Promoter Hypomethylation Results in Increased Expression of Protein Phosphatase 2A in T Cells from Patients with Systemic Lupus Erythematosus

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The catalytic subunit α isoform of protein phosphatase 2A (PP2Acα) activity, protein, and mRNA have been found increased in systemic lupus erythematosus (SLE) T cells and to contribute to decreased IL-2 production. The PP2Acα promoter activity is controlled epigenetically through the methylation of a CpG within a cAMP response element (CRE) motif defined by its promoter. We considered that hypomethylation may account for the increased expression of PP2Acα in patients with SLE. Using bisulfite sequencing, we found that SLE T cells displayed decreased DNA methylation in the promoter region compared with normal T cells. More importantly, we found that the CRE-defined CpG, which binds p-CREB, is significantly less methylated in SLE compared with normal T cells, and the levels of methylation correlated with decreased amounts of DNA methyltransferase 1 transcripts. Methylation intensity correlated inversely with levels of PP2Ac mRNA and SLE disease activity. Chromatin immunoprecipitation assays revealed more binding of p-CREB to the CRE site in SLE T cells, resulting in increased expression of PP2Acα. We propose that PP2Acα represents a new methylation-sensitive gene that, like the previously reported CD70 and CD11a, contributes to the pathogenesis of SLE. The Journal of Immunology, 2011, 186: 4508–4517.

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Abbreviations used in this article: 5-azaC, 5-azacytidine; ChIP, chromatin immunoprecipitation; CRE, cAMP response element; DNMT1, DNA methyltransferase 1; MECP, methyl cytosine-binding protein; PP2A, protein phosphatase 2A; PP2Ac, catalytic subunit of protein phosphatase 2A; PP2Acα, α isoform of protein phosphatase 2A catalytic subunit; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index.

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In this study, we investigated DNA methylation patterns in the PP2Aca promoter region in SLE T cells and compared it to that in normal T cells. We show evidence that the PP2Aca promoter is hypomethylated in SLE T cells due to reduced DNMT1 expression and allows enhanced binding of p-CREB, which results in the overexpression of this molecule. Thus, PP2Aca represents a new methylation-sensitive gene, which, like the previously reported CD70, CD40L and CD11a, and probably others, contributes to the pathogenesis of SLE.

Materials and Methods

 Patients and T lymphocyte purification

A total of 24 SLE patients (23 female and 1 male) who fulfilled at least 4 of the 11 revised criteria of the American College of Rheumatology for the classification of SLE (35) were enrolled in this study.

The demographics of the patients are shown in Table I. Nineteen patients were studied at different time points, and their demographics are shown in Table II. The patients shown in Table II were studied in multiple occasions. Thus, total sample number from lupus patients studied in this work is 34.

SLE disease activity was assessed by the SLE disease activity index (SLEDAI) (36). Age ranged between 19 and 49 y (average: 35), and SLEDAI ranged between 0 and 36 (average: 7). We classified samples from patients into two groups: the high disease activity group was defined when the SLEDAI score was >6 (n = 19), and the low disease activity group was defined when the SLEDAI score was â‰¤6 (n = 15). Appropriate age-, ethnicity-, and sex-matched 16 healthy volunteers were also used as controls. Studies were approved by the Human Use Committee of our institution.

CD3+ T lymphocytes were purified using a rosette T cell purification kit (Stem Cell Technologies) as described before (34). Subsequently, both RNA and DNA were extracted from T cells (3 × 10^6) using the AllPrep RNA/DNA/Protein mini kit (Qiagen) according to the manufacturer’s protocol.

DNMTs plasmid transfection

After the purification of CD3+ T lymphocytes from normal individuals’ peripheral blood, a plasmid encoding DNMT1 (Invivogen) and DNMT3a (Invivogen) or corresponding empty vector (pORF9) were transfected into peripheral blood, a plasmid encoding DNMT1 (Invivogen) and DNMT3a were transfected into normal T cells (Fig. 1A). We determined whether DNA methyltransferase DNMT1 (maintenance methyltransferase) and DNMT3a (de novo methyltransferases) regulate the promoter region in SLE T cells and compared it to that in normal T cells.

