Chapter 11
Epigenetics in Primary Sjögren's Syndrome

Anne Bordron, Valérie Devauchelle-Pensec, Christelle Le Dantec, Arthur Capdeville, Wesley H. Brooks, and Yves Renaudineau

Abstract Primary Sjögren's syndrome (SjS) is a chronic and systemic autoimmune epithelitis with predominant female incidence, which is characterized by exocrine gland dysfunction. Incompletely understood, the etiology of SjS is multi-factorial and evidence is growing to consider that epigenetic factors are playing a crucial role in its development. Independent from DNA sequence mutations, epigenetics is described as inheritable and reversible processes that modify gene expression. Epigenetic modifications reported in minor salivary gland and lymphocytes from SjS patients are related to (i) an abnormal DNA methylation process inducing in turn defective control of normally repressed genes involving such matters as autoantigens, retrotransposons, and the X chromosome in women; (ii) altered nucleosome positioning associated with autoantibody production; and (iii) altered control of microRNA. Results from epigenome-wide association studies have further revealed the importance of the interferon pathway in disease progression, the calcium signaling pathway for controlling fluid secretions, and a cell-specific cross talk with risk factors associated with SjS. Importantly, epigenetic modifications are reversible thus opening opportunities for therapeutic procedures in this currently incurable disease.

Keywords Sjögren's syndrome · Epithelial cells · Genetics · Epigenetics · DNA methylation · microRNAs · Histone

A. Bordron · V. Devauchelle-Pensec · C. Le Dantec · A. Capdeville · Y. Renaudineau (✉)
INSERM U1227, «Lymphocyte B et autoimmunité», Labex Igo "Immunotherapy Graft, Oncology", Réseau épigénétique du cancéropole Grand Ouest,
Université de Brest, Brest, France
e-mail: yves.renaudineau@univ-brest.fr

V. Devauchelle-Pensec
Department of Rheumatology, CHU Cavale Blanche,
Brest University Medical School, Brest, France

W. H. Brooks
Department of Chemistry, University of South Florida, Tampa, FL, USA

Y. Renaudineau
Laboratory of Immunology and Immunotherapy, Brest University Medical School,
CHU Morvan, Brest, France

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11.1 Introduction

Primary Sjögren's syndrome (SjS) is an autoimmune systemic disease characterized by lymphocytic infiltration of the exocrine glands with tropism for the salivary, lacrimal, upper respiratory, and vaginal glands of women (Brito-Zeron et al. 2016a). The clinical picture of SjS is based on evidence of xerostomia and xerophthalmia, which define a syndrome of dryness, and association with intense fatigue and widespread pain that leads to a profound alteration in the patients' quality of life (Ramos-Casals et al. 2012). In one-third of patients, there are systemic manifestations, i.e., extra-glandular, which can affect the kidneys, liver, lungs, and thyroid.

The severity of the disease is generally associated with visceral abnormalities and the development of B lymphoma in 5% of patients (Brito-Zeron et al. 2016b; Nocturne and Mariette 2018). The prevalence of SjS ranges from 0.01 to 0.72% (Kabasakal et al. 2006; Maldini et al. 2014). In its primary form, SjS primarily affects women (9:1), with an average age of onset of about 50 years (Ramos-Casals et al. 2015). The secondary forms (50%), generally of lesser intensity, coexist with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), thyroiditis, and even primary biliary cirrhosis.

In addition to the fact that SjS is characterized by an autoimmune epithelitis, its physiopathology raises numerous questions (Brito-Zeron et al. 2016a; Sandhya et al. 2017). Based on data acquired from the analysis of the exocrine glands, in particular minor salivary glands (MSG), several steps were highlighted. First of all, activation of the epithelium, which leads to lymphocyte infiltration, consisting mainly of T cells and more particularly $CD4^+$ T cells (Christodoulou et al. 2010; Verstappen et al. 2018). Then, and concomitantly with epithelial activation and disease progression, new cell populations appear in MSG such as follicular T cells, TH17 cells, dendritic (interferon producing) cells, and B lymphocytes which gradually become predominant and organize themselves into ectopic germinal centers. B lymphocyte hyperactivation is accompanied by local production of autoantibodies (auto-Ab) and, in particular, of sicca syndrome type A (SSA/Ro) or type B (SSB/La) auto-Ab.

At a peripheral level, the detection of anti-SSA/SSB auto-Ab is frequently associated with the detection of rheumatoid factor, hypergammaglobulinemia, and hypocomplementemia that reflects an active immunological profile (Capaldo et al. 2016). This hyperactivation is accompanied by abnormalities of peripheral B lymphocyte subpopulations, reflecting the attraction of memory B cells into tissues (Alonso et al. 2010; Cornec et al. 2014; Simonin et al. 2016; Renaudineau 2017). In the MSG, there is also an increase in the size of the glands and significant ultra-sonographic changes (Le Goff et al. 2017).

The etiology and pathogenesis of SjS are multi-factorial and consist of an aggregate of genetic predispositions with environmental factors (Renaudineau and Ballestar 2016). Recent data further support the important contribution of epigenetic mechanisms in SjS no longer solely to manage epithelial system development, but also as key regulators for controlling cell cycle, cell differentiation, immune system

recruitment, and activation and, last but not least, to control the response to external factors such as various treatment modalities. From the cellular point of view, the definition of epigenetics has advanced and now includes adjustments in gene expression that do not entail modifications in the DNA sequence and are inheritable and reversible. This chapter summarizes implications of epigenetic modifications in SjS and their consequences.

