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The Epigenetic Face of Systemic Lupus Erythematosus

Esteban Ballestar, *Manel Esteller, * and Bruce C. Richardson†

Systemic lupus erythematosus (SLE) is an archetypical systemic, autoimmune inflammatory disease characterized by the production of autoantibodies to multiple nuclear Ags. Apoptotic defects and impaired removal of apoptotic cells contribute to an overload of autoantigens that become available to initiate an autoimmune response. Besides the well-recognized genetic susceptibility to SLE, epigenetic factors are important in the onset of the disease, as even monozygotic twins are usually discordant for the disease. Changes in DNA methylation and histone modifications, the major epigenetic marks, are hallmark in genes that undergo epigenetic deregulation in disease. In SLE, global and gene-specific DNA methylation changes have been demonstrated to occur. Moreover, histone deacetylase inhibitors reverse the skewed expression of multiple genes involved in SLE. In the present study, we discuss the implications of epigenetic alterations in the development and progression of SLE and how epigenetic drugs constitute a promising source of therapy to treat this disease. The Journal of Immunology, 2006, 176: 7143–7147.

Although the existence of a genetic determinant of many diseases is well established, it is generally accepted that environmental factors, including diet and lifestyle, modulate the susceptibility, in part, through epigenetic changes (1). However, although many studies have corroborated these ideas and led to the identification of the genetic defects involved in many diseases, virtually no epigenetic information has been routinely or systematically collected at the genome level. In recent years, we have witnessed an increasing interest in the role of epigenetic modifications in the etiology of human disease.

Epigenetics determines the identity and function of cells

Epigenetics comprises the stable and heritable (or potentially heritable) changes in gene expression that do not entail a change in DNA sequence. Whereas genetic information is homogeneous in an organism regardless of the cell type (excepting differences due to somatic mutations), epigenetic modifications are characteristic of different cell types and, in fact, play a key role in defining the transcriptome, which determines the identity of each cell type (2).

Two major groups of changes contribute to defining the epigenome of a cell: DNA methylation and histone modifications.

The most common form of DNA methylation occurs at the 5′ position of cytosine in the context of CpG dinucleotides, which are unevenly distributed throughout the genome. Particularly remarkable are CpGs clustered in so-called CpG islands (3), many of which coincide with the promoter of protein–protein-coding genes, as well as those present in repetitive sequences. CpG methylation has functional consequences, such as transcriptional repression, when it occurs in CpG islands. In fact, a number of tissue-specific genes are silenced by promoter methylation.

Posttranslational modifications that occur in histones make up a second group of epigenetic modifications. Core histones are organized in octamers that are intimately associated with DNA in nucleosomes, the repeating subunit of chromatin. lysine acetylation and methylation and serine phosphorylation, among other modifications, regulate many nuclear functions. For instance, reversible acetylation of histone lysines is generally associated with transcriptional activity. The functional consequences of lysine or arginine methylation depend on the specific site that is modified. Recently, it has been proposed that different combinations and sequences of posttranslational modifications make up a sort of code that is read by nuclear factors to mediate different processes (4).

DNA methylation and histone modifications are coupled through different machineries, including DNA methyltransferases (DNMTs)3 and different families of methylated DNA binding proteins that associate with histone modifying enzymes in multiprotein complexes (5) (Fig. 1).

Besides a direct effect on nuclear processes such as transcriptional activity, DNA methylation and histone modifications also play a key role in organizing nuclear architecture (6, 7), which in turn is also involved in regulating transcription and other nuclear processes. Therefore, epigenetic modifications are essential for defining the cellular transcriptome at several levels. Aberrant changes in the pattern of epigenetic modifications

3 Abbreviations used in this paper: DNMT, DNA methyltransferase; ICF, immunodeficiency, centromeric region instability, and facial anomaly; SLE, systemic lupus erythematosus; HAT, histone acetyltransferase.