Results

 Cotransfection of DNMT1 and DNMT3a into normal T cells reduced mRNA expression of PP2Aca by blocking p-CREB binding to methylated promoter region

We previously showed that 5-azac, a DNA methylation inhibitor, influenced the methylation pattern in PP2Aca promoter region and induced increased activity (34). In this study, we examined whether DNA methyltransferase DNMT1 (maintenance methyltransferase) and DNMT3a (de novo methyltransferases) regulate PP2Aca expression by modifying the methylation pattern on the PP2Aca core promoter region. First, we confirmed that the DNA methylation levels at a specific site that is essential for the binding of transcription factor p-CREB were significantly elevated when the DNMTs were transfected into normal T cells (Fig. 1A). We compared the methylation effect in T cells with DNMT1 or DNMT3a alone or in combination. Cells transfected with both DNMTs produced the strongest methylation effect, and these were used in all subsequent experiments.

Next, we determined the levels of p-CREB binding to the CRE motif in the PP2Aca promoter region in T cells transfected with DNMTs. As shown in Fig. 1B, using ChIP assay, we found that overexpression of DNMTs inhibited p-CREB binding to the region. We also quantified PP2Aca mRNA expression levels and

Reverse transcription and real-time PCR

Total RNA (300 ng) was transcribed in cDNA in a conventional thermocycler using AMV reverse transcriptase and oligo(dT) primer (RT-PCR kit; Promega). Real-time RT-PCR was performed in duplicate for every sample with a LightCycler 480 System and using SYBR green (Roche) as the reaction mixture. Primers were used: DNMT1 forward 5'-GTTGGGGGAC-TTGTTCTCTGTTG-3' and reverse 5'-TGAAGGTGCTGTCCTCA-3'; PP2Aca forward 5'-TGGAGTTGGTGGAGGCGAG-3' and reverse 5'-GGTCAAGGAGTGTTACTGTTCTA-3'; and GAPDH forward 5'-CAACTACAGG TTACATGTCCT-3' and reverse 5'-GGACTGGTGCGTA TGCTCTCT-3'. The average cycle threshold values of each reaction derived from the target gene, determined with LightCycler 480 System software (Roche), were normalized to GAPDH levels. Cycle threshold values were used to calculate relative mRNA expression by the ∆ΔCt relative quantification method.

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) analysis was done using ChIP Assay kit (Upstate Biotechnology) according to the manufacturer’s instructions. T cells (3 × 10^8) were used per immunoprecipitating Ab. The cells were fixed with 1% formaldehyde for 10 min, lysed, and sonicated. The DNA–protein complexes were immunoprecipitated with anti–p-CREB Ab (Upstate Biotechnology) or its control Ab (rabbit normal IgG, Santa Cruz Biotechnology), and, after a series of washing steps, the cross-linking was reversed, and the protein was digested with proteinase K (Qiagen). The DNA was extracted by the QIAquick PCR Purification kit (Qiagen). Immunopurified and precipitated DNA was analyzed by PCR using the primers F (–468) and R (–83) described as above, which are specific for the human PP2Aca promoter and were used to amplify a promoter fragment containing a CRE motif. The corresponding nonimmunoprecipitated DNA (input DNA) was also analyzed. PCR products were semiquantified using the same method as above in the methylation-specific PCR.

Statistics

Data are presented as mean value ± SEM. The paired two-tailed Student t test and the Pearson product moment correlation coefficient were used for statistical analysis. Statistical significance was defined as p < 0.05.
found them decreased in cells transfected with DNMTs (Fig. 1C). These results showed that DNMTs have an important role in promoting hypermethylation in the promoter region and silencing the methylation-sensitive gene PP2Acα.

