Epigenetic Modifications in the Pathogenesis of Systemic Sclerosis

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Abstract: Systemic sclerosis is a rare chronic autoimmune disease, which mainly manifests as immune disorders, vascular damage, and progressive fibrosis. The etiology of SSc is complex and involves multiple factors. Both genetic and environmental factors are involved in its pathogenesis. As one of the molecular mechanisms of environmental factors, epigenetic regulation plays an important role in the occurrence and development of systemic sclerosis, which involves DNA methylation, histone modification and non-coding RNA regulation. This review summarizes research advances in epigenetics, including exosomes, lncRNA, and mentions possible biomarkers and therapeutic targets among them.

Keywords: systemic sclerosis, pathogenesis, epigenetics, biomarkers

Introduction

Systemic sclerosis (SSc) is an chronic autoimmune disease involving immune system disorders, microvascular injury, and increasing organ fibrosis. The cause of SSc is still unclear, and may be related to factors such as genetics,[1] environmental factors (air pollution, viral infection, chemical substances such as silicon, etc.),[2] female hormones,[3] cellular and humoral immune abnormalities.[4] The pathogenesis of SSc remains ambiguous, and main processes currently recognized includes: immune disorders cause the body to produce a large amount of autoantibodies and cytokines to exacerbate autoimmunity; abnormal structure and function of arterioles or microcirculation, leading to fibrosis of the tube wall; fibroblasts dysfunction leads to excessive accumulation of collagen and other matrix components. These three processes are related to each other.[5] SSc is divided into three subtypes: limited cutaneous SSc (lcSSc), diffuse cutaneous SSc (dcSSc) and SSc without cutaneous involvement.[6] This disease can affects multiple organs throughout the body, and once severe pulmonary hypertension and scleroderma renal crisis occur, the situation is life-threatening. Because the current treatments are still limited, the prognosis and quality of life of SSc patients are not optimistic. And early diagnosis of SSc without skin lesions is challenging. Therefore, finding suitable biomarkers to assist in diagnosis or targeted therapy is particularly important.

Studies have shown that if a person has a positive family history of SSc, then his/her incidence rate of SSc will be higher than others, with the relative risk of siblings increases by 15–19 times, and first-degree relatives increases by 13–15 times.[7] Compared with the general population, certain ethnic groups have an increased prevalence of SSc and different clinical manifestations.[8] All of the above have confirmed that genetic factors play a part in the pathogenesis of SSc. However, it cannot fully explain the onset of this complex disease, as well illustrated by the largely incomplete concordance among identical twins.[9] Besides, environmental factors can cause phenotypic changes, indicating that it may be involved in the pathogenesis of SSc. As one of the hot research mechanisms of environmental factors, the growing evidence implicates that epigenetics may provide additional explanations for immune system disorders including SSc (Figure 1).
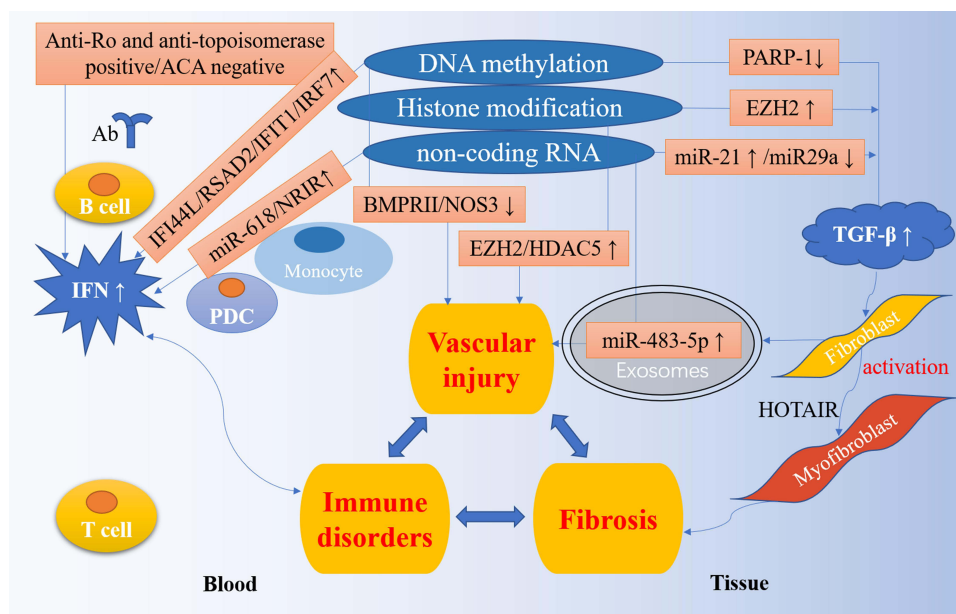
Figure 1 Epigenetic regulation in the pathogenesis of SSc. The pathogenesis of SSc is characterized by three processes: immune disorders, vascular damage and fibrosis. A large number of studies have shown that epigenetics plays a vital role in the pathogenesis of SSc. *IFI44L, RSAD2, IFIT1, IRF7* and other IFN related genes are highly expressed in the blood due to hypomethylation; anti-Ro and anti-topoisomerase positive and ACA negative are related with IFN highly expression; the up-regulation of miR-618 and *NRIR* are also associated with IFN production. These controls have led to the high IFN expression pattern of SSc. The down-regulation of PARP-1 and miR-29a, and the up-regulation of EZH2 and miR-21 participate in the TGF-β pathway and activate fibroblasts into myofibroblasts. In addition, the decrease of BMPRII and *NOS3*, the increase of EZH2, HDAC5, and miR-483-5p are involved in vascular injury.

