Available online at www.ijpbr.net

DOI: http://dx.doi.org/10.18782/2394-3726.1099

ISSN: 2394 - 3726

Int. J. Phar. & Biomedi. Rese. (2020) *7*(4), 6-16

International Journal of Pharmacy & Biomedical Research

Peer Reviewed, Refereed, Open Access Journal

Review Article

Role of Micro RNA in Therapy of Atherosclerosis and Skin Fibrosis: A Review

Anusree S.[1*], Silvia Navis[2] and Prasobh G. R.[3]

[1]Assistant Professor, Sreekrishna College of Pharmacy and Research Centre, Kerala

[2]HOD Department of Pharmacology, Sreekrishna College of Pharmacy and Research Centre, Kerala

[3]Principal, Sreekrishna College of Pharmacy and Research Centre, Kerala

*Corresponding Author E-mail: anusarath07@gmail.com

Received: 15.06.2020 | Revised: 22.07.2020 | Accepted: 25.07.2020

ABSTRACT

MiRNAs are highly conserved class of small (19-25 nucleotides), non-coding RNAs, which regulate post-transcriptional gene expression by binding to target mRNAs and result in gene silencing. In the genome, miRNAs can be found situated in the exons of non-coding genes, introns of coding and non-coding genes and the intragenic regions. Small interfering RNAs are approximately 22 nucleotides in length and mediate RNA interference (RNAi). miRNAs control endothelial cell, vascular smooth muscle cell and macrophage functions, and thereby regulate the progression of atherosclerosis. miRNAs expression is modulated by different stimuli involved in every stage of atherosclerosis and conversely miRNAs modulates several pathways implicated in plaque development such as cholesterol metabolism. miRNAs are involved in the regulation of key processes that contribute to skin fibrosis, including TGF-beta signaling, ECM deposition, fibroblast proliferation and differentiation, and epithelial to mesenchymal transition or transformation. Some miRNAs are profibrotic and their upregulation favors these processes contributing to fibrosis, while anti-fibrotic miRNAs inhibit these processes and may be reduced in fibrosis.

Keywords: *Micro RNA, Atherosclerosis, Skin fibrosis, Fibroblast, Plaque*

INTRODUCTION

RNA molecules are single stranded nucleic acids composed of nucleotides. RNA was thought to play a very minor role in gene expression by converting genetic information from DNA into functional proteins upon receiving an appropriate signal. In the late 1960s, a subset of RNAs was found to control gene expression by stating which genes should turn on and which should turn off (Condorelli & Dimmeler, 2008). These non-coding RNAs, rightly named because they do not code for a protein, are of distinct classes distinguished based on their function and origin. These include microRNA, small temporal RNA, short interfering RNA, short hairpin RNA, small nuclear RNAs, small nucleolar RNAs, transfer RNAs and ribosomal RNAs (Bahadori, 2008).

Cite this article: Anusree, S., Navis, S., & Prasobh, G.R. (2020). Role of Micro RNA in Therapy of Atherosclerosis and Skin Fibrosis: A Review, *Int. J. Phar. & Biomedi. Rese. 7*(4), 6-16. doi: http://dx.doi.org/10.18782/2394-3726.1099

MiRNAs are highly conserved class of small (19-25 nucleotides), non-coding RNAs, which regulate post-transcriptional gene expression by binding to target mRNAs and result in gene silencing. In the genome, miRNAs can be found situated in the exons of non-coding genes, introns of coding and non-coding genes and the intragenic regions. Small interfering RNAs are approximately 22 nucleotides in length and mediate RNA interference (RNAi). Both miRNAs and siRNAs mediate the down-regulation of gene expression; however, their biogenesis and method of gene silencing differs significantly (Bentwich et al., 2007). The human genome may encode over 1000 miRNAs which are abundant in many mammalian cell types and appear to target about 60% of the genes of humans and other mammals and are involved in various biological processes including cellular proliferation, development, differentiation, growth control, and apoptosis. MicroRNA controls gene expression mainly by binding with messenger RNA in the cell cytoplasm. Instead of being translated quickly into a protein, the marked mRNA will be either destroyed and its components recycled, or it will be preserved and translated later. MicroRNAs are now recognized to play a pivotal role in the regulation of certain processes related to development in all eukaryotes and because of their potential role as agents controlling cell growth and differentiation, cellular proliferation.

