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# Role of microRNA in the pathogenesis of systemic sclerosis tissue fibrosis and vasculopathy

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ABSTRACT

Systemic Sclerosis (SSc) pathogenesis involves multiple immunological, vascular and fibroproliferative abnormalities that contribute to a severe and complex clinical picture. Vasculopathy and fibroproliferative alterations are two hallmark pathological processes in SSc that are responsible for the most severe clinical manifestations of the disease and determine its clinical outcome and mortality. However, the pathogenesis of SSc vasculopathy and of the uncontrolled SSc fibrotic process remain incompletely understood. Recent investigations into the molecular pathways involved in these processes have identified an important role for epigenetic processes that contribute to overall disease progression and have emphasized microRNAs (miRNAs) as crucial epigenetic regulators. MiRNAs hold unique potential for elucidating SSc pathogenesis, improving diagnosis and developing effective targeted therapies for the disease. This review examines the important role that miRNAs play in the development and regulation of vascular and fibroproliferative alterations associated with SSc pathogenesis and their possible participation in the establishment of pathogenetic connections between these two processes. This review also emphasizes that further understanding of the involvement of miRNA in SSc fibrosis and vasculopathy will very likely provide novel future research directions and allow for the identification of groundbreaking therapeutic interventions within these processes. MiR-21, miR- 31, and miR-155 are of particular interest owing to their important involvement in both SSc vasculopathy and fibroproliferative alterations.

## 1. Introduction

Systemic Sclerosis (SSc) is a heterogeneous systemic autoimmune disease of unknown etiology displaying a variety of clinical presentations. The most severe clinical and pathologic manifestations of the disease result from an uncontrolled fibroproliferative vasculopathy and from the exaggerated and disorderly accumulation of fibrotic tissue in the skin, microvasculature and various internal organs [1–4]. These processes are accompanied by the occurrence of innate, humoral, and cellular immunologic alterations, creating the full clinical disease picture [5–8]. SSc pathogenesis is highly complex, and the exact mechanisms involved remain poorly understood. It has become apparent, however, that the development of the severe and often progressive systemic fibroproliferative process characteristic of the disease, is a crucial mechanism in SSc pathogenesis. Extensive studies have shown that this intricate process involves alterations in numerous cellular processes, molecular mediators, and growth factors occurring within a host with a genetically permissive background [9–12].

It has recently been proposed that SSc, in its essence, is a vascular disease with an initial endothelial insult preceding the hallmark fibroproliferative change and intertwining immunological abnormalities [13]. Multiple pathways have been implicated in triggering the widespread endothelial cell dysregulation that underlies early SSc pathogenesis [14–18]. However, the exact relationship between endothelial dysregulation, immunologic dysfunction, and the fibroproliferative alterations remains undisclosed. Recent investigations have been performed to connect these disease processes in an attempt to fully understand SSc pathogenesis and develop effective therapies.

Numerous mechanistic connections between SSc fibrosis, autoimmunity, and vasculopathy have been proposed, including possible roles for adipokines and platelet activation [18,19]. Recent efforts have also been focused on exploring the impact of epigenetic factors,

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particularly microRNAs (miRNAs) and other non-coding genetic elements, on SSc pathogenesis [20–32]. Besides expanding current frameworks of the molecular mechanisms and regulatory alterations in SSc, miRNAs also hold potential as novel therapeutic targets that may be less likely to induce undesirable collateral side effects and may offer a more tailored approach to SSc treatment. This is a distinct advantage that miRNAs may hold over more traditional targets such as cellular signaling molecules or cytokines. The concept became very apparent in a clinical trial of a TGF-β inhibitory antibody that had to be stopped as a result of severe and unexpected side effects [33]. As evidenced by this trial, without a complete understanding of the mechanisms that drive growth factor and cytokine expression alterations and signaling pathway abnormalities in SSc, it becomes highly challenging to use them as therapeutic targets. However, if the underlying mechanisms leading to the fibrotic processes including TGF-β dysfunction in SSc are precisely understood, therapies can be more specifically targeted [34]. MiRNAs provide a possible route to elucidate those underlying mechanisms and target them therapeutically. The specificity of identifying and targeting the miRNA interactions involved in the pathogenesis of numerous diseases, including multiple malignant disorders, has already led to promising therapeutic advances in other pathological processes [35]. Thus, connecting the major components of SSc disease pathophysiology through miRNAs could provide therapeutic targets with greater specificity for the underlying pathogenesis as a whole, rather than narrowly focused on its individual components. This approach has gained substantial results in the treatment of other diseases, including multiple types of cancer [36].

Although existing literature has extensively reviewed the role of miRNAs in SSc fibroproliferative and immunologic alterations, less is presently reported on how miRNAs contribute to SSc vasculopathy. In terms of our present understanding, these components of SSc pathogenesis exist as separate entities, and miRNAs have been studied primarily within the individual processes. Fully elucidating the intertwining functions of miRNA between the vasculopathy and tissue fibrosis pathways could improve our understanding and provide groundbreaking information about SSc pathogenesis that can then be used to develop novel diagnostic and therapeutic approaches to the disease.

The purpose of this review is to explore the role of miRNA in the pathogenesis of tissue fibrosis and vasculopathy in SSc and to identify potential mechanistic connections between these two crucial disease components that could hold both diagnostic and therapeutic value. We describe and emphasize here the most promising mechanistic connections between SSc fibroproliferation and vasculopathy, and explore in great depth the role of miRNA in the development of these two crucial SSc components. Owing to the focus of our review on these SSc components, we have not included the extensive pre-existing scientific literature regarding miRNA involvement in the SSc immunologic abnormalities.

## 2. Role of microRNA in SSc pathogenesis

### 2.1. MicroRNA gene expression regulatory effects

MiRNAs are small (~22 nucleotides), evolutionarily conserved non-coding RNA which play important roles regulating the expression of protein-coding genes at the post-transcriptional level [37,38]. The mechanisms involved are highly complex and require the sequence-specific complementary binding to the 3′ or 5′ untranslated region (UTR) of target mRNAs leading to either mRNA translation inhibition or facilitating mRNA degradation [38–43]. Extensive studies have demonstrated that an individual miRNA may regulate the expression levels of more than one target mRNA and may have regulatory functions on gene expression for numerous physiological processes involving growth, differentiation, immunity, and metabolism [37,42,44–48]. The role of miRNA has also been established in the development of many disease processes including cancer, fibrosis, and autoimmunity [49–60].

### 2.2. MicroRNA biogenesis

The biogenesis of miRNA is quite complex and is highly regulated in a cell-specific and context-dependent process [37,42,61]. MiRNA genes are transcribed predominantly by RNA Polymerase II, producing primary miRNA (Pri-miRNA). Following transcription, Pri-miRNA is cleaved by a microprocessor complex comprised of the intranuclear RNAse enzyme, Drosha and the RNA-binding protein encoded by DiGeorge syndrome chromosomal region 8 (DGCR8). The DGCR8 protein forms a complex with Drosha required to initiate cleavage from Pri-miRNA to Pre-miRNA in the nucleus. The Pre-miRNA is secreted from the nucleus through Exportin 5, which is required for Pre-miRNA transport to the cytoplasm. In the cytoplasm, pre-miRNA is cleaved by the cytoplasmic RNAse Dicer in association with the transactivating response RNA binding protein (TRBP) to yield miRNA duplex [37,42,61]. The miRNA duplex can then bind an Argonaute (Ago) protein to form a mature RNA-induced silencing complex known as RISC. RISC assembly includes two important steps. The first step, known as wedging, causes separation of the two strands of the miRNA duplex, and is mediated by Ago. The second step is the elimination of the passenger strain, which is discharged from Ago. The mature RISC induces repression of the specific mRNA target [62]. The steps involved in miRNA biogenesis, RISC assembly and downregulatory effects on target mRNAs are diagrammatically illustrated in Fig. 1.

Regulation of miRNA expression occurs at multiple levels, including gene transcription, with the miRNA gene promoter utilizing a mRNA-similar structure to control RNA Pol II activity [63], as well as post-transcriptionally. This is a highly complex process involving numerous transcriptional, post-transcriptional and processing regulators. Following transcription and processing, miRNA activity can be modified by direct inhibition as well as by end-target modifications that prevent miRNA binding to its corresponding mRNA targets [64].

The half-life of an individual miRNA may be as long as 5+ days although certain miRNA subsets degrade rapidly [65]. Multiple miRNA degradation pathways have been identified, and they also vary based on unique miRNA stability characteristics that are controlled by several mechanisms including adenylation, uridylation, microvesicle packaging, and interactions with various binding proteins [42,66]. Recent investigations have demonstrated that following their biogenesis, miRNAs are not confined to remain intracellularly, but they can be packaged, secreted, and transported within exosomal and other microvesicles to exert paracrine effects on neighboring and distant cellular targets [67]. This ability may be a crucial mechanism mediating the extension and propagation of SSc alterations to non-affected tissues.

### 2.3. MicroRNA and SSc vascular alterations

The effect of miRNA-dependent gene expression modulation on endothelial cell (EC) functions has been extensively studied and specific miRNAs that play important regulatory functions within numerous physiologic and disease-related vascular processes have been identified [68]. The most relevant molecular pathways suggested to play a role in the EC alterations in SSc and in the development and progression of SSc vasculopathy will be briefly reviewed in the following sections with an emphasis on the role of miRNAs involved.