Epigenetics

Epigenetics is the subject of studying the heritable changes in genes without changing their nucleotide sequences. There are a lot of research in various disciplines, and it has developed into an increasingly diverse field of scientific inquiry. It is mainly composed of three parts: DNA methylation, histone modification and non-coding RNA-mediated regulation. It typically involves changes in gene expression levels, chromatin structural stability and other functions.[10] More and more studies have shown that epigenetics is a biological process, and its disorder is related to the occurrence of some autoimmune diseases.[11]

DNA methylation is an important research content in epigenetics. In this biological process, the role of DNA methyltransferase (DNMT) is crucial.[12] Since this process mainly occurs in the promoter region of the gene CpG island, its effect will inhibit gene transcription.[13] DNMTs mainly includes DNMT1, DNMT3a and DNMT3b. Different DNMTs have different functions. For example, DNMT1 is ubiquitous and maintains DNA methylation level, while DNMT3a and DNMT3b promote the re-methylation process, thus ensuring methylation during embryonic development.[14]

Besides DNA methylation, histone modification is another epigenetic mechanism, which is a covalent post-translational regulation, including ubiquitination, phosphorylation, methylation, acetylation, ADP ribosylation, sulfony-lation, etc.[15] By changing the electric charge of histones and the conformation of chromatin, the expression of genes can be regulated. Among them, acetylation and methylation are the most studied.[16] Histone acetylases (HATs) and histone deacetylases (HDACs) are the two main enzymes involved in the process of histone acetylation. By recruiting or removing acetyl groups on histones, histone acetylation is at different levels, thereby activating or inhibiting gene transcription.[17] While histone methyltransferase (HMT) and demethylase (HDM) are the other two main enzymes that catalyze histone methylation. How histone methylation affects gene expression depends on the position of amino acid residues and the number of methyl groups.[18] The results of histone modifications are affected by many aspects, and there are also interrelationships among various modifications.

In addition to the above two processes, non-coding RNA is also one of the most studied epigenetic mechanisms, consisting of microRNA (miRNA) and long non-coding RNA (lncRNA). Among them, miRNA are about 22 nucleotides, while lncRNA is more than 200 nucleotides.[19] miRNA usually binds to the 3'-UTR of target mRNA, thereby regulating

gene expression after transcription, and then preventing gene translation through mRNA cleavage and degradation.[20] It regulates a variety of key pathways, and dysregulation can lead to inflammation, fibrosis, angiogenesis, etc.[21] lncRNA is a group of newly identified molecules and current research on it is still at an early stage. Relevant studies have shown that lncRNA may participate in various gene regulatory mechanisms, including gene transcription and may act a pivotal part in regulating the development of the disease.[22]

The aim of this review is to summarizing the research results of predecessors in epigenetics and the latest research progress in recent years, and strive to further understand the pathogenesis of SSc and find the most possible biomarkers.

