses, the epigenetic regulation of CD4+ T cell polarization may be an important factor for the occurrence of psoriasis. Genome-wide histone methylation maps in CD4+ T cells and polarized T cells have been reported [21]. The expression of T helper type 2 (Th2) cytokines such as interleukin (IL)-4 was shown to be regulated by DNA methyltransferase-1 in CD4+ T cells [15]. Previously, we reported that the methylation changes in naive CD4+ T cells of patients with psoriasis were strongly associated with the pathogenesis of psoriasis [11]. These data suggested that modulation of the epigenetic status on DNA or histones of CD4+ T cells could lead to changes in cytokine production and differentiation.

Studies have investigated global DNA methylation in psoriasis [19, 30, 31]. They analyzed the methylation status of genes in skin samples from lesions and non-lesional skin of patients with psoriasis and healthy controls. And there is a brief report about the global DNA methylation analysis using the peripheral blood samples of psoriasis patients [29]. However, despite the importance of immune cells in the pathology of psoriasis, the methylation changes in total CD4+ T cells of patients with psoriasis have not been well studied. In this study, we investigated the methylation differences of the genome-wide methylation status in total CD4+ T cells between psoriasis patients and healthy controls using MeDIP-Seq. Among the most hypermethylated genes in the promoter regions in psoriasis patients, we identified four genes whose expression was significantly decreased in total CD4+ T cells of psoriasis patients compared with that in healthy controls. The association between methylation status and gene expression of the genes was assessed by methylation inhibition experiments and bisulfite sequencing.

Materials and methods

Subjects

Peripheral blood samples were obtained from 15 patients with plaque-type psoriasis and 11 healthy controls. The restricted conditions of the patients were applied as described previously [11]. Detailed information of the subjects included in the study is listed in Online resource 1. For controls, healthy subjects who had no history of psoriasis, atopic dermatitis, allergic rhinitis, and asthma were enrolled. This study was approved by the Catholic University of Korea, College of Medicine, and written informed consent was obtained from all participants according to the Declaration of Helsinki.

Isolation of total CD4+ T cells

Total CD4+ T cells were obtained from peripheral blood mononuclear cells (PBMCs) of patients and controls and were isolated by Ficoll-PaqueTM PLUS (GE Healthcare Bio-Science, Uppsala, Sweden) density gradient centrifugation using a total CD4+ T cell isolation kit (Miltenyl Biotec, Bergisch Gladbach, Germany).

Preparation of affinity-purified methylated DNA for Solexa sequencing

The entire process for preparation of methylated genomic DNA and Solexa sequencing was performed as described previously [11]. Briefly, genomic DNA was isolated from total CD4+ T cells using a QIAamp® DNA mini blood kit (Qiagen, Hilden, Germany), and then each set of samples was pooled. The DNA was fragmented using the *MseI* (NEB, Beverley, MA, USA) endonuclease. The methylated DNA was immunoprecipitated with the glutathione *S*-transferase-conjugated methyl-CpG-binding domain. The precipitated DNA was ligated to a pair of Solexa adaptors for Illuminar Genome Analyzer sequencing and amplified by polymerase chain reaction (PCR). Cluster generation and sequencing were performed after elution from 175- to 225-bp fragments of amplified methylated genomic DNA. The sequence tags were mapped to the human genome using 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).

RNA isolation and real-time quantitative reverse transcription (RT)-PCR

Total RNA was isolated from total CD4+ cells using the RNeasy Mini Kit (Qiagen), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR was performed using a Rotor-Gene 3000 (Qiagen) and the KAPA SYBR fast qPCR Kit (KAPA Biosystems, Boston, MA, USA). The results were normalized to expression of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. The PCR conditions were as follows: one cycle at 95 °C for 5 min followed by 50 cycles at 96 °C for 20 s, 55 °C for 20 s and 72 °C for 20 s, and ending with one cycle at 72 °C for 5 min. Primers used in this experiment were as follows (forward and reverse, respectively): *PPAPDC3*: 5′-AGGTGCTC ATGAATCTGCTC-3′ and 5′-GGAAGGCGTAGA TGT CCATG-3′; DelaN p73 (*TP73*): 5′-GTTTACAAGA AAGCGGAGCAC-3′ and 5′-GTCA TCCACATAC

TGCGAGAG-3′; cation channel, sperm associated 2 (*CATSPER2*): 5′-ACCT CCAAGGTCAGCTTCAA-3′ and 5′-GGGTCCTTGAG TGTCCTCTCT-3′; fibronectin type III and ankyrin repeat domains 1 (*FANK1*): 5′-AG-TGGTTCAGGTTCTC GATTG-3′ and 5′-TGACCTT-CAGGCGAAATCTG-3′.

5-Azacytidine treatment of total CD4+ T cells

Total CD4+ T cells purified from the patients were cultured with RPMI medium supplemented with 10 % fetal bovine serum and 100 U/ml of IL-2. The cultured cells were incubated in the medium in the presence or absence of 10 μM 5-azacytidine, a DNA methyltransferase inhibitor. After 24 or 48 h, the cells were harvested and subjected to quantitative real-time PCR for each gene.

