Review

Epigenetic Alterations in Cellular Immunity: New Insights into Autoimmune Diseases

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Key Words

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Abstract

Epigenetic modification is an additional regulator in immune responses as the genome-wide profiling somehow fails to explain the sophisticated mechanisms in autoimmune diseases. The effect of epigenetic modifications on adaptive immunity derives from their regulations to induce a permissive or negative gene expression. Epigenetic events, such as DNA methylation, histone modifications and microRNAs (miRNAs) are often found in T cell activation, differentiation and commitment which are the major parts in cellular immunity. Recognizing the complexity of interactions between epigenetic mechanisms and immune disturbance in autoimmune diseases is essential for the exploration of efficient therapeutic targets. In this review, we summarize a list of studies that indicate the significance of dysregulated epigenetic modifications in autoimmune diseases while focusing on T cell immunity.

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Introduction

Epigenetic modification is known as a gene-environmental term that characterized by the process of regulating gene expression as well as cellular function without changing DNA sequence [1]. Epigenetic dysregulation activate a range of human diseases and also has been linked to diverse pathological processes, especially in autoimmunity [2]. Due to the epigenetic bridge which links the gene and environment factors to contribute to immunity, the studies have shown the emergence of more epigenetic regulations in autoimmune diseases. Immune events occur in the presence of epigenetic modifications, owing to their molecular mechanisms in immune system by influencing overreactive immune cell functions in autoimmunity.

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DNA methylation

DNA methylation as a stable component in the epigenome has been a topic of intense interest. DNA methylation is implicated to cause gene repression, reversible promoter silencing and chromosomal instability [3, 4]. As a mechanism for altering transcription activity through certain modifications, DNA methylation is of great importance among epigenetic mechanisms, by which gene expression can be manipulated. The methylation level in the exon 1, which is in the downstream of the transcriptional start sites (TSS), is firmly connected to gene silence [5]. In addition, the methylation in TSS is activated by some dsRNAs through RNA-directed DNA methylation (RdDM) pathway, eventually, leading transcriptional gene silencing (TGS) [6]. In mammalian genomes, the methylated cytosine is clustered in GC-rich regions, named CpG islands [7]. DNA methylation is characterized by cytosine methylation mediated by de novo DNA methyltransferase enzymes (DNMT) [8], including DNMT1, DNMT3a, DNMT3b, and DNMT3L. DNMT1 is essential for remethylation of hemimethylated CpGs in mammalian development, while DNMT3a and DNMT3b are responsible for de novo methylation and carry out new methylations [9, 10]. DNA demethylation, in conjunction with a group of permissive translation activities, promotes the combination of several transcription factors binding to distinct gene loci.

Histone modification

Histone modifications adjust chromatin structure and target gene expression by recruiting remodeling enzymes and using energy from ATP hydrolysis to reposition the nucleosomes [11]. The most common histone modifications include acetylation, methylation, ubiquitination, phosphorylation, sumoylation, citrullination, ADP ribosylation, and proline isomerization [12]. Among them, acetylation is typically related to either active or repressive gene expression activities by two enzyme families with relative regulations, histone acetyltransferases (HATs) and histone deacetylases (HDACs), therefore, is suggested to be reversible and dynamic. On the contrary, methylation is found to be more stable with long-term maintenance of the expression pattern in the genomic regions [13]. The process of histone methylation is alternative, depending on the two opposite enzymes that catalyze different biochemical reactions, histone methylases and demethylases. For example, H3K4 trimethylation is suggested to upregulate gene expression; while H3K9 methylation and H3K27 trimethylation are known to downregulate gene expression. Convincing evidence shows that histone posttranslational alterations are significant regulators in the development of different cell lineages.

