Chapter 6
Epigenetics of Autoimmune Diseases

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Abstract This chapter provides several examples of epigenetic deregulation in autoimmune diseases, a heterogeneous group of human conditions characterized by a deregulated immune response against the body own organs and tissues. Early studies based on the candidate gene approach have been flanked by genome-wide screenings in the last few years, revealing global changes in DNA methylation or histone tail modifications, as well as deregulated methylation and/or expression of hundreds of genes and microRNAs in cells from patients affected by those disorders. This chapter will focus on epigenetic deregulations observed in systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, psoriasis, multiple sclerosis, systemic sclerosis, and autoimmune thyroid diseases, even though epigenetic modifications are increasingly being observed in many other autoimmune diseases. By contrast, only a few environmental factors have been shown or suspected to induce the observed epigenetic changes. Epigenetic drugs and RNA silencing experiments have often reversed autoimmune disease-like phenotypes in rodents or cell cultures, leading researchers to debate on their potential use in the treatment of these human conditions.

Keywords Epigenetics • Autoimmune diseases • Systemic lupus erythematosus • Rheumatoid arthritis • Sjögren's syndrome • Psoriasis • Multiple sclerosis • Systemic sclerosis

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N. Maulik and T. Karagiannis (eds.), *Molecular Mechanisms and Physiology of Disease: Implications for Epigenetics and Health*, DOI 10.1007/978-1-4939-0706-9_6,

6.1 Introduction

Autoimmune diseases include over 100 conditions characterized by an inappropriate immune response against the body own tissues and falling into two general types: systemic autoimmune diseases that damage many organs and systems such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SjS), and systemic sclerosis or scleroderma (SSc) among others, and diseases characterized by an autoimmune response against a specific organ or tissue such as psoriasis, Hashimoto's thyroiditis (HT), Graves' disease (GD), and many others (Selmi 2012). It is now clear that while many of these pathologies can be influenced by heritable factors, also environmental factors such as drugs, ultraviolet light, infectious agents, and diet play a role, and recent genome-wide association studies revealed that genomics alone cannot fully explain the individual susceptibility to those disorders (Selmi 2012). Increasing evidence points to additional mechanisms linking the individual susceptibility with environmental factors, and epigenetics is increasingly recognized as one of the most promising missing links (De Santis and Selmi 2012). Indeed, since epigenetic mechanisms are sensitive to external stimuli, several environmental effects on immune responses could be mediated by epigenetic changes (Costenbader et al. 2012).

The term epigenetics comprises heritable and reversible modifications that alter gene expression without resulting from direct changes in the primary DNA sequence. Several epigenetic mechanisms are known, including DNA methylation, covalent modifications of histone tails, and nucleosome positioning, all interacting to determine chromatin folding and the relative accessibility of a given genetic locus to activating and suppressing transcription factors (Martín-Subero 2011). Noncoding RNAs affecting gene expression are also largely recognized as epigenetic mechanisms (Esteller 2011). All those mechanisms are exhaustively described in Chap. 1 of this book.

Most of the studies performed so far have focused on epigenetic modifications in SLE and rheumatoid arthritis (Quintero-Ronderos and Montoya-Ortiz 2012), but there is increasing evidence of epigenetic changes in other autoimmune disorders such as SjS, multiple sclerosis, systemic sclerosis, psoriasis, autoimmune thyroid diseases (AITDs), and others (Meda et al. 2011; Zhang et al. 2011; Quintero-Ronderos and Montoya-Ortiz 2012). This chapter will provide a summary of the most relevant evidence of epigenetic deregulation in autoimmune disorders.

6.2 Systemic Lupus Erythematosus

SLE is a systemic autoimmune disorder characterized by the production of autoantibodies directed against nuclear self-antigens. The disease can target many organs, including the skin and joints, but also the heart, the kidneys, the nervous system, and others. It afflicts both sexes but occurs more frequently in women. Genetic association studies, genome-wide association studies, and discordance in disease

inheritance in twins, have revealed that genetics alone does not completely account for disease heritability (Deapen et al. 1992; Cunninghame Graham 2009). Early studies of SLE epigenetics showed that CD4+ T cells treated with the DNA methylation inhibitor 5-azacytidine respond to the presentation of self antigens (Richardson 1986), and their injection in mice caused a lupus-like syndrome (Quddus et al. 1993). Those studies suggested that impairments of DNA methylation might be involved in autoimmunity.

6.2.1 DNA Methylation in SLE

DNA methylation represents one of the most studied epigenetic mechanisms for gene regulation and consists of the addition of a methyl group to the 5′ position of the cytosine pyrimidine ring (5-methylcytosine) mediated by DNA methyltransferase enzymes (DNMTs) using *S*-adenosylmethione (SAM) as the methyl donor compound (Jones 2012). There are multiple families of DNMTs in mammals. Among them DNMT1 is primarily involved in the maintenance of DNA methylation patterns during development and cell division, whereas DNMT3a and DNMT3b are the de novo methyltransferases and establish DNA methylation patterns during early development (Jones and Liang 2009). Methylated DNA can be specifically recognized by a set of proteins called methyl-CpG binding proteins (MBPs), including MECP2 (methyl-CpG binding protein 2) and MBD proteins (methyl-CpG binding domain proteins), that contain a transcription repression domain to interact with other proteins and enhance DNA methylation-mediated transcriptional repression (Fournier et al. 2012).

