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REVIEW

The role of epigenetic variation in the pathogenesis of systemic lupus erythematosus

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Abstract

The focus of the present review is on the extent to which epigenetic alterations influence the development of systemic lupus erythematosus. Lupus is a systemic autoimmune disease characterized by the production of autoantibodies directed at nuclear self-antigens. A DNA methylation defect in CD4+ T cells has long been observed in idiopathic and drug-induced lupus. Recent studies utilizing high-throughput technologies have further characterized the nature of the DNA methylation defect in lupus CD4+ T cells. Emerging evidence in the literature is revealing an increasingly interconnected network of epigenetic dysregulation in lupus. Recent reports describe variable expression of a number of regulatory microRNAs in lupus CD4+ T cells, some of which govern the expression of DNA methyltransferase 1. While studies to date have revealed a significant role for epigenetic defects in the pathogenesis of lupus, the causal nature of epigenetic variation in lupus remains elusive. Future longitudinal epigenetic studies in lupus are therefore needed.

Introduction

Systemic lupus erythematosus (SLE) is a prototypic auto-immune disease characterized by the production of autoantibodies directed against nuclear self-antigens. The role of genetic variation has been extensively investigated in lupus, revealing polymorphisms throughout the genome correlated with disease [1-3]. Although studies investigating common genetic variants in lupus have revealed a number of susceptibility loci, the cumulative effect size of these loci accounts for a small fraction of disease heritability, approximately 15 to 20% [4]. The inability of present genetic association studies to comprehensively account for disease heritability has led

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many to postulate that the missing heritability of complex human disease may broadly reside in epigenetic mechanisms [5].

Epigenetics refers to heritable modifications that regulate gene expression without changes to DNA sequence [6]. Common epigenetic mechanisms include DNA methylation and histone modification. MicroRNAs are also involved in epigenetic regulation. The most widely researched epigenetic modification to date is DNA methylation, which is known to have diverse regulatory function, governing gene expression patterns in a cell- and tissue-specific manner. Broadly speaking, DNA methylation is a negative regulator of gene expression whereby higher levels of promoter and CpG island methylation are typically observed to correlate with lower levels of gene expression. Aberrant DNA methylation and gene expression are observed in a number of conditions and diseases, including cancer, neuro-psychiatric disorders, and autoimmunity.

DNA methylation patterns are established during development and maintained throughout the life course by a class of enzymes known as DNA methyltransferases [7]. Further, widespread epigenomic differences are observed throughout cellular development [8,9]. Once established, patterns of DNA methylation are heritably preserved from parent to daughter cells during replication by DNA methyltransferase 1 (DNMT)1. DNMT1 associates with replication fork complex proteins where it transfers methylation patterns from the template strand to the incipient copy.

Histone proteins act to spatially organize chromatin and determine the extent to which chromatin is accessible for active transcription. Covalent modification of histone proteins represents another facet of epigenetic variation through which gene expression and chromatin structure are regulated. Methylation and acetylation of specific histone lysine residues act to regulate the transcriptional state of chromatin in *cis*. Locus-specific chromatin architecture varies between cell types, as genes are differentially expressed among different cells at specified times.

Epigenetic regulation acts to program gene expression patterns in the various cell types that comprise the tissues

and organ systems of the human body. In effect, epigenetic modification facilitates the differentiation of diverse cell types from static genomic DNA. The possibility for epigenetic variation underlying the disease process of lupus is intriguing as patients experience periods of calm punctuated by disease flares. Because epigenetic states vary with time and between cell types, it is in the context of disease activity that epigenetic variation in lupus is likely to stand in sharpest relief against the epigenome of normal tissues.

Epigenetic regulation in normal lymphocytes

The human immune system is composed of a number of cell types that serve specific functions in establishing and maintaining cell-mediated and humoral immunity. The diversity of lymphocyte populations that comprise the human immune system is established and maintained through epigenetic modification. Distinct and highly conserved epigenetic profiles of DNA methylation are observed in normal B and T lymphocytes [10,11]. While DNA methylation patterns are broadly conserved within cell types, widespread differences are observed between lymphocyte subtypes. DNMT1 function and DNA methylation patterns are essential in ensuring T cell differentiation and function [12]. DNA methylation and histone modifications reinforce repressive and permissive chromatin states at key regulatory loci in CD4+ T cells lineages [13,14].

Demethylation and changes in chromatin architecture at transcription factor and cytokine genes accompany effector cell differentiation in response to cytokine signaling among lymphocyte populations. Chromatin remodeling of the *IL17* locus is observed upon differentiation of Th17 cells [15]. Likewise, DNA demethylation and histone acetylation of the *IFNG* (IFN-γ) and the *IL4-IL5-IL13* genetic loci, upon Th1 and Th2 differentiation, respectively, is mediated by T cell lineage-specific transcription factors following T-cell receptor signaling [16]. Indeed, Th2-specific chromatin remodeling and demethylation takes place in a locus control region that flanks a number of Th2 cytokines [17]. Further, these epigenetic changes are accompanied by changes in long-range interactions and chromatin architecture at cytokine gene loci under Th1 and Th2 polarizing conditions [18].