Cardiovascular diseases often of atherosclerotic origin, are the leading cause of death worldwide. Death is typically due to a rupture of the atherosclerotic plaque and the ischemia caused by the formed thrombus in the vital arteries of the heart or the brain. Atherosclerosis is a chronic inflammatory disease that leads to atherosclerotic lesion/plaque development and narrowing of the arterial lumen. Plaque rupture and thrombosis cause myocardial infarction and stroke, the leading causes of death. Initially there are generally no symptoms. When severe it can result in coronary artery disease, stroke, peripheral artery disease, or kidney problems depending on the arteries which are affected. Symptoms, if they occur, generally do not begin until middle age. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. The narrowing of arteries limits the flow of oxygen-rich blood to parts of the body. Diagnosis is based upon a physical exam, electrocardiogram, and exercise stress test among others. Lifestyle changes, such as eating a healthy diet and exercising, are often the most appropriate treatment for atherosclerosis. Sometimes, medication or surgical procedures may be recommended as well.

Skin fibrosis is excessive scarring of the skin, and is a result of a pathologic wound healing response. There is a wide spectrum of fibrotic skin diseases: scleroderma, nephrogenic fibrosing dermopathy, mixed connective tissue disease, scleromyxedema, scleroderma, and eosinophilic fasciitis (Chen et al., 2009).

MICRO RNA

A microRNA (miRNA, miR) is a non-coding RNA molecule containing about nucleotides found in plants, animals and some viruses that function in RNA silencing and post-transcriptional regulation of gene expression. While the majority of miRNAs are located within the cell, some miRNAs, commonly known as circulating miRNAs or extracellular miRNAs, have also been found in extracellular environment, including various biological fluids and cell culture media.

The first miRNA was discovered in the early 1990s by a group led by Ambros and including Lee and Feinbaum and identified the first miRNA, *lin-4*, *Caenorhabditiselegans.* The field of miRNA research has since flourished with over 17,000 miRNAs discovered to date in 142 species, including more than 1900 in humans (Asakura & Karino, (1990). MiRNA plays multiple role in plant and animal development and in many other biological processes. As discovery of human

miRNAs increased, the research focus was gradually shifted towards functional characterization of miRNAs, particularly in the context of human disease. Certain miRNA expression patterns could be disease-specific and hold great prognostic value. MicroRNAs are involved in several developmental, physiological and pathological processes where they alter and modulate the expression of different proteins. They silence genes either by initiating the cleavage of their respective target mRNA or by inhibiting gene translation after complete or only partial binding to their target sequence. Each miRNA has the potential to target many genes. Over the past decade, miRNAs have emerged as major transcriptional regulators of gene expression for critical biological processes including neuronal developmental, differentiation and synaptic plasticity in the central nervous system. At the molecular level, deregulation of several neurotransmitters, ion channels and proteins are reported to contribute to the development of central and peripheral sensitization (Fasanaro et al., 2010).

MicroRNAs can be transported between cells and tissues. Both membrane-free miRNAs and miRNAs associated with vesicles can be found in the blood. The non –vesicle-associated miRNAs are thought to be stabilized by protein complexes, such as the RISC protein Argonaute 2 and nucleophosmin 1. How miRNAs are released from cells is still unclear, but they may have been released into the bloodstream as the consequence of a passive release of the cell content in necrosis. Membrane-bound miRNAs have been found in apoptotic bodies, exosomes, and microvesicles. Circulating miRNAs are involved in cell-to-cell communication and potentially, to have a role in disease progression. MicroRNAs are now recognized to play a pivotal role in the regulation of certain processes related to development in all eukaryotes and because of their potential role as agents controlling cell growth and differentiation, they have been proposed to be good candidates for cancer therapy (Ambros, 2004).

MECHANISM OF BIOGENISIS

In the nucleus, miRNA is transcribed as a long primary miRNA transcript from miRNA gene by RNA polymerase II. This pri-miRNA is then processed into a stem loop structure of about 70-80 nucleotides known as precursor miRNA by a microprocessor enzyme comprising of a double-strand (ds)-RNA-specific ribonuclease Dorsha, along with its partner DGCR8 also known as Pasha. This pre-miRNA is transported into the cytoplasm by exportin-5-RanGTP dependent mechanism. In the cytoplasm, pre-miRNA is digested by a second dsRNA-specific ribonuclease called Dicer into 18-25 nucleotide mature double-stranded miRNA with the help of trans-activation response RNA binding protein and Argonaute 2. The guide strand or mature miRNA is incorporated into a miRNA-induced silencing complex, which carries the miRNA strand with sequence complementary to specific target mRNA. The RNA-induced silencing complex is the effector complex of the miRNA function and is comprised of miRNA, AgO2 proteins, and other RNA binding proteins (Wightman et al., 1993).