Several studies have demonstrated that DNMT1 is reduced in SLE patients (25–27), but we confirmed this observation using T cells from SLE patients and corresponding control subjects. The demographics of the patients used in the experiments are all shown in Tables I and II. We divided the samples from patients into two groups: the high disease activity group included patients with an SLEDAI score of >6, and the low disease activity group included patients with an SLEDAI score of ≤6. Fig. 1D showed that T cells from patients with active disease have a reduced amount of DNMT1 compared with inactive patients or normal subjects. There was a negative correlation between SLEDAI and DNMT1 expression ($r = -0.398$, $p = 0.0192$; $n = 34$).

These data suggested that decreased levels of DNMT1 in SLE T cells may account for the global DNA hypomethylation and contribute to regulation and expression of the methylation-sensitive gene.
SLE T cells display a highly hypomethylated pattern around the CRE motif defined by the PP2Ac promoter

To investigate PP2Ac transcriptional mechanisms through epigenetic mechanisms, we focused on the essential regulatory region of this gene. As shown in Fig. 2A, the region that started at the −517 position from the translation initiation start site (ATG) was characterized by a high GC content and revealed the existence of several potential stable protein 1 transcription factor sites and a complete CRE motif located around the −238 position. In this region, there are 63 CpG. Among them, one of the CpGs is located at the center of the CRE motif (−238 position).

We first searched for differences in the DNA methylation pattern between T cells from three SLE patients and three matched control subjects. By bisulfite sequence analysis, both groups showed a global hypomethylated pattern; however, SLE T cells displayed a relatively lower methylation pattern than normal T cells, especially around the CRE motif shown in Fig. 2B and 2C. The total percentage of DNA methylated fragments in this region (−519 to +29) was significantly decreased in SLE T cells compared with normal T cells shown in Fig. 2D.

This method, although very useful in determining global gene methylation patterns, is limited when quantification is required. Therefore, we used a methylation-sensitive PCR to quantify the levels of DNA methylation at the CpG in the CRE motif (−238 position). Briefly, we used the methylation-sensitive restriction enzyme AatII, which recognizes only unmethylated CRE motifs, and applied PCR using two sets of primers to distinguish unmethylated and methylated status at this deoxycytosine (dC) in the CRE motif. A band can be detected only when deoxymethylcytosine exists in the CRE motif because it cannot be digested by AatII. A control band was generated by another set of primers defining an area of the PP2Ac promoter, which did not contain any AatII-sensitive motifs. We measured the densitometric intensity for each band, and the ratio of the methylation-sensitive

Table I. Patient demographics and treatment

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Race</th>
<th>Sex</th>
<th>SLEDAI</th>
<th>Predonisolone (mg)</th>
<th>Others</th>
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<td>36</td>
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<td>50</td>
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<tr>
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<td>F</td>
<td>8</td>
<td>0</td>
<td>H400 + M3</td>
</tr>
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<td>None</td>
</tr>
<tr>
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<td>African American</td>
<td>F</td>
<td>6</td>
<td>30</td>
<td>M3</td>
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<td>4</td>
<td>10</td>
<td>H400 + M2,5</td>
</tr>
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<td>F</td>
<td>3</td>
<td>2</td>
<td>H400 + A125</td>
</tr>
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<td>17.5</td>
<td>M3</td>
</tr>
</tbody>
</table>

A, azathioprine (mg); C, cyclosporine (mg); Cyc, cyclophosphamide (i.v.); F, female; H, hydroxychloroquine (mg); M, mycophenolate mofetil (g); MTX, methotrexate (mg).