Following the observation that inhibitors of DNA methylation were able to induce autoimmune reactions and lupus-like symptoms in animals (Richardson 1986, Quddus et al. 1993), several investigators analyzed DNA methylation levels in SLE patients (Table 6.1). One of the most replicated findings is a global DNA hypomethylation observed in the CD4+ T cells of these patients (Balada et al. 2007a). DNA hypomethylation has been often linked to a reduction of DNMT1 mRNA levels in those cells (Zhu et al. 2011; Qin et al. 2013), but data on DNMT1, DNMT3a, and DNMT3b expression levels are still conflicting (Balada et al. 2008; Liu et al. 2011; Zhu et al. 2011). Also an increased expression of MBD2 and an inverse correlation between MBD2 expression and DNA methylation levels was often observed in CD4+ T cells of SLE patients (Balada et al. 2007b; Qin et al. 2013).

Apart from studies on global DNA methylation levels, the analysis of gene-specific promoter methylation by means of candidate gene approaches led researchers to identify several genes that are hypomethylated and therefore over-expressed in CD4+ T cells of SLE patients, some examples are *ITGAL*, *CD40LG*, *PRF1*, *TNFSF7*(*CD70*), and *KIR* family genes (reviewed in Hughes and Sawalha 2011). Many of those genes encode proteins involved in immune function and inflammation, and it is believed that their overexpression leads to lupus T cell autoreactivity and subsequent induction of autoreactive B cell immunoglobulin production

Table 6.1 Some examples of epigenetic deregulation in systemic lupus erythematosus

DNA methylation		
Human CD4+ T cells	Global hypomethylation	Balada et al. (2007a)
	Reduced DNMT1 and increased MBD2 levels	Zhu et al. (2011), Qin et al. (2013)
	Demethylated genes: *ITGAL, CD40LG, PRF1, TNFSF7*(*CD70*), *KIR2DL4*	Hughes and Sawalha (2011)
	LINE-1 hypomethylation	Nakkuntod et al. (2011)
	Whole-genome approaches: 232 hypomethylated genes and 104 hypermethylated genes	Jeffries et al. (2011)
Histone tail modifications		
Animal models: MRL/lpr mice	Global hypoacetylation of histone H3 and H4	Garcia et al. (2005)
	Overexpression of histone deacetylase SIRT1	Hu et al. (2009)
	Treatment with HDACi reversed the lupus-like phenotype	Reilly et al. (2011)
Human CD4+ T cells	Global hypoacetylation of histone H3 and H4	Hu et al. (2008)
	Overexpression of histone deacetylase SIRT1	
	Global H3K9 hypomethylation	
MicroRNA expression		
Human CD4+ T cells	Upregulation of miR-21, miR-148a, miR-126, miR-224	Amarilyo and La Cava (2012), Lu et al. (2013)
	Downregulation of miR-142-3p, miR142-5p, miR-145, miR-146a	Chan et al. (2012), Ding et al. (2012), Lu et al. (2013)

(Hughes and Sawalha 2011). Recently, Balada and coworkers determined the expression levels of *ITGAL*, *PRF1*, *KIR2DL4*, *TNFSF7*(*CD70*), and *CD40LG* genes in CD4+ T cells of patients with SLE and performed correlations with the global DNA methylation status and the levels of DNMT and MBD proteins. SLE patients had significantly elevated transcript levels of *ITGAL*, *PRF1*, and *TNFSF7*(*CD70*), and those levels correlated with global DNA hypomethylation as well as with the expression of most of the DNA methylation-related genes, reinforcing the hypothesis of an epigenetic deregulated network in SLE (Balada et al. 2012).

The analysis of methylation of repetitive elements in SLE patients revealed hypomethylation of LINE-1 but not Alu in CD4+ T lymphocytes, CD8+ T lymphocytes, and B lymphocytes (Nakkuntod et al. 2011).

The candidate gene approach analysis has been paralleled in recent years by whole-genome methylation studies that are revealing hundreds of genes differentially methylated and expressed in SLE patients with respect to controls. One of such approaches performed on monozygotic twins discordant for SLE featured widespread changes in the DNA methylation status of a significant number of genes associated with immune function that occurred in parallel with a global decrease in the 5-methylcytosine content in the affected twins (Javierre et al. 2010). A subsequent genome-wide DNA

methylation analysis in CD4+ T cells from SLE patients revealed 236 hypomethylated and 105 hypermethylated CpG sites (representing 232 and 104 genes, respectively). Hypomethylated genes in lupus T cells included, among others, many involved in autoimmunity, while genes involved in folate biosynthesis, required for SAM production and DNA methylation, resulted hypermethylated (Jeffries et al. 2011). Another whole-genome methylation analysis of SLE revealed that hypomethylation of interleukin *IL10* and interleukin receptor *IL1R2* promoters is associated with disease activity (Lin et al. 2012).

