Aberrant Epigenetic Regulation in the Pathogenesis of Systemic Lupus Erythematosus and Its Implication in Precision Medicine

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DNA methylation/demethylation · Epigenetic regulation · Histone modification · miRNA · Pathogenesis · Precision medicine · Systemic lupus erythematosus

Abstract
Great progress has been made in the last decades in understanding the complex immune dysregulation in systemic lupus erythematosus (SLE), yet the efforts to pursue an effective treatment of SLE proved to be futile. The pathoetiology of SLE involves extremely complicated and multifactorial interaction among various genetic and epigenetic factors. Multiple gene loci predispose to disease susceptibility, and the interaction with epigenetic modifications mediated through sex, hormones, and the hypothalamo-pituitary-adrenal axis complicates susceptibility and manifestations of this disease. Finally, certain environmental and psychological factors probably trigger the disease via epigenetic mechanisms. In this review, we summarize and discuss recent epigenetic studies of SLE and suggest a personalized approach to the dissection of disease onset and therapy or precision medicine. We speculate that in the future, precision medicine based on epigenetic and genetic information could help guide more effective targeted therapeutic intervention.

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease with a diverse array of clinical manifestations [Fairhurst et al., 2006; Tsokos, 2011]. SLE is characterized by the presence of anti-nuclear autoantibodies produced by uncontrolled over-activated B cells [Javierre and Richardson, 2011]. Deposit of the autoantibodies bound to their autoantigen causes chronic inflammation and tissue damage in many parts of the body. Although the exact etiology of SLE is unknown, it is clear that genetic predisposition is involved in its etiopathology [Anaya et al., 2006]. Extensive studies reported that genes of the major histocompatibility complex and human leukocyte antigen genes are strongly associated with SLE [Lie and Thorsby, 2005; Relle and Schwarting, 2012]. Other susceptibility genes such as IL10, TNF, STAT4, PTPN22, BANK1, and ICAM3 also show extensive involvement in major immune response pathways, both innate and adaptive immune systems, and in the pathogenesis of SLE [Lund et al., 2004; Wu et al., 2005; Blenman et al., 2006; Aringer and Smolen, 2008; Han et al., 2009; Yang et al., 2010; Okada et al., 2012; Piotrowski et al., 2012; Zhernakova et al., 2013; Deng and Tsao, 2014; Lee et al., 2014].

Genetic factors clearly create a predisposition towards SLE, whereas the incomplete disease concordance between identical twins suggests that environmental factors
also have an essential role in the onset and progression of SLE [Javierre et al., 2010].

Epigenetics refers to inheritable modifications that regulate gene expression and subsequently affect cellular functions without changing the DNA sequence [Bird, 2007; Wilson, 2008]. The research efforts accumulated in the last decades have been able to track the pathogenic origin of SLE to both genetic susceptibility and epigenetic variations arising from environmental factors [Sestak et al., 2007; Morel, 2010; Hedrich and Tsokos, 2011; Hughes and Sawalha, 2011; Deng and Tsao, 2014]. For example, environmental factors such as smoking, nutrition, viral infection, and the exposure to chemicals can trigger immunologic events to eventually result in SLE via epigenetic mechanisms [Costenbader et al., 2012]. Also, genetic aberrations in SLE and the epigenetic modifications often interact and complicate SLE progress by dysregulating various pro-inflammatory gene expressions [Maciejew ska Rodrigues et al., 2009; Brooks et al., 2010]. Therefore, identifying detailed epigenetic mechanisms and searching for therapy for SLE have been of significant interest in SLE research. The complexity of SLE dictates a more personalized method of therapy. In this review, we summarize and discuss recent scientific advancements in epigenetics of SLE, with a focus on prospective candidates for epigenetic therapy and personalized methods.

**Epigenetic Mechanisms of SLE**

So far, there are 3 widely accepted key epigenetic mechanisms playing an important role in the pathogenesis of SLE, including DNA methylation, histone modifications and altered miRNA profiling [Hedrich and Tsokos, 2011] (fig. 1).

**DNA Methylation/Demethylation**

DNA methylation involves the addition of a methyl group to the 5' carbon position of the pyrimidine or the purine ring. In general, DNA methylation represses gene

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**Fig. 1.** Key epigenetic mechanisms including DNA methylation, histone modifications, and altered miRNA profiling play an important role in the pathogenesis of SLE. The mechanisms are discovered in CD4+ T cells, B cells, PBMCs, or monocytes. One representative nucleosome cartoon is given as an example of genes that are found epigenetically modified in SLE patients. For specific affected genes, please refer to tables 1 and 2.
transcription in vivo by blocking the accessibility of transcription factors and RNA polymerases [Wolffe and Matzke, 1999]. The reaction is catalyzed and maintained by the DNA methyltransferases family (DNMTs). Among them, DNMT1 mainly contributes to maintaining the methylation status during cell division; DNMT3A and 3B are de novo DNMTs responsible for new methylation during embryonic development; DNMT3L is a cofactor helping DNMT3A/B activity [Rountree et al., 2001].

DNMT1 function and the DNA methylation pattern are essential in ensuring repressive and permissive epigenetic states at important gene loci in CD4+ T cells. Global hypomethylation in promoters of genes such as CD40L, CD70, IL10, IL13, PRF1, CSF3R, TNFSF7, ITGAL (CD11A), and IFNGR2 is observed in CD4+ T cells of SLE patients [Richardson et al., 1990; Kaplan et al., 2004; Oelke et al., 2004; Lu et al., 2007; Zhao et al., 2010b; Zhu et al., 2011; Lin et al., 2012] concurrently associated with decreased expression of methylation-related genes such as DNMT1 [Richardson et al., 1990]. This hypomethylation occurs in CD4+ T cells before, throughout, and even beyond active stages of SLE disease. A genome-wide DNA methylation study in lupus patients and healthy controls found significant hypomethylation in interferon (IFN)-regulated genes in naïve T cells including IFIT1/3, IFI44L, USP18, TRIM22, MX1, STAT1, and BST2 [Coit et al., 2013]. Possibly due to lack of transcription factors in naïve T cells, these hypomethylated genes were not found overexpressed there, in contrast to their overexpression in CD4+ T cells from lupus patients. Nevertheless, these hypomethylated IFN-regulated genes may explain the hyper-responsiveness to type I IFN in lupus T cells. The degree of hypomethylation correlates with disease activity of SLE (table 1). The genes that are affected are often involved in the type I IFN pathway, autoantibody production, and tissue damage, all playing an important role in the pathogenesis of SLE [Kato and Fujita, 2015].