11.2 Environmental, Genetic, and Epigenetic Factors

11.2.1 Environmental Factors

Due to the frequent presence of severe fatigue and arthralgia at diagnosis, a viral cause is suspected, notably Herpes family members such as Epstein–Barr virus (EBV) and human herpes virus 6 (HHV6) (Lucchesi et al. 2014). Several indirect arguments favor this direction, such as the evidence of a higher prevalence of EBV- and HHV6-specific IgG in this disease (Kivity et al. 2014). A cross-reactivity is described for these two viruses with the SSB/La protein due to molecular mimicry (Haaheim et al. 1996; Hajjar et al. 1995), and a correlation between anti-SSB/La auto-Ab and IgG levels against the early antigens of EBV was recently reported (Agmon-Levin et al. 2017). In this study, a correlation was also reported between anti-Ro/SSA auto-Ab and IgG/M directed against EBV, cytomegalovirus (CMV), and toxoplasma.

However, no direct evidence has been reported on the direct role of these viruses which are widely distributed in the general population, and several studies did not find this association. As a result, other viruses have been implicated, again without direct evidence, including HTLV1 (human T cell lymphotropic virus 1), hepatitis B and C viruses, retrovirus, and coxsackievirus (Hajjar et al. 1995; Wattiaux et al. 1995; Brauner et al. 2017; Nakamura et al. 2018). Another explanation is related to the capacity of SjS patients to have a higher immune response to infectious agents as observed with the H1N1 influenza vaccine (Brauner et al. 2017). An alteration of the microbiome is also reported in SjS with dysbiosis reported in the intestinal tract, and in ocular and oral flora that are suspected of influencing the immune system (De Luca and Shoenfeld 2018; Tsigalou et al. 2018).

Other factors associated with SjS include tobacco, vitamin D deficiency, ultraviolet radiation, and chemical agents whose exposure appears to be correlated with the appearance of SjS (Busche et al. 2015; Garcia-Carrasco et al. 2017). The demographic components of SjS reinforce the involvement of environmental factors with a higher reported prevalence of SjS in Nordic countries (Shapira et al. 2010) Another argument in support of this hypothesis is based on the study of homozygous twins for whom the concordance rate with SjS, which defines the genetic share of a disease, ranges between 15 and 25% (Brooks et al. 2010).

11.2.2 Genetic Factors

With the development of GWAS (genome-wide association study) projects, more than 40 risk factors have been associated with SjS but they have only a slight effect on the risk of developing SjS (Lessard et al. 2012; Konsta et al. 2014). First, there are risk factors involved in antigen presentation with certain regions of the HLA class I and II system or upstream with factors involved in the regulation and expression of HLA molecules. The presence of anti-SSA/SSB auto-Ab and labial salivary gland focus score is associated with HLA-DQA1 and HLA-DQB1 in Europeans, an association not confirmed in Asians, which contrasts with HLA-DPB1 that was observed in both ethnic groups (Taylor et al. 2017).

For the other risk factors associated with SjS, there are risk factors involved in innate and acquired immunity such as genes associated with interferon (IFN) signatures (*irf5* [interferon regulatory factor 5], *il12*a [interleukin 12A], *ncr3* [natural cytotoxicity triggering receptor 3], and *stat4* [signal transducer and activator of transcription 4]), genes associated with T and B cell functions (*tnfaip3* [TNF-alpha induced protein 3], *tnip1* [TNFAIP3-interacting protein 1], *cxcr5* [C-X-C chemokine receptor type 5], *blk* [B lymphocyte kinase], *baff* [B cell activating factor], *ebf1* [early B cell factor 1], *gtf2i* [general transcription factor IIi], *tnsff4* [Tumor Necrosis Factor Superfamily Member 4], *lta* [Lymphotoxin-α], and *ccl11* [C-C motif chemokine 11]), and genes with other functions (*htt* [solute carrier family 6 member 4]) (Lessard et al. 2013; Burbelo et al. 2014; Konsta et al. 2015; Teos and Alevizos 2017). The mode of action of these SjS associated risk factors is complex because few of them have been attributed to the normal functions of the proteins. However, recent studies revealed that they are not randomly located but present in cell-specific and epigenetic regulatory zones to control transcription (Konsta et al. 2015). Consequently, a better understanding of the cross talk between risk factors and epigenetic factors to control cell-specific gene expression can lead to a better understanding of the pathophysiology of the disease.

11.2.3 Epigenetic Factors

Epigenetics plays an essential role in acting at key stages of differentiation and activation of the immune system. But there are actually several epigenetic mechanisms involved, such as post-translational histone modifications (to alter DNA compaction or decondensation), non-coding RNAs (which can modulate gene expression via sense/anti-sense interactions with other transcripts), and DNA methylation (modulates actions of transcription factors and gene repressors) (Fig. 11.1). This latter mechanism occurs by the transfer of a methyl group on the 5th carbon of the CpG dinucleotide cytosine of DNA (5-mCyt) under the action of DNA methyltransferases (DNMTs) with S-adenosylmethionine (SAM) being the key source of the methyl groups (Renaudineau and Youinou 2011; Brooks and Renaudineau 2015). Important modifications of these processes, specific for a given cell type, have been observed in