6.2.2 Histone Tail Modifications in SLE

The chromatin state represents another important modulator of gene expression profiles. Histone tail acetylation on lysine residues is mediated by histone acetyltransferases (HATs) and represents one of the most studied modifications associated with chromatin relaxation and transcriptional activation (Berger 2007). Another frequently studied modification of histone tails is methylation on either lysine or arginine residues, mediated by protein methyltransferases. Methylation of histone tails can be associated with either condensation or relaxation of the chromatin structure, since several sites for methylation are present on each tail thus allowing many combinations (Martin and Zhang 2005). Most of our current knowledge on histone tail modifications in SLE derives from studies based upon in vitro cell cultures and in vivo studies in murine models of lupus demonstrating that histone deacetylase inhibitors (HDACi) reversed the expression of multiple genes involved in autoimmunity and SLE pathogenesis (reviewed in Reilly et al. 2011). For example a global hypoacetylation of histone H3 and H4 was observed in a mouse model of lupus (MRL/lpr mice) compared to control mice, and the administration of the HDACi trichostatin A reversed histone hypoacetylation with improvement of disease phenotype (Garcia et al. 2005). Others observed an aberrant expression pattern of HATs and histone deacetylases (the enzymes responsible for histone deacetylation) in CD4+ T cells of MRL/lpr mice, among which the overexpression of histone deacetylase SIRT1 was implicated in lupus pathogenesis (Hu et al. 2009). Suppression of SIRT1 expression by means of RNA silencing in the animals resulted in an increase of global histone H3 and H4 acetylation levels and mitigated the disease-related phenotype (Hu et al. 2009).

Studies in humans revealed global histone H3 and H4 hypoacetylation and increased SIRT1 mRNA levels in active lupus CD4+ T cells of SLE patients compared with controls, as well as global histone H3K9 hypomethylation in both active and inactive lupus CD4+ T cells (Hu et al. 2008). There is also evidence that aberrant histone modifications within the *TNFSF7*(*CD70*) promoter may contribute to the development of lupus by increasing CD70 expression in CD4+ T cells (Zhou et al. 2011). More than 100 different auto-antibodies to nuclear antigens were found in patients with SLE, some of which recognize insoluble nuclear antigens (chromatin, DNA, histones, and RNA), leading researchers to formulate

the hypothesis of a potential relationship between auto-antibodies production in SLE with changes in epigenetic patterns, such as DNA methylation and histone tail modifications (Thabet et al. 2012).

6.2.3 RNA-Mediated Epigenetic Mechanisms in SLE

Among noncoding RNAs, microRNA (miRNAs) are a group of small noncoding RNAs of about 22 nucleotides in length that bind to the 3′ untranslated region (3′-UTR) of target mRNAs and mediate their posttranscriptional regulation leading to either degradation or translational inhibition, depending on the degree of sequence complementarities. MiRNAs target about 60 % of all genes (Sato et al. 2011), and a complex network of interactions exists among miRNAs and other epigenetic mechanisms, such as DNA methylation and histone modification processes, to organize the whole gene expression profile.

In 2007 Dai and coworkers published the first report of a difference in miRNA expression between SLE patients and healthy controls (Dai et al. 2007). Since then studies profiling miRNA expression in blood cells, body fluids, and target tissues from SLE patients revealed unique miRNA signatures when compared with healthy individuals or those with other autoimmune diseases (Amarilyo and La Cava 2012; Shen et al. 2012). Among over-expressed miRNAs in lupus CD4+ T cells, miR-21, miR-148a, and miR-126 lead to DNMT1 downregulation by directly targeting its transcript (miR-148a and miR-126) or transcripts of genes that operate in the Ras–MAPK pathway upstream of DNMT1 (miR-21), and resulting in DNMT1 inhibition, DNA hypomethylation, and altered expression of genes involved in both pro-inflammatory and anti-inflammatory processes (Amarilyo and La Cava 2012). Other miRNAs were found to be up-regulated or down-regulated in cells of SLE patients; some examples include miR-142-3p and miR-142-5p that are down-regulated in SLE CD4+ T cells, causing T cell over-activation and B cell hyperstimulation (Ding et al. 2012). By contrast miR-146a, a negative regulator in immune and inflammatory responses, is down-regulated in SLE patients (Chan et al. 2012). A recent study profiled the expression of 270 human miRNAs in T cells from five SLE patients and five healthy controls, and identified under-expressed miR-145 and over-expressed miR-224 as well as down-regulated expression of their target genes linked to accelerated T cell activation-induced cell death (Lu et al. 2013).

6.2.4 Environmental Factors and Their Potential Epigenetic Properties in SLE

The demethylating agent 5-azacytidine was the first drug to be identified to cause lupus-like symptoms in rodents by altering DNA methylation levels (Richardson 1986; Quddus et al. 1993). Since then procainamide and hydralazine also have been

suspected of causing SLE by inhibiting DNA methylation and inducing T cells autoreactivity. Procainamide is a competitive inhibitor of DNMT1 enzymatic activity and hydralazine inhibits T and B cell ERK pathway (Cornacchia et al. 1988). Other chemicals, such as air pollutants, have been suspected to act on DNA methylation and other epigenetic mechanisms in SLE (De Santis and Selmi 2012). Among physical agents, it has been suggested that UV light might induce the overexpression of autoimmunity-related genes through aberrant T cell DNA demethylation (Li et al. 2010). Additional factors suspected to epigenetically contribute to the incidence of autoimmune diseases are increasing age and infectious agents (De Santis and Selmi 2012).

6.3 Rheumatoid Arthritis

RA is a systemic autoimmune disease primarily characterized by chronic inflammation of the joints and ultimately leading to joint destruction. Both genetic and environmental factors are involved in disease pathogenesis, but increasing evidence (Klein et al. 2012) supports a role for epigenetic modifications (Table 6.2). RA synovial fibroblasts (RASFs) play a major role in the initiation and perpetuation of the disease; they are the most common cell type at the site of invasion and active contributors in joint damage due to their ability to secrete cytokines, chemokines, and joint-damaging enzymes. Moreover, RASFs show tumoral behavior including invasiveness and resistance to apoptosis. Epigenetic mechanisms have been largely investigated as contributors of RASFs aggressiveness and those cells are the best-characterized ones for epigenetic alterations in RA (Klein et al. 2012; Nakano et al. 2013).