Table II. Time course of SLEDAI and treatment from nine different patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (y)</th>
<th>Race</th>
<th>Sex</th>
<th>Date</th>
<th>SLEDAI</th>
<th>Predonisolone (mg)</th>
<th>Others</th>
</tr>
</thead>
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<td>40</td>
<td>M1</td>
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<tr>
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<td>02/11/2009</td>
<td>10&quot;*</td>
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<td>M1.5</td>
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<td></td>
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<tr>
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<td>04/16/2009</td>
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<tr>
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<td>Female</td>
<td>06/26/2009</td>
<td>0</td>
<td>20</td>
<td>H200 + Cyc</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>Indian</td>
<td>Female</td>
<td>06/16/2009</td>
<td>4</td>
<td>0</td>
<td>H200 + A100</td>
</tr>
<tr>
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<td>Female</td>
<td>09/29/2009</td>
<td>10</td>
<td>40</td>
<td>H400 + A100</td>
</tr>
<tr>
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<td>03/30/2010</td>
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<td>40</td>
<td>M3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Female</td>
<td>03/18/2009</td>
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<td>5</td>
<td>H400</td>
</tr>
<tr>
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<td>African American</td>
<td>Male</td>
<td>02/17/2009</td>
<td>2</td>
<td>20</td>
<td>H400 + A150</td>
</tr>
<tr>
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<td>02/11/2010</td>
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<td>H400 + A150</td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>52</td>
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<td>Female</td>
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<tr>
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<td>Female</td>
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<td>37</td>
<td>01/23/2009</td>
<td>10</td>
<td>40</td>
<td>H400</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*This sample was excluded in prospective analysis in Fig. 6.

A, azathioprine (mg); C, cyclosporine (mg); Cyc, cyclophosphamide (i.v.); F, female; H, hydroxychloroquine (mg); M, mycophenolate mofetil (g); MTX, methotrexate (mg).
FIGURE 2. Global hypomethylation within the essential PP2Ac promoter region in T cells from SLE patients. A, Nucleotide sequence within bp −517/+29 of the 5′-flanking region of the human PP2Ac gene. The translation initiation codon, ATG, is indicated as +0. The major transcription factor is located at −211 position. Each primer sequence for round II in nested PCR is shown in italics. The essential promoter region for gene regulation, which we previously determined is located from −280 to −218, is shown with arrows. At the center, there is a complete CRE motif surrounded by dashed box where the transcription factor CREB/p-CREB can bind. Whole region qualifies as a CpG island using CpG finder at the European Bioinformatics Institute (http://www.ebi.ac.uk/). All dC in CpG included in this region are highlighted with underlining. Within this region, there are 63 dC in CpG. Among them, a dC in CpG included CRE motif exists at −238 position.

B, One example of PP2Ac promoter methylation pattern in normal (upper panel) or SLE T cells (lower panel). T cells were isolated from a lupus patient and a matched control individual, and DNA was treated by sodium bisulfite. The region shown in Fig. 1A was amplified by PCR, cloned, and 10 colonies were picked up and sequenced from each subject. The fragment number is shown on the y-axis and the location of each CpG pair on the x-axis. Open circles, unmethylated cytosines; closed circles, methylated cytosines.

C, Summary of the promoter DNA methylation pattern in T cells isolated from three different normal individuals (left panel) or three SLE patients (right panel). The value of y-axis showed

D, Methylation fraction (%), within the region, SLE (n = 3), P = 0.048
产品到的控制被计算为半定量DNA甲基化状态在CRE的推手。如图3A, T细胞来自SLE患者显著较低水平的DNA甲基化在该核苷酸与来自健康控制的。这是在高（SLEDAI＞6）疾病活动度间有显著低甲基化状态比较，与SLEDAI活动度（≤6）及健康控制。我们也可找到适度负相关于SLEDAI及DNA甲基化水平（r = -0.352, p = 0.040; n = 34）。

在添加中，我们确定了3个表达的DNA表达甲基化水平的PP2Ac反映DNA甲基化在SLE中。数据显示增加的SLE疾病活动度在DNA表达甲基化结果于减少的DNMT1表达及p-CREB结合到CRE模里，特别在定义该CRE的位点。

p-CREB binding to the CRE motif in the PP2Ac promoter was stronger in lupus T cells than in normal T cells

Next, we investigated predicted differences of p-CREB binding to the PP2Ac promoter in SLE and normal T cells. ChIP assays revealed that p-CREB bound to PP2Ac promoter more intensely in SLE T cells compared with normal T cells (Fig. 4). Previously, we had noted increased p-CREB binding to the PP2Ac promoter (area defining the CRE motif) in T cells treated with the DNA methylation inhibitor 5-azaC (33), suggesting that disease flares initiate a process that leads to inhibition of DNA methylation. In this study, we confirmed the same phenomena between SLE T cells and T cells treated with DNA methylation inhibitor.

