

# Possible Role of DNA Methylation in the Induction of Systemic Lupus Erythematosus

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**Abstract:** Recent studies on epigenetics, including DNA methylation and its regulatory enzymes, seem to contribute to elucidation of the pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE), although the relationship between DNA methylation and SLE has long been the subject of investigation. To obtain a deeper understanding of the role of DNA methylation in the induction of SLE, we reviewed the relationship between DNA methylation and SLE based on findings reported in the literature and our own data. Various studies, including ours, have indicated the possible importance of DNA methylation, especially hypomethylation, in the etiology of SLE. These epigenetic studies may give us clues towards elucidation of the pathogenesis of SLE and development of new therapeutic strategies for this disease.

**Keywords:** Systemic lupus erythematosus, DNA methylation, CpG motif, human endogenous retroviruses, toll-like receptor.

## INTRODUCTION

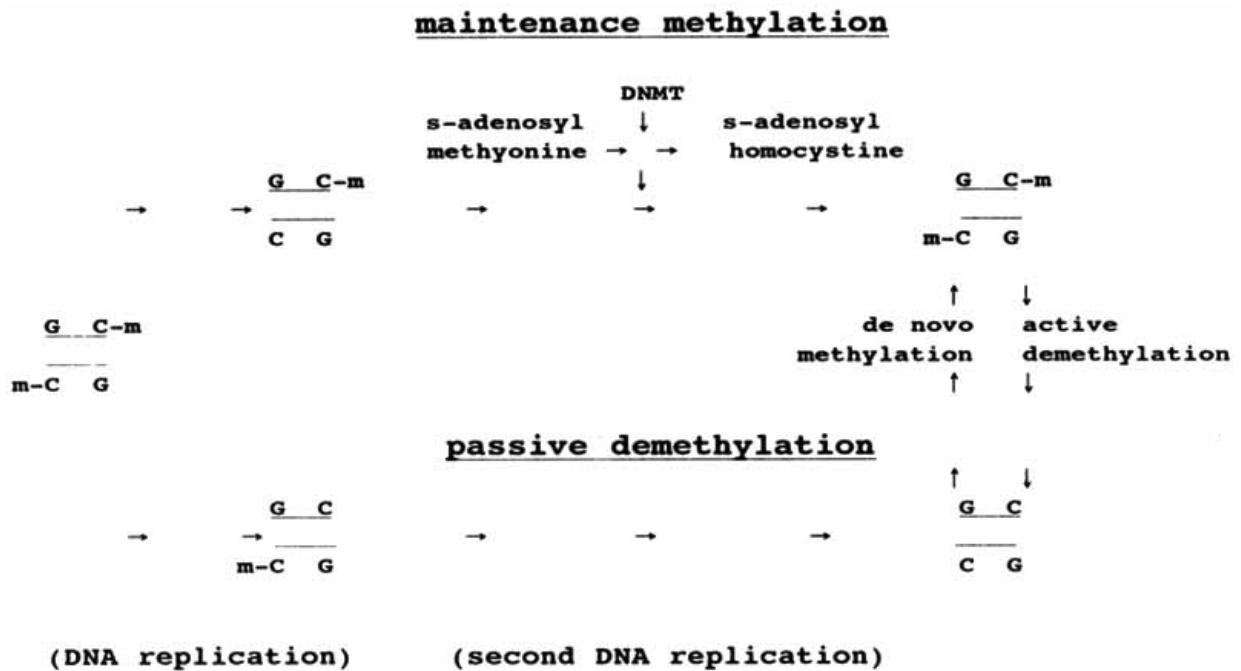
Epigenetics is defined as the investigation of "heritable changes in gene expression that occur without a change in DNA sequence," with DNA methylation and histone acetylation being involved in such changes [1]. Recent epigenetic investigations have contributed to exploration of the pathogenesis of several disorders, including cancer, immunodeficiency, and autoimmunity. Cytosine methylation of the regulatory sequences of DNA is an epigenetic mechanism that is associated with the transcriptional inactivation of genes, while hypomethylation contributes to the activation of transcription [2,3]. Several lines of evidence have indicated that abnormalities of DNA methylation may contribute to the development of SLE. Here we review the possible role of DNA methylation in the pathogenesis of SLE using the literature and our own findings regarding the transcription of human endogenous retroviruses (HERV) in SLE patients.