6.3.1 DNA Methylation in RA

In 1991, Corvetta and coworkers observed reduced global DNA methylation in peripheral blood, synovial mononuclear cells, and synovial tissues from RA patients (Corvetta et al. 1991). Others observed hypomethylation and overexpression of LINE 1 retrotransposable elements in RASFs, affecting the expression of other genes likely contributing to cell activation (Neidhart et al. 2000; Kuchen et al. 2004). A subsequent study confirmed those previous findings and revealed that proliferating RASFs were deficient in DNMT1, and that the demethylating agent 5-azacytidine reproduced the activated phenotype of RASFs in normal synovial fibroblasts, with upregulation of over 100 genes including growth factors and receptors, extracellular matrix proteins, adhesion molecules, and matrix-degrading enzymes (Karouzakis et al. 2009). The search for specific genes regulated by DNA methylation in RASFs revealed other demethylated and over-expressed ones,

Table 6.2 Some examples of epigenetic deregulation in rheumatoid arthritis

DNA methylation		
Human synovial fibroblasts (RASFs)	Global hypomethylation	Corvetta et al. (1991), Karouzakis et al. 2009
	LINE-1 hypomethylation	Neidhart et al. 2000, Kuchen et al. 2004
	Reduced DNMT1 levels	Karouzakis et al. (2009)
	Demethylated genes: *CXCL12*	Karouzakis et al. (2011)
	Hypermethylated genes: *DR3*	Takami et al. (2006)
	Whole-genome approaches: 207 hypomethylated or hypermethylated genes	Nakano et al. (2013)
Human CD4+ T cells	Demethylated genes: *CD40LG*	Liao et al. (2012)
Human peripheral blood mononuclear cells	Demethylated genes: *IL-6, IL-10*	Nile et al. (2008), Fu et al. (2011)
Histone tail modifications		
Human synovial fibroblasts (RASFs)	Overexpression of histone deacetylase HDAC1	Horiuchi et al. (2009)
Human peripheral blood mononuclear cells	Overexpression of histone deacetylase HDAC1	Gillespie et al. (2012)
Animal models	Treatment with HDACi reversed the RA-like phenotype	Cantley et al. (2012) De Santis and Selmi (2012)
MicroRNA expression		
RA synovial cells	Upregulation of miR-146a, miR-155, miR-223	Amarilyo and La Cava (2012), Lu et al. (2013)

such as for example *CXCL12* that contributes to the expression of matrix metalloproteinases (Karouzakis et al. 2011). Other genes were found to be hypermethylated in those cells, including the promoter of the death receptor 3 (*DR3*) gene, whose downregulation in RASFs was linked to resistance to apoptosis (Takami et al. 2006). Impairments of DNA methylation were also observed in peripheral blood mononuclear cells of RA patients, some examples are demethylation of CpG sites in interleukin-6 (*IL-6*) and interleukin-10 (*IL-10*) gene promoters (Nile et al. 2008; Fu et al. 2011). The incidence of both RA and SLE is higher in females than in males, and the *CD40LG* gene on the X chromosome was found to be demethylated and overexpressed in CD4+ T cells from female RA and SLE patients (Lian et al. 2012; Liao et al. 2012).

A recent genome-wide approach in RASFs revealed 207 hypermethylated or hypomethylated genes, with hypomethylation increased in multiple pathways related to cell migration (Nakano et al. 2013), and recent studies in RA animal models showed an increased expression of MeCP2 in synovium and fibroblast-like synoviocytes, suggesting that MeCP2 could participate in RA pathogenesis through silencing of certain genes (Miao et al. 2013).

6.3.2 Histone Tail Modifications in RA

Several investigators observed increased overexpression and activity of histone deacetylases, and particularly of HDAC1, in RASFs and peripheral blood mononuclear cells of RA patients, suggesting a role for histone tail modifications in disease pathogenesis (Horiuchi et al. 2009; Gillespie et al. 2012). Moreover, HDACi, such as for example trichostatin A, were potent inhibitors of tumor necrosis factor and IL-6 production in those cells (Gillespie et al. 2012; Grabiec et al. 2012). There is also evidence from studies in vitro and in animal models that HDACi have the potential to suppress bone destruction in chronic inflammatory diseases such as RA (Cantley et al. 2012). These are only some of many examples showing anti-inflammatory properties of HDACi in RA models, whose beneficial effects are exerted through reduced production of cytokines, chemokines, and related receptors (De Santis and Selmi 2012).

6.3.3 RNA-Mediated Epigenetic Mechanisms in RA

MiRNAs have been largely investigated in the pathogenesis of RA, and some of them, such as for example miR-146a, miR-155, and miR-223, are of particular interest in disease pathogenesis (Ammari et al. 2013). Mir-146a is a negative regulator in immune and inflammatory responses up-regulated in several tissues of RA patients, including RASFs and peripheral blood mononuclear cells, and associated with tumor necrosis factor alpha production and disease activity (Xu et al. 2012). MiR-155 is up-regulated in synovial membrane and synovial fluid macrophages from RA patients (Kurowska-Stolarska et al. 2011), and has a powerful regulatory potential in a wide variety of immune cells through targeting specific mRNAs (Leng et al. 2011). MiR-223 is intensely expressed in RA synovium, and its overexpression suppresses osteoclastogenesis in vitro (Shibuya et al. 2013). Those miRNAs are currently investigated as potential therapeutic targets in RA, and recent integrated analyses of DNA methylation and miRNA expression profiling in RASFs are revealing novel markers of DNA methylation and sets of miRNAs that are controlled by DNA methylation, as well as genes that are regulated by DNA methylation and are targeted by miRNAs with a potential use as clinical markers (de la Rica et al. 2013).