### 2.4. MicroRNA regulation of Nitric Oxide (NO) release

There has been intense interest in the study of NO and of endothelial NO synthase (eNOS) in the pathogenesis of SSc vascular alterations, and it has been suggested that ECs in SSc have an intrinsic defect in NO production, likely mediated by decreased eNOS activity. The relevant role of eNOS in SSc pathogenesis was documented in studies

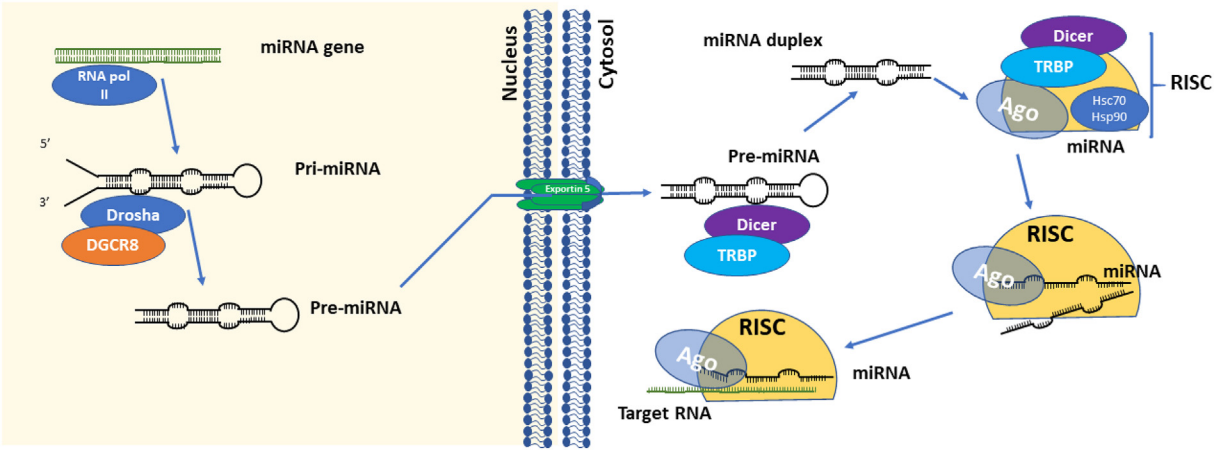


**Fig. 1.** Canonical pathway of miRNA processing. MiRNA genes are predominantly transcribed by RNA Polymerase II to yield Pri-miRNA. Pri-miRNA is cleaved by a DGCR8- Drosha complex within the nucleus to yield Pre-miRNA. Pre-miRNA is then transported across the nuclear membrane via Exportin 5. Pre-miRNA is cleaved in the cytoplasm by Dicer, forming a miRNA duplex. A complex termed RNA-induced silencing complex (RISC) formed by Argonaut protein (Ago), Transactivating response RNA binding protein (TRBP), Dicer, and HSC70/HS90 disassembles the miRNA duplex and mediates sequence-specific target mRNA silencing, stabilization, or degradation.

demonstrating lower eNOS mRNA levels in SSc dermal microvascular ECs (MVECs) compared to normal MVECs [69]. These studies showed that NO release was decreased in the SSc MVECs highlighting eNOS pathway dysfunction as a potential early contributor to SSc vasculopathy [69]. In a related study on the miRNA regulation of eNOS, Suarez et al. found that the loss of Dicer-dependent miRNA did not induce a change in eNOS mRNA levels, however, it resulted in increased eNOS protein levels and NO release [70]. Differences between mRNA and protein levels in these studies suggest that miRNA involvement in the eNOS pathway occurs at translational and/or post-translational levels. However, the role of miRNA in these regulatory mechanisms is highly complex and miRNA effects alone would not explain the decreased eNOS mRNA levels observed in SSc MVECs [69]. Indeed, it has been proposed that pre-translational alterations in the eNOS pathway at the level of DNA methylation may occur in SSc based on several studies that have shown that DNA methyltransferase 1 (DNMT1) interacts with miR-152 within the eNOS pathway. This mechanism's potential within SSc pathogenesis has been discussed in a recent review by Altorok et al. describing studies that demonstrated miR-152 downregulation in SSc MVECs and the resulting increase in DNMT1, as well as miR-152 driven hypermethylation of the *NOS3* gene encoding eNOS in SSc [71].

### 2.5. MicroRNA regulation of Wnt signaling

Wnt signaling plays crucial roles during embryonic development, in the maintenance of normal vascular and EC functions, and is altered in numerous diseases [72,73]. In the context of SSc, most investigations have focused on the profibrotic end-effects of Wnt/β-catenin signaling in fibroblasts [74–77]. Although excessive fibroblast signaling through this pathway contributes to the fibrotic pathology [74–77], it has been clearly demonstrated that Wnt/β-catenin pathway activity is required in ECs for proper functioning and regulation [78,79]. A downregulation of Wnt signaling in ECs could actually contribute to, if not trigger the pathogenesis of SSc vascular alterations [78,79]. Numerous miRNAs have been identified as potentially connected to Wnt- mediated endothelial dysfunction in SSc. A study by Zhou et al. showed that culture of human MVECs with serum from SSc patients resulted in altered expression of a specific group of miRNAs related to Toll-like receptor (TLR) signaling, TGF-β pathway activation, and Wnt signaling [80]. These alterations included overexpression of miR-146b, 130b, 21, 31 and 34a, and underexpression of miR-145 [80]. MiR-34a is an identified suppressor of canonical Wnt signaling [81], and its overexpression has also been associated with EC senescence [82]. A related study compared miR-34a levels in patients with primary Raynaud's Phenomenon, SSc, and healthy controls [83]. The results demonstrated that miR-34a levels were elevated only in samples from SSc patients. Furthermore, miR-34a levels correlated with vascular manifestations such as digital ulcers [83]. Given that the presence of digital ulcers in SSc patients is associated with an increased risk of mortality [84], miR-34a levels may be a potential marker for SSc severity and mortality prognosis. Abnormal miR-34a expression is not specific to SSc, however, if further investigations demonstrate that its alterations are shown to occur in the context of SSc epigenetic dysregulation, then miR-34a could become a target for novel SSc diagnostic and therapeutic approaches.

Another miRNA linked to Wnt signaling, various EC functions, and immunologic and inflammatory processes related to SSc pathogenesis is the multifunctional miR-155. MiR-155 has been found to be overexpressed in the serum of SSc patients compared to healthy controls [83], and in affected skin of SSc patients [85]. Other studies have confirmed the elevated levels of miR-155 in serum [86], and in fibroblasts from SSc patients [87]. The study by Yan et al. suggested that the effect of miR-155 in SSc might be independent of vasculopathy, since miR- 155 was shown to activate Wnt/β-catenin signaling in fibroblasts [85]. Considering the role of Wnt/β-catenin signaling in ECs, this finding is perhaps not surprising and further supports the specific, contrasting complexities of miRNA influence on ECs and fibroblasts in SSc. MiR-155 is also an important target in understanding the fibrotic processes of SSc that will be discussed in a subsequent section of this review.

### 2.6. MicroRNA effects on Plasminogen Activation (PA)

Another pathway of importance to SSc vasculopathy involves the urokinase-type plasminogen activator (uPA) and its receptor (uPAR). Under physiological conditions, activated uPA proteolysis is responsible for many integral cellular functions related to vascular growth, development and remodeling [88–90]. Based on its defined function, a compromised uPA-uPAR pathway can seriously impair angiogenic and vascular repair processes with devastating effects in SSc [91]. D'Alessio et al. linked uPA-uPAR pathway dysfunction to SSc vasculopathy [92]. They demonstrated overexpressed matrix metalloproteinase 12 (MMP-12) and the occurrence of MMP-12-dependent cleavage of uPAR in SSc MVECs. They further showed that treatment of normal MVECs with culture medium from MVECs from SSc patients impaired uPA-uPAR dependent cellular proliferation, a defect that was corrected by anti-

MMP-12 antibodies [92]. Besides the MMP-12-mediated cleavage of uPAR, a pathologic change to uPA has also been identified in SSc MVECs as shown more recently by Iwamoto et al., who found that miR-193b was downregulated in skin biopsies and dermal fibroblasts from SSc patients and that its downregulated levels caused a concurrent upregulation of uPA [93]. The results from these studies established two concurrent pathological changes to the uPA-uPAR pathway contributing to vasculopathy in SSc. The decrease in uPAR induces apoptosis and impairs angiogenesis whereas the increase in uPA inhibits apoptosis and stimulates smooth muscle proliferation [93,93]. The identification of miR-193b in this process [93], indicates that this miRNA may serve as an important target for therapeutic intervention for the vascular alterations in SSc.

### 2.7. MicroRNA regulation of endothelin-1 production

Endothelin-1 (ET-1) is an endogenous vasoconstrictor polypeptide that acts on one of two endogenous receptors: ETA and ETB [94]. ET-1 is implicated in vascular proliferation and fibrosis and has been shown to be elevated in the serum of SSc patients [95–97]. Furthermore, elevated ET-1 levels and differential expression of ETA and ETB appear to be associated with SSc pathogenesis and the highly frequent occurrence of pulmonary arterial hypertension (PAH) in SSc [98–101]. Recent studies have examined the regulatory role of miRNAs on the molecular mechanisms of ET-1 signaling. Kang et al. identified miR-98 as an inhibitor of ET-1 and showed that a decrease in miR-98 increased expression of ET-1 [102]. They also found that PPARγ and miR-98 are intimately involved in the regulation of ET-1 expression in pulmonary artery EC since EC-targeted PPARγ knockout displayed a marked miR-98 reduction causing a marked increase in ET-1 levels in these cells [102]. A related study showed that ET-1 stimulates miR- 27a/b expression which triggers a reduction in PPARγ, resulting in the subsequent proliferation of vascular smooth muscle cells, thus completing a positive feedback loop towards PAH development [103]. Similar observations by Bertero et al. identified a contributory role for the miR-130/301 families in PPARγ-driven pulmonary vascular proliferation and the subsequent development of PAH [104,105]. A second identified trigger of ET-1 expression is TGF-β, which closely associates ET-1 within SSc pathogenesis. Along these lines of investigation, Luo et al. used TGF-β to induce expression of miR-130b and demonstrated a subsequent downregulation of PPARγ [106]. Overexpression of miR-130b and reduced PPARγ levels were found in SSc skin biopsies [106]. With its intertwining functions in both vascular alterations and tissue fibrosis, ET-1 is a key contributor to SSc pathogenesis. Further understanding of miRNA-related modulation of ET-1 expression will certainly uncover novel therapeutic pathways for SSc fibrosis and vasculopathy including PAH.

