Epigenetic alterations in autoimmune rheumatic diseases

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Abstract | The potential roles of epigenetic alterations in the pathogenesis of autoimmune rheumatic diseases are raising great expectations among clinicians and researchers. Epigenetic mechanisms regulate gene expression and are sensitive to external stimuli, bridging the gap between environmental and genetic factors. Considerable evidence of epigenetic changes, particularly altered patterns of DNA methylation, exists in diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. The importance of such changes in the pathology of rheumatic diseases has been demonstrated by examining the relationship between gene-specific methylation and SLE in monozygotic twins discordant for the disease, in whom genetic variability is excluded as a cause for discordance. Several studies have highlighted the importance of the tissue-specificity of DNA methylation changes, an aspect which—in contrast with genetic analysis—must be considered when designing epigenetic studies. Here I discuss the proposed mechanisms and implications of DNA methylation changes in the pathogenesis of autoimmune rheumatic diseases, the prospects for future epigenetic studies in rheumatology, the relevance of specific DNA methylation markers and the potential use of drugs with an epigenetic effect in the clinical management of these diseases.

Ballestar, E. *Nat. Rev. Rheumatol.* **7**, 263–271 (2011); published online 22 February 2011; doi:10.1038/nrrheum.2011.16

Introduction

The complex etiopathology of autoimmune rheumatic disorders has been attributed to crosstalk between genetic predisposition and environmental factors. The first genetic studies in autoimmune disease revealed a strong association between genes within the major histocompatibility complex (MHC), particularly human leukocyte antigen (HLA) genes, and several autoimmune diseases.[1] More recently, many other susceptibility genes have been uncovered, some of which, such as *PTPN22*, *IRF5*, and *STAT4*, are also associated with the development of several autoimmune rheumatic diseases,[2] suggesting overlap in their pathogenesis. Complexity arises from the fact that each of these susceptibility genes make small and interlinked contributions to the overall risk of disease. For example, a haplotype of *IRF5* that is associated with risk for systemic lupus erythematosus (SLE) has been linked to increased production of interferon (IFN),[3] and increased sensitivity to IFN-α in patients with SLE is caused by a variant of *STAT4* that is associated with autoimmune disease.[4] Over and above the expression of basic genetic variability, the contribution of genetic factors to disease risk can be modulated by the environment (Figure 1). A number of internal and external environmental factors have been associated with the etiopathology of rheumatic disorders, including viral infection, nutrition, and exposure to chemicals and radiation.[5,6] Such factors influence or modify the profile of epigenetic modifications, which, in turn, have a direct relationship with the regulation of gene expression, and ultimately the function of the immune system.

Rapid progress in understanding epigenetic alterations in cancer has enabled us to determine the general mechanisms of epigenetic deregulation, identify clinical markers of epigenetic change, and embark on the development of novel therapeutic drugs.[7] By contrast, advances in understanding epigenetic mechanisms in the context of rheumatic diseases, as well as in other disorders, have been much slower, and studies remain confined to a small number of laboratories. Nevertheless, evidence of important roles for these types of alterations in autoimmune diseases is increasing. Furthermore, novel technologies that facilitate gene identification and the systematic search for novel epigenetically deregulated genes support the investment of research in this area. This article summarizes and discusses the evidence for epigenetic mechanisms in autoimmune rheumatic diseases, with a focus on changes in DNA methylation, and outlines the future steps to be taken in the field.

Epigenetics and gene function

Epigenetic gene regulation has an essential role in determining individual gene function and activity, and, at the genomic level, determines which sets of genes are functional in each specific cell type. Such regulation takes the form of small chemical group additions to DNA or to the protein complex around which DNA is wrapped, particularly core histones. In brief, the two major types of epigenetic modification are DNA methylation and histone post-translational modifications. Both types of

Competing interests
The author declares no competing interests.

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Key points

- Autoimmune rheumatic disorders are complex diseases that involve genetic and environmental components—these facets are linked by epigenetic modifications, which control gene expression and are subject to environmental influences
- Monozygotic twins discordant for autoimmune disease provide an opportunity to specifically study epigenetic changes that lead to the development of autoimmunity, because genetic variability is excluded
- Candidate gene approaches have identified a small set of genes that undergo aberrant DNA demethylation and overexpression in systemic lupus erythematosus and rheumatoid arthritis
- High-throughput approaches are necessary for screening epigenetic alterations in autoimmune disease, and it is essential to screen the specific tissue and cell types that are relevant to disease pathogenesis
- Identification of cell-specific targets of epigenetic deregulation in autoimmune rheumatic disorders will provide clinical markers for diagnosis, disease progression and response to therapies