Cell transfection and luciferase analysis

Each promoter region of the selected genes was amplified by PCR. The DNA fragments were ligated using the pGL3-basic luciferase plasmids (Promega, Madison, WI, USA). Serially deleted promoters of each gene were constructed by PCR using specific primers. Each construct was transfected into Jurkat T cells, human immortalized T lymphocytes, using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). Cells were harvested 48 h after transfection, and luciferase activity was measured using the Dual luciferase assay kit (Promega). Transfection efficiency was normalized by *Renilla* luciferase activity.

In vitro methylation assay

The promoter constructs of *PPAPDC3* (−228) and *TP73* (−184), as well as empty vectors, were methylated using CpG methyltransferase (*M. SssI*) and *S*-adenosyl methionine (SAM; NEB) at 37 °C for 16 h. Control methylation was performed in the absence of *M. SssI* and SAM. The methylation status of each construct was determined using the methylation-sensitive restriction enzyme *NotI* (NEB). The methylated constructs were transfected into Jurkat T cells, and the promoter activity was measured by luciferase analysis.

Bisulfite sequencing

Genomic DNA was isolated as described above, and bisulfite conversion was performed using the EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's protocol. The corresponding promoter region from +100 to −100 around the transcription start site of *PPAPDC3* was amplified by PCR. The fragment was cloned into pLPS-T vectors (ELPIS, Daejeon, Korea), and ten independent clones from each of five patients and five normal controls were sequenced for the amplified fragments. The following primers were used (forward and reverse, respectively): *PPAPDC3* BS: 5′-GGTTTAGAGGGAAGTTGGTAAA AG-3′ and 5′-TCTACCACCCAACTCCAAAAA A-3′.

Statistical analysis

Data are presented as mean values ± standard deviation. Unpaired Student's *t* test was used to compare values. The *p* values less than 0.05 were considered to be statistically significant. Statistical analysis for Solexa sequencing reads and MES scores was performed as described previously [11].

Results

Genome-wide analysis of total CD4+ T cells in patients with psoriasis

To understand the epigenetic changes in CD4+ T cells from patients with psoriasis, we performed MeDIP-Seq analysis. Total CD4+ T cells were collected from 12 patients with psoriasis and 10 normal control volunteers. Genomic DNA pooled by each set of samples was used for whole genome DNA methylation profiling. Local methylation enrichment, which was calculated using methylation enrichment scores (MESs) [1, 11], was measured for each sample set, and differential MESs (dMESs) were calculated by subtracting the MESs of normal controls from those of psoriasis patients.

To evaluate the genome-wide differences in DNA methylation of total CD4+ T cells from those of patients with psoriasis, we calculated the averaged dMESs in gene promoters and bodies for psoriasis patients relative to normal controls, summarized them by chromosome, and finally displayed them as box and scattered plots (Figs. 1, 2). The figures showed that the methylation status in all chromosomes trended toward hypermethylation in psoriasis patients compared with normal controls in the promoter region; however, in gene body regions, the methylation status barely changed. In addition, it was also found that the unique regions ranging from 10 to 70 kb in size with dMESs were markedly higher in 10-kb intervals along each chromosome in psoriasis patients than in normal controls (Online Resource 2).

Expression analysis of hypermethylated genes in the promoter region

Using genome-wide analysis, we listed the most hypermethylated genes in the promoter region (Table 1). Generally, because expression of the hypermethylated genes in

