Introduction
Systemic sclerosis (SSc) is a chronic, heterogeneous multi-system autoimmune connective tissue disease. It is characterized by three pathological processes: vascular injury and endothelial dysfunction, resulting in vascular intimal proliferation and remodeling, vasoconstriction, and defective angiogenesis; immune dysregulation, resulting in cell-mediated immunity and autoantibodies production; and fibroblast (FB) activation, resulting in excessive collagen and extracellular matrix (ECM) production and accumulation in the skin and other organs (1-4). Of note, vascular manifestations can precede other disease manifestations by several years. The pathogenesis of SSc is still unclear. The disease occurs in a multi-step process involving interaction between genetic and environmental factors in a genetically susceptible individual. This process starts with microvascular endothelial cell dysfunction and overexpression of adhesion molecules and chemokines, attracting diverse types of immune cells, including T cells and activated B cells. These cells release their cytokines when they accumulate in the tissue, stimulating FBs to produce excessive amounts of collagen and other ECM components (5).

SSc is classified as limited cutaneous SSc (lcSSc) when it affects limited portions of skin and has minimal systemic involvement and as diffuse cutaneous SSc (dcSSc) when it affects large portions of skin and involves multiple internal organs. SSc is more prevalent in women with an overall female-to-male ratio of 3:1 or greater and marked ethnic differences (1, 6). There is no clear causative factor for SSc. Genetics plays an important role in the pathogenesis of SSc; however, despite the identification of multiple genetic risk loci such as the major histocompatibility complex (MHC) II (7), which are associated with increased susceptibility to the disease, genetic factors alone do not explain the occurrence of the disease (1-4, 8). Of note, vascular manifestations can precede other disease manifestations by several years. The pathogenesis of SSc is still unclear. The disease occurs in a multi-step process involving interaction between genetic and environmental factors in a genetically susceptible individual. This process starts with microvascular endothelial cell dysfunction and overexpression of adhesion molecules and chemokines, attracting diverse types of immune cells, including T cells and activated B cells. These cells release their cytokines when they accumulate in the tissue, stimulating FBs to produce excessive amounts of collagen and other ECM components (5).

Epigenetics is defined as heritable variations in gene expression patterns without alteration in the DNA sequence. These modifications are accomplished through various mechanisms, including DNA methyl-
DNA methylation

DNA methylation is the process by which a -CH$_3$ (methyl) group, derived from S-adenosyl-L-methionine, covalently binds to position 5 in the cytosine ring within the CpG dinucleotides in the DNA. This process is catalyzed by DNA methyltransferases (DNMTs), namely DNMT1, DNMT3a, and DNMT3b. Even DNMT3L belongs to this group; however, it has no catalytic activity. Nonetheless, it stimulates de novo methylation of cytosine by DNMT3α and DNMT3β (15). DNMT3α and DNMT3β play important roles in the de novo methylation and generation of specific DNA methylation patterns. DNMT3α has a significant role in genomic imprinting during gametogenesis (17-19), whereas DNMT3β has an important role in embryonic development. DNMT1 is responsible for the maintenance of DNA methylation patterns during replication, ensuring faithful inheritance of epigenetic changes during replication and mitotic divisions (20). Therefore, any abnormalities or changes in the inherited methylation patterns can be attributed to DNMT1 dysfunction (21). DNA methylation is the proposed mechanism for genomic imprinting and X chromosome inactivation (21, 22).

DNA methylation of the cytosine in the CpG sites marks the heterochromatin closed structure, resulting in transcriptional silencing by direct physical interference with the binding of transcriptional factors or by binding to methyl-CpG-binding proteins such as MeCP2 and methylated DNA-binding domain (MBD)-containing proteins (23). DNA methylation can be reversed by a demethylation process that can be active or passive. Active demethylation is mediated by 10-11 translocation oxidases and passive demethylation occurs through replication in the absence of DNMT1 activity (2). The demethylation of CpG islands in the gene promoter region results in increased transcriptional activity.

DNA methylation plays a significant role in SSC pathogenesis as described above in the three key cells associated with disease pathogenesis—FBs, endothelial cells, and lymphocytes. These cells mediate the major SSC manifestations of fibrosis, vasculopathy, and immune dysregulation.