Abnormal DNA Methylation
DNA Methylation and Immune-Related Cells

Previous studies have confirmed that *IFI44L* was hypomethylated and together with its overexpression in the blood in immune-related diseases such as systemic lupus erythematosus (SLE) and Sjogren's syndrome (SS), suggesting that *IFI44L* methylation differences may serve as a serum biomarker for these common autoimmune diseases.[23] Similarly, *IFI44L* was overexpressed in SSc blood, as were *RSAD2* and *TYROB*P.[24–26] More recently, Ramos et al found 27 differentially expressed genes by detecting the whole genome DNA methylation in whole blood, and newly discovered *CYFIP1* gene hypomethylation, *STAT3, TNFRSF1A, TNXB, TLE3* gene hypermethylation. Correspondingly, *TNXB* expression was low in SSc blood.[27] Another research by Chen et al also newly found that *IFIT1, IRF7, MX1, OAS1* and *USP18* in CD4+T cells of SSc patients were significantly hypomethylated.[28] The above *IFI44L, RSAD2, IFIT1, IRF7* and other genes were all type I interferon (IFN) related genes. Dysfunction of type I IFN has been studied in various immune-related diseases such as SSc and SLE.[29] Further study has reported that some SSc patients had high expression of IFN-induced genes,[30] and one of the possible explanations is that the serum of most anti-topoisomerase positive SSc patients could induce IFN production.[31] SSc serum antibodies might have a gradient relationship with IFN markers,[32] which will provide favorable evidence for the research of the pathogenesis and therapeutic targets of SSc.

Wang et al has observed that hypermethylation and decreased expression of *FOXP3* in CD4+T cells in patients with SSc, the promoter methylation status and expression level of *FOXP3* were closely related to disease activity.[33] Subsequent studies also confirmed that *FOXP3* was hypermethylated in the peripheral blood of SSc, besides, *PRF1, ITGAL* hypermethylation and *CDKN2A* and *CD70* hypomethylation were also found.[34] Compared with healthy women, women with SSc had lower DNA regulatory sequence methylation levels and significantly higher *CD40L* mRNA expression in CD4+T cells.[35] This might be an explanation for female susceptibility. Moreover, abnormal methylation changes might cause the differential expression of genes in peripheral blood mononuclear cells (PBMC) and also participated in the aberrant activation, proliferation and migration of immune cells in SSc patients. Simultaneously, four methylation-regulated genes *F2R, FYN, PAG1* and *PRKCH* were differentially expressed between SSc with and without interstitial lung disease (ILD).[36] These differentially methylated sites further revealed epigenetic alterations in the pathogenesis of SSc.

DNA Methylation and Fibroblasts

Altorok et al found that *COL8A1, COL16A1, COL29A1* in dcSSc fibroblasts and *COL1A1, COL6A3, COL12A1* in lcSSc fibroblasts were all hypomethylated by detecting genome-wide DNA methylation in skin fibroblasts from SSc patients. Furthermore, *PAX9* and *TNXB* were hypomethylated in both dcSSc and lcSSc, which are related to collagen synthesis and extracellular matrix (ECM) glycoproteins.[37] Similarly, the methylation level of SSc fibroblasts decreased, and the level of DNA demethylases *TET1* mRNA increased; in the hypoxic environment, TET1 expression in SSc fibroblasts was abnormally regulated and was accompanied by overall DNA hypomethylation.[38] Recently, studies demonstrated that the enzyme poly(ADP-ribose) polymerase-1 (PARP-1) negatively regulated transforming growth factor β (TGF-β) signal in cutaneous fibrosis.[39] The decreased expression of PARP-1 was due to DNA hypermethylation in its promoter region, and decreased PARP-1 might be directly participate in the excessive activation of TGF-β signaling and the sustained activation of fibroblasts.[39] Recent studies have reported hypomethylation of *DLX5* and *TMEM140* in skin fibroblasts from African-American SSc patients, accompanied by overexpression of these genes.[40] It also provided further strong evidence that people

of African ancestry were more likely to develop SSc, but the specific factors behind racial differences remained elusive, and epigenetics may provide a more scientifically plausible platform for unraveling this mystery.