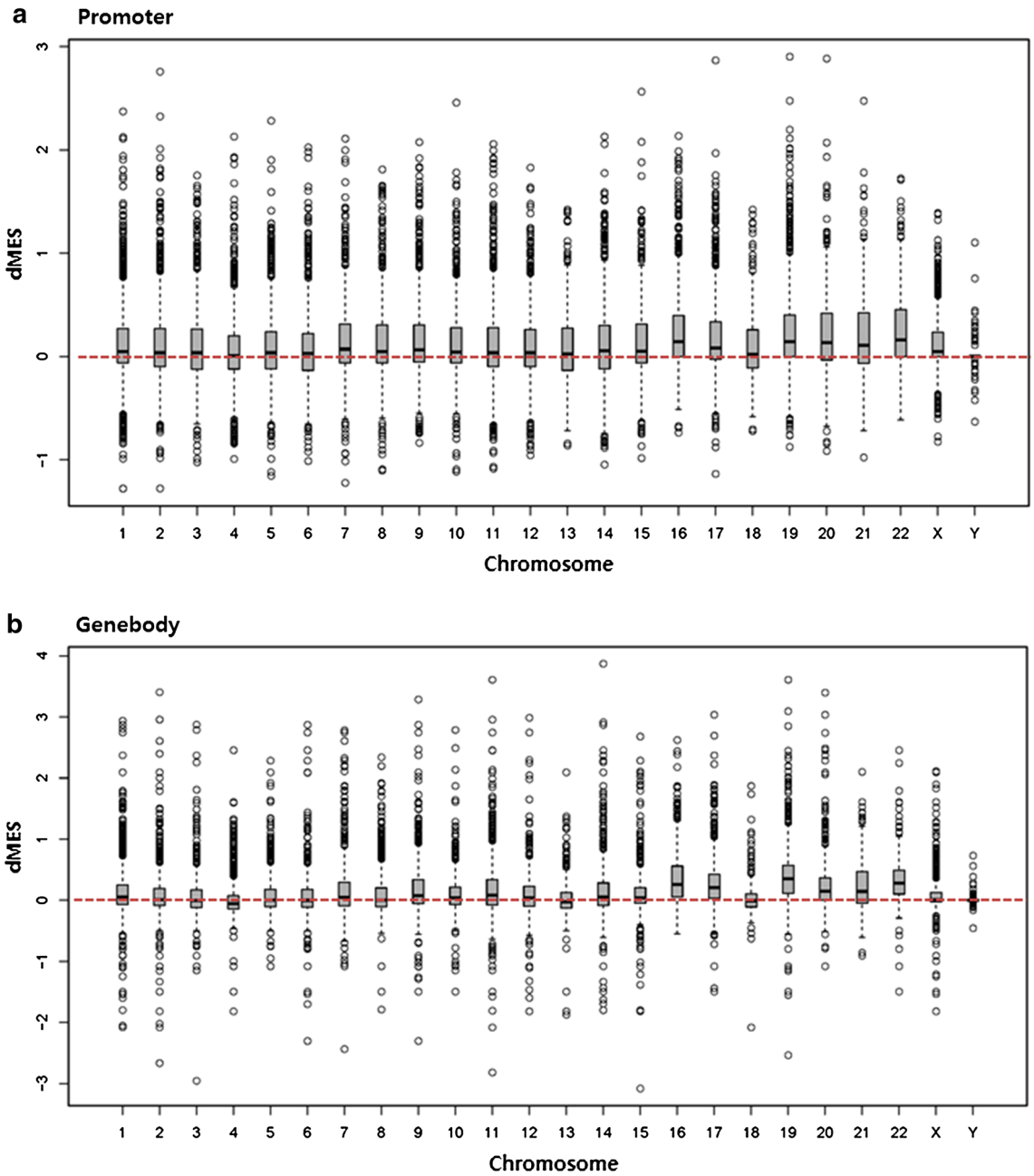

Fig. 1 DNA methylation changes summarized by chromosomes in total CD4+ T cells in **a** the promoter and **b** gene body from psoriasis patients and normal control subjects. The distribution of dMESs for each promoter and gene body region is presented as *box plots*. *Box plots* display differences between populations without statistical distribution. Each figure shows the dMES values (on the *y-axis*) of genes in each chromosome (on the *x-axis*). The more distribution of the *circles* above the 0 line (*red dotted line*), the more methylated genes located in the chromosome in CD4+ T cells from psoriasis patients. A promoter was defined as the region of genomic DNA from −1,000 to +600 bp relative to the transcription start site

the promoter region is negatively regulated, we performed quantitative real time RT-PCR using total RNA isolated from CD4+ T cells of nine patients with psoriasis and eight normal controls to select the genes that were significantly down-regulated in the CD4+ T cells in patients with psoriasis. After analysis of the gene expression levels of the selected genes, we classified three genes—*PPAPDC3*, *TP73*, and *FANK1*—as being significantly down-regulated (Fig. 3a). *CATSPER2* also had a tendency toward down-regulated, although the trend was not statistically significant. To verify the association between DNA methylation of the promoter regions and suppressed gene expression of the four genes, the CD4+ T cells purified from patients with psoriasis were cultured with 5-azacytidine, a DNA

