sclerosis

MDPI

Review

Non-Coding RNA Networks in Systemic Sclerosis: Roles in Pathogenesis, Biomarkers, and Therapeutic Opportunities

Daisuke Uematsu, Mizuki Nakatsuka and Masatoshi Jinnin *

Department of Dermatology, Wakayama Medical University, Wakayama 641-0012, Japan; m18017du@jichi.ac.jp (D.U.); nktkmizu@wakayama-med.ac.jp (M.N.)
* Correspondence: mjin@wakayama-med.ac.jp

Abstract

Non-coding RNAs have emerged as gene-regulatory molecules acting at epigenetic, transcriptional, and post-transcriptional levels. Among them, microRNAs are short RNA molecules consisting of approximately 22 nucleotides. Increased microRNA expression suppresses target protein expression, whereas decreased microRNA expression relieves this repression and can induce overexpression of target proteins. microRNAs integratively regulate highly complex biological processes, including cell proliferation, differentiation, apoptosis, morphogenesis, and immune responses. Therefore, alterations in microRNA expression profiles may be directly linked to the fundamental pathogenesis of systemic sclerosis (SSc), an autoimmune and fibrotic disease involving multiple organs. This focused narrative review examines microRNAs involved in SSc fibrosis, vasculopathy, and immune dysregulation; extracellular-particle-associated microRNAs; and circulating and hair-derived microRNAs as candidate biomarkers. It also summarizes evidence on long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), yRNAs, and PIWI-interacting RNAs (piRNAs). Finally, therapeutic perspectives, including microRNA mimics, antisense inhibitors, and broader RNA-targeted strategies, are discussed together with key challenges in validation and clinical translation.

Keywords: microRNA; non-coding RNA; long non-coding RNA

1. Introduction

The pathogenesis of systemic sclerosis (SSc) is extremely complex and is thought to be triggered when certain environmental factors act on individuals with genetic predisposition [1]. In recent years, epigenetics has emerged as a "missing link" connecting "genetics" and "environment". Alongside DNA methylation and histone modifications, abnormalities in non-coding RNAs are increasingly considered to play a role in SSc onset and progression.

Completion of the Human Genome Project and subsequent advances in comprehensive transcriptome analyses using next-generation sequencing have brought about a paradigm shift in life science. Specifically, protein-coding regions occupy only a small fraction of the human genome, whereas a large proportion of the transcriptome consists of non-coding RNAs that generally lack conventional protein-coding annotation. Moreover, these non-coding RNAs, once regarded as functionless "junk" generated during evolution, are now recognized as major regulators of gene expression that act at transcriptional and post-transcriptional levels. In epigenetic systems that dynamically and precisely switch mRNA–protein expression on and off in response to environmental factors without rewrit-

Check for updates

Academic Editor: Carol M. Artlett

Received: 1 April 2026
Revised: 22 August 2026
Accepted: 2 September 2026
Published: 7 September 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

ing the DNA sequence, non-coding RNAs play indispensable roles in modulating cellular functions and generating disease diversity (Figure 1).

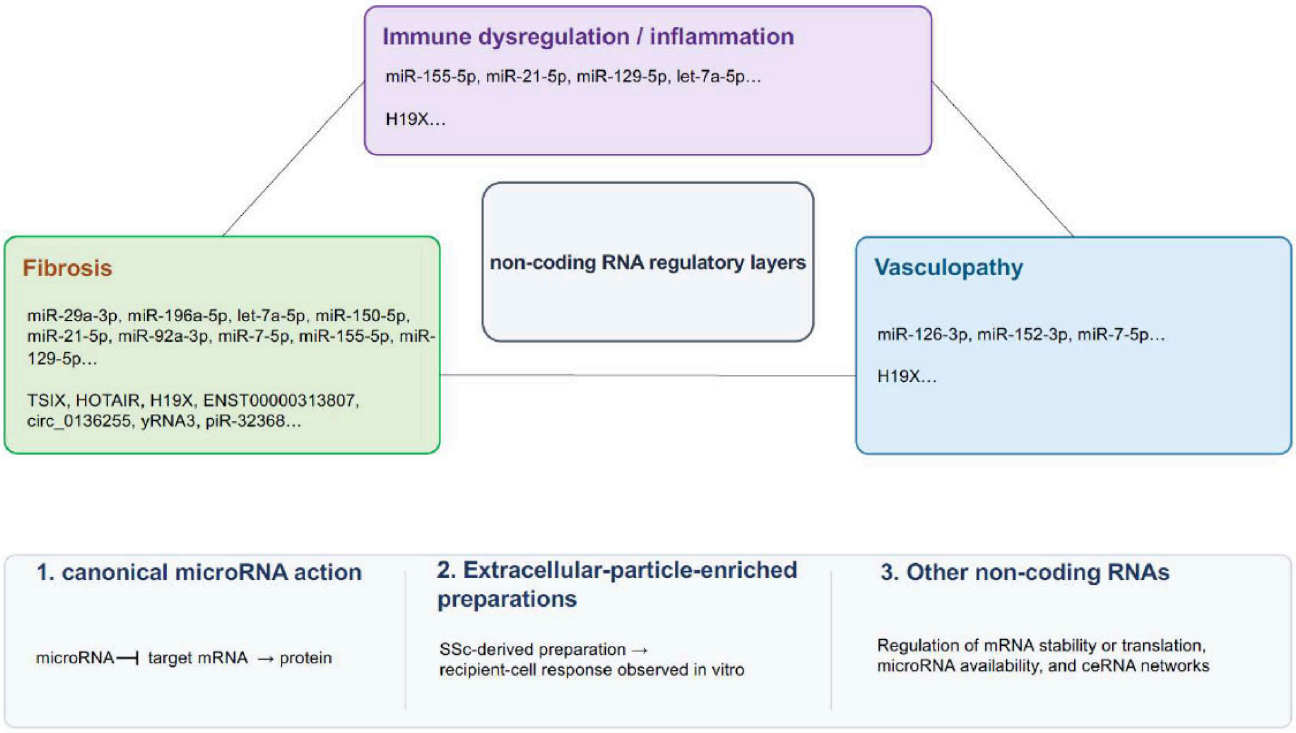


Figure 1. Non-coding RNA networks integrate the three pathogenic axes of SSc. This conceptual schematic summarizes representative non-coding RNAs involved in fibrosis, vasculopathy, and immune dysregulation/inflammation. Connecting lines indicate biological interdependence rather than proven direct causality. The lower panel illustrates three regulatory modes: canonical microRNA-mediated repression, recipient-cell responses to SSc-derived extracellular particle-enriched preparations, and regulation by other non-coding RNAs through mRNA stability, translation, microRNA availability, and ceRNA networks. Abbreviation: ceRNA, competing endogenous RNA.

This article is a focused narrative review, rather than a systematic or scoping review, and aims to integrate mechanistic, clinical, and translational evidence concerning non-coding RNAs in SSc, including, but not limited to, studies from our group. To update and contextualize the literature, targeted searches of PubMed/MEDLINE and Google Scholar were performed from database inception to June 2026, using combinations of "systemic sclerosis" or "scleroderma" with "non-coding RNA", "microRNA", "miRNA", "long non-coding RNA", "lncRNA", "circular RNA", "circRNA", "yRNA", "piRNA", "extracellular particle", "exosome", "biomarker", and "therapy". Reference lists of relevant articles were also examined. Original human, cellular, animal, and translational studies directly relevant to SSc were considered, together with selected reviews for background. Non-SSc studies were included only when required to interpret mechanisms not yet examined directly in SSc. No formal risk-of-bias assessment or quantitative weighting was performed.

2. The Roles of microRNAs in SSc

Non-coding RNAs can be broadly categorized into small and long non-coding RNAs; among small non-coding RNAs, the prototypical class is microRNAs of approximately 19–25 nucleotides. Since their first discovery in 1993 as lin-4, which controls developmental timing in *Caenorhabditis elegans* (*C. elegans*), it has been confirmed that more than

2500 human mature microRNA sequences have been annotated, and many protein-coding mRNAs are estimated to be regulated by microRNAs [2,3].

Throughout this review, human microRNA gene loci are denoted by italicized HGNC symbols (e.g., *MIR146A*), primary and precursor transcripts by pri-miR and pre-miR nomenclature, respectively (e.g., pri-miR-146a and pre-miR-146a), and mature products by miRBase names with 5p or 3p arm designations where applicable (e.g., miR-146a-5p and miR-146a-3p). The species prefix "hsa-" is omitted for readability. DNA sequence variants are identified by their rs numbers and are not referred to as mature microRNAs [4].

For microRNAs to function as mature, active molecules, they must undergo a multi-step biogenesis pathway that spans from the nucleus to the cytoplasm. In the first step, primary microRNAs, which are transcripts ranging from several hundred to several thousand nucleotides, are transcribed mainly by RNA polymerase II from non-coding genomic regions or intronic regions [5,6]. Next, the microprocessor complex (composed of Drosha, a cleavage enzyme of the RNase III family, and its essential cofactor DGCR8, a double-stranded RNA-binding protein) recognizes and cleaves the primary microRNA [7,8]. This processing generates precursor microRNAs with a double-stranded, hairpin-shaped stem–loop structure of approximately 60–70 nucleotides. The resulting precursor microRNAs are actively transported from the nucleus to the cytoplasm via an Exportin-5/Ran-GTP-dependent mechanism through the nuclear pore [9]. After translocation to the cytoplasm, Dicer cleaves both strands of the pre-microRNA stem near the terminal loop, releasing an approximately 22-nucleotide microRNA duplex. These double-stranded microRNAs are incorporated into the RNA-induced silencing complex (RISC) containing Argonaute proteins [6,10]. One strand is termed the "passenger strand" (formerly "microRNA*"), and is typically degraded and eliminated, although some passenger strands can also have biological functions. The remaining "guide strand" becomes the mature microRNA that searches for and suppresses target mRNA expression and/or protein output.

Mature microRNAs primarily bind complementary sequences in the 3′ untranslated region (3′UTR) of target mRNAs, although binding to the 5′UTR and open reading frame has also been reported [11,12]. The seed region—nucleotides 2–7 or 2–8 from the 5′ end of the microRNA—is the primary determinant of target mRNA recognition [13–15]. The degree of complementarity between the microRNA and the target mRNA influences the mode of repression; in plants, microRNAs are often fully or nearly fully complementary to their targets, and Argonaute-mediated slicing of the target mRNA tends to occur [16]. In mammals, in contrast, imperfect pairing centered on the seed region is typical, and translational repression (including inhibition of translation initiation) followed by deadenylation, decapping, and mRNA decay is frequently induced [6,17]. Overall, as a seesaw-like relationship, increased microRNA expression often suppresses target protein expression, whereas decreased microRNA expression releases repression and can induce overexpression of the target proteins.

Notably, a single microRNA can target multiple distinct mRNAs, and a single mRNA can be coordinately regulated by multiple microRNAs in a network. Through such complex hub-like architectures, microRNAs integratively control highly complex biological phenomena including cell proliferation, differentiation, apoptosis, morphogenesis, and immune responses. Thus, changes in microRNA profiles may be directly linked to the fundamental pathogenesis of systemic autoimmune and fibrotic diseases involving multiple organs. Multiple web databases based on computational algorithms, including DIANA-microT, MiRanda, PicTar, and TargetScan, have been developed to predict and rank microRNA target genes using indices such as seed-sequence conservation and thermodynamic binding stability [18]. This provides a research framework that enables in silico identification of

molecular pathways disrupted by disease-specific changes in microRNA levels, paving the way for subsequent validation through in vitro and in vivo experiments.

Because the pathogenesis of SSc comprises three major components (fibrosis, vasculopathy, and immune dysregulation) that are temporally and spatially intertwined, the involvement of microRNAs in each is described below (Table 1).