## DNA METHYLATION

Epigenetic regulation is a mechanism by which gene function is selectively activated or inactivated. Recently, various proteins that regulate gene expression, such as DNA methyltransferases, methyl-CpG binding protein, and histone-modifying enzymes, have been identified [1-3]. Methylation of DNA helps to stabilize chromatin in an inactive configuration and inhibits gene transcription. Mechanisms of

DNA methylation and demethylation are summarized in Fig. 1. In mammalian cells, the term DNA methylation refers to the postsynthetic methylation of deoxycytidine (dC) residues at the 5-position to form deoxymethylcytosine (dmC). dmC is synthesized by the enzyme-catalyzed transfer of methyl groups from S-adenosylmethionine to dC residues in DNA, producing dmC and S-adenosylhomocystine. Nearly all dmC residues are found in the cytosines that precede guanines in DNA strands (CpG dinucleotides), and approximately 60-90% of all CpG sequences in the genome are methylated, while unmethylated CpG dinucleotides are mainly clustered in the CpG-rich sequence (CpG island) of the promoter region of a gene. Normally, both the core promoter and the transcription start site are included within the CpG island, and gene expression is completely repressed when this region becomes hypermethylated [2]. The organic bases that participate in the formation of DNA can undergo methylation under the influence of methylation-regulating enzymes. The methylation status of DNA is mediated by at least three classes of enzymes: maintenance methyltransferases, de novo methyltransferases, and demethylases [2,3]. DNA methyltransferase (DNMT)-1 preferentially methylates hemimethylated DNA and is a maintenance methyltransferase that provides the methylation pattern for newly replicated daughter strands during mitosis, based on that of the parent strand [4]. DNMT-3a and -3b mainly add a methyl group to unmethylated CpG base pairs, resulting in the creation of a new hemimethylated and then fully methylated CpG. This de novo methylation is thought to be implicated in cell growth and differentiation, and in altered methylation during tumorigenesis. The function of DNMT-2, which is another member of the DNMT family, remains still unclear. The role of demethylases, which are capable of removing

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**Fig. (1).** Mechanisms of DNA methylation and demethylation. During cell mitosis, the pattern of DNA methylation are replicated by DNMT-1. DNMT-1 can bind the replication fork and catalyze the transfer of methyl groups from S-adenosylmethionine to dC residues in CG pairs only if the parent strand is methylated (maintenance methylation). Passive demethylation will occur on the newly synthesized strand if this methylation step is inhibited during replication, and the second round of replication will then result in demethylation of both strands. DNMT-3a and -3b can mediate de novo methylation, raising the possibility that DNA can be methylated in non-dividing cells. A DNA demethylase that actively removes methylcytosine has been also identified (active demethylation).

dmC residues, is also still not well characterized, but these enzymes may be involved in modifying the methylation pattern of in nondividing cells [2,3].

A change of methylation status has been reported to contribute to the development of several diseases [2,3]. Certain hereditary disorders, such as Fragile X syndrome and immunodeficiency-centromeric instability-facial anomalies syndrome (ICF syndrome), are known to be associated with abnormal methylation patterns [5,6]. Hypomethylation of protooncogenes has been reported in liver tumors, leukemia, and colorectal cancer, and it may promote malignant transformation by increasing protooncogene expression [7]. Hypomethylation can also lead to an increase of the mutation rate [8]. In contrast, the methylation of normally unmethylated CpG islands has also been associated with a variety of human tumors such as retinoblastoma [7]. In addition to these findings, a possible important role of methylation abnormalities has been reported in the development of autoimmune diseases, especially SLE.