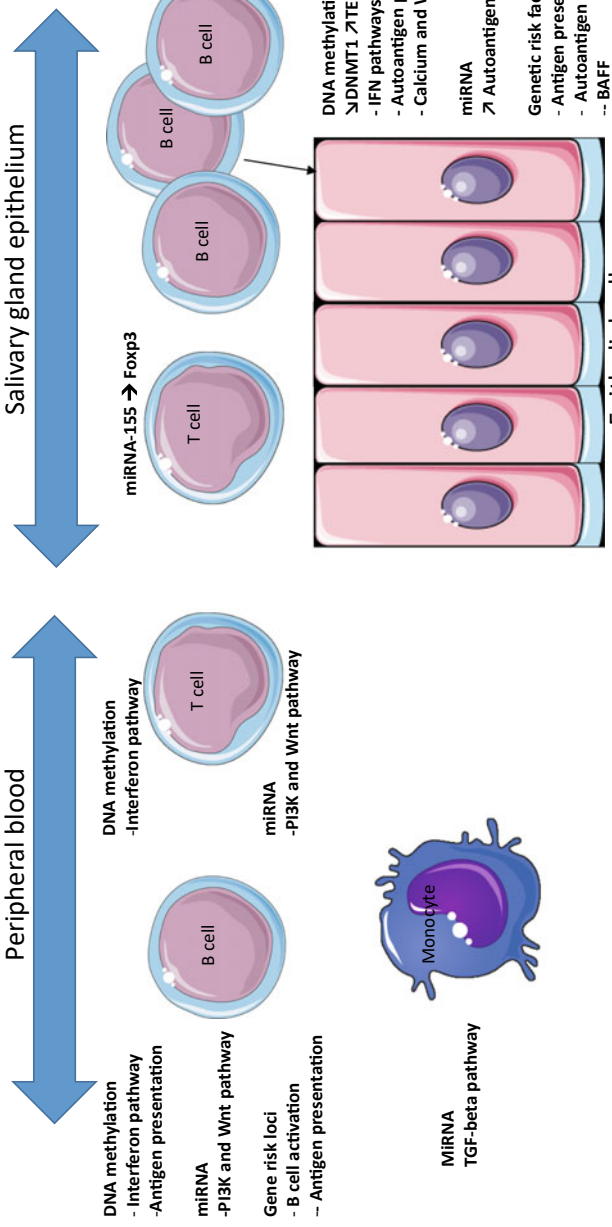

Fig. 11.1 Specific epigenetics and genetic risk factors of cells implicated in the pathogenesis of Gougerot-Sjogren. Figures of cells were obtained from the Servier database

pathology and in particular in cancers and autoimmune diseases (Brooks et al. 2010; Bagacean et al. 2017a, b). The first argument implicating epigenetic mechanisms in SjS derives from the observation that oral administration of DNA demethylating drugs such as hydralazine or isoniazid promotes sicca development in mice treated over several weeks together with immunological elements of an SLE-like disease (Bordron et al. 2018). From these initial experiments, it was also reported that the animal strain, age, and sex were important for the pathological process, and that this effect disappeared when the demethylating drug was withdrawn.

Next, both the Richardson and Zouali teams demonstrated that forcing DNA hypomethylation in both $CD4^+$ T cells and $CD19^+$ B cells promotes autoreactivity (Quddus et al. 1993; Mazari et al. 2007). Indeed, when $CD4^+$ T cells or $CD19^+$ B cells pretreated with DNA methyltransferase inhibitors are passively transferred into mice, the engrafted mice produce auto-Ab including anti-dsDNA Ab. One further step was to characterize in SLE patients the defective pathways that lead to decreased DNMT1 expression in both $CD4^+$ T cells and $CD19^+$ B cells, which includes the MAPk/Erk pathways (Gorelik et al. 2015). From the epigenetic point of view, the most important difference between SLE and SjS is related to the fact that epithelial cells, and to a lesser extent lymphocytes, have a defective DNA methylation process (Dantec et al. 2015).

11.3 Epigenetic Defects

11.3.1 DNA Methylation

11.3.1.1 Global Methylation Analysis

1. **Demethylation of DNA in SjS**

Overall, DNA methylation acts on gene transcription either directly by preventing transcription factors from binding or indirectly by recruiting enzymes responsible for chromatin compaction such as histone deacetylases (HDAC) and histone methyltransferases (HMT). In mammals, chromatin is normally methylated and compacted and it is the regulatory and transcriptionally active zones that are demethylated. The DNA demethylation process is either passive during cell division or active and is in this case initiated by TET (ten-eleven translocation) oxidation enzymes (Bagacean et al. 2018). TETs oxidize 5-mCyt into 5-hydroxymethylcytosine (5-hmCyt) and subsequently, in a less efficient manner, into 5-formylcytosine (5-fCyt) and 5-carboxylcytosine (5-CaCyt) with the use of α-ketoglutarate (α-KG), molecular oxygen and iron as cofactors. The overall rate of 5mCyt found in mammals varies according to cell type from 70 to 80%, with low levels in immune system cells.

Analysis of histological sections from MSG and epithelial cells cultured and isolated from patients' MSGs, as well as analysis of peripheral B and T lymphocyte populations (Table 11.1) and analysis of the overall DNA methylation state made it possible to highlight in SjS: (i) a significant reduction in overall 5-mCyt status in the

Table 11.1 Epigenetic defects observed in Gougerot-Sjogren's Syndrome or in relation with this disease