The levels of the PP2Ac transcript are increased in T cells from patients with active SLE and reflects DNA methylation of CRE

Previously, we had determined that mRNA and protein levels of PP2Ac were higher in SLE T cells compared with normal T cells, yet we had failed to show a correlation with disease activity (29). In this study, we focused on the major isoform of PP2Ac, PP2Ac, and re-examined the expression levels of its transcripts. As shown in Fig. 5A, the expression levels of PP2Ac mRNA were significantly higher in the patient group with a high SLEDAI score (>6), but not in the group of patients with a low SLEDAI score (≤6) compared with normal controls. There was a positive correlation between SLEDAI and PP2Ac expression level (r = 0.369, p = 0.0308; n = 34). In addition, we noted a significant negative correlation between the levels of transcripts and the levels of DNA methylation (Fig. 5B). Our data, taken together, indicate that disease activity affects DNA methylation status within the PP2Ac promoter, which can result in the upregulation of its expression.

Prospective study of PP2Ac promoter methylation status in patients with SLE

Because our data pointed out that DNA hypomethylation is primarily observed in patients with active disease, we wanted to confirm this observation in the same patients as their disease activity changed over time. The demographics of nine patients used in this experiment are shown in Table II. We defined the time point at which disease activity was low as phase 1 (SLEDAI was ranged between 0 and 10, average; 1.78) and the time point when the disease activity was relatively high compared with phase 1 as phase 2 (SLEDAI ranged between 4 and 35; average: 15). The difference of SLEDAI between phase 1 and phase 2 was at least 4

![FIGURE 3.](http://www.jimmunol.org/)
DNA methylation and related pathology (19, 26, 42–45).

Factors including UV light and drugs contribute to the expression of active genes, in which expression requires a chromatin conformation permissive of transcription factor binding (10, 38). Thus, abnormalities in DNA methylation could disturb the normal regulation of gene silencing, X chromosome inactivation, and lineage specification (10–13, 39–41). The relationship between abnormalities involved in the pathogenesis of the disease by providing useful clues on the mechanisms whereby environmental factors including UV light and drugs contribute to the expression of autoimmunity and related pathology (19, 26, 42–45).

Epigenetic mechanisms have been implicated in the pathogenesis of various disorders, including cancer, immunodeficiency, and autoimmunity (19, 24, 37). DNA methylation involves the covalent modification of the fifth carbon in cytosine residues of CG dinucleotides. Most CG pairs in mammalian DNA are methylated, with exceptions in areas that are located in or near the promoters of active genes, in which expression requires a chromatin configuration permissive of transcription factor binding (10, 38).

DNM1 is a major DNA methyltransferase responsible for DNA methylation following DNA replication during cell division (26, 27, 46), which has been found to be decreased in SLE T cells and claimed to be involved in the immunopathogenesis of the disease. Defective ERK signaling has been demonstrated to account for the decreased DNM1 expression in SLE T cells (27). In addition, a defect in the phosphorylation of protein kinase Cδ, which is located downstream of ERK, results in DNA hypomethylation and overexpression of methylation-sensitive genes such as CD70 (47). SLE T cells have been reported to express higher amounts of microRNAs 21 and 148a, and these are known to downregulate DNM1 (48). In addition, DNM1 polymorphisms, V120L in exon 4, have been associated with the production of anti-La Ab in SLE patients (49).