MicroRNA transcripts

MiRNAs are small noncoding single-stranded RNAs, consisting of a class of 21 to 23 nucleotide. The small noncoding RNAs as post-transcriptional and post-translational regulators are involved in gene expression by binding to target mRNAs. More than half of the protein-coding genes of mammals are found to be encoded through thousands of miRNAs that corporately affect gene expression. By targeting the complementary sequences within 3' UTR of a transcript, miRNAs inhibit transcription activity of target gene, and/or reduce mRNA stability, thus alternatively regulating protein expression [14]. MiRNAs serve as a guardian at every checkpoint genetically, modulate effector cell function through the regulation of specific signaling pathway, or target genes. Dysregulation of miRNAs will lead to varieties of dysfunctions or disorders, such as malignancy to immune diseases, implicating that miRNAs are involved in the maintenance of homeostasis, more specifically, participating in hematopoietic development, and cell activation and differentiation [15]. Additionally, miRNAs have also been identified as fine-tuning regulators to manipulate diverse biological processes at post-transcriptional level [16].

T helper cell lineages

In the immune system, the differentiation and function of T cell is a good model to study epigenetic regulation [17]. Increasing evidence suggests that epigenetic reprogramming participates in T cell activation, differentiation, and development, thus defines cell identity and function to environmental challenges [18]. Additionally, epigenome analyzing studies performed a range of high-resolution transcriptomic profiling of different kinds of immune cell including CD4+ T cells, and thus providing us with more knowledge of the uncovered story of T cell epigenetics [19].

Upon antigen stimulated, naïve T cell start their long term to travel to different T cell lineages. T helper 1(Th1) cell is important for controlling intracellular bacterial infection while T helper 2(Th2) cell initiates antibody response against extracellular pathogen. Tbet and Gata3 are major transcription factors for the induction of IFN- γ and IL-4 gene expression respectively. T helper 17 (Th17) cell is characterized by the expression of the key transcription factor retinoic orphan receptor γ t (ROR γ t) and the production of IL-17A, IL-17F, IL-21 and IL-23 cytokines [20]. Th17 cell is considered to be protective against infections at mucosal surfaces as a distinct CD4+ effector lineage [21, 22]. It was mentioned by Sakaguchi et al. in 1995 that Regulatory T (Treg) cell could express Forkhead box P3 (Foxp3) and produce IL-10 and TGF- β [23]. Treg cell is associated with peripheral tolerance and homeostasis in the immune system. Foxp3 is a transcriptional factor required to modulate the certain signaling mechanisms, and is considered to organize a perplexed transcriptional network which can stabilize the features and functions of Treg cell lineages [24]. T helper 9 (Th9) cell and T follicular helper (Tfh) cell are two subsets and were identified recently. Th9 cell is characterized by secreting IL-9 cytokine. It is not until recently have the studies found that Th9 is regulated by ETS family transcription factor PU.1, as well as IRF4 and GATA-3 [25-28]. Follicular helper T (Tfh) cell is featured as they localized in germinal centers to help B cells produce antibodies [29]. These Tfh cells express IL-21 cytokine (which is believed to be secreted only by Th17 cells) in addition to Bcl-6 (which is important for the development of B cells) [30, 31].

DNA methylation displayed a range of dynamic methylation/demethylation in T cell lineages

Th1 and Th2 cell. Studies have shown that DNA methylation guides the Th1 and Th2 differentiation by regulating their Th1 cytokine (IFN- γ) and Th2 cytokine (IL-4, IL5 and IL-13) gene expression, respectively [32]. In Th1 cells, IFN- γ gene locus achieves permissive modifications such as DNA demethylation and histone H3 acetylation or trimethyl-histone H3 lysine 4 (H3K4me3), while Th2 cytokine loci obtain repressive histone modifications and DNA methylation. However, those epigenetic patterns are negatively displayed in Th2 cells. Th2 cytokine gene loci receive allowable histone marks but the IFN- γ gene locus receives DNA methylation and repressive histone marks including trimethylation of histone H3 lysine 27 (H3K27me3) (Fig. 1) [33]. HS V is found to be DNA methylated in naïve T cells and de novo methylated during Th1 differentiation [34]. T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) has been found occasionally Th1 cell, which implies that some potential mechanisms may be linked with the TIM-3 and Th1 cell. DNA methylation analysis determines that a CpG island within the TIM-3 promoter is controlled by the regulation of DNA methylation. *In vitro* induced differentiation of Th0, 1 and 2 cell all displayed a DNA demethylation in that specific region of TIM-3 promoter, thus suggesting an increased expression of certain genes that encoded by the region of TIM-3 promoter [35].