Similarly, in other cell types such as B cells and peripheral blood mononuclear cells (PBMCs), promoters of genes such as CD5, HRES1, and lipocalin-2 (LCN2) were also reported to be hypomethylated in SLE patients [Garaud et al., 2009; Fali et al., 2014] (table 1). Hypomethylation of CD5 could unlock its inhibitory effect on B cell activation, whereas that of HRES1 could lead to induction of cross-reactive autoantibodies. A recent genome-wide DNA methylation study generated maps to document interindividual epigenetic variation from a cohort of healthy individuals by using neutrophils [Chatterjee et al., 2015]. In this study, 12,851 autosomal interindividually variably methylated fragments (iVMFs) were identified and localized in different elements of the genome such as promoters, gene body, and regions far upstream of the gene. These iVMFs were integrated with chromatin marks such as histone marks, enhancers, and transcription factor binding sites as well as repetitive elements to investigate the potential role of these methylation variations in genome regulation. The authors found that the highest DNA methylation variation lay in gene body and upstream regions, whereas the lowest was observed in promoters. The iVMFs were mainly associated with tran-

### Table 1. Aberrant DNA methylation in SLE

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Gene affected</th>
<th>DNA modifications</th>
<th>Associated function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td>PRF1, CSF3R, CD40L, CD70, IFNGR2</td>
<td>hypomethylation</td>
<td>monocyte killing, antigen presentation, female predelection of the disease, overproduction of IgG, development of idiopathic SLE, increased IFNa and IFN-inducible genes, autoantibody production and tissue damage</td>
<td>Kaplan et al., 2004; Javierre and Richardson, 2011; Oelke et al., 2004; Lu et al., 2002; Coit et al., 2013; Zhao et al., 2010b; Hedrich et al., 2014</td>
</tr>
<tr>
<td>B cells</td>
<td>CD5, HRES1</td>
<td>hypomethylation</td>
<td>increased B cell activation, induction of cross-reactive autoantibodies</td>
<td>Garaud et al., 2009; Fali et al., 2014</td>
</tr>
<tr>
<td>PBMCs</td>
<td>IFNGR2, LCN2</td>
<td>hypomethylation</td>
<td>lymphadenopathy and kidney damage, a common marker for lupus nephritis</td>
<td>Schwarting et al., 1998; Javierre et al., 2010; Javierre et al., 2010</td>
</tr>
<tr>
<td>Whole blood</td>
<td>IFIG44L</td>
<td>hypomethylation</td>
<td>a highly specific and sensitive biomarker</td>
<td>Zhao et al., 2016b</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>MX1, IFIG44L, IFITM1, PARP9, IFIT3, DDX60, LY6E, ISG15</td>
<td>hypomethylation</td>
<td>increased production of type I IFN driving abnormal B cell differentiation</td>
<td>Coit et al., 2015b</td>
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scription regulation, responsive function, and signal transduction pathways, and positively correlated with inclusion of the exon when found at differentially expressed exons. Neutrophils are also found to play an important role in the pathogenesis of lupus. Coit et al. (2015b) recently performed an epigenome-wide study between lupus patients and controls and identified 293 differentially methylated CG sites in neutrophils, of which the majority (68%) were hypomethylated. Among the differentially methylated sites, IFN signature genes were found demethylated in a robust and consistent way in lupus neutrophils compared to controls (table 1), suggesting a pathogenic role for neutrophils in lupus.

Most recently, our lab discovered significant hypomethylation of 2 CpG sites within the IFI44L promoter in patients with SLE (Zhao et al., 2016b). The significant cut-off methylation level of these 2 sites was highly specific and sensitive for SLE. Due to the distinct differences in the methylation levels of the IFI44L promoter in SLE patients, other than those in healthy subjects and other autoimmune disease patients, it was suggested to be a highly sensitive and specific diagnostic marker for SLE.