Drug-induced lupus points to epigenetic dysregulation in autoimmunity

While approximately 90% of lupus cases are idiopathic, prolonged exposure to certain medications is known to induce lupus. It was eventually discovered that procainamide and hydralazine, medications that can cause lupus, inhibit DNA methylation in T cells [19]. It was further shown that *in vitro* treatment of Th2 cells with procainamide or 5-azacytidine (a DNA methylation

inhibitor) leads to their autoreactivity, and upon adoptive transfer these cells are capable of inducing autoimmunity in mice [20].

A better understanding of the mechanisms through which drug-induced lupus occurs has provided insight into the epigenetic mechanisms and pathways involved in idiopathic lupus. Global hypomethylation of genomic DNA is observed in CD4+ T cells of lupus patients [21]. This decline is attributable to a reduction in methyltransferase activity. Procainamide acts as a competitive inhibitor of DNMT1 [22]. Hydralazine reduces DNA methylation via inhibiting extracellular signal-regulated kinase (ERK) pathway signaling [23], which regulates the expression of DNMT1 [24,25]. The ERK pathway further ensures proper immune cell development and function [26].

Reduced ERK pathway signaling in lupus CD4+ T cells

An ERK pathway signaling defect has been observed in lupus CD4+ T cells [27,28] and has been attributed to originate from decreased signaling through protein kinase C delta in lupus CD4+ T cells [29]. Decreased protein kinase C signaling may further account for defective T cell activation in lupus [30]. Additionally, mice lacking the protein kinase C delta gene display germinal center formation in the absence of stimulation and develop autoimmunity [31].

A reduction of ERK pathway signaling leading to reduced expression of DNMT1 has been shown to lead to T cell autoreactivity. Mice that express a dominant-negative form of the ERK pathway component MEK show reduced ERK pathway signaling and reduced expression of DNMT1. This leads to overexpression of methylation-sensitive genes, anti-double stranded DNA antibody production, and activation of IFN-regulated signaling pathways similar to lupus patients [32].

Sex-chromosome complement and skewed X chromosome inactivation in lupus

Lupus is a sexually dimorphic autoimmune disease with a 9:1 female bias, a fact that makes sex chromosome complement and skewed X chromosome inactivation interesting candidates for investigating the origins of the disease. Under normal circumstances, females have only one X chromosome from which genes are actively transcribed, while the additional X chromosome remains transcriptionally inactive through epigenetic repression. As previously stated, widespread demethylation of genomic DNA is observed in lupus CD4+ T cells, and this, it is thought, might lead to the reactivation of methylation-sensitive genes on the normally inactive X chromosome. An example of the reactivation of a gene on the inactive X chromosome in lupus CD4+ T cells is

that of CD40 ligand (*CD40LG*). CD40LG is a B-cell co-stimulatory molecule that, upon binding with CD40, promotes B cell activation, plasma cell differentiation, immunoglobulin production and antibody class switching [33,34]. Overexpression of CD40LG is observed in lupus CD4+ and CD8+ T cells as well as lupus B cells [35]. CD4+ T cells from women but not men overexpress CD40LG when treated with 5-azacytidine, while the overexpression of autosomal methylation-sensitive genes is comparable between the sexes [36]. CD40LG-transfected normal human T cells lead to autologous B cell activation and plasma cell differentiation in co-culture assays [37]. Further, T cells from women but not men increase B cell IgG production when treated with DNA methylation inhibitors, which can be inhibited by anti-CD40LG antibody [37].

There is evidence for skewed X chromosome inactivation in a number of other autoimmune diseases apart from lupus, including autoimmune thyroiditis and scleroderma, many of which also have a strong female bias [38]. Further evidence for the role of sex chromosome complement in the pathogenesis of lupus comes from Klinefelter's syndrome (47, XXY), a condition in which phenotypic male individuals possess an additional X chromosome. It was found that lupus is approximately 14 times more frequent among men with Klinefelter's syndrome than it is among (46, XY) men, a rate similar to that observed among women [39].

Aberrant methylation and gene expression in lupus CD4+ T cells

Global reduction of DNA methylation is a hallmark of CD4+ T cells in lupus (Figure 1). Several autosomal genes are known to be hypomethylated in lupus CD4+ T cells. Many of these genes encode proteins involved in processes related to immune function and inflammation. It is thought that the overexpression of methylation-sensitive genes leads to lupus T cell autoreactivity and subsequent induction of autoreactive B cell immunoglobulin production.