Th17 and Treg cell. As discussed previously, similar histone remodeling and DNA demethylation occurs at IL17A and IL17F during the development of mouse Th17 cells, as the epigenetic regulation of *Ifng* locus and Th2 cytokine gene loci in the differentiation of Th1 and Th2 cells [36-38]. *In vivo*-isolated human Th17 cells are featured by DNA demethylation in IL17A and RORC loci, which is associated with specific expression in these cells [37, 39].

Many researches mentioned the critical roles of DNA methylation, in sustaining the steadily expressing of Foxp3 gene in association with Treg cell function. For example, Foxp3

Fig. 1. Epigenetic modifications in Th1 and Th2 cell differentiation have been extensively analyzed at the Th1 (IFN- γ) and Th2 gene cytokine loci. In Th1 cells, Th1 cytokine gene (Ifng) gains hypomethylation and permissive histone modifications (H3 and H4 acetylation, H3K4me3) whereas Th2 cytokine genes gain DNA methylation and repressive histone modifications. Conversely, in Th2 cells, the Th2 cytokine locus gains active histone modifications and DNA hypomethylation while the IFN γ locus gains repressive histone modifications and DNA methylation.

gene demethylation within Treg cells has been found in both human and animals (Fig. 2). However, those *in vitro* TGF- β -induced Treg cells (without epigenetic control) are identified to lose the stable expression of Foxp3 as well as the immunosuppressive function [40]. In a recent study, it has been noted that TET enzyme family do have a positive correlation with the long term expression of FOXP3 in TGF- β -induced Treg cells. Targeting TET enzymes by some certain molecular such as Vitamin C that promote the oxidation process of 5mC to 5hmC which is tightly associated with DNA demethylation that induced FOXP3 gene overexpression [41]. Treg-specific demethylated region (TSDR) has known to be a characterized fragment within the Foxp3 gene. Emerging evidences proved that TSDR can be only detected in stable Treg cell phenotypes. Study has also found that a global demethylation profile is gathered in this region in natural Tregs but effector T cells [42]. Thus, these data performed a hallmark of TSDR makes it an effective biomarker in detecting Treg cell [43]. Therefore, the hypomethylation pattern in Treg cells plays a pivotal role in maintaining the lineage commitment, stabilization, and suppressive function of Treg cells. Recent studies have deciphered a series of hypomethylated genes (Foxp3, Ctla4, Ikzf4, and Ikzf2, which are prerequisites for the Treg phenotype) are also under the regulation of permissive histone modifications in Foxp3 promoter region [44-47]. A recent study conducted an interesting experiment which induced the production of human CD4+CD25^{hi}FOXP3+ T cell from normal CD4+CD25- T cells in the presence of DNA methyltransferase inhibitor 5-azacytidine (5-Aza) as well as a less suitable TCR stimulation. The successful induction of CD4+CD25+FOXP3+Treg cell under DNA methylation regulation implies that the differentiation process from CD4+ T cells to iTreg cell is controlled by DNA hypomethylation which may increase the expression of several regulatory genes, such as FOXP3 [48].

Histone acetylation and H3K4 methylation are critical regulators in determining differentiation directions

Th1 and Th2 cell. Th1 and Th2 cell differentiation are controlled by histone modifications in the IL4 locus with the involvement of cis-regulatory elements [49]. NFAT is a transcription factors of activated T-cells, which obtains an alternative and lineage-specifying function to selectively promote Th1 or Th2 cell differentiation due to different epigenetic regulations [49, 50]. Histone hyperacetylation is suggested to be related with an open chromatin state, which is found in the promoter region of Ifng and IL4 gene in memory Th1 and Th2 cells, separately (Fig. 1) [51, 52]. A large amount of studies have reported the important role of the combination of Histone H3 acetylation, Lys-4 tri-methylation (H3K4me3), and Trithorax G (TrxG) (a well-characterized epigenetic modulator in Th2 cell differentiation) [53, 54].