Equally important and coupled with DNA methylation is DNA demethylation, a reaction of removing a methyl group from 5-methylcytosine in DNA. DNA demethylation can be either passive or active (Bhutani et al., 2011). 5-Methylcytosine can be oxidized to 5-hydroxymethylcytosine (5hmC), then to 5-formylcytosine and finally to 5-carboxycytosine by the ten-eleven translocation (TET) family enzymes which are also known as DNA hydroxymethylases and include TET1, TET2, and TET3 (Tahiliani et al., 2009; Booth et al., 2012). 5hmC can then be actively reverted to cytosine through iterative oxidation and thymine DNA glycosylase-mediated base excision repair (He et al., 2011). Deletion of TET2 in T cells resulted in decreased cytokine expression by Th1 and Th17 cells, a consequence of reduced 5hmC and key transcription factor binding (Ichiyama et al., 2015). Recently, our lab has found that in SLE CD4+ T cells, the level of overall 5hmC and hydroxymethylation in the promoters of most of the genes were increased (Zhao et al., 2016a). Of the genes, 2,748 fall in the categories of neurotrophin, MAPK, calcium or mTOR signaling pathway. 131 of the genes are immune-related, such as SOCS1, NR2F6, and IL15RA. A transcription factor, CCCTC-binding factor (CTCF), was involved in regulating the promoter hydroxymethylation of the genes. The increased 5hmC and hydroxymethylation may partly account for the overexpression of the genes. These results provide a novel mechanism to explain the critical role that hydroxymethylation plays in aberrantly regulating gene transcription in SLE. Early work revealed that different DNA methylation inhibitors like 5-azacytidine, procarinamide, and hydralazine all induce SLE or lupus-like disease (Gorelik et al., 2007; Hedrich and Tsokos, 2011; Ivanov et al., 2012). Demethylation is sufficient to induce SLE progression, since injection of demethylated CD4+ T cells into syngeneic hosts causes a lupus-like disease (Patel and Richardson, 2013). Demethylation of CD40L, a B lymphocyte co-stimulatory molecule on the female inactive X chromosome, upregulates its expression and may partially explain why SLE dominantly occurs in females. Since CD40L is demethylated and overexpressed in females with active SLE (Lu et al., 2007; Hewagama et al., 2013), 5-azacytidine treatment of female CD4+ cells causes hypomethylation and overexpression of CD40L (Lu et al., 2007), while a similar treatment did not induce CD40L in male CD4+ cells due to the fact that the male X chromosome was already demethylated at physiological conditions. Significantly enhanced expression of estrogen receptor (ER) α in lupus patients is associated with DNA demethylation of its gene in the promoter region (Liu et al., 2014). DNA hypomethylation can not only be passively induced by low expression of DNMTs, but also actively by activation-induced cytidine deaminase (AICDA). The AICDA gene is highly expressed in germinal center B cells. Its promoter region is hypermethylated resulting in gene suppression in naïve B cells (Fujimura et al., 2008). Activation of naïve B cells leads to demethylation of the AICDA gene and thus promotes activation of B cells and generation of IgG anti-nucleosomal antibodies of murine SLE (Fritz et al., 2013; Detanico et al., 2015).

Histone Modifications

Inside the nucleus, chromatin is organized as DNA coiled around core histones composed of H2A, H2B, H3, and H4. The core histones are predominantly globular except for their N-terminal tails. The unstructured N-terminal tails are flexible. Their accessibility to the transcription machinery is important for gene expression regulation. The accessibility is regulated by various post-translational modifications, including acetylation/deacetylation, methylation/demethylation, ubiquitination, phosphorylation, sumoylation, ADP-riboseylation, deamination, and proline isomerization (Hewagama and Richardson, 2009; Maciejewska Rodrigues et al., 2009). These post-translational modifications on histones are dynamic and rapidly changing. Gene expression in SLE regulated by histone acetylation/deacetylation and methylation/demethylation is extensively investigated.
Histone acetylation is catalyzed by histone acetyltransferase, adding an acetyl group to the lysine residue in the N-terminus of the protein. Generally, H3 and H4 hyperacetylated regions are more actively transcribed. Histone deacetylases (HDACs) remove acetyl groups from histone tails. Deacetylation usually tightens the chromatin structure leading to gene non-transcription [Kouzarides, 2007]. T cells and monocytes are both implicated in SLE pathogenesis and demonstrated abnormal H3 or H4 acetylation patterns in SLE patients (table 2). SLE CD4+ T cells had decreased overall acetylation of both H3 and H4, and the H3 acetylation level correlated inversely with SLE disease activity [Zhang et al., 2010]. SLE CD4+ T cells also had increased H3 acetylation at lysine 18, causing upregulation of IL10 [Hedrich et al., 2014]. Global H4 hyperacetylation resulted in upregulation of genes such as IRF1, RFX1, and BLIMPI (PRDM1) in SLE monocytes [Zhang et al., 2010].

Histone methylation also plays an important role in gene expression regulation. It adds 1–3 methyl groups to the lysine or arginine residues in the histone tails by histone methyltransferases [Clarke, 1993]. Histone methylation is a major contributor of epigenetic modification in regulating gene transcription and DNA repair [Bannister and Kouzarides, 2005]. The effect of histone methylation on gene regulation is dependent on the residues modified and the number of methyl groups that are added to the modified residues [Scharf and Imhof, 2011]. Methylation of histone H3 lysine 4 (H3K4) or histone H3 lysine 36 (H3K36) enhances gene expression, whereas H3K27 trimethylation (H3K27me3) is repressive [Berger, 2002]. Demethylation removes methyl groups through histone demethylases. Generally, histone methylation turns `on' gene transcription by loosening histone tails allowing for access of transcription factors, whereas demethylation acts in the opposite direction [Ho and Crabtree, 2010].

Hypomethylation at H3K9 has been reported in the CD4+ T cells of SLE patients (table 2). The expression level of the corresponding methyltransferases SUV39H2 and EZH2 was also decreased in the SLE CD4+ T cells [Hu et al., 2008]. Additionally, studies from hematopoietic progenitor kinase 1 (HPK1) knockout mice provided persuasive evidence for regulation of histone methylation in SLE. HPK1 was previously suggested to regulate mitogen-activated protein kinases (MAPK), nuclear factor-kB (NF-kB), and play a role in mouse CD4+ T-cell apoptosis. Studies revealed that HPK1 knockout mice developed severe autoimmune disease, similar to human SLE. In SLE patients, the repressive H3K27me3 mark was found enriched in the promoter region of HPK1 (MAP4K1) from CD4+ T cells [Gray, 2013] (table 2). Consequently, HPK1 expression levels in CD4+ T cells were significantly decreased in SLE patients, pointing to a possible role of HPK1 in the pathogenesis of SLE [Gray, 2013]. CD70 is an important T-cell co-stimulatory protein that is overexpressed in SLE. Permissive histone H3 acetylation and H3K4me2 were significantly elevated in patients with SLE. Interestingly, these modifications were positively correlated with CD70 mRNA levels in SLE CD4+ T cells and disease activity.

miRNAs

miRNAs are a group of short, ~22 nucleotide-long, noncoding RNA molecules, which mostly negatively regulate gene expression at post-transcriptional and post-translational levels. miRNAs execute gene silencing by complementary binding to the 3’ untranslated region (3’ UTR) of target mRNA [Carthew and Sontheimer, 2009]. This interaction leads to mRNA translational repression, arrest, and mRNA degradation. It is known that there are up to 1,000 miRNAs which control over one-third of human genes and are involved in a wide array of cellular functions.