Endothelial cells: One of the major functions of microvascular endothelial cells (MVECs) is to control the vascular tone by maintaining a healthy balance between vasoconstrictors and vasodilators. Nitric oxide (NO) is one of the most important vasodilators and is synthesized by endothelial nitric oxide synthase (eNOS) that is encoded by the gene NOS3. NO has vasodilatory, antiatherogenic, antiplatelet, and antioxidant properties (27). Significant downregulation of NOS3 expression was observed in SSC MVECs. In addition, substantial methylation of the CpG sites in the promoter region of NOS3 was identified in association with gene repression. The addition of 5-azacytidine reversed CpG sites methylation, leading to normalization of NOS3 expression. This observation may explain the propensity for vasospasm and platelet activation in SSC (1, 28). The bone morphogenetic protein receptor II (BMPRII) promoter region was found to be intensely methylated in SSC MVECs (29). BMPRII is a member of the transforming growth factor-β (TGF-β) superfamly that coordinates cell proliferation, differentiation, and survival. Signaling through BMPRII in MVECs results in apoptosis resistance and promotes the survival of MVECs. Downregulation of BMPRII expression was observed in SSC MVECs with enhanced response to apoptotic signals, including growth factor withdrawal (29). The heightened response to apoptosis induction was reversed with exposure to 5-azacytidine. This mechanism may enhance endothelial apoptosis in SSC.

Recent work demonstrated significant genome-wide DNA methylation anomaly in SSC MVECs, characterized by differential methylation of 2,455 CpG sites, representing 1,301
Table 1. Summary of epigenetic modifications in SSc.

| Gene/Pathway | Epigenetic modification | Cell type | Consequences                                                                 | Reference number |
|--------------|-------------------------|-----------|-------------------------------------------------------------------------------|------------------|
| NOS3         | Hypermethylation        | MVECs     | Reduced NOS activity in MVECs                                                  | (20)             |
|              |                         |           | Increased expression of proinflammatory and vasospastic genes                 |                  |
| FL1          | Hypermethylation        | FB        | Overexpression of collagen genes in SSc FBs                                    | (24)             |
| DNA demethylase | Downregulation       | MVECs     | Hypermethylation                                                               | (24)             |
| MBD1         | Overexpression          | FB        | Recruitment of HDACs, resulting in unfavorable chromatin structure              | (24)             |
| DNMT1        | Overexpression          | FB, MVECs | Increased expression of DNMT1 could contribute to hypermethylation of certain genes such as FLI1 | (24, 25)         |
| BMPRII       | Hypermethylation        | MVECs     | Failure of the inhibitory mechanism for cell proliferation and induction of apoptosis. | (29)             |
| DNMT1        | Downregulation          | CD4+ T cells | Global hypomethylation in CD4+ T cells                                 | (32)             |
| FOXP3        | Hypermethylation        | CD4+ T cells | Decreased FOXP3 expression leads to quantitative defects in Tregs that may contribute to autoimmunity | (39)             |
| CD40L        | Hypomethylation         | CD4+ T cells | May contribute to female susceptibility in SSc                                | (41)             |
| CD70 (TNFSF7) | Hypomethylation        | CD4+ T cells | Co-stimulatory molecule, role is not clear in SSc                            | (43)             |
| CD11a        | Hypomethylation         | CD4+ T cells | Overexpression of CD11a leads to increased proliferation of CD4+ T cells and increased production of IgG antibodies by B cells | (44)             |
| ACTA         | Hypomethylation         | MVECs     | Failure of the inhibitory mechanism for cell proliferation and induction of apoptosis. | (24)             |
| CTNNA3, CTNND2 | Hypomethylation       | FB^c       | Involved in the Wnt/b-catenin pathway activation                               | (46)             |
| COL1A1, COL6A3, COL12A1 | Hypomethylation | FB^c       | May contribute to collagen overexpression                                     | (46)             |
| SOX20T       | Hypermethylation        | FB^c       | Encodes Inc-RNAs                                                              | (46)             |
| PDGF-C       | Hypermethylation        | FB^c       | PDGF-C is a profibrotic factor, contributes to FB activation and their transformation to myofibroblasts | (46)             |
| CDH11        | Hypermethylation        | FB^c       | Overexpression of CDH11 leads to increased cadherin-11 that facilitates myofibroblast differentiation | (46)             |
| TNXB         | Hypermethylation        | FB^c       | May contribute to matrix maturation by increasing the expression of tenascin family of glycoproteins | (46)             |
| PAX9         | Hypomethylation         | FB^c       | Overexpression of pro-α 2 chain of type I collagen                            | (46)             |
| ADAM12       | Hypermethylation        | FB^c       | Contributes to fibrosis through activating the TGF-β signaling pathway         | (46)             |
| ITGA9        | Hypomethylation         | FB^c       | Overexpression of ITAG9 leads to TGF-β upregulation                           | (46)             |
| C8ORF4       | Hypermethylation        | Lung FB    | Decreased expression of C8ORF4 may contribute to the decreased capacity of fibrotic lung FBs to produce COX-2 and PEG2 | (47)             |
| KLF5         | Hypermethylation        | FB^c       | Downregulation of KLF5 and FLI1 works synergistically to enhance the expression of connective tissue growth factors | (48)             |
| DKK1, SFRP1  | Hypermethylation        | FB^b/Bech^abc | Decreased expression of DKK1 and SFRP1 results in aberrant Wnt signaling | (49)             |
| RORC1, RORC2 | Hypermethylation        | PBMCs^a    | Hypermethylation of RORC1 and RORC2 correlated with inflammatory marker and Scl-70+ | (50)             |
### Table 1. Summary of epigenetic modifications in SSc (Continue).