Table 1. Study-level evidence supporting the proposed roles of microRNAs in SSc. This table summarizes representative microRNAs according to their reported direction of alteration, proposed target and pathogenic domain, evidence source, validation, replication, and demonstrated mechanistic or clinical findings. "Up" and "down" indicate the direction reported in SSc-derived samples relative to the relevant controls and may differ among tissues. Evidence and validation codes describe the type of supporting data. Independent replication indicates confirmation by another group in a separate human cohort. Evidence-source codes: HT, human SSc tissue; PDC, patient-derived cells; AM, animal model; NS, non-SSc experimental model. Validation codes: E, differential expression or association; F, gain/loss-of-function in vitro; D, direct target assay; R, rescue experiment; V, in vivo validation. Abbreviations: dcSSc, diffuse cutaneous SSc; ECM, extracellular matrix; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; MVEC, microvascular endothelial cell; NA, not applicable; TSK, tight skin.

microRNA/ Entity	Direction/ Entity Type	Reported or Proposed Target	Proposed Pathogenic Domain	Evidence Source/ SSc Population/ Cohort Size	Validation/ Replication	Demonstrated Mechanistic Finding/ Actual Patient-Level Clinical Association
miR-29a-3p	down	COL1A1, COL1A2, COL3A1	Fibrosis	HT: lesional SSc skin, PDC: primary dermal fibroblasts from dcSSc, AM: bleomycin-induced skin fibrosis.	HT:E, PDC:E+F+D, AM:E, independent human replication	Reduced miR-29 family expression in SSc, restoration suppresses ECM/collagen-related programs.
miR-196a-5p	down	COL1A1, COL1A2	Fibrosis	HT: 5 dcSSc + 5 lcSSc and 5 controls, PDC: dermal fibroblasts from 5 early dcSSc (<2 years) and 5 matched controls, AM: TSK/+ skin (n = 3/group).	HT:E, PDC:E+F+D, AM:E single-study evidence	Reduced expression in SSc, mimic reduced collagen overexpression in SSc fibroblasts, inhibitor induced collagen in normal fibroblasts.
let-7a-5p	down	COL1A1, COL1A2	Fibrosis	HT: SSc skin (n = 7), PDC: SSc dermal fibroblasts, AM: bleomycin-induced skin fibrosis.	HT:E, PDC:E+F+D, AM:V single-study evidence	Reduced expression in SSc, mimic reduced collagen overexpression, inhibitor induced collagen in normal fibroblasts.
miR-150-5p	down	Integrin β3	Fibrosis	HT: 5 SSc and 5 controls, PDC: dermal fibroblasts from 5 early dcSSc (<2 years) and 5 matched controls, AM: TSK/+ skin.	HT:E, PDC:E+F+D, AM:E single-study evidence	Reduced expression in SSc, which induces integrin β3/TGF-β activation axis
miR-21-5p	up	Smad7	Fibrosis	HT: lcSSc and dcSSc skin in a preceding same-group expression study, PDC: SSc dermal fibroblasts, AM: bleomycin-induced skin fibrosis with bortezomib treatment.	HT:E, PDC:E+F+D, AM:E follow-up report from the same investigative group	TGF-β-induced miR-21-5p suppresses Smad7, and amplifies TGF-β/Smad signaling in fibroblasts.
miR-92a-3p	up	MMP-1	Fibrosis—potential intracellular role	PDC: primary dermal fibroblasts from early dcSSc (<2 years) and matched controls.	PDC:E+F single-study evidence	Increased expression in SSc, forced overexpression in normal fibroblasts reduced MMP-1 expression.

Table 1. *Cont.*

microRNA/ Entity	Direction/ Entity Type	Reported or Proposed Target	Proposed Pathogenic Domain	Evidence Source/ SSc Population/ Cohort Size	Validation/ Replication	Demonstrated Mechanistic Finding/ Actual Patient-Level Clinical Association
miR-126-3p	down	SPRED1, PIK3R2/ p85-β	Vasculopathy	HT: SSc and matched-control skin, PDC: 6 SSc-derived and 6 matched-control MVEC lines.	HT:E, PDC:E+F+R single-study evidence	Reduced expression in SSc MVECs, inhibitor impaired angiogenic responses in normal MVECs, mimic restored tube formation/migration in SSc MVECs, SPRED1 and PIK3R2 changed reciprocally.
miR-152-3p	down	DNMT1	Vasculopathy—preliminary	PDC: SSc-derived and healthy-control MVECs in a short symposium report.	PDC:E+F single-study evidence	Preliminary association with DNMT1 up-regulation and NOS3 repression.
miR-7-5p	up	COL1A2	Fibrosis	HT: lesional skin from 6 dcSSc and 8 controls, PDC: dermal fibroblasts from 5 early dcSSc (<2 years) and 5 matched controls.	HT/PDC:E, PDC:F+R single-study evidence	Increased expression interpreted as negative feedback related to TSP-2/COL1A2 regulation.
miR-155-5p	up	NLRP3	Fibrosis/immune dysregulation	HT: skin from 12 SSc patients, 7 patients with morphea, and 9 controls, NS: primary murine dermal fibroblasts, AM: bleomycin model with miR-155 knockout or topical antagomiR treatment, PDC: lung fibroblasts from 9 SSc-ILD patients and 5 controls, dermal fibroblasts from 5 SSc patients and 6 controls, NS: wild-type, NLRP3-knockout, and miR-155-knockout murine fibroblasts.	HT:E, PDC:E+F, NS:F+D+R, AM:V multiple studies, no independent clinical validation	Overexpression/requirement linked to NLRP3 inflammasome-mediated collagen synthesis, skin expression correlated positively with the extent of skin sclerosis.
miR-129-5p	down	COL1A1	Fibrosis/immune dysregulation	PDC: dermal fibroblasts from 5 early dcSSc (<2 years) and 5 matched healthy controls.	PDC:E+F+D single-study evidence	Disrupted IL-17A-miR-129-5p-COL1A1 suppressive axis promotes collagen accumulation in fibroblasts.
MIR146A rs2910164 C/G	NA—DNA variant within pre-miR-146a hairpin	NA	Vascular phenotype association	Human DNA association study: 52 Japanese patients with SSc and 107 healthy controls.	E single-study evidence	Associated with telangiectasia among patients.

2.1. Roles in Fibrosis

2.1.1. Excess Collagen Production Due to Depletion of Antifibrotic microRNAs

The best-known microRNA associated with SSc fibrosis is the miR-29 family (miR-29a, -29b, -29c) [19]. The family is referred to as a "master fibro-microRNA" and functions to suppress expression of major extracellular matrix (ECM) component genes such as type I, III, and IV collagens, fibronectin, and laminin [1]. In tissues and cultured dermal fibroblasts derived from skin lesions of SSc, miR-29 family expression is constitutively decreased [19]. The depletion of miR-29a-3p in cultured dermal fibroblasts is reported to be associated with stimulation by fibrosis-related cytokines such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF)-B, and interleukin (IL)-4 [19]. Reduced levels of miR-29 family members not only directly increase collagen production but also releases

repression of TGF-β-activated kinase 1 binding protein 1 (TAB1), a molecule related to the TGF-β signaling pathway, and up-regulates tissue inhibitor of metalloproteinases 1 (TIMP-1), which inhibits matrix metalloproteinases (MMPs) [20]. This creates a dual vicious cycle of enhanced ECM production and suppressed ECM degradation. Similarly, previous studies have indicated that miR-196a-5p and let-7a-5p are also antifibrotic microRNAs that regulate both α1(I) collagen (COL1A1) and α2(I) collagen (COL1A2) [3]. In cultured fibroblasts derived from lesional skin of early diffuse cutaneous SSc (dcSSc), expression of both microRNAs is suppressed by TGF-β signaling. In vitro, forced expression of miR-196a-5p mimics or let-7a-5p mimics in cultured dcSSc fibroblasts down-regulated collagen overexpression, whereas introducing inhibitors of these microRNAs into normal cultured fibroblasts induced collagen expression. These findings suggest that decreased expression of these microRNAs may be a direct cause of the pathological phenotype. In addition, miR-150-5p, which targets integrin β3, is also constitutively decreased in cultured early dcSSc fibroblasts [3]. Overexpression of integrin β3 reportedly promotes activation of latent TGF-β [21].

2.1.2. Aberrant Increase of Profibrotic microRNAs

While antifibrotic microRNAs decrease, overexpression of profibrotic microRNAs that actively drive fibrosis also exacerbates SSc pathogenesis. A representative example is miR-21-5p [1,22]. miR-21-5p, whose expression is induced by TGF-β1 stimulation in cultured dermal fibroblasts, targets and suppresses Smad7, an endogenous inhibitory factor of the TGF-β/Smad signaling pathway [22]. Furthermore, serum and fibroblast miR-92a-3p levels were increased in SSc, and forced miR-92a overexpression in normal dermal fibroblasts reduced MMP-1 expression [23]. These findings suggest a potential intracellular profibrotic role through reduced matrix degradation, although direct target validation and in vivo causality remain limited.

2.2. Roles in Vasculopathy

MicroRNAs also serve regulatory roles in maintaining endothelial cell function and angiogenesis. For example, miR-126-3p, one of the "angio-microRNAs" important for vascular homeostasis, promotes endothelial angiogenesis by suppressing negative regulators of the VEGF pathway, including SPRED1 and PIK3R2/p85-β [24]. Several previous studies have demonstrated that this microRNA is decreased in cultured endothelial cells of SSc patients, which may induce SSc-specific vascular abnormalities.

In addition, decreased miR-152-3p in cultured microvascular endothelial cells of SSc induces overexpression of its target DNMT1 [25]. Excess DNMT1 causes abnormal hypermethylation of gene promoter regions, suppressing the expression of genes involved in vasodilation and cell survival, and may trigger endothelial cell apoptosis, abnormal vasoconstriction, and even activation of surrounding fibroblasts in this disease. The main hypermethylated genes are endothelial nitric oxide synthase (eNOS; encoded by NOS3) and bone morphogenetic protein receptor type 2 (BMPR2), which in turn may lead to a decrease in NO synthesis, and a decrease in BMPR2 may increase apoptosis of endothelial cells [26–28]. Of note, the involvement of the miR-152-3p–DNMT1 axis in endothelial cells has mainly been observed in other diseases [26]; in SSc, it has only been reported in a short symposium publication [29]. Thus, the evidence for miR-152-3p–DNMT1–NOS3/BMPR2 is still preliminary in SSc.

Another mechanism that has recently attracted attention in SSc vasculopathy is endothelial-to-mesenchymal transition (EndoMT), in which endothelial cells acquire a fibroblast-like phenotype and begin secreting ECM. Although direct evidence in SSc remains limited, several microRNAs have been identified as mediators participating in

EndoMT [30]. For instance, increased expression of miR-148b-3p promotes angiogenic capacity, migration, and proliferation in cultured endothelial cells, whereas silencing this microRNA increases TGF-β-induced EndoMT [30,31], suggesting potential relevance to SSc. Moreover, miR-181b-5p is down-regulated in a rat model of pulmonary arterial hypertension (PAH). In that model, overexpression of miR-181b-5p reduces pulmonary vascular thickening, right ventricular remodeling, and EndoMT [30].

Compensatory network changes have also been observed. For example, in cultured early dcSSc fibroblasts, overexpression of miR-7-5p targeting COL1A2 is observed as a negative feedback response to decreased intracellular thrombospondin-2, which is involved in angiogenesis and ECM regulation [32].

In addition, single-nucleotide polymorphisms within microRNA gene sequences themselves (miRSNPs) correlate with clinical phenotypes. The MIR146A rs2910164 C/G miRSNP is located within the pre-miR-146a hairpin and is distinguished from the mature products miR-146a-5p and miR-146a-3p. The rs2910164 genotype in DNA derived from lesional skin was associated with telangiectasia [33]. Although this variant has been shown in non-SSc experimental systems to affect precursor processing and mature miR-146a production, this mechanism was not established in the SSc association study. Separately, mature miR-146a-5p has been suggested to influence cellular senescence by targeting NADPH oxidase 4 (NOX4), an enzyme that can generate reactive oxygen species (ROS) in the vascular wall [25].

2.3. Roles in Immune Dysregulation

miR-155-5p targets molecules such as the angiotensin II type 1 receptor (AT1R) and vascular endothelial growth factor (VEGF) receptor type 2, and controls angiogenesis in vitro [25]. At the same time, the microRNA is involved in inflammatory responses in macrophages and lymphocytes [34,35], and contributes to collagen expression in cultured dermal and lung SSc fibroblasts through the NLRP3 inflammasome [36]. Accordingly, miR-155-5p is considered one of the therapeutic target microRNAs in SSc animal models [37].