6.3.4 Environmental Factors and Their Potential Epigenetic Properties in RA

Among environmental factors, cigarette smoke condensate was shown to up-regulate gene and protein expression of pro-inflammatory cytokines in human fibroblast-like synoviocytes (Shizu et al. 2008), and tobacco smoke is recognized

among environmental RA risk factors (Karlson and Deane 2012). However, an epigenetic effect of tobacco smoke in RA is at present only speculative (De Santis and Selmi 2012).

6.4 Other Autoimmune Diseases

Epigenetic studies in autoimmune diseases other than SLE and RA are increasing in recent years (Tables 6.3, 6.4, 6.5, 6.6, and 6.7). Within this paragraph we describe some of the most recent examples.

6.4.1 Epigenetics of SjS

SjS is a systemic autoimmune disease characterized by chronic inflammation leading to reduced secretion of the exocrine salivary and lacrimal glands. Epigenetic studies in SjS are still in their infancy (Table 6.3); however, hypomethylation and overexpression of *TNFSF7*(*CD70*) were observed in CD4+ T cells of SjS patients (Yin et al. 2010), and hypermethylation of *BP230*, coding for a protein involved in the anchorage of salivary gland cells, was observed in labial salivary glands in SjS (González et al. 2011). The methylation profile of the gene coding for the interferon regulatory factor 5 (*IRF5*) was investigated in CD4+ T cells, B lymphocytes, and monocytes from patients with SjS, but the observed methylation levels were similar to those observed in cells from controls (Gestermann et al. 2012).

Abnormal distribution of aquaporin 5 (AQP5) in salivary gland acini is likely to contribute to the deficiency of fluid secretion in SjS, and the tumor necrosis factor alpha plays an important role in the destruction of acinar structures in exocrine glands,

Table 6.3 Some examples of epigenetic deregulation in Sjögren's syndrome

DNA methylation		
Human CD4+ T cells	Demethylated genes: *TNFSF7*(*CD70*) Hypermethylated genes: *BP230*	Yin et al. (2010) González et al. (2011)
Histone tail modifications		
Human salivary gland cells	Deacetylation of histone H4 in the promoter of *AQP5* gene and inhibition of aquaporin 5 expression	Yamamura et al. (2012)
MicroRNA expression		
Human salivary gland cells	Deregulation of miR-547 and miR-768-3p expression: the expression of miR-768-3p increases, whereas the expression of miR-574 decreases with increasing focus scores	Alevizos et al. (2011)
Human peripheral blood mononuclear cells	Upregulation of miR-146a and miR-146b	Pauley et al. (2011), Zilahi et al. (2012)

Table 6.4 Some examples of epigenetic deregulation in psoriasis

DNA methylation		
Human skin samples	Demethylated genes: *SHP-1*	Ruchusatsawat et al. (2006)
	Whole-genome approaches: differential methylation of 1,108 sites between normal and psoriatic tissues	Roberson et al. (2012)
Human CD4+ T cells	Whole-genome approaches: hypomethylation of 26 regions of the genome, most of them pericentromeric	Han et al. (2012)
	Hypermethylation of 121 genes on the X chromosome	Han et al. (2012)
Human ematopoietic cells	Hypomethylation of genes coding for p16, p21, and p53	Zhang et al. (2007, 2009)
Histone tail modifications		
Human skin samples	Overexpression of histone deacetylase HDAC1	Tovar-Castillo et al. (2007)
Human peripheral blood mononuclear cells	Global histone H4 hypoacetylation	Zhang et al. (2011)
MicroRNA expression		
Human skin samples	Deregulation of 98 canonical and 15 noncanonical miRNAs, including upregulation of miR-203, miR-21, and miR-31	Joyce et al. (2011), Xia et al. (2013)

Table 6.5 Some examples of epigenetic deregulation in multiple sclerosis

DNA methylation		
Human white matter samples	Demethylated genes: *PAD2*	Mastronardi et al. (2007)
Human peripheral blood mononuclear cells	Demethylated genes: *PAD2*	Calabrese et al. (2012)
Histone tail modifications		
Human white matter samples	Increased histone H3 acetylation	Pedre et al. (2011)
MicroRNA expression		
Human blood cells	Upregulation of miR-21, miR-146a, miR-146b, and miR-326	Fenoglio et al. (2012)
Human brain regions	Upregulation of miR-155, miR-326, and miR-34a	Fenoglio et al. (2012)
Human B lymphocytes	Dowregulation of 49 miRNAs	Sievers et al. (2012)

and inhibits *AQP5* gene expression in human salivary gland acinar cells by suppression of acetylation of histone H4 in the promoter region (Yamamura et al. 2012).

Also some miRNAs were found to be deregulated in SjS salivary glands (miR-547 and miR-768-3p) and/or in peripheral mononuclear cells (miR-146a and miR-146b) (Alevizos et al. 2011; Pauley et al. 2011; Zilahi et al. 2012). Moreover, a recent study has shown that the SjS antigen B is a pre-miRNA-binding protein that regulates miRNA processing in vitro (Liang et al. 2013).