During biogenesis of miRNAs, loading of the guide strand into the miRISC makes it functional and ready to regulate posttranscriptional gene expression. The miRISC-mediated translational inhibition has been reported to arise from 3 putative mechanisms: site specific cleavage, enhanced mRNA decay and translational inhibition. The mechanism and the effectiveness of this regulation are dependent on the characteristics of the miRNA and target mRNA interaction. In metazoans, extensive base pairing has been demonstrated to induce mRNA cleavage, whereas, in mammals, imperfect binding leads to target repression through translational inhibition and/or mRNA destabilization.er RNA binding proteins (Ji et al., 2003).

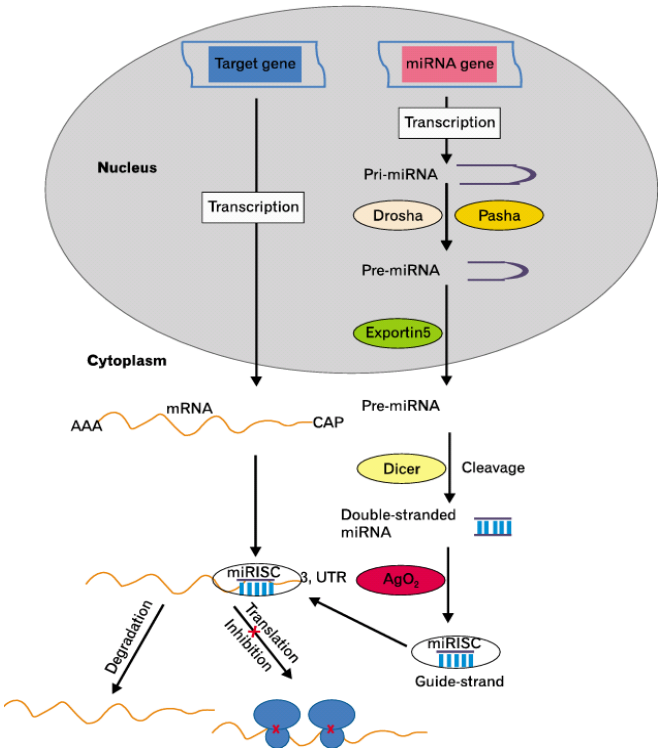

Figure 1: MicroRNA biogenesis pathway: In the nucleus, miRNAs are expressed as long hairpin RNAs called primiRNAs. Primary miRNAs are cleaved by Dorsha and Pasha into 70-100 nucleotide hairpin RNAs known as precursor miRNAs. Precursor miRNAs are exported by exportin-5 to the cytoplasm, then another ribonuclease Dicer cleaves them into 18-25 nucleotides long mature double-stranded miRNA. The guide strand of the mature miRNA is then incorporated into a miRNA-induced silencing complex. This complex binds to the 3' UTR of the target gene through partial complementarity and prevents mRNA from translation into protein. If the miRNA carries the exact complementary sequence to an mRNA, it will cleavage the target mRNA.

FUNCTIONS OF MICRORNA

- They are thought to play a role in specifying tissue identity since they are involved in the process of differentiation into specific tissue. Thus, the expression of miRNA in a specific cell type can be a useful marker for identifying the particular cell type (Arroyo et al., 2020).
- Specific codes of miRNAs have been identified which regulate cell differentiation and are required for tooth patterning; size, shape and number determination.
- MiRNAs have been implicated in controlling the fate and behavior of stem cells. Down-regulation of miR-21, which are essential for stem cell self-renewal. Embryonic stem cell differentiation is promoted by miR-296 and inhibited by miR-22.
- In humans, the balance between apoptosis and proliferation is vital for homeostasis maintenance. Some miRNAs are oncogenes and some are tumor suppressor miRNAs. MiRNAs play a vital role in evaluating the development, progression, prognosis, diagnosis and treatment response in cancer patients. The first human disease known to be associated with miRNA deregulation was chronic lymphocytic leukemia (Han et al., 2006).
- miRNAs are found to regulate immune response, immune cell development and prevention of autoimmunity. A possible role has been suggested for miRNA in the development of autoimmune diseases such as Rheumatoid Arthritis, Systemic Lupus Erythematosus. Distinctive miRNA expression patterns have been linked to salivary gland dysfunction in patients and these miRNAs can serve as potential biomarkers for the disease.
- Altered expression of miRNAs in diabetes causes malfunctions in insulin release and

insulin resistance by regulating cellular membrane electrical excitability, insulin granule exocytosis, insulin synthesis in β-cells, and β-cell fate and islet mass formation. miRNAs in body fluid appears be useful as biomarkers for monitoring the development and progression of diabetes mellitus.

