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REVIEW

Critical role of DNA methylation in the pathogenesis of systemic lupus erythematosus: new advances and future challenges

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Systemic lupus erythematosus (SLE) is a systemic multi-organ autoimmune disease with different immunological characteristics and clinical manifestations characterized by an autoantibody response to nuclear and cytoplasmic antigens; the etiology of this disease remains largely unknown. Most recent genome-wide association studies demonstrate that genetics significantly predispose to SLE onset, but the incomplete disease concordance rates between monozygotic twins indicates a role for other complementary factors in SLE pathogenesis. Recently, much evidence strongly supports other molecular mechanisms involved in the regulation of gene expression ultimately causing autoimmune disease, and several studies, both in clinical settings and experimental models, have demonstrated that epigenetic modifications may hold the key to a better understanding of SLE initiation and development. DNA methylation changes the structure of chromatin, being typically able to modulate the fine interactions between promoter-transcription factors and encoding genes within the transcription machinery. Alteration in DNA methylation has been confirmed as a major epigenetic mechanism that may potentially cause a breakdown of immune tolerance and perpetuation of SLE. Based on recent findings, DNA methylation treatments already being used in oncology may soon prove beneficial to patients with SLE. We herein discuss what we currently know, and what we expect in the future. *Lupus* (2014) **23,** 730–742.

Key words: Systemic lupus erythematosus; DNA methylation; DNA methyltransferase; microRNA; autoimmune disease

Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by multisystem involvement and the production of autoantibodies against nuclear, cytoplasmic, and cell-surface antigens.[1,2] The pathogenesis of this disease is complex and multifactorial, and the molecular mechanisms responsible for the breakdown of immune tolerance and autoantigen generation are not fully understood, but abnormal activation of B cells, T cells, and antigen-presenting cells during SLE development have been described.[3,4] Over the years, disease-related genes have been extensively investigated in order to reveal the molecular mechanisms of immune dysregulation in SLE, and this work has been successful in identifying several predisposing loci or regions, such as *BANK1*, *PDCD1*, *MECP2*, *STAT4*, *TNFSF4*, *IRF5*, and *BLK*.[5–7] However, disease-related gene studies have not identified a complete list of genes that participate in the pathogenesis of SLE, and accumulating evidence suggests that environmental factors and other mechanisms are involved in disease susceptibility and development.[8,9]

Recent studies have revealed that epigenetic regulations can alter phenotype without changing the sequence of encoding genes, thereby leading to changes that result in gene activation or gene silencing. In SLE, as well as in other autoimmune diseases, interactions of genetic and environmental factors may contribute to disease susceptibility, and influence pathogenic processes.[10,11] Recent evidence shows that DNA methylation, a major form of epigenetic modification, participates in the pathogenesis of SLE in which environmental and genetic factors are involved.[12,13] Twin studies

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confirm the important role of DNA hypomethylation in human lupus. When comparing DNA methylation in leukocytes from identical twins discordant for SLE, dermatomyositis and rheumatoid arthritis, only the DNA of twins discordant for SLE has widespread changes in DNA methylation, with 49 low-methylated genes.[14–16] Hypomethylation was associated with low expression of DNA methyltransferases (DNMTs), leading to a high expression of SLE-associated genes during SLE development.[17] Based on recent findings, we discuss the critical role of DNA methylation in the pathogenesis of SLE, with special emphasis on new progress and future challenges.

DNA methylation

DNA methylation is a known mechanism for the direct modification of DNA sequences after their synthesis, and this modification will change the function of target genes dramatically.[18,19] In contrast to various epigenetic modifications of histone, DNA methylation is relatively simple and represents the only known alteration of the chemical composition of DNA.[20] In prokaryotes, methylation is confined to adenosine and cytosine bases, whereas the addition of methyl groups generally occurs at cytosine bases within CpG dinucleotides in multicellular eukaryotes.[21] In DNA sequences, the CpG dinucleotides are clustered in so-called CpG islands, which are mainly located in 5′ regulatory sites of genes, leading to a rich GC content in DNA. DNA methylation is catalyzed by DNMTs, which use the methyl donor S-adenosylmethionine to specifically methylate the fifth carbon atom of the cytosine ring.[22] According to their function in the methylation process, mammalian DNMTs can be divided into two different groups. Among DNMTs, DNMT1 recognizes established methylation marks and copies them to the newly synthesized DNA strands, leading to the maintenance of the DNA methylation pattern during cell division. DNMT3A and DNMT3B act as de novo methyltransferases by introducing methyl groups into previously unmethylated CpG dinucleotides.[23,24] It is commonly accepted that increased methylation in DNA sequences results in transcriptional silencing of target genes, and two mechanisms are involved in the process. One is that methylation of cytosine bases in target genes directly attenuates the affinity for binding of transcription factors; another is the recruitment of methyl-CpG-binding domain proteins (MBDs) to methyl groups in promoter regions, leading to a co-suppression of transcription silencing.[25,26]