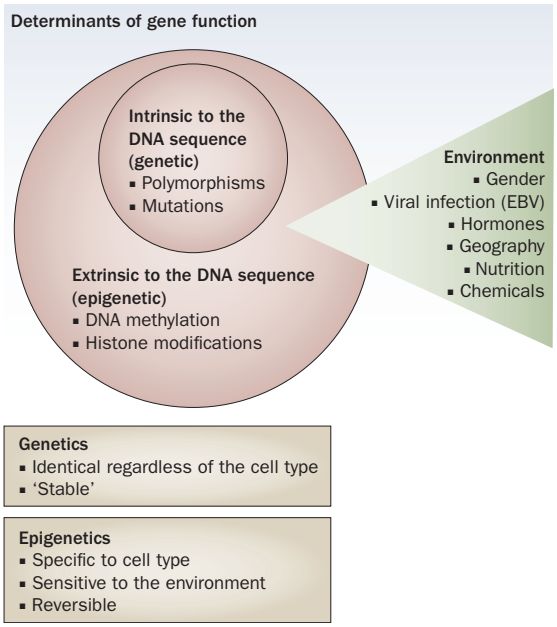

Figure 1 | Genetic and epigenetic components determine gene function in health and disease. DNA sequence changes (including polymorphisms and mutations) can be considered intrinsic to the DNA sequence, whereas DNA methylation and histone modifications, that is, the major epigenetic modifications, are extrinsic to the DNA sequence. Epigenetic modifications are far more sensitive to environmental stimuli than the sequence of DNA. Abbreviation: EBV, Epstein–barr virus.

modifications influence gene expression, especially when occurring at or near a promoter sequence.[8] Furthermore, epigenetic marks also help to regulate other processes, such as DNA repair[9] and replication.[10] To cover the entire range of cellular processes in which epigenetic regulation is involved, therefore, a general definition that describes epigenetics as mechanisms that register, signal or perpetuate gene activity states has been proposed.[11]

Roles of DNA methylation

DNA methylation mainly occurs at cytosine–guanine (CpG) dinucleotides. Under-represented in the genome, these dinucleotides are concentrated in a variety of repetitive sequences, as well as in regions known as CpG islands, many of which overlap with promoters. From the point of view of gene regulation, CpG methylation at promoters is generally associated with transcriptional repression (Figure 2), an outcome that applies not only to CpG island-associated promoters, but also to promoters with a low density of CpGs. Promoter methylation has been best studied in CpG island-containing gene promoters, which are often present in housekeeping or ubiquitous genes.[12] It has also been well studied in the context of allele-specific DNA methylation, which occurs in imprinted genes, where monoallelic expression and methylation is maternally or paternally determined, and in X chromosome inactivation, a mechanism that compensates for the unequal copy number of X-linked genes between males and females. Recent studies have shown that methylation in regions adjacent to CpG islands, known as CpG island shores, can also be associated with changes in gene expression.[13] Methylation also occurs in repetitive sequences, which are in fact the main location of 5-methylcytosine in vertebrate cells.

In all cases, CpG methylation is established and maintained by a group of enzymes known as DNA methyltransferases (DNMTs),[14] which use S-adenosylmethionine as the methyl group donor. DNA demethylation occurs or is induced by inhibition of DNA methyltransferase activities (Figure 2). This mechanism, also known as passive DNA demethylation, provides the basis for several drugs that are used as therapeutic compounds with the aim of erasing aberrant hypermethylation.[15] Alternatively, active demethylation has been described, particularly in cell (de)differentiation and reprogramming processes, and in the context of the activation of immune cells.[16,17] The identities of the enzymes involved in active demethylation are less clear. Several candidates have been proposed, including methyl-CpG-binding domain protein 2 (MBD2), or the cyclical activity of DNA methyltransferases, but their roles are controversial.[18] Recently, there has been more general agreement that active demethylation might depend on the activity of cytosine deaminases, such as activation-induced cytidine deaminase (AICDA) (Figure 2).[16,19] AICDA-dependent demethylation reportedly requires the participation of additional elements such as the G/T mismatch-specific thymine DNA glycosylase, a member of the methyl-CpG binding domain protein family, and the p53-effector gene *GADD45A*, which encodes growth arrest and DNA damage-inducible protein GADD45α.[20] Interestingly, *Gadd45α* knockout mice develop a lupus-like syndrome.[21] In a related advance, TET proteins have recently been shown to catalyze the conversion of 5-methylcytosine to hydroxymethylcytosine, raising the possibility that demethylation might be a TET-mediated process, without excluding a role for AICDA.[22,23]

Histone modifications

Histone post-translational modifications are another major source of epigenetic information,[24] and occur in the form of lysine acetylation, methylation of lysine or