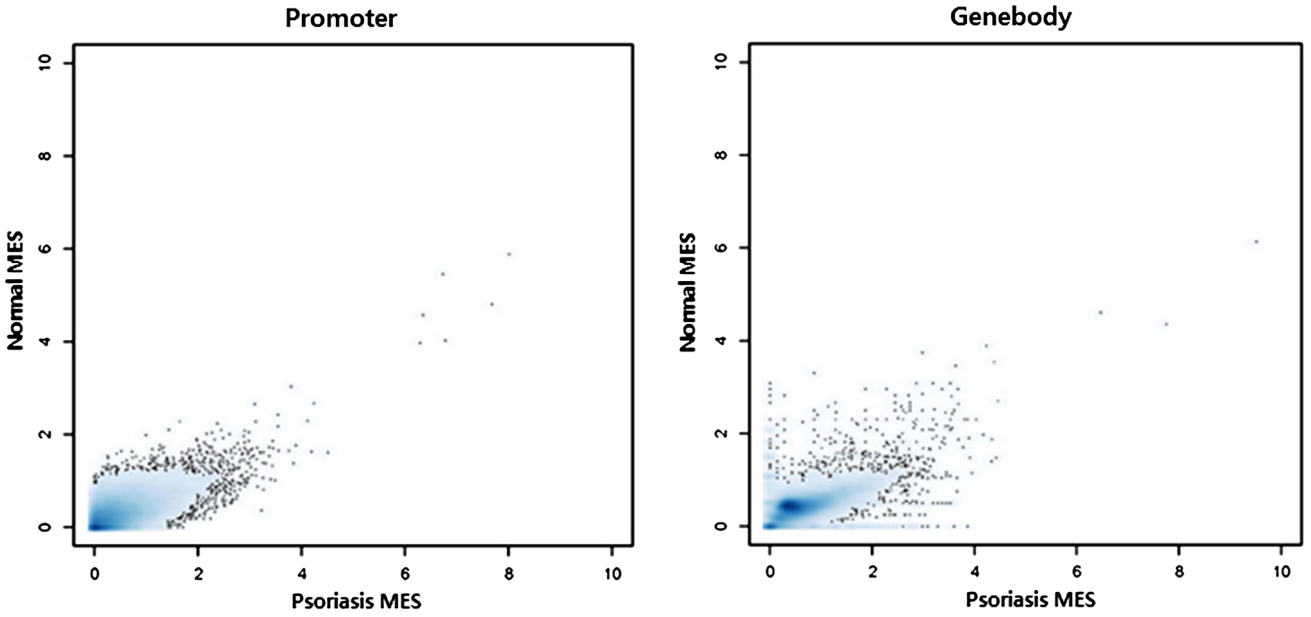

Fig. 2 Scattered plotting of averaged MESs for the promoter (*left panel*) and gene body (*right panel*) in total CD4+ T cells from patients and normal controls. Each figure shows a scatter plot of the averaged MES values calculated from samples of psoriasis patients (on the *x-axis*) and normal controls (on the *y-axis*). The plotting pattern shows the shift in MES values toward to psoriasis patients in the promoter region. A promoter was defined as the region of genomic DNA from −1,000 to +600 bp relative to the transcription start site

methyltransferase inhibitor. As shown in Fig. 3b, expression levels of all four genes were increased after 24- and 48-h incubation with 5-azacytidine. These results suggested that the decreased gene expression of the four genes, including *CATSPER2*, in CD4+ T cells from psoriasis patients closely correlated with the hypermethylated score values of the promoter regions of the genes.

In vitro methylation analysis of the selected genes to demonstrate the effect of methylation on gene expression

According to the less significant gene expression result of *CASPERS2* (Fig. 3a) and in vitro methylation assay of *FANK1* (Online Resource 3), we focused on the analyses of *PPAPDC3* and *TP73*. Next, to verify the involvement of DNA methylation on gene expression, we cloned the promoter regions of the *PPAPDC3* and *TP73* to the luciferase reporter pGL3-basic. To determine whether these recombinant pGL3-luciferase vectors defined active promoters, we transiently transfected them into Jurkat T cells using the empty vector and a *Renilla* construct as controls. Compared with the basal activity of the empty vector, the promoter activities of *PPAPDC3* and *TP73* were higher in Jurkat T cells (Fig. 4, *left panels*). We also tested the promoter activity of several constructs that have serially deleted promoters. The minimal regions of the *PPAPDC3* and *TP73* promoters with considerable activities resided from nucleotide −228 to +167 and −438 to +53 from the transcription start sites of *PPAPDC3* and *TP73*, respectively (Fig. 4a, b).

Using these minimal constructs, we conducted in vitro methylation experiments to determine the effect of DNA methylation on promoter activity. The minimal constructs and the empty vector were methylated by *M. SssI* and transfected into Jurkat T cells. As shown in Fig. 4a, b (*right panels*), the promoter activities displayed by the methylated constructs were significantly suppressed compared with those of the unmethylated constructs. The same experiments for the *CATSPER2* gene also showed similar results, but *FANK1* showed a reverse result (Online Resource 3).

Validation of the methylated DNA by direct bisulfite sequencing

Bisulfite sequencing allows methylation analysis of the CpG sites in a certain region of a promoter. We performed cloning-based sequencing for bisulfite sequencing to validate the reliability of the MeDIP-Seq and gene expression analysis. *PPAPDC3* and *TP73* were analyzed for methylation validation in total CD4+ T cells from patients with psoriasis. Based on comparison of the dMES values with 200-bp intervals in the promoter region of *PPAPDC3*, we chose the region between nucleotides −100 to +100 of *PPAPDC3* for bisulfite sequencing. After PCR using specific primers followed by bisulfite conversion of genomic

Table 1 Top 35 hypermethylated genes in total CD4+ T cells of patient with psoriasis