Accumulating evidence suggests that DNA methylation plays a pivotal role in the pathogenesis of human diseases. For example, there is evidence showing that the expression of IL-2 is silenced by DNA methylation in its promoter region in resting naïve T cells. When T cells are activated by T-cell receptor-dependent stimulation, the methylation level of the *IL-2* promoter is reduced, leading to a rapid increase in IL-2 production.[27] In T cells from patients with SLE, the expression of IL-4 and IL-6 is significantly higher in comparison with controls. Treatment of healthy T cells with 5-azacitidine (5-azaC) demethylates the CpG islands in the promoters of *IL-4* or *IL-6* and up-regulates their expression. Hypomethylation of the *IL-4* and *IL-6* promoters contributes to the high expression of IL-4 and IL-6 in SLE and is associated with the disease severity.[28] Perforin, a cytotoxic effector molecule, is overexpressed in CD4 + T cells from patients with subacute cutaneous lupus erythematosus (SCLE) and has a role in the pathogenesis of this disease. Recent evidence suggests that perforin overexpression in SCLE is due to the hypomethylation of its promoter region.[29] CD40 ligand (CD40L), encoded on the X chromosome, has been reported to be overexpressed in SLE T cells. Both female lupus CD4 + T cells and CD4 + T cells treated with demethylating agents show overexpressed CD40L mRNA, and this is due to DNA demethylation in the CD40LG promoter.[30] In the past few years, accumulated evidence suggests a pivotal role for DNA methylation in the pathogenesis of SLE; however, the precise mechanisms remain incompletely understood.

Aberrant DNA methylation in SLE

Global DNA hypomethylation in SLE

The global level of DNA methylation is significantly decreased in peripheral blood mononuclear cells (PBMCs) of patients with SLE in comparison with that in controls. However, the expression of DNMT1 and MBD2 are up-regulated during SLE development, and there is no significant difference in the methylation levels and the expression of DNMT1 and MBD2 between patients with active and inactive SLE. A positive correlation between expression of DNMT1 and MBD2 has also been found in patients with SLE.[31,32]

DNA methylation in B cells may provide a new therapeutic target to control autoreactive B cells in

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SLE. Emerging evidence supports the fact that DNA methylation at CpG dinucleotides controls B-cell development and the elimination or inactivation of autoreactive B cells during SLE development.[33] DNA methylation in B cells also controls the expression of B-cell-specific factor Pax5, cytokine production and rearrangement of the B-cell receptor (BCR).[34,35] Among B cells, the CD5 + B cell is a kind of B-cell sub-population with hypomethylation status in its DNA sequence that leads to activation of normally repressed genes, such as the human endogenous retrovirus (HERV).[36] In patients with SLE, autoreactive B cells are characterized by their inability to induce DNA methylation that prolongs their survival, and this is confirmed by the finding that treating B cells with demethylating drugs can increase their autoreactivity.[37,38]

Accumulating evidence shows that the levels of DNA methylation in T cells are down-regulated during SLE development.[39–41] To understand the extent and nature of dysregulated DNA methylation in T cells of SLE, Jeffries and colleagues performed a genome-wide DNA methylation study in CD4 + T cells from SLE patients compared with healthy controls. In 27,578 CG sites located within the promoter regions of 14,495 genes, 236 hypomethylated and 105 hypermethylated CG sites in SLE CD4 + T cells were identified, consistent with widespread DNA methylation changes in SLE T cells. Of interest, several genes with known involvement in SLE pathogenesis, such as *CD9*, *MMP9* and *PDGFRA*, were significantly hypomethylated. Importantly, the *BST2* gene, which codes for an interferon-inducible membrane-bound protein that helps restrict the release of retroviral particles, was also hypomethylated in CD4 + T cells from patients with SLE. In addition, the transcription factor *RUNX3* was hypermethylated in SLE patients, indicating an impact on T-cell maturation. Protein–protein interaction maps identified that the transcription factor HNF4a displayed as a regulatory hub affecting a number of differentially methylated genes and that apoptosis was also an overrepresented ontology.[42]

Patients with SLE have significant global DNA hypomethylation when compared with healthy controls, and the global DNA methylation is inversely correlated with the SLE Disease Activity Index (SLEDAI), indicating that global DNA hypomethylation plays a critical role in SLE pathogenesis.[43] There is evidence suggesting that DNMT1 expression in patients with SLE is lower than that in controls, and lower expression of DNMT1, which is not the exclusive regulation factor of global DNA hypomethylation of SLE, may contribute to the development of SLE.[44] There is no direct correlation between DNMT3A expression and SLE pathogenesis. Furthermore, DNMT3B expression is down-regulated during SLE development, and there is no difference between patients with SLE and healthy controls. Global DNA hypomethylation in SLE has been confirmed, but its mechanism is complicated.[45] T lymphocytes from patients with SLE have a global decrease in DNA methylation level. Previous studies have provided evidence of hypomethylation in the promoters of several genes, but there is limited research into interspersed repetitive sequences (IRSs). Nakkuntod and colleagues demonstrated that hypomethylation of long interspersed nuclear element 1 s (LINE-1) but not Alu was found in CD4 + T lymphocytes, CD8 + T lymphocytes, and B lymphocytes of SLE patients. LINE-1 hypomethylation was more significantly distinguished in both CD4 + and CD8 + T lymphocytes of patients with active SLE when compared with an inactive group. The hypomethylation in each lymphocyte subset of SLE patients was IRSs specific, mainly found in LINE-1 rather than Alu.[46] DNA hypomethylation of CD4 + T cells may contribute to the development of drug-induced and idiopathic human lupus. Much evidence shows that inhibiting DNA methylation in mature CD4 + T cells causes MHC-specific autoreactivity in vitro, and the lupus-inducing drugs hydralazine and procainamide also inhibit T-cell DNA methylation and induce autoreactivity.[47] T cells from patients with active lupus have hypomethylated DNA and a similarly autoreactive T-cell subset, and T cells treated with DNA methylation inhibitors demethylate the same sequences in T cells from patients with inactive SLE.[48] Furthermore, mice receiving CD4 + T cells demethylated by a variety of agents including procainamide and hydralazine develop a lupus-like disease.[49] Transgenic mice with an inducible T-cell DNA methylation defect also develop lupus-like autoimmunity.[50]