Table 6.6 Some examples of epigenetic deregulation in systemic sclerosis

DNA methylation		
Human scleroderma fibroblasts	Hypermethylation of *FLI1* gene	Wang et al. (2006)
Human CD4+ T cells	Global hypomethylation	Lei et al. (2009)
	Reduced DNMT1, MBD3, and MBD4 mRNA levels	Lei et al. (2009)
	Demethylated genes: *CD40LG, TNFSF7*(*CD70*)	Jiang et al. (2012) Lian et al. (2012)
Human peripheral blood mononuclear cells	Different methylation profiles of genes on the X chromosome in monozygotic twins discordant for the disease	Selmi et al. (2012)
Histone tail modifications		
Human skin tissues	Overexpression of histone acetyltransferase p300, and histone H4 acetylation of the *COL1A2* locus	Ghosh et al. (2013)
Cultured fibroblasts	Involvement of histone H3 methylation on lysine 27 in regulation of fibroblast activation	Krämer et al. (2012)
MicroRNA expression		
Human skin tissues	Upregulation of miR-23b, and let-7	Li et al. (2012)
	Dowregulation of miR-125b, miR-133a, miR-206, and miR-140-5p	
Human skin tissues and fibroblasts	Upregulation of miR-21	Maurer et al. (2010), Zhu et al. 2012
	Dowregulation of miR-145 and miR-29	
Human dermal fibroblasts	Dowregulation of miR-150	Honda et al. (2013)

Table 6.7 Some examples of epigenetic deregulation in autoimmune thyroid diseases

DNA methylation		
Blood DNA	*MTHFR* C677T polymorphism associated with reduced risk of Graves' disease in women	Mao et al. (2010)
	DNMT1 32204GG genotype associated with DNA hypomethylation and response to treatment in Graves' disease	Arakawa et al. (2012)
	MTRR A66G polymorphism associated with the severity of Hashimoto's thyroiditis	Arakawa et al. (2012)
MicroRNA expression		
Human peripheral blood mononuclear cells	Downregulation of miR-154*, miR-376b, and miR-431* in early stages Graves' disease	Liu et al. (2012)
Human thyroid tissues	Downregulation of miR-146a1 in Graves' disease	Bernecker et al. (2012)
	Downregulation of miR-155_2 and upregulation of miR-200a1 in Hashimoto's thyroiditis	Bernecker et al. (2012)

6.4.2 *Epigenetics of Psoriasis*

Psoriasis is an organ-specific autoimmune disease triggered by an active immune system causing cells to build up rapidly on the surface of the skin, resulting in thick, white, silvery, or red patches that are sometimes painful. The pathology of psoriasis is complex, involving both genetic and environmental components (Zhang et al. 2012), and increasing evidence supports a role for epigenetic modifications (Table 6.4). Early studies on DNA methylation revealed *SHP-1* promoter methylation in normal epithelial tissues and demethylation in psoriasis. SHP-1 is a tyrosine phosphatase and has been proposed as a candidate tumor suppressor gene in lymphoma, leukemia, and other cancers, as it functions as an antagonist to the growth-promoting and oncogenic potentials of tyrosine kinases (Ruchusatsawat et al. 2006). A reduced proliferative activity has been detected in the hematopoietic cells from patients with psoriasis and linked to hypomethylation of the genes coding for p16, p21, and p53 (Zhang et al. 2007, 2009). More recent genome-wide approaches are revealing hundreds of novel methylation markers of the disease, thereby strengthening the contribution of epigenetics in psoriasis. The methylation levels at 27,578 CpG sites in skin samples from individuals with psoriasis and unaffected individuals revealed different methylation of 1,108 sites (Roberson et al. 2012). Similarly, differences in DNA methylation were found in CD4+ T cells of monozygotic twins discordant for psoriasis (Gervin et al. 2012). A genome-wide DNA methylation profiling of naïve CD4+ T cells showed distinct hypomethylation in 26 regions of the genome ranging in size from 10 to 70 kb (most of them pericentromeric) in patients with psoriasis with respect to healthy controls. Conversely, the promoter regions of 121 genes, and particularly of immune-related genes, on the X chromosome were hypermethylated in psoriasis patient T cells compared to those from healthy controls (Han et al. 2012).

Concerning histone tail modifications, the HDAC-1 mRNA resulted overexpressed in psoriatic skin samples compared with skin specimens from healthy subjects (Tovar-Castillo et al. 2007). Moreover, global histone H4 hypoacetylation was observed in peripheral blood mononuclear cells from psoriasis patients, and there was a negative correlation between the degree of histone H4 acetylation and disease activity (Zhang et al. 2011).

A comprehensive analysis of the normal and psoriatic skin miRNAome with next-generation sequencing revealed 80 known and 18 novel miRNAs that were differentially expressed in psoriatic skin. Of particular significance was the 2.7-fold upregulation of a novel miRNA derived from the antisense strand of the miR-203 locus, which plays a role in epithelial differentiation. Other differentially expressed miRNAs included hematopoietic-specific miRNAs such as miR-142-3p and miR-223/223*, and angiogenic miRNAs such as miR-21, miR-378, miR-100, and miR-31, which was the most highly up-regulated miRNA in psoriatic skin (Joyce et al. 2011). Subsequent functional studies of those miRNAs revealed that miR-21 suppresses apoptosis in activated T cells, and thus, overexpression of miR-21 may contribute to T cell-derived psoriatic skin inflammation (Meisgen et al. 2012), while miR-31 modulates inflammatory

cytokine and chemokine production in keratinocytes via targeting serine/threonine kinase 40 (Xu et al. 2013). Moreover, the analysis of more than 670 million qualified reads from 67 small RNA libraries, revealed 21 novel, noncanonical miRNAs (3 small nuclear RNA-derived, 2 tRNA-derived miRNAs, and 16 miRtrons) and 39 novel endo-siRNAs that were expressed in skin, and 15 of them were significantly differentially expressed in psoriatic versus normal skin (Xia et al. 2013).