Fig. 2. Dynamic epigenetic changes are involved in the differentiation of Th17, iTregs, Th9, and Tfh cells, such epigenetic marks are permissive and especially regulated in their active genes. H3/H4 acetylation and H3K4me3 are specifically increased in the IL-17, FOXP3, PU.1 and BCL-6 gene promoters, which permit the differentiation from Naïve T cell into different effector T cells.

The production of TrxG complex happens when STAT6 is activated at the promoter region of GATA3, which is an important transcription factor in the Th2 cell development and differentiation [53]. Growth factor independence 1 (Gfi1) is a transcriptional inhibitor that enhances Th2 cell but suppresses Th17 and Treg cell differentiation. Studies recently target Gfi1 to explore its role in Th1 cell and a negative correlation has been found between Th1-related transcription factors Gfi1. Gfi1 combines with those transcription factors of Th1 cell and a decreased expression of histone H3K4 methylation that is tightly associated with gene repression [55].

Th17 and Treg cell. The synergistic role of histone modifications in promoting chromatin accessibility for transcription is deciphered. H3K4me3 is specifically correlated with IL-17 and IL-17F gene promoters in Th17 cell subset. Upon stimulated, histone acetylation of IL17 gene is initiated by the combination of TGF- β and IL-6 [36]. The role of H3K27 demethylase Jmjd3 is important in regulating Th17 cell differentiation since the combination of Jmjd3 with H3K27me3 within Rorc promoter promotes the process of H3K27 demethylation. An increased expression of Jmjd3 is induced by activating naïve CD4+ T cell to differentiation into T helper cells. Reversely, silencing Jmjd3 gene in CD4+ T cell caused a decreased number of Th17 cells. *In vitro* experiment of EAE mouse model in Jmjd3 knockout mice showed an alleviated symptoms and clinical scores. Therefore, Th17 cell differentiation is regulated by Jmjd3 induced H3K27 demethylation and deficiency of which may be carried out to inhibit the immune responses mediated by Th17 cells [56].

CBP and p300 bromodomains are two histone acetyltransferases which have been proved to play significant role in stabilizing Treg cell lineage commitment and differentiation [57]. The bromodomains interacts with histone acetylation thus increasing FOXP3 gene expression and sustain the Treg cell function and stability [58]. Histone/protein deacetylases (HDACs) are important enzymes in generating histone deacetylation, thus play significant roles in regulating immune cells. HDAC5 deficient mice displayed a reduced production of Treg cells as well as less Treg cells induced when lack of HDAC5 in naïve CD4⁺ T cell upon Treg polarizing conditions [59]. Aforementioned, TSDR region is also regulated by a group of permissive histone marks, such as histone H3 acetylation and H3K4me3 [40, 60].

Th9 and Tfh cell. A recent study has deciphered that via inhibiting repressive histone methylation induce Th9-specific PU.1 expression, which conversely, preventing histone acetylation to diminish PU.1 expression after IL-9-inducing stimulation. This result suggests that epigenetic modifications of PU.1 are unique for regulating Th9 differentiation [61]. Smad2 and Smad4, two transcriptional factors initiated by TGF-beta signaling pathway, are required for Th9 differentiation *in vitro*. Impaired IL-9 expression can be induced by Smad2 or Smad4 deficiency in T cells, and this may be caused by the repressive chromatin modification histone H3K27 trimethylation and enhanced EZH2 binding to the IL9 locus

[62]. A recent study identified a demand of ETS transcription factor ETS variant 5 (ETV5) to augment IL-9+ Th9 cells with an enrichment of histone acetyltransferases (HATs) within the IL-9 gene promoter. *In vitro* study showed that a reduction of p300 in the absence of ETV5, accompanied by low level of histone H3 acetylation and H4K16 acetylation enriched in the IL9 promoter [63].