Targets of DNA methylation in SLE

HERV

Several lines of evidence suggest that global hypomethylation and demethylation in the promoters of several genes in T cells from patients with active SLE may contribute to the pathogenesis of this disease.[51] However, data are scarce on methylation of retroelements in patients with SLE. Nakkuntod and colleagues investigated the methylated levels

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of human endogenous retroviruses (HERV)-E and HERV-K in SLE and normal CD3+CD4+T lymphocytes, CD8+T and B lymphocytes using combined bisulfite restriction analysis–IRSs. The results showed that HERV-E LTR2C methylation levels in CD3+CD4+T lymphocytes from active SLE were significantly lower when compared with inactive SLE and normal controls. However, HERV-K LTR5_Hs methylation levels in CD3+CD4+T lymphocytes from patients with inactive SLE were significantly lower than those in active SLE and normal controls. Furthermore, the hypomethylation of HERV-E LTR2C in active SLE was positively correlated with lymphopenia, whereas the hypomethylation of HERV-K LTR5_Hs was significantly correlated with SLEDAI score and complement activity.[52]

IL10

Recent evidence suggests that the etiology of SLE involves a complex interaction of genetic and environmental factors. Investigations have shown that environmentally driven epigenetic changes participate in the pathogenesis of SLE. IL-10 plays an important role in Th2 cell differentiation and autoantibody production in patients with SLE, and the mechanisms leading to IL10 overexpression in SLE patients are unclear. DNA methylation level within the *IL-10* gene promoter is reduced in SLE CD4+T cells when compared with healthy controls, and the reduced DNA methylation level negatively correlates with IL10 expression.[53] Lin and colleagues investigated the DNA methylation changes between 12 pairs of SLE patients and healthy controls. The results showed that the methylation statuses of *IL-10* genes were significantly reduced in the SLE patient samples when compared with healthy controls, and that there was a trend toward SLE patients having hypomethylated *IL-10* genes accompanied by greater disease activity.[54]

IL13

IL-13 plays an important role in Th2 cell differentiation and autoantibody production in patients with SLE, and the mechanisms leading to IL13 overexpression in SLE patients are unclear. During SLE development, *IL-13* mRNA in CD4+T cells is up-regulated, and the protein level in the serum of patients with SLE is also increased. The level of DNA methylation within the *IL-13* gene promoter is reduced in SLE CD4+T cells when compared with healthy controls, and the reduced DNA methylation level negatively correlates with IL13 expression.[53,55]

IL1R2

Lin and colleagues[54] hypothesized that aberrant DNA methylation might contribute to the activation of the abnormal immune response and trigger SLE disease activity. They investigated the DNA methylation changes between 12 pairs of SLE patients and healthy controls, and the results were further confirmed in 66 SLE patients and 102 healthy controls. The results showed that the methylation status of the *IL1R2* gene was significantly reduced in the SLE patient samples when compared with healthy controls, and that there was a trend toward SLE patients having hypomethylated *IL1R2* genes accompanied by greater disease activity. Thus, DNA hypomethylation should be associated with the pathogenesis of SLE, and the hypomethylated IL1R2 may provide a potential diagnostic marker and therapeutic target for SLE.[54]

CD5

A defect in DNA methylation favors the development of SLE by preventing the effects of SHP-1. Based on current findings, CD5-positive B cells have been considered to play a paradoxical role in preventing, rather than inducing, autoimmunity during disease development.[56] There is evidence that the membrane expression of CD5 can be regulated, and the molecular mechanism has been described, based on the selection between exon 1A and exon 1B. The full-length protein variant, encoded by exon 1A-cd5, translocates the phosphatase SHP-1 to the vicinity of the BCR, leading to an inhibition of B-cell autoreactivity. In contrast, the truncated variant, encoded by exon 1B-cd5, remains in the cytoplasm and retains the SHP-1 translocation. Normally, exon 1B expression is silenced by DNA methylation and its product is degraded by proteosomes, indicating the critical role of gene methylation in SLE pathogenesis.[57]

CD70

CD70 (TNFSF7) is a B-cell costimulatory molecule that contributes to excessive B-cell activation, and increased CD70 in the CD4+T cells of patients with SLE promotes development of SLE. There is evidence which shows that DNA demethylation in *CD70* promoter sequences regulates the expression of CD70 in SLE CD4+T cells. Furthermore, the demethylation of promoter sequences contributes to the overexpression of CD70 in CD4+T cells from patients with SCLE.[58] Zhang et al. provided a novel approach for measuring the methylation status of *ITGAL* and *CD70* promoter sequences. The procedure combines bisulfite sequencing PCR

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(BSP) with high-throughput oligonucleotide microarrays that allows for rapid quantification of deoxycytosine and deoxymethylcytosine content in bisulfite-treated DNA samples. The microarray is first used to generate a standard curve from fully methylated and fully unmethylated DNA samples in the form of a one-dimensional linear equation that calculates fluorescence emission as methylation levels; the microarray prediction is found to be highly accurate when compared with BSP. The new microarray-based assay may provide a rapid, reliable, and effective diagnostic and prognostic service for patients with SLE.[59]

PP2Acα

The expression of the catalytic subunit α isoform of protein phosphatase 2A (PP2Acα) is up-regulated in T cells from patients with SLE, and increased PP2Acα contributes to decreased IL-2 production.[60] *PP2Acα* promoter activity is controlled through the methylation of the cAMP response element (CRE) motif defined by its promoter, and hypomethylation may account for the increased expression of PP2Acα in patients with SLE. The CRE-defined CpG, which binds p-CREB, is significantly less methylated in SLE T cells compared with normal controls, and the level of methylation correlates with decreased amounts of DNMT1 transcripts. Methylation intensity correlates inversely with expression level of PP2Acα and SLE disease activity.[61]