## DNA METHYLATION IN SLE

### Human SLE Studies

Various studies on the relationship between methylation and SLE and/or autoimmunity have been reported in mice and humans. The main literature covered in this manuscript is summarized chronologically in Table 1. A series of experiments by Richardson *et al.* have revealed the important role of methylation status in the induction of SLE [9-17]. They have shown that DNA extracted from the T cells of

SLE patients is hypomethylated when compared to DNA from normal T cells [9]. Treatment of cloned T cells with 5-aza-deoxycytidine (5-aza C; a demethylating agent) results in a change of activation requirements, so that previously antigen-specific cells respond to self major histocompatibility complex determinants (HLA-D molecules) without the relevant antigen, thus becoming autoreactive [10]. This increase of autoreactivity is correlated with the expression of leukocyte function-associated antigen-1 (LFA-1; CD11a/CD18), an adhesion molecule involved in T cell activation, which is probably induced by 5-aza C-mediated demethylation. LFA-1 overexpression seems to be sufficient to cause autoreactivity, as observed in SLE patients [11]. In fact, T cells transfected with LFA-1 can also induce a similar disease to SLE *in vivo* [12]. It has been also reported that the overexpression of perforin on SLE-T cells is induced by hypomethylation and this may account in part for the increase of T cell mediated-apoptotic cells in the patients [13]. Adoptive transfer of cloned or polyclonal T cells treated with 5-aza C into mice can cause various autoimmune manifestations in mice, such as anti-DNA antibody, immune complex-mediated glomerulonephritis, central nervous system lesions resembling human SLE, and pulmonary alveolitis [14,15]. Recent evidences have revealed that DNA hypomethylation in SLE patients may be regulated by a decrease in signaling through the ras-mitogen-activated protein kinase (ras-MAPK) pathway [16]. In contrast, it has been reported that hypermethylation of neutrophils and B cells is observed in SLE patients and this may be related to the low expression of certain molecules (such as HLA-DR on B cells), while SLE serum enhances methylation activity [18,19].

**Table 1. DNA Methylation and SLE or Autoimmunity**

Authors	Year	Ref.	Summary
1. Richardson B <i>et al.</i>	1986	[10]	5-aza C-mediated autoreactive T cell clone.
2. Sano H <i>et al.</i>	1985	[18]	Hypermethylation-related low expression of HLA-DR on B cells and -related autoimmunity in SLE.
3. Evans JT <i>et al.</i>	1987	[21]	Hypomethylation-induced c-myb overexpression in MRL/lpr-T cells.
4. Niwa Y <i>et al.</i>	1988	[19]	High methyltransferase activity in SLE lymphocyte membranes and enhancement of methylation of normal cells by SLE serum.
5. Eleftheriades EG <i>et al.</i>	1989	[22]	Methylation is unrelated to overexpression of c-myc in SLE.
6. Yoshida H <i>et al.</i>	1990	[24]	Protective effect of 5-aza C on the development of autoimmunity in MRL/lpr mice.
7. Richardson B <i>et al.</i>	1990	[9]	Impaired DNA methylation in SLE T cells.
8. Schauenstein K <i>et al.</i>	1991	[25]	Autoimmune thyroiditis in chickens is induced 5-aza C treatment.
9. Richardson B <i>et al.</i>	1992	[11]	Similar phenotypic features to SLE, such as LFA-1 expression, by on 5-aza C-treated normal human T cell clones.
10. Quddus J <i>et al.</i>	1993	[14]	Induction of autoimmunity by adoptive transfer of 5-aza C or procainamide-treated T cells in mice.
11. Yung RL <i>et al.</i>	1995	[15]	Induction of SLE-like manifestations <i>in vivo</i> and <i>in vitro</i> by 5-aza C- or procainamide-treated murine T cells.
12. Mizugaki M <i>et al.</i>	1997	[23]	Different changes of DNA methylation levels in MRL/lpr organs with aging.
13. Deng C <i>et al.</i>	2001	[16]	DNA hypomethylation of SLE-T cells may be regulated by ras-MAPK pathway.
14. Okada M <i>et al.</i>	2002	[51]	Important role of hypomethylation in the transcription of HERV in SLE.