MeCP2 protein is the main component of the methylated CpG binding domain (MBD) family. It inhibits the transcription of its downstream target genes through specific binding to methylated DNA and is an important transcription inhibitor. Compared with the control group, the levels of MeCP2, MBD-1, MBD-2, DNMT1 of SSc fibroblasts increased significantly, and CpG islands in the promoter region of the collagen suppressor gene *FLI1* of SSc skin fibroblasts were hypermethylated.[41] Besides the overall DNA methylation differences in SSc dermal fibroblasts, the transcription factors genes *RUNX1, RUNX2* and *RUNX3* of dcSSc and lcSSc were also hypomethylated,[37,42] and DNA methylation was also involved in the effect of the TGF-β pathway.[42] The study of Henderson et al showed that MeCP2 highly expressed in SSc fibroblasts enhanced Wnt signal transduction and collagen synthesis.[43] However, the study of He et al unveiled that the overexpression of MeCP2 in skin fibroblasts inhibited the differentiation, proliferation and migration of myofibroblasts. Genes such as *PLAU, NID2*, and *ADA* partially mediated the anti-fibrotic effect of MeCP2.[44] Although the reasons for the inconsistency between the two studies were not clear, these results all illustrated that abnormal DNA methylation was closely related to the pathogenesis of SSc, and its specific molecular mechanisms deserved further study.

DNA Methylation and Endothelial Cells

Microvascular endothelial cells (MVEC) played a critical role in regulating the contraction and expansion of normal blood vessels, and many studies have confirmed the damage of endothelial cells in SSc.[45–48] Wang et al have demonstrated that extensive methylation of CpG sites in the promoter region of bone morphogenetic protein receptor type II receptor (BMPRII) leads to its reduced expression, which resulted in MVECs of SSc being more prone to apoptosis than healthy controls. Corresponding, adding 2-deoxy-5-azacytidine and trichostatin A (TSA) to SSC MVECs normalized BMPRII expression level.[49] Nitric oxide (NO) has been previously reported to be one of the most important vasodilators, synthesized by endothelial nitric oxide synthase (eNOS) encoded by the *NOS3* gene. A significant down-regulation of *NOS3* expression was observed in SSc MVECs and the CpG site in the promoter region of *NOS3* was extensively methylated.[50] Recent studies have pointed out that there were significant differences in genome-wide DNA methylation in SSc MVEC, mainly at 2455 CpG sites. The hypermethylated genes identified in SSc MVEC included *NOS1, DNMT3A, DNMT3B, HDAC4* and *ANGPT*2.[51] This further proved that the methylation of genes related to angiogenesis was involved in the occurrence and development of scleroderma.

DNA Methylation and Serum Cytokine

In a study by Zhu et al, the DNA methylation changes of serum cytokines were analyzed in combination with the genome-wide methylation expression profile, and it was displayed that the transcription initiation regions of IL-10, IL-12P70, IL-1β, IL-1ra and VEGF all have DNA hypomethylation, suggesting that they may all be associated with DNA methylation.[52]

Histone Modification
Histone Modification and Immune-Related Cells

Previous research by Wang et al has shown that the overall histone H4 hyperacetylation and the overall histone H3K9 hypomethylation in the B cells of SSc patients. Overall histone H4 acetylation was negatively correlated with HDAC2 expression. At the same time, histone modifications in SSc B cells were related to skin thickness and disease activity, indicating that it might become a valuable epigenetic marker.[53] Ciechomska et al have proven that under certain conditions, histone demethylation in monocytes promoted transdifferentiation of fibroblasts in SSc.[54] In addition to B cells, histone modifications at the whole genome level were also associated with changes in the phenotype of SSc monocytes. Van der Kroef et al have reported that there were abnormal histone methylation modifications at multiple gene sites. The expression of some genes was directly related to the level of histone methylation near the transcription start point. Genes related to histone modification were mainly involved in immune, IFN and antiviral pathways.[55]