Epstein–Barr virus

Epstein–Barr virus (EBV) is a human herpesvirus found in the memory B cells of the majority of the world population, and it has been suggested that plays it a role in the pathogenesis of SLE. Generally, EBV infection will cause infectious mononucleosis, a self-limiting disease, and viral latency is associated with many types of cancer, some of which occur in immune-suppressed individuals. However, virus production does not occur in strict latency. Several lines of evidence suggest that DNA methylation and histone modifications control the expression of latent viral oncoproteins and non-translated RNAs that result either in a complete silencing of the EBV genome in memory B cells, or in a cell type-dependent usage of some latency promoters in lymphoblastoid cells, germinal center B cells and tumor cells.[62,63] Importantly, lytic EBV proteins can elicit a strong immune response, and enhanced lytic replication will result in new infection and transformation events, and thus is a risk factor both for malignant and autoimmune diseases. Increased viral replication and changed presentation of lytic or latent EBV proteins that cross-react with cellular antigens may trigger pathogenic processes through molecular mimicry that result in SLE.[64]

Mechanisms of DNA methylation in SLE

DNMTs in SLE

DNMT1

In the past few years, much evidence has shown that global DNA hypomethylation in CD4+T cells may play a critical role in the pathogenesis of SLE. However, the underlying mechanisms of global DNA hypomethylation in patients with SLE remain unclear. Recently, Qin and colleagues[65] explored the relationship between DNA hypomethylation patterns and transcript levels of DNMT1 and MBD2 in CD4+T cells from patients with SLE by real-time PCR and the Methyflash DNA methylation quantification kit. The global DNA methylation levels in SLE patients were significantly lower when compared with controls, and global DNA hypomethylation was inversely correlated with the SLEDAI. During SLE development, DNMT1 expression was significantly down-regulated and there was no correlation between DNMT1 levels and SLEDAI, but there was a positive correlation between DNMT1 expression and global DNA methylation. However, MBD2 was significantly up-regulated compared with controls in this process. There was a positive correlation between MBD2 expression and SLEDAI and an inverse correlation between *MBD2* mRNA level and global DNA methylation. It seems that aberrant expression of DNMT1 and MBD2 should be noted as important causes of the global hypomethylation observed in CD4+T cells in patients with SLE.[65] In recent years, emerging evidence has shown that different blood cell populations of patients with SLE are characterized by a global DNA hypomethylation. The DNA hypomethylation is generally associated with defects in the ERK signaling pathway and DNMT1 down-regulation. DNA methylation inhibitors are also known to induce autoreactivity in vitro and cause the SLE disease in vivo. Among the identified targets undergoing demethylation are genes involved in autoreactivity (*ITGAL*), antigen presentation (*CSF3R*), inflammation (*MMP14*), B-cell and T-cell interaction (*CD70* and *CD40L*), cytokine pathways (*CSF3R*, *IL-4*, *IL-6* and *IFNGR2*) and osmotic lysis and apoptosis (*PRF1*, *MMP14* and *LCN2*).[66]

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DNMT3
DNA methylation participates in the pathogenesis of SLE, and there is emerging evidence that DNMTs are involved. For example, DNMT3A expression is up-regulated in SLE patients when compared with age-matched healthy controls, and this increase is associated with a higher SLEDAI. More striking is that the expression levels of DNMT3A for African-American women are higher than for European-American women in SLE populations, and a subset of African-American women on dehydroepiandrosterone therapy showed a significant decrease in DNMT3A expression in comparison with SLE patients not on the therapy.[67] DNMT3B expression is also up-regulated significantly in patients with SLE when compared with age-matched healthy controls, but no significant difference is noted in DNMT1 expression between SLE patients and healthy controls. Thus, African-American women have a higher incidence of SLE; however, the effects of dehydroepiandrosterone therapy on DNMT3A expression in African-American women need further investigation in a larger population.[68]

Regulatory factors

CREM alpha
The transcriptional regulator cAMP responsive element modulator α (CREM alpha) is seen as a key molecule that drives autoimmunity. In addition to its regulatory functions on gene promoters in T lymphocytes, recent evidence indicates that CREM alpha alters the epigenetic conformation of cytokine genes by interacting with enzymes that control CpG methylation as well as histone methylation and acetylation.[69]