In addition to Th9, epigenetics have also established roles in Tfh differentiation. A great mapping of epigenetic profiles addresses that Tbx21, Gata3, and Rorc loci present permissive translational activities in Tfh cells. Conversely, a finding of H3K4me3 accumulation within Bcl6 gene locus in most non-Tfh cell implies the function of T helper cell lineages to display characteristic factors of Tfh cells upon stimulation [62]. (Fig. 2)

miRNAs

Th1 and Th2 cells. The studies of miRNAs in T helper cell were first identified in Dicer-deficient CD4+ T cells the studies with that Th cell differentiation was regulated by miRNA. The secretion of Th1 cell specific cytokine IFN γ and transcription factor T-bet in Dicer-deficient Th2 cells strongly suggest that miRNAs play negative roles during the differentiation stage, [64] since Dicer is a RNase III enzymes essential for obtaining mature miRNA [65]. Increased expression of miR-29 in naive T cells has substantially compromised Th1 cell differentiation by targeting several important factors, such as Tet (Tbx21) and eomesodermin (Eomes). In addition, reduced expression of miR-29a in transgenic mice is proved to elevate the serum IFN γ levels, suggesting that miR-29a perform the function by regulating IFN γ mRNA expression. Compared with Th1 cells, regulation of Th2 cell differentiation by miRNA still needs more investigation. The study has also confirmed the role of miR-21 in T cells to promote Th2 cell differentiation *in vitro* [66], whilst IL-4 and IL-5 secretion are reduced under the regulation of miR27 or miR128 [67].

Th17 cell. miRNA has an ancillary role in Th17 cell differentiation. It has been identified that several noncoding sequences in the IL-17/IL-17F locus were kept across species.[38] Studies have found that miR-155 play a active role in promoting Th1 cells and Th17 cells differentiation during the induction phase of experimental autoimmune encephalomyelitis (EAE), which is a disease model of the Multiple sclerosis. MiR-326 proves to suppress the expression of Ets-1– a negative regulator of Th17 differentiation, thus promoting differentiation [68]. These together imply that miRNAs are associated with the generation and growth of both Th1/17 cells and Tregs. The RORC gene, which is required for Th17 development, is associated with prominent H3K4me3 epigenetic marks in Th17 cells [36], and is proved to be marked at the Il17 and Il17f locus [38]. The methylation in the Il17a promoter represses its combination with the STAT3, thus inhibiting the transcription regulation during the Th17 cell differentiation [39].

Treg cell. Dicer deletion in CD4-cre model induces the decreasing numbers of Tregs [69], implying that microRNAs play central roles in Treg proliferation. MiRNA-155 defects in Treg cells of mice, indicating a reduction in numbers of Tregs due to the Foxp3 regulation of miR155 represses SOCS-1 thus increasing sensitivity of Tregs. Otherwise, miR-21 acts as a positive regulator of Foxp3 expression, therefore, contributes a prominent role in Treg cells [70].

The differentiation of Tfh cells is differentially regulated by a list of microRNAs due to their variant functions in transcriptional factors or regulatory elements. The miRNA events contributes a significant part to determine Tfh cell differentiation with two enhancers as miR-17~92 [71, 72] and miR-155 [73] as well as an dampened character miR-146a [74], thus establishing a comprehensive adjusting system to induced Tfh cell. (Fig. 1, 2)

Autoimmune diseases and epigenetic modifications. The risk factors provide us a variety of landscapes in explaining the etiology of autoimmune diseases, however, discordance rates between monozygotic twins remind us that many epigenetic mechanisms are still undetermined and are waiting us to explore in autoimmune diseases. (Fig. 3).

Fig. 3. Epigenetic mechanism of T Cells in the Pathogenesis of Autoimmune Diseases. These mechanisms include DNA methylation, histone post-translational modifications, and small interfering RNAs, which can alter genome and produce a different gene expression profile. This figure deciphers a schematic representation of some aberrant epigenetic patterns involved in autoimmune diseases.

Systemic lupus erythematosus (SLE)

SLE is a common autoimmune disease especially occurred in women and caused by multifactorial elements that produce autoantibodies and inflammatory responses in organs and tissues. Desregulated T lymphocytes in SLE generate a series of aberrant immune dysfunctions that make the disease complicated and hard to handle. Although gene susceptibility have revealed an part of the profound mechanisms of SLE, the concordance rates in monozygotic twins of lower than 50% [75] strongly suggests that epigenetics and environmental problems are involved in the onset and progression of the disease [76].