Lymphocyte function-associated antigen 1 (LFA-1) is a multi-unit, integrin family protein involved in lymphocyte adhesion that is composed of the products of two genes, *CD11a* and *CD18*. DNA methylation and local chromatin architecture regulate the tissue-specific expression of *CD11a* [40]. Promoter hypomethylation of the *ITGAL* (*CD11a*) gene is observed in lupus CD4+ T cells [41]. This leads to overexpression of LFA-1 among an autoreactive T-cell subset. Further, ectopic expression of *CD18* recapitulates the effects of treatment with any of the multiple DNA methylation inhibitors in inducing T-cell autoreactivity [42]. LFA-1 overexpression is a driver of autoreactivity directly linked to reduced levels of DNA methylation.
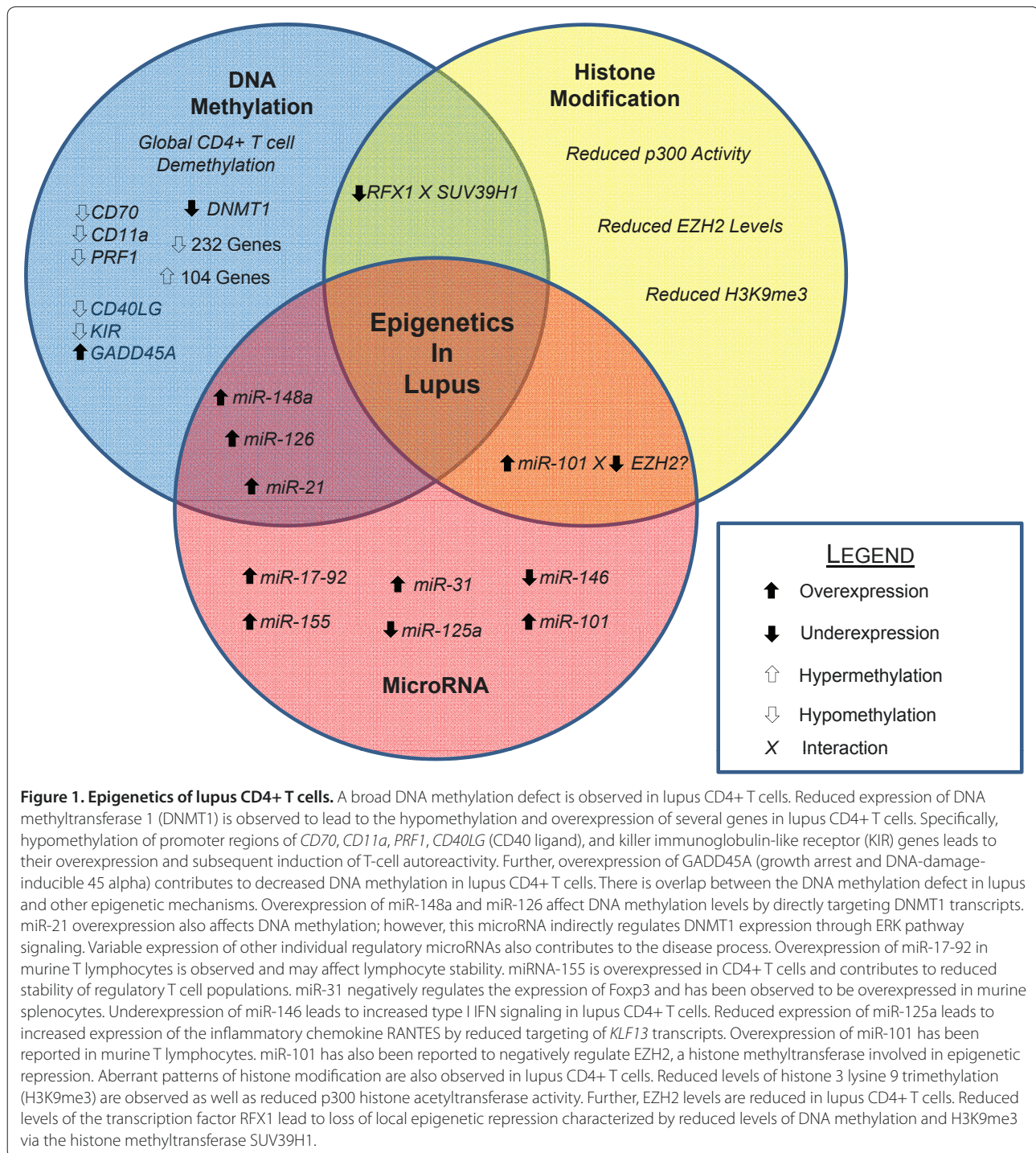
CD70 (TNFSF7) is a membrane bound, B-cell co-stimulatory protein involved in regulating B-cell immunoglobulin production. CD70 is overexpressed in lupus CD4+ T cells and T cells treated with DNA methylation inhibitors, and this overexpression stimulates B cell IgG production *in vitro* [43]. Indeed, the promoter sequence of *CD70* that demethylates upon treating normal CD4+ T cells with DNA methylation inhibitors is similarly demethylated in lupus CD4+ T cells [44]. Reduced methylation of a CpG island in the *CD70* promoter was also observed to correspond with higher CD70 expression and reduced *DNMT1* mRNA levels in MRL/*lpr* mice at 16 weeks when autoimmunity is established [45]. Overexpression of CD70 in response to reduced promoter methylation is also observed in patients with subacute cutaneous lupus erythematosus. Increased expression was observed at both the mRNA and protein level by flow cytometry, and although the overall proportion of CD70-expressing cells was unchanged, the mean florescent intensity was greater among subacute cutaneous lupus erythematosus patient samples [46].

Many transcription factors are known to influence local chromatin architecture through specific interaction with DNA and histone methyltransferases. Decreased levels of RFX1 at *CD11a* and *CD70* promoters leads to decreased methylation and increased expression of both of these genes in lupus CD4+ T cells [47]. Moreover, this change in DNA methylation and expression is reinforced by concomitant reductions in histone 3 lysine 9 trimethylation (H3K9me3) at these promoters in a RFX1-SUV39H1 interaction-dependent manner [48].