6.4.3 Epigenetics of Multiple Sclerosis

Multiple sclerosis is an autoimmune demyelinating disease and a common cause of neurodegeneration and disability in young adults. Disease discordance in monozygotic twins indicates environmental importance in its pathogenesis, but a genome-wide DNA methylation study in CD4+ lymphocytes of monozygotic twins discordant for MS failed to find significant differences, thereby dampening research expectations (Baranzini et al. 2010). However, the promoter of the peptidyl arginine-deiminase 2 (*PAD2*) gene was hypomethylated in the white matter from MS patients, resulting in increased synthesis of PAD2 protein that is responsible for the increased amount of citrullinated myelin basic protein, which in turn results in loss of myelin stability in MS brain (Mastronardi et al. 2007). Similar results were observed in peripheral blood mononuclear cells of MS patients, where *PAD2* overexpression was associated with promoter demethylation (Calabrese et al. 2012).

If data on DNA methylation alterations are scarce in MS, an increased histone H3 acetylation associated with increased levels of transcriptional inhibitors of oligodendrocyte differentiation was observed in the white matter of patients with chronic MS (Pedre et al. 2011), and a number of miRNAs have been found to be dysregulated in blood cells from MS patients, in brain lesions, as well as in biological fluids such as serum and plasma (Table 6.5). Some examples are miR-326 that was found to be up-regulated in MS blood and promoted T-helper CD4+ cells differentiation, miR-21, miR-146a and miR-146b up-regulated in peripheral blood mononuclear cells of MS patients as compared with controls, and miR-155, miR-326, and miR-34a that were found to be up-regulated in active MS brain lesions and targeted CD47, a regulatory membrane protein (reviewed in Fenoglio et al. 2012). These are only some of several examples of miRNAs deregulation in MS tissues, and recent large-scale studies are revealing dozens of novel markers, such as for example an expression profiling of 1,059 miRNAs in B lymphocytes that revealed 49 miRNAs down-regulated in untreated MS patients compared with healthy controls (Sievers et al. 2012). A recent integration of miRNAs databases revealed that the miRNAs associated with MS according to different studies are able or predicted to target about 1,500 different genes many of which play a role in T cell activation and signaling, or have transcription factor activity (Angerstein et al. 2012).

Among environmental factors considerable evidence has linked past Epstein–Barr virus (EBV) infection to an increased risk of MS, and, since a complete silencing of the EBV genome in memory B cells is under epigenetic control via DNA

methylation and histone tail modifications, some authors have suggested that an epigenetic dysregulation of the EBV latency might contribute to the development of MS and other autoimmune diseases (Niller et al. 2011).

6.4.4 Epigenetics of Systemic Sclerosis

SSc is a systemic autoimmune disease characterized by deposition of collagen in the skin and, less commonly, in other tissues with progressive vasculopathy. Early studies on DNA methylation (Table 6.6) revealed association between enhanced type I collagen expression and epigenetic repression (hypermethylation) of the *FLI1* gene in scleroderma fibroblasts (Wang et al. 2006). Subsequent studies revealed that CD4+ T cell DNA from patients with SSc was significantly hypomethylated relative to controls, and DNMT1, MBD3, and MBD4 mRNAs were significantly decreased in the SSc group (Lei et al. 2009). Demethylation of *TNFSF7*(*CD70*) was observed to contribute to CD70 overexpression in CD4+ T cells from patients with SSc (Jiang et al. 2012). Moreover, SSc occurs more frequently in females than males, suggesting that epigenetic modifications of genes on the X chromosome might be involved. Particularly, demethylation of *CD40LG* regulatory elements on the inactive X chromosome contributed to CD40L overexpression in CD4+ T cells from female patients with SSc, but no significant difference was observed in the expression of CD40L between male patients with SSc and male control subjects (Lian et al. 2012). A recent methylation profile of all X chromosome genes in peripheral blood mononuclear cells from monozygotic twins discordant for SSc revealed sites with an elevated probability to be consistently hypermethylated ($n=18$) or hypomethylated ($n=25$) in affected twins. Identified genes include transcription factors and surface antigens, and pathway analysis suggests their involvement in cell proliferation, apoptosis, inflammation, and oxidative stress (Selmi et al. 2012).

Increasing evidence suggests the involvement of histone tail modifications in fibrosis (Table 6.6), the hallmark of SSc, characterized by a persistent fibroblast activation triggered by transforming growth factor-β (TGF-β). Indeed, it was observed that the expression of the HAT p300 is markedly elevated in SSc skin biopsies and is induced by TGF-β in explanted normal skin fibroblasts. Moreover, TGF-β enhanced both p300 recruitment and histone H4 acetylation at the *COL1A2* (collagen, type I, α2) locus, suggesting that p300-mediated histone acetylation could represent a fundamental epigenetic mechanism in fibrogenesis (Ghosh et al. 2013). Similarly, inhibition of trimethylation of histone H3 on lysine 27 (H3K27me3), induced by treatment with 3-deazaneplanocin A, stimulated the release of collagen in cultured fibroblasts in a time and dose-dependent manner and was sufficient to induce fibrosis, suggesting that trimethylation of histone H3 on lysine 27 acts as a negative regulator of fibroblast activation (Krämer et al. 2012).