Histone Modification and Fibroblasts

Histone modifications have a role in regulating the expression of genes associated with fibroblast activation of myofibroblasts, governing their fate, as there are persistent histone DNA methylation and deacetylation in SSc fibroblasts.[56] The study by Wang et al has shown that adding HDAC inhibitors (TSA) significantly reduced the expression of type I collagen in SSc fibroblasts; compared with the control group, the levels of HDAC1 and HDAC6 of SSc fibroblasts increased significantly. The acetylation of histones H3 and H4 in the promoter region of *FLI1* gene of SSc fibroblasts was significantly reduced besides.[41] Recently, the effect of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) has been studied in SSc fibroblasts. EZH2 mediated the trimethylation of histone H3 lysine 27 (H3K27me3) to inhibit transcription. In a previous study, Kramer et al demonstrated that inhibition of H3K27me3 stimulated collagen release from fibroblasts in a time and dose-dependent manner, and treatment with DZNep (an inhibitor of EZH2 and H3K27me3) exacerbated fibrosis induced by bleomycin or by overexpression of TGF-βI.[57] Moreover, inhibition of H3K27me3 induced the expression of the profibrotic transcription factor *Fra-2* both in vitro and in vivo, and addition of *Fra-2* siRNA reduced the profibrotic effect of DZNep.[57] These all suggested inhibiting H3K27me3 could induce fibrosis. However, another study by Tsou et al showed the opposite, DZNep dose-dependently reduced the expression of *COL1A1, TGF-β, LRRC16A, Fra-2* and other fibrosis-related genes in SSc fibroblasts, and inhibited the migration activity of SSc fibroblasts.[58] In addition, EZH2 inhibitors prevented bleomycin-induced skin fibrosis, and overexpression of EZH2 accelerated wound healing, while high-dose GSK126 (another EZH2 inhibitor) significantly inhibited wound healing; furthermore, hypermethylation and low expression of *LRRC16A* gene were associated with EZH2-mediated cell migration.[58] The above showed that EZH2 inhibitors could prevent fibrosis in vitro and in vivo. Meanwhile, the study by Wasson et al found that inhibiting EZH2 with GSK126 also inhibited the increase in collagen and α-SMA expression in SSc dermal fibroblasts, confirming former work of Tsou et al.[59] One possible explanation for this inconsistent result is that the upregulation of H3K27me3 in SSc fibroblasts may be a compensatory attempt to neutralize the increased expression of profibrotic genes in SSc fibroblasts under different disease conditions. The regulation of fibrosis by H3K27me3 may be related to the diversity of histone methylases and the complexity of the molecular mechanisms of transcriptional regulation. H3K27me3 may be a central regulator of SSc fibrosis, and the exact role requires further studies to clarify.

Histone Modification and Endothelial Cells

In another study by Tsou et al, it was reported that the expression of HDAC5 in endothelial cells of SSc patients was significantly higher than that of healthy controls. Silencing HDAC5 in SSc endothelial cells restored normal angiogenesis. *CYR61, PVRL2* and *FSTL1* were key genes involved in angiogenesis and fibrosis regulated by HDAC5. Knockdown of HDAC5 and *CYR61, PVRL2* or *FSTL1* at the same time inhibited the angiogenesis of SSC endothelial cells. On the contrary, overexpression of these genes alone resulted in an increase in angiogenesis in SSc, indicating that these genes regulated by HDAC5 played a functional role in the angiogenesis disorder of SSc.[60] The use of HDAC inhibitors has also shown promising anti-fibrosis effects both in vivo and in vitro.[41,61] Previous studies have proven that EZH2 affected the regulation of genes related to endothelial cells function, especially genes related to cell adhesion and migration.[62] The overexpression of EZH2 in normal dermal endothelial cells inhibited angiogenesis, while the decrease of EZH2 led to an increase in angiogenesis. EZH2 was elevated in SSc endothelial cells, knocking down EZH2 in SSc endothelial cells significantly increased angiogenesis compared to the control. Similarly, treatment of SSc endothelial cells with DZNep reduced the expression of EZH2 in SSc endothelial cells by approximately 50%, thereby significantly enhancing angiogenesis. These data indicated that EZH2 might inhibit angiogenesis in SSc endothelial cells by inhibiting pro-angiogenic genes or activating anti-angiogenic genes. Further research reported that inhibition of EZH2 in endothelial cells restored the normal angiogenesis of SSc by activating the *NOTCH* pathway, especially by up-regulating *NOTCH* ligand DLL4.[58]