	Minor salivary glands	Salivary gland epithelial cells	Salivary gland cell lines	B cells	T cells	Monocytes
DNA methylation	↘5mCyt (Thabet et al. 2013; Konsta et al. 2016b) ↘DNMT1 and DNMT3b (Mavragani et al. 2018) ↗5hmCyt and ↗TET2 (Lagos et al. 2018)	↘5mCyt, ↘DNMT1 and ↗GAD45-alpha (Thabet et al. 2013; Konsta et al. 2016b)	↘5mCyt and ↘Erk/DNMT1 pathway in co-culture with B cells (Thabet et al. 2013) ↗5hmCyt and ↗TET2 in the presence of IFN-γ and TNF-α (Lagos et al. 2018)	Normal 5mCyt levels (Thabet et al. 2013)	Normal 5mCyt levels (Thabet et al. 2013)	
Histone modifications	↗Histone in saliva (Hall et al. 2017)		↘ H4 acetylation in the presence of TNF-α (Yamamura et al. 2012)			
miRNA modifications	↗hsa-miR146a-5p (Pauley et al. 2011) ↗hsa-miR181a and has-miR155 (Le Dantec et al. 2012) ↗hsa-miR-16 (Gourzi et al. 2015) ↗hsa-miR-768-3p ↘hsa-miR-574 (Alevizos et al. 2011) ↗miR-126 and miR-335-5p (Martini et al. 2017) ↗ebv-miR-BART13-3p (Gallo et al. 2016)	↗hsa-miR-200b-3p (Gourzi et al. 2015)		hsa-let-7d-3p, hsa-miR-155-5 p, hsa-miR-222-3 p, hsa-miR-30c-5p, hsa-miR-146a-5p, hsa-miR-378a-3p, and hsa-miR-28-5 p (Wang-Renault et al. 2018) ↗ebv-miR-BART13-3p (Gallo et al. 2016)	hsa-miR-378a-3p, hsa-miR-222-3 p, hsa-miR-26a-5p, hsa-miR-30b-5p, and hsa-miR-19b-3p (Wang-Renault et al. 2018)	miR-34b-3p, miR-4701-5p, miR-609, miR-300, miR-3162-3p, and miR-877-3p (Williams et al. 2016)

SGEC: salivary gland epithelial cells; DNMT1: DNA methyltransferase 1; HSG: Human salivary gland cell line

MSG and which predominates in epithelial cells; (ii) that this defect was preserved in culture and that it was associated with suppression of expression of the methylation enzyme DNMT1 as reported in long term purified salivary gland epithelial cells (SGEC); and (iii) conversely, no difference in overall DNA methylation could be observed in B and T cells of patients' and controls' peripheral blood, pointing out that epigenetic changes preferentially affect the epithelial cells.

MSG 5-mCyt reduction in SjS and DNMT1/3b reductions in SjS patients with lymphoma were recently confirmed (Lagos et al. 2018; Mavragani et al. 2018), and Lagos et al. have further underlined an active DNA demethylation process based on the observation that 5hmCyt and TET2 levels are increased in epithelial cells from MSG sections.

2. **Involvement of lymphocytes and pro-inflammatory cytokines in the demethylation of DNA from epithelial cells of minor salivary glands**

The presence of a significant lymphocyte infiltration appears to be associated with 5-mCyt reduction in MSG since the methylation level is inversely proportional to the Tarpley and focus scores which reflects the inflammatory state of MSG (Konsta et al. 2016b; Lagos et al. 2018). An inverse association with anti-SSB/La auto-Ab positivity is also reported in these two studies. On one hand, the role of B cells is suspected to interfere with the Erk/DNMT1 pathway leading to 5-mCyt reduction in the HSG (human salivary gland) cell line when this cell line is co-cultivated with B cells (Thabet et al. 2013). This process could be reversed as observed in MSG from patients that have recovered a normal DNA methylation level 4 months after receiving a treatment with rituximab, an anti-B lymphocyte immunotherapy (Thabet et al. 2013). On the other hand, HSG stimulation with the pro-inflammatory cytokines interferon (IFN)-γ and the tumor necrosis factor (TNF)-α promote TET2 overexpression that in turn increases 5-hmCyt and decreases 5-mCyt (Lagos et al. 2018).

11.3.1.2 Complete Methylome Analysis

1. **Methylation analysis of CpG units of peripheral blood cell DNA**

Several teams have used the HM450K technology from Illumina to study DNA methylation across the genome in peripheral blood mononuclear cells (PBMCs) which include a mixture of T, B cells, and monocytes, or better by using purified T cells (total and naive) and B cells of patients with SjS (Altorok et al. 2014; Imgenberg-Kreuz et al. 2016; Miceli-Richard et al. 2016). Apart from the different methods of analysis, we can highlight several points. First, hypermethylated regions of DNA are found in HLA type I and II antigen presentation genes but also on certain genes regulated by interferon type I (*IFI44L*, *IFITMI*). Second, specific signaling pathways are found in T cells (solute-carrying proteins and the transcription factor RUN2X) and in B cells (B cell receptors, developmental genes). Third, the effect predominates in patients with anti-SSA/SSB auto-Ab and in B cells because the methylation changes are 50 times greater than in T cells (Miceli-Richard et al. 2016).

2. **Methylation analysis of CpG units of minor salivary gland DNA**

Two teams analyzed DNA methylation using HM450K chips in MSG of patients with SjS (Cole et al. 2016; Imgenberg-Kreuz et al. 2016). However, the limitation of this approach, as reported for PBMC, is related to the heterogeneity of the samples since the glands studied include both acinar and tubular epithelial cells which are found in patients and controls, but also immune cells present in patients. Despite this limitation, the importance of the IFN pathway is reported, again with, in particular, the detection of a gene inducible by the IFN such as *oas2*, which is demethylated. The other differentially methylated regions concern *psmd8* (26S proteasome non-ATPase regulatory subunit 8), *tap1* (antigen peptide transporter 1), and microRNAs as well as a wide variety of genes involved in cell activation, antigen presentation and autoantigen production (Cole et al. 2016; Renaudineau and Ballestar 2016).

3. **Methylation analysis of CpG units of epithelial cells in minor salivary glands**

As previously indicated, the study of CpG patterns on MSG was carried out on a cellular mixture, and therefore does not fully reflect the impact of DNA modifications on MSG epithelial cells. This limitation was removed by using SGEC from MSG cultured for 3–4 weeks to obtain a pure cell population to use as the biological source (Charras et al. 2017). Significant differences were observed when comparing SjS patients with a control population using HM450K chips. These differences concern a large number of genes regulated by IFN (61% of genes). In addition, the calcium pathway (involved in salivary flow control) was demethylated while the Wnt pathway (involved in epithelial cell survival and differentiation) was methylated in this study (Fig. 11.2). From such analysis, the phosphatidyl inositol (PI3)-kinase pathway was also associated with hydroxychloroquine intake.