A role for the *Gadd45A* (growth arrest and DNA-damage-inducible 45 alpha) gene in autoimmunity has long been suggested. Initial knock-out studies of *Gadd45a* reported by Salvador and colleagues revealed a protective role for the gene product, as knockout Gadd45A-/- mice grew to develop a lupus-like systemic autoimmune disease [49]. More recently, transfection of HEK293 cells with a *Gadd45A* overexpression vector was shown to result in both site-specific and global DNA demethylation. Further, increased expression of *Gadd45A* following exposure to ultraviolet light was shown to lead to reduced genomic DNA methylation, a trend partially counteracted by knockdown of *Gadd45A* [50]. These findings were recapitulated in recent work in CD4+ T cells in which overexpression of *Gadd45A* and exposure to UV-B light radiation led to increased expression and promoter demethylation of *CD70* and *CD11a* [51].

Perforin is a protein encoded by the *PRF1* gene, expressed primarily by CD8+ T lymphocytes and natural killer cells. Perforin acts to promote target cell lysis by promoting the formation of pores in the target cell membrane. Initially, overexpression of perforin in T cells was observed in response to treatment with DNA

Figure 1. Epigenetics of lupus CD4+ T cells. A broad DNA methylation defect is observed in lupus CD4+ T cells. Reduced expression of DNA methyltransferase 1 (DNMT1) is observed to lead to the hypomethylation and overexpression of several genes in lupus CD4+ T cells. Specifically, hypomethylation of promoter regions of *CD70*, *CD11a*, *PRF1*, *CD40LG* (CD40 ligand), and killer immunoglobulin-like receptor (KIR) genes leads to their overexpression and subsequent induction of T-cell autoreactivity. Further, overexpression of GADD45A (growth arrest and DNA-damage-inducible 45 alpha) contributes to decreased DNA methylation in lupus CD4+ T cells. There is overlap between the DNA methylation defect in lupus and other epigenetic mechanisms. Overexpression of miR-148a and miR-126 affect DNA methylation levels by directly targeting DNMT1 transcripts. miR-21 overexpression also affects DNA methylation; however, this microRNA indirectly regulates DNMT1 expression through ERK pathway signaling. Variable expression of other individual regulatory microRNAs also contributes to the disease process. Overexpression of miR-17-92 in murine T lymphocytes is observed and may affect lymphocyte stability. miRNA-155 is overexpressed in CD4+ T cells and contributes to reduced stability of regulatory T cell populations. miR-31 negatively regulates the expression of Foxp3 and has been observed to be overexpressed in murine splenocytes. Underexpression of miR-146 leads to increased type I IFN signaling in lupus CD4+ T cells. Reduced expression of miR-125a leads to increased expression of the inflammatory chemokine RANTES by reduced targeting of *KLF13* transcripts. Overexpression of miR-101 has been reported in murine T lymphocytes. miR-101 has also been reported to negatively regulate EZH2, a histone methyltransferase involved in epigenetic repression. Aberrant patterns of histone modification are also observed in lupus CD4+ T cells. Reduced levels of histone 3 lysine 9 trimethylation (H3K9me3) are observed as well as reduced p300 histone acetyltransferase activity. Further, EZH2 levels are reduced in lupus CD4+ T cells. Reduced levels of the transcription factor RFX1 lead to loss of local epigenetic repression characterized by reduced levels of DNA methylation and H3K9me3 via the histone methyltransferase SUV39H1.

methylation inhibitors. Promoter demethylation and perforin overexpression were accompanied by changes in chromatin architecture as evidenced by the gain of a DNAse hypersensitive site upstream of the *PRF1* transcription start site [52]. Further, this trend of *PRF1* promoter demethylation leading to increased perforin expression was then confirmed in lupus CD4+ T cells,

where increased perforin was observed via flow cytometry in CD4+ T cells of lupus patients with active disease [53].

Killer immunoglobulin-like receptors (KIRs) comprise a diverse set of polymorphic genes involved in HLA type I stimulatory and inhibitory signals in natural killer cells and CD4+ CD28- T cells [54]. Expression of KIR genes is regulated by methylation of their promoter regions, and

treatment of T cells with 5-azacytidine leads to activation of a number KIR genes [55]. Interestingly, overexpression of KIR genes is also observed in lupus CD4+ T cells, and this overexpression is seen to correspond to disease activity [56].

DNA methylation in lupus B cells

Several genes are observed to be differentially regulated in lupus B cells. Surface expression of CD5, which is important in maintaining B cell anergy [57], is known to be reduced in lupus B cells. IL-6 has been observed to alter CD5 expression levels in lupus B cells by inhibiting DNA methylation [58]. Increased exposure to IL-6 leads to ERK pathway inhibition, decreased DNMT1 levels, and increased expression of CD5-1EB, the cytoplasmic form of CD5, which in turn limits the expression of CD5 on the cell surface. This trend was observed in lupus B cells and confirmed among B cells from healthy controls exposed to increased levels of IL-6 or treated with DNA methylation inhibitors [58].