In addition, miR-21-5p, as described above, is a profibrotic microRNA that amplifies TGF-β signaling; it also contributes to Th1/Th17 balance and to a shift toward M2 macrophages in non-SSc experimental models [38,39]. Thus, there is a possibility that miR-21-5p is involved in both immune abnormalities and fibrosis, although evidence for its role in the pathogenesis of SSc is not adequate. Other pathways linking the immune system and fibrosis include abnormalities in miR-129-5p. Under normal conditions, IL-17A signaling induces miR-129-5p expression to suppress COL1A1 production. However, because IL-17 receptor expression is decreased in cultured dcSSc fibroblasts, this IL-17A–miR-129-5p antifibrotic suppressive axis is disrupted, thereby promoting collagen accumulation [40].

Furthermore, for example, serum let-7a-5p expression is associated with a lower frequency of anti-centromere antibody positivity [3], suggesting that changes in specific epigenetic states may be linked to particular autoantibody-production pathways.

3. microRNAs Associated with Extracellular Particles in the Pathogenesis of SSc

Extracellular vesicles (EVs) such as exosomes and apoptotic bodies are now recognized. In recent years, according to the Minimal Information for Studies of Extracellular Vesicles 2023 guidelines (MISEV2023), the term “extracellular particles” (EPs) is used as an umbrella term encompassing extracellular vesicles (EVs) and non-vesicular EPs (NVEPs). EVs are particles released from cells that are delimited by a lipid bilayer and cannot replicate on their own, whereas NVEPs are released multimolecular assemblies that lack a lipid bilayer.

When size-based terminology is required, "small EVs" and "large EVs" are operational terms, often referring to particles smaller than and larger than approximately 200 nm, respectively; however, measured diameter depends on the characterization method, and these terms should therefore be used cautiously. "Exosome" is a biogenesis-based term indicating origin from the endosomal system and should generally be used only when this subcellular origin has been demonstrated [41]. Because commonly used separation procedures may recover mixed preparations containing EVs, NVEPs, lipoproteins, ribonucleoprotein complexes, and soluble proteins, microRNAs measured in such fractions are referred to here as EP-associated unless their intravesicular localization has been directly demonstrated. Similarly, a biological response to an EP-enriched preparation cannot be attributed to a specific vesicular microRNA without appropriate fractionation, cargo-delivery assays, and microRNA-specific perturbation experiments [41].

Nakamura et al. reported altered particle abundance and dysregulated collagen-related microRNAs in preparations recovered from conditioned media of SSc dermal fibroblasts [42]. Exposure of normal dermal fibroblasts to preparations recovered from SSc fibroblast cultures also increased type I collagen expression. However, these parallel compositional and functional observations are consistent with, but do not by themselves establish, microRNA-mediated intercellular signaling.

Furthermore, Wermuth et al. reported that serum EP-enriched preparations from patients with SSc showed increased levels of six candidate profibrotic microRNAs—let-7g-5p, miR-17-5p, miR-23b-5p, miR-155-5p, miR-215-5p, and miR-503-5p—and decreased levels of ten candidate antifibrotic microRNAs—let-7a-5p, miR-29b-3p, miR-92a-3p, miR-129-5p, miR-133a-3p, miR-140-5p, miR-145-5p, miR-196a-5p, miR-200a-3p, and miR-223-3p [43]. These microRNAs are not specific to EPs; reduced let-7a-5p, miR-196a-5p, and miR-129-5p and increased miR-155-5p are broadly consistent with the tissue or intracellular findings described above, whereas several others show compartment-dependent differences, possibly reflecting selective loading into EPs or different cellular origins. In a separate functional experiment, exposure of normal dermal fibroblasts to the complete preparations increased the expression of ECM components, actin α2, smooth muscle (ACTA2), TGF-β, and connective tissue growth factor (CTGF). However, the active component or components of the preparations were not identified.

The terms "profibrotic" and "antifibrotic" in the original report were assigned from prior literature and do not represent functional testing of each microRNA in the EP-enriched preparations. These classifications are therefore context-dependent. In particular, although miR-92a-3p was decreased in the EP-enriched fraction and was categorized as antifibrotic in that report, intracellular fibroblast evidence in SSc suggests a potential profibrotic effect through suppression of MMP-1 expression [23]. EP-associated abundance may not mirror intracellular abundance or biological function, and no microRNA-specific experiment established that EP-associated miR-92a-3p mediated or opposed the fibroblast response.

In addition, when microRNA profiles within serum EPs were comprehensively compared by next-generation sequencing between patients of Raynaud's phenomenon with positive and negative antinuclear antibodies, multiple microRNAs with significantly different expression between the two groups were identified [44]. These findings support further evaluation of microRNAs measured in EP-enriched preparations as exploratory biomarkers.

4. Current Evidence for microRNA Biomarker Candidates in SSc

In general, RNA molecules are rapidly degraded by RNases when present in extracellular body fluids. However, circulating microRNAs present in body fluids such

as serum, plasma, urine, and saliva maintain high stability. This may be because microRNAs are protected from physical and chemical degradation by being encapsulated within lipid-bilayer-delimited EVs or by forming tight complexes with proteins such as Argonaute-2 or with high-density lipoprotein (HDL) [3]. In addition, compared with protein markers, microRNAs, whose expression is regulated at the transcriptional level, often respond more rapidly and sensitively to environmental changes. Therefore, circulating levels of microRNAs have attracted worldwide attention as promising candidate biomarkers for early diagnosis, risk stratification, and assessment of therapeutic responses.

On the other hand, one of the clinical challenges in SSc is the lack of reliable biomarkers that can objectively and early evaluate disease progression and severity. SSc-specific autoantibodies are useful for diagnosis and subgroup classification, but their titers are insufficient to reflect real-time changes in disease activity. Because SSc fibrosis often persists even after treatment, diagnosis and therapeutic intervention are desired at a very early stage before irreversible fibrosis and tissue remodeling are established (Figure 2).

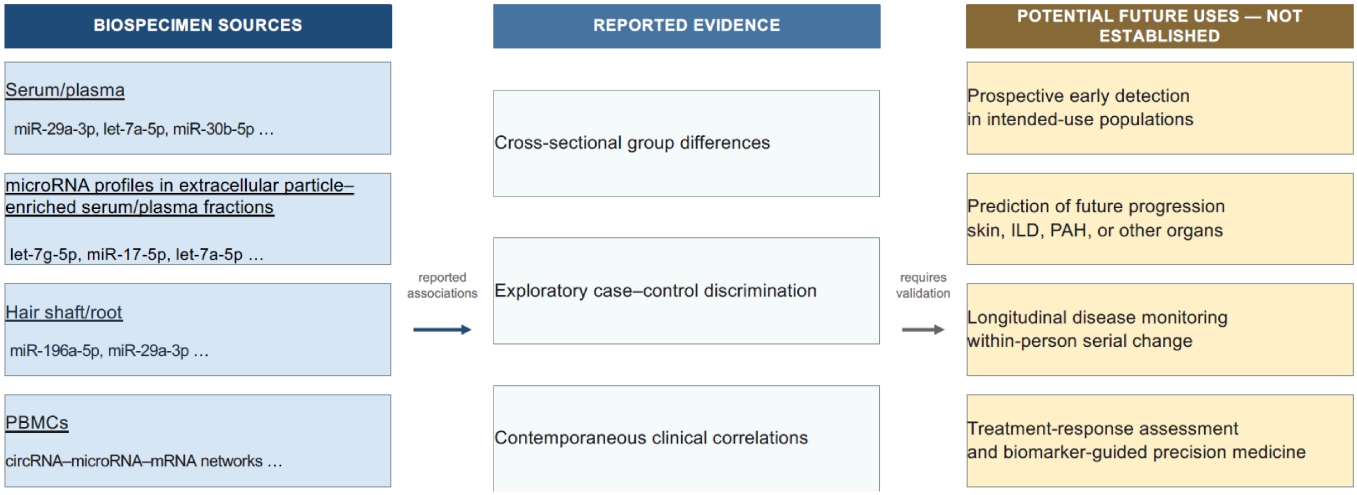


Figure 2. Candidate non-coding RNA biomarkers in SSc: current evidence and unvalidated future uses. The diagram distinguishes biospecimens and analytes investigated to date (**left**) from reported evidence (**center**) and potential clinical applications that remain unestablished (**right**). Current evidence consists mainly of cross-sectional group differences, exploratory case–control discrimination, and contemporaneous clinical correlations. Prospective and longitudinal validation in intended-use populations is required before these candidates can be applied to early detection, prediction of progression, disease monitoring, or treatment-response assessment. Abbreviation: ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; PBMC, peripheral blood mononuclear cell.

4.1. Circulating microRNA Biomarkers of SSc in Blood

The levels of specific microRNAs in circulating blood may reflect severity and specific clinical features of SSc (Table 2). In patients with scleroderma spectrum disorder (SSD), who do not fulfill the criteria of SSc but are considered at risk of developing SSc [45–47], serum miR-29a-3p levels were found to be significantly decreased compared with healthy controls and patients classified as having SSc [3]. The finding may indicate early dysregulation, but longitudinal studies are required to determine whether serum miR-29a-3p predicts progression to classified SSc.

Table 2. Study-level evidence for circulating and hair-shaft microRNA biomarker candidates in SSc. This table compares candidate microRNAs by specimen, cohort size, validation, independent replication, observed patient-level associations, and potential future uses. The final column lists applications that remain unvalidated. Because most studies are small and cross-sectional, and independent replication is limited, these candidates remain exploratory. Independent replication denotes confirmation by another group in a separate human cohort. Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; HC, healthy control; SSD, scleroderma spectrum disorder; dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; LSc, localized scleroderma; DM, dermatomyositis; SLE, systemic lupus erythematosus; ACA, anti-centromere antibody; TLCO, transfer factor of the lung for carbon monoxide; RVSP, right ventricular systolic pressure; mRSS, modified Rodnan skin score; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension.

Candidate/Specimen	Cohort Size	Validation Type	Independent Replication	Actual Patient-Level Clinical Association	Potential Future Use—Not Established
miR-29a-3p/serum	Cross-sectional: 61 SSc (23 dcSSc, 38 lcSSc), 12 SSD, and 20 HC.	Cross-sectional expression or clinical association only	Independent serum studies exist, but the SSD-specific decrease and RVSP association have not been replicated, reported directions are inconsistent.	Serum miR-29a was lower in SSD than in HC, lcSSc, or dcSSc. Within SSc, reduced levels were associated with higher Doppler-estimated RVSP.	Prospective prediction of transition from SSD to classifiable SSc, prediction of pulmonary vascular outcomes, serial monitoring.
let-7a-5p/serum	Cross-sectional: 39 SSc (20 dcSSc, 19 lcSSc), 17 HC, disease controls: 32 LSc, 8 SLE, and 8 DM.	Cross-sectional expression or clinical association only	No independent human replication identified.	Serum let-7a was lower in SSc and LSc than in HC. Among SSc, the low-level group had higher mRSS, more dcSSc, and lower frequency of ACA positivity than the high-level group.	Longitudinal tracking of skin fibrosis, treatment-associated change, or prognosis.
miR-30b-5p/serum	Cross-sectional: 50 SSc (18 dcSSc, 32 lcSSc) and 24 age- and sex-matched HC.	Cross-sectional expression or clinical association only	No independent human replication identified.	Serum level was lower in SSc than in HC and lower in dcSSc than in lcSSc. It correlated inversely with mRSS and was not associated with ILD.	Prediction of future skin progression, within-person monitoring of mRSS or treatment response.
miR-126-3p/serum	Cross-sectional: 45 SSc (18 dcSSc, 27 lcSSc, median disease duration 6 years) and 57 matched HC.	Cross-sectional expression or clinical association only	Later independent case-control data are discordant, the TLCO association has not been independently replicated.	No significant SSc-HC difference and substantial interindividual variability. A modest correlation with hemoglobin-corrected TLCO % predicted was observed, but levels did not differ by TLCO <70% vs. ≥70%, and the correlation was absent after dcSSc/lcSSc stratification.	Prediction of future TLCO decline, SSc-ILD/PAH progression, or treatment response.
miR-206 + miR-21-5p/serum	Pilot case-control derivation cohort: 15 SSc and 15 HC.	Cross-sectional expression or clinical association ROC in the same derivation cohort, no internal or external validation	No independent human validation of the two-microRNA classifier identified.	The six individual serum microRNAs did not differ significantly between groups. Apparent AUC was 0.70 for miR-206 and 0.73 for the miR-206/miR-21-5p combination.	Diagnostic validation in a clinically relevant intended-use population, prospective prognosis, longitudinal monitoring.