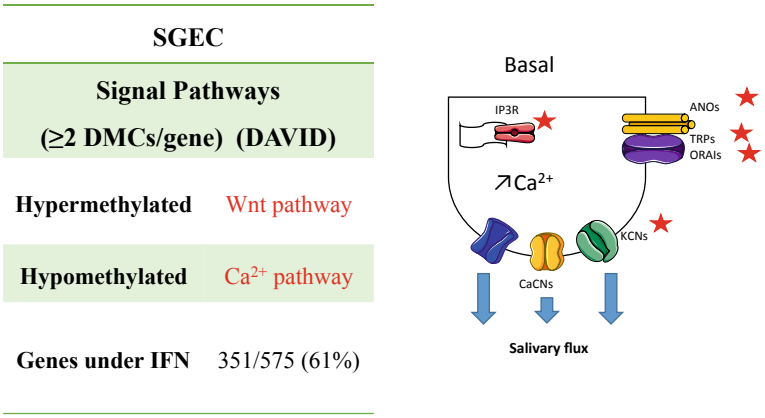

Fig. 11.2 Implication of DNA methylation in the pathogenesis of Sjogren's syndrome (Charras et al. 2017)

11.3.2 Histones

Histones are small globular proteins (11–15 kD) with flexible N-terminal tails that project from the nucleosome core. Histone N-terminal tails play an important role in controlling gene transcription and expression and this is regulated by post-translational modifications at lysine, arginine, and serine residues. Modifications at these residues can be acetylation, methylation, phosphorylation, ubiquitination/sumoylation, ADP ribosylation, deimination/citrullination, protein conjugation, or β-N-acetylglucosamination.

Using a chromatin immunoprecipitation (ChIP) approach a decrease in H4 acetylation was observed at the AQP5 (aquaporin 5) promoter and there was overexpression from the gene in human salivary gland acinar cells after treatment with TNF-α (Yamamura et al. 2012). Such an observation is in line with the report of Imgenberg-Kreuz et al. in which they investigated the distribution of hypomethylated, hypermethylated, and differentially methylated cytosines (DMC) in T and B cells. In both cell types, hypomethylated DMCs are located within areas of H3K4me1 and H3J27Ac, while it is in association with H3K26 that is reported for hypermethylated DMCs (Imgenberg-Kreuz et al. 2016). Interestingly, the main alteration in the salivary proteome of SjS patients is related to the abnormal presence of histones (Hall et al. 2017), probably through an accelerating apoptotic process, explaining why anti-histone auto-Ab are reported in SjS (Hu et al. 2011).

In addition, treating the SjS non-obese diabetic (NOD) mouse model with resveratrol, which enhances NAD(+)-dependent histone deacetylase activity through sirtuin 1, improves saliva secretion and expression of the anti-inflammatory cytokine IL-10 in salivary glands without affecting inflammatory cell infiltration (Inoue et al. 2016). SAHA (suberoylanilide hydroxamic acid, a specific histone deacetylase inhibitor) reduces inflammation in dry eye disease and this may have applications in SjS (Ratay et al. 2018). Such discrepancies may be explained in part by the fact that the two drugs are not acting on the same cell subset.

11.3.3 miRNA

Small non-coding and single-strand RNA of 19–22 nucleotides in length, microRNAs (miRNAs) adjust gene expression at the post-transcriptional level and are crucial in a wide array of physiological and pathological processes. In the nucleus, fundamental miRNA transcripts are generated through RNA polymerase II, and cleaved by an RNAse III enzyme, referred to as Drosha. After cleavage, miRNAs are transported to the cytoplasm via exportin 5 for processing using Dicer to generate mature miRNA duplexes. Duplexes are then separated into single strands at the core of the multiprotein RNA-induced silencing complex (RISC) which includes argonaute proteins. Most miRNAs bind to the 3′ untranslated vicinity (UTR) of the targeted mRNAs which leads to mRNA translation or repression or degradation (Zare-Shahabadi et al. 2013). Auto-Ab to the miRNA-binding protein argonaute 2 (Su antigen) enriched

in the cytoplasmic GW/P bodies are described in 10–20% of patients with SjS and neurological diseases (Bhanji et al. 2007; Satoh et al. 2013).

miRNA expression profile analysis reveals distinct profiles when comparing MSG from control subjects and SjS patients. Alevizios et al. have also reported that miRNA upregulation is more important in the group with decreased salivary functions (Alevizos et al. 2011). In addition, let7b, miR16, miR181a, miR223, and miR483-5p levels are positively correlated with Ro52/TRIM21-mRNA in MSG, while in SGEC miR181a and miR200b-3p are negatively correlated with Ro52/TRIM21 and Ro60/TROVE2 mRNAs, respectively, whereas let7b, miR200b-5p, and miR223 are associated with La/SSB-mRNA (Gourzi et al. 2015).

With regards to PBMCs instead of PBMC's subsets, a limited number of miRNAs, including miR-146a and miR-155, are reported when comparing SjS patients with sicca-complaining controls (Pauley et al. 2011; Peng et al. 2014; Shi et al. 2014; Gourzi et al. 2015). In contrast, when considering purified T and B cells from SjS patients, there are major differences corresponding to 21 miRNAs in T cells (9 upregulated and 12 downregulated) and 24 miRNAs in B cells (11 upregulated and 12 downregulated) (Wang-Renault et al. 2018). In this study, regulation through DNA methylation at promoters was excluded and differential expression patterns were observed according to the anti-SSA auto-Ab status. The most interesting pathways associated with differentially expressed miRNAs are related to the PI3K pathway (T and B cells), the transforming growth factor (TGF)-β pathway (T cells), and the Wnt pathway (B cells).