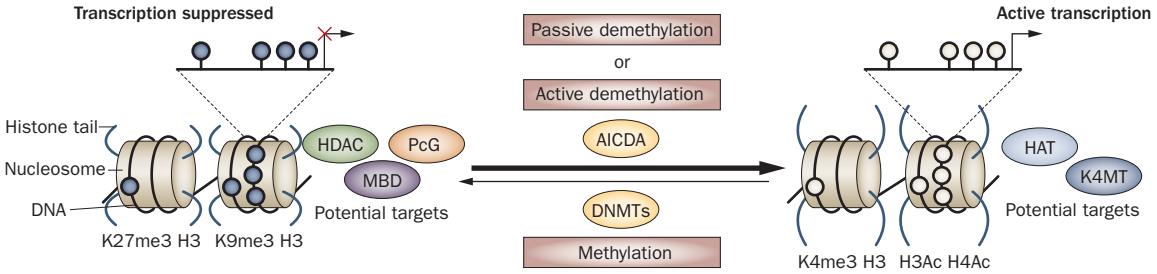

Figure 2 | DNA methylation, histone modification and gene expression changes in autoimmune disease. Two nucleosomes (represented as cylinders) covering approximately 400 base pairs are shown. CpG methylation is represented with filled black circles, open circles denote unmethylated CpGs. Histone modifications, which occur at protruding N-terminal tails, are related to DNA methylation status. On the left, two modifications of histone H3 associated with DNA methylation and the absence of gene expression are shown: K9me3 H3 and K27me3 H3. On the right, histone marks associated with hypomethylation and active transcription are shown: H3Ac, H4Ac and K4me3 H3. Most epigenetic changes identified in autoimmune rheumatic diseases are loss of CpG methylation leading to increased expression, which can result from induction of active demethylation pathways, for instance involving AICDA, or passive demethylation, by loss of DNMT activity. Elements of chromatin that might participate in this process are shown in association with the nucleosomes. Abbreviations: AICDA, activation-induced cytidine deaminase; CpG, cytosine–guanine dinucleotide; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; H3Ac, hyperacetylated histone H3; H4Ac, hyperacetylated histone H4; K4me3 H3, histone H3 trimethyl lysine 4; K4MT, histone H4 lysine 4 methyltransferase; K9me3 H3, histone H3 trimethyl lysine 9; K27me3 H3, histone H3 trimethyl lysine 27; MBD, methyl-CpG binding domain proteins; PcG, Polycomb group proteins.

arginine, phosphorylation of serine or threonine, and other less frequent modifications. Such changes have been detected in at least 60 different amino acid residues.[24] Creating further complexity, lysine and arginine methylation can comprise the addition of one, two or three methyl groups in a symmetric or asymmetric form. Different combinations of histone modifications are associated with specific functional states, including transcriptional activation or repression, transcriptional elongation, or competence for DNA replication. This variety provides an enormous potential for functional outcomes, in association with different histone modification profiles.[24] In some cases, the association of specific histone modifications with gene expression is very well defined, such as for histone H3 trimethyl-lysine9 (K9me3 H3) or trimethyl-lysine27 (K27me3 H3), which are characteristic of repressed genes, or H3 and H4 hyperacetylation (H3Ac, H4Ac) and trimethyl-lysine4 H3 (K4me3 H3), which are present in many active genes (Figure 2). In common with DNA methylation, histone modifications are enzymatically reversible, although—in contrast to DNA demethylation—the enzymes that remove chemical groups from amino acid residues are well characterized.[25] Histone lysine and arginine methyltransferases and demethylases, and histone lysine acetyltransferases and deacetylases, are all involved in establishing and maintaining the balance of histone modifications. All these enzymes constitute a potential source of new targets for developing therapeutic compounds.[26]

The epigenome

Strictly speaking, for a given functional situation, each cell type in an organism is characterized by a specific epigenomic profile composed of a related set of histone modifications and DNA methylation patterns (Box 1), with an associated transcriptome. Major efforts are being made to map the profiles of epigenetic modifications in different cell types, physiological situations and pathological contexts. We remain, however, far from understanding the relevance and meaning of the complex epigenetic profiles of different cell types, including which modifications are functionally relevant. Furthermore, the epigenome acts as an interface between the genome and the environment that may be considered at two levels: the cellular environment and the environment surrounding an entire organism.

The enzymes and factors that comprise the epigenetic machinery are associated with, and targeted to, specific genomic regions through several elements, including hormone—and other ligand—receptors,[27] as well as association with other transcription factors, such as those involved in cell differentiation.[28] Thus, the targeting and setting of epigenetic marks is dependent on the availability of such elements, and, therefore, a variety of events can lead to the disruption of epigenetic profiles at particular genomic regions. For instance, hormone-dependent recruitment of histone modification enzymes is, obviously, affected by hormonal levels.[29] Similarly, changes in the stability, levels, efficiency or affinity of transcription factors that are associated with histone modification enzymes can influence epigenetic modifications at a local level. Such changes can, for example, be due to genetic mutations or polymorphisms that disrupt the correct function of the factors.[30]

Mechanisms of epigenetic deregulation

Epigenetic alterations are a source of potential defects that lead to gene malfunction in a pathological context, particularly in so-called genetically complex diseases. In contrast to genetic defects, epigenetic errors occur without a direct change in the genetic sequence and are potentially reversible. Understanding how epigenetic alterations arise in cancer provides a useful reference for investigating this type of defect in other diseases, such as autoimmune