Table 2. *Cont.*

Candidate/Specimen	Cohort Size	Validation Type	Independent Replication	Actual Patient-Level Clinical Association	Potential Future Use—Not Established
miR-196a-5p/hair shaft	Cross-sectional: 11 SSc and 13 HC, occipital scalp sampling.	Cross-sectional expression or clinical association only	No independent human replication identified.	Hair-shaft level was lower in SSc, whereas serum and hair-root levels did not differ.	Diagnostic accuracy, longitudinal disease/treatment monitoring.
miR-29a-3p/hair shaft	Cross-sectional: 20 SSc, 13 HC, and 5 DM.	Cross-sectional expression or clinical association only	No independent human replication identified.	Hair-shaft level was lower in SSc than in HC or DM. Lower levels were associated with a higher prevalence of phalangeal contractures. Hair, skin, and serum levels did not show strong concordance.	Prediction of contracture progression, serial disease or treatment-response monitoring.

Decreased serum let-7a-5p levels in SSc patients were confirmed to correlate inversely with the modified Rodnan skin thickness score (mRSS), a validated semiquantitative clinical measure of skin thickness [3]. In addition, miR-30b-5p was identified as one of the most markedly decreased microRNAs in the sera of SSc patients. The microRNA is a direct inhibitor of PDGF receptor-β, which is involved in vascular function and fibrosis. Its serum level also shows an inverse correlation with mRSS [48], suggesting its potential utility as a marker of skin fibrosis severity rather than as an established diagnostic or prognostic biomarker.

In one cross-sectional study of 45 patients with SSc and 57 healthy controls, serum miR-126-3p did not differ significantly between SSc and control groups and showed substantial interindividual variability [49]. A modest positive correlation with hemoglobin-corrected transfer factor of the lung for carbon monoxide (TLCO) percentage predicted was observed within the SSc cohort ($r = 0.37$, $p = 0.02$); however, miR-126-3p levels did not distinguish patients with TLCO values below versus above 70%. This result does not establish serum miR-126-3p as a diagnostic or prognostic biomarker of pulmonary involvement, but may represent an exploratory association.

On the other hand, because the discriminatory performance of individual circulating microRNAs may be limited, combinations of candidate microRNAs have also been explored. In a pilot study, Koba et al. measured six serum microRNAs selected based on previous studies (let-7g-5p, miR-21-5p, miR-29b-3p, miR-125b-5p, miR-145-5p, and miR-206) by real-time PCR in 15 patients with SSc and 15 healthy controls, and the area under the curve (AUC) according to the receiver operating characteristic (ROC) curve was calculated. In this exploratory cohort, miR-206 had the highest AUC (0.70), whereas the combination of miR-206 and miR-21-5p yielded an apparent AUC of 0.73 for distinguishing patients with SSc from healthy controls, yielding a numerically higher apparent AUC [50]. However, the original report did not provide 95% confidence intervals, a formal statistical comparison between the AUCs, sufficient details to reproduce the combined score, or internal or external validation. Therefore, the modest numerical difference should not be interpreted as established incremental diagnostic performance. The miR-206/miR-21-5p combination should instead be regarded as a preliminary, unvalidated diagnostic, rather than prognostic, biomarker. Its ability to predict subsequent disease progression or clinical outcomes has not been evaluated.

4.2. Hair-Shaft microRNAs as Exploratory Biomarker Candidates

Due to narrowing of peripheral blood vessels and skin sclerosis, blood collection from peripheral venipuncture can be difficult in SSc patients. Because hair shafts are highly keratinized, largely anucleate structures, it was traditionally assumed that hair shafts contain little intact nuclear material. However, there is a possibility that microRNAs (perhaps protected by EPs or similar carriers) exist in a stable manner. Hair-shaft samples can be collected repeatedly without venipuncture and have therefore been explored as an alternative biospecimen. Although evidence for hair-shaft microRNAs in SSc remains limited to small cross-sectional reports from a single investigative group, for example, Wang et al. analyzed one hair root and five hair-shaft segments collected from the occipital scalp of 11 patients with SSc and 13 healthy controls at the time of serum sampling. Serum and hair-root miR-196a-5p levels did not differ significantly between the groups, whereas hair-shaft miR-196a-5p levels were lower in patients with SSc [51]. Also, hair-shaft miR-29a-3p levels were lower in 20 patients with SSc than in 13 healthy controls and five patients with dermatomyositis, and lower levels were associated with a higher prevalence of phalangeal contractures [52]. Because these reports evaluated different microRNAs and do not constitute independent replication, the findings should be regarded as preliminary and hypothesis-generating rather than as evidence for established diagnostic, prognostic, or longitudinal monitoring performance.

The original authors proposed that a growing hair shaft may temporally integrate small changes in circulating microRNAs and molecular alterations in adjacent skin or microvascular tissue. However, this hypothesis has not been experimentally demonstrated. The cellular source and mode of incorporation of hair-shaft microRNAs, the relationship between shaft position and calendar time, and the concordance of segmental hair measurements with longitudinal serum or tissue measurements remain unknown. Standardized segmental sampling, paired longitudinal measurements in hair, blood, and skin, validated contamination controls, extraction and normalization procedures, repeated within-person sampling, and independent multicenter cohorts are required before the clinical utility of hair-shaft microRNAs can be assessed.

5. Other Non-Coding RNAs in the Pathogenesis of SSc

With advances in next-generation sequencing technologies, not only microRNAs but also other classes of non-coding RNAs—long non-coding RNAs (lncRNAs) longer than 200 nucleotides, circular RNAs (circRNAs) with circular structures, yRNAs or PIWI-interacting RNAs (piRNAs)—have been indicated to be involved in the complex pathogenesis of SSc. These RNAs may function independently and also form complex networks with one another to comprehensively tune gene expression.

5.1. Epigenetic Regulation by lncRNAs

lncRNAs regulate gene expression through diverse mechanisms: nuclear lncRNAs can modify chromatin accessibility or recruit chromatin-modifying complexes, whereas cytoplasmic lncRNAs can regulate mRNA stability, translation, and microRNA availability. Thus, lncRNAs may connect fibrosis, vasculopathy, and immune activation through different subcellular mechanisms, as described below (Table 3).

Table 3. Study-level evidence for non-microRNA non-coding RNAs in SSc. This table summarizes representative lncRNAs, circRNAs, yRNAs, and piRNAs according to their proposed pathogenic domains, evidence sources, validation, replication, and demonstrated mechanistic or patient-level findings. The codes distinguish human observations from functional, animal, non-SSc, and computational evidence. Evidence-source codes: HT, human SSc tissue; CS, circulating sample; PDC, patient-derived cells; AM, animal model; NS, non-SSc experimental system; CP, computational prediction. Validation codes: E, differential expression or association; F, gain/loss-of-function in vitro; D, direct target assay; R, rescue or epistasis; V, in vivo validation. Abbreviations: AUC, area under the curve; ceRNA, competing endogenous RNA; ECM, extracellular matrix; EP, extracellular particle; ILD, interstitial lung disease; PBMC, peripheral blood mononuclear cell; PRESS, Prospective Registry of Early Systemic Sclerosis; ROC, receiver operating characteristic.

Molecule/Class	Proposed Pathogenic Domain	Evidence Source/ SSc Population/ Cohort Size	Validation Type	Independent Replication	Demonstrated Finding/ Actual Patient-Level Clinical Association
TSIX/lncRNA	Fibrosis	HT: 8 dcSSc + 5 lcSSc, 5 healthy controls, and 3 SLE disease controls. PDC: dermal fibroblasts from 3 female dcSSc and matched female healthy-control donors.	HT:E, PDC:E+F	single-study evidence, no independent human replication.	TSIX was increased in SSc skin and fibroblasts, and was induced by TGF-β1. siRNA knockdown reduced COL1A1/COL1A2 expression, type I collagen, and collagen-mRNA stability.
HOTAIR/lncRNA	Fibrosis	Full-thickness forearm biopsies from 12 adults with recent-onset SSc. Key expression experiments compared SSc skin/fibroblasts with healthy controls, a same-group follow-up used SSc dermal fibroblasts.	HT/PDC:E, PDC:F+R	same-group follow-up only, no independent human replication.	HOTAIR was increased in SSc skin and myofibroblasts. Ectopic HOTAIR increased collagen, ACTA2/α-SMA, and CTGF. EZH2 or Notch inhibition and restoration of miR-34a attenuated the profibrotic phenotype, the same-group follow-up linked this axis to GLI2.
H19X/lncRNA—fibroblast study	Fibrosis	Skin cohorts: 5 active untreated early dcSSc + 5 matched controls, PRESS 48 early dcSSc + 33 controls, 14 SSc + 6 controls, and 6 SSc + 6 controls. Lung: 11 end-stage SSc-ILD + 11 controls. Primary SSc and control dermal fibroblasts were used for functional studies.	HT:E, PDC:E+F+D+R	single-study evidence, expression reproduced across four skin cohorts and one SSc-ILD lung cohort within the same publication, no external independent replication.	H19X was up-regulated in SSc skin and lung and was induced by TGF-β. ASO silencing reduced ECM synthesis, myofibroblast differentiation, contractility, and survival. Chromatin-interaction assays implicated DDIT4L, DDIT4L knockdown partly reversed the antifibrotic effect of H19X silencing. In PRESS skin samples, higher H19X expression was significantly positively correlated with the fibroblast gene-expression signature.
H19X/lncRNA—endothelial study	Proposed vasculopathy/ immune dysregulation	Single-cell skin dataset: 27 dcSSc and 10 healthy controls. Functional experiments used healthy human dermal microvascular endothelial cells and mononuclear-cell adhesion assays.	HT:E, NS:F	single-study evidence, no independent human replication.	H19X was increased in SSc endothelial cells and induced by type I and II interferons. H19X knockdown in healthy endothelial cells reduced VCAM1 and other adhesion molecules, contractility, soluble VCAM1 release, and mononuclear-cell adhesion.

Table 3. *Cont.*

Molecule/Class	Proposed Pathogenic Domain	Evidence Source/ SSc Population/ Cohort Size	Validation Type	Independent Replication	Demonstrated Finding/ Actual Patient-Level Clinical Association
ENST00000313807/ lncRNA	Putative ceRNA network/fibrosis	Plasma EP-enriched preparations from 20 SSc and 20 age- and sex-matched healthy controls, discovery sequencing subset n = 6/group, RT-qPCR, ROC, and clinical analyses n = 20/group. Dual-luciferase assays were performed in 293T cells.	CS:E+CP, NS:D, same-cohort ROC	single-study evidence, no independent cohort or external classifier validation.	ENST00000313807 and COL1A1 were increased and miR-29-family members decreased in EP-enriched plasma fractions. Bioinformatics and dual-luciferase assays supported ENST00000313807-miR-29a-3p and miR-29a-3p-COL1A1 binding, Same-cohort combined AUC was 0.94, Higher ENST00000313807 levels showed significant positive correlations with higher HRCT scores and higher anti-Scl-70.
circ_0136255/ circRNA	Fibrosis-related network	PBMCs from 32 patients with active SSc and matched healthy controls. A bleomycin pulmonary-fibrosis model was used to study the murine homolog mmu_circ_0005373.	CS:E+CP, AM:E+F+V	single-study evidence, no independent human replication.	circ_0136255 was reduced in SSc PBMCs. The proposed human circ_0136255-miR-330-3p-TNFAIP3 network was computational. Functional and in vivo evidence for mTOR/4E-BP1 signaling pertained to the murine homolog. In human, circ_0136255 levels showed significant cross-sectional associations with the degree of lung involvement and MEF25.
yRNA3/yRNA	Fibrosis	Cultured dermal fibroblasts from patients with dcSSc and healthy-control donors.	PDC:E+F	single-study evidence, no independent replication.	yRNA3 was increased in cultured SSc dermal fibroblasts. siRNA-mediated yRNA3 knockdown reduced COL1A1 expression.
piR-32368/piRNA	Fibrosis	PDC: dermal fibroblasts from 3 early dcSSc patients (<2 years from onset of skin thickening) and 3 healthy controls.	PDC:E+F, CP	single-study evidence, no independent replication.	piR-32368 was increased in SSc fibroblasts and induced by TGF-β1. Synthetic overexpression in normal fibroblasts reduced COL1A1 mRNA and protein but not COL1A2.