Studies of miRNA expression profiles in SLE patients revealed unique miRNA signatures related to disease activity and major organ involvement. These aberrantly expressed miRNAs are mainly involved in 3 types of bio-

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Histone modifications</th>
<th>Gene affected</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td>overall H4 hyperacetylation</td>
<td>IRF1, RFX1, BLIMPI</td>
<td>Zhang et al., 2010</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>decreased overall acetylation of H3 and H4</td>
<td>179 genes</td>
<td>Zhang et al., 2010</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>increased H3 acetylation at lysine 18</td>
<td>IL10</td>
<td>Hedrich et al., 2014</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>increased H3 dimethylation at lysine 4 (H3K4me2) and H3 acetylation</td>
<td>CD70</td>
<td>Zhou et al., 2011</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>increased H3 trimethylation at lysine 27</td>
<td>HPK1</td>
<td>Gray, 2013</td>
</tr>
</tbody>
</table>
logical processes relevant to SLE pathogenesis (fig. 1): (1) hyperactivation of the type I IFN pathway; (2) down-regulation of DNA methylation by directly or indirectly inhibiting DNMTs; (3) exacerbation of inflammatory responses by promoting cytokine/chemokine secretion.

miR-146a expression level is reduced in PBMCs in SLE patients [Tang et al., 2009]. Ablation of the miR-146a gene in mice led to several severe immune-related phenotypes and eventually premature death [Boldin et al., 2011]. Reduction of miR-146a contributes to abnormal activation of the type I IFN pathway in human SLE by directly targeting IFN regulatory factor 5 (IRF5) and STAT1 [Tang et al., 2009]. miR-21 and miR-148a were found overexpressed in the mouse lupus model, MRL/lpr mice [Pan et al., 2010; Garchow et al., 2011]. miR-21 suppresses expression of programmed cell death 4 (PDCD4) and PTEN respectively, contributing to aberrant T cell responses and B cell hyperactivation in SLE [Stagakis et al., 2011; Wu et al., 2014]. miR-21, miR-148a, and miR-126 also reduce DNMT1 activity, which subsequently causes upregulation of methylation-sensitive genes, such as CD70 and LFA1 [Pan et al., 2010]. miR-29b directly influences DNA methylation by regulating DNMT3A and DNMT3B expression [Garzon et al., 2009; Hedrich and Tsokos, 2011; Qin et al., 2013]. miR-155 plays an important role in anti-dsDNA antibody production by targeting ETS1 in SLE patients [Jeong et al., 2009]. Deletion of miR-155 in the MRL/lpr mouse resulted in decreased serum IgG anti-dsDNA autoantibodies and alleviated lupus nephritis [Thai et al., 2013]. Most recently, our lab discovered that miR-1246 was significantly decreased in B cells from SLE patients by specifically targeting the early B cell factor 1 (EBF1) mRNA through interacting with its 3′ UTR to regulate its expression [Luo et al., 2015]. Transfection of miR-1246 inhibitors into healthy B cells could upregulate the expression of EBF1 and increase B cell surface co-stimulatory molecules such as CD10, CD80, and CD86, eventually enhancing B cell function.

Interaction among Epigenetic Regulations

All these aforementioned epigenetic mechanisms do not regulate gene expression separately but cross-talk to each other at the same time. For example, as mentioned above, DNA methylation is mainly established and maintained by DNMTs. It is also modified by methyl-CpG-binding domain proteins through recruiting silencing complexes containing HDACs and histone methyltransferases to participate in transcriptional repression [Ehrlich and Lacey, 2013]. DNA methylation and histone acetylation in CD4+ T cells of SLE patients are regulated by RFX1 by recruiting DNMT1 and HDAC1 to the CD11A (ITGAL) and CD70 promoters, and thereby their expression is repressed [Zhao et al., 2010a]. In mouse embryonic stem cells, the ADD domain of DNMT3L selectively binds to the unmethylated lysine 4 of histone H3 (H3K4) to trigger DNA methylation [Jeong et al., 2009]. On the other hand, DNMT3A and DNMT3B were found to be stably associated with nucleosomes containing high levels of methylated DNA in somatic cells, essential for regulating cellular levels of DNMT3A and DNMT3B [Sharma et al., 2011]. A cross-talk between DNA methylation or histone acetylation and miRNAs has also been observed in CD4+ T cells of SLE patients. For example, dysregulated miRNAs such as miR-126 and miR-142-3P contribute to DNA hypomethylation and self-reactivity of lupus T cells [Zhao et al., 2011; Ding et al., 2012]. HDAC1 and HDAC4 are targeted by miR-449a and miR-1, respectively [Chen et al., 2006; Noonan et al., 2009].

It is worth noting that epigenetics should not be only restricted to a static genetics-based perspective. We should also consider sections of chromatin between genes that are dynamic and can be changed by cellular stress that can have far-reaching impact on gene packaging and DNA conformation. Cellular stress can generate some new DNA conformations which are transient components of the nucleolus. Upon DNA stress, the transiently appearing DNA conformation can be stabilized by polyamines, highly charged polycations that serve many essential functions in the cell and assist with nucleoprotein complex assembly in the nucleolus increase [Moinard et al., 2005; Pegg, 2009; Igarashi and Kashiwagi, 2010]. Polyamines are particularly important during cellular stress when the inactive X chromosome could be engulfed by the nucleolus [Brooks and Renaudineau, 2015]. Since SLE dominantly occurs in females, epigenetic control over polyamines may be more relevant.