Box 1 | Associations between epigenetic marks

Epigenetic modifications at a given genomic sequence are not fully independent, and various mechanisms couple the establishment and maintenance of different epigenetic marks. Indeed, histone modifications are associated with DNA methylation via several routes. For example, members of the polycomb group (PcG) protein family of transcription factors, responsible for the repression of key genes involved in cell differentiation and development, form part of complexes containing histone modification enzymes and are strongly associated with genomic regions of methylated DNA.[34,35,87] The exact mechanism by which this connection occurs is not yet understood, but it denotes a context-dependent collaboration between PcG proteins and DNA methylation. Similarly, methyl-CpG binding domain (MBD) proteins interact directly with methylated DNA, and are associated with several histone modification and remodeling complexes.[88,89] Many transcription factors are also associated with such complexes. As well as DNA methylation having an influence on histone modifications, the inverse relationship can occur; it has been shown that *de novo* methyltransferases bind to chromatin that contains unmethylated histone H3 lysine 4.[90]

As well as the connections between histone and DNA modifications, different histone modifications are also linked. Multiple feedback loops exist, in which enzymes that are responsible for the establishment of a particular histone modification specifically interact with chromatin characterized by another, or the same, histone modification. These interactions occur because the modifications provide specific binding sites for protein domains. Histone modification and chromatin remodeling enzymes are among the factors that contain such domains, including the bromodomain, which binds acetyl-lysine, and the chromodomain, which binds methyl-lysine.[91]

rheumatic disorders. Rapid progress in cancer epigenetics has also contributed to the development of novel strategies, platforms and approaches for investigating and screening for epigenetic alterations in human disease.[31]

The analysis of epigenetic alterations in cancer has revealed that DNA methylation is profoundly disrupted. For instance, cancer cells display hypermethylation at the promoter CpG islands of many genes including, but not limited to, tumor suppressor and other cancer-relevant genes.[7] In parallel, cancer cells undergo a global decrease in the content of 5-methylcytosine as a consequence of hypomethylation at repetitive elements.[32] Hypomethylation of mobile elements such as retrotransposons is of particular interest as it can play a major part in genomic instability.[33]

To explain how aberrant promoter hypermethylation in cancer is targeted to specific sites, it has been proposed that DNA methyltransferases associate with factors that are involved in repressing genes in a tissue-specific manner, as is the case with the Polycomb group proteins, which are normally involved in cell differentiation.[34,35] The tissue-type-specific association of Polycomb group proteins enables the generation of hypermethylation profiles that are specific to cancer type.[35] In parallel, several cellular mechanisms result in the generation of aberrant profiles of histone modifications, although their tumor-type specificity has been less well studied. For example, in leukemia, many fusion proteins—resulting from chromosomal translocations—contain a histone modification enzyme fused to another factor that binds DNA in a sequence-specific manner.[36] Also, aberrant CpG island hypermethylation leads to the recruitment of methyl-CpG binding domain proteins, which are integral subunits of histone modification complexes.[37]

Epigenetic changes, which are well studied in the case of cancer, have been less widely investigated in other diseases. In contrast to the genome, which is almost identical in all cell types of an organism, profiles of epigenetic modifications, both normal and pathological, are tissue-specific and can be differentially influenced by external factors. When investigating epigenetic alterations, therefore, it is essential to focus on a pure or enriched cell type. Such specificity is relatively easy to achieve in the case of cancer, because the clonal nature of tumors allows relatively pure populations of highly proliferating cells, and cell model systems, to be analyzed. When investigating other diseases, such as autoimmune rheumatic diseases, it is important to analyze cell types relevant to the pathogenesis, although the capacity to do so is usually limited by the number of cells that can be obtained.

Epigenetics in rheumatic diseases

One of the most striking pieces of evidence of a role for environmental effects in the development of autoimmune rheumatic diseases is the high discordance rate observed in monozygotic twins, who share their genetic information and therefore possess identical genetic susceptibility variants. For instance, rheumatoid arthritis (RA) shows 12–35% concordance between twins, while the figure for SLE is 20–40%.[38,39] These rates, which are considerably higher than for non-identical siblings but which fall far short of 100% concordance, indicate the dual genetic and environmental origin of rheumatic disorders. Recently, environmental influences have been shown to be more directly associated with epigenetic deregulation than it was previously possible to demonstrate, due to the great progress made in the study of epigenetics in the past decade, and in the light of several studies that have addressed the relevance of epigenetic changes against a background of phenotypic divergence (or similarity) between twins,[40,41] and in cloned animal models.[42,43] Furthermore, the recognition that the environment can indeed directly affect DNA methylation and histone modification patterns has strengthened the notion of environmental influence.[44,45]