IL-17A is a proinflammatory cytokine that is produced by specialized T helper cells and contributes to the development of several autoimmune diseases such as SLE. Transcription factor CREM alpha displays up-regulated levels in T cells from patients with SLE, and has been described to account for aberrant T-cell function in the pathogenesis of SLE. There is evidence that CREM alpha physically binds to a cAMP responsive element, CRE, within the proximal region in the human *IL-17A* promoter and increases its transcriptional activity. Activated naïve CD4 + T cells and T cells from patients with SLE display increased CREM alpha binding to this site compared with controls, and the histone H3 modifications at the CRE site and neighboring conserved non-coding sequences within the human *IL-17A* gene locus provide an accessible chromatin structure for activated gene expression, including H3K27 hypomethylation and H3K18 hyperacetylation in activated naïve CD4(+) T cells and SLE T cells. Furthermore, H3K27 hypomethylation is accompanied by DNA methylation in these regions in SLE T cells, and reporter studies confirm that DNA methylation of the *IL-17A* promoter indeed abrogates its inducibility. It is confirmed that CREM alpha has an important role in the pathogenesis of SLE because it contributes to the disorder of DNA methylation in histone H3.[70] The proinflammatory cytokines IL-17A and IL-17F are primarily produced by Th17 lymphocytes, and both are involved in host defense mechanisms against bacterial and fungal pathogens. Recent evidence suggests that IL-17F contributes to the development of various autoimmune diseases, such as SLE, rheumatoid arthritis, and systemic sclerosis.[71,72] T lymphocytes from patients with SLE display increased expression of transcription factor CREM alpha, which has been documented to participate in the pathogenesis of SLE and account for aberrant T-cell function in SLE patients. During SLE development, IL-17F expression is reduced in SLE T cells, and CREM alpha binds to a yet-unidentified CRE site within the proximal promoter. However, reduced IL-17F expression in SLE T lymphocytes is independent of activating epigenetic patterns, including CpG-DNA demethylation, increased histone H3 Lys-18 acetylation, and reduced histone H3 Lys-27 trimethylation. Thus, CREM alpha is involved in cytokine dysregulation in SLE by contributing to a disrupted balance between IL-17A and IL-17F, and an increased IL-17A/IL-17F ratio may contribute to the proinflammatory phenotype of SLE (Figure 1).[73]

IL-2 is a key cytokine during the proliferation and activation of T lymphocytes, and the absence of IL-2 has been linked to the development of autoimmune disease in humans and animals. SLE is a multifactorial autoimmune disease and is characterized by dysregulation of transcription factor and cytokine expression, lymphocyte function, and antigen presentation. Reduced IL-2 expression is a hallmark of SLE T lymphocytes, and its down-regulation results in decreased numbers of regulatory T lymphocytes which play an important role in preventing autoimmunity.[74] Furthermore, down-regulation of IL-2 expression leads to overproduction of the transcription regulatory factor CREM alpha in T lymphocytes from patients with SLE and subsequent CREM alpha binding to a CRE site within the *IL-2* promoter. Increased CREM alpha binding to the *IL-2* promoter has been reported to result in *IL-2* silencing that is mediated by several

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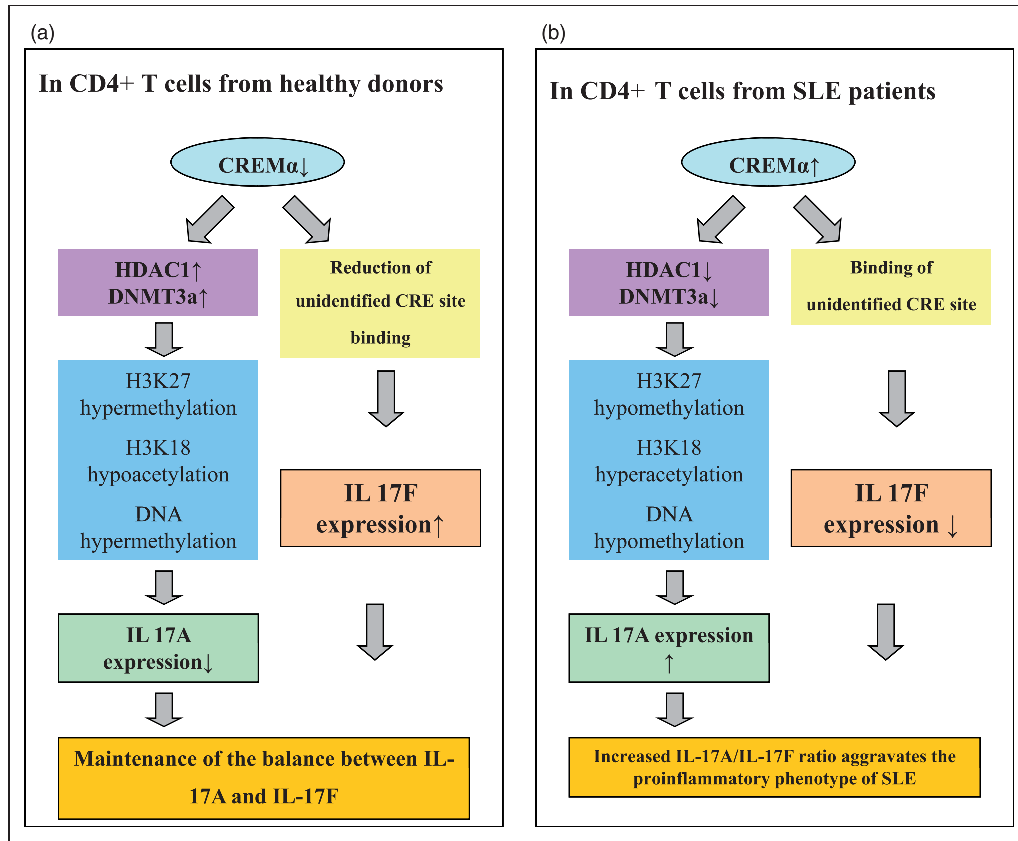

Figure 1 CREM alpha displays increased expression levels in T cells from SLE patients, and CREM alpha physically binds to a cAMP responsive element, CRE (−111/−104), within the proximal human IL17A promoter and increases its activity. The histone H3 modification pattern at the CRE site and neighboring conserved non-coding sequences within the IL17A gene suggests an accessible chromatin structure (H3K27 hypomethylation and H3K18 hyperacetylation) in SLE T cells. H3K27 hypomethylation is accompanied by decreased CpG-DNA methylation in these regions in SLE T cells. Decreased recruitment of histone deacetylase 1 and DNMT3a to the CRE site account for the observed epigenetic alterations. In addition, IL 17F expression is reduced in SLE T cells, and CREM alpha binds to a yet-unidentified CRE site within its proximal promoter. CREM alpha contributes to a disrupted balance between IL 17A and IL 17F and increased IL 17A/IL 17F ratio may aggravate the proinflammatory phenotype of SLE.