On the other hand, hypomethylated DNA and histone modifications are not only directly involved in SLE by regulating the susceptibility genes, but also directly linked to production of autoantibodies to nuclear antigens, such as DNA, chromatin, nucleosomes, histones, non-histone DNA binding proteins, and ribonucleoproteins, along with epigenetically modified ribonucleoprotein-derived peptides [Tan, 1989; Thabet et al., 2012]. Future work is needed to elucidate which epigenetic changes contribute to which subset of autoantibodies.
Prospects for Epigenetic Therapy

Possible Triggers of SLE

SLE is a complex disease. Genetic variations create the predisposition towards SLE, while the onset of the disease and disease progression are often possibly triggered by certain environmental factors and psychological and social stress, respectively, via epigenetic modifications.

Environmental factors, such as ultraviolet light, silica, infections, toxic chemicals, drugs, cigarette smoke, and dietary components, produce free radicals and cause oxidative stress which modifies the immune system to cause lupus flares via epigenetic modifications [Cañas et al., 2016]. For example, oxidizing agents decreased DNMT1 in human CD4$^+$ T cells, which resulted in demethylation and overexpression of genes in the ERK signaling pathway and induced anti-dsDNA antibody and glomerulonephritis [Somers and Richardson, 2014]. Recently, oxidants including H$_2$O$_2$ and ONOO$^-$ were found to decrease DNMT1 expression in CD4$^+$ T cells and suppress gene expression by DNA methylation in animal models of SLE [Strickland et al., 2015]. Following UV-B exposure, patients with active SLE showed decreased DNA methylation and DNMT1 mRNA expression levels in T cells [Zhu et al., 2013]. Exposure to environmental estrogen also poses a threat to SLE susceptible people because of estrogen's natural role in stimulating immune response [Sthoeger et al., 2003]. Estrogen binding of its receptors facilitates ER's recruitment of various cofactors and regulation of downstream gene expression. Many of these cofactors are histone-modifying enzymes, such as HDAC, pCAF, p300, and CBP [Sthoeger et al., 2003].

Many SLE patients experience high levels of emotional distress. Depression is the most common symptom, while anxiety and fear feeling are also frequently developed in SLE patients. More often than not, these profound negative psychological and social stresses exacerbate disease progression and cause an unpredictable clinical course of SLE. The hypothalamic-pituitary-adrenal (HPA) axis is the central mechanism of the stress response system [Segerstrom and Miller, 2004]. Evidence points to a defective HPA axis that is associated with SLE in both human and mouse models [Mok and Lau, 2003]. Persuasive evidence suggests that increased prenatal stress levels correlate with higher levels of DNA methylation at the glucocorticoid receptor promoter and lower levels of methylation at the promoter of hypothalamic corticotropin-releasing hormone in the HPA axis, resulting in a defect in stress response [Monk et al., 2012]. Chronic social defeat stress profoundly increases the levels of the repressive histone mark H3K27me2 at the promoter regions of BDNF, while antidepressant treatment boosts the activating histone mark H3 acetylation and H3K4me2 [Renthal and Nestler, 2009]. Chronic social defeat stress also induced DNMT3A expression while chronic antidepressant reduced it.

Existing Drugs with Epigenetic Effects

Drugs Not Primarily Targeting Epigenetic Mechanisms

So far, SLE patients are generally treated with antimalarial agents, immunosuppressive medications, and steroids. These drugs were designed not primarily targeting epigenetic mechanisms. They usually have considerable side effects and are only partially effective in SLE patients. Although the precise mechanisms of their anti-inflammatory effect have not been fully elucidated, they do exert effect on epigenetic factors.

Hydroxychloroquine, an antimalarial agent, is now an essential therapeutic element in treatment of SLE. It prevents disease flares, damage accrual, and the occurrence of vascular and thrombotic events [Wallace, 2001; Fessler et al., 2005]. Pharmacogenomics studies showed that hydroxychloroquine may alter miRNA expression in female NZB/W lupus mice and in the kidneys of SLE patients [Dai et al., 2009; Chafin et al., 2013a, b].

Glucocorticoids (GCs) are small lipophilic compounds that target the intracellular GC receptor to directly or indirectly regulate immune gene transcription. GCs are widely used in the treatment of SLE because of their rapid anti-inflammatory and immunosuppressant effects. However, they fail to maintain disease remission in the majority of SLE patients [Zhao et al., 2013]. SLE patients treated with GCs had increased E4BP4 expression in CD4$^+$ T cells [Zhao et al., 2013]. Because E4BP4 binds to the promoter of CD40L and inhibits gene expression by altering histone acetylation and methylation levels, it is suggested that GCs may exert their therapeutic effect via indirectly influencing histone acetylation and methylation.

Tamoxifen is an antagonist of ER in many tissues and modulates ER function. Tamoxifen significantly reduced autoantibody production in a mouse SLE model [Sthoeger et al., 2003]. In separate studies, tamoxifen-induced suppression of the typical estrogen responsive gene is perfectly consistent with the recruitment of the HDAC complexes to the target promoter and the subsequent deacetylation of histone H3 and H4 tails [Sthoeger et al., 2003].

Mycophenolic acid (MPA), the principal active metabolite of mycophenolate mofetil, has been widely used for the treatment of SLE since it is more effective and safe-
er than other immunosuppressants [Iaccarino et al., 2007; Dooley et al., 2011; Liu et al., 2012]. MPA is a noncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase. This enzyme is a rate-limiting biosynthetic enzyme in the de novo synthesis of purine nucleotides, required by lymphocytes. MPA is found capable to prevent the secretion of inflammatory cytokines that contribute to the pathogenesis of SLE, such as IL10, IL17, and IFN-γ [Jonsson and Carlsten, 2002]. Recently, our lab found that MPA is capable of reversing hypoacetylation in lupus CD4+ T cells by downregulating HDACs and upregulating histone acetyl transferase expression, in addition to suppressing CD40L expression by downregulating its histone acetylation in the promoter region [Yang et al., 2015]. Our lab also discovered that MPA increased expression of miR-142-3p/5p and miR-146a in lupus CD4+ T cells in vitro [Tang et al., 2015]. The upregulation of these 2 miRNAs is due to alteration of histone H4 or H3 acetylation.