Epigenome study uncover the epigenetic spectrum in SLE T cells

Epigenetic studies of SLE started from the discovery of globally reduced DNA methylation in lupus T cells.[77, 78] The association between wide-ranged DNA hypomethylation in lupus and disease activity is on the basis of the study that applying DNA methylation inhibitors in CD4+ T cells would lead to a lupus-like syndrome. Recent studies applying sequence analysis technology have gained meaningful results that provide us with more information about lupus heritability [79], ethnicity variance [80] and renal involvement with DNA methylome analysis in CD4+ T cells [81]. Coit et al. confirmed a group of interferon-related genes that are aberrantly hypomethylated, including IFN-induced protein 44-like (IFI44L), IFN-induced protein with tetratricopeptide (IFIT1), IFIT3, MX dynamin-like GTPase 1 (MX1), signal transducer and activator of transcription 1 (STAT-1), bone marrow stromal cell antigen 2 (BST2) and tripartite motif containing 22 (TRIM22), in lupus CD4+ T cells [82]. Furthermore, a newly identified IFN-related gene IFI44L has been deciphered in SLE, and the study also emphasized its potential role as a diagnostic biomarker [83]. Furthermore, the globally reduced histone acetylation in lupus CD4+ T cells has been confirmed, implicating a number of aberrantly regulated genes [84-86]. In addition, a global histone H3K9 hypomethylation has also been found in CD4+ T cells. However, there is no evidence to connect the histone modifications with disease activity [84].

Methylation-sensitive genes in lupus CD4+T cells

In SLE, a wide range of hypomethylated genes in T lymphocytes have been reported, the methylation sensitive genes such as CD11a (ITGAL), perforin (PRF1), CD70 (TNFSF7), and CD40LG (TNFSF5) are significantly increased [87]. The promoter regions of CD11a and CD70 are recruited with decreased DNA methylation and increased histone acetylation which contributes to gene overexpression. The function of the two regulatory genes is noted

with their autoimmune responses in CD4+ T cells of patients with SLE [88]. Relevant study has shown that perforin overexpression in CD4+ T cells from patients with active lupus were also caused by DNA demethylation [89]. Although the reason why systemic lupus erythematosus primarily affects women still remains undetermined, demethylation of CD40LG may contribute to the female susceptibility of this disease [90]. Reducing Regulatory factor X1 (RFX1) and the Nuclear factor interleukin-3-regulated protein (NFIL3, also known as E4BP4) were also differentially methylated as in our previous study in CD4+ T cells. Reduced Regulatory factor X1 (RFX1) levels lead to CD4+ T cell autoreactivity by promoting CD11a and CD70 overexpression with less DNMTs as well as more histone methyltransferase SUV39H1 recruitment in CD4+ T cells [91, 92]. Overexpression of the transcription regulator E4BP4 (NFIL3) generates a protective mechanisms in CD4(+)T cells through inhibiting CD40L expression, therefore downregulating the autoimmune responses in SLE patients [93]. Enhanced expression of IL-10 in T cells from SLE is regulated by aberrant activation of Stat3 with histone acetyltransferase p300. Therefore, overexpression of IL10 contributes to autoantibody production and tissue damage [94]. Similarly, in SLE T-cells, Sunahori et al. demonstrated that inhibiting catalytic subunit of protein phosphatase 2A (PP2Ac) resulted in increased MEK/ERK phosphorylation, elevated DNA methylation enriched with DNMTs, as well as decreased expression of CD70 gene. Disturbance of the methylation-sensitive genes are tightly associated with SLE pathogenesis [95]. Likewise, expression level of the growth arrest and DNA damage-induced 45a (GADD45a) is increased. Our previous work showed a negative correlation between the GADD45a and DNA methylation in human lupus CD4+ T cells [96]. Killer-cell immunoglobulin-like receptor (KIR) genes are specifically expressed on NK cells. KIR promoter region is enriched with methylation regulated loci and studies have found that KIR gene is overexpressed in lupus CD4+ T cells with DNA demethylation regulation, thus promoting the generation of inflammatory cells and cytokines [97, 98]. The human endogenous retrovirus type E (HERV-E) promoter recruits repressive modifications as DNA methylation thus suppressing its expression in lupus CD4+ T cells, which are implied to be linked with lupus progression [99].