Non-Coding RNA-Mediated Regulation

miRNAs in the Blood

The expression of miR-483-5p increased in the serum, fibroblasts and endothelial cells of SSc patients, and regulated the expression of fibrosis-related genes. It was worth noting that miR-483-5p was also up-regulated in localized scleroderma,

but it was not up-regulated in other autoimmune diseases (such as SLE and SS), suggesting that it has a potential role as a unique and precise biomarker and mediator of SSc fibrosis.[63] In addition, Koba et al reported that combined detection of serum miR-206 and miR-21 levels was more helpful to distinguish scleroderma patients from normal people than detection of miR-206 or miR-21 alone.[64] Furthermore, miR-5196 in monocytes and serum of SSc patients increased significantly, and was positively correlated with CRP levels. Exogenous transfection of miR-5196 reduced the expression of TIMP-1 that promoted fibrosis, suggesting that it may be a potential regulator of SSc fibrosis, and miR-5196 was considered to be a potential biomarker for SSc.[65] Plasmacytoid dendritic cells (PDCs) were an important source of I type IFNs and participated in the occurrence and maintenance of autoimmunity. The expression of miR-618 in peripheral blood PDCs of SSc patients was up-regulated, including those with early disease without skin fibrosis. IFN regulatory factor 8 was a key transcription factor for PDC formation and activation, and it has been identified as a target of miR-618. The up-regulation of miR-618 inhibited the development of PDCs and enhanced their ability to secrete IFN-α, which may be one of the reasons for the type I IFN characteristics of SSc patients. Therefore, miR-618 may be an important epigenetic target in SSc immune regulation.[66] In PBMC of SSc-ILD, the expression of miR-155 was closely related to lung function.[67] Moreover, the level of miR-200C in PBMC of SSc-ILD patients was negatively correlated with forced vital capacity and forced expiratory volume in 1 second, and the level of miR-200C was positively correlated with the severity of SSc-ILD. Thus miR-200C could be used as a biomarker of the severity of ILD in SSc patients.[68] And circulating miRNA profiles were different between lcSSc and dcSSc patients and between patients with different autoantibodies.[69]

miRNAs in the Fibroblasts

The study by Liakouli et al found that EGFL7 (Epidermal Growth Factor Like-domain 7), which played an important role in angiogenesis, and its specific negative regulator miR-126 might be related to the pathogenesis of SSc vascular disease and fibrosis.[70] In addition, the serum expression level of miR-126 could distinguish RA patients from SSc patients in the study of Stypinska et al.[71] In both dcSSc and TGF-β treated fibroblasts, miR-21 increased while miR-29a decreased. MiR-21 and TGF-β had a synergistic effect in inducing collagen production, while miR-29a reduced the collagen production of dermal fibroblasts induced by TGF-β.[72] Other studies have also confirmed the pro-fibrotic effect of miR-21 and the anti-fibrotic effect of miR-29a.[73–75] Besides being involved in the production of collagen, miR-21 and miR-29a also played a role in the apoptosis of myofibroblasts. Previous studies showed that the overexpression and inhibition of miR-29a and miR-21 respectively induced apoptosis of dermal fibroblasts.[76,77] These results suggested that miR-21 and miR-29a may be potential therapeutic targets or useful prognostic or diagnostic markers. Several other related miRNAs in SSc fibroblasts were miR-145, miR-155, miR-196a, let-7a, miR-29b-3p, miR-138-5p and miR-146b-5p, which were associated with immune responses and fibroblast activation.[78–85]