mechanisms, such as HDAC1 recruitment through CREM alpha, the failure to activate the histone acetyltransferase p300 by CREM alpha, and trans-repression of the IL2 promoter. Still, the involvement of these mechanisms in IL-2 silencing in SLE T lymphocytes has not yet been demonstrated. Hedrich and colleagues[75] demonstrated that the IL2 gene in T lymphocytes from SLE patients had undergone epigenetic modifications at different levels. CREM alpha mediates gene-wide histone deacetylation in primary T lymphocytes from SLE patients, and increased CREM alpha-mediated DNA methylation and histone H3K27 trimethylation through *DNMT3A* were demonstrated.[75] Moderate expression of IL-2 plays a pivotal role during the development and differentiation of T cells, and a tight balance between IL-2 and the effector cytokine IL-17A is essential for immune homeostasis in health and disease. It is suggested that DNA methylation plays a critical role in cytokine regulation during T-cell lineage commitment, but the molecular mechanisms that induce chromatin remodeling are less well understood. Hedrich and colleagues investigated epigenetic regulators that mediated the diametric expression of IL-2 and IL-17A in naive, central memory, and effector memory CD4+T cells and found that CREM alpha contributed to epigenetic remodeling of IL2 in effector memory T cells through the recruitment of *DNMT3A*. Interestingly, CREM alpha expression can be regulated at the epigenetic level by DNA methylation, which allows increased CREM alpha expression in effector memory CD4+T cells. As we know, T cells from patients with SLE express increased CREM alpha and exhibit a phenotype that is similar to effector memory CD4+T cells with epigenetically predetermined expression patterns of IL-2 and

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IL-17A. Thus, CREM alpha controls IL2 and IL17A expression during CD4 lineage commitment and subset distribution in SLE.[76]

RFX1

In healthy controls, regulatory factor X-box 1 (RFX1) can recruit DNMT1 and histone deacetylase 1 (HDAC1), resulting in a silencing of target genes. Down-regulated RFX1 contributes to DNA hypomethylation and histone H3 hyperacetylation at the *cluster of differentiation (CD) 11a* and *CD70* promoters in CD4+T cells from patients with SLE, leading to ITGAL and CD70 overexpression and autoimmune response hyperactivation.[77,78] Zhao and colleagues found that RFX1 was involved in regulating histone 3 lysine 9 (H3K9) trimethylation at the *ITGAL* and *CD70* promoters in SLE CD4+T cells.[78] In CD4+T cells from SLE patients, RFX1 recruited histone methyltransferase suppressor of variegation 3–9 (Drosophila) homolog 1 (SUV39H1) at the *ITGAL* and *CD70* promoters and promoted the methylation of these genes. During SLE development, decreased RFX1 resulted in reduction the H3K9 trimethylation levels within the *ITGAL* and *CD70* promoter regions in CD4+T cells. RFX1 expression correlated with H3K9 trimethylation levels, as well as ITGAL and CD70 expression levels in SLE CD4+T cells. These findings suggest that RFX1 will decrease local H3K9 trimethylation levels and promote disease development when it cannot interact with SUV39H1 in the promoter regions of the *ITGAL* and *CD70* genes in SLE CD4+T cells.[78]

GADD45α

Demethylation of *ITGAL* and *CD70* regulatory regions in CD4+T cells contributes to the autoimmune reaction and autoantigen generation in SLE, and the growth arrest and DNA damage-induced 45α(GADD45α) reduces the epigenetic silencing of target genes by reducing the methylation levels.[79] Investigating whether GADD45α contributed to SLE pathogenesis by inducing DNA hypomethylation in T cells from SLE patients, Li and colleagues[80] showed that increased GADD45A expression and global DNA hypomethylation were observed in CD4+T cells from patients with SLE. *GADD45A* gene expression was inversely proportional to the levels of DNA methylation, and positive correlations were found between GADD45A and the levels of ITGAL and CD70. Moreover, transfection of CD4+T cells with GADD45A significantly up-regulated the expression of ITGAL, and CD70, and autoreactivity and excessive B-cell stimulation was increased at the same time. Transfection of GADD45A small interfering RNA significantly inhibited the autoreactivity of SLE CD4+T cells and resulted in significant increases of methylation levels in the *ITGAL* and *CD70* promoter sequences.[80]

CD40L

CD40L, which is encoded on the X chromosome, has been reported to be overexpressed in T cells from patients with SLE. Both female lupus CD4+T cells and CD4+T cells treated with demethylating agents show overexpressed CD40L and overstimulate B cells to produce IgG. This is due to DNA demethylation and thereby reactivation of the inactive X chromosome in females.[81] Recently, another report showed that the expression of ITGAL, PRF1 and CD70 was significantly elevated in CD4+T cells from patients with SLE, and there was a positive correlation between CD40L and ITGAL as well as between CD40L and PRF1. These findings suggest that the tight association of CD40L with ITGAL and PRF1 probably contributes to the pathogenesis of the disease.[82]