### Animal Models

Several studies regarding methylation have also been performed in mouse models of SLE, especially MRL/lpr mice which produce various autoantibodies and develop severe lupus glomerulonephritis associated with massive lymphadenopathy due to the expansion of a unique T cell subset. Autoimmunity-related defective deletion of self-reactive T cells (*via* apoptosis) in the MRL/lpr-thymus can be induced by abnormal Fas expression, which is mediated by insertion of an endogenous retrovirus-like gene into lpr gene [20]. Overexpression of various protooncogenes in SLE is well known, and c-myc gene expression by a unique T cell subset in MRL/lpr mice is promoted by hypomethylation of the gene [21]. However, hypomethylation is not observed in human SLE lymphocytes with overexpression of the c-myc gene [22]. In these mice, DNA methylation of genes in thymus and axillary lymph node cells decreases and methylation of spleen cell genes increases with aging and is related to the progression of disease activity. However, there is no significant change of methylation with aging in the peripheral blood of the mice [23]. Administration of 5-aza C to MRL/lpr mice strongly promotes their longer survival, decreases lymphadenopathy/splenomegaly, and reduces circulating autoantibodies. This treatment also reduces DNA methylation in the axillary lymph nodes and spleen. However, similar effects were not observed in another lupus-prone strain, male BXSB/MjP mice [24]. It is possible that the sensitivity of methylation to 5-aza C differs between the lymphoid organs in these mice and that there are particular mechanisms for maintaining DNA methylation in each organ or lupus-prone strain. In contrast, 5-aza C treatment has been reported to cause autoimmune diseases such as thyroiditis in chickens [25].

Demethylation or hypomethylation results in an increase of gene transcription, so the hypothesis may be raised that hypomethylation of SLE-associated genes results in the ex-

pression or overexpression of these genes and subsequent development of SLE. As described above, the extent of methylation of each specific gene or organ may be quite different in both humans and mice. However, based on some compelling evidence, DNA hypomethylation (especially in T cells) seems to be a plausible contributor to the induction of SLE [17].

### Hypomethylated Plasma DNA in SLE

Several recent studies have highlighted the possible important role of hypomethylated plasma DNA in the pathogenesis of SLE and the main literature is summarized in Table 2. Bacterial and viral DNA, which are both rich in CpG dinucleotides and are hypomethylated, can induce various immune changes *in vivo* and *in vitro* like those observed in SLE, including 1) polyclonal B cell activation (PBA) and production of autoantibodies such as anti-DNA antibodies in mice and humans, 2) interleukin (IL)-6 secretion, and 3) resistance to apoptosis (the mechanism that is thought to be responsible for maintaining self tolerance, thereby potentially allowing the survival of autoreactive cells) [26-28]. In fact, aggravation of SLE after bacterial or viral infection is well known to occur [29,30]. Similarly, certain synthetic oligodeoxyribonucleotides (ODNs) containing an unmethylated CpG motif can induce PBA and anti-DNA antibodies [31]. Huck *et al.* have reported that the combination of accelerated apoptosis with the defective clearance of apoptotic cells results in the increased release of nucleosomes with abnormally methylated, CpG-rich DNA and provides autologous stimulation that could bypass self tolerance in SLE [32]. Supporting these findings, several reports have indicated that SLE patients without infection have elevated levels of circulating plasma low-molecular-weight DNA, which is enriched in hypomethylated CpGs [33-35]. Recent studies have revealed that ODN with a CpG motif in serum from SLE patients can promote an increase of cytokine