An increasing number of miRNAs was found to be deregulated in SSc samples (Table 6.6). For example, a miRNA array analysis in skin tissues from SSc patients and healthy controls revealed 24 miRNAs that were differentially expressed in

patients with SSc and six miRNAs that may be correlated with the pathogenesis of SSc. Particularly, miR-23b and let-7 were up-regulated, while miR-125b, miR-133a, miR-206, and miR-140-5p were down-regulated (Li et al. 2012). Others observed that in comparison with the normal skin tissues, miRNAs were aberrantly expressed in limited cutaneous scleroderma and diffuse cutaneous scleroderma skin tissues, and identified six miRNAs whose expressions were correlated with SSc fibrosis: miR-21, miR-31, miR-146, miR-503, miR-145, and miR-29b. Particularly, the study confirmed that miR-21 was increased whereas miR-145 and miR-29b were decreased both in the skin tissues and in fibroblasts. As predicted target genes, *SMAD7*, *SAMD3*, and *COL1A1* were regulated by these tree miRNAs (Zhu et al. 2012). Previous results had shown that miR-29a was strongly down-regulated in SSc fibroblasts and skin sections as compared with the healthy controls, and that this miRNA acts as a key regulator of collagen expression in SSc (Maurer et al. 2010). Overall, miRNA-29 is a recently discovered class of miRNAs which is related to fibrotic disease and a potential therapeutic target for systemic sclerosis (Peng et al. 2012). More recently, it was found that miR-150 downregulation contributes to the constitutive type I collagen overexpression in SSc dermal fibroblasts via the induction of integrin β3 (Honda et al. 2013).

6.4.5 Epigenetics of AITDs

AITDs comprise Graves' disease and Hashimoto's thyroiditis, both organ-specific autoimmune diseases characterized by female preponderance, and in which the autoimmune attack of the thyroid takes place by infiltration of lymphocytes of the glandule. A possible role of skewed X chromosome inactivation, mediated by epigenetic mechanisms, has been suggested in the etiology of AITD to partially explain the female preponderance (Brix et al. 2005; Chabchoub et al. 2009).

A few studies have been performed to clarify the association between factors regulating DNA methylation and the prognosis of AITDs (Table 6.7). Particularly, those studies focused on polymorphisms in genes encoding DNMTs, methylenetetrahydrofolate reductase (MTHFR), and methionine synthase reductase (MTRR), which are all enzymes essential for DNA methylation reactions. The *MTHFR* C677T polymorphism was associated with reduced GD risk in women (Mao et al. 2010), while the *DNMT1* 32204GG genotype was correlated with DNA hypomethylation and with the intractability of GD, and the *MTRR* 66AA genotype with the severity of HD (Arakawa et al. 2012). Albeit in their infancy, those studies suggest that those genes might account for AITD susceptibility, severity, and response to treatment, partially mediated by changes in DNA methylation (Mao et al. 2010; Arakawa et al. 2012).

Also the few available studies on miRNA profiling in AITD tissues (Table 6.7) suggest deregulated networks in those disorders. Liu and coworkers showed that the expression of miR-154*, miR-376b, and miR-431* was suppressed in peripheral blood mononuclear cells from initial GD patients, and that their expression levels were recovered in GD patients in remission (Liu et al. 2012). Another group showed

that miR-146a1 was significantly decreased in the thyroid tissue of GD patients, in comparison with the control group (Bernecker et al. 2012). Similarly, miR-155_2 was significantly decreased and miR-200a1 was significantly increased in the thyroid of HT patients, with respect to the control tissues (Bernecker et al. 2012). Albeit preliminary, those studies suggest a potential role of miRNA deregulations in AITDs that warrants further research.

6.5 Concluding Remarks

In the present chapter we described some examples of epigenetic deregulation in human autoimmune diseases. This field of research has gained tremendous attention in the last 2–3 years and it is now emerging that epigenetic modifications play a role, or are supposed to do it, in several autoimmune disorders, including but not limited to those detailed in this chapter. Indeed, evidence of an epigenetic contribution is increasing also in inflammatory bowel diseases (Jenke and Zilbauer 2012), type 1 diabetes (Dang et al. 2013), immune thrombocytopenic purpura (Khorshied and El-Ghamrawy 2012), and many other inflammatory and/or autoimmune diseases. Despite this, only few environmental factors have been suggested to epigenetically contribute to those disorders, some examples are drugs, air pollutants, ultraviolet light, cigarette smoke, and microbial infections, but for most of them the epigenetic link is still only speculative (De Santis and Selmi 2012). Several authors have suggested that epigenetic deregulations of genes on the X chromosome might account for gender differences, i.e., female predominance, in the incidence of many autoimmune diseases (Lian et al. 2012; Liao et al. 2012), and age-related epigenetic changes might also be of interest (De Santis and Selmi 2012).

Early epigenetic studies in autoimmune diseases, based on the candidate gene approach, have been paralleled and/or replaced in recent years by whole-genome approaches, that are revealing dozens, or even hundreds of genes or miRNAs that are deregulated in the affected tissues as well as in peripheral tissues of the patients (Tables 6.1–6.7). This is leading to a better understanding of the networks involved in disease pathogenesis, thereby opening the way for potential diagnostic and prognostic tools, as well as for epigenetic interventions based on miRNA silencing or chromatin remodeling agents, such as HDAi (Garchow et al. 2011; Reilly et al. 2011).

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