Environment exposure driven epigenetics in lupus T cell

Environmental factors such as UV exposure, smoking, vitamin D and infections are long been discussed in SLE [100]. Here, we put more emphasize on some newly identified elements that contribute to lupus.

Even if UV exposure is not a novel factor in SLE, the mechanisms altered dynamic in different studies. UV exposure produces an increased expression of gadd45A and CD11a/CD70, which suggested that ultraviolet, may create epigenetic changes since gadd45A is demethylated after UV-B irradiation [101]. High salt is a diver in many autoimmune diseases. Recently a study confirmed its role in SLE. High salty may be involved in SLE pathogenesis by promoting Tfh cell differentiation and commitment, which are significant players in lupus [102]. Exposed to oxidative stress may generated a lupus-like syndromes with increased serum anti-dsDNA antibody and glomerulonephritis in mice, due to the reason that oxidant is an associated with DNA demthylation [103]. Icaritin (ICT) is an extract from a Chinese medicine that is efficient in immune responses due to its role to maintain the balance of Th17 and Treg cells. Studies further identified that ICT may be used as a drug in lupus for stabilizing Treg cell protective role in autoimmune reactions [104].

Distinct microRNAs independently or combined with DNA methylation to affect lupus pathogenesis

Several studies have begun to unravel the contributions of noncoding RNAs to lupus pathogenesis [105]. Indeed, seemingly dysfunctions are observed in aberrant immune responses in SLE. miR-21 and miR-148a, miR-126 are three methylation regulated microRNAs that are combined with a decreased expression of DNMTs in CD4+ T cells of SLE. More specifically, depletion of miR-21 in chronic graft-versus-host disease (cGVHD) model of SLE alleviates a series of autoimmune symptoms in lupus. In this model, miR-21 also affects

T cell proportions with a reduced Th17 cell but an enhanced Treg cell [106, 107]. Previous study confirmed the mir-155 overexpression in Treg cells of MRL/lpr mice [108]. Further, mir-155 has been reported to have a regulation of T cell distribution as mir-155 deficiency mice displayed a reduced serum IL4 and IL17A which are two specific cytokines secreted by Th2 and Th17 cell separately. Similar to mir-21, lupus relevant clinical manifestations in the absence of mir-155 are milder than their controls [109]. miR-17~92 exhibits a controversial role in regulation Tfh cell differentiation. Previous study believed mir-17~92 limits Tfh cell differentiation since Bcl-6 could reverse the suppression role of mir-17~92 that inhibits CXCR5 expression in Tfh cells [110]. However, recent study doubted the thesis while lack of mir-17~92 promotes Tfh cell accumulation by targeting phosphatase PHLPP2 which is significant for T cell migration [72]. Accordingly, depletion or overexpression of mir-17~92 would lead a correspondence Tfh cell quantity as accompanied by subsequent autoimmune responses. mir-146a is a negative regulator in Tfh cell production, thus mir-146a deficiency mice may be resistant to lupus like symptoms [74].

Rheumatoid arthritis (RA)

RA is a chronic inflammatory diseases that specifically damage joints. Genome-wide study of DNA methylation in RA CD4+ T cells displayed a widely methylation variance in several regulatory genes or CpG sites. The specific mechanism of methotrexate (MTX) in RA has been revealed as MTX accumulates protective Treg cells by promoting the FOXP3 expression through promoter demethylation. Further to Treg cell, recent study identified that MTX treatment also reverse the hypomethylated status in PBMC cells of RA patients [111, 112]. Similarly, all-trans retinoic acid performs its function as to stabilize FOXP3+ Treg cell efficacy under DNA methylation controlling [113].