**Table 2. Hypomethylated DNA and CpG Motif in SLE**

Authors	Year	Ref	Summary
1. Sano H <i>et al.</i>	1989	[33]	Detection of cytosine in low molecular DNA in SLE plasma.
2. Krapf F <i>et al.</i>	1989	[34]	Hypomethylation of plasma DNA in SLE.
3. Corvetta A <i>et al.</i>	1991	[35]	Low DNA methylcytosine in blood, synovial cells, and tissue in SLE.
4. Gilkenson GS <i>et al.</i>	1993	[26]	Anti-DNA antibody production <i>in vivo</i> by bacterial CpG.
5. Krieg AM <i>et al.</i>	1995	[27]	Induction of SLE-like autoimmunity by bacterial CpG.
6. Huck S <i>et al.</i>	1996	[28]	Autoreactive B cell induction by unmethylated bacterial CpG.
7. Liang H <i>et al.</i>	1996	[31]	Induction of anti-DNA antibody production and PBA by several ODNs.
8. Huck S <i>et al.</i>	1999	[32]	SLE-like autoimmunity is induced by abnormally methylated CpG-rich DNA from apoptotic cells.
9. Sato Y <i>et al.</i>	1999	[36]	Detection of DNA sequences containing CpG motifs in serum from SLE patients and induction of immune abnormalities.
10. Vallin H <i>et al.</i>	1999	[38]	Induction of IFN- $\gamma$ by anti-DNA antibodies and unmethylated CpG DNA.
11. Magnusson M <i>et al.</i>	2001	[37]	Activation of IFN- $\alpha$ -producing cells by SLE-related ODNs.
12. Anders HJ.	2005	[39]	Possible important role of TLR9 in the induction of CpG-related autoimmunity.

mRNAs and enhance the production of cytokines such as IL-12, interferon (IFN)- $\alpha$  and - $\gamma$ , with such changes possibly being related to the induction of SLE [36,37]. Formation of complexes between anti-DNA antibodies and DNA with an unmethylated CpG motif is also suggested to promote the production of IFN- $\alpha$ , which is correlated with disease activity in SLE [38]. Thus, ODN enriched with hypomethylated-CpGs that is probably derived from apoptotic cells may induce several SLE-related autoimmune manifestations, as well as bacterial CpG motifs.

### Drug Induced Lupus

Drug-induced lupus is interesting when we consider the possible important role of DNA methylation in SLE [39,40]. The occurrence of drug-induced lupus syndromes, during chronic treatment with certain drugs, such as procainamide and hydralazine, is well documented. For example, over 90% of patients undergoing treatment with procainamide for 1 to 2 years develop antinuclear antibodies and ~20% of them also develop lupus-like symptoms [32]. These drugs, as well as ultraviolet (UV) light, are inhibitors of DNA methylation and prokaryotic DNA exposed to such SLE triggers exhibits an abnormal pattern of DNA methylation such as low levels of dmC [29]. In fact, murine T cells that have become autoreactive after exposure to procainamide or hydralazine can induce SLE-like disorders *in vivo* that are identical to those caused by 5-aza C [41].

### Recognition of Hypomethylated DNA

The ability of both innate and adaptive immune systems has been largely attributed to the family of Toll-like receptors (TLRs) [42]. ODNs containing an hypomethylated CpG motif and microbacterial DNA have been known to be recognized through TLR9. Several reports have indicated the possible important role of the TLR9 in the induction of autoantibody production such as anti-DNA antibody and anti-RNP antibody [42-44]. The DNA component of immune complexes purified from SLE patients can induce proliferation of self-reactive B cells and cytokine production such as

IFN- $\alpha$  by plasmacytoid dendric (pDC) cells in a TLR9-dependent manner [44]. In contrast, a certain study using a murine model of SLE having with genetic deficiency of TLR9 has suggested that engagement of a TLR9-independent DNA activation pathway promote autoimmunity, while TLR9 signaling can ameliorate SLE-like immune pathology *in vivo* [45]. In addition, a recent genetic experiment has indicated that there is no evidence that common alleles of the TLR9 gene contribute significantly to the genetic risk involved in susceptibility to SLE or lupus nephritis [46]. Thus, the contribution of the TLR system to autoimmune disorders remains controversial. This seems to be due to the functional difference of TLR in cells. In fact, it has been known that TLR9 found in the dendric cells and B cells produce differential outcome in response to structurally distinct CpG-ODNs. While one class of CpG-ODN activates B cells and produce immunoglobulin, other can either redirect pDC cells to secrete high levels of IFN- $\alpha$  or myeloid dendric cells (mDC) to produce T helper (Th)-1 like cytokines [47]. Further investigations are required to elucidate the relationship between autoimmunities and CpG-ODN activation through TLR9 system.