| II-Histone modification | III-miRNAs anomaly |
|-------------------------|---------------------|
| H3, H4 acetylation | mir-29 |
| H3, H4 hypoacetylation | Downregulation |
| FLI1 | FB |
| Reduction | Antifibrotic factor, putative target is type I collagen (24) |
| KLF5 | mir-let-7a |
| H3, H4 hypoacetylation | Downregulation |
| FRA2 | mir-196a |
| H3K27me3 inhibition | Downregulation |
| VEGF, FG2, DNMT1, DNMT3A, MECP2 | FB |
| H3K27me3 inhibition | Inhibition of H3K27me3 results in downregulation of pro-angiogenic genes and genes involved in DNA methylation (24) |
| TGF-β | mir-150 |
| Unclear | Downregulation |
| Global H4 acetylation, H3K methylation | FB |
| WIF1 | mir-129-5p |
| Histone hypoacetylation | Overexpression |
| Increased H4 acetylation, decreased H3K methylation | FB |
| COL1A2 | mir-145 |
| H4 hyperacetylation | Overexpression |
| shafts | mir-146 |
| miR-152 | Overexpression |
| Downregulation | FB |
| All cell types are human in origin, unless otherwise specified. diffuse SSc. limited SSc. ACTA: actin, alpha 2, smooth muscle, aorta; BMPRII: bone morphogenetic protein type II receptor; CDX-2: prostaglandin-endoperoxide synthase 2; DNA: deoxyribonucleic acid; DNMT1: DNA (cytosine-5-) -methyltransferase 1; ECM: extracellular matrix; FLI1: Friend leukemia integration 1; FB: fibroblast; H3K27me3: trimethylation of histone H3 on lysine 27; HDACs: histone deacetylases; IgG: immunoglobulin G; MBD1: methyl-CpG-binding domain protein 1; MeCP2: methyl-CpG-binding protein 2; MMP: matrix metalloproteinase; PBMC: peripheral blood mononuclear cell; RNA: ribonucleic acid; RORC: RAR-related orphan receptor C; SMAD: intracellular proteins that transduce extracellular signals from TGF-β ligands; SSc: systemic sclerosis; TGF-β: transforming growth factor beta; Tregs: regulatory T cells. |
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...and its gene promoter region was found to be hypomethylated (44).

Other examples of hypomethylated genes include ACTA (45), CTNNN3, CTNNND2, COL1A1, COL6A3, COL12A1, PDGF-C, TNXB, Pax9, ADAM12, and ITGA9 (46). Examples of hypomethylated genes are C8ORF4 (47), KLFS (48), SOX20 (46), DKK1, SFRP1 (49), and RORC1 and RORC2 (50). Table 1 summarizes the biological consequences of these modifications.