In monocytes from SjS patients, miRNAs are upregulated and they preferentially target the TGF-β signaling pathway and, to a lesser extent, the Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling cascades (Williams et al. 2016). The EBV-specific xeno-miRNA, EBV-miR-BART13-3p, can be transferred from SjS B cells, through exosomes, to SGEC (Gallo et al. 2016). This xeno-miRNA controls calcium signaling and salivary flux by targeting the stromal interacting molecule 1 (STIM1) (Mukherjee et al. 2017).

11.4 Epigenetic Reprogramming and Consequences

11.4.1 Methylation Modifications

11.4.1.1 Retrotransposons

More than 50% of our genome is composed of retrotransposons that correspond to DNA sequences that have the ability of multiplying to spread in the genome. Retrotransposons can be divided into different families, including Alu (10%), LINE (17%), and endogenous human retroviruses (HERV, 8%). In order to protect themselves from the action of these elements, they are regulated by DNA methylation.

Alu transcripts are found to be increased in SjS, and interferon type I allows Alu production as well as that of other pro-inflammatory cytokines (Mavragani and Crow 2010; Hung et al. 2015). Alu elements are also capable of binding to the SSA/Ro60 auto-Ag that can lead to the formation of an immune complex when associated with anti-Ro60 auto-Ab that is widely found in SjS and SLE. As a consequence this provides an explanation for the SSA/Ro60-associated RNA complex to initiate Toll-like receptors (TLR)-7/8 dependent pro-inflammatory cytokine release (Reed and Gordon 2016). Similar observations were made with LINE-1 which is upregulated in MSG from SjS patients, in relation to a defective DNA methylation process as recently reported by Mavragani et al. (2018). As a consequence, inappropriate LINE-1 expression in SjS is believed to contribute to the pathophysiology of SjS through the activation of the TLR-7/8-IFN-type I pathway as observed in plasmacytoid dendritic cells transfected with LINE-1 sequences (Balada et al. 2010; Mavragani et al. 2016).

In MSG (Table 11.2), HERV have been found to be overexpressed, such as HERV-K113, HERV-5, and the HERV-E family including clone 4-1 (Moyes et al. 2005; Le Dantec et al. 2012). For the most part, HERV genes have evolved to become non-functional due to deletions, presence of frame shifts or stop codons. However, a few copies have retained their functionality to generate viral proteins and to promote the expression of fusion transcripts with neighboring genes (Renaudineau et al. 2005; Le Dantec et al. 2015). For this reason, HERV elements are repressed by DNA methylation as demonstrated with HRES-1 (human T cell leukemia related endogenous retrovirus), inserted at the long arm of chromosome 1 at position 1q42 (Fali et al. 2014). When this control is lost, the HRES-1 Gag p38 auto-Ag is expressed and induces the production of anti-Gag p38 autoantibodies (Banki et al. 1992). Auto-Ab against HRES-1 Gag p38 is detected in 10% of patients with SjS versus 1.5% in healthy controls. Another HERV-E element overexpressed in SjS is HERV-E clone 4-1. HERV 4-1 p30 gag auto-Ab has been detected in 35% of SjS sufferers and is absent in healthy donors (Hishikawa et al. 1997).

11.4.1.2 Autoantigens

Demethylation of the SSB/La promoter is observed in patients with SjS, which generates overexpression of the transcript and protein (Konsta et al. 2016b). This effect is even more true in those SjS patients that express anti-SSB/La auto-Ab and can be reproduced by treating the HSG cell line with 5-azacytidine (5-Aza). Similar observations have been made with ICA1, another autoantigen (Charras et al. 2017).

11.4.1.3 Other Genes

Treatment with 5-Aza increases the expression of cytokeratin 19 in the HSG line (Konsta et al. 2016a) and also the aquaporin 5 gene (*aqp5*) with an increase in salivary flow in a human ductal salivary gland line (Motegi et al. 2005). Analysis by bisulfite sequencing of the *aqp5* promoter shows hypermethylation of the CpG islets at the Sp1 transcription factor binding sites, sites that can be demethylated with 5-Aza, allowing the Sp1 transcription factor to bind to DNA and initiate transcription of this gene.

Table 11.2 Epigenetic reprogramming and consequences in Gougerot-Sjogren's syndrome