Several genes involved in the DNA methylation pathway have been linked to the expression of SLE. RFX1, a transcription factor that affects DNA methylation and histone acetylation by recruiting the corepressors DNM1 and histone deacetylase 1 to the promoters of methylation-sensitive genes, was reported to be decreased in SLE T cells (50). Gene polymorphisms of methionine
transcript levels of DNMT1 and PP2Ac associated with the development of SLE (54). MECP2 single nucleotide polymorphisms were reported to be chromatin structure inaccessible for transcription (52, 53). Some deacetylase, which increases chromatin density and induces a mechanism by preventing the binding of transcription factors to moter regions is known to represent an indirect gene-silencing methyl cytosine-binding proteins (MECPs) to demethylated promoter regions (52, 53). MECP2 also recruits histone deacetylase, which increases chromatin density and induces a chromatin structure inaccessible for transcription (52, 53). Some MECP2 single nucleotide polymorphisms were reported to be associated with the development of SLE (54).

CD4+ T cells form active SLE patients as well as T cells treated with DNA methylation inhibitors displayed increased expression of integrin αL (CD11a), which was linked to patchy demethylation within the promoter (47). Hypomethylated, autoreactive CD4+ T cells in SLE patients also spontaneously kill autologous macrophages, causing release of antigenic nucleosomes through mechanisms including demethylation and subsequent overexpression of the perforin 1 gene (20). The costimulatory molecules CD70 (TNF superfamily member 7) and CD40L are also known as methylation-sensitive genes, and their overexpression leads to increased stimulation of autoreactive B cells and increased Ab production in patients with SLE (17, 18, 55). Demethylation of CD40L on the inactive X chromosome may also contribute to the striking female predilection of lupus (18). In addition, some cytokines such as IL-4, IL-6, and IFN-γ are regulated by DNA methylation, and abnormal demethylation may disturb appropriate T cell subset development and normal immune response in SLE (23, 25). Moreover, the abundance of hypomethylated DNA derived from apoptotic cells in lupus patients would possibly contribute to autoimmunity via TLR9 stimulation, which is activated by hypomethylated CpG DNA (56–58).

The catalytic subunit of PP2A has been identified as a player in the pathogenesis of SLE. Increased PP2Ac activity results in reduced binding of p-CREB to the IL-2 core promoter and decreased production of IL-2 (29). Abnormal overexpression of PP2Ac in T cells from patients with SLE also contribute to decreased expression of CD3ζ-chain and increased expression of FcRγ-chain by dephosphorylation of eukaryotic transcription elongation factor, which becomes part of the CD3 complex and contributes to aberrant signaling (59).

PP2A is a major serine/threonine phosphatase with complex composition and is involved in many essential aspects of cell function. The heterodimeric PP2A core enzyme consists of a well-conserved 36-kDa catalytic subunit (C subunit; PP2Ac) and a 65-kDa scaffold subunit (A subunit). To gain full activity toward specific substrates, the PP2A core enzyme associates with a variable regulatory subunit (B subunit) to form a heterotrimeric holoenzyme (30–32). PP2Ac also regulates histone deacetylase, which increases chromatin density and induces a chromatin structure inaccessible for transcription (52, 53). Some MECP2 single nucleotide polymorphisms were reported to be associated with the development of SLE (54).

CD4+ T cells form active SLE patients as well as T cells treated with DNA methylation inhibitors displayed increased expression of integrin αL (CD11a), which was linked to patchy demethylation within the promoter (47). Hypomethylated, autoreactive CD4+ T cells in SLE patients also spontaneously kill autologous macrophages, causing release of antigenic nucleosomes through mechanisms including demethylation and subsequent overexpression of the perforin 1 gene (20). The costimulatory molecules CD70 (TNF superfamily member 7) and CD40L are also known as methylation-sensitive genes, and their overexpression leads to increased stimulation of autoreactive B cells and increased Ab production in patients with SLE (17, 18, 55). Demethylation of CD40L on the inactive X chromosome may also contribute to the striking female predilection of lupus (18). In addition, some cytokines such as IL-4, IL-6, and IFN-γ are regulated by DNA methylation, and abnormal demethylation may disturb appropriate T cell subset development and normal immune response in SLE (23, 25). Moreover, the abundance of hypomethylated DNA derived from apoptotic cells in lupus patients would possibly contribute to autoimmunity via TLR9 stimulation, which is activated by hypomethylated CpG DNA (56–58).