### 2.8. MicroRNA effects on endothelial to mesenchymal transition

An important process recently considered in the endothelial triggering of SSc pathogenesis is endothelial to mesenchymal transition (EndoMT) [107,108]. The complete elucidation of the molecular mechanisms regulating EndoMT still requires further investigation, however, TGF- β and canonical Wnt signaling have both been suggested as important regulators. As acknowledged previously, TGF-β stimulation of Wnt signaling is responsible for fibroblast activation, and this connection implicates increased Wnt signaling in fibroproliferative change [109,110]. A contrasting process occurs in ECs, with an inhibition of Wnt signaling potentially contributing to endothelial senescence and SSc pathogenesis as demonstrated by Cheng et al. who induced Dickkopf-related protein 1 (Dkk-1) inhibition of Wnt signaling in aortic ECs and found that Wnt signaling inhibition enhances EndoMT in these cells [111]. Another signaling pathway associated with the development of vasculopathy in SSc is the interferon (IFN) pathway [112]. It was recently recognized that IFNs activate both the JAK/STAT and NF-κB pathways. These pathways both directly regulate the expression of miR-21, a promoter of EndoMT [113]. EndoMT has been studied in the setting of multiple fibrotic diseases, including cardiac fibrosis and pulmonary hypertension among others [114–116]. ECs isolated from SSc-affected lung tissue have been found to co-express both endothelial and mesenchymal cell markers, establishing the presence of EndoMT in SSc pathogenesis [117]. The contribution of EndoMT to SSc vasculopathy and overall pathogenesis has been previously reviewed and continues to be the subject of intense investigation [108].

MiRNA regulation of EndoMT could have future therapeutic potential, particularly related to TGF-β and Wnt signaling. Many miRNAs have been identified as stimulators and inhibitors of EndoMT [116,118], and the most relevant are discussed in greater detail in the "Connecting SSc Vasculopathy and Fibrosis Through miRNA" section.

The NOTCH signaling pathway has been identified in physiological cardiovascular development as well as pathway activation leading to pathological EndoMT [119]. The impact of NOTCH signaling on fibroblast activation in SSc has been studied but the possible effect on EndoMT is less established [120]. While low levels of miR-18a-5p have been shown to cause Notch2 induced EndoMT in aortic valve ECs, further research is necessary to determine the endothelial relevance of NOTCH signaling in SSc [121]. NOTCH signaling remains an important component of SSc fibrogenesis research and its potential utility as a therapeutic target has been previously identified [122].

### 2.9. MicroRNA and tissue fibrosis in SSc

Given the crucial role of fibrotic alterations in SSc pathophysiology and clinical manifestations, it has become important to determine whether the previously discussed miRNAs involved in SSc vasculopathy may also participate in the regulation of SSc fibrosis. Numerous miRNAs have been identified in SSc-associated fibrosis, displaying either profibrotic or antifibrotic effects, as well as modulating profibrotic gene expression mediated by TGF-β pathways [123–136]. Some of these pathways are diagrammatically shown in Fig. 2. Targets of the relevant miRNA have been identified at multiple junctions along the mechanistic path of SSc-associated fibroproliferation. The miRNA-29 family is of substantial relevance to SSc- pathogenesis, because these miRNAs regulate the expression of genes for various collagens and several fibrosis-related transcription factors. Indeed, it has been shown that miR-29a exhibits potent antifibrotic effects and is one of the more extensively studied miRNAs involved in SSc fibrosis [126,128]. Maurer et al. found that miR-29a was markedly down-regulated in SSc dermal fibroblasts and skin [128]. They also found that overexpression of miR-29a in SSc fibroblasts decreased mRNA and protein levels of type I and type III collagens, supporting a posttranscriptional effect for miR-29a. Furthermore, when TGF-β and PDGF-B pathways were inhibited in a bleomycin model of skin fibrosis, levels of miR-29a were restored [128]. Expanding on these findings, Gallant-Behm et al. recently reported the intriguing antifibrotic potential of a miR-29 mimic that was capable of preventing injury-induced cutaneous fibroplasia in normal human volunteers [137]. Further miR-29 antifibrotic effects have been demonstrated in lung, kidney, and cardiac fibrosis [138–140].

Several members of the let-7 family of miRNAs have been shown to be involved in the dysregulated fibrotic processes associated with SSc. Makino et al. identified the α1 and α2 type I collagen mRNAs as direct targets of miR-let-7a. It was further shown that miR-let-7a inhibition resulted in type I collagen upregulation and they concluded that miRNA-let-7a downregulation contributed to the abnormally increased expression of type I collagen in SSc. These investigators also measured miR-let-7a levels in serum from SSc patients and found that these levels were significantly decreased compared to levels in normal serum [141]. A relevant mechanistic study on breast cancer stem cell expansion demonstrated that Wnt/β-catenin signaling repressed let-7 miRNAs [142]. This finding is of particular relevance for SSc pathogenesis given that Wnt pathway hyperactivity in SSc fibroblasts is important for tissue

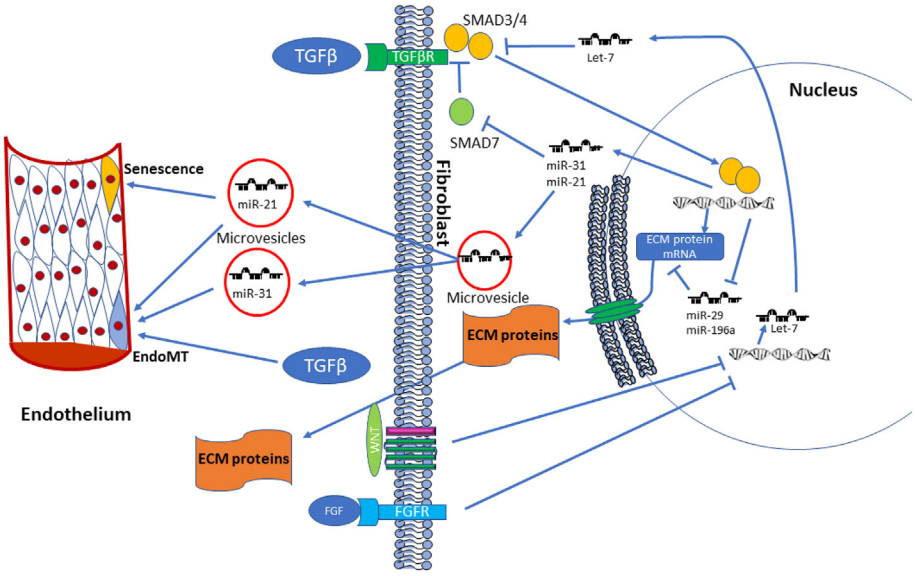


**Fig. 2.** MiRNA regulation of a proposed pathway connecting SSc vasculopathy and fibroproliferation. MiR-21 and miR-31 are involved in the triggering of EndoMT and EC senescence. TGF-β upregulates expression of miR-21, also creating a positive feedback loop on the system. Activation of TGF-β canonical pathways through SMAD3/4 in fibroblasts upregulates the expression of miR-21. MiR-21 inhibits SMAD7, a negative regulator of the SMAD3/4 pathway, promoting continued activation of canonical TGF- β pathways. Other negative regulators of SMAD pathways such as Let-7 can be inhibited by Wnt and FGFR activation. This results in an increased production of ECM proteins and phenotypic transformation of ECs into myofibroblasts. MicroRNAs can also reach the systemic circulation following their packaging into microvesicles and are able to exert both paracrine and distant effects in ECs and other target cells. MiR-21 exerts an important effect in endothelial cell senescence and along with miR-31 promotes Endo-MT.

fibrosis [143]. The role of let-7 miRNAs in tissue fibrosis was also examined in a murine model of cutaneous fibrosis. These studies showed that the intraperitoneal administration of miR-let-7a, to mice with bleomycin- induced skin fibrosis, improved the cutaneous fibrotic process supporting the use of miR-let-7a analogs as antifibrotic therapeutics [141]. A related study demonstrated that the profibrotic Fibroblast Growth Factor decreased the levels of let-7 miRNAs, leading to activation of TGF-β profibrotic pathways including the stimulation of TGF-β-driven EC conversion into myofibroblasts through EndoMT [144].

Given the pleiotropic and essential effects of TGF-β in numerous physiological and pathological processes there has been strong interest in the study of the role of miRNAs in the modulation of TGF-β effects. Two miRNAs that are closely related to the TGF-β signaling SMAD pathways are miR-21 and miR-145. MiR-21 downregulates the expression of the inhibitory antifibrotic SMAD7 and was found to be upregulated in SSc [127]. In contrast, the antifibrotic miR-145 inhibits SMAD3 and is downregulated in SSc [145]. Together, the upregulation of miR-21 and the downregulation of miR-145 promote fibrosis by a dual effect of upregulating profibrotic SMAD3 and downregulating antifibrotic SMAD7. Noncanonical TGF-β signaling pathways are also regulated by miRNAs, as recently demonstrated by Xia et al. who showed that miR-21 within microvesicles could induce myocardial fibrosis through AKT stimulation, another important TGF-β-related pathway [146].

There are additional miRNAs that have been implicated in SSc fibrosis, including miR- 483-5p, which was recently found to be upregulated and packaged within exosomal microvesicles in SSc serum [147]. Additional pathways and associated miRNAs involved in SSc fibrosis are discussed further in the subsequent section “Connecting SSc Vasculopathy and Fibrosis Through miRNA.”

### 2.10. Connecting SSc vasculopathy and fibrosis through miRNA

Numerous miRNAs have been individually studied in the setting of vascular/EC or fibroproliferative dysregulation in SSc, although the miRNA participating in both processes have not been studied as extensively. The effects of the miRNAs involved in both endothelial dysfunction and tissue fibrosis are of great interest when considering the overall SSc pathogenesis. However, as evidenced by studies on miR-155, many of these miRNAs do not neatly fit into either the EC senescence or the fibroproliferative components of SSc pathogenesis. Thus, an approach combining the existing evidence of the individual role of each miRNA and identifying their potentially cumulative or synergistic/antagonistic effects will allow the establishment of a strong pathogenic association between miRNA-dependent vasculopathy and fibroproliferative changes in early SSc development and progression. Thus, in this section, miRNA involved in both SSc vasculopathy and associated fibrotic alterations are discussed. As will be outlined, combining the existing evidence to develop an overall picture of miR-21, miR-31 and miR-155 functioning creates a theoretical association between miRNA-dependent vasculopathy and fibroproliferative alterations in early SSc progression.