Gene accession number	Symbol	Chrom	Start	End	Ave. normal MES	Ave. psoriasis MES	dMES
NM_000697	ALOX12	17	6840107	6854779	0.3630	3.2292	2.8661
NM_052997	ANKRD30A	10	37454790	37561501	0.8091	3.2653	2.4561
NM_019102	HOXA5	7	27147520	27149812	1.2659	3.6642	2.3982
NM_138568	EXOC3L2	19	50407718	50429309	1.0021	3.2869	2.2848
NM_199261	TPTE	21	9928613	10012791	1.3767	3.6241	2.2474
NM_001122772	AGAP2	12	56405260	56418296	1.2183	3.4406	2.2223
NM_145658	SPESP1	15	67009918	67026202	0.6596	2.8447	2.1850
NM_153046	TDRD9	14	1.03E+08	1.04E+08	1.7678	3.8949	2.1271
NM_002402	MEST	7	1.3E+08	1.3E+08	0.7597	2.8713	2.1118
NM_001136503	C19orf77	19	3425404	3431540	0.4880	2.5763	2.0882
NR_024569	LOC100130872	4	1179571	1192750	0.6127	2.6940	2.0813
NR_024627	KCNQ1DN	11	2847838	2849909	0.5937	2.6746	2.0810
NM_001085401	C6orf201	6	4024438	4075998	1.1449	3.1979	2.0530
NM_001126240	TP73	1	3597095	3640327	1.0488	3.0903	2.0415
NM_152771	C19orf34	19	1903525	1905548	1.0414	3.0802	2.0387
NM_001029955	DCAF4L1	4	41678469	41683241	0.9425	2.9808	2.0382
NR_027020	NCRNA00164	2	1.33E+08	1.33E+08	4.3572	6.3273	1.9701
NM_004732	KCNAB3	17	7766751	7773478	0.4877	2.4450	1.9573
NM_145235	FANK1	10	1.28E+08	1.28E+08	4.4831	6.4291	1.9460
NM_001012415	SOHLH1	9	1.38E+08	1.38E+08	0.7183	2.6563	1.9379
NM_001042690	C4orf44	4	3220564	3228140	1.3819	3.3099	1.9280
NM_001127464	ZNF469	16	87021379	87034666	0.4370	2.3539	1.9169
NM_003353	UCN	2	27383768	27384634	0.3746	2.2875	1.9129
NM_001362	DIO3	14	1.01E+08	1.01E+08	0.5616	2.4644	1.9028
NM_001143980	CCDC154	16	1424390	1434491	0.9060	2.7602	1.8542
NM_207320	OTUD6A	X	69199065	69200754	0.3746	2.2173	1.8426
NM_005325	HIST1H1A	6	26125238	26126019	0.5101	2.3519	1.8418
NM_001007533	DYSFIP1	17	77384656	77386215	0.4370	2.2722	1.8352
NM_032728	PPAPDC3	9	1.33E+08	1.33E+08	1.0451	2.8790	1.8338
NM_173853	KRTCAP3	2	27518736	27520667	0.9636	2.7812	1.8176
NM_177478	FTMT	5	1.21E+08	1.21E+08	0.9579	2.7705	1.8125
NM_054020	CATSPER2	15	41710063	41728331	0.6022	2.3873	1.7850
NM_012283	KCNG2	18	75724655	75760804	2.0522	3.8201	1.7679
NM_020185	DUSP22	6	237100	296355	0.8000	2.5608	1.7607
NM_138574	HDGFL1	6	22677656	22678729	0.7705	2.5111	1.7405

Chrom chromosome, *Ave* average

DNA samples from five patients with psoriasis and genomic DNA samples from five normal controls, each PCR product was cloned into a TA cloning vector. Ten individual positive clones from each sample were sequenced, and each CpG site was assessed for methylation status (Fig. 5a). Among the CpG sites in the region of *PPAPDC3*, four continuous CpG sites (+36, +60, +84, +98) were more hypermethylated in the samples from psoriasis patients than in normal controls (Fig. 5b). Although the differences in the percent methylation scores between psoriasis patients and normal samples were relatively small, the hypermethylation level was more significantly pronounced in psoriasis patient samples than in normal controls ($p < 0.05$, Fig. 5b). In case of TP73, we performed same as PPAPDC3 with several regions based on comparison of the dMES, however, we could not find the significant hypermethylated CpG sites.

Discussion

The importance of T-cell responses in psoriasis pathology is highly significant, and signaling molecules for T-cell activation have been considered targets for psoriasis