miRNAs in the Lungs

Previous studies have reported that miR-155 and miR-143 in lung tissue of SSC-ILD patients were closely related to the high-resolution CT score.[67] Moreover, He et al identified 26 differentially expressed miRNAs by comparing SSc-ILD samples with healthy control lung tissue samples. Among them, 2 miRNAs (hsa-let-7e and hsa-miR-455) in SSc-ILD samples were significantly up-regulated, while the other 24 miRNAs were down-regulated. The top 10 dysregulated miRNAs were hsa-miR-4459, hsa-miR-4507, hsa-miR-4417, hsa-miR-3687, hsa-miR-1246, hsa-miR4668, hsa-miR-4530, hsa-miR-4299, hsa-miR-4516 and has-miR-663b, mainly associated with inflammation and fibroblast regulation related signaling pathways, such as ECM-receptor interaction, TGF-β signaling pathway, Wnt signaling pathway, and cell adhesion connection. *CX3CL1, FOSL1* and *FOSB* were the target genes predicted by these miRNAs.[86] A large cohort study showed that *CX3XL1* was associated with anti-topoisomerase I antibodies and pulmonary fibrosis, as well as the progression of ILD.[87] The expression of *FOSL1* in the lung mediated anti-fibrosis effects by regulating the expression of proinflammatory, profibrotic and fibrotic genes, providing a potential biomarker for poor prognosis and treatment options for pulmonary fibrosis.[88] However, these studies have some limitations due to the limited number of samples and lack of sufficient in vivo and in vitro experimental verification. Therefore, its clinical significance needs to be further studied.

lncRNAs

So far, only a few lncRNAs that regulate immune response have been reported in SSc. *NRIR* in monocytes of patients with SSc was closely related to IFN response, which further supports the abnormal phenomenon of IFN in SSc.[89] Abd-Elmawla et al analyzed the plasma expression profile of lncRNA, and studied its relationship with disease types and related clinical indicators, and proposed plasma *SPRY4-IT1, HOTTIP, ANCR* and *TINCR* as new candidate biomarkers for SSc.[90] Further, the study by Takata et al unveiled that *OTUD6B-AS1* regulated cell proliferation and apoptosis through the expression of *cyclinD1* independent of the sense gene, revealing its potential value in regulating the apoptosis of fibroblasts and vascular smooth muscle cells, and may participate in the pathogenesis of SSc.[91] Another study by Pachera et al showed that the expression of *H19X* was dose-dependent with the concentration of TGF-β and could effectively induce fibroblast differentiation and survival, enhance ECM production and tissue progressive fibrosis.[92] Of note, the study by Wang et al demonstrated that intrinsic TGF-β activation may result in increased expression of *TSIX* in the serum of patients with SSc, and that *TSIX* was able to regulate collagen production.[93] Since increased levels of *TSIX* appear to correlate with levels of skin fibrosis, it has the potential to act as a novel disease biomarker that may also serve as a potential therapeutic target.[94] Recently, *HOTAIR* was found to induce skin fibroblasts differentiation in vitro, driving EZH2-dependent myofibroblast activation in SSc through miRNA 34a-dependent activation of *NOTCH*.[59] Apart from Notch signaling, *HOTAIR* can also induce fibrosis through Hedgehog signaling.[95] The differentially expressed ncRNA00201 and *PSMB8-AS1* in blood and *MGC12916* in fibroblasts were also confirmed to be important players in the pathogenesis of SSc.[40,96,97] The involvement of lncRNAs in the pathogenesis of scleroderma is intricate and complicated, which may be through the epigenetic regulation of the gene itself, interacting with miRNAs, or affecting the secretion of important cytokines such as IL-6 and TNFα,[97] and participating in regulating the expression of fibrosis-related genes. At present, the field of lncRNA is still in its infancy, and related research is still limited but proved to be effective. Therefore, this is a promising prospect and it is worthwhile to further research the epigenetic mechanism of SSc.

Exosomes

Exosomes are thought to mediate cell-to-cell communication by transferring their contents (protein, RNA, miRNA, lncRNA, circRNA and DNA) to target cells. The study by Wermuth et al have shown that compared with the normal control group, 6 fibrotic miRNAs in serum exosomes isolated from SSc patients increased, and 10 anti-fibrotic miRNAs decreased. In addition, serum exosomes isolated from SSc patients stimulated normal human skin fibroblasts to express fibrosis genes and type I collagen, and produced and secreted fibronectin in a dose-dependent manner.[98]