	Minor salivary glands	Salivary gland epithelial cells	Salivary gland cell lines	B cells	T cells	PBMC	Monocytes
DNA methylation	↗HERV (Le Dantec et al. 2015) ↗oas2 (Imgenberg-Kreuz et al. 2016) ↗SSB/La (Konsta et al. 2016b) ↗cytokeratin 19 (Konsta et al. 2016a) ↗*LINE1* (Mavragani et al. 2016) Stress and immune response (Cole et al. 2016)	↗SSB/La (Konsta et al. 2016b) ↗cytokeratin 19 (Konsta et al. 2016a) ↗ICA1 (Charras et al. 2017) IFN, calcium, Wnt, PI3K pathways (Charras et al. 2017)	↗SSB/La (Konsta et al. 2016b) ↗cytokeratin 19 (Konsta et al. 2016a) ↘ire1α, xbp-1, dst (Gonzalez et al. 2011; Sepulveda et al. 2018) ↗aquaporin 5 (Yamamura et al. 2012)	IFN pathway, antigen presentation and lymphoproliferative disorders (Imgenberg-Kreuz et al. 2016; Miceli-Richard et al. 2016)	IFN pathway (Altorok et al. 2014) ↗CD70 (Yin et al. 2010) ↘Foxp3 (Yu et al. 2013)		
Histone modifications			↗aquaporin 5 (Motegi et al. 2005)	DNA demethylation marks overlap with H3K4me1 and H3K27ac (Imgenberg-Kreuz et al. 2016)	DNA demethylation marks overlap with H3K4me1 and H3K27ac (Imgenberg-Kreuz et al. 2016)		
Pathways associated with miRNA dysregulation	Positive correlation with Ro52/TRIM21 (Gourzi et al. 2015) Calcium pathway (Gallo et al. 2016)	Negative correlation with Ro52/TRIM21 and Ro60/TROVE2 (Gourzi et al. 2015)		PI3K and TGF-ß pathways (Wang-Renault et al. 2018) BAFF (Wang-Renault et al. 2018)	PI3K and Wnt pathways (Wang-Renault et al. 2018) FoxP3 (Wang-Renault et al. 2018)	NF-κB pathway (Zilahi et al. 2012)	TGF-ß and Jak/STAT pathways (Williams et al. 2016)

SGEC: salivary gland epithelial cells; HSG: Human salivary gland cell line; MSG: minor salivary gland

Other gene promoters have been analyzed, in particular the *ire1α* (inositol-requiring enzyme 1α), the *xbp-1* (X box-binding protein 1 (XBP-1), and the *dst* (dystonin) genes (Gonzalez et al. 2011; Sepulveda et al. 2018). Decreased mRNA levels for IRE1α and XBP-1 can be explained in part by an IFN-dependent hypermethylation pathway controlling their promoters. When demethylated, the *dst* promoter leads to the low expression of an epithelial alternative splicing variant, an auto-Ag, the bp320 pemphigoid bullous antigen 1. In $CD4^+$ T cells of patients with SjS, demethylation at CD70 (TNFSF7) promoter contributes to CD70 overexpression (Yin et al. 2010), while hypermethylation in the FOXP3 promoter leads to its repression (Yu et al. 2013).

11.4.1.4 X Chromosome in SjS

In women, in order to balance X-linked gene dosage, one of the two X chromosomes is inactivated in each somatic cell. This epigenetic control is suspected to be defective in SjS (Brooks and Renaudineau 2015). Several arguments support this assertion such as the predominant female sex bias observed in SjS and reports showing that trisomy X (47, XXX), a superb female phenotype (mosaic of XXXXX/XXXX/XXX/XX/XO), or Klinefelter's syndrome (47, XXY) increase the chance of developing SjS (Harris et al. 2016; Liu et al. 2016; Sharma et al. 2017). Observing that the X-linked CD40 ligand (CD40L, Xq26.3) was overexpressed in $CD4^+$ T cells from SjS females, Belkhir et al. failed to link CD40L expression levels with the DNA methylation status of its regulatory areas, in contrast to what is observed in SLE (Lu et al. 2007; Belkhir et al. 2014).

X chromosome inactivation (XCI) occurs early in female mammalian development to transcriptionally silence all but one of the X chromosomes in each cell through increased DNA methylation, thereby achieving dosage equivalency with the one X chromosome in males (XY). As a consequence, differences in X-linked gene expression between SjS and controls may underlie an abnormal control of genes following X chromosome inactivation (XCI) in SjS women with normal 46, XX genotype, as proposed first by Brooks et al. in response to disruption by a nearby nucleolus during stress (Brooks 2010, 2017) and validated in silico by Mougeot et al. (2018).

The X inactivation specific transcript (XIST), a lncRNA, along with LINE-1 genes in the X chromosome are involved in establishing XCI. However, XIST and LINE-1 sequences, two demethylation sensors, are overexpressed in SjS as well as the polycomb repressive complexes (PRC)2 genes EED and EZH1 that can be recruited by XIST to silence target genes. Altogether, these suggest an active but probably ineffective XCI process and difficulties in maintaining XCI during SjS, a defect that can be explained in part by the X chromosome nucleolus nexus hypothesis (Brooks 2017).

11.4.2 Histone Modifications in SjS

Imgenberg-Kreuz et al. demonstrated that several differentially methylated CpG (DMC) sites were hypomethylated and enriched in histone enhancers (H3K4me1 and H3K27ac) allowing access to the chromatin. On the other hand, hypermethylated DMCs in patients were underrepresented in enhancer regions (H3K4me3) but instead were enriched in the histone marker (H3K36) for actively transcribed genes (Imgenberg-Kreuz et al. 2016).

11.4.3 miRNA in SjS

11.4.3.1 miRNA in Minor Salivary Glands from SS Patients

miRNAs have been investigated in MSG revealing that miRNA expression is differentially expressed between SjS patients and controls, and that miRNA is involved in the control of salivation through neurologic pathways. Downregulation of the miRNA let-7b in MSG from SjS patients is also suspected of contributing to the lack of transcriptional control of the auto-Ag SSA/Ro and SSB/La.

11.4.3.2 miRNA in PBMC and Exosomes from SjS Patients

The two main miRNAs associated with SjS, miR146a, and miR155, are upregulated in response to the adaptive immune response when testing PBMC from SjS patients and from SS-prone mouse models (Pauley et al. 2011). Authors have shown, in SjS patients, that miR146a expands prior to disease onset in PBMCs, and at a more advanced stage in MSG from SjS patients. In addition, detected in PBMC and MSG, miRNAs are also present in exosomes which are microvesicles secreted by a large variety of cells including lymphocytes.