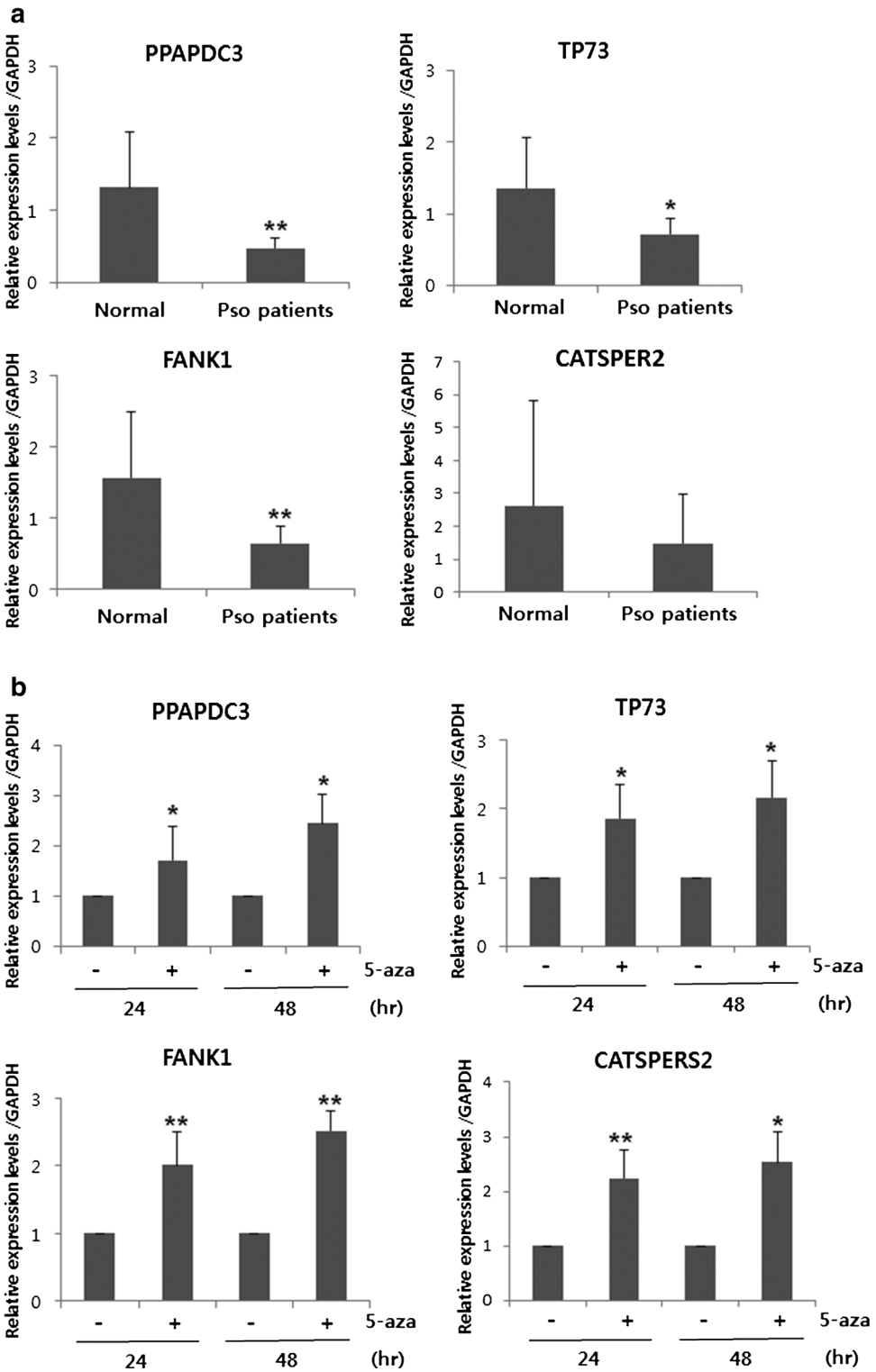

Fig. 3 Gene expression analysis of *PPAPDC3, TP73, CATSPER2*, and *FANK1* by real-time quantitative RT-PCR. **a** Nine CD4+ T samples from psoriasis patients and eight samples from normal controls were used for real-time quantitative RT-PCR using specific primers for each gene. All mRNA levels were significantly down-regulated in psoriatic CD4+ T cell samples (psoriasis versus normal samples, $*p < 0.05$; $**p < 0.01$), except *CATSPER2* ($p = 0.19$). **b** Four CD4+ T cell samples of psoriasis patients were collected and cultured with IL-2 (100 U/ml) in the presence or absence of 5-azacytidine (10 μM). After 24 or 48 h of incubation, the cells were subjected to real-time quantitative RT-PCR to examine the expression levels of each gene. The expression levels of the four genes were induced by treatment with 5-azacytidine at both incubation times (5-azacytidine-treated versus non-treated, $*p < 0.05$; $**p < 0.01$)

treatment; however, epigenetic studies of CD4+ T cells in the pathogenesis of psoriasis has not been well studied. Previously, we reported the increased DNA methylation pattern in the promoter region of genes on the X-chromosome through genome-wide analysis in naive CD4+ T cells from patients with psoriasis [11]. In the present study, we performed genome-wide analysis of DNA methylation in total CD4+ T cells from patients with psoriasis and healthy controls. In the psoriatic lesions, individual genes were studied concerning their epigenetic