MiR-155 is an identified inhibitor of EndoMT [148,149]. Bijkerk et al. found that miR-155 inhibits TGF-β-induced EndoMT via RhoA signaling modulation [149]. The interplay between miR-155 and early SSc vasculopathy is counterintuitive, as the elevated serum miR-155 levels in early SSc found by Alivernini et al. [83] would theoretically protect against EndoMT if interaction with ECs occurs. The findings of Yan et al., discussed previously, however, support a possible role of miR-155 linking vasculopathy and fibrosis [85]. To further elucidate this possible connection, it is important to first understand the roles of miR-21 and -31. Zhou et al. found that miR-21 and -31, which both stimulate EndoMT [150–153], are overexpressed in MVECs stimulated with SSc serum [80]. In the setting of atherosclerotic EC damage, Zuo et al. linked overexpression of miR-21 with decreased EC proliferative capacity and increased TGF-β signaling, which they identified as potentially responsible for the EC effects observed [154]. These findings, linking miR-21 with EC senescence, TGF-β signaling, and EndoMT, underscore its significance in SSc pathogenesis. MiR-31 has also been shown to stimulate EndoMT through a positive modulation of TGF-β signaling [150]. Considering miR-21, -31 and -155, the underlying question behind TGF-β-induced EndoMT and SSc pathogenesis is fundamentally a question of what process occurs first. Is EndoMT a result of fibrotic alterations or is there an initial insult that triggers EndoMT and subsequent/simultaneous fibrotic change? At least relative to miR-31, a study by Katsura et al. suggests the latter. Using a murine MVEC model, miR-31 was found to positively regulate TGF-β-induced EndoMT, but importantly, TGF-β did not induce miR-31 expression [150]. This is in contrast with the observations by Zhu et al., who found that TGF-β indeed regulated the expression of miR-21 [127].

Returning to miR-155, a preceding trigger of EndoMT, such as that induced by miR-21 and miR-31, could support its presence and role independent of vasculopathy early in SSc. Essentially, if vasculopathy occurs before fibrosis, it may explain the elevated levels of miR-155 despite its protective effects against EndoMT and EC senescence. This

discrepancy emphasizes the need to clarify the mechanisms that trigger upregulation of miR-155 and when this occurs in SSc pathogenesis. Related studies by Yan et al. found that miR-155 exerts its profibrotic effects via induction of Wnt/β-catenin signaling in fibroblasts [85]. It has been recently reported that the initial expression of miR-155 in fibroblasts is triggered by IL-1 mediated NLRP3 inflammasome activation, and miR-155 then exerts a positive feedback on the system [87]. This chronology suggests that miR-155 upregulation does not occur until after a fibroproliferative phenotype has been triggered. Adding this finding to what is known regarding miR-21 and miR- 31 strongly supports a pathogenetic mechanism of vasculopathy preceding fibrotic change and provides a cogent explanation of the elevated miR-155 levels found early in SSc. Finally, this proposed mechanism relies on a direct connection between miR-21/miR-31 driven vasculopathy and miR-155 upregulation, which could be provided via NLRP3 inflammasome activation.

Inactive NLRP3 is produced under the stimulation of pathogen-associated/damage- associated molecular patterns (PAMPs/DAMPs) and NF-κB. NF-κB is subsequently oligomerized with ASC and pro-caspase 1 into a complex termed the inflammasome. This complex then triggers the activation of IL-1. Paracrine stimulation of IL-1 receptors results in MyD88-mediated ERK/JAK upregulation of TGF-β [87]. Both NLRP3 and NLRP1 overexpression have been identified in SSc [155,156]. Interestingly, when examining angiotensin II-induced liver fibrosis, Ning et al. found that miR-21 was responsible for mediating NLRP3 inflammasome activation [157]. In related studies, Sun et al. were able to establish the same finding in pulmonary fibroblasts, with upregulated miR-21 activating NLRP3 [158]. In the previously discussed Zhou et al. study [80], MVECs and skin fibroblasts were stimulated by serum from SSc patients, and miRNA levels were measured. MiR-21 had the largest early increase in relative expression of any miRNA in both MVECs and fibroblasts [80]. As mentioned, miR-21 also stimulates EndoMT and is inducible by TGF-β. Microvesicle packaging could account for miR-21 from a single source exerting both vascular and fibrotic effects, and, in fact, exosomal miR-21-5p was found to be significantly elevated in the serum of patients with diffuse SSc compared to healthy controls [159]. Together, these findings implicate miR-21 as both an early mediator of SSc vasculopathy, along with miR-31, and as a contributing trigger of fibroproliferation via NLRP3 activation. This would then induce and be propagated through miR-155 upregulation. Thus, increased TGF-β signaling (linked to stimulation via NLRP3) [87,160], would potentially be sustained via the positive feedback loop created by miR-155 mediation before a larger positive feedback loop is created by TGF-β induced expression of miR- 21, contributing to widespread vasculopathy and fibrosis.

In summary, the previously cited studies have established a potential mechanistic connection between SSc vasculopathy and fibroproliferation, initiated by the upregulation of miR-21 and miR-31. Both of these miRNAs are capable of triggering vasculopathy and have been shown to be among the most highly elevated miRNAs in early SSc disease pathogenesis. MiR-21 was separately found to trigger the activation of the NLRP3 inflammasome, which has recently been implicated in the development of SSc tissue fibrosis. The activation of the NLRP3 inflammasome has also been shown to mediate the upregulation of miR-155, in addition to its contribution to fibrotic change. MiR-155 is upregulated in SSc and is directly vascular-protective, but the outlined chronology suggests that it is not upregulated until after vascular damage has been initiated and established. MiR-155 is a very important regulator and stimulator of NLRP3 activation, which then exerts positive feedback on miR-21 via TGF-β. This creates a cyclic feedback loop connecting vasculopathy and fibroproliferation. Given its inhibition by TGF-β within this framework, miR-31 may serve as an important initial trigger of disease progression. A depiction of this theoretical framework is presented in Figs. 2 and 3.

The functions of miR-21 within SSc pathogenesis and other processes of immune dysregulation are highly complex [127]. Similarly, the activation and role of inflammasomes in SSc pathogenesis is more involved than what has been explored in this review and remains incompletely understood. The potential mechanistic interplay between miR-21 and -155 has been previously reported as has the therapeutic potential of miR-155 in the context of SSc-associated pulmonary fibrosis [161]. Further investigation of these miRNAs as part of a larger connection between vasculopathy and fibrosis should provide great value in understanding SSc pathogenesis and developing novel therapeutic targets. Clarifying the role of miRNAs involved in EndoMT relative to TGF-β signaling in the setting of SSc could further support a model of SSc pathogenesis initiated and triggered by endothelial dysfunction.

## 3. Future directions

### 3.1. MicroRNA as therapeutic targets for SSc vasculopathy and fibroproliferative alterations

Collectively, the pathways described in this review offer a very high potential to further support ongoing efforts aimed at a better understanding of SSc pathogenesis. The identified involvement of miRNAs in each pathway's dysfunction adds particular therapeutic value that warrants further investigation. This approach more closely addresses the underlying root of SSc pathogenesis, and of SSc's most severe clinical manifestations, and could reach valuable success through the exploration and targeting of miRNAs.

Beyond SSc research, miRNAs are being studied as potential therapies for numerous pathologies, including cancers and cardiovascular diseases [162,163]. The understanding of their role within these various pathologies offers highly specific therapeutic targets, accounting for the recent increase in scientific interest [164]. Effectively applying miRNA within the development of novel SSc therapies will likely require a preceding and precise knowledge of how their functioning underlies SSc pathogenesis. The currently incurable status of SSc is, in part, a reflection of our inability to connect the vascular, fibrotic, and immunological changes that account for its morbidity and mortality. Future research efforts aimed at using miRNAs to connect these pathogenetic components may provide a pathway to find success in understanding and treating SSc.

### 3.2. MicroRNA as diagnostic and prognostic biomarkers for SSc vasculopathy and fibroproliferative alterations

Besides their potential as therapeutic targets, miRNAs have been investigated as diagnostic and prognostic biomarkers for numerous diseases. Many pathologic processes, including SSc, have been shown to display unique miRNA biomarker profiles, providing excellent diagnostic value. MiRNAs are stable in serum and other biological fluids and have proven to be reliable disease markers [165]. As with their therapeutic value, the utility of miRNA as biomarkers is related to the sensitivity and specificity they show for a given disease process. A miRNA profile, once identified, can serve as an efficient, non-invasive, and easily testable disease signature [166]. There is substantial scientific literature reporting the potential utility of miRNAs as diagnostic biomarkers in SSc [30,123,126]. Continued research into this area of SSc disease management will greatly improve our diagnostic and prognostic capabilities. Table 1 presents a list of selected miRNA biomarkers that have been found to have important vascular implications in SSc. Similarly, miRNA biomarkers involved in SSc fibroproliferative alterations are presented in Table 2.