Mir146a is important for control of the phagocytic process and to repress inflammatory cytokine production in human monocytic THP1 cells. Mir146a is activated through the transcription factor NF-kappa B that controls the TLR/INF pathway through TNF-associated component 6 (TRAF6), IL-1 receptor-associated kinase (IRAK1), STAT1, and IRF5. Additionally, Zilahi et al. have measured the expression of miR146a and miR146b, and their target genes IRAK1, IRAK4, TRAF6 in PBMCs of patients with SjS and from controls (Zilahi et al. 2012). By quantitative RT-PCR they identified miR146a/b and the gene of TRAF6, as being overexpressed in SjS patients, whereas the expression of IRAK1 was significantly decreased. They proposed that the TRAF6 gene contributes to the increased activation of the NF-κB pathway by the involvement of PKCξ present typically in the disease, and that possibly the TRAF6 gene is a new biomarker of SjS.

Experiments for miR155 have shown an influence on the response of toll-like receptors (TLRs) and interleukin-1 receptors (TIRs) that can have an additional

effect on the immune response. FoxP3 transcription factor, which is detected in a subset of T cells infiltrating SjS MSG, has been shown to result in miR155 expression (Wang-Renault et al. 2018).

The expression of B cell activating factor (BAFF) is increased in B cells from SjS patients and such expression is inversely correlated with miR-30b expression The utilization of an antagomir (miRNA inhibitor) for miR-30 increases BAFF expression after transfection as observed in the THP-1 cell line (Wang-Renault et al. 2018).

11.5 Genetic Risk Factors Associated with SjS and Related to Epigenetic Factors

11.5.1 Methylation Modification

Recent data demonstrate highly significant correlations between DNA methylation modifications and the most important risk factors associated with SjS (Table 11.3). This was described for the HLA region and IRF5-TNPO3 locus with methylation quantitative trait loci (metQTL) by Imgenberg-Kreuz et al. using PBMC (Imgenberg-Kreuz et al. 2016, 2018). The same observation between DNA methylation and genetic risk loci was reported by Miceli-Richard et al. using peripheral B cells from SjS patients for HLA-DRA, HLA-DQB1, HLA-DQA1, HLA-DPB1, IRF5, CXCR5, BLK, PRDM1, ITSN2, GTF2I, and COL11A2 (Miceli-Richard et al. 2016). LTA and GSTM1 were reported by Altorok et al. in $CD4^+$ T cells, and CXCR5, BLK, LTA, and BAFF in MSGs (Altorok et al. 2014).

In SGEC, we have reported that seven SjS genetic risk factors presented at least one differentially methylated site: CXCR5, GTF2I, ICA1, NRLP3, SLC25A10, TNF, and MBL2 (Charras et al. 2017). However, it should be kept in mind that the demonstration of correlations may be difficult to establish as reported by Gestermann et al. who have tried to establish a link between the CpG polymorphism in the promoter of *irf5* and DNA methylation by comparing $CD4^+$ T cells, B lymphocytes, and monocytes from 19 SjS patients and 24 healthy controls (Gestermann et al. 2012).

11.5.2 Histone Modifications

In general, single nucleotide polymorphisms (SNP) related to SjS risk factors are enriched at 29.2% in promoters (RNA polymerase 2A site), at 56.9% in enhancers (H3K27Ac, H3K36me3, and H3K27me3), and at 6.9% in insulators (CTCF binding) (Konsta et al. 2015). In addition, we have also reported a cell-specific effect between SjS risk factors and the histone markers in monocyte enhancers (H3K36me3) and in B cell promoters (H3K4me2, H3K4me3, and H3K9Ac) and enhancer (H3K36me3) cells.

Table 11.3 Genetic risk factors associated with Gougerot-Sjogren's Syndrome and related to epigenetic factors (Konsta et al. 2015)

	Minor salivary glands	Salivary gland epithelial cells	T cells	B cells	PBMCs	Monocytes
DNA methylation	CXCR5, BLK, LTA, BAFF (Cole et al. 2016)	CXCR5, GTF2I, ICA1, NRLP3, SLC25A10, TNF, and MBL2 (Charras et al. 2017)	LTA, GSTM1 (Altorok et al. 2014) Lack of association with IRF5 (Gestermann et al. 2012)	HLA-DRA, HLA-DQB1, HLA-DQA1, HLA-DPB1, IRF5, CXCR5, BLK, PRDM1, ITSN2, GTF2I, and COL11A2 (Miceli-Richard et al. 2016) Lack of association with IRF5 (Gestermann et al. 2012)	HLA region and IRF5-TNPO3 locus (Imgenberg-Kreuz et al. 2016)	Lack of association with IRF5 (Gestermann et al. 2012)
Histone modifications				Overlap with promoter and enhancer histone marks (Konsta et al. 2015)		Overlap with enhancer histone marks (Konsta et al. 2015)
miRNA modifications	SSA/Ro52/TRIM21 (Gourzi et al. 2015; Yang et al. 2016)			BAFF (Wang-Renault et al. 2018)		

11.5.3 miRNA Modification

Targets of miRNA may include SjS risk factors such as BAFF, which is overexpressed in B cells and whose 3′ UTR is targeted by hsa-miR-30b-5p (Wang-Renault et al. 2018). Another example is TRIM21 as reported in Table 11.2 (Gourzi et al. 2015; Yang et al. 2016).

11.6 Conclusions

Epigenetic research conducted on SjS in the last decade has contributed to better understanding of this complex disease. More breakthroughs are expected in the near future. Future research may be focused on selecting pure cell subsets for analysis, understanding the mechanisms that control epigenetic defects, and coupling epigenetic analysis with other OMIC approaches (RNA-Seq, GWAS, proteomic). Finally, epigenetic research provides us the opportunity to develop new drugs in order to prevent/cure not only SjS but also lymphoma associated with SjS.

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