Widespread DNA methylation differences between lupus patients and healthy controls

Studies in disease-discordant monozygotic twins are useful when investigating epigenetic aspects of complex human disease. Monozygotic twin studies enable the control of many of the confounding influences of genetic variation, although there is evidence for copy number variation among monozygotic twins [59], while focusing on disease-relevant, inter-individual epigenetic variation. Monozygotic twins display significant life course variation in epigenetic modifications, and this variation, it is thought, may drive disease pathogenesis [60]. A recent twin study in lupus revealed significant epigenetic variation in white blood cells between disease-discordant monozygotic twins [61]. The study reported and validated a number of genes found to possess promoter methylation differences. Specifically, 49 genes were found to be hypomethylated in lupus patients, highlighting ontologies relevant to autoimmunity. In addition, variable methylation of ribosomal genes was reported whereby 18S and 28S ribosomal subunit genes are hypomethylated in lupus individuals of discordant pairs and hypomethylated in both individuals of concordant lupus twin pairs examined.

A recent case-control methylation study utilizing high-throughput methylation arrays identified a number of differentially methylated genes and pathways in lupus CD4+ T cells. The methylation status of over 27,000 CpG sites in promoter regions of nearly 15,000 genes was collected and analyzed for 12 female lupus cases and 12 controls. In the study, Jeffries and colleagues report hypomethylation of 236 CpG sites and hypermethylation of another 105 (representing 232 and 104 genes, respectively). Among hypomethylated genes, ontology analysis
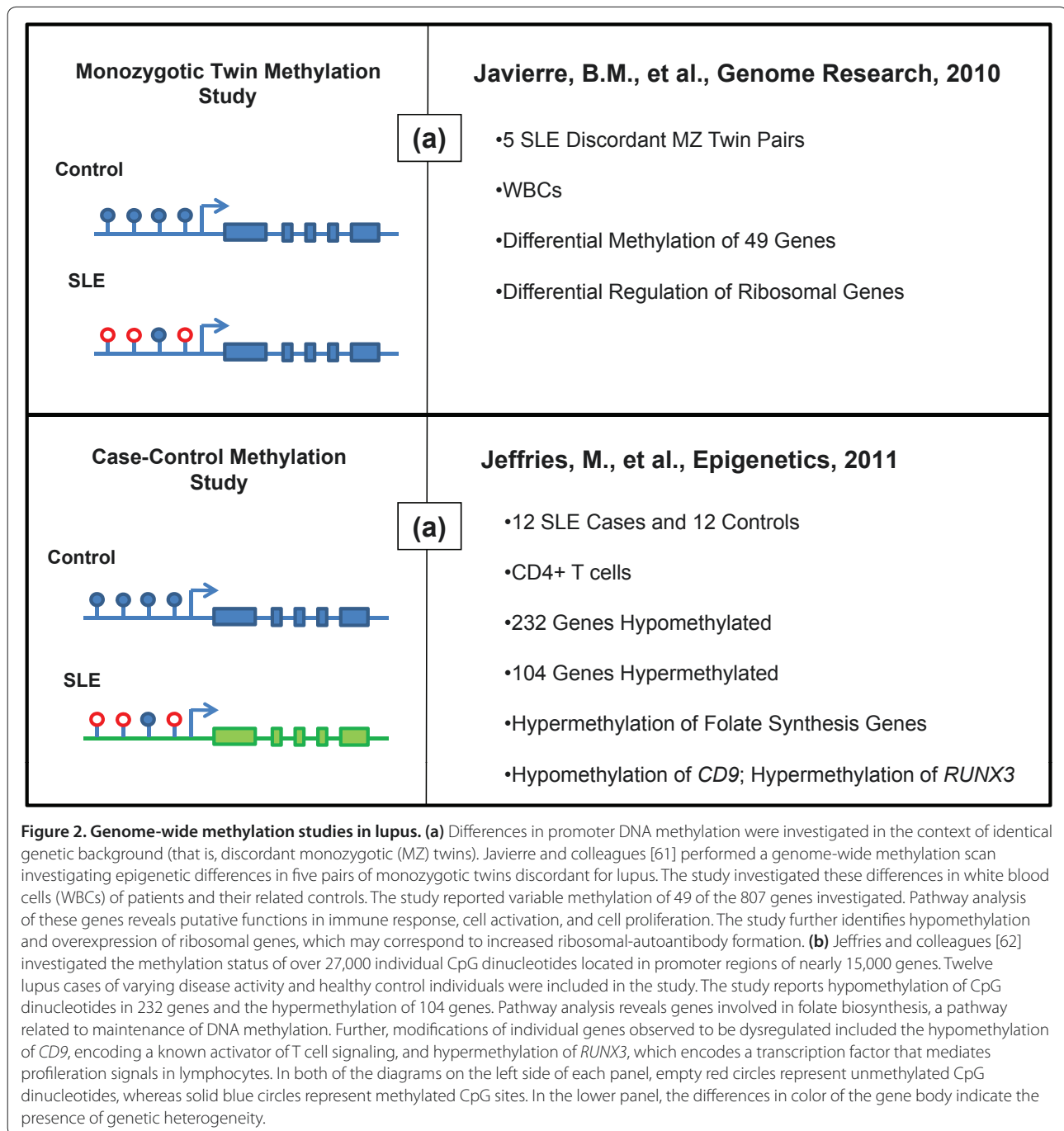
highlighted genes involved in connective tissue development, including *ADAMTS1*, *ALX4*, *CD9*, *ESRRA*, *FGF8*, *HOXA13*, *HOXD11*, *MMP9*, *MSX1*, *PDGFRA*, and *SOX5* [62]. Hypermethylation of genes involved in folate biosynthesis, a pathway related to maintenance of DNA methylation, was observed [62]. Interestingly, *RUNX3*, which belongs to a transcription factor family involved in mediating cell proliferation signals [63], was found to be hypermethylated in lupus CD4+ T cells. Protein interaction network analysis also revealed differential methylation of several apoptosis-associated genes, and genes involved in cell growth, tissue development, and cell division. This study also provided data to support a potential role for some specific DNA methylation changes as novel biomarkers for lupus disease activity, with the caveat that these need to be first validated and then examined in the setting of a clinical trial before being considered of any clinical benefit (Figure 2).