During the past twenty years, epigenetic studies in rheumatology have been restricted to a few diseases, and most of the data are limited to SLE and RA. The first suggestions of a potential role for DNA methylation in autoimmune disease came from studies in which small compounds that result in decreased DNA methylation, such as 5-azacytidine, hydralazine or procainamide, induced symptoms that are associated with autoimmune disease. For example, these drugs induce autoreactivity in $CD4^+$ T cells, or antinuclear factors in both human and mouse models.[46–48] Azacytidine-treated cells were later found to cause SLE-like disease when transferred to mice.[49,50] Furthermore, direct administration of 5-azacytidine and procainamide induced a lupus-like syndrome in mice,[49,51] and 5-azacytidine-treated T cells were shown to be phenotypically and functionally similar to T cell subsets from patients with active SLE.[52] All these data point to the role of epigenetically altered T cells in causing autoimmunity.

Moving beyond pharmacological induction of demethylation, we have known for some time that T cells from patients with SLE or RA, as well as synovial fibroblasts from individuals with RA, have a lower content of 5-methylcytosine than their healthy equivalents.[53,54] One common approach to identify sequences that undergo DNA methylation changes is the analysis of genes that are known to be relevant for a particular cell type. The so-called candidate sequence DNA methylation analysis has identified genes and other types of sequences that are hypomethylated in SLE and RA. Several pieces of evidence support the notion that hypomethylation could be relevant in other cell types, including B cells in SLE,[55] and the specific targets, pathways and cell types involved are discussed in further detail in the following sections.

Global and high-throughput assessments of the DNA methylation profiles of white blood cells from twins discordant for SLE have recently revealed hypomethylation in the twins with SLE in comparison with their healthy siblings.[56] A number of specific sites of hypomethylation were identified in this first high-throughput analysis; indeed, 49 genes were hypomethylated in the twins with SLE. Furthermore, a decrease in the DNA methylation status of the 18S and 28S sequences of ribosomal RNA was found. Although the functional relevance of hypomethylation in these elements has yet to be determined, these findings reinforce the notion that SLE, and perhaps other autoimmune rheumatic diseases, are associated with global and sequence-specific decreases in methylation, which cause overexpression of affected genes.

Mechanisms of loss of DNA methylation

Several mechanisms are thought to participate in the hypomethylation of cells in SLE, which are the best studied example of this change in rheumatic disease. A potential source of epigenetic deregulation comes from the effects of aging on DNA methylation patterns, which can explain variations and dynamics of epigenetic profiles within and between tissues.[57,58] Early studies showed that DNA methylation decreases during aging in several tissue types,[59] and cells cultured to senescence undergo a progressive loss of methylation.[60] It is thought that loss of DNA methylation during aging results from passive demethylation, as a result of decreased efficacy of DNMTs. An alternative mechanism proposed to explain both global and sequence-specific hypomethylation is based on the finding that levels of DNMTs are decreased in T cells in individuals with SLE (Figure 2),[61] perhaps due to decreased extracellular signal-regulated kinase (ERK) pathway signaling.[62] In this sense, decreased levels of DNMTs might result in failure to maintain DNA methylation patterns throughout mitosis and therefore contribute to SLE pathogenesis. Another proposed mechanism that might explain decreased levels of DNMTs in SLE involves microRNAs (miRNAs), a class of small noncoding RNAs that regulate gene expression by pairing with their target mRNAs and that are often deregulated in cancer and other human diseases.[63] Specifically, two miRNAs, miR-21 and miR-148a, have recently been identified that are overexpressed in $CD4^+$ T cells of both patients with SLE and lupus-prone MRL/*lpr* mice. Data from this mouse model show that the miRNAs promote hypomethylation by repressing the expression of DNMT1.[64]

On the other hand, it has also been suggested that active mechanisms of DNA demethylation might be involved (Figure 2). Although the identities of bona fide DNA demethylases remain elusive, several factors that have been reported to participate in active demethylation are altered, in terms of activity and/or expression, in the context of autoimmune diseases. Such factors might include GADD45α, which occurs at high levels in $CD4^+$ T cells of patients with SLE.[65] Interestingly, recent data indicate that suppression of AICDA, which is currently one of the best candidate enzymes for promoting active demethylation, results in a significant decrease of autoantibody production in an SLE mouse model.[66]

Targets and pathways

Candidate gene studies have revealed several pathways in which aberrant gene expression due to DNA demethylation is linked with the development of SLE (Table 1). Most of the targets identified by genetic approaches in SLE come from studies of $CD4^+$ T cells. Hypomethylation and subsequent overexpression have been observed in genes from several pathways. Most of the genes for which DNA hypomethylation has been reported are from the cluster of differentiation (CD) group, including *ITGAL* (also known as *CD11A*),[67] which is important for cell–cell adhesion, *CD70* (encoding CD70, also known as tumor necrosis factor ligand superfamily member 7),[68] which is required for T cell proliferation, clonal expansion and the promotion of effector T cell formation, and *CD40LG* (encoding CD40 ligand),[69] which stimulates B cell IgG overproduction. Nevertheless, other factors, such as the gene encoding perforin 1 (*PRF1*),[70] which contributes to autoreactive killing of macrophages and release of apoptotic material, are also hypomethylated in $CD4^+$ T cells from individuals with SLE (Table 1).