The catalytic subunit of PP2A has been identified as a player in the pathogenesis of SLE. Increased PP2Ac activity results in reduced binding of p-CREB to the IL-2 core promoter and decreased production of IL-2 (29). Abnormal overexpression of PP2Ac in T cells from patients with SLE also contribute to decreased expression of CD3ζ-chain and increased expression of FcRγ-chain by dephosphorylation of eukaryotic transcription elongation factor, which becomes part of the CD3 complex and contributes to aberrant signaling (59).

PP2A is a major serine/threonine phosphatase with complex composition and is involved in many essential aspects of cell function. The heterodimeric PP2A core enzyme consists of a well-conserved 36-kDa catalytic subunit (C subunit; PP2Ac) and a 65-kDa scaffold subunit (A subunit). To gain full activity toward specific substrates, the PP2A core enzyme associates with a variable regulatory subunit (B subunit) to form a heterotrimeric holoenzyme (30–32). PP2Ac has two isoforms, α and β, but α represents the major isoform because of the evidence that the activity of the PP2Ac promoter and mRNA levels are 7–10-fold higher than those of β isoform (60). The PP2Ac gene is composed of seven exons and six introns encoded on chromosome 5q23-q31. It is characterized by an extremely GC-rich sequence
and the lack of TATA and CCAAT boxes that are frequently found in many housekeeping genes (61). The expression of PP2Ac is tightly controlled through autoregulation to ensure the presence of relatively constant levels of PP2A (62). One mechanism of this regulation involves p-CREB, which regulates gene expression and is also dephosphorylated by PP2Ac itself (34, 63, 64). However, the autoregulation of PP2Ac appeared to be disturbed in T cells from SLE patients because the expression levels of this molecule are significantly higher in SLE T cells than in normal T cells. The control of the expression of PP2Ac levels in SLE T cells is broken at the methylation level of the CRE motif of its promoter. In addition, because PP2A can dephosphorylate p-ERK and p-JNK, which control DNTM1 expression, it may lead to further positive regulation of itself (positive feedback) (65). We previously reported that total PP2Ac (PP2Acα and PP2Acβ isoforms) is increased in SLE T cells compared with T cells from healthy individuals; yet, we had failed to find a correlation with disease activity. In the current study, we have focused on the regulation of the promoter activity in the PP2Acα, the major isoform of the enzyme, and we have recorded its levels rather than total levels of PP2Ac. The fact that the levels of PP2Ac correlate with disease activity suggests the importance of this isoform in the expression of the disease and that our previous data (29) were confounded by the indiscriminate determination of both isoforms together. In addition, in the current study, we used mRNA samples to examine the correlation between expression levels of PP2Ac and disease activity because we focused on the regulation of the transcription of the PP2Ac gene rather than on the translation of the gene. The regulation of PP2Ac mRNA translation is still unclear and remains to be investigated fully in future (29). In this work, we demonstrated that abnormal hypomethylation of this promoter in lupus T cells allows the overexpression of this molecule by increasing accessibility of p-CREB to the CRE motif in the promoter. In conclusion, we presented the evidence that hypomethylation of the CRE site of the PP2Acα promoter enables amble binding of p-CREB, which results in increased expression of PP2Ac in SLE T cells. Our results add PP2Ac to the list of genes that are altered epigenetically in patients with SLE. The fact that PP2Ac promoter paralleled disease activity urges a larger prospective study to explore its potential to serve as a disease biomarker.

Disclosures

The authors have no financial conflicts of interest.

References