It is interesting to note hyper- and hypo-methylation patterns in different cells that are likely to contribute to SSC pathology. The epigenetic modification differs depending on the cell type (hypermethylation in FBs and MVECs and hyper- and hypo-methylation in CD4+ T cells). Mapping all the patterns of epigenetic modifications in cells is essential to completely understand the role of DNA methylation in the pathogenesis of SSC.

Histone modification

Histones are an essential part of the eukaryotic nucleosomes and are the key building blocks of chromatin (16). There are five different types of histones, which are divided into two main groups: core histones (H2A, H2B, H3, and H4) and linker histones (H1 and H5) (9). Post-translational modifications of histones occur on their N-terminal domains. These modifications include methylation, acetylation, phosphorylation, citrullination, ubiquitination, and sumoylation (2). The most studied modifications are histone acetylation and methylation. Histone acetylation results from the transfer of an acetyl group from acetyl coenzyme A to the histones and the negatively charged DNA, as class 3 HDAC (4). Histone acetylation relaxes the chromatin structure by reducing the interaction between the positively charged histones and the negatively charged DNA, as acetylation removes the positive charge on histones. Thus, it allows transcription factors to gain access to the promoter region and initiate transcription activation (22). On the contrary, histone deacetylation represses transcription. The general acetylation state of histones is based on the balance between HATs and HDACs (19). Histone deacetylation can be catalyzed by HDACs such as HDAC1, 2, and 4; HDACs; and sirtuins such as SIRT1-7, which is also known as class 3 HDAC (4). Histone acetylation relaxes the chromatin structure by reducing the interaction between the positively charged histones and the negatively charged DNA, as acetylation removes the positive charge on histones. Thus, it allows transcription factors to gain access to the promoter region and initiate transcription activation (22). On the contrary, histone deacetylation represses transcription. The general acetylation state of histones is based on the balance between HATs and HDACs (19). Histone acetylation can be catalyzed by HDACs such as HDAC1, 2, and 4. Histone acetylation increases gene expression, whereas that of H3K9 and H3K27 induces gene repression (3, 4, 19). It is important to recognize that DNA methylation and histone modification are linked (24). Accordingly, when MBD proteins bind methylated cytosines, they recruit HDACs, resulting in heterochromatin conformation that inhibits the transcription machinery (1, 3, 54).

The role of histone methyltransferase, enhancer of zeste homolog 2 (EZH2), was recently studied in SSC FBs and endothelial cells (55). EZH2 catalyzes the trimethylation of histone H3 lysine 27 (H3K27me3) to repress transcription. This enzyme has a role in T cell differentiation, endothelial cell angiogenesis (56), and myofibroblast transformation. The levels of expression of EZH2 and H3K27me3 are elevated in SSC FBs when compared with control cells. Inhibition of EZH2 by DZNep decreases fibrosis both in vitro and in vivo. DZNep decreases the expression of EZH2, H3K27me3, COL1A1, TGFβ, FRA2, and LRRN16A in a dose-dependent manner. Similarly, DZNep decreases the expression of DNMT1, DNMT3A, and MECPP2, resulting in reduced DNA methylation. In addition, DZNep and GSK126 (another EZH2 inhibitor) prevent bleomycin-induced skin fibrosis. Exposure of SSC FBs to GSK126 results in decreased matrix gel contraction, indicating decreased myofibroblast contractility. The effect of overexpression of EZH2 in normal FBs was analyzed using a wound closure model. The results showed that the overexpression of EZH2 resulted in increased wound closure, supporting a role of EZH2 in myofibroblast contraction. Migration of FBs was negatively affected when EZH2 was overexpressed in LRRN16A (a gene encoding cell membrane cytoskeleton-associated protein) knockdown FBs, indicating a significant role of LRRN16A in EZH2-mediated FB migration.

Similarly, the collagen suppressor gene FLII was found to have more acetylated H3 and H4 and more methylated regions in its promoter region as compared with controls. The addition of a HDAC inhibitor (trichostatin A, TSA) and a DNA methyltransferase inhibitor normalized the expression of type I collagen in SSC FBs (24). Moreover, TSA can reduce TGF-β-induced FB activation by decreasing the nuclear translocation of SMAD3/SMAD4 and DNA binding of SMAD transcription factors (57). As TSA is a broad deacetylase inhibitor, its clinical use is limited by its safety profile; therefore, more specific HDAC inhibitors are required for clinical use. Specifically inhibiting HDAC7 using small interfering RNA resulted in decreased TGF-β-induced accumulation of type I and type III collagen (58). Another HDAC inhibitor is suberoylanilide hydroxamic acid (SAHA), which
was found to prevent TGF-β-induced collagen deposition and FB activation (59).