Few studies have addressed methylation changes in B cells in SLE, but in 2009 Garaud and colleagues[71] reported that the E1B promoter of *CD5* is hypomethylated in resting SLE B cells. This study also showed that high levels of interleukin (IL)-6 in SLE B cells, which is known to be positively associated with SLE disease activity, reduce the expression of DNMT1. Reduced methyltransferase activity affects methylation and expression of *CD5*, resulting in impaired B cell receptor signaling, relevant to SLE pathogenesis. Given the importance of B cells in the pathogenesis of SLE, it is likely that more studies will soon concentrate on B cell subtypes.

In a related finding, IL-6 has also been shown to be hypomethylated in peripheral blood mononuclear cells (PBMCs) from individuals with RA.[72] Given the mixed nature of these samples, further analysis of isolated B cells is necessary to confirm whether deregulated IL-6 expression occurs in these cells as a consequence of epigenetic changes in RA. Nevertheless, altered methylation of *IL6* in RA reinforces the notion of the importance of applying epigenetic studies to the investigation

Table 1 | Genetic elements that are hypomethylated in autoimmune rheumatic diseases

Genetic element	Disease	Cell type	Product and/or function	Reference
Genes				
ITGAL	SLE	$CD4^+$ T cell	Integrin α-L, important for cell–cell adhesion	Lu *et al*. (2002)[67]
CD70	SLE	$CD4^+$ T cell	CD70 antigen, required for T cell proliferation	Oelke *et al*. (2004)[68]
CD40LG	SLE	$CD4^+$ T cell	CD40 ligand, stimulates overproduction of IgG by B cells	Lu *et al*. (2007)[69]
PRF1	SLE	$CD4^+$ T cell	Perforin 1, involved in autoreactive killing	Kaplan et al. (2004)[70]
CD5	SLE	$CD19^+$ B cell	T-cell surface glycoprotein CD5, associated with production of several interleukins, negative regulator of BCR signaling	Garaud *et al*. (2009)[71]
IFNGR2	SLE	PBMC	IFN-γ receptor 1, proinflammatory activity through interaction with different cell types	Javierre *et al*. (2010)[56]
MMP14	SLE	PBMC	MMP-14, involved in tissue destruction and inflammation	Javierre *et al*. (2010)[56]
LCN2	SLE	PBMC	Neutrophil gelatinase-associated lipocalin, iron transporter and marker for SLE	Javierre *et al*. (2010)[56]
KIR3DL1	SLE	SLE	Killer cell Ig-like receptor, three domains, long cytoplasmic tail, 1, modulates NK cell-mediated killing	Basu *et al*. (2009)[86]
IL6	RA	PBMC	IL-6, participates in B cell response	Nile *et al*. (2009)[72]
miRNAs				
miRNA203	RA	SF	Repressor of several MMPs and IL-6	Stanczyk *et al.* (2010)[75]
Repetitive elements				
rDNA (18S, 28S)	SLE	PBMC	Constitutive elements of ribosomal particles	Javierre *et al.* (2010)[56]
L1 elements	RA	SF	Fine tuning of expression for nearby or enveloping genes	Karouzakis *et al.* (2009)[74]

Abbreviations: BCR, B cell receptor; IFN, interferon; Ig, immunoglobulin; IL, interleukin; miRNA, microRNA; MMP, matrix metalloproteinase; NK, natural killer; RA, rheumatoid arthritis; rDNA, ribosomal DNA; SF, synovial fibroblast; SLE, systemic lupus erythematosus; PBMC, peripheral blood mononuclear cell.

of pathways that are affected in rheumatic diseases. So far, few data are available on epigenetic changes in RA. As the disease is characterized by the progressive destruction of joints by invasive synovial fibroblasts, most of the studies that do exist have focused on this cell type. Remarkably, increased expression of retrotransposable L1 elements, one of the major classes of repetitive elements that are interspersed in the genome, has been found to occur in association with global hypomethylation in synovial tissue.[73] Hypomethylation of specific CpG sites upstream of an L1 open-reading frame has since been reported to play an essential part in the irreversible phenotypic changes that occur in synovial fibroblasts.[74] More recently, DNA demethylation-associated deregulation of one miRNA has also been associated with the deregulation of target genes that seem to have a key role in RA pathogenesis (Table 1).[75]