Variable regulation of microRNAs in lupus

MicroRNAs represent a post-transcriptional regulatory mechanism whereby single-stranded RNAs 20 to 24 bp in length homologously bind to mRNA transcripts, effectively blocking their subsequent translation [64]. MicroRNA regulatory networks work to fine-tune immune function. Further, differential regulation of microRNAs appears to play a role in the disease process of lupus.

MiR-146, which plays a role in negatively regulating innate immune responses [65], has been observed to be intrinsically underexpressed in lupus CD4+ T cells. Reduced expression of miR-146 leads to induction of type I interferon signaling, and miR-146 expression levels negatively correlate with disease activity. Interestingly, IFN signatures of lupus patient peripheral blood mononuclear cells were alleviated upon transfection with a miR-146 expression vector [66]. Zhao and colleagues observed targeting of the 3' UTR of the *KLF13* gene by miR-125a in a dose-dependent manner. Underexpression of miR-125a was also reported in lupus CD4+ T cells leading to increased levels of the inflammatory chemokine RANTES [67]. Normalization of miR-125a expression in CD4+ T cells of lupus patients resulted in reduced RANTES levels.

Investigation of microRNA patterns in three models of murine lupus reveals common microRNA pathways. The study investigated common defects in regulatory microRNAs among splenic T and B lymphocytes in B6/*lpr*, MRL/*lpr*, and NZB/W mice when compared with their respective age-matched control mice. Dai and colleagues report a number of regulatory microRNAs, including miR-155, miR-150, miR-182-96-183, miR-31, miR-127, and miR-379, to be significantly increased in splenic B and T cells of MRL/*lpr* mice. Significantly, upregulation of miR-146, miR101a, and miR-17-92 in

Figure 2. Genome-wide methylation studies in lupus. (a) Differences in promoter DNA methylation were investigated in the context of identical genetic background (that is, discordant monozygotic (MZ) twins). Javierre and colleagues [61] performed a genome-wide methylation scan investigating epigenetic differences in five pairs of monozygotic twins discordant for lupus. The study investigated these differences in white blood cells (WBCs) of patients and their related controls. The study reported variable methylation of 49 of the 807 genes investigated. Pathway analysis of these genes reveals putative functions in immune response, cell activation, and cell proliferation. The study further identifies hypomethylation and overexpression of ribosomal genes, which may correspond to increased ribosomal-autoantibody formation. **(b)** Jeffries and colleagues [62] investigated the methylation status of over 27,000 individual CpG dinucleotides located in promoter regions of nearly 15,000 genes. Twelve lupus cases of varying disease activity and healthy control individuals were included in the study. The study reports hypomethylation of CpG dinucleotides in 232 genes and the hypermethylation of 104 genes. Pathway analysis reveals genes involved in folate biosynthesis, a pathway related to maintenance of DNA methylation. Further, modifications of individual genes observed to be dysregulated included the hypomethylation of *CD9*, encoding a known activator of T cell signaling, and hypermethylation of *RUNX3*, which encodes a transcription factor that mediates profileration signals in lymphocytes. In both of the diagrams on the left side of each panel, empty red circles represent unmethylated CpG dinucleotides, whereas solid blue circles represent methylated CpG sites. In the lower panel, the differences in color of the gene body indicate the presence of genetic heterogeneity.

MRL/*lpr* mice was observed exclusively among T lymphocytes [68]. Mice with increased miR-17-92 expression display uncontrolled expansion and autoimmunity characterized by reductions in the proapoptotic protein Bim and the tumor suppressor PTEN (phosphatase and tensin homolog) [69]. Interestingly, genomic loss of miR-101 has been associated with overexpression of the repressive histone methyltransferase EZH2 in human prostate cancer cells [70]. EZH2, the histone methyltransferase that mediates H3K27me3, functionally associates with DNMT1 [71]. Overexpression of miR-101 might contribute to the loss of repression observed in lupus CD4+ T cells. Moreover, downregulation of EZH2 in CD4+ T cells has been reported in lupus patients with active disease [72].

A systematic, microarray-based investigation of micro-RNA expression in Epstein-Barr virus-transformed B cell lines of lupus patients with nephritis and controls in

Caucasian and African-American populations observed reproducible overexpression of a number of microRNAs across ethnicities [73]. This study also included a discordant monozygotic twin pair in which the broader microRNA expression patterns were partially conserved.

More recently, analysis of microRNA expression profiles in lupus peripheral blood mononuclear cells compared to controls revealed the differential expression of 27 microRNAs out of 365 analyzed [74]. Importantly, the levels of miR-21, miR-25, miR-106b and miR-148b correlated with disease activity in lupus patients, while miR-196a and miR-379 expression inversely correlated with disease activity, suggesting a potential role for microRNA profiling as disease biomarker in lupus [74].