Studies attempting to explore the functions of microRNAs on RA pathogenesis are gaining momentum [114]. Li et al. developed a microRNA expression profile analysis indicating the expression variance of microRNAs in CD4+ T cells of RA patients. The upregulation of miR-146a as well as downregulation of miR-363 and miR-498 has been recorded [115]. Further study confirmed the increased expression of miR-146a in CD4+ T cell of RA patients [115]. However, there are different opinions when a study drew an opposite conclusion that miR-146a and miR-155 are decreased in Treg cells in response to T cell stimulation of RA patients [116]. Also, mir-126a expression is elevated in RA patients, which in turn increased expression of CD11a and CD70 by inducing DNA hypomethylation in their promoter region [117]. Reduced expression of miR-21 has been found in RA patients and study perceives that the decreased mir-21 would contribute Th17/Treg cell imbalance [118]. Further, increased expression of miR-21 has been found and it was believed to increase the accumulation of Treg cells in RA patients synovial fibroblasts [119].

Systemic sclerosis (SSc)

SSc is an autoimmune connective tissue disease with a high mortality. Early studies had shown whole genome wide DNA methylation signature of a list of target genes in CD4+ T cells. The decreased methylation level is induced by the downregulation of functional enzymes such as DNMT1, MBD3, and MBD4 [120]. Many methylation sensitive genes are involved in SSc pathogenesis and induced by DNA hypomethylation in their promoter sites. Overexpression of CD40L, CD11a and CD70 has been demonstrated as a predominant feature of SSc due to global reduced DNA methylation [121-123]. Despite the wide-ranged hypomethylation, Foxp3 promoter region gains hypermethylation mark that limits proliferation and functional activity of Treg cells [124]. Posttranslational modifications of CD4+ T cells in SSc exhibit globally reduced H3K27me3 thus resulting in accumulation of JMJD3 in SSc CD4+ T cells [125].

Type 1 diabetes mellitus (T1DM)

T1D is a chronic autoimmune disorder leading to the destruction of the beta cells with a strong inflammatory response through a mechanism mediated with T cells. It has

been reported that regulatory regions of FOXP3 in CD4⁺T cells was hypermethylated in T1D, resulting in reduced FOXP3 expression and fail to generate regulatory T cells [126]. In addition, Wang et al. claims that FOXP3 hypermethylation was induced by Toll like receptor 9 (TLR9), in conjugation with a decreasing binding activity of Interferon regulator factor 7 (IRF-7) [127]. Emerging evidence has indicated that the aberrant histone modifications were also involved in T1D. CLTA4 is a T1DM susceptibility gene. The enrichment of histone H3 lysine 9 dimethylation (H3K9me₂) in the promoter of CLTA4 was tightly correlated with T cells activation [128]. It has been suggested that Trichostatin A (TSA), an HDAC inhibitor, promotes IFN- γ secretion and enhances the transcription activity of Tbx21 in T lymphocytes, therefore relieving the inflammatory cell infiltration of islets [129]. Moreover, T1DM exhibits a wide range of miRNA expression profiles. Studies have found an upregulation of miRNA-510 as well as a downregulation of miRNA-342 and miRNA-191. In addition, when comparing Tregs and other types of effector T cells, the results revealed a differential expression of miR-146a and decreased expression of eight targeting miRNAs (20b, 31, 99a, 100, 125b, 151, 335, and 365) which support the involvement of microRNAs in T1D patients' Treg cells [130].

Conclusion

Inspired by overwhelming achievements in autoimmune diseases, a great deal of interest in immune cell and autoimmune reactions has focused on epigenetic determinants that are linked to three crucial areas of progress. Recent epigenetic therapies, such as DNA methyltransferase inhibitors, Histone deacetylase inhibitors, or BET bromodomain inhibitors [131] based on epigenetic mechanisms suggest the great potential for disease control and clinical relieve. HDAC inhibitors (HDACi) modulate immune and inflammatory processes, with a special emphasis on T-cell biology, consist of activating and differentiating naive T cells and developing featured T-cell subsets, particularly, Foxp3(+) Treg cells [132]. However, even though the translation of epigenetic mechanisms into clinical drug has been applied in cancer or other diseases, autoimmune diseases still lack the successful attempts. Whatever, the ability of epigenetic modification specifically regulates target regulatory region of a certain gene as well as monitor immune responses is promising for this technique in the treatment of autoimmune diseases.

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Disclosure Statement

No potential conflicts of interest were disclosed.

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