Many susceptibility genes have been identified for several autoimmune rheumatic diseases, but it is not yet known whether epigenetic changes affect some of these genes, or other genes for which no disease links have been established, or both. It seems unlikely that genes for which certain haplotypes have been found to confer disease risk will be the same as those that undergo altered epigenetic regulation, because the mechanisms and processes that generate genetic and epigenetic variation are entirely different. High-throughput DNA methylation and histone modification analyses will surely contribute to systematic identification of the relationships between genetic and epigenetic alterations in pathological pathways. In this sense, array-based analysis of twins discordant for SLE[56] has revealed new pathways and groups of factors that might be relevant to epigenetic deregulation in autoimmune disease (Table 1). These potential culprits include an interleukin receptor gene (*IFNGR2*), a matrix metalloproteinase (MMP), and a molecule involved in cellular transport (encoded by *LCN2*).[56] Although twins discordant for disease provide a unique model for studying epigenetic involvement in pathogenesis, given that genetic variability is excluded as a cause for discordance, genome-wide analyses of epigenetic changes are becoming easier and cheaper to perform, and will increasingly be carried out with larger collections of individuals with rheumatic disease. Such analyses are of clinical interest for two reasons: firstly, they might provide key information to enable classification of patients with respect to their clinical phenotype or their response to different biologic therapeutic agents, and secondly, they might reveal novel targets for potential epigenetic therapeutic treatment.

The therapeutic potential of compounds directed against epigenetic changes is obviously a primary line of research. In other pathological contexts, such as in hematological malignancies, several inhibitors of DNA methylation and histone deacetylation are now used for the clinical treatment of patients.[15,76] In autoimmune rheumatic disease, inhibiting DNA methylation would not be appropriate to revert DNA methylation changes (as the changes identified to date are hypomethylation, not hypermethylation, Table 1), and agents should be designed to specifically increase methylation (no such agent yet exists). This need highlights the importance of the identification of mechanisms that promote

both global and sequence-specific hypomethylation. Inhibition of histone deacetylases, which has been shown to alleviate renal disease in a mouse model of SLE, does have potential in the treatment of autoimmune rheumatic diseases.[77,78] In line with this, it has been recently reported that histone deacetylase is efficient in the treatment of juvenile idiopathic arthritis.[79] Identification of novel epigenetic targets, a better understanding of the epigenetic mechanisms and development of novel compounds directed against them will surely open novel therapeutic approaches in rheumatic disease.

Investigating the correct cell or tissue type

As mentioned previously, the study of epigenetic alterations requires analysis of the appropriate cell or tissue types. The reasons for this are threefold: firstly, each cell type is characterized by a particular epigenetic profile, or epigenome, that is exquisitely associated with a specific gene expression profile; secondly, different cell types can undergo epigenetic alterations independently (because they are exposed to the environment in distinct manners, have different underlying metabolic and signaling pathways, and are thus altered by divergent pathways); and finally, different cell types have distinct roles in the pathogenesis of each disease.

The complexity of interactions between different immune cell types in rheumatic diseases (Figure 3) has meant that our concept of the cell types that are involved in pathology has evolved along with our understanding of innate and adaptive immunity. Historically, research in rheumatic diseases focused on T cells, but now the role of B cells is regarded as important for the development of therapeutic agents and, in fact, some of the most effective therapies in the treatment of RA (comparable in efficacy to antagonists of tumor necrosis factor), are directed towards depleting this cell type.[80] In SLE, despite the failure of several of these anti-B cell therapies, renewed vigor has come from the latest promising results using agents that inhibit B-cell-activating factor (BAFF). Mouse studies have demonstrated a role for BAFF in lupus pathogenesis, as well as improvement in response to BAFF blockade,[81] and results of phase III clinical trials are encouraging.[82] Furthermore, DNA demethylating agents such as hydralazine have been shown to subvert B lymphocyte tolerance, and to contribute to the generation of pathogenic autoreactivity.[83] In RA, synovial fibroblasts have attracted the attention of clinicians and researchers because they acquire aberrant phenotypic features commonly associated with those of transformed cells. In the pre-genomic era and even very recently, most DNA methylation and histone modification analyses—including those listed in Table 1—involved the use of candidate sequence-based strategies. Furthermore, many of the studies focused on specific tissue and cell types according to contemporary perceptions of their potential role in pathogenesis. These approaches have probably biased the identification to date of targets and cell types that undergo epigenetic deregulation in autoimmune diseases. For instance, for many years, most of the DNA methylation analyses in SLE focused on the study of T cells. These studies have contributed greatly