TSIX: TSIX is an antisense transcript at the X-inactive specific transcript (XIST) locus. TSIX is overexpressed in cultured dermal fibroblasts from dcSSc [53]. This high TSIX expression was induced by TGF-β1. Experimental knockdown of TSIX using siRNA decreased collagen mRNA stability and consequently suppressed excessive type I collagen expression in cultured early dcSSc fibroblasts. Serum TSIX levels are also increased in patients with SSc. Taken together, these findings support a profibrotic role for TSIX through stabilization of collagen mRNA in SSc fibroblasts. However, the above study does not directly demonstrate disruption of X-chromosome inactivation or explain female susceptibility to SSc. Any link between TSIX dysregulation, altered X-chromosome inactivation, and the female predominance of SSc proposed in the paper should therefore be regarded as hypothesis-generating and requires direct validation.

HOX transcript antisense RNA (HOTAIR): HOTAIR is a lncRNA with increased expression in both cultured fibroblasts and lesional tissues from SSc patients [54]. HOTAIR

promotes EZH2-dependent repression of miR-34a, thereby activating NOTCH signaling and the profibrotic phenotype [54].

H19X: H19X (MIR503HG), a lncRNA induced by TGF-β, stimulates differentiation of myofibroblasts [55]. In addition, H19X modifies chromatin accessibility near DDIT4L and supports TGF-β-driven ECM production. Furthermore, single-cell RNA sequencing data showed that H19X expression is higher in SSc endothelial cells than in healthy-control endothelial cells [56]. Because H19X also promotes endothelial VCAM1 expression and mononuclear-cell adhesion [56], H19X may be involved in vasculopathy and immune dysfunction as well as tissue fibrosis.

Competing endogenous RNA: A competing endogenous RNA (ceRNA) network can form in which lncRNAs, microRNAs, and ultimately target mRNAs competitively regulate one another's expression levels [57]. In SSc pathogenesis, abnormalities in such a ceRNA network were identified in a 2023 multi-omics study. Specifically, high expression of lncRNA ENST00000313807 in plasma EPs from SSc patients was confirmed [58]. ENST00000313807 may act as a sponge that sequesters and inactivates miR-29a-3p, a master fibro-microRNA in SSc. As a result, repression of COL1A1 mRNA by miR-29a-3p may be released, leading to increased COL1A1 mRNA, excessive collagen production, and tissue fibrosis. Although causal perturbation/rescue was not shown in SSc-relevant systems, abnormalities of this putative "ENST00000313807–hsa-miR-29a-3p–COL1A1 axis" correlated with several clinical and immunological indices, including the extent of lung lesions assessed by high-resolution computed tomography (HRCT), Scl-70 antibody levels, and CRP. ROC curve analyses further indicated that considering the entire ceRNA network as a combined biomarker showed a higher apparent AUC in the same cohort than using a single marker.

5.2. Functions of circRNAs

circRNAs are commonly generated by back-splicing, in which a downstream 5′ splice donor is joined to an upstream 3′ splice acceptor. This circular structure makes them less susceptible to exonuclease-mediated degradation, conferring higher stability than linear RNAs in blood and tissues [59,60]. In addition, circRNAs may also interact with RNA-binding proteins, regulate transcription or splicing, and undergo translation in selected cases.

A frequently discussed function of circRNAs is that they can act as "microRNA sponges (decoys)" by harboring multiple binding sites complementary to specific microRNA sequences (microRNA binding sites), thereby sequestering and inhibiting intracellular microRNAs. Physiologically relevant ceRNA activity also depends on RNA abundance, binding-site number, and stoichiometry. For example, circ_0136255 is decreased in peripheral blood mononuclear cells in SSc, and its levels showed significant cross-sectional associations with the degree of lung involvement [61]. The putative circ_0136255/miR-330-3p/TNFAIP3 ceRNA axis was computationally proposed. The circRNA may correlate with eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)/mTOR signaling, although direct functional validation was lacking.

5.3. Involvement of yRNAs and piRNAs

yRNA: yRNAs are small non-coding RNAs of several tens to hundreds of nucleotides that are evolutionarily highly conserved from bacteria to humans. They possess conserved sequences at the 5′ and 3′ ends and form a characteristic stem–loop structure. Ro60 protein targeted by autoantibodies in systemic lupus erythematosus or Sjögren's disease binds to part of this stem–loop, and La proteins also bind to a poly-U structure at the 3′ end, maintaining the structural stability of yRNAs. Although the detailed functions of yRNAs had been unknown, studies have shown that they are required for initiation

of chromosomal DNA replication in experimental systems [62], and for regulating the functions of other non-coding RNAs. In SSc, yRNA3 is up-regulated in cultured dermal fibroblasts [63]. Experimental knockdown of yRNA3 using siRNA suppressed COL1A1 expression in cultured dcSSc fibroblasts, suggesting that yRNA3 contributes to pathways enhancing collagen expression. In contrast, yRNA4 levels are down-regulated in the sera of SSc patients compared with healthy controls, indicating that elucidation of functional partitioning and secretion mechanisms for yRNAs between tissues and circulation is needed. In addition, extracellular yRNA fragments can influence innate immune signaling [64,65]. However, such immune functions have not yet been established in SSc.

piRNA: piRNAs are a class of small non-coding RNAs discovered relatively recently [66]. piRNAs are generated through a specific biogenesis process from primary transcripts containing piRNA clusters, interact with PIWI proteins, bind specific target molecules, and can recruit chromatin-modifying factors to enable transcriptional repression [67,68]. Aberrant expression of PIWI proteins and piRNAs has been reported in human cancers; PIWI/piRNA complexes are implicated in tumorigenesis and associated with prognosis. In the study by Tanaka et al., no differences were observed in PIWI-like proteins (PIWIL)1–4 levels between cultured normal dermal fibroblasts and cultured SSc dermal fibroblasts [66]. On the other hand, among piRNAs predicted to target SSc-related molecules by web-based target gene prediction, the study identified a significant increase in piR-32368 in cultured dcSSc dermal fibroblasts, which was thought to result from endogenous TGF-β signaling. Forced overexpression of piR-32368 in normal cultured dermal fibroblasts decreased COL1A1 expression at both mRNA and protein levels but did not affect COL1A2. Therefore, piR-32368 overexpression in SSc fibroblasts may function as a negative feedback response to collagen overexpression and suggests that piRNAs may be candidate regulators requiring validation. However, PIWI loading, endogenous loss-of-function, and chromatin effects of piRNA have not been demonstrated in this disease.

Both yRNA3 and piR-32368 regulated only COL1A1. These findings indicate that the experimental manipulation selectively affected COL1A1 but not COL1A2; the underlying mechanism remains to be determined.

Mechanistically, the non-microRNA classes discussed above should not be regarded merely as additional examples of non-coding RNAs, but as regulatory layers that surround and interact with microRNA-mediated gene silencing. First, although not determined in SSc, nuclear lncRNAs and selected PIWI–piRNA complexes can regulate chromatin accessibility, histone modifications, DNA methylation, enhancer–promoter communication, and transcriptional activity [69–73]. Second, similar to ceRNAs, cytoplasmic lncRNAs and circRNAs can modulate post-transcriptional regulation by altering mRNA stability, RNA-binding protein availability, and microRNA activity [60,73–76]. Third, several non-coding RNA classes can influence translation either indirectly, by releasing mRNAs from microRNA-mediated translational repression, or directly, by interacting with translational regulators and by serving as templates for small peptides [6,60,73]. Lastly, extracellular non-coding RNAs, including EP-associated microRNAs, lncRNAs, circRNAs, and yRNA fragments, may contribute to immune signaling [64,65,77,78]. Therefore, the biological impact of microRNA and other non-coding RNAs in SSc should be interpreted as a network effect that integrates chromatin regulation, RNA stability, and translation.

Importantly, these mechanisms are not mutually exclusive. For example, profibrotic lncRNAs can simultaneously recruit chromatin-modifying proteins, stabilize collagen-related transcripts, and compete with antifibrotic microRNAs [53,54,58]. Likewise, a circRNA may function as a microRNA sponge in one context but as an RNA-binding protein scaffold, transcriptional regulator, or immune-stimulatory RNA in another [60,75,79,80].

This mechanistic diversity is particularly relevant to SSc, in which fibrosis, vasculopathy, and immune activation are tightly interconnected.

6. Future Perspectives and Applications to Therapeutic Interventions

Taken together, non-coding RNAs act at distinct but interconnected regulatory levels. Beyond the non-coding RNA classes discussed above, additional regulatory layers may contribute to SSc. Disease-associated non-coding variants can alter enhancer activity, lncRNA expression or structure, and long-range enhancer–promoter communication within the three-dimensional genome [81–83]. As one example of disease-associated SNPs affecting lncRNA expression and interactions, rs11117420 was reported as an expression quantitative trait locus for lncRNA RP11-542M13.3 [84]. In SSc skin, cell-type-resolved chromatin-accessibility profiling showed that accessible elements in skin-resident dendritic cells were enriched for SSc risk variants and associated with the extent of skin fibrosis [85]. Active enhancers may also generate enhancer RNAs that contribute to transcriptional regulation, although their functional relevance in SSc remains unknown [86,87]. RNA modifications such as N6-methyladenosine can influence RNA splicing, stability, localization, and translation; current evidence in SSc remains largely preclinical [88,89]. Alternative polyadenylation provides a more direct SSc example: reduced CFIm25, encoded by NUDT21, in SSc skin and fibroblasts promotes 3′-UTR shortening of profibrotic transcripts, which can reduce microRNA-mediated repression and increase protein expression [90]. Rare non-coding variants may also perturb polyadenylation signals and generate tissue-specific alternative-polyadenylation outliers [91]. Future integration of genomic, single-cell epigenomic, chromatin-conformation, long-read RNA-sequencing, and epitranscriptomic data may clarify how these regulatory layers converge in disease-relevant cell states.

Non-coding RNA research is not only deepening our understanding of pathogenesis in human diseases but is also pointing toward fundamental disease-modifying therapies using RNA-based approaches. For example, an antisense oligonucleotide targeting miR-122-5p, which is essential for hepatitis C virus replication (miravirsen), has already demonstrated sustained antiviral efficacy and safety in a randomized, placebo-controlled phase 2a trial, providing proof of concept for microRNA-targeted therapy [92]. On the other hand, in the cardiovascular field, an anti-miR-132-3p agent (CDR132L) in a phase 1b trial for patients with heart failure showed decreased plasma miR-132-3p and trends toward improvement in clinical/biomarker measures such as reduced NT-proBNP [93]. More recently, the randomized phase 2 trial showed pharmacodynamic target engagement and acceptable tolerability, but no significant improvement in the primary endpoint or principal secondary endpoints compared with placebo [94], illustrating that pharmacodynamic target engagement does not necessarily translate into clinical benefit.

In SSc-related preclinical studies, systemic let-7a supplementation and inhibition of profibrotic microRNAs such as miR-155 have attenuated bleomycin-induced skin fibrosis, whereas restoration of miR-29 has primarily been supported by functional studies in SSc fibroblasts and fibrotic models outside SSc [3,19,37,95]. Compared with conventional molecular targeted drugs (small molecules or biologics) that basically inhibit only a single protein or receptor, the greatest advantage of microRNA-based therapy is that a single microRNA may simultaneously and comprehensively normalize multiple pathogenic targets, including collagens, components of the TGF-β signaling pathway, and even angiogenic factors. This is a favorable feature for treating SSc, a multifactorial disease.