Another important profibrotic factor is the HAT p300 that is regulated by SIRT1 (60). P300 modifies transcription factors affecting the regulatory region of the collagen gene. Levels of SIRT1 are significantly decreased in SSc dermal FBs compared with controls. A SIRT1 activator resulted in decreased response of FBs to TGF-β stimulation and reduced collagen production (61). However, another study revealed opposite effects of the SIRT1 activator on FB response (62); more studies are required to clarify the effects of SIRT1.

The overexpression of EZH2 in SSc endothelial cells affected cell adhesion and migration (63-65). The knockdown of EZH2 in SSc endothelial cells significantly increased angiogenesis, which is similar to the effect of the addition of DZNep to cell cultures. The treatment with DZNep upregulated the expression of notch ligands JAG1, JAG2, DLL4, notch receptor NOTCH2, and notch target gene HES1, whereas it downregulated the expression of notch signaling inhibitors NOTCH1, NOTCH3, NUMB, and FBXW7. These results suggest that EZH2 activates certain genes and inhibits others. When compared with normal endothelial cells, SSc endothelial cells showed increased levels of JAG2 and NUMB, and decreased levels of DLL4, HES1 and HEY2. Moreover, it was found that EZH2 inhibited SSc endothelial cells tube formation by repressing the notch ligand DLL4 through increased binding of EZH2 and H3K23me3 at the promoter region of DLL4 (55). The effect on tube formation was reversed when endothelial cells were treated with an EZH2 inhibitor.

Histone modifications such as increased H4 acetylation and decreased H3K methylation were associated with activating B cell genes that are responsible for the production of antibodies (66).

Histone deacetylases regulate the proliferation and migration of endothelial cells. The expression of HDACs, an antiangiogenic factor, is significantly increased in SSc MVECs, and it may play a significant role in SSc vasculopathy. Vascular damage in SSc is an early event that occurs before the onset of tissue fibrosis (67). The proposed mechanism for HDACs in inhibiting angiogenesis is that HDAC5 represses pro-angiogenic genes. The pro-angiogenic genes identified after HDAC5 was knockdown were FGFR2, SLIT2, EPHB4, PVR2L (cell adhesion molecule that improves angiogenic ability of MVECs), FSTL1 (plays a role in fibrosis and MVEC proliferation and tube formation), and CYR61 (a member of the CCN protein family that supports angiogenesis). Moreover, knockdown of HDACs increased the levels of bFGF, which is encoded by FGFR2 and increased the expression of FSTL1. Although these observations are interesting, the results infer limited potential clinical utility of HDAC inhibitors as antiangiogenic therapy because of their detrimental effects on MVECs that could potentially contribute to SSc vasculopathy. The ideal HDAC inhibitors as a potential therapeutic agent must have a specific target profile with no effects on multiple genes and multiple processes in different cell types.

Other histone modifications such as histone hypoacetylation in WIF1 (68), H3 and H4 hyperacetylation in NR4A1 (69), and H4 hyperacetylation in COL1A2 (60) were observed. Table 1 summarizes the biological consequences.

Non-coding RNA mechanisms

Non-coding RNAs (ncRNAs) are functional RNA molecules that are transcribed from DNA but not translated into proteins. These RNA molecules are biologically active and can affect gene expression, epigenetic modulation, and post-translational modification throughout the body (4, 70). The ncRNA molecules are divided into different groups based on the number of nucleotides: long ncRNAs (lncRNAs) that have more than 200 nucleotides and can be present in both the nucleus and the cytoplasm; medium-sized ncRNAs (<200 nucleotides) that include small nucleolar RNAs ( snoRNAs) and promoter-associated small RNAs (PASRs); and small ncRNAs (<50 nucleotides) that include miRNAs and PIWI-interacting RNAs (piRNAs) (4).