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genetic alterations accumulate gradually over an individual's disease (1). Moreover, recent findings have indicated that epigenetic dependence and quantitative nature, and the mechanism by which the environment modulates genetic predisposition to several characteristics of many diseases, including their age dependence are not shown. Changes in the chromatin of these genes lead to a change in the transcriptional status. Demethylation is compatible with gene transcription (np) and methylation leads to gene silencing. A number of nuclear factors associate with the methylated promoter: DNMTs and methyl-CpG binding domain proteins (MBDs) that associate with histone methyltransferases (HMTs) and histone deacetylases (HDACs). Histone methyltransferases (HMTs K9 H3) and histone acetyltransferases (HATs) are associated with unmethylated promoters and transcriptional activity (np).

would result in altered nuclear activity, and thereby an altered transcriptome, which would transform the identity of the cell.

In recent years, increasing evidence has demonstrated the role of epigenetic alterations in the etiology of many diseases. For instance, unscheduled hypermethylation of CpG islands of tumor suppressor genes and the resulting transcriptional silencing are associated with malignant transformation in cancer (8). These changes are associated with aberrant patterns of histone modifications (9). In addition, cancer cells suffer a loss of methylation and alterations in the histone modification pattern of repetitive sequences that appear to be involved in chromosomal instability in cancer (10, 11). Other diseases with well-recognized epigenetic defects include immunodeficiency, centromeric region instability, and facial anomalies (ICF) syndrome, caused by mutations in the DNMT 3B, Prader-Willi and Beckwith-Wiedemann syndromes, resulting from the absence of transcriptional regulation of the paternal or maternal copy of an imprinted gene, and Rett syndrome, involving mutations in the methylation-dependent transcriptional repressor MeCP2 (12, 13). The epigenetic component of many other diseases remains to be identified. In fact, the epigenetic framework could explain several characteristics of many diseases, including their age dependence and quantitative nature, and the mechanism by which the environment modulates genetic predisposition to disease (1). Moreover, recent findings have indicated that epigenetic alterations accumulate gradually over an individual’s lifetime (14, 15). In fact, the comparison of epigenetic modifications in genetically identical monozygotic twins has revealed that environmental factors, including diet and lifestyle, contribute significantly to the phenotype by changing the epigenetic profile. This could explain discordance in the development of disease (15). Individual epigenetic peculiarities that modulate susceptibility could therefore explain the apparent complexity in the patterns of inheritance.

The role of apoptosis in systemic lupus erythematosus (SLE)

A remarkable example of a disease for which patterns of inheritance are extremely complex is SLE, an autoimmune disease characterized by the production of a variety of Abs against nuclear components that causes inflammation and injury of multiple organs. The disease primarily affects women in their reproductive years, and its prevalence is estimated to vary between 12 and 64 cases per 100,000 in European-derived populations and to be higher in non-European-derived populations. The presence of autoantibodies directed against a set of cell constituents has meant that lupus is classified as an autoimmune disease. In fact, autoantibodies to nuclear components, including nucleosomes, and their individual components, DNA and histones, are typically present years before the diagnosis of SLE and progressive accumulation precedes the onset of the disease (16).

Several studies indicate that the presence of autoantibodies in SLE patients is associated with two major alterations: increased rate of apoptosis in circulating lymphocytes and monocytes, and abnormal recognition of autoantigens released during apoptosis (Fig. 2).

Nucleosomes are released in vivo exclusively by endonuclease digestion of chromatin during apoptosis (17), and therefore, acceleration of this type of cell death, or an impaired clearance of apoptotic cells, provides a mechanism for disrupting peripheral self-tolerance. The current dogma is that clearance of intact dying cells prevents secondary necrosis of apoptotic cells and release of dangerous enzymes into the microenvironment and is therefore anti-inflammatory. However, recent findings have
suggested that, under special circumstances, cells dying by apoptosis may also trigger a specific immune response (18, 19). Indeed, dying cells are capable of triggering the release of chemokines and proapoptotic molecules by macrophages. Apoptosis under certain conditions appears to be proinflammatory, triggering the caspase-1-mediated release of inflammatory cytokines from dying cells (20).