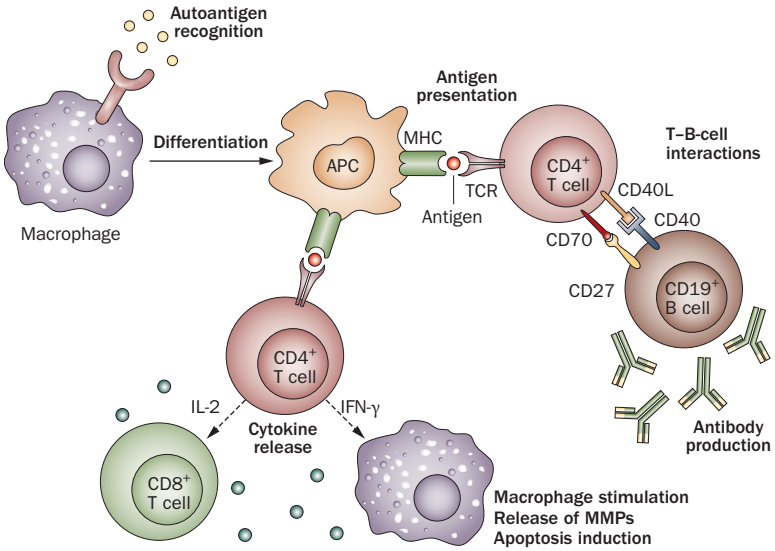

Figure 3 | Complex interactions between cells of the immune system, their targets and products underlie the pathology of autoimmune rheumatic diseases and might be epigenetically deregulated. Several processes that are known to be deregulated in these diseases are depicted, including autoantigen recognition, antigen presentation, cytokine release, release of MMPs, induction of apoptosis and production of autoantibodies. Alterations affect several cell types, including B cells, T cells (both $CD4^+$and $CD8^+$) and macrophage-derived APCs. Some of the molecules that participate in immune interactions relevant to rheumatic disease have already been demonstrated to be hypomethylated and overexpressed in RA and SLE, including CD40LG,[46] CD70,[45] and MMPs.[39] The epigenetic statuses of many other factors in autoimmune rheumatic disease remain to be analyzed. Abbreviations: APC, antigen presenting cell; CD, cluster of differentiation; CD40LG, CD40 ligand; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; TCR, T cell receptor.

to the recognition of DNA methylation alterations in SLE. However, given that B cells have a central role in the pathogenesis of autoimmune diseases, including the presentation of autoantigens, activation of $CD4^+$ and $CD8^+$ cells and secretion of proinflammatory cytokines, it is relevant to investigate epigenetic deregulation in this cell type. Indeed, recent studies have shown that B cells also display alterations in the DNA methylation status of certain genes in SLE.[71] Similarly, in the case of RA, analyses have tended to focus on synovial fibroblasts, due to their aberrant phenotype and the major role that synovial tissue is thought to have in disease progression.

The importance of studying the 'correct' tissue is illustrated by studies of multiple sclerosis (MS), an autoimmune disorder that shares some features with autoimmune rheumatic diseases. A recent study compared $CD4^+$ T cells of monozygotic twins discordant for MS but found no substantial differences in DNA methylation,[84] whereas a candidate gene study has shown hypomethylation at the *PAD2* promoter in normal-appearing white matter from MS patients.[85] Although the small number of DNA methylation studies in MS limits the confidence that can be placed in the conclusions, these results suggest that perhaps microglia or another cell type should be studied, and emphasizes the importance of selecting the tissue type for study.

Conclusions

Greater efforts are needed to understand the molecular mechanisms that contribute to the pathogenesis of autoimmune rheumatic diseases. Disorders of this type share many features, and genetic studies have revealed the existence of several common susceptibility genes. However, genetic variation represents only half of the story. Gene function depends not only on DNA sequence, but also on epigenetic modifications, including both DNA methylation and histone post-translational modifications. These modifications are influenced by environmental factors and are known to contribute to the pathogenesis of several autoimmune diseases. Most importantly at present, epigenetic alterations can be used as clinical markers of disease progression or response to therapy. Furthermore, epigenetic alterations can be pharmacologically reverted. This potential to tackle aberrant changes opens the possibility of developing novel therapies, some of which are already used for the clinical treatment of hematological malignancies. Most efforts to identify the epigenetic alterations that occur in autoimmune rheumatic disease have focused on SLE and RA, and have served to identify both global and sequence-specific hypomethylation and overexpression of key genes in immune function. Studies have made use of candidate-gene methylation analysis and have concentrated their attention on a few cell types. We now face several challenges: to make use of high-throughput approaches, to systematically analyze all potential specific cell types relevant to disease pathogenesis, to perform prospective studies to better understand the extent and role of these alterations in these diseases, and to find the best way of using this information in a clinical setting.

Review criteria

The studies included in this Review were identified by searching PubMed using the phrases "autoimmune AND epigenetics", "lupus AND epigenetics", "lupus AND methylation", "arthritis AND epigenetics", or "arthritis AND methylation". The searches were restricted to full-text papers in the English language, without limitation of publication date, and were completed on January 17th, 2011. In addition, a few selected key chromatin and epigenetics Reviews, as well as well as a few seminal papers on epigenetic regulation have been included. Papers cited in this Review were selected based on the author's view of their direct relevance to the concepts illustrated.

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Acknowledgments

E. Ballestar is supported by PI081346 (FIS) grant from the Spanish Ministry of Science and Innovation (MICINN) and 2009SGR184 grant from AGAUR (Catalan Government).