With future research and development, non-invasive biomarker panels (microRNA signatures) using circulating EPs or hair may enable early diagnosis and prediction of future organ involvement (skin sclerosis, interstitial lung disease, PAH). This could realize

precision medicine in which "personalized nucleic acid therapeutics" are administered according to each patient's epigenetic disruption profile during the window of opportunity before irreversible fibrosis is established.

7. Limitations and Future Issues

Because this paper was a focused narrative synthesis rather than a systematic review, the literature coverage was not exhaustive.

Most of the findings summarized in this review are supported by only a single study, and do not have enough validation and evidence, with the exception of a very small number of microRNAs, including miR-29. Their reproducibility has not been sufficiently demonstrated, and it would be desirable to build up a body of research. There is currently limited evidence, and they await validation by other researchers. Also, findings concerning lncRNA, circRNA, yRNA, and piRNA remain preliminary and require independent validation.

Furthermore, circulating microRNA levels should currently be regarded as exploratory biomarker candidates. Clinical translation requires that multiple issues be addressed, including independent cohorts, longitudinal sampling, disease controls, pre-analytical variables, normalization strategies, hemolysis control, isolation protocols for EPs, assay reproducibility, and performance against existing clinical measures such as autoantibodies, nailfold capillaroscopy, mRSS, HRCT, and pulmonary function testing. For example, measured circulating RNA levels are strongly affected by pre-analytical and analytical factors, which may contribute to inconsistent findings among studies. Serum and plasma are not interchangeable because coagulation, hemolysis, residual platelets or blood cells, anticoagulants, processing delays, centrifugation, storage, and freeze–thaw cycles can alter RNA profiles. Future studies should therefore report sample type and handling conditions, and use a consistent, validated extraction protocol, as RNA recovery also varies with input volume, carrier use, and extraction method [96–98]. In addition, normalization remains challenging because biofluids contain little RNA and lack a universally stable housekeeping transcript. Synthetic non-human spike-ins can monitor extraction, reverse transcription, and amplification efficiency, but do not correct for biological variation or cellular contamination. Endogenous controls such as miR-16 or U6 should be validated in the relevant sample type and cohort, preferably using multiple stable reference microRNAs or a prespecified data-driven approach [96,99,100]. Small-RNA sequencing enables broad discovery but is affected by low-input and library-preparation biases, whereas reverse transcription quantitative polymerase chain reaction (RT-qPCR) is suitable for targeted validation but depends on assay efficiency, reference selection, and the handling of undetected values. Sequencing candidates should therefore be confirmed using an orthogonal method and an independent cohort. Finally, samples from cases and controls should be randomized and balanced across extraction, assay, library-preparation, and sequencing batches, with technical controls and batch metadata used to identify and adjust for batch effects.

Hair-shaft microRNA measurements may be influenced by matrix-specific biological and pre-analytical factors that were not comprehensively evaluated as determinants of the reported SSc associations. Cosmetic hair treatments, including dyeing, bleaching, perming, and chemical straightening, as well as topical hair or scalp products, may affect shaft integrity, surface contamination, or RNA recovery and should therefore be documented. Sampling should be standardized with respect to anatomical site, the distance of the analyzed segment from the scalp, segment length, and, where ascertainable, hair-growth phase. External contamination and decontamination procedures, extraction efficiency from highly keratinized shafts, and the choice and matrix-specific stability of normalization controls also require validation. Age, ethnicity, hair characteristics, and local scalp disease or

alopecia may confound between-group comparisons and should be recorded and addressed through matching, exclusion criteria, stratification, or multivariable analysis.

The therapeutic findings summarized above should be regarded as proof-of-concept rather than evidence for clinically actionable treatment for SSc. Sample size limitations or correlative (rather than causal) evidence remain major hurdles to be addressed. In addition, optimization of drug-delivery systems to safely and efficiently deliver nucleic acids to target organs and cells (e.g., skin and fibroblasts), the control of unexpected adverse effects due to off-target actions, variability across SSc subsets, dosing, immunogenicity, chemical modifications, biodistribution, or durability remain major issues for clinical application. Non-coding RNA research may eventually contribute to improved disease stratification and therapy, but substantial mechanistic and clinical validation is still required.

Author Contributions: Validation, M.N. and M.J.; data curation, M.N.; writing—original draft preparation, D.U. and M.N.; writing—review and editing, M.J.; visualization, M.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; α-SMA, alpha-smooth muscle actin; ACTA2, actin alpha 2, smooth muscle; AT1R, angiotensin II type 1 receptor; AUC, area under the curve; BMPR2, bone morphogenetic protein receptor type 2; ceRNA, competing endogenous RNA; circRNA, circular RNA; CTGF, connective tissue growth factor; dcSSc, diffuse cutaneous systemic sclerosis; ECM, extracellular matrix; EndoMT, endothelial-to-mesenchymal transition; eNOS, endothelial nitric oxide synthase; EP, extracellular particle; EV, extracellular vesicle; HDL, high-density lipoprotein; HOTAIR, HOX transcript antisense RNA; HRCT, high-resolution computed tomography; IL, interleukin; lncRNA, long non-coding RNA; miRSNP, microRNA-related single-nucleotide polymorphism; MMP, matrix metalloproteinase; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; PDGF, platelet-derived growth factor; piRNA, PIWI-interacting RNA; PIWIL1–4, PIWI-like proteins 1–4; RISC, RNA-induced silencing complex; ROC, receiver operating characteristic; ROS, reactive oxygen species; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SNP, single-nucleotide polymorphism; SSc, systemic sclerosis; SSD, scleroderma spectrum disorder; TAB1, TGF-β-activated kinase 1-binding protein 1; TGF-β, transforming growth factor beta; TIMP-1, tissue inhibitor of metalloproteinases 1; TLCO, transfer factor of the lung for carbon monoxide; TSIX, XIST antisense RNA; UTR, untranslated region; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2.

References

1. Jimenez, S.A.; Mendoza, F.A.; Piera-Velazquez, S. A review of recent studies on the pathogenesis of Systemic Sclerosis: Focus on fibrosis pathways. *Front. Immunol.* **2025**, *16*, 1551911. [CrossRef]
2. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **1993**, *75*, 843–854. [CrossRef]
3. Jinnin, M. Various applications of microRNAs in skin diseases. *J. Dermatol. Sci.* **2014**, *74*, 3–8. [CrossRef]
4. Kozomara, A.; Birgaoanu, M.; Griffiths-Jones, S. miRBase: From microRNA sequences to function. *Nucleic Acids Res.* **2019**, *47*, D155–D162. [CrossRef]
5. Lee, Y.; Kim, M.; Han, J.; Yeom, K.H.; Lee, S.; Baek, S.H.; Kim, V.N. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* **2004**, *23*, 4051–4060. [CrossRef]
6. Bartel, D.P. Metazoan MicroRNAs. *Cell* **2018**, *173*, 20–51. [CrossRef]

7. Lee, Y.; Ahn, C.; Han, J.; Choi, H.; Kim, J.; Yim, J.; Lee, J.; Provost, P.; Rådmark, O.; Kim, S.; et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature* **2003**, *425*, 415–419. [CrossRef]
8. Han, J.; Lee, Y.; Yeom, K.H.; Kim, Y.K.; Jin, H.; Kim, V.N. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* **2004**, *18*, 3016–3027. [CrossRef]
9. Lund, E.; Güttinger, S.; Calado, A.; Dahlberg, J.E.; Kutay, U. Nuclear export of microRNA precursors. *Science* **2004**, *303*, 95–98. [CrossRef]
10. Hutvágner, G.; McLachlan, J.; Pasquinelli, A.E.; Bálint, E.; Tuschl, T.; Zamore, P.D. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* **2001**, *293*, 834–838. [CrossRef]
11. Lytle, J.R.; Yario, T.A.; Steitz, J.A. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 9667–9672. [CrossRef]
12. Qin, W.; Shi, Y.; Zhao, B.; Yao, C.; Jin, L.; Ma, J.; Jin, Y. miR-24 regulates apoptosis by targeting the open reading frame (ORF) region of FAF1 in cancer cells. *PLoS ONE* **2010**, *5*, e9429. [CrossRef]
13. Brennecke, J.; Stark, A.; Russell, R.B.; Cohen, S.M. Principles of microRNA-target recognition. *PLoS Biol.* **2005**, *3*, e85. [CrossRef]
14. Friedman, R.C.; Farh, K.K.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* **2009**, *19*, 92–105. [CrossRef]
15. Sheu-Gruttadauria, J.; Xiao, Y.; Gebert, L.F.; MacRae, I.J. Beyond the seed: Structural basis for supplementary microRNA targeting by human Argonaute2. *EMBO J.* **2019**, *38*, e101153. [CrossRef]
16. Brodersen, P.; Sakvarelidze-Achard, L.; Bruun-Rasmussen, M.; Dunoyer, P.; Yamamoto, Y.Y.; Sieburth, L.; Voinnet, O. Widespread translational inhibition by plant miRNAs and siRNAs. *Science* **2008**, *320*, 1185–1190. [CrossRef]
17. Baek, D.; Villén, J.; Shin, C.; Camargo, F.D.; Gygi, S.P.; Bartel, D.P. The impact of microRNAs on protein output. *Nature* **2008**, *455*, 64–71. [CrossRef]
18. Maragkakis, M.; Alexiou, P.; Papadopoulos, G.L.; Reczko, M.; Dalamagas, T.; Giannopoulos, G.; Goumas, G.; Koukis, E.; Kourtis, K.; Simossis, V.A.; et al. Accurate microRNA target prediction correlates with protein repression levels. *BMC Bioinform.* **2009**, *10*, 295. [CrossRef]
19. Maurer, B.; Stanczyk, J.; Jüngel, A.; Akhmetshina, A.; Trenkmann, M.; Brock, M.; Kowal-Bielecka, O.; Gay, R.E.; Michel, B.A.; Distler, J.H.; et al. MicroRNA-29, a key regulator of collagen expression in systemic sclerosis. *Arthritis Rheum.* **2010**, *62*, 1733–1743. [CrossRef]
20. Ciechomska, M.; O'Reilly, S.; Suwara, M.; Bogunia-Kubik, K.; van Laar, J.M. MiR-29a reduces TIMP-1 production by dermal fibroblasts via targeting TGF-beta activated kinase 1 binding protein 1, implications for systemic sclerosis. *PLoS ONE* **2014**, *9*, e115596. [CrossRef]
21. Asano, Y.; Ihn, H.; Yamane, K.; Jinnin, M.; Mimura, Y.; Tamaki, K. Increased expression of integrin αvβ3 contributes to the establishment of autocrine TGF-β signaling in scleroderma fibroblasts. *J. Immunol.* **2005**, *175*, 7708–7718. [CrossRef]
22. Zhu, H.; Luo, H.; Li, Y.; Zhou, Y.; Jiang, Y.; Chai, J.; Xiao, X.; You, Y.; Zuo, X. MicroRNA-21 in scleroderma fibrosis and its function in TGF-beta-regulated fibrosis-related genes expression. *J. Clin. Immunol.* **2013**, *33*, 1100–1109. [CrossRef]
23. Sing, T.; Jinnin, M.; Yamane, K.; Honda, N.; Makino, K.; Kajihara, I.; Makino, T.; Sakai, K.; Masuguchi, S.; Fukushima, S.; et al. microRNA-92a expression in the sera and dermal fibroblasts increases in patients with scleroderma. *Rheumatology* **2012**, *51*, 1550–1556. [CrossRef]
24. Wang, Y.; Sun, J.; Kahaleh, B. Epigenetic down-regulation of microRNA-126 in scleroderma endothelial cells is associated with impaired responses to VEGF and defective angiogenesis. *J. Cell. Mol. Med.* **2021**, *25*, 7078–7088. [CrossRef]
25. Rusek, M. Angiogenic microRNAs in Systemic Sclerosis: Insights into Microvascular Dysfunction and Therapeutic Implications. *Genes* **2025**, *16*, 1057. [CrossRef]
26. Huang, J.; Wang, Y.; Guo, Y.; Sun, S. Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1. *Hepatology* **2010**, *52*, 60–70. [CrossRef]
27. Wang, Y.; Kahaleh, B. Epigenetic repression of bone morphogenetic protein receptor II expression in scleroderma. *J. Cell. Mol. Med.* **2013**, *17*, 1291–1299. [CrossRef]
28. Wang, Y.; Altorok, N.; Kahaleh, B. Potential beneficial role for endothelin in scleroderma vasculopathy: Inhibition of endothelial apoptosis by type B endothelin-receptor signaling. *J. Scleroderma Relat. Disord.* **2016**, *1*, 213–219. [CrossRef]
29. Wang, Y.; Kahaleh, B. Epigenetic Repression of eNOS in Scleroderma (SSc) Microvascular Endothelial Cells (MVECs) is Related to the Downregulation of MicroRNA-152 by Enhanced DNA Methyltransferase 1 (Dnmt1) Expression. *Translation* **2023**, *11*, e1–e2. [CrossRef]
30. Di Benedetto, P.; Ruscitti, P.; Berardicurti, O.; Vomero, M.; Navarini, L.; Dolo, V.; Cipriani, P.; Giacomelli, R. Endothelial-to-mesenchymal transition in systemic sclerosis. *Clin. Exp. Immunol.* **2021**, *205*, 12–27. [CrossRef]
31. Miscianinov, V.; Martello, A.; Rose, L.; Parish, E.; Cathcart, B.; Mitić, T.; Gray, G.A.; Meloni, M.; Al Haj Zen, A.; Caporali, A. MicroRNA-148b Targets the TGF-beta Pathway to Regulate Angiogenesis and Endothelial-to-Mesenchymal Transition during Skin Wound Healing. *Mol. Ther.* **2018**, *26*, 1996–2007. [CrossRef]