Signaling pathway

Serotonin system

The occurrence of SLE involves a gene–environment interaction, and epigenetic modifications, such as DNA methylation, may contribute to the etiology of SLE. Accumulating evidence shows that the neurotransmitter serotonin regulates the activation and proliferation of B cells and T cells via the 5-HT1A receptor, participating in SLE pathogenesis. Xu and colleagues[83] showed a significant hypomethylation of PR-HTR1A in patients with SLE compared with healthy controls, and SLE patients also showed a significantly higher *HTR1A* mRNA level than did the controls. Relatively higher expression of anti-histone antibodies in methylated SLE patients was found compared with unmethylated patients. These results indicate that DNA methylation via the serotonin system may contribute to SLE occurrence and development, and reveal the link between the brain and the immune system.[83]

Notch signaling

T lymphocytes in patients with SLE display a severely altered phenotype with several molecular and functional aberrations, including defective capacities to up-regulate the Notch-1 receptor expression upon T-cell receptor activation. Notch signaling constitutes an evolutionarily conserved

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pathway that transduces signals between neighboring cells and determines major decisions in cell proliferation, differentiation and survival, and Notch signaling has been shown to play a critical role in T lymphocyte differentiation and function.[84] In various T-cell subpopulations from patients with active SLE, Notch-1 expression is decreased at the mRNA and protein levels, and the down-regulated *Notch-1* level inversely correlates with disease activity in SLE patients. Rauen and colleagues[85] suggested that DNA methylation in the human *Notch-1* promoter contributed to the decreased *Notch-1* expression in T cells from patients with SLE. Furthermore, CREM alpha, a key regulator of epigenetic patterns and gene transcription, was up-regulated in SLE T cells, and increased CREM alpha was bound to the *Notch-1* promoter, which eventually resulted in significantly reduced *Notch-1* promoter activity and gene transcription.[85]

ERK signaling

SLE is a poorly understood autoimmune disease, characterized by autoantibody production against nuclear antigens and immune complex deposition in organs such as the kidney. Some lines of evidence suggest that the extracellular signal-regulated kinase (ERK) has an important role in the pathogenesis of SLE.[86,87] For example, hydralazine, a lupus-inducing drug, can decrease T-cell DNA methylation by inhibiting the activity of the ERK signaling pathway, replicating the disease characteristics found in SLE T cells. These observations suggest that defective ERK signaling in SLE T cells contributes to SLE pathogenesis by inhibiting DNA methylation. Understanding the mechanism causing decreased ERK pathway signaling in lupus may help us understand the mechanisms contributing to SLE pathogenesis in genetically predisposed people.[88]

X chromosome

SLE is less common in men than women, and the molecular mechanism for this is incompletely understood. Several lines of evidence suggest that SLE will develop when genetically predisposed individuals encounter environmental factors that trigger the disease, and that the environmental effect is mediated at least in part by T-cell DNA hypomethylation. The *PRF1* and *KIR2DL4 (KIR)* promoters were previously reported to demethylate in proportion to lupus disease activity as measured by the SLEDAI,[89,90] and Sawalha and his colleagues[91] therefore compared the degree of DNA methylation in these gene promoters in the same patients. The men tended to have slightly lower methylation levels than the women in the *KIR* promoter, but the difference was not statistically significant (p ¼ 0.16). In the *PRF1* promoter, there was a trend for the men to have lower methylation levels, but the difference was not significant (p ¼ 0.20). Since overall lupus severity correlated with increased total genetic risk, and the degree of T-cell DNA demethylation correlated with the severity of individual flares as determined by the SLEDAI,[89,90] Sawalha et al. compared the ratio of (genetic risk)/(DNA methylation) in each of the men and women with lupus at the same level of SLEDAI. After controlling for SLEDAI, the mean (genetic risk)/(KIR methylation) ratio overall is 0.0515 lower for women than men, with a p-value of 0.010, and the mean (genetic risk)/(PRF1 methylation) overall is 0.0757 lower for women than men, with a p-value of 0.0054. Thus, for both genes, combining the total genetic risk with the degree of DNA methylation in each subject demonstrates that women require a greater degree of DNA methylation and/or a lower total genetic risk to achieve a lupus flare equal in severity to men. This difference is not explained by a difference in the genetic risk or T-cell DNA hypomethylation alone, suggesting a genetic–epigenetic interaction. The interaction of genetic risk and T-cell DNA hypomethylation in patients with SLE influences disease severity, and men will require a higher genetic risk and greater degree of T-cell DNA hypomethylation to achieve a lupus flare equal in severity to women.[91] Based on the finding of Liao and his colleagues,[92] DNA demethylation contributes to CD40L expression in CD4 + T cells from patients with rheumatoid arthritis, and may in part explain the female preponderance of this disease. In addition, they have demonstrated that DNA demethylation of CD40L on the X chromosome is responsible for female susceptibility to SLE.[92]

There has been recent evidence that estrogen, environmental exposure and two X chromosomes are required for disease development in the females.[93] SLE is an autoimmune disease primarily afflicting women, and the reason for the gender bias is unclear, but estrogen, genetic susceptibility and environmental agents appear to play pivotal roles in its pathogenesis.[94] Strickland and colleagues[95] used (C57BL/6xSJL) F1 mice transgenic for a dominant-negative MEK (dnMEK) that was previously shown to be selectively expressed in T cells from patients with SLE. In this doxycycline-induced model, T-cell ERK signaling and DNMT expression were suppressed, leading to DNA

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demethylation in T cells, and the expression of immunity gene ITGAL, CD70 and anti-dsDNA antibody were up-regulated. It was confirmed that doxycycline induced IgG anti-dsDNA antibodies in placebo-treated control female but not male transgenic mice, and that glomerular IgG was deposited in the kidneys of female but not male transgenic mice, and not in the absence of doxycycline. In addition, estrogen enhanced anti-dsDNA IgG antibodies only in transgenic and ERK-impaired female mice, and decreased ERK activation also led to overexpression and demethylation of the X-linked methylation-sensitive gene CD40L in females, consistent with demethylation of the second X chromosome in the females. Thus, both female gender and estrogen contribute to the female predisposition in SLE susceptibility through hormonal, epigenetic X chromosome effects and the ERK signaling.[95]