A recent comprehensive review on the role of micro-RNA in the pathogenesis of lupus and other autoimmune disease has been recently published [75].

Crosstalk between microRNAs and DNA methylation in lupus

Targeting of DNA methyltransferases by regulatory micro-RNAs is observed in both development and disease. MicroRNAs appear to play a critical role in regulation of *de novo* methylation in mouse embryonic stem cells [76]. Targeting of DNMT3a and DNMT3b by miR-29 family members is observed in human lung cancer [77]. Two recent studies demonstrate downregulation of DNMT1 as a result of variable expression of regulatory microRNAs in lupus.

Pan and colleagues [78] demonstrated a connection between aberrant regulation of certain microRNAs and reduced expression of DNMT1. A genome-wide micro-RNA scan in both lupus patients and MRL/*lpr* lupus-prone mice revealed increased expression of both miR-21 and miR-148a. miR-21 targets and inhibits RSGRP1, a signaling molecule involved in the ERK signaling pathway, through which it is able to indirectly downregulate DNMT1 expression. MiR-148a, however, directly targets the DNMT1 transcript.

MiR-126 is among a number of microRNAs observed to be variably regulated in lupus CD4+ T cells. Zhao and colleagues [79] report overexpression of miR-126 in lupus CD4+ T cells, which correlates with decreased DNMT1 expression. They further demonstrate that miR-126 targets a 3' UTR region in *DNMT1* and reduces DNMT1 protein expression. Furthermore, ectopic over-expression of miR-126 in CD4+ T cells from healthy donors led to hypomethylation and overexpression of the methylation sensitive genes *CD11a* and *CD70* and induction of T cell autoreactivity [79].

Variable histone protein modification in lupus

Covalent modification of histone protein represents another layer of epigenetic regulation. Acetylation of histone tails leads to an open conformation of chromatin that is permissive to gene expression. The acetyltransferase activity of p300 in B cells is crucial in preventing autoimmunity. Mice with a B cell-specific knock-in mutant version of p300 that lacks only histone acetyltransferase activity develop a lupus-like autoimmune disease characterized by splenomegaly, nephritis, and vasculitis [80]. Reduced global levels of H3K9me3 and histone 3 acetylation have been reported in lupus CD4+ T cells, and differences in the activity of histone acetyltransferases have been observed in lupus CD4+ T cells [72].

Sullivan and colleagues [81] initially reported variability in histone 4 acetylation at the tumor necrosis factor alpha (TNF-α) locus in lupus monocytes. This same group further studied histone 4 acetylation at the genome level in lupus monocytes where they found hyperacetylation and overexpression of several genes with modest overlap with the acetylation and expression patterns of IFN-α treated cells [82]. These findings point to widespread changes in chromatin architecture in lupus monocytes.

The altered epigenetic state of regulatory T cell populations in murine lupus

Foxp3+ CD25high regulatory T cells broadly serve to suppress autoimmunity. Expression of the transcription factor Foxp3 regulates the differentiation of the regulatory T cell lineage [83]. Mice that lack Foxp3 display a paucity of regulatory T cells and subsequently develop fatal autoimmunity [84]. Epigenetic modifications regulate the emergence and durability of regulatory T cells. Demethylation of the *Foxp3* promoter accompanies the emergence of the regulatory T cell lineage in both mice and humans [85,86]. Foxp3 expression is also governed post-transcriptionally by microRNAs. Specifically, miR-31 is a negative regulator of Foxp3 expression, while miR-21 positively regulates Foxp3 [87].

Regulatory T cells of lupus patients with active disease are reduced in quantity commensurate to disease severity and display decreased suppressive function [88,89]. Divekar and colleagues [90] have recently reported a regulatory T cell defect of murine lupus whereby regulatory T cells exhibit reduced suppressive potential in MRL/*lpr* mice that is further characterized by decreased dicer expression and increased miR-155 expression. Interestingly, miR-155 plays a critical role in the regulation of germinal center formation and is critical in maintaining proper immune function [91,92]. Divekar and colleagues suggest that increased expression of miR-155 may account for the regulatory T cell defect observed in MRL/*lpr* mice. These findings further coincide with previous reports in which dicer deficiency among regulatory T cells led to diminished regulatory T cell populations and autoimmune pathology [93,94].