## 4. Conclusion

MiRNAs are integral regulators of gene expression that have also been implicated in the pathogenesis of numerous diseases. In the setting of SSc, miRNAs have been investigated as both pathogenic contributors

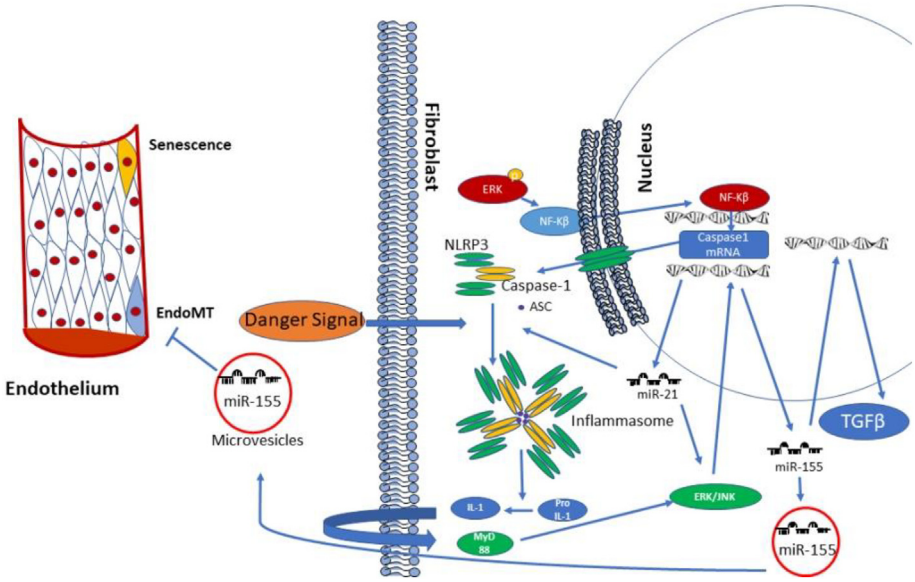


**Fig. 3.** Role of MiRNA in inflammatory and NLRP3 pathways involved in SSc vasculopathy and fibroproliferation. MiR-21 is a potent activator of the NLRP3 inflammasome within fibroblasts. The NLRP3 inflammasome contributes to tissue fibrosis through TGF-β upregulation, among other processes. NLRP3 activation upregulates miR-155 expression. MiR-155 upregulation creates a positive feedback loop through NLRP3 activation. MiR-21 can also stimulate the ERK/NF-κβ pathway, and stimulate the production of NLRP-3 inflammasome. NLRP3 activation subsequently stimulates the production of IL-1. IL-1, through effects mediated by MyD-88-activated pathways, induces the increased production of miR-155. MiR-155 is a potent negative regulator and inhibitor of Endo-MT.

**Table 1**
MicroRNA implicated in SSc vascular alterations.

| miRNA | Physiologic effect | Levels in SSc | |
|---|---|---|---|
| 21 | ↑EndoMT | Overexpressed[a] | [80] |
| 31 | ↑EndoMT, ↑TGF-β | Overexpressed[a] | [80] |
| 34a | ↓Wnt signaling | Overexpressed | [81] |
| 130b | ↓PPARγ | Overexpressed | [106] |
| 152 | ↑eNOS expression | Underexpressed | [31] |
| 155 | ↑Wnt signaling, ↓EndoMT | Overexpressed | [83] |
| 193b | ↓uPA | Underexpressed | [93] |

[a] Overexpressed when stimulated with serum from SSc patients.

**Table 2**
MicroRNA implicated in SSc fibrotic alterations.

| miRNA | Physiologic effect | Levels in SSc | |
|---|---|---|---|
| Let-7a | ↓Collagen | Underexpressed | [141] |
| 21 | ↓SMAD7 | Overexpressed | [127] |
| 26a-5p | ↓Collagen | Underexpressed | [167] |
| 29a | ↓Collagen | Underexpressed | [128] |
| 30b | ↓PDGFR-β | Underexpressed | [168] |
| 31 | ↑EndoMT, ↑TGF-β | Overexpressed[a] | [80] |
| 92a | ↓MMP-1 | Overexpressed | [130] |
| 129-5p | ↓Connective Tissue Growth Factor | Underexpressed | [169] |
| 142-3p | ↑Integrin αV | Overexpressed | [170] |
| 150 | ↓Integrin β3 | Underexpressed | [131] |
| 155 | ↑Wnt signaling, ↓EndoMT | Overexpressed | [83] |
| 196a | ↓Collagen | Underexpressed | [129] |
| 483-5p | ↑Collagen | Overexpressed | [147] |

[a] Overexpressed when stimulated with serum from SSc patients

as well as diagnostic and prognostic biomarkers. Vasculopathy associated with early SSc pathogenesis and triggering the fibrotic process in SSc is an area of particular interest, and a full understanding of the role of miRNAs in these processes could become highly important to allow their identification as therapeutic targets for this disease. While testing of this framework is admittedly difficult, future studies that aim to identify miRNAs and other epigenetic factors connecting the primary pathogenic processes of SSc may provide valuable results. The growing knowledge base of miRNA biogenesis has offered tremendous therapeutic advances in other diseases and current evidence indeed suggests that the same progress is possible, if not highly likely in SSc. MiRNAs as therapeutic targets are a relatively new development and must be extensively explored for their potential application to SSc therapy. MiR-21, -31 and -155 hold particular potential for future investigation given their participation in both endothelial and fibroproliferative processes occurring in SSc. Future research that clarifies the role of miRNAs in the overall pathogenesis of SSc holds particular potential for their use as valuable diagnostic and prognostic biomarkers, and for the development of novel, effective, and targeted therapies for this currently incurable disease.

### 4.1. Take home messages

- The pathogenesis of SSc is complex and involves immune response dysregulation, progressive vasculopathy, and excessive accumulation of extracellular matrix proteins in skin and multiple target organs.
- The pathogenetic connections between vasculopathy and tissue fibrosis in SSc are poorly understood despite intensive investigations.
- MiRNAs are crucial epigenetic regulators that modulate endothelial cell and fibroblast gene expression and are likely to play a role connecting both pathways of SSc pathogenesis.
- MiRNAs are promising targets for the development of antifibrotic and vasculopathy therapies and they can serve as highly sensitive and specific biomarkers for SSc diagnosis, and for the assessment of SSc progression and eventual clinical outcome.

## Declaration of Competing Interest

The authors declare no conflicts of interests.

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## References

[1] Allanore Y, Simms R, Distler O, Trojanowska M, Pope J, Denton CP, et al. Systemic sclerosis. Nat Rev Dis Prim. 2015;1:15002.
[2] Asano Y. Systemic sclerosis. J. Dermatol. 2018;45:128–38. https://doi.org/10.1111/1346-8138.14153.