### ROLE OF METHYLATION IN THE TRANSCRIPTION OF HERV, A POSSIBLE CAUSATIVE AGENT OF SLE

#### Human Endogenous Retroviruses (HERV)

Numerous reports have suggested a possible important role of HERV as autoantigens in the etiology of SLE through the several mechanisms such as molecular mimicry between HERV and autoantibodies [48], although this is still controversial and the precise role of these HERV remains unclear [49]. HERV account for approximately 8 % of human DNA, although their transcription and translation are blocked by several interrupters such as termination codons, deletions, and methylation sites in normal persons. Our recent results have indicated that transcription and translation of the gene for HERV clone 4-1 (which belongs to the HERV-K family) are markedly increased in SLE patients compared with nor-

mal controls, while serum autoantibodies to this HERV and expression of its antigens on lymphocytes are detected in SLE patients, but not normal controls [48,50-53]. In addition, synthetic peptides derived from HERV clone 4-1 can induce the immune abnormalities observed in SLE patients, such as T cell activation, cytokine production, and polyclonal B cell activation [54].

### Transcription of HERV in SLE

The increased transcription of HERV clone 4-1 in SLE patients is partially regulated by epigenetic mechanisms, as is the case for other HERV [50,51,55]. In normal individuals, the level of transcription HERV clone 4-1 transcription is increased by demethylating agents such as 5-aza C that inhibit DNMT-1, and the level of DNMT-1 activity in SLE patients is lower than in normal controls [50,51,56]. The expression (especially transcription) of endogenous autoantigens such as HERV seems to be promoted by DNA hypomethylation, which is implied by low DNMT-1 activity.

Besides DNA methylation, there are several epigenetical regulations of DNA expression such as histone modifications, including acetylation, phosphorylation, and ubiquitination [57]. p300/CBP-associated factor (PCAF) is one of the representative histone acetyltransferase that plays an important role in the remodeling of chromatin and the regulation of gene expression [58]. Our recent studies using the real-time quantitative-polymerase chain reaction (RQ-PCR) have suggested that there are no differences of the quantitative expression of PCAF mRNA in peripheral blood mononuclear cells (PBMC) between SLE and healthy person, and are no influences of steroid therapy in SLE on the expression of PCAF mRNA (data not shown). Thus, there seemed to be no strong influence of PCAF-mediated histone acetylation on the activation of gene transcriptions in SLE, differently from DNMT-1.

Abnormalities of DNMT-1 in SLE may contribute to the production of hypomethylated CpGs as a potential etiologic factor of the disease (as described above), as well as an increase of transcription of HERV. Thus, a possible new therapeutic strategy may exist in a regulation of DNMT-1-related hypomethylation and the resultant suppression of HERV transcription or hypomethylated-CpGs production. Human T cell leukemia virus (HTLV)-1 transgenic mice have been known to develop an autoimmune disorder resembling human rheumatoid arthritis [59]. Therefore, the creation of transgenic mice using SLE-derived HERV clone 4-1 and the effect of methylation regulating agents such as 5-aza C on such model mice may help to clarify the role of HERV clone 4-1 and/or DNA methylation in the development of autoimmune diseases including SLE.

### CONCLUSION

Several investigations have suggested the etiologic importance of DNA methylation in the development of SLE. There are differences in the degree of methylation among specific genes or organs in both murine and human SLE. However, DNA hypomethylation seems to play an important role in the etiology of SLE and this is supported by the following evidence: 1) Induction of SLE-like autoimmunity by the hypomethylated CpG motif and detection of such a motif

in serum from SLE patients, 2) evidence of hypomethylation of SLE lymphocytes (especially T cells), including reduced expression of DNMT-1 mRNA, 3) induction of SLE-like autoimmunity *in vivo* and *in vitro* by demethylating agents such as 5-aza C, 4) effect on DNA methylation of SLE triggering drugs (drug-induced lupus), and 5) an important role of hypomethylation in the transcription of HERV, which are probably related to the etiology of SLE.

Recent extensive studies on epigenetics should contribute to elucidation of the pathogenesis of SLE. Further studies on the DNA methylation status, especially hypomethylation, in SLE may provide to light important clues to assist in the development of new treatments for SLE as well as a deeper understanding of the etiology of this disease.

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