32. Kajihara, I.; Jinnin, M.; Yamane, K.; Makino, T.; Honda, N.; Igata, T.; Masuguchi, S.; Fukushima, S.; Okamoto, Y.; Hasegawa, M.; et al. Increased accumulation of extracellular thrombospondin-2 due to low degradation activity stimulates type I collagen expression in scleroderma fibroblasts. *Am. J. Pathol.* **2012**, *180*, 703–714. [CrossRef]
33. Sakoguchi, A.; Jinnin, M.; Makino, T.; Kajihara, I.; Makino, K.; Honda, N.; Nakayama, W.; Inoue, K.; Fukushima, S.; Ihn, H. The miR-146a rs2910164 C/G polymorphism is associated with telangiectasia in systemic sclerosis. *Clin. Exp. Dermatol.* **2013**, *38*, 99–100. [CrossRef]
34. O'Connell, R.M.; Taganov, K.D.; Boldin, M.P.; Cheng, G.; Baltimore, D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 1604–1609. [CrossRef]
35. Hu, J.; Huang, S.; Liu, X.; Zhang, Y.; Wei, S.; Hu, X. miR-155: An Important Role in Inflammation Response. *J. Immunol. Res.* **2022**, *2022*, 7437281. [CrossRef]
36. Artlett, C.M.; Sassi-Gaha, S.; Hope, J.L.; Feghali-Bostwick, C.A.; Katsikis, P.D. Mir-155 is overexpressed in systemic sclerosis fibroblasts and is required for NLRP3 inflammasome-mediated collagen synthesis during fibrosis. *Arthritis Res. Ther.* **2017**, *19*, 144. [CrossRef]
37. Yan, Q.; Chen, J.; Li, W.; Bao, C.; Fu, Q. Targeting miR-155 to Treat Experimental Scleroderma. *Sci. Rep.* **2016**, *6*, 20314. [CrossRef]
38. Murugaiyan, G.; da Cunha, A.P.; Ajay, A.K.; Joller, N.; Garo, L.P.; Kumaradevan, S.; Yosef, N.; Vaidya, V.S.; Weiner, H.L. MicroRNA-21 promotes Th17 differentiation and mediates experimental autoimmune encephalomyelitis. *J. Clin. Investig.* **2015**, *125*, 1069–1080. [CrossRef]
39. Xue, J.; Xiao, T.; Wei, S.; Sun, J.; Zou, Z.; Shi, M.; Sun, Q.; Dai, X.; Wu, L.; Li, J.; et al. miR-21-regulated M2 polarization of macrophage is involved in arsenicosis-induced hepatic fibrosis through the activation of hepatic stellate cells. *J. Cell. Physiol.* **2021**, *236*, 6025–6041. [CrossRef]
40. Nakashima, T.; Jinnin, M.; Yamane, K.; Honda, N.; Kajihara, I.; Makino, T.; Masuguchi, S.; Fukushima, S.; Okamoto, Y.; Hasegawa, M.; et al. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts. *J. Immunol.* **2012**, *188*, 3573–3583. [CrossRef]
41. Welsh, J.A.; Goberdhan, D.C.I.; O'Driscoll, L.; Buzas, E.I.; Blenkiron, C.; Bussolati, B.; Cai, H.; Di Vizio, D.; Driedonks, T.A.P.; Erdbrügger, U.; et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J. Extracell. Vesicles* **2024**, *13*, e12404. [CrossRef]
42. Nakamura, K.; Jinnin, M.; Harada, M.; Kudo, H.; Nakayama, W.; Inoue, K.; Ogata, A.; Kajihara, I.; Fukushima, S.; Ihn, H. Altered expression of CD63 and exosomes in scleroderma dermal fibroblasts. *J. Dermatol. Sci.* **2016**, *84*, 30–39. [CrossRef]
43. Wermuth, P.J.; Piera-Velazquez, S.; Jimenez, S.A. 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*, 21–30.
44. Piera-Velazquez, S.; Fortina, P.; Jimenez, S.A. Comparison of serum exosome miRNA from patients with Raynaud's phenomenon with positive and negative serum antinuclear antibodies. *Clin. Exp. Rheumatol.* **2024**, *42*, 1629–1634. [CrossRef]
45. Maricq, H.R.; McGregor, A.R.; Diat, F.; Smith, E.A.; Maxwell, D.B.; LeRoy, E.C.; Weinrich, M.C. Major clinical diagnoses found among patients with Raynaud phenomenon from the general population. *J. Rheumatol.* **1990**, *17*, 1171–1176.
46. Maricq, H.R.; Weinrich, M.C.; Keil, J.E.; Smith, E.A.; Harper, F.E.; Nussbaum, A.I.; LeRoy, E.C.; McGregor, A.R.; Diat, F.; Rosal, E.J. Prevalence of scleroderma spectrum disorders in the general population of South Carolina. *Arthritis Rheum.* **1989**, *32*, 998–1006. [CrossRef]
47. Ihn, H.; Sato, S.; Tamaki, T.; Soma, Y.; Tsuchida, T.; Ishibashi, Y.; Takehara, K. Clinical evaluation of scleroderma spectrum disorders using a points system. *Arch. Dermatol. Res.* **1992**, *284*, 391–395. [CrossRef]
48. Tanaka, S.; Suto, A.; Ikeda, K.; Sanayama, Y.; Nakagomi, D.; Iwamoto, T.; Suzuki, K.; Kambe, N.; Matsue, H.; Matsumura, R.; et al. Alteration of circulating miRNAs in SSc: miR-30b regulates the expression of PDGF receptor β. *Rheumatology* **2013**, *52*, 1963–1972. [CrossRef]
49. Wajda, A.; Walczyk, M.; Dudek, E.; Stypińska, B.; Lewandowska, A.; Romanowska-Próchnicka, K.; Chojnowski, M.; Olesińska, M.; Paradowska-Gorycka, A. Serum microRNAs in Systemic Sclerosis, Associations with Digital Vasculopathy and Lung Involvement. *Int. J. Mol. Sci.* **2022**, *23*, 10731. [CrossRef]
50. Koba, S.; Jinnin, M.; Inoue, K.; Nakayama, W.; Honda, N.; Makino, K.; Kajihara, I.; Makino, T.; Fukushima, S.; Ihn, H. Expression analysis of multiple microRNAs in each patient with scleroderma. *Exp. Dermatol.* **2013**, *22*, 489–491. [CrossRef]
51. Wang, Z.; Jinnin, M.; Kudo, H.; Inoue, K.; Nakayama, W.; Honda, N.; Makino, K.; Kajihara, I.; Fukushima, S.; Inoue, Y.; et al. Detection of hair-microRNAs as the novel potent biomarker: Evaluation of the usefulness for the diagnosis of scleroderma. *J. Dermatol. Sci.* **2013**, *72*, 134–141. [CrossRef]
52. Takemoto, R.; Jinnin, M.; Wang, Z.; Kudo, H.; Inoue, K.; Nakayama, W.; Ichihara, A.; Igata, T.; Kajihara, I.; Fukushima, S.; et al. Hair miR-29a levels are decreased in patients with scleroderma. *Exp. Dermatol.* **2013**, *22*, 832–833. [CrossRef]