- Modulation of miRNAs has potential to address the therapeutic deficiency in the management of skin fibrosis and may emerge a novel treatment modality. Several miRNAs are involved in the regulation of key processes that contribute to skin fibrosis, including TGF-beta signaling, ECM deposition, fibroblast proliferation and differentiation, and epithelial to mesenchymal transition or transformation (Chendrimada et al., 2005).

MICRORNA THERAPEUTICS IN ATHEROSCLEROSIS

The nascent field of miRNA therapeutics relies on modulating gene expression through miRNA mimics or miRNA antagonists. A miRNA mimic is a molecule that mimics the function of a particular miRNA thereby downregulating the expression of its target genes; conversely, a miRNA antagonist disrupts the function of a specific miRNA causing an upregulation in the expression of its target genes. Since an individual miRNA can regulate multiple genes in a pathway or several pathways simultaneously, although the effect of a single miRNA on a single gene (Hutvagner & Zamore, 2002) is mild, simultaneous regulation of multiple targets especially those in the related pathways may have far-reaching biological effects. This "one drug/multiple target" model of miRNA therapeutics would provide a natural means of normalizing the expression of disease genes and achieve outstanding potency while potentially avoiding the toxicity, drug resistance, or activation of alternative pathways caused by switching a single target on or off. miRNA antagonists are typically chemically modified single stranded oligonucleotides with sequences complementary to the miRNAs of interest, which competitively bind to the endogenous miRNAs. The commonly used chemical modifications include cholesterol-conjugated 2′-*O*-methyl modification (Oral, 2014) and LnA modification. miRNA "sponges" contain complementary binding sites to miRNAs of interest and expressed in an expression vector. miRNA mimics can be chemically modified double-stranded miRNAs or miRNAs expressed by plasmid or viral vectors.

1. MICRO RNA IN LIPID METABOLISM AND ATHEROGENISIS

Although atherosclerosis is a multifactorial disease, high serum cholesterol level is unique among atherosclerotic risk factors in being sufficient to drive lesion development in the absence of other known factors. This makes cholesterol regulation a prime target for therapeutic intervention. Modified LDLs promote atherogenesis at almost all stages, from recruitment of the inflammatory cells to the lesion to macrophage and VSMC foam cell formation. Conversely, high-density lipoproteins transfer cholesterol from peripheral tissue to the liver for excretion and exert anti-inflammatory functions. Increasing plasma hDL has been shown to hinder atherosclerotic plaque progression and promote regression (Zhang, 2008).

Recent studies have discovered key roles of miRNAs in regulating lipid metabolism in atherosclerosis. mir-33a and mir-33b (simplified as mir-33) are intronic miRNAs cotranscribed with their host genes, sterol-response-element-binding protein genes *SREBF2* and *SREBF1*, respectively, and are found to be critical regulators of cholesterol and fatty acid homeostasis *SREBF1* and *SREBF2* genes code for transcriptional factors SrEBP1 and cell formation. Conversely, high-density lipoproteins transfer cholesterol from peripheral tissue to the liver for excretion and exert anti-inflammatory functions. mir-33 prevents cholesterol efflux by downregulating ATP-binding cassette transporter A1 which is responsible for apolipoprotein A1-mediated transportation of cholesterol out of cells to

generate nascent hDL particles (a process called reverse cholesterol transport or cholesterol efflux) (Hatfield & Ruohola-Baker, 2008). mir-33 also decreases fatty acid oxidation and increases VLDL by targeting fatty acid oxidation genes *carnitine O-octanoyltransferase*, *hydroxyacylCoA-dehydrogenase*, and *carnitine palmitoyltransferase 1A*. More excitingly, inhibiting mir-33 expression in mice has shown strong atheroprotective capability and has actually reversed the atherosclerotic phenotypes as indicated by reduction in plaque size and lipid content, increased markers of plaque stability, and decreased inflammatory gene expression. These elegant discoveries strongly suggest mir-33 antagonists as potential therapeutics for atherosclerosis and related metabolic diseases (Chen et al., 2014).