In vitro, apoptotic material has been demonstrated to activate T cells and B cells. In fact, coinoculation of autologous apoptotic material with freshly isolated PBMCs leads to the expansion of histone-specific T cell clones that could help B cells to form Abs.

The loss of immune tolerance to self-components is the basis of SLE, so many genes encoding proteins with regulatory or adaptive functions in the immune system have been considered as candidates predisposing to development of the disease (21). These include genes of the MHC, FcγRs, complement components, CTL Ag-4, and programmed cell death-1. Moreover, the analysis of polymorphisms suggests that, besides gene-gene interactions, many modifying environmental effects influence the disease phenotype.

An interesting line of evidence that environmental factors influence the development of SLE comes from studies of identical monozygotic twins. The highest estimated concordance rate in twins is <60% (22, 23), so the involvement of nongenetic factors in the pathogenesis of SLE must be invoked. As mentioned above, epigenetic alterations are accumulated during an individual’s lifetime (15). The high incidence of twin pairs in which only one of the siblings has developed SLE supports the notion that environmental factors and their involvement in epigenetic modifications could affect the onset of the disease. Epigenetic alterations could influence the onset of SLE at several levels. The first is the epigenetic deregulation of genes that contribute to, or increase the activation of, apoptosis. Moreover, apoptotic-released nuclear material with specific epigenetic patterns may contribute to aberrant overexpression of various genes. These aberrant changes occur in genes like perforin (37), whose demethylation could contribute to monocyte killing. CD70 is also overexpressed in CD4+ lupus T cells by demethylation (38). In this case, CD70 overexpression contributes to excessive B cell stimulation in lupus. Another example is the demethylation of ITGAL regulatory sequences, which may also contribute to the development of lupus (39).

Systematic analysis of methylation and demethylation events in different stages and the extent of SLE manifestation will surely contribute to our understanding of the hierarchy and relevance of these methylation events in the development of SLE.

Epigenetic alterations in SLE: DNA methylation changes

As mentioned above, DNA methylation is a fundamental determinant of chromatin structure, with potent suppressive effects on gene expression. Abnormalities in the DNA methylation system result in the aberrant increase or decrease in gene expression, which is implicated in the development of a variety of diseases. The first evidence of the role of aberrant changes in the DNA methylation patterns in the development of SLE was that T cells from patients with active lupus were shown to exhibit globally hypomethylated DNA (27). More recent studies have demonstrated an association between DNA hypomethylation in SLE and a decrease in the enzymatic activity of DNMTs (28), implying a possible mechanism to explain DNA hypomethylation. Additional evidence of the role of methylation changes in the development of SLE comes from studies with DNA demethylating drugs. One of the most common demethylating drugs used to induce SLE in mice is 5-azacytidine, a cytosine analog that contains a nitrogen atom at the 5’ position of the pyrimidine ring and is incorporated into newly synthesized DNA. Treatment with 5-azacytidine causes genome-wide hypomethylation, resulting in the altered expression of many genes. Other demethylating drugs used to induce SLE are procainamide (29), a competitive DNMT inhibitor, and hydralazine, whose demethylating activity has been explained as an indirect result of the inhibition of the ERK pathway signaling, decreasing DNMT1 and DNMT3a levels during mitosis (30). In all cases, exposing T cells to demethylating drugs results in the demethylation-dependent induction of lupus-like disease (31–34).

Histone modifications in SLE

As with the role of DNA methylation alterations in SLE, our knowledge about the alterations in the histone modification patterns in SLE is incomplete. Since DNA methylation and histone modifications are mechanistically linked (see Fig. 1), it is likely that different changes in histone modifications are associated with DNA methylation changes in SLE.

Similarly to studies on DNA methylation and lupus, most of the evidence about the role of changes in histone modifications in SLE comes from the use of epigenetic drugs. In the case of histone modification, the use of histone deacetylase inhibitors suggests that deacetylation is involved in the skewed expression of certain genes that are associated with the disease.