[3] Denton CP, Khanna D. Systemic sclerosis. Lancet 2017;390:1685–99.
[4] Stern EP, Denton CP. The pathogenesis of systemic sclerosis. Rheum. Dis. Clin. N. Am. 2015;41:367–82.
[5] Greenblatt MB, Aliprantis AO. The immune pathogenesis of scleroderma: context is everything. Curr. Rheumatol. Rep. 2013;15:297.
[6] Brown M, O'Reilly S. The immunopathogenesis of fibrosis in systemic sclerosis. Clin. Exp. Immunol. 2019;195:310–21.
[7] Chizzolini C, Boin F. The role of the acquired immune response in systemic sclerosis. Semin. Immunopathol. 2015;37:519–28.
[8] Dowson C, Simpson N, Duffy L, O'Reilly S. Innate immunity in systemic sclerosis. Curr. Rheumatol. Rep. 2017;19:2.
[9] Cutolo M, Soldano S, Smith V. Pathophysiology of systemic sclerosis: current understanding and new insights. Expert. Rev. Clin. Immunol. 2019:1–12.
[10] Ho YY, Lagares D, Tager AM, Kapoor M. Fibrosis–a lethal component of systemic sclerosis. Nat. Rev. Rheumatol. 2014;10:390–402.
[11] Pattanaik D, Brown M, Postlethwaite BC, Postlethwaite AE. Pathogenesis of systemic sclerosis. Front. Immunol. 2015;6:272.
[12] Wynn TA. Cellular and molecular mechanisms of fibrosis. J. Pathol. 2008;214:199–210.
[13] Matucci-Cerinic M, Kahaleh B, Wigley FM. Review: evidence that systemic sclerosis is a vascular disease. Arthritis Rheum. 2013;65:1953–62.
[14] Cipriani P, Benedetto PD, Ruscitti P, Liakouli V, Berardicurti O, Carubbi F, et al. Perivascular cells in diffuse cutaneous systemic sclerosis overexpress activated ADAM12 and are involved in myofibroblast transdifferentiation and development of fibrosis. J. Rheumatol. 2016;43:1340–9.
[15] Cipriani P, Benedetto PD, Ruscitti P, Capece D, Zazzeroni F, Liakouli V, et al. The endothelial-mesenchymal transition in systemic sclerosis is induced by endothelin-1 and transforming growth factor-β and may be blocked by macitentan, a dual endothelin-1 receptor antagonist. J. Rheumatol. 2015;42:1808–16.
[16] Rajkumar VS, Sundberg C, Abraham DJ, Rubin K, Black CM. Activation of microvascular pericytes in autoimmune Raynaud's phenomenon and systemic sclerosis. Arthritis Rheum. 1999;42:930–41.
[17] Dolcino M, Tinazzi E, Puccetti A, Lunardi C. In systemic sclerosis, a unique long non coding RNA regulates genes and pathways involved in the three main features of the disease (vasculopathy, fibrosis and autoimmunity) and in Carcinogenesis. J. Clin. Med. 2019;8.
[18] Żółkiewicz J, Stochmal A, Rudnicka L. The role of adipokines in systemic sclerosis: a missing link? Arch. Dermatol. Res. 2019;311:251–63.
[19] Ntelis K, Bogdanos D, Dimitroulas T, Sakkas L, Daoussis D. Platelets in systemic sclerosis: the missing link connecting vasculopathy, autoimmunity, and fibrosis? Curr. Rheumatol. Rep. 2019;21:15.
[20] Luo Y, Wang Y, Wang Q, Xiao R, Lu Q. Systemic sclerosis: genetics and epigenetics. J. Autoimmun. 2013;41:161–7.
[21] Luo Y, Wang Y, Shu Y, Lu Q, Xiao R. Epigenetic mechanisms: an emerging role in pathogenesis and its therapeutic potential in systemic sclerosis. Int. J. Biochem. Cell Biol. 2015;67:92–100.
[22] Makino T, Jinnin M. Genetic and epigenetic abnormalities in systemic sclerosis. J. Dermatol. 2016;43:10–8.
[23] Tsou P-S, Sawalha AH. Unfolding the pathogenesis of scleroderma through genomics and epigenomics. J. Autoimmun. 2017;83:73–94.
[24] Walczyk M, Paradowska-Gorycka A, Olesinska M. Epigenetics: the future direction in systemic sclerosis. Scand. J. Immunol. 2017;86:427–35.
[25] Broen JCA, Radstake TRDJ, Rossato M. The role of genetics and epigenetics in the pathogenesis of systemic sclerosis. Nat. Rev. Rheumatol. 2014;10:671–81.
[26] Murdaca G, Contatore M, Gulli R, Mandich P, Puppo F. Genetic factors and systemic sclerosis. Autoimmun. Rev. 2016;15:427–32.
[27] Bergmann C, Distler JH. Epigenetic factors as drivers of fibrosis in systemic sclerosis. Epigenomics 2017;9:463–77.
[28] Angiolilli C, Marut W, van der Kroef M, Chouri E, Reedquist KA, Radstake TRDJ. New insights into the genetics and epigenetics of systemic sclerosis. Nat. Rev. Rheumatol. 2018;14:657–73.
[29] Aslani S, Sobhani S, Gharibdoost F, Jamshidi A, Mahmoudi M. Epigenetics and pathogenesis of systemic sclerosis; the ins and outs. Hum. Immunol. 2018;79:178–87.
[30] Zhang Z, Zhang R. Epigenetics in autoimmune diseases: pathogenesis and prospects for therapy. Autoimmun. Rev. 2015;14:854–63.
[31] Altorok N, Kahaleh B. Epigenetics and systemic sclerosis. Semin. Immunopathol. 2015;37:453–62.
[32] Mazzone R, Zwergel C, Artico M, Taurone S, Ralli M, Greco A, et al. The emerging role of epigenetics in human autoimmune disorders. Clin. Epigenetics 2019;11:34.
[33] Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007;56:323–33.
[34] Wei J, Bhattacharyya S, Tourtellotte WG, Varga J. Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. Autoimmun. Rev. 2011;10:267–75.
[35] Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat. Rev. Drug Discov. 2017;16:203–22.
[36] Mollaei H, Safaralizadeh R, Rostami Z. MicroRNA replacement therapy in cancer. J. Cell. Physiol. 2019;234:12369–84.
[37] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[38] Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and function. Thromb. Haemost. 2012;107:605–10.
[39] Cannell IG, Kong YW, Bushell M. How do microRNAs regulate gene expression? Biochem. Soc. Trans. 2008;36:1224–31.
[40] Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu. Rev. Biochem. 2010;79:351–79.
[41] Eulalio A, Huntzinger E, Izaurralde E. Getting to the root of miRNA-mediated gene silencing. Cell 2008;132:9–14.
[42] O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA biogenesis, mechanisms of actions, and circulation. Front. Endocrinol. 2018;9.
[43] Xu W, San Lucas A, Wang Z, Liu Y. Identifying microRNA targets in different gene regions. BMC Bioinforma. 2014;15(Suppl. 7):S4.
[44] Flowers E, Froelicher ES, Aouizerat BE. MicroRNA regulation of lipid metabolism. Metabolism 2013;62:12–20.
[45] Sharma S. Immunomodulation: a definitive role of microRNA-142. Dev. Comp. Immunol. 2017;77:150–6.
[46] Sun M, Lu Q. MicroRNA regulation of airway smooth muscle function. Biol. Chem. 2016;397:507–11.
[47] Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, et al. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 2006;108:3068–71.
[48] Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer is required for embryonic angiogenesis during mouse development. J. Biol. Chem. 2005;280:9330–5.
[49] Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. a comprehensive review. EMBO Mol. Med. 2017;9:852.
[50] Lages E, Ipas H, Guttin A, Nesr H, Berger F, Issartel J-P. MicroRNAs: molecular features and role in cancer. Front. Biosci. Landmark Ed. 2012;17:2508–40.
[51] Jiang X, Tsitsiou E, Herrick SE, Lindsay MA. MicroRNAs and the regulation of fibrosis. FEBS J. 2010;277:2015–21.
[52] Vettori S, Gay S, Distler O. Role of MicroRNAs in Fibrosis. Open Rheumatol. J. 2012;6:130–9.
[53] Patel V, Noureddine L. MicroRNAs and fibrosis. Curr. Opin. Nephrol. Hypertens. 2012;21:410–6.
[54] Bowen T, Jenkins RH, Fraser DJ. MicroRNAs, transforming growth factor beta-1, and tissue fibrosis. J. Pathol. 2013;229:274–85.
[55] Stepicheva NA, Song JL. Function and regulation of microRNA-31 in development and disease. Mol. Reprod. Dev. 2016;83:654–74.
[56] Jimenez SA, Piera-Velazquez S. Potential role of human-specific genes, human-specific microRNAs and human-specific non-coding regulatory RNAs in the pathogenesis of systemic sclerosis and Sjögren's syndrome. Autoimmun. Rev. 2013;12:1046–51.
[57] Singh RP, Massachi I, Manickavel S, Singh S, Rao NP, Hasan S, et al. The role of miRNA in inflammation and autoimmunity. Autoimmun. Rev. 2013;12:1160–5.
[58] Churov AV, Oleinik EK, Knip M. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. Autoimmun. Rev. 2015;14:1029–37.
[59] Chen J-Q, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmun. Rev. 2016;15:1171–80.
[60] Nziza N, Duroux-Richard I, Apparailly F. MicroRNAs in juvenile idiopathic arthritis: can we learn more about pathophysiological mechanisms? Autoimmun. Rev. 2019;18(8):796–804.
[61] Graves P, Zeng Y. Biogenesis of mammalian microrna: a global view. Genomics Proteomics Bioinforma. 2012;10:239–45.
[62] Kobayashi H, Tomari Y. RISC assembly: coordination between small RNAs and argonaute proteins. Biochim. Biophys. Acta 2016;1859:71–81.
[63] Ozsolak F, Poling LL, Wang Z, Liu H, Liu XS, Roeder RG, et al. Chromatin structure analyses identify miRNA promoters. Genes Dev. 2008;22:3172–83.
[64] Davis BN, Hata A. Regulation of microRNA biogenesis: a miRiad of mechanisms. Cell. Commun. Signal 2009;7:18.
[65] Gantier MP, McCoy CE, Rusinova I, Saulep D, Wang D, Xu D, et al. Analysis of microRNA turnover in mammalian cells following Dicer1 ablation. Nucleic Acids Res. 2011;39:5692–703.
[66] Baraban JM, Shah A, Fu X. Multiple pathways mediate microRNA degradation: focus on the translin/trax RNase complex. Adv. Pharmacol. San. Diego. Calif. 2018;82:1–20.
[67] Bayraktar R, Van Roosbroeck K, Calin GA. Cell-to-cell communication: microRNAs as hormones. Mol. Oncol. 2017;11:1673–86.
[68] Fernández-Hernando C, Suárez Y. MicroRNAs in endothelial cell homeostasis and vascular disease. Curr. Opin. Hematol. 2018;25:227–36.
[69] Romero LI, Zhang DN, Cooke JP, Ho HK, Avalos E, Herrera R, et al. Differential expression of nitric oxide by dermal microvascular endothelial cells from patients with scleroderma. Vasc. Med. 2000;5:147–58.
[70] Suárez Y, Fernández-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ. Res. 2007;100:1164–73.
[71] Altorok N, Wang Y, Kahaleh B. Endothelial dysfunction in systemic sclerosis. Curr. Opin. Rheumatol. 2014;26:615–20.
[72] Logan CY, Nusse R. The wnt signaling pathway in development and disease. Annu. Rev. Cell Dev. Biol. 2004;20:781–810.
[73] Masckauchán TNH, Shawber CJ, Funahashi Y, Li C-M, Kitajewski J. Wnt/β-catenin signaling induces proliferation, survival and interleukin-8 in human endothelial cells. Angiogenesis 2005;8:43–51.
[74] Königshoff M, Eickelberg O. WNT signaling in lung disease. Am. J. Respir. Cell Mol. Biol. 2010;42:21–31.
[75] Bergmann C, Distler JHW. Canonical wnt signaling in systemic sclerosis. Lab. Investig. 2016;96:151–5.
[76] Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell 2017;169:985–99.
[77] Burgy O, Königshoff M. The WNT signaling pathways in wound healing and