SLE is a predominantly female autoimmune disease that affects multiple organ systems, and over the years several X chromosome-related genes have been extensively investigated in order to reveal the molecular mechanisms of the immune dysregulation in SLE. Sawalha et al.[96] found that *MECP2* was located on chromosome Xq28 and encoded for a protein that played a critical role in epigenetic transcriptional regulation of methylation-sensitive genes. Utilizing a candidate gene association approach, they genotyped 21 single nucleotide polymorphisms within and around MECP2 in SLE patients and controls, and identified the association between SLE and the genomic element containing MECP2 in two independent SLE cohorts from two ethnically divergent populations.[96] A combined forward and reverse genetic approach was undertaken to test the candidacy of interleukin-1 receptor associated kinase-1 (IRAK1) as an X chromosome-encoded risk factor for SLE. Using congenic mouse models bearing the disease loci Sle1 or Sle3, IRAK1 deficiency abrogated all lupus-associated phenotypes, including IgG and IgM autoantibodies, renal disease, and lymphocytic activation in both models. Moreover, the absence of IRAK1 reversed the dendritic cell ‘hyperactivity’ associated with Sle3. These findings suggest that the X chromosome gene *IRAK1* may be a disease susceptibility factor in human SLE, which raises the possibility that the gender difference in SLE may in part be attributed to sex chromosome genes.[97] Shen and his colleagues[98] identified and replicated association of a *Toll-like receptor 7 (TLR7)* 3’UTR SNP, rs3853839 (G/C), with SLE in 9274 Eastern Asians, with a stronger effect in male than female subjects. The results showed that G-allele carriers had increased TLR7 transcripts and a more pronounced IFN signature than C-allele carriers. Furthermore, heterozygotes had 2.7-fold higher transcripts of G-allele than C-allele. These data suggest a functional polymorphism in type I IFN pathway gene *TLR7* predisposing to SLE, especially in Chinese and Japanese male subjects.[98]

Conclusions and future perspectives

With all of the previously discussed research in mind, how may we now begin to develop a research program for an epigenetic-based treatment for patients with SLE? First, and possibly most straightforward, those genes known to be aberrantly methylated may be considered as effective therapeutic targets. Since these genes may be targeted with a number of gene-specific approaches and altered gene expression may affect a series of genes, a therapeutic approach targeting methylation-sensitive genes should face insurmountable technical obstacles. However, several attempts are promising for SLE treatment in the future.[99,100] For example, an immunosuppressant monoclonal antibody designed by Genentech, efalizumab (Raptiva®), targets ITGAL and is marketed as a treatment for psoriasis. Unfortunately, this drug was eventually withdrawn from the market due to adverse effects, such as viral meningitis, bacterial sepsis and progressive multifocal leukoencephalopathy.[101] A second approach involves epigenetic modification as a means of therapy. For example, specific transfection of CD4 + T cells with vectors encoding a methylase (perhaps DNMT1) under the control of a strong promoter will raise global methylation levels. However, increased DNMTs inevitably up-regulate global hypermethylated states. Similar states have been detailed described in leukemias and lymphoma, precisely the same cells to be targeted by a SLE treatment. However, this treatment approach for patients with SLE seems to be more tantalizing. 5-azadC is a kind of DNMT inhibitor that can inhibit the methyl group transfer to hemimethylated DNA strands during gene replication, resulting in DNA demethylation and up-regulation of gene expression.[102] 5-azadC is a promising cancer treatment drug and has been used in the experimental study of cancer treatment.[103,104] However, 5-azadC will also up-regulate global DNA hypomethylation in the treatment process. This difficulty is the main obstacle for the clinical application of 5-azadC. Site-directed DNA remethylation via fusion proteins consisting of

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methylase domains linked to site-specific DNA binding domains would remedy many of these issues, provided they had minimal off-target effects. In light of minimal off-target effects, site-directed remethylation via fusion proteins consisting of DNA methylase domains may be an alternative approach.[105]

MiRNAs are small, non-coding, 21–23 nucleotide-long RNAs, which mediate posttranscriptional silencing of target genes. Generally, miRNAs bind to partially complementary sites in the 3' untranslated region of target mRNAs, and efficient mRNA translation inhibition requires continuous base-pairing of miRNA nucleotides 2 to 8, the so-called 'seed sequence'.[106] In this way, miRNAs can regulate target gene expression by either mRNA degradation, translational inhibition, or both. It has been confirmed that aberrant miRNAs participate in the pathogenesis of human diseases, such as SLE, but the molecular mechanisms are unclear. Recent work has suggested that there is a crosstalk between miRNAs and DNA methylation in SLE and other autoimmune diseases, including rheumatoid arthritis, and the association between miRNAs and DNA methylation provides an insight into the role of DNA methylation in SLE CD4 + T-cell hypomethylation and the pathogenesis of SLE.[107]

Recent progress has been made in our understanding of DNA methylation mechanisms in SLE. Despite this, many unanswered questions remain. A growing body of literature supports the concept that aberrant DNA methylation in B cells and T cells, caused by environmental and genetic factors, contributes to SLE pathogenesis by altering the expression of methylation-associated genes in patients with SLE. Thus, preventing or correcting the altered methylation patterns, or designing therapies directed at SLE-associated genes, may be effective therapeutic approaches for SLE patients in the future.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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