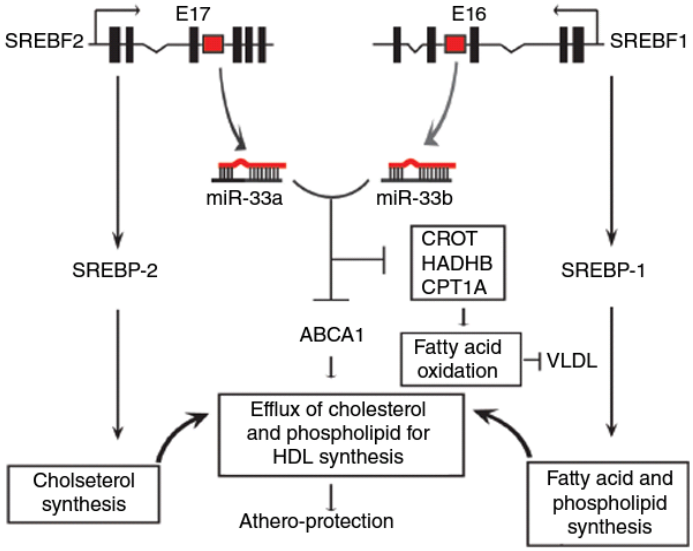

FIGURE 2: mir-33 regulates lipid metabolism in atherosclerosis: mir-33a and mir-33b exist as introns within the SREBF2 and SREBF1, respectively. The SREBFs encode proteins that activate cholesterogenic and lipogenic pathways. mir-33 represses cholesterol and phospholipid efflux for hDL synthesis through targeting ABCA1 protein and decreases fatty acid oxidation and therefore increases VLDL by targeting CroT, CPT1A, and hADhB. Inhibition of mir-33 protects from atherosclerosis by increasing hDL while decreasing VLDL levels. These exemplify the cooperation of miRNAs and their host genes to regulate cholesterol and fatty acid metabolism. Of note, mir-33a is present in all mammals, while mir-33b exists in mammals except rodents (Yang et al., 2007).

2. MICRO RNA IN EARLY LESION DEVELOPMENT

The key events of early atherosclerotic lesion development include endothelial dysfunction, lipid accumulation in the arterial wall, and monocyte infiltration which leads to a profusion of cholesterol-laden macrophage foam cells. Atherosclerosis develops preferentially in arterial branches and curvatures where local flow is disturbed. Laminar shear flow tends to maintain endothelial cells in a quiescent state, contributing to the atheroprotection in the straight portions of the arteries, while endothelial dysfunction induced by disturbed laminar flow or injury may lead to the upregulation of several cell adhesion molecules, including vascular cell adhesion molecule-1, E-selectin, and P-selectin, which are involved in leukocyte adhesion and infiltration into the arterial wall. miRNAs have been implicated in early atherosclerotic lesion development. A list of miRNAs has been shown to be significantly regulated in laminar flow-treated ECs (Glass & Witztum, 2001). Among them, mir-19 and mir-23b were upregulated by laminar flow and contribute to shear stress induced growth arrest. Downregulation of mir-10a in athero-prone endothelium in vivo could contribute to

inflammation in ECs in the athero-prone arteries through upregulation of monocyte chemotactic protein-1, interleukin-6, IL-8, VCAM-1, and E-selectin. mir-92a is downregulated more in ECs exposed to atheroprotective pulsatile shear flow compared to athero-prone oscillatory shear flow, which in turn increases the expression of kruppel-like factor 2, a critical mediator of shear stress and atheroprotective endothelial function. mir-126, an EC-enriched miRNA, has been shown to be induced by kLF2 (at least in zebrafish) and repress leukocyte adhesion to ECs through targeting VCAM-1. mir-21 is upregulated by both nilateral shear stress and athero-prone oscillatory shear stress mir-21 upregulation by unilateral shear stress has been reported to protect EC while mir-21 upregulation by athero-prone oscillatory shear stress increases adhesion of mir-21 has been implicated in the initial oxidation responsible for increasing oxLDL levels.

3. MICRO RNA IN ADVANCED ATHEROSCLEROTIC LESION/PLAQUE

The transition from the fatty streak to advanced lesion/plaque is characterized by the immigration of VSMCs from the media to the intima and the formation of a fibrous cap, followed by the formation of necrotic core through the accumulation of apoptotic and necrotic cholesterol-laden macrophages and VSMCs in the advanced lesion. VSMC phenotypic switching from a contractile state to a proliferative state is a significant contributor to neointima formation in atherosclerosis. Several specific miRNAs with a well-characterized role in VSMC phenotype alteration include mir-21, mir-26a, mir-133, mir-143/mir-145, and mir-221/mir-222. mir-133 expression is inversely correlated with VSMC proliferation, and its overexpression reduces VSMC proliferation and migration in vitro and in vivo. mir-26a promotes VSMC proliferation/migration while inhibiting differentiation by targeting SMAD-1 and SMAD-4 in the TGF-β pathway (Overview of atherosclerosis, 2010). mir-221 expression in