53. Wang, Z.; Jinnin, M.; Nakamura, K.; Harada, M.; Kudo, H.; Nakayama, W.; Inoue, K.; Nakashima, T.; Honda, N.; Fukushima, S.; et al. Long non-coding RNA TSIX is upregulated in scleroderma dermal fibroblasts and controls collagen mRNA stabilization. *Exp. Dermatol.* **2016**, *25*, 131–136. [CrossRef]
54. Wasson, C.W.; Abignano, G.; Hermes, H.; Malaab, M.; Ross, R.L.; Jimenez, S.A.; Chang, H.Y.; Feghali-Bostwick, C.A.; Del Galdo, F. Long non-coding RNA HOTAIR drives EZH2-dependent myofibroblast activation in systemic sclerosis through miRNA 34a-dependent activation of NOTCH. *Ann. Rheum. Dis.* **2020**, *79*, 507–517. [CrossRef]
55. Pachera, E.; Assassi, S.; Salazar, G.A.; Stellato, M.; Renoux, F.; Wunderlin, A.; Blyszczuk, P.; Lafyatis, R.; Kurreeman, F.; de Vries-Bouwstra, J.; et al. Long noncoding RNA H19X is a key mediator of TGF-beta-driven fibrosis. *J. Clin. Investig.* **2020**, *130*, 4888–4905. [CrossRef]
56. Tirelli, F.; Pachera, E.; Gmür, S.; Lafyatis, R.; Huang, M.; Zulian, F.; Camarillo Retamosa, E.; Kania, G.; Distler, O. Long non-coding RNA H19X as a regulator of mononuclear cell adhesion to the endothelium in systemic sclerosis. *Rheumatology* **2024**, *63*, 2846–2855. [CrossRef]
57. Ala, U. Competing Endogenous RNAs, Non-Coding RNAs and Diseases: An Intertwined Story. *Cells* **2020**, *9*, 1574. [CrossRef]
58. Sun, X.; Ding, T.; Wang, B.; Chang, Z.; Fei, H.; Geng, L.; Wang, Y. Identification of lncRNA-miRNA-mRNA networks in circulating exosomes as potential biomarkers for systemic sclerosis. *Front. Med.* **2023**, *10*, 1111812. [CrossRef]
59. Milov, A.; Nikolova, M.; Mandadzhieva, S.; Doncheva, N.; Milova, N.; Yordanov, A. CircRNAs-Potential Diagnostic Biomarkers and Therapeutic Targets for Receptive and Cancerous Endometrium. *Epigenomes* **2025**, *9*, 47. [CrossRef]
60. Kristensen, L.S.; Andersen, M.S.; Stagsted, L.V.W.; Ebbesen, K.K.; Hansen, T.B.; Kjems, J. The biogenesis, biology and characterization of circular RNAs. *Nat. Rev. Genet.* **2019**, *20*, 675–691. [CrossRef]
61. Sun, X.; Wang, B.; Ding, L.; Ding, T.; Wang, Y.; Xu, M. Mmu_circ_0005373 and hsa_circ_0136255 participate in the pulmonary fibrosis of systemic sclerosis. *Int. Immunopharmacol.* **2024**, *139*, 112690. [CrossRef]
62. Christov, C.P.; Gardiner, T.J.; Szüts, D.; Krude, T. Functional requirement of noncoding Y RNAs for human chromosomal DNA replication. *Mol. Cell. Biol.* **2006**, *26*, 6993–7004. [CrossRef]
63. Tani, S.; Kaminaka, C.; Nishiyama, K.; Yariyama, A.; Nakatani, Y.; Inaba, Y.; Kunimoto, K.; Yamamoto, Y.; Makino, K.; Makino, T.; et al. yRNA3 up-regulation in cultured dermal fibroblasts and yRNA4 down-regulation in the sera of scleroderma patients. *J. Dermatol. Sci.* **2021**, *102*, 68–71. [CrossRef]
64. Hizir, Z.; Bottini, S.; Grandjean, V.; Trabucchi, M.; Repetto, E. RNY (YRNA)-derived small RNAs regulate cell death and inflammation in monocytes/macrophages. *Cell Death Dis.* **2017**, *8*, e2530. [CrossRef]
65. Cambier, L.; de Couto, G.; Ibrahim, A.; Echavez, A.K.; Valle, J.; Liu, W.; Kreke, M.; Smith, R.R.; Marbán, L.; Marbán, E. Y RNA fragment in extracellular vesicles confers cardioprotection via modulation of IL-10 expression and secretion. *EMBO Mol. Med.* **2017**, *9*, 337–352. [CrossRef]
66. Tanaka, M.; Inaba, Y.; Yariyama, A.; Nakatani, Y.; Kunimoto, K.; Kaminaka, C.; Yamamoto, Y.; Makino, K.; Fukushima, S.; Jinnin, M. Expression of collagen-related piRNA is dysregulated in cultured dermal fibroblasts derived from patients with scleroderma. *Intractable Rare Dis. Res.* **2023**, *12*, 241–245. [CrossRef]
67. Brower-Toland, B.; Findley, S.D.; Jiang, L.; Liu, L.; Yin, H.; Dus, M.; Zhou, P.; Elgin, S.C.; Lin, H. Drosophila PIWI associates with chromatin and interacts directly with HP1a. *Genes Dev.* **2007**, *21*, 2300–2311. [CrossRef]
68. Czech, B.; Hannon, G.J. One Loop to Rule Them All: The Ping-Pong Cycle and piRNA-Guided Silencing. *Trends Biochem. Sci.* **2016**, *41*, 324–337. [CrossRef]
69. Aravin, A.A.; Sachidanandam, R.; Bourc'his, D.; Schaefer, C.; Pezic, D.; Toth, K.F.; Bestor, T.; Hannon, G.J. A piRNA pathway primed by individual transposons is linked to de novo DNA methylation in mice. *Mol. Cell* **2008**, *31*, 785–799. [CrossRef]
70. Le Thomas, A.; Rogers, A.K.; Webster, A.; Marinov, G.K.; Liao, S.E.; Perkins, E.M.; Hur, J.K.; Aravin, A.A.; Tóth, K.F. Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state. *Genes Dev.* **2013**, *27*, 390–399. [CrossRef]
71. Kopp, F.; Mendell, J.T. Functional Classification and Experimental Dissection of Long Noncoding RNAs. *Cell* **2018**, *172*, 393–407. [CrossRef]
72. Czech, B.; Munafò, M.; Ciabrelli, F.; Eastwood, E.L.; Fabry, M.H.; Kneuss, E.; Hannon, G.J. piRNA-Guided Genome Defense: From Biogenesis to Silencing. *Annu. Rev. Genet.* **2018**, *52*, 131–157. [CrossRef]
73. Statello, L.; Guo, C.J.; Chen, L.L.; Huarte, M. Gene regulation by long non-coding RNAs and its biological functions. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 96–118. [CrossRef]
74. Salmena, L.; Poliseno, L.; Tay, Y.; Kats, L.; Pandolfi, P.P. A ceRNA hypothesis: The Rosetta Stone of a hidden RNA language. *Cell* **2011**, *146*, 353–358. [CrossRef]
75. Hansen, T.B.; Jensen, T.I.; Clausen, B.H.; Bramsen, J.B.; Finsen, B.; Damgaard, C.K.; Kjems, J. Natural RNA circles function as efficient microRNA sponges. *Nature* **2013**, *495*, 384–388. [CrossRef]

76. Denzler, R.; Agarwal, V.; Stefano, J.; Bartel, D.P.; Stoffel, M. Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol. Cell* **2014**, *54*, 766–776. [CrossRef]
77. Driedonks, T.A.P.; Nolte-'t Hoen, E.N.M. Circulating Y-RNAs in Extracellular Vesicles and Ribonucleoprotein Complexes; Implications for the Immune System. *Front. Immunol.* **2019**, *9*, 3164. [CrossRef]
78. O'Brien, K.; Breyne, K.; Ughetto, S.; Laurent, L.C.; Breakefield, X.O. RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 585–606. [CrossRef]
79. Yang, Y.; Fan, X.; Mao, M.; Song, X.; Wu, P.; Zhang, Y.; Jin, Y.; Yang, Y.; Chen, L.L.; Wang, Y.; et al. Extensive translation of circular RNAs driven by N(6)-methyladenosine. *Cell Res.* **2017**, *27*, 626–641. [CrossRef]
80. Chen, Y.G.; Kim, M.V.; Chen, X.; Batista, P.J.; Aoyama, S.; Wilusz, J.E.; Iwasaki, A.; Chang, H.Y. Sensing Self and Foreign Circular RNAs by Intron Identity. *Mol. Cell* **2017**, *67*, 228–238.e5. [CrossRef]
81. Kumar, V.; Westra, H.J.; Karjalainen, J.; Zhernakova, D.V.; Esko, T.; Hrdlickova, B.; Almeida, R.; Zhernakova, A.; Reinmaa, E.; Võsa, U.; et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. *PLoS Genet.* **2013**, *9*, e1003201. [CrossRef]
82. López-Isac, E.; Acosta-Herrera, M.; Kerick, M.; Assassi, S.; Satpathy, A.T.; Granja, J.; Mumbach, M.R.; Beretta, L.; Simeón, C.P.; Carreira, P.; et al. GWAS for systemic sclerosis identifies multiple risk loci and highlights fibrotic and vasculopathy pathways. *Nat. Commun.* **2019**, *10*, 4955. [CrossRef]
83. Li, T.; Ortiz-Fernández, L.; Andrés-León, E.; Ciudad, L.; Javierre, B.M.; López-Isac, E.; Guillén-Del-Castillo, A.; Simeón-Aznar, C.P.; Ballestar, E.; Martin, J. Epigenomics and transcriptomics of systemic sclerosis CD4+ T cells reveal long-range dysregulation of key inflammatory pathways mediated by disease-associated susceptibility loci. *Genome Med.* **2020**, *12*, 81. [CrossRef]
84. González-Serna, D.; Shi, C.; Kerick, M.; Hankinson, J.; Ding, J.; McGovern, A.; Tutino, M.; Villanueva-Martin, G.; Ortego-Centeno, N.; Callejas, J.L.; et al. Identification of Mechanisms by Which Genetic Susceptibility Loci Influence Systemic Sclerosis Risk Using Functional Genomics in Primary T Cells and Monocytes. *Arthritis Rheumatol.* **2023**, *75*, 1007–1020. [CrossRef]
85. Liu, Q.; Zaba, L.C.; Satpathy, A.T.; Longmire, M.; Zhang, W.; Li, K.; Granja, J.; Guo, C.; Lin, J.; Li, R.; et al. Chromatin accessibility landscapes of skin cells in systemic sclerosis nominate dendritic cells in disease pathogenesis. *Nat. Commun.* **2020**, *11*, 5843. [CrossRef]
86. Li, W.; Notani, D.; Ma, Q.; Tanasa, B.; Nunez, E.; Chen, A.Y.; Merkurjev, D.; Zhang, J.; Ohgi, K.; Song, X.; et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* **2013**, *498*, 516–520. [CrossRef]
87. Andersson, R.; Gebhard, C.; Miguel-Escalada, I.; Hoof, I.; Bornholdt, J.; Boyd, M.; Chen, Y.; Zhao, X.; Schmidl, C.; Suzuki, T.; et al. An atlas of active enhancers across human cell types and tissues. *Nature* **2014**, *507*, 455–461. [CrossRef]
88. Roundtree, I.A.; Evans, M.E.; Pan, T.; He, C. Dynamic RNA Modifications in Gene Expression Regulation. *Cell* **2017**, *169*, 1187–1200. [CrossRef]
89. Shen, L.; Yu, Y.; Jiang, M.; Zhao, J. Alteration of the m(6)A methylation landscape in a mouse model of scleroderma. *Epigenomics* **2021**, *13*, 1867–1883. [CrossRef]
90. Weng, T.; Huang, J.; Wagner, E.J.; Ko, J.; Wu, M.; Wareing, N.E.; Xiang, Y.; Chen, N.Y.; Ji, P.; Molina, J.G.; et al. Downregulation of CFIm25 amplifies dermal fibrosis through alternative polyadenylation. *J. Exp. Med.* **2020**, *217*, e20181384. [CrossRef]
91. Zou, X.; Zhao, Z.; Chen, Y.; Xiong, K.; Wang, Z.; Chen, S.; Chen, H.; Wei, G.H.; Xu, S.; Li, W.; et al. Impact of rare non-coding variants on human diseases through alternative polyadenylation outliers. *Nat. Commun.* **2025**, *16*, 682. [CrossRef]
92. Janssen, H.L.; Reesink, H.W.; Lawitz, E.J.; Zeuzem, S.; Rodriguez-Torres, M.; Patel, K.; van der Meer, A.J.; Patick, A.K.; Chen, A.; Zhou, Y.; et al. Treatment of HCV infection by targeting microRNA. *N. Engl. J. Med.* **2013**, *368*, 1685–1694. [CrossRef]
93. Täubel, J.; Hauke, W.; Rump, S.; Viereck, J.; Batkai, S.; Poetzsch, J.; Rode, L.; Weigt, H.; Genschel, C.; Lorch, U.; et al. Novel antisense therapy targeting microRNA-132 in patients with heart failure: Results of a first-in-human Phase 1b randomized, double-blind, placebo-controlled study. *Eur. Heart J.* **2021**, *42*, 178–188. [CrossRef]
94. Bauersachs, J.; Solomon, S.D.; Anker, S.D.; Antorrena-Miranda, I.; de Boer, R.A.; Filippatos, G.; Friede, T.; Hauke, W.; Ponikowski, P.; Vardeny, O.; et al. The microRNA inhibitor CDR132L in patients with reduced left ventricular ejection fraction after myocardial infarction: A randomized phase 2 trial. *Nat. Med.* **2026**, *32*, 2654–2661. [CrossRef]
95. Gallant-Behm, C.L.; Piper, J.; Lynch, J.M.; Seto, A.G.; Hong, S.J.; Mustoe, T.A.; Maari, C.; Pestano, L.A.; Dalby, C.M.; Jackson, A.L.; et al. A MicroRNA-29 Mimic (Remlarsen) Represses Extracellular Matrix Expression and Fibroplasia in the Skin. *J. Investig. Dermatol.* **2019**, *139*, 1073–1081. [CrossRef]
96. McDonald, J.S.; Milosevic, D.; Reddi, H.V.; Grebe, S.K.; Algeciras-Schimnich, A. Analysis of circulating microRNA: Preanalytical and analytical challenges. *Clin. Chem.* **2011**, *57*, 833–840. [CrossRef]
97. Willeit, P.; Zampetaki, A.; Dudek, K.; Kaudewitz, D.; King, A.; Kirkby, N.S.; Crosby-Nwaobi, R.; Prokopi, M.; Drozdov, I.; Langley, S.R.; et al. Circulating microRNAs as novel biomarkers for platelet activation. *Circ. Res.* **2013**, *112*, 595–600. [CrossRef]
98. The exRNAQC Consortium. Blood collection tube and RNA purification method recommendations for extracellular RNA transcriptome profiling. *Nat. Commun.* **2025**, *16*, 4513. [CrossRef]

99. Kok, M.G.; Halliani, A.; Moerland, P.D.; Meijers, J.C.; Creemers, E.E.; Pinto-Sietsma, S.J. Normalization panels for the reliable quantification of circulating microRNAs by RT-qPCR. *FASEB J.* **2015**, *29*, 3853–3862. [CrossRef]
100. Marabita, F.; de Candia, P.; Torri, A.; Tegnér, J.; Abrignani, S.; Rossi, R.L. Normalization of circulating microRNA expression data obtained by quantitative real-time RT-PCR. *Brief. Bioinform.* **2016**, *17*, 204–212. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.