For instance, SLE Th cells exhibit increased and prolonged expression of cell-surface CD40L (CD154), spontaneously overproduce IL-10 (IL-10), but underproduce IFN-γ (IFN-γ). The histone deacetylase inhibitor trichostatin A (TSA) significantly reverses this skewed expression of these gene products (40). It is likely that this reversion is the result of modification of...
the histone acetylation status, although alteration of the acetylation levels of transcription factors cannot be discarded. At any rate, this result not only suggests that histone acetylation might account for this aberrant expression but also that this pharmacological agent may be a candidate for the treatment of this autoimmune disease.

Similar results have been obtained with other histone deacetylase inhibitors, such as suberoylanilide hydroxamic acids, that are able not only to revert the aberrant expression of certain genes but also to modulate renal disease (41).

Detailed information is therefore needed about the type and extent of histone modifications at the global and gene-specific scales in SLE T cells. This information will not only serve to identify genes that suffer aberrant deregulation but also help to develop specific epigenetic drugs.

**Toward a definition of SLE as an epigenetic disease: perspectives and therapies**

Many approaches have been used with partial success to determine the genetic determinants of SLE, including analyses based on candidate genes, genome-wide linkage studies, and family-based classical linkage studies. All the evidence points to the existence of a complex system in which multiple genes may participate in the determination of SLE predisposition. However, since epigenetics could play a key role in defining or modulating the onset of the pathogenesis for genetically predisposed individuals, it is of inherent interest to analyze epigenetic modifications systematically to understand the etiology of SLE. This will surely help not only to elucidate the pathway of the disease but also to determine whether epigenetic therapies can be used to treat or prevent the disease.

A number of analytical techniques are now available for studying epigenetic modifications in a genome-wide manner. These techniques have already proved to be useful for identifying epigenetic alterations in other diseases, such as various types of cancer. These include methods that identify changes in the methylation profile of specific sequences, such as differential methylation hybridization or amplification of intermethylated sites (42). Both techniques are based on the use of restriction endonucleases that are sensitive to methylation status followed by hybridization on genomic microarrays or sequencing and database analysis. Other techniques, such as chromatin immuno-precipitation assays coupled to hybridization of genomic microarrays (ChIP-on-chip) (9), allow the identification of sequences with different patterns of histone modifications. The systematic use of these genomic techniques will serve to provide a full profile of epigenetic deregulation in SLE.

Unlike genetic alterations, which are permanent, epigenetic alterations are reversible. This opens up the possibility of using epigenetic drugs to reverse the pattern of epigenetic alterations to relieve the phenotype. To exploit potential it is important to define the full range of epigenetic modifications in SLE.

Several drugs that reverse the pattern of epigenetic modifications have already been used to treat SLE. To date, histone deacetylase inhibitors such as suberoylanilide hydroxamic acid and TSA have proved to be useful for relieving SLE disease in mice (26). For instance, TSA suppresses the expression of the TCR c-chain gene, whereas it up-regulates the expression if its homologous gene Fc(ε)RI g-chain. On the other hand, TSA suppresses the expression of the IL-2 gene (43). The effects of TSA on human T cells are predominantly immunosuppressive and reminiscent of the signaling aberrations that have been described in patients with SLE. However, histone deacetylation inhibitors and DNA-demethylating drugs could also be a promising source of epigenetic therapies to treat SLE. Current evidence indicates that most of the genes that exhibit aberrant patterns of DNA methylation are hypomethylated, although gene-gene-specific hypermethylation cannot be ruled out. Therefore, a detailed analysis of DNA methylation at the gene level will serve to evaluate how useful DNA demethylating drugs could be.

In addition, increasing efforts are being made with other compounds with histone methyltransferase inhibiting activity, which could also be useful in epigenetic therapy.

We truly believe that the future in the treatment of SLE depends greatly on the ability to revert epigenetic alterations, which are in fact the factors that determine that a genetically prone individual actually develops the disease or not. In this sense, the precise determination of the array of epigenetic alterations on a gene-gene-specific level is absolutely required. In parallel, the generation of compounds able to revert or modify epigenetic patterns in a gene-targeted fashion will ensure the development of effective therapies to cure SLE.

**References**