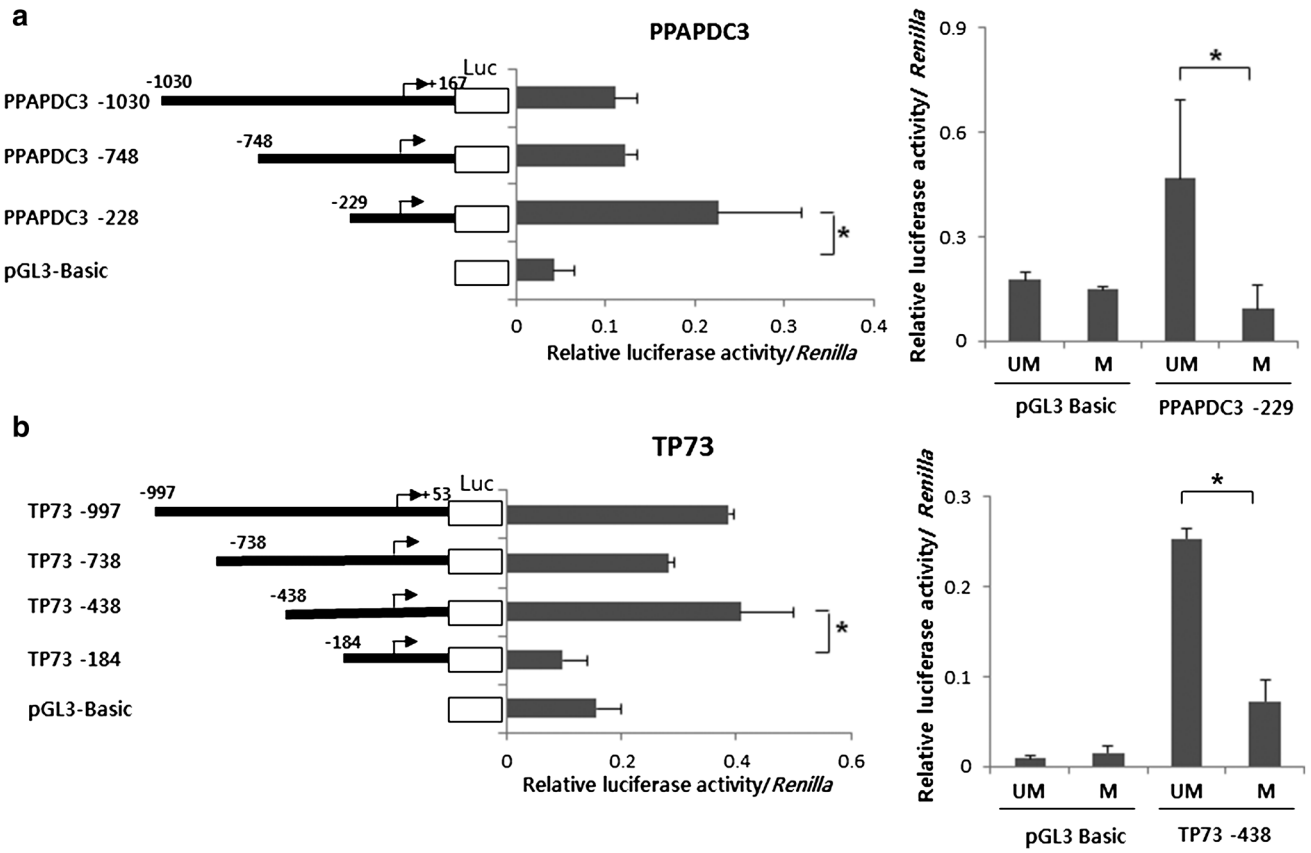

Fig. 4 Functional analysis of the *PPAPDC3* and *TP73* promoters. The putative promoter regions within −1,030 to +167 bp, and −997 to +53 bp of **a** PPAPDC3 and **b** TP73 were cloned into the pGL3-basic vector, respectively. After serially deleted constructs of each promoter were established, each construct or empty vector was transfected into Jurkat T cells with the pRL-TK plasmid as a control of transfection efficiency. Luciferase activity was normalized to *Renilla* activity. Compared with the basal activity of the empty vector, the constructs displaying the highest promoter activity of each construct showed significantly increased activity (empty vector versus promoter constructs, $*p < 0.05$, (**a**) and (**b**) *left panels*). To examine the methylation effect of the promoter activity, the two constructs of each gene, *PPAPDC3*-229 and *TP73*-438, as well as the empty vector, were treated with or without *M. SssI* and its substrate SAM. The promoter activity of each construct was analyzed as described above. The promoter activities decreased significantly following DNA methylation (unmethylated versus methylated, $*p < 0.05$; *UM* unmethylated, *M* methylated; (**a**) and (**b**) *right panels*)

regulation. The promoter regions of p15 and p21 genes are hypomethylated in psoriasis [27]. It has also been reported that the methylation level of the p16 gene promoter was higher in marrow mononuclear cells from patients with psoriasis than in normal controls, and the severity was correlated with the methylation status of p16 [28]. There have been reports concerning the epigenetic differences in psoriatic-involved skin compared with normal skin [19, 31]. Roberson et al. demonstrated that the methylation status of psoriasis-related genes was closely correlated with their expression in psoriatic lesions using microarray analysis. Zhang et al. reported that they used MeDIP-Seq analyses to characterize whole-genome DNA methylation patterns in skin lesions from patients with psoriasis. These reports suggested that the number of hypermethylated genes was much higher than that of hypomethylated genes in psoriatic skin samples. We also observed the tendency of differences toward DNA hypermethylation in the CD4+ T cells of psoriasis patients compared with that in the CD4+ T cells of healthy controls, particularly in promoter regions (Figs. 1, 2).

The regulation of gene expression is a complex process that is achieved through the function of transcription factors and epigenetic regulatory mechanisms. Epigenetic alterations such as DNA methylation and histone modifications are correlated with gene expression changes [18, 25]. The evidence to support an epigenetic contribution in gene regulation resulted from experiments using drugs that affect the status of epigenetic information. In T-cell regulation, the DNA methyltransferase inhibitor 5-azacytidine induced the production of interferon-γ [3], and HDAC inhibitors increased the expression of Th1 and Th2 cytokines [5, 32]. In the present study, we performed experiments using 5-azacytidine and CD4+ T cells from patients and showed that the expression levels of the four genes were induced (Fig. 3b). This result implied that the DNA methylation