Drugs Targeting the Major Components of the Epigenetic Mechanisms

As reviewed above, epigenetics plays an essential role in the onset and progression of SLE. Epigenetic drugs have been a focus among recent SLE drug discovery. So far, HDAC inhibitors are the most widely studied epigenetic therapeutics for SLE. These inhibitors have demonstrated anti-inflammatory and immunosuppressive effects [Grabiec et al., 2010, 2012].

Trichostatin A (TSA) is a specific and reversible HDAC inhibitor in vitro and active at low concentrations in vivo [Yoshida et al., 1990]. TSA and a structurally related compound named suberoylanilide hydroxamic acid are the first 2 HDAC inhibitors used to treat SLE. Cytokines produced by Th1 and Th2 lymphocytes such as IL6, IL10, IL12, and IFN-γ largely contribute to pathogenesis of both human and murine SLE [Dean et al., 2000]. HDACs were found to regulate Th1 and Th2 cytokines and play a key role in SLE. In the MRL-lpr/lpr murine model of lupus, treatment with TSA and suberoylanilide hydroxamic acid downregulates IL6, IL10, IL12, and IFN-γ in both mRNA and protein levels in the splenocytes [Mishra et al., 2001]. This downregulation was temporally related to enhanced acetylation of histone H3 and H4 [Cornacchia et al., 1988; Mishra et al., 2001, 2003]. As a result, TSA-treated MRL-lpr/lpr mice exhibited a significant reduction in proteinuria, glomerulonephritis, and spleen weight. Other than Th1 and Th2 cells, Th17 and Treg cells are the other 2 major subsets that CD4+ cells can differentiate into [Zhu et al., 2010]. SLE patients also tend to have increased levels of Th17 cells leading to overproduction of IL17, which causes tissue damage [Crispin et al., 2010; Apostolidis et al., 2011; Joshi et al., 2011; Miyake et al., 2011]. Treg cells regulate proliferation of the other immune cell subsets. Depletion of Treg cells caused healthy mice to develop autoimmune disease [Hayashi et al., 2005; Wahl and Chen, 2005; Wenzel et al., 2005; Liu et al., 2008]. Differentiation of CD4+ cells into Treg cells requires FOXP3 transcription factor. FOXP3 expression is directly regulated by HDACs [Akimova et al., 2010; Beier et al., 2011]. ITF2357 is a hydroxamic acid-derived compound, which is a specific class I and II HDAC inhibitor. Treatment with ITF2357 decreased the IL17 level and increased the percentage of Treg cells through elevating FOXP3 acetylation [Regna et al., 2014]. An increased number of Treg cells may reduce autoantibody production [Regna et al., 2014]. Given the improvement in the similar diseases mentioned above, ITF2357 may have a therapeutic benefit in SLE. Of note, even for a very enzyme-specific HDAC inhibitor, it still unavoidably alters expression of many genes and possibly causes unacceptable toxicity [Kroesen et al., 2014]. Therefore, in order to minimize toxicity, targeting proteins downstream of HDAC may be a safe strategy for therapy.

As mentioned above, gene-specific or aberrant hypomethylation is quite often observed in promoters of genes in T cells of SLE patients that are involved in pathogenesis of SLE or lymphocytic invasion pathways and mitochondria dysfunction [Richardson et al., 1990; Kaplan et al., 2004; Oelke et al., 2004; Lu et al., 2007; Zhao et al., 2010b; Zhu et al., 2011; Lin et al., 2012; Absher et al., 2013; Zhao et al., 2014; Talaat et al., 2015]. These genes all have shown overexpression in lupus CD4+ T cells, contributing to the overproduction of autoantibodies by B cells after their activation by autoreactive T cells. Targets that can upregulate DNMT expression in the relevant genes mentioned above would be useful to increase DNA methylations. The catalytic subunit of protein phosphatase 2A is overexpressed in T cells of SLE patients. It controls DNMT1 expression through the MEK/ERK signaling pathway and thus affects DNA methylation in both normal and SLE T cells [Sunahori et al., 2013]. It probably can serve as a potential drug target to indirectly regulate DNA methylation in T cells for SLE patients. Also, an anti-IL6 receptor monoclonal antibody named itolizumab was found to reverse the effect that overexpressed IL6 in SLE B cells prevents them from inducing DNMT1 to methylate DNA [Garaud et al., 2009]. In other words, this antibody drug against IL6 receptor
miRNAs play a central role in regulating the immune system and have been extensively investigated as potential disease biomarkers and therapeutic targets [Shen et al., 2012; Ma and Liu, 2013; Yan et al., 2014]. Around 9 miRNAs have been shown to be able to modulate the pathogenesis of autoimmune diseases including SLE through their effects on T and B cell functions [Zhang et al., 2011]. miR-146a was found to be positively correlated with SLE disease activity index and glomerular filtration rate [Wang et al., 2012]. Reduced levels of miR-146a increase the induction of type I IFN in PBMCs from SLE patients. When conjugated with virus-like particles, miR-146a could be effectively transferred into various cells and tissues to induce a low toxicity [Pan et al., 2012a]. Administration of miR-146a in BXSB lupus-prone mice resulted in decreased autoantibody and plasma cytokine production [Pan et al., 2012b]. Most recently, our lab has discovered that miR-1246 specifically targeted EBF1 which plays a central role in the development of B cells and led to aberrant B cell over-activation [Luo et al., 2015]. Reduced levels of miR-1246 are found in B cells of SLE patients. Thus, it acts as a negative regulator of B cell activation and a protective factor against SLE. Therefore, it is tempting to speculate that recovering miR-1246 expression using virus-like particles may be a potential and effective method to treat SLE. On the other hand, for miRNAs that are aberrantly overexpressed in SLE patients, the strategy of therapy would be to bring the expression level down to normal through either trapping the miRNA or degrading it. The miRNA ‘sponge’ strategy has been employed in several studies where an endogenous target miRNA can be spliced to multiple artificial miRNA-binding sites to be selectively depleted [Ebert et al., 2007]. This strategy was applied in the study of miR-326 in patients with multiple sclerosis [Du et al., 2009]. Small molecule inhibitors can also be employed to target each step in miRNA assembly and function, from transcription of primary miRNAs to formation of miRNA-induced silencing complex and its interaction with target mRNA. For example, azobenzene showed an inhibitory effect in miR-21 expression [Gumireddy et al., 2008]. The miRNA-targeting antisense oligonucleotides bind to and specifically suppress target miRNAs function and induce degradation of target miRNAs. For example, anti-miR-223 mice showed incidence of arthritis, attenuation of osteoclastogenesis, and bone erosion in joints [Li et al., 2012].