MicroRNAs are a group of small non-coding RNAs, ranging from 18 to 22 nucleotides that are synthesized initially as a longer precursor, which is degraded to miRNAs (71). miRNAs are involved in post-transcriptional silencing and regulation of gene expression (23, 72, 73) by binding to the complementary sequence in the 3’ prime region of the mRNA, resulting in translational repression or mRNA degradation (74-76). Therefore, the upregulation of miRNAs results in gene repression, whereas their downregulation results in gene activation. The expression of miRNAs is regulated through epigenetic mechanisms; for example, miRNAs can be silenced by DNA methylation. miRNAs differ from siRNAs in that siRNAs target a single gene, whereas miRNAs can target multiple genes (77, 78). The involvement of miRNAs in tissue fibrosis was initially reported in cardiac fibrosis after myocardial infarction. It was found that the expression of the miR-29 family decreased in the cardiac cells adjacent to the infarct area (79).

As miR-29 regulates fibrosis-related genes, its downregulation resulted in tissue fibrosis (9).

In 2010, the first study focusing on miRNA levels in SSc dermal FBs found that miR-29a was downregulated. The same finding was observed in bleomycin-induced skin fibrosis (80). Interestingly, downregulating miR-29a in normal dermal FBs increased the formation of collagen types I and III, and overexpressing it in SSc FBs decreased the expression of collagen. Furthermore, miR-29a plays an important role in liver (81) and kidney fibrosis (82). Profibrotic cytokines, such as TGF-β1 and IL-4, decrease the levels of miR-29a (9). Further analysis of miRNAs showed that in SSc skin, 9 miRNAs were upregulated and 15 miRNAs were downregulated. Of these, the expression of miR-206, miR-125b, and let-7g was confirmed by real-time polymerase chain reaction (PCR). As miR-125b functions as a regulator of multiple molecules involved in SSc pathology, including SMAD5, interleukin (IL)-1F10, IL-6R, and IL-13, its downregulation results in increased levels of these molecules. Moreover, the expression of multiple collagen-related miRNAs is decreased in SSc FBs and TGF-β-stimulated normal dermal FBs (83). Alpha 1 and 2 type I collagen are regulated by miR-196a (84) and let-7a, the expression of both is reduced in SSc FBs, both in vivo and in vitro (9). Transfection by miR-196a and let-7a inhibitors resulted in increased expression of α1 and α2 type I collagens, whereas transfection with their mimics resulted in decreased expression. It is suggested that the activation of TGF-β in SSc dermal FBs results in miR-196a and let-7a downregulation, which in turn upregulates the expression of collagen. Interestingly, levels of miR-196a decreased in shafts of hairs obtained from patients with SSc (85).

Another miRNA with an important role in SSc is miR-150 that is underexpressed in SSc (86). miR-150 is a regulator of integrin-β3, a known inducer of TGF-β. Interestingly, the overexpression of miR-150 in SSc dermal FBs resulted in decreased integrin-β3, phosphorylated SMAD3, and type I collagen deposition. Knocking down miR-150 resulted in opposite changes.

IL-17A is known to have antifibrogenic effects; it stimulates the overexpression of miR-129-5p, which in turn downregulates the production of α1 type I collagen. IL-17 receptor is downregulated in SSc FBs in association with decreased expression of miR-129-5p and overproduction of α1 type I collagen (87).

Circulating levels of miRNAs are proposed as sensitive biomarkers of disease activity, as changes in miRNAs appear earlier than those in...
levels were associated with higher mRSS scores. Similarly, it was found that lower serum let-7a (mRSS), indicating more extensive skin fibrosis, was associated with higher modified Rodnan skin score (mRSS). Lower serum miR-196a levels in SSc were associated with higher in vivo collagen production, resulting in tissue/organ fibrosis; and endothelial cell injury and vascular dysfunction. The figure was created using BioRender.com.