Table 1. Epigenetic perturbations observed in lupus T cells

Gene/pathway	Epigenetic defect	Cell type	Consequence	Reference
ERK pathway	Defective signaling	Human and murine T cells	Reduced DNMT1 expression and reduced T cell DNA methylation	[23,32]
PRF1	Hypomethylation	CD4+ T cells	Increased perforin expression	[52,53]
ITGAL	Hypomethylation	CD4+ T cells	Increased CD11a expression	[40,41]
CD40LG	Hypomethylation	CD4+ T cells	B-cell hyperactivation	[36]
KIR2DL4 (KIR)	Hypomethylation	CD4+ T cells	Increased KIR expression	[55,56]
TNFSF7	Hypomethylation	CD4+ T cells	Increased CD70 expression	[43-45]
GADD45A	Overexpression	CD4+ T cells	Decreased DNA methylation	[51]
Folate biosynthesis	Hypermethylation	CD4+ T cells	Impaired DNA methylation?	[62]
RUNX3	Hypermethylation	CD4+ T cells	Impaired T cell proliferation?	[62]
CD9	Hypomethylation	CD4+ T cells	Increased T cell activation?	[62]
MMP9	Hypomethylation	CD4+ T cells	?	[62]
PDGFRA	Hypomethylation	CD4+ T cells	?	[62]
SOX5	Hypomethylation	CD4+ T cells	?	[62]
miR-126	Overexpression	CD4+ T cells	Downregulation of DNMT1	[79]
miR-146	Underexpression	CD4+ T cells; murine T splenocytes	Increased type I IFN	[66,68]
miR-17-92	Overexpression	Murine T splenocytes	Lymphocyte stability	[68]
miR-155	Overexpression	CD4+ T cells; CD4+ CD25+ T cells	Reduced Treg cell stability	[90]
miR-101a	Overexpression	Murine T splenocytes	Reduced EZH2?	[68]
miR-31	Overexpression	Regulatory T cells; murine T splenocytes	Repression of FOXP3	[62,87]
miR-21	Overexpression	CD4+ T cells	Targets ERK pathway leading to lower DNMT1	[78]
miR-125a	Underexpression	CD4+ T cells	Targets KLF13 leading to increased RANTES	[67]
miR-148a	Overexpression	CD4+ T cells	Downregulates DNMT1	[78]
RFX1	Underexpression	CD4+ T cells	Reduced H3K9me3 and DNA methylation	[47,48]

CD40LG, CD40 ligand; DNMT, DNA methyltransferase; ERK, extracellular signal-regulated kinase; GADD45A, growth arrest and DNA-damage-inducible 45 alpha; H3K9me3, histone 3 lysine 9 trimethylation; KIR, killer immunoglobulin-like receptor; Treg, regulatory T cell.

Epigenetic variation in lupus: cause or consequence?

This review offers clear evidence of the involvement of epigenetic variation in lupus. The studies mentioned herein describe widespread lupus-associated epigenetic variation, yet association and causation are not interchangeable terms. Many questions regarding the causal nature of epigenetic variation in lupus remain to be answered. At what stage of disease are epigenetic manifestations first present? In what cell type(s) do epigenetic perturbations first appear in lupus? Does epigenetic variation merely reflect prior canalization of disease, or are these modifications ultimately causal? Is disease-associated epigenetic variation a proximate or an ultimate cause of disease?

The causal nature of epigenetic variation in lupus is a complicated question. In our view, large longitudinal cohort studies that will allow the epigenome to be examined before and after disease will be crucial to answer this question. Of course, these types of studies will be complicated to design, and samples from specific cell types will have to be maintained and subsequently examined. The evidence that inhibiting T cell ERK pathway signaling using a mouse model results in decreased DNMT1 expression, overexpression of methylation sensitive genes similar to lupus patients, the development of anti-double stranded DNA antibody, and an interferon signature-like gene expression profile represents the strongest argument for a causal role of DNA methylation in lupus to date [32]. Recent studies have shown that epigenetic variation in lupus occurs along multiple axes. Unraveling the causal web of lupus epigenetics has become an increasingly complicated task as recent studies have revealed a complex, interconnected network of epigenetic regulation that leads to altered gene expression in lupus. These studies pose new questions about the nature of complexity of regulatory events involved in lupus pathogenesis. Future epigenetic studies

investigating the origins of complex human disease will therefore need to incorporate multiple-level systems modeling and complexity science to uncover disease etiology in complex and dynamic biologic systems.

Determining the environmental-epigenetic interactions that contribute to lupus is important in understanding the ultimate cause of lupus. There is much reference made to environmental factors in the lupus epigenetics literature, yet these typically refer to a few examples or are invoked as an unknown explanatory variable. With a few notable exceptions, the specific mechanisms through which environmental triggers operate remain largely uncharacterized to date. Further characterization of the putative environmental-epigenetic tipping point between active and inactive disease is therefore needed.

Conclusion

Studies to date have revealed a substantial epigenetic component of disease in lupus, particularly in T cells (Table 1, Figure 1), yet ultimately the causal nature of disease associated epigenetic variation remains ambiguous. Although the etiology of lupus is indeterminate, some generalizations about the origins of lupus can be made: first, there is a significant genetic component to the disease; second, known genetic variation does not comprehensively describe the origin, development, and even the heritability of the disease; third, epigenetic variation correlates with variable expression of a number of genes in pathways involved in disease pathogenesis; and fourth, lupus is the result of complex interactions between genetic susceptibility, environmental exposure, and epimutation. The path forward in understanding lupus is not merely in the clear delineation of the various components of disease but in understanding emergent phenotypes that result from their interaction over time.

Abbreviations

CD40LG, CD40 ligand; DNMT, DNA methyltransferase; ERK, extracellular signal-regulated kinase; GADD45A, growth arrest and DNA-damage-inducible 45 alpha; H3K9me3, histone 3 lysine 9 trimethylation; IFN, interferon; IL, interleukin; KIR, killer immunoglobulin-like receptor; LFA-1, lymphocyte function-associated antigen 1; UTR, untranslated region.

Competing interests

The authors declare that they have no competing interests.

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