fibrosis. Matrix Biol. J. Int. Soc. Matrix Biol. 2018;68–69:67–80.
[78] Dejana E. The role of wnt signaling in physiological and pathological angiogenesis. Circ. Res. 2010;107:943–52.
[79] Reis M, Liebner S. Wnt signaling in the vasculature. Exp. Cell Res. 2013;319:1317–23.
[80] Zhou B, Zuo XX, Li YS, Gao SM, Dai XD, Zhu HL, et al. Integration of microRNA and mRNA expression profiles in the skin of systemic sclerosis patients. Sci. Rep. 2017;7:42899.
[81] Kim NH, Kim HS, Kim N-G, Lee I, Choi H-S, Li X-Y, et al. p53 and microRNA-34 are suppressors of canonical wnt signaling. Sci. Signal. 2011;4(197):ra71.
[82] Ito T, Yagi S, Yamakuchi M. MicroRNA-34a regulation of endothelial senescence. Biochem. Biophys. Res. Commun. 2010;398:735–40.
[83] Alivernini S, Bosello SL, Luca GD, Canestri S, Mario CD, Gigante MR, et al. A3.21 MicroRNA-34a and microRNA-155 in systemic sclerosis: possible epigenetic biomarkers of endothelial dysfunction in VEDOSS and long-standing disease. Ann. Rheum. Dis. 2014;73:A50.
[84] Meunier P, Dequidt L, Barnetche T, Lazaro E, Duffau P, Richez C, et al. Increased risk of mortality in systemic sclerosis-associated digital ulcers: a systematic review and meta- analysis. J. Eur. Acad. Dermatol. Venereol. 2018;33:405–9.
[85] Yan Q, Chen J, Li W, Bao C, Fu Q. Targeting miR-155 to treat experimental scleroderma. Sci. Rep. 2016;6:203–14.
[86] Dolcino M, Pelosi A, Fiore PF, Patuzzo G, Tinazzi E, Lunardi C, et al. Gene profiling in patients with systemic sclerosis reveals the presence of oncogenic gene signatures. Front. Immunol. 2018;9:449.
[87] Artlett CM, Sassi-Gaha S, Hope JL, Feghali-Bostwick CA, Katsikis PD. Mir-155 is overexpressed in systemic sclerosis fibroblasts and is required for NLRP3 inflammasome- mediated collagen synthesis during fibrosis. Arthritis Res. Ther. 2017;19.
[88] Tkachuk VA, Plekhanova OS, Parfyonova YV. Regulation of arterial remodeling and angiogenesis by urokinase-type plasminogen activator. Can. J. Physiol. Pharmacol. 2009;87:231–51.
[89] Alfano D, Franco P, Vocca I, Gambi N, Pisa V, Mancini A, et al. The urokinase plasminogen activator and its receptor: role in cell growth and apoptosis. Thromb. Haemost. 2005;93:205–11.
[90] Fibbi G, Caldini R, Chevanne M, Pucci M, Schiavone N, Morbidelli L, et al. Urokinase- dependent angiogenesis in vitro and diacylglycerol production are blocked by antisense oligonucleotides against the urokinase receptor. Lab. Investig. J. Tech. Methods. Pathol. 1998;78:1109–19.
[91] Kanno Y. The role of fibrinolytic regulators in vascular dysfunction of systemic sclerosis. Int. J. Mol. Sci. 2019;20.
[92] D'Alessio S, Fibbi G, Cinelli M, Guiducci S, Rosso AD, Margheri F, et al. Matrix metalloproteinase 12–dependent cleavage of urokinase receptor in systemic sclerosis microvascular endothelial cells results in impaired angiogenesis. Arthritis Rheum. 2004;50:3275–85.
[93] Iwamoto N, Vettori S, Maurer B, Brock M, Pachera E, Jüngel A, et al. Downregulation of miR-193b in systemic sclerosis regulates the proliferative vasculopathy by urokinase-type plasminogen activator expression. Ann. Rheum. Dis. 2016;75:303–10.
[94] Schiffrin EL, Touyz RM. Vascular biology of endothelin. J. Cardiovasc. Pharmacol. 1998;32(Suppl. 3):S2–13.
[95] Cozzani E, Javor S, Laborai E, Drosera M, Parodi A. Endothelin-1 levels in scleroderma patients: a pilot study. ISRN Dermatol. 2013;2013:4. 125632.
[96] Yamane K, Miyauchi T, Suzuki N, Yuhara T, Akama T, Suzuki H, et al. Significance of plasma endothelin-1 levels in patients with systemic sclerosis. J. Rheumatol. 1992;19:1566–71.
[97] Hajialilo M, Noorabadi P, Tahsini Tekantapeh S, Malek Mahdavi A. Endothelin-1, α- Klotho, 25(OH) Vit D levels and severity of disease in scleroderma patients. Rheumatol. Int. 2017;37:1651–7.
[98] Abraham DJ, Vancheeswaran R, Dashwood MR, Rajkumar VS, Pantelides P, Xu SW, et al. Increased levels of endothelin-1 and differential endothelin type A and B receptor expression in scleroderma-associated fibrotic lung disease. Am. J. Pathol. 1997;151:831–41.
[99] Shi-Wen X, Denton CP, Dashwood MR, Holmes AM, Bou-Gharios G, Pearson JD, et al. Fibroblast matrix gene expression and connective tissue remodeling: role of endothelin-1. J. Invest. Dermatol. 2001;116:417–25.
[100] Hachulla E, Coghlan JG. A new era in the management of pulmonary arterial hypertension related to scleroderma: endothelin receptor antagonism. Ann. Rheum. Dis. 2004;63:1009–14.
[101] Morelli S, Ferri C, Polettini E, Bellini C, Gualdi GF, Pittoni V, et al. Plasma endothelin-1 levels, pulmonary hypertension, and lung fibrosis in patients with systemic sclerosis. Am. J. Med. 1995;99:255–60.
[102] Kang B-Y, Park KK, Kleinhenz JM, Murphy TC, Green DE, Bijli KM, et al. Peroxisome Proliferator-Activated Receptor γ and microRNA 98 in hypoxia-induced Endothelin-1 signaling. Am. J. Respir. Cell Mol. Biol. 2016;54:136–46.
[103] Xie X, Li S, Zhu Y, Liu L, Pan Y, Wang J, et al. MicroRNA-27a/b mediates endothelin-1- induced PPARγ reduction and proliferation of pulmonary artery smooth muscle cells. Cell. Tissue. Res. 2017;369:527–39.
[104] Bertero T, Cottrill K, Krauszman A, Lu Y, Annis S, Hale A, et al. The microRNA-130/301 family controls vasoconstriction in pulmonary hypertension. J. Biol. Chem. 2015;290:2069–85.
[105] Bertero T, Lu Y, Annis S, Hale A, Bhat B, Saggar R, et al. Systems-level regulation of microRNA networks by miR-130/301 promotes pulmonary hypertension. J. Clin. Invest. 2014;124:3514–28.
[106] Luo H, Zhu H, Zhou B, Xiao X, Zuo X. MicroRNA-130b regulates scleroderma fibrosis by targeting peroxisome proliferator-activated receptor γ. Mod. Rheumatol. 2015;25:595–602.
[107] Jimenez SA, Piera-Velazquez S. Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of Systemic Sclerosis-associated pulmonary fibrosis and pulmonary arterial hypertension. Myth or reality? Matrix Biol. 2016;51:26–36.
[108] Jimenez SA. Role of Endothelial to mesenchymal transition in the Pathogenesis of the vascular alterations in systemic sclerosis. ISRN Rheumatol. 2013:835948.
[109] Zhang P, Cai Y, Soofi A, Dressler GR. Activation of wnt11 by transforming growth factor-β drives mesenchymal gene expression through non-canonical wnt protein signaling in renal epithelial cells. J. Biol. Chem. 2012;287:21290–302.
[110] Akhmetshina A, Palumbo K, Dees C, Bergmann C, Venalis P, Zerr P, et al. Activation of canonical wnt signalling is required for TGF-β-mediated fibrosis. Nat. Commun. 2012;3:735.
[111] Cheng S-L, Shao J-S, Behrmann A, Krchma K, Towler DA. Dkk1 and MSX2-Wnt7b signaling reciprocally regulate the endothelial-mesenchymal transition in aortic endothelial cells. Arterioscler. Thromb. Vasc. Biol. 2013;33:1679–89.
[112] George PM, Oliver E, Dorfmuller P, Dubois OD, Reed DM, Kirkby NS, et al. Evidence for the involvement of type i interferon in pulmonary arterial hypertension. Circ. Res. 2014;114:677–88.
[113] Pfeffer LM. The role of nuclear factor κB in the interferon response. J. Interf. Cytokine Res. 2011;31:553–9.
[114] Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat. Med. 2007;13:952–61.
[115] Arciniegas E, Frid MG, Douglas IS, Stenmark KR. Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. Am. J. Phys. Lung Cell. Mol. Phys. 2007;293:L1–8.
[116] Piera-Velazquez S, Jimenez SA. Endothelial to mesenchymal transition: role in physiology and in the Pathogenesis of Human Diseases. Physiol. Rev. 2019;99:1281–324.
[117] Mendoza FA, Piera-Velazquez S, Farber JL, Feghali-Bostwick C, Jimenez SA. Endothelial cells expressing endothelial and mesenchymal cell gene products in Systemic Sclerosis-associated interstitial lung disease lung tissues. Arthritis. Rheumatol. 2016;68:210–7.
[118] Piera-Velazquez S, Mendoza FA, Jimenez SA. Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of human fibrotic diseases. J. Clin. Med. 2016;5:E45.
[119] Noseda M, McLean G, Niessen K, Chang L, Pollet I, Montpetit R, et al. Notch activation results in phenotypic and functional changes consistent with endothelial-to-mesenchymal transformation. Circ. Res. 2004;94:910–7.
[120] Dees C, Tomcik M, Zerr P, Akhmetshina A, Horn A, Palumbo K, et al. Notch signalling regulates fibroblast activation and collagen release in systemic sclerosis. Ann. Rheum. Dis. 2011;70:1304–10.
[121] Geng H, Guan J. MiR-18a-5p inhibits endothelial-mesenchymal transition and cardiac fibrosis through the Notch2 pathway. Biochem. Biophys. Res. Commun. 2017;491:329–36.
[122] Beyer C, Dees C, Distler JHW. Morphogen pathways as molecular targets for the treatment of fibrosis in systemic sclerosis. Arch. Dermatol. Res. 2013;305:1–8.
[123] Zhu H, Luo H, Zuo X. MicroRNAs: their involvement in fibrosis pathogenesis and use as diagnostic biomarkers in scleroderma. Exp. Mol. Med. 2013;45:E41.
[124] Miao C, Xiong Y, Yu H, Zhang X, Qin M, Song T, et al. Critical roles of microRNAs in the pathogenesis of systemic sclerosis: New advances, challenges and potential directions. Int. Immunopharmacol. 2015;28:626–33.
[125] Bagnato G, Roberts WN, Roman J, Gangemi S. A systematic review of overlapping microRNA patterns in systemic sclerosis and idiopathic pulmonary fibrosis. Eur Respir Rev Off J Eur Respir Soc 2017;26.
[126] Peng W-J, Tao J-H, Mei B, Chen B, Li B-Z, Yang G-J, et al. MicroRNA-29: a potential therapeutic target for systemic sclerosis. Expert Opin. Ther. Targets 2012;16:875–9.
[127] Zhu H, Luo H, Li Y, Zhou Y, Jiang Y, Chai J, et al. MicroRNA-21 in scleroderma fibrosis and its function in TGF-β-regulated fibrosis-related genes expression. J. Clin. Immunol. 2013;33:1100–9.
[128] Maurer B, Stanczyk J, Jüngel A, Akhmetshina A, Trenkmann M, Brock M, et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. Arthritis Rheum. 2010;62:1733–43.
[129] Honda N, Jinnin M, Kajihara I, Makino T, Makino K, Masuguchi S, et al. TGF-β-mediated downregulation of microRNA-196a contributes to the constitutive up-regulated type I collagen expression in scleroderma dermal fibroblasts. J. Immunol. 2012;188:3323–31.
[130] Sing T, Jinnin M, Yamane K, Honda N, Makino K, Kajihara I, et al. microRNA-92a expression in the sera and dermal fibroblasts increases in patients with scleroderma. Rheumatol. Oxf. Engl. 2012;51:1550–6.
[131] Honda N, Jinnin M, Kira-Etoh T, Makino K, Kajihara I, Makino T, et al. miR-150 down- regulation contributes to the constitutive type I collagen overexpression in scleroderma dermal fibroblasts via the induction of integrin β3. Am. J. Pathol. 2013;182:206–16.
[132] Makino K, Jinnin M, Aoi J, Hirano A, Kajihara I, Makino T, et al. Discoidin domain receptor 2-microRNA 196a-mediated negative feedback against excess type I collagen expression is impaired in scleroderma dermal fibroblasts. J. Invest. Dermatol. 2013;133:110–9.
[133] Martin J, Jenkins RH, Bennagi R, Krupa A, Phillips AO, Bowen T, et al. post-transcriptional regulation of transforming growth factor beta-1 by MicroRNA-744. PLoS One 2011;6:e25044.
[134] Jafarinejad-Farsangi S, Gharibdoost F, Farazmand A, Kavosi H, Jamshidi A, Karimizadeh E, et al. MicroRNA-21 and microRNA-29a modulate the expression of collagen in dermal fibroblasts of patients with systemic sclerosis. Autoimmunity 2019:1–9.
[135] Li Y, Zhang J, Lei Y, Lyu L, Zuo R, Chen T. MicroRNA-21 in skin fibrosis: potential