Figure 1. An overview of the effect of epigenetics on immune cells (T and B cells), fibroblasts, and endothelial cells that contribute to the pathogenesis of SSC. Histone acetylation switches chromatin configuration from condensed to relaxed, permitting the transcription machinery to access the DNA to initiate transcription. This process is catalyzed by histone acetyltransferases and reversed by histone deacetylases. DNA methylation results in transcription repression, as the addition of methyl groups to DNA prevents transcription factors from accessing the DNA. This process is catalyzed by DNA methyltransferases and reversed by DNA demethylases. Inhibition of gene expression by miRNAs through translational repression and degradation of mRNA. MiRNAs can upregulate profibrotic molecules or downregulate antifibrotic molecules. The results of epigenetic alterations in SSc are the activation of the immune system leading to autoimmunity; increased collagen and ECM production resulting in tissue/organ fibrosis; and endothelial cell injury and vascular dysfunction. The figure was created using BioRender.com.

proteins. However, miRNAs are unstable when present extracellularly; they are rapidly degraded by RNases despite multiple proposed conditions to preserve their extracellular stability. Nonetheless, levels of few miRNAs are lower in SSc than in controls, and those of others are the same. However, the rank order is different, indicating different expression patterns. An example of different rank order is the levels of miR-7g, miR-21, miR-29b, miR-125, miR-145, and miR-206 between SSc and controls (71, 80, 88, 89).

MiR-196a was measured by PCR in cultured skin FBs and sera from patients with SSc and controls. Levels of miR-196a were significantly lower in SSc FBs compared with control FBs. However, there were no significant differences in the levels in the serum. Intriguingly, the lower serum miR-196a levels in SSc were associated with higher modified Rodnan skin score (mRSS), indicating more extensive skin fibrosis. Similarly, it was found that lower serum let-7a levels were associated with higher mRSS scores.

Furthermore, it was found that patients with lower let-7a levels had a lower frequency of antitumorigenic antibodies, proposing a potential role for let-7a in regulating the immune system (9). Lower miR-30b serum levels were also associated with higher mRSS scores. Of note, miR-30b is a negative regulator of platelet-derived growth factor beta (PDGFB), and its lower levels may increase the levels of PDGFB (90).

Levels of other miRNAs vary depending on the disease phenotype; for example, miR-7 was found to be overexpressed in SSc FBs both in vivo and in vitro (91), whereas it was found to be downregulated in lcSSc FBs in vivo (92).

The use of bortezomib, a proteasome inhibitor that downregulates miR-21 (profibrotic mRNA that is upregulated in SSc dermal FBs), blocked TGF-β-induced fibrosis in an SSc animal model (93). Moreover, the topical application of miR-155 antagonist decreased the production of collagen in a mouse model (94).

The potential use of miRNAs in SSc therapy is emerging but is still in the experimental stage. A therapeutic role for let-7a in bleomycin-induced skin fibrosis has been shown. The intraperitoneal injection of let-7a combined with atelocollagen, for the protection of let-7a from in vivo degradation by RNases, resulted in the overexpression of let-7a in the skin with a concomitant decrease in collagen production (83).

Other examples of overexpressed miRNAs in SSc include miR-142 (95), miR-92a (96), and miR-483-5p (97). An underexpressed miRNA is miR-152 (98), Table 1 summarizes their biological consequences.

The future of miRNAs as a therapeutic option for SSc is promising. However, this approach needs to overcome several obstacles. The most troubling is the potential for miRNAs to alter the function of several genes that may result in undesirable outcomes. Moreover, appropriate dosing and the method of delivery are other obstacles in this emerging field.

Conclusion
In this review, we provided evidence for a key role of epigenetic regulation in the pathogenesis of SSc involving disparities in DNA methylation, anomalies in the histone code, and altered expression of miRNAs in different tissues and cell types (Figure 1). Although it is likely that environmental cues trigger epigenetic regulatory mechanisms, this needs to be confirmed in detail, possibly in a longitudinal cohort study starting with epigenetic profiling of individuals at risk of developing SSc and repeating the epigenetic profile for those who develop the disease. This would provide a better understanding of how environmental stimuli interact and trigger the epigenetic regulatory mechanisms. Furthermore, this approach will provide us a better understanding of whether these epigenetic variations among individuals are a cause or a result of the disease process. In addition, we should develop an experimental model of SSc that we can use for further analysis to obtain an epigenetic map for each cell type involved in the disease process, including endothelial cells, T cells, and FBs. A huge collaborative effort, similar to genome-wide association studies, is required to reveal the epigenetic map. With the ever-expanding discoveries of epigenetic targets, understanding the epigenetic basis of SSc is important for finding potential therapeutics. It is possible that in the near future, epigenetic research may lead to the development of epigenomic tools that can both uncover the risk and offer effective therapeutic options.
The authors have no conflict of interest to declare.
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