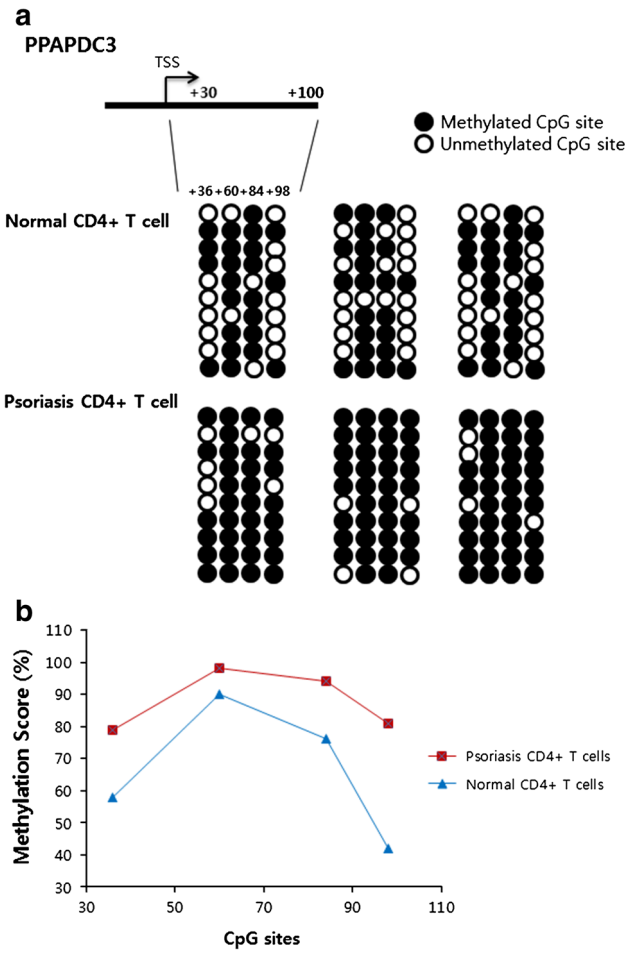

Fig. 5 Bisulfite sequencing analysis of the *PPAPDC3* promoter. **a** Schematic diagram showing the gene region analyzed relative to the transcription start site (TSS). Three representative sequencing data are shown for total CD4+ T cells from normal control and psoriasis patients. The methylation status of a CpG dinucleotide is represented by circles, with a *filled circle* representing a methylated dinucleotide or an *open circle* indicating an unmethylated CpG site. **b** DNA methylation scores of each CpG site of *PPAPDC3*. The scores were averaged from the bisulfite sequencing of five normal controls and psoriasis patients, respectively. Each CpG site was more significantly hypermethylated in patients with psoriasis than in normal controls (normal control versus psoriasis sample; +36, +60: $p < 0.05$; +84, +98: $p < 0.01$)

mechanisms might be involved in the regulation of the genes in CD4+ T cells of patients with psoriasis.

By expression analysis of the listed genes in Table 1, relatively few genes showed a correlation between methylation and gene expression. Two possible explanations exist. First, the expression results originate from the complex combination of regulatory mechanisms in addition to methylation. Second, the sample sizes were too low to detect expression and methylation correlations. Among the genes listed as being hypermethylated in the promoter regions as determined by differential MES values, we examined the expression levels of each in the CD4+ T cells from patients with psoriasis and normal controls. As such, we selected four genes for further studies. *PPAPDC3* (also called *NET39*), a nuclear transmembrane protein, has been known to function as a negative regulator of myoblast differentiation [14]. *PPAPDC3* acts, in part, through effects on mTOR signaling, which plays regulatory roles in IL-22-induced psoriatic inflammatory diseases and can provide target molecules for the therapeutic agents against psoriasis [8, 16]. *TP73* is considered an oncogenic protein and appears to be implicated in various human cancers [6]. The *TP73* promoter functions in human tonsil B cells using epigenetic regulatory mechanisms [4]. *CATSPER2* is an important regulator in male fertility, and *FANK1* is highly expressed in the testis and functions as an anti-apoptotic protein [23, 24]. However, it has not been reported whether the *CATSPER2* and *FANK1* genes are controlled by epigenetic regulation regarding their expression. Additionally, the relationship between the four selected genes and skin diseases has not been described to date. According to the results (Fig. 3a and online resource 3), *CATSPER2* showed a tendency with slightly higher *p* value and *FANK1* promoter activity from in vitro methylation assay showed increased activity. Therefore, in addition to methylation, we could not rule out the participation of the other regulation mechanisms of their expression in psoriasis patients. Thus, we focused on the *PPAPDC3* and *TP73* for the further analyses of the genes. For the bisulfite sequencing, we tried to find the appropriate promoter region of *PPAPDC3* and *TP73* based on the dMES information. In case of TP73, we could not find the significantly hypermethylated CpG sites of the *TP73* promoter region. But, since we searched the several parts (200 bp interval) of the promoter, we could not rule out the existence of hypermethylated CpG sites in the unanalyzed parts of the TP73 promoter or beyond the confined promoter region (−1,000 and +600 bp).

To our knowledge, genome-wide DNA methylation changes in psoriatic total CD4+ T cells versus normal control CD4+ T cells have not previously been reported. Here, we report for the first time the results of epigenetic and gene expression studies using total CD4+ T cells of psoriasis patients. The results from bisulfite sequencing analysis of PPAPDC3 were in accordance with those from MeDIP-Seq (Fig. 5), and the mRNA expression levels of the four selected genes were consistent with the DNA methylation status of the genes (Figs. 3, 4). Taken together, our results suggest that *PPAPDC3, TP73, CATSPER2*, and *FANK1* may be associated with the pathogenesis of psoriasis. Further studies need to be carried out to elucidate the possible mechanisms of the relationships.

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