for diagnosis and treatment. Mol. Diagn. Ther. 2017;21:633–42.
[136] Deng Z, He Y, Yang X, Shi H, Shi A, Lu L, et al. microrna-29: a crucial player in Fibrotic Disease. Mol. Diagn. Ther. 2017;21:285–94.
[137] Gallant-Behm CL, Piper J, Lynch JM, Seto AG, Hong SJ, Mustoe TA, et al. A microRNA-29 mimic (remlarsen) represses extracellular matrix expression and fibroplasia in the skin. J. Invest. Dermatol. 2018;139:1073–81.
[138] Sassi Y, Avramopoulos P, Ramanujam D, Grüter L, Werfel S, Giosele S, et al. Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. Nat. Commun. 2017;8:1614.
[139] Xiao J, Meng X-M, Huang XR, Chung AC, Feng Y-L, Hui DS, et al. miR-29 inhibits bleomycin-induced pulmonary fibrosis in mice. Mol. Ther. 2012;20:1251–60.
[140] Wang B, Komers R, Carew R, Winbanks CE, Xu B, Herman-Edelstein M, et al. Suppression of microRNA-29 expression by TGF-β1 promotes collagen expression and renal Fibrosis. J. Am. Soc. Nephrol. 2012;23:252–65.
[141] Makino K, Jinnin M, Hirano A, Yamane K, Eto M, Kusano T, et al. The down-regulation of microRNA let-7a contributes to the excessive expression of type I collagen in systemic and localized scleroderma. J. Immunol. 2013;190:3905–15.
[142] Cai W-Y, Wei T-Z, Luo Q-C, Wu Q-W, Liu Q-F, Yang M, et al. The Wnt–β-catenin pathway represses let-7 microRNA expression through transactivation of lin28 to augment breast cancer stem cell expansion. J. Cell Sci. 2013;126:2877–89.
[143] Wei J, Fang F, Lam AP, Sargent JL, Hamburg E, Hinchcliff ME, et al. Wnt/β-catenin signaling is hyperactivated in systemic sclerosis and induces Smad-dependent fibrotic responses in mesenchymal cells. Arthritis Rheum. 2012;64:2734–45.
[144] Chen P-Y, Qin L, Barnes C, Charisse K, Yi T, Zhang X, et al. FGF regulates TGFβ signaling and endothelial-to-mesenchymal transition via control of let-7 miRNA expression. Cell. Rep. 2012;2:1684–96.
[145] Zhu H, Li Y, Qu S, Luo H, Zhou Y, Wang Y, et al. MicroRNA expression abnormalities in limited cutaneous scleroderma and diffuse cutaneous scleroderma. J. Clin. Immunol. 2012;32:514–22.
[146] Xia YW, Wang SB. Microvesicles containing microRNA-21 induce myocardial fibrosis via AKT pathway. Eur. Rev. Med. Pharmacol. Sci. 2018;22:4634–41.
[147] Chouri E, Servaas NH, Bekker CPJ, Affandi AJ, Cossu M, Hillen MR, et al. Serum microRNA screening and functional studies reveal miR-483-5p as a potential driver of fibrosis in systemic sclerosis. J. Autoimmun. 2018;89:162–70.
[148] Wang J, He W, Xu X, Guo L, Zhang Y, Han S, et al. The mechanism of TGF-β/miR-155/c-Ski regulates endothelial-mesenchymal transition in human coronary artery endothelial cells. Biosci. Rep. 2017;37.
[149] Bijkerk R, de Bruin RG, van Solingen C, Duijs JMGJ, Kobayashi K, van der Veer EP, et al. MicroRNA-155 functions as a negative regulator of RhoA signaling in TGF-β-induced endothelial to mesenchymal transition. MicroRNA 2012;1:2–10.
[150] Katsura A, Suzuki HI, Ueno T, Mihira H, Yamazaki T, Yasuda T, et al. MicroRNA-31 is a positive modulator of endothelial–mesenchymal transition and associated secretory phenotype induced by TGF-β. Genes Cells 2016;21:99–116.
[151] Guo Y, Li P, Bledsoe G, Yang Z-R, Chao L, Chao J. Kallistatin inhibits TGF-β-induced endothelial-mesenchymal transition by differential regulation of microRNA-21 and eNOS expression. Exp. Cell Res. 2015;337:103–10.
[152] Kumarswamy R, Volkmann I, Jazbutyte V, Dangwal S, Park D-H, Thum T. Transforming growth factor-β-induced endothelial-to-mesenchymal transition is partly mediated by microRNA-21. Arterioscler. Thromb. Vasc. Biol. 2012;32:361–9.
[153] Kwon O-S, Kim K-T, Lee E, Kim M, Choi S-H, Li H, et al. Induction of MiR-21 by stereotactic body radiotherapy contributes to the pulmonary Fibrotic response. PLoS One 2016;11:e0154942.
[154] Zuo K, Li M, Zhang X, Lu C, Wang S, Zhi K, et al. MiR-21 suppresses endothelial progenitor cell proliferation by activating the TGFβ signaling pathway via downregulation of wwp1. Int. J. Clin. Exp. Pathol. 2015;8:414–22.
[155] Dieudé P, Guedj M, Wipff J, Ruiz B, Riemekasten G, Airo P, et al. NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis. Ann. Rheum. Dis. 2011;70:668–74.
[156] Martínez-Godínez MA, Cruz-Domínguez MP, Jara LJ, Domínguez-López A, Jarillo-Luna RA, Vera-Lastra O, et al. Expression of NLRP3 inflammasome, cytokines and vascular mediators in the skin of systemic sclerosis patients. Isr. Med. Assoc. J. 2015;17:5–10.
[157] Ning Z-W, Luo X-Y, Wang G-Z, Li Y, Pan M-X, Yang R-Q, et al. MicroRNA-21 Mediates Angiotensin II-Induced Liver Fibrosis by Activating NLRP3 Inflammasome/IL- 1β Axis via Targeting Smad7 and Spry1. Antioxid Redox Signal. 2017;27:1–20.
[158] Sun N-N, Yu C-H, Pan M-X, Zhang Y, Zheng B-J, Yang Q-J, et al. Mir-21 Mediates the Inhibitory Effect of Ang (1–7) on AngII-induced NLRP3 Inflammasome Activation by Targeting Spry1 in lung fibroblasts. Sci. Rep. 2017;7:14369.
[159] Wermuth PJ, Piera-Velazquez S, Jimenez SA. Exosomes isolated from serum of systemic sclerosis patients display alterations in their content of profibrotic and antifibrotic microRNA and induce a profibrotic phenotype in cultured normal dermal fibroblasts. Clin. Exp. Rheumatol. 2017;35(Suppl. 106):21–30.
[160] Tian R, Zhu Y, Yao J, Meng X, Wang J, Xie H, et al. NLRP3 participates in the regulation of EMT in bleomycin-induced pulmonary fibrosis. Exp. Cell Res. 2017;357:328–34.
[161] Christmann RB, Wooten A, Sampaio-Barros P, Borges CL, Carvalho CRR, Kairalla RA, et al. miR-155 in the progression of lung fibrosis in systemic sclerosis. Arthritis Res. Ther. 2016;18.
[162] Berindan-Neagoe I, Monroig P, Pasculli B, Calin GA. MicroRNAome genome: a treasure for cancer diagnosis and therapy. CA Cancer J. Clin. 2014;64:311–36.
[163] Philippen LE, Dirkx E, Wit JBM, Burggraaf K, de Windt LJ, da Costa Martins PA. Antisense MicroRNA therapeutics in cardiovascular disease: Quo Vadis? Mol. Ther. 2015;23:1810–8.
[164] Henderson J, Distler J, O'Reilly S. The role of epigenetic modifications in systemic sclerosis: A druggable target. Trends Mol. Med. 2019;25:395–411.
[165] Ferracin M, Negrini M. Micromarkers 2.0: an update on the role of microRNAs in cancer diagnosis and prognosis. Expert. Rev. Mol. Diagn. 2015;15:1369–81.
[166] Imani S, Zhang X, Hosseinifard H, Fu S, Fu J. The diagnostic role of microRNA-34a in breast cancer: a systematic review and meta-analysis. Oncotarget 2017;8:23177–87.
[167] Li H, Yang R, Fan X, Gu T, Zhao Z, Chang D, et al. MicroRNA array analysis of microRNAs related to systemic scleroderma. Rheumatol. Int. 2012;32:307–13.
[168] Tanaka S, Suto A, Ikeda K, Sanayama Y, Nakagomi D, Iwamoto T, et al. Alteration of circulating miRNAs in SSc: miR-30b regulates the expression of PDGF receptor β. Rheumatol Oxf Engl 2013;52:1963–72.
[169] Nakashima T, Jinnin M, Yamane K, Honda N, Kajihara I, Makino T, et al. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts. J. Immunol. 2012;188:3573–83.
[170] Makino K, Jinnin M, Kajihara I, Honda N, Sakai K, Masuguchi S, et al. Circulating miR- 142-3p levels in patients with systemic sclerosis. Clin. Exp. Dermatol. 2012;37:34–9.