Epigenetic Mechanisms Complicate SLE Progression and Implicate Personalized Medicine for SLE

The Complexity of Pathogenesis

The development of new drugs to treat SLE has been quite slow, largely due to the heterogeneity of the disease. For example, rarely do any 2 patients exhibit the same clinical manifestations, making it difficult to measure outcome of clinical trials. This issue is worsened even more by differences in disease activity, clinical signs and prevalence among different ethnic groups, when examined at a large scale among populations.

Genome-wide association studies (GWASs) revolutionized the study of human genetics, including the genetics of SLE. It has led to the definitive identification of multiple genomic loci which contribute to SLE. Together with studies in mouse models, it has greatly advanced our understanding of SLE. Many of the findings direct the focus to a mechanistic standpoint: most of these genomic loci and genes identified have important roles in immune regulation [Brooks et al., 2010; Hughes and Sawalha, 2011]. For example, multiple SNP alleles of the Stat4 locus have been linked to SLE susceptibility, and persuasive evidence supports that deletion of Stat4 blocks SLE progression in the mouse model. But it is well appreciated that a single mutation of STAT4 would not cause SLE in human [Gorissen et al., 2011]. Similarly, aberrant DNA methylation has also been identified in other SLE contributory genes, including CD40L, IL10, IL13, CTLA4, and IL2 [Absher et al., 2013; Coit et al., 2013]. PBMCs express high levels of IL10, and the serum level of potent B cell stimulator IL10 correlates with disease activity and severity; it is believed that IL10 may be upregulated in SLE due to hypomethylation of its promoter region in CD4+ cells [Hedrich et al., 2014]. This elevated circulating IL10 together with other lupus contributory proteins further activate humoral immune response and trigger SLE disease. The knowledge learned from SLE so far supports a multifactorial disease model: multidimensional subtle modification of immune regulatory genes progressively compromises the resilience of the immune system. Those modifications, either genetic variation or epigenetic changes add together until a certain breakpoint of the immune system, and then SLE is triggered. Based on this model, it becomes clear why each SLE patient is different and an effective treatment is so difficult to find.

Due to the heterogeneous nature of the disease, it does not seem feasible to identify a clear and classic genetic and generic mechanism that would explain all the clinical manifestations. Researchers are now starting to dissect the clin-
ical manifestations and identify manifestation-specific epigenetic modifications in SLE. These efforts have led to the discovery of novel regulation mechanisms and targets that are involved in different sets of clinical manifestations. For example, Coit et al. [2015a] identified 191 CG sites and 121 genes that were only differentially methylated in lupus patients with renal involvement. They identified a type I IFN master regulator gene, IRF7, that was only hypomethylated and regulated a few loci demethylated only in lupus patients with renal involvement. These loci include CD80, HERC5, IFI44, IRF7, ISG15 or 20, ITGAX, and PARP12. Within the 191 CG sites, CHST12 showed high sensitivity and specificity for stratifying lupus patients with renal involvement and could be a potential biomarker for the diagnosis. Renauer et al. [2015] from the same lab identified 36 and 37 unique differentially methylated regions that contribute to epigenetic susceptibility to malar rash and discoid rash, respectively, in naïve CD4+ T cells. These genes are involved in the pathway for TAP-dependent exogenous antigen processing and MHC-I cross-presentation. Hypomethylation of novel target genes such as MIR886 and TRIM69 as well as hypermethylation of RNF39 were specific for SLE patients with malar rash. Hypomethylation of RHOJ was specific for SLE patients with discoid rash. In addition, as mentioned above, our new findings on increased 5hmC and hydroxymethylation in SLE CD4+ T cells provide a novel mechanism to explain the critical role that hydroxymethylation plays in aberrantly regulating gene transcription in SLE [Zhao et al., 2016a].

Personalized Medicine of SLE Comes to the Rescue

SLE is a complex disease, which involves multiple genetic and epigenetic aberrations creating a disposition toward the disease. Environmental and psychological factors often introduce additional epigenetic intervention and bring uncertainty to the disease onset and/or progression. The high heterogeneity in SLE patients makes it extremely challenging to find effective treatments since each SLE patient is different by the nature of etiology or pathogenesis. Personalized medicine (or precision medicine) is often described as providing ‘the right patient with the right drug at the right dose at the right time.’ Based on the complexity of SLE, personalized medicine seems to be the right solution. Specifically, personalized medicine applied to SLE patients could be developed on the following aspects.