Interestingly, many miRNAs were encapsulated in exosomes for circulation, such as miR-483-5p mentioned earlier. These findings suggest that miRNAs in exosomes may be involved in the transmission of fibrotic signals, and related study has further confirmed this view.[99]

Conclusion

Epigenetics has been in development for decades and has been implicated in many autoimmune diseases. In recent years, with the in-depth understanding of various pathway mechanisms, it has been found that epigenetics plays a pivotal role in the occurrence and development of systemic scleroderma. This review summarizes the recent advances in epigenetics in the pathogenesis of SSc and attempts to find suitable disease-related biomarkers and therapeutic targets (Table 1). However, up to now, the etiology of SSc is still under research, and studies on the specific mechanisms of tissue fibrosis, vascular lesions, and immune disorders in SSc are limited. Moreover, the results of some experiments are inconsistent, which also shows that the epigenetic mechanism of SSc is inscrutable, which requires further exploration.

With the progress of research, more and more epigenetically related molecules have been excavated for their medical value, and agonists or inhibitors of the above molecules are also a promising choice for future targeted therapy of SSc. If the non-invasive detection of the above-mentioned biological molecules can achieve the effects of judging the condition, assisting the treatment, and evaluating the prognosis, this will become more scientifically meaningful, but many obstacles need to be overcome. As mentioned earlier in the text, many genes related to the interferon pathway undergo epigenetic changes and appear to be associated with different antibodies in SSc, which provides the possibility of targeted therapy in

Table 1 Epigenetic Regulation in the Pathogenesis of SSc

Epigenetics	Type	Change	Expression	Cell/Tissue	Reference
DNA methylation	IFI44L	Hypomethylation	Increased	Blood	[24–26]
	RSAD2	Hypomethylation	Increased	Blood/CD4+T	[24–26]
	TYROBP	Hypomethylation	Increased	Blood	[26]
	FOXP3	Hypermethylation	Decreased	CD4+T	[33]
	CD40L	Hypomethylation	Increased	CD4+T	[35]
	PAX9	Hypomethylation		Fibroblast	[37]
	TNXB	Hypomethylation		Fibroblast	[37]
	TET1	Hypomethylation	Increased	Fibroblast	[38]
	PARP-1	Hypermethylation	Decreased	Fibroblast	[39]
	DLX5	Hypomethylation	Increased	Fibroblast	[40]
	TMEM140	Hypomethylation	Increased	Fibroblast	[40]
	BMPRII	Hypermethylation	Decreased	Endothelial	[49]
	NOS3	Hypermethylation	Decreased	Endothelial	[50]
Histone modification	Global histone H4	Hyperacetylation		B cell	[53]
	FLI1	Deacetylation	Decreased	Fibroblast	[41]
	H3K27me3		Increased	Fibroblast/endothelial	[57,58]
	HDAC5		Increased	Endothelial	[60]
Non-coding RNA	miR-483-5p		Increased	Serum/fibroblast/endothelial	[63]
	miR-5196		Increased	Serum/monocyte	[65]
	miR-618		Increased	Peripheral blood PDC	[66]
	miR-200C		Increased	PBMC	[68]
	miR-21		Increased	Fibroblast	[72]
	miR-29a		Decreased	Fibroblast	[72]
	NRIR		Increased	Monocyte	[89]
	SPRY4-IT1		Increased	Plasma	[90]
	HOTTIP		Increased	Plasma	[90]
	ANCR		Decreased	Plasma	[90]
	TINCR		Increased	Plasma	[90]
	OTUD6B-AS1		Decreased	Fibroblast, human pulmonary artery smooth muscle cells	[91]
	H19X		Increased	Fibroblast	[92]
	TSIX		Increased	Fibroblast, serum	[94]
	HOTAIR		Increased	Fibroblast	[59]
	ncRNA00201		Decreased	PBMC	[96]
	PSMB8-AS1		Increased	PBMC	[97]
	MGC12916		Decreased	Fibroblast	[40]

the clinic. If the above-mentioned epigenetically related molecules can be developed into novel dressings with percutaneous absorption or micro sustained release, it may also be a future direction for the treatment of localized scleroderma, but it also requires a lot of follow-up and research.

Ethics Statement
The authors confirm to have complied with ethical standards.

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Disclosure

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