1. Collect All Genetic and Epigenetic Aberrations for Diagnosis and Tailor Medical Treatment to the Individual Characteristics. The ENCODE project defined epigenomic elements across the entire genome [ENCODE Project Consortium, 2004]. The project also inferred the existence of a large amount of enhancer-like regions that regulate gene expression at long range in the mammalian genome. Each cell type is regulated by around 20,000–40,000 enhancers. GWASs have identified more than 50 robust loci and genetic variations associated with SLE susceptibility [Han et al., 2009; Yang et al., 2010; Okada et al., 2012; Lee et al., 2014]. Only few of the variants (SNPs) lead to gain or loss of function of the encoded proteins, whereas the majority falls in noncoding regions [Deng and Tsao, 2014]. For example, IRF5, 7, and 8 are transcription factors required for activating transcription of IFN-α and IFN-inducible genes. Genetic variants in or near these 3 genes are associated with SLE susceptibility [International Consortium for Systemic Lupus Erythematosus Genetics et al., 2008; Fu et al., 2011]. Through locating summits of chromatin marks including histone modifications, DNA accessibility such as DNase I hypersensitive sites and common epigenetic features such as CTAC-binding sites, Trynka et al. [2013] found that SNPs associated with the same trait overlap with chromatin marks in the same cell type and chromatin marks are cell type-specific. For example, H3K4me3 peaks overlapped with 31 disease-associated SNPs for rheumatoid arthritis within CD4+ regulatory T cells. Thus, cell type-specific chromatin marks of different sets or combinations might be used to connect phenotypes to specific cell types and map phenotype/trait-associated SNPs to potential regulatory variants. The NIH Roadmap Epigenomics Mapping Consortium has utilized broad sets of chromatin marks to investigate key functional elements controlling gene expression in 127 human tissue and cell types from healthy individuals and those with diseases [Dixon et al., 2015; Farh et al., 2015; Gjoneska et al., 2015; Leung et al., 2015; Polak et al., 2015; Roadmap Epigenomics Consortium et al., 2015; Tsankov et al., 2015; Ziller et al., 2015]. The project extended the chromatin marks to include 5 histone methylation marks, 2 histone acetylation marks, DNase hypersensitivity, DNA methylation, and RNA expression assays. Ziller et al. [2015] and Tsankov et al. [2015] found that different sets of transcription factors bound to promoters and enhancers of different cell lineages and regulatory elements controlling genes are often also epigenetically modified in parental cells. The most recent paper from the NIH Roadmap Epigenomics Consortium generated the largest collection of 111 reference human epigenomes [Roadmap Epigenomics Consortium et al., 2015]. In this study, 1,821 histone modification data sets, 360 DNA accessibility data sets, 277 DNA methylation data sets, and 166 RNA-seq data sets are identified, profiled and mapped out. With the
unprecedented large scale of data, this study found that histone modification marks are highly informative of the methylation, 5% of each epigenome marked by enhancer or promoter signatures on average, and coordinated activity patterns of enhancer regions in biologically meaningful cell types. This study also confirmed that through an unbiased sampling across the GWAS catalog, diverse immune disease- and trait-associated genetic variants were enriched in immune-specific enhancers and expansive enhancer clusters in tissues of SLE patients along with other autoimmune diseases. This implicates gene regulatory processes in disease etiology and provides an important resource for understanding the molecular basis of human diseases including SLE, such as mechanistic insights into the likely relevant cell types underlying genome-wide significant loci. For example, Seumois et al. [2014] discovered that asthma-specific enhancers in T cells that differed between healthy individuals and those with asthma and also gained H3K4me2 mark during Tm2 cell development showed the highest enrichment for asthma-associated SNPs. This strategy can be applied to any accessible cell type that is relevant to human disease and allows for enhancer profiling. Combining this biggest reference of human epigenomes with several other companion papers that further explored the datasets in the context of autoimmune diseases [Seumois et al., 2014; Farh et al., 2015], a most extensive and SLE-specific reference human epigenome can be generated. With the cost of next-generation sequencing further decreased, various diagnostic methods based on this method become powerful and standard procedures to collect individual disease-causing genes or regulatory gene variants including epigenetic variations [Jones et al., 2014]. Individual genetic and epigenetic profiling can be based on the comparison between SLE-specific reference human epigenomes with individual epigenomes in the tissue of choice. This profiling can help guide personalized and precise treatment.

(2) Discover More Epigenetic Markers for Early Detection and Disease Prognostics. Many miRNAs that are aberrantly expressed in SLE patients and are correlated with SLE pathogenesis circulate in plasma released from diseased tissue or exist in PBMCs. This character makes them ideal candidates for biomarkers in diagnostics and prognostics. For example, miR-126 as well as newly discovered miR-1246 may potentially become biomarkers for SLE [Luo et al., 2015]. A recent study has validated a ‘top 4’ miRNA-based score for SLE risk in circulating miRNA in SLE patients [Carlsen et al., 2013]. However, sensitivity and specificity of diagnostic strategy should be developed further by large sample clinical trials. In the future, more miRNA biomarkers will emerge to detect changes in every step along SLE development, such as increased monocytes and plasmacytoid dendritic cells, activation of autoreactive B and T cells, product of autoantibodies, and secretion of proinflammatory cytokines and chemokines. These biomarkers will be essential to help predict, monitor, and reflect individual response to a particular treatment, acting as an essential part of personalized treatment.

(3) Combine Target Medication with Its Epigenetic Regulators. The universal toxicity of steroids confirms the need for more targeted interventions. Advancement in biotechnology has made personalized medicine possible today. When belimumab, a human monoclonal antibody against BLyS, became the first approved new treatment for SLE, an army of biotech or pharmaceutical companies has put huge efforts in the development of various biological or small molecules to target the IFN pathway from innate immunity, BLyS pathway, co-stimulatory pathways, and other T-cell modulators within B and T cells [Thanou and Merrill, 2014]. Current drug candidates in the pipelines of major pharmaceutical companies are mainly targeting CD22, CD4 modulator, JAK1 inhibitor, modified chaperonin 10, IL6, IFNα and FcγRIIB [Thanou and Merrill, 2014]. However, one medication against one target will not fit all patients. Flexibility is needed in choosing medications in combination with other agents as well as dosing for different individuals to achieve optimal treatment efficacy. For example, because the drug targets are all tightly regulated by epigenetic mechanisms, if an epigenetic regulation, for instance, site-specific hypoacetylation of H3 and H4 is found in the drug target’s promoter region from the patient’s sequencing profiling, the drug effect may be improved by including a HDAC inhibitor such as TSA.

Taken together, with the rapid development of drugs with epigenetic effects such as HDAC inhibitors and DNMT enhancers as well as miRNA targeting therapeutics by mimics or inhibitors, combining target medication in immune system with a therapy of appropriate epigenetic mechanisms, it may not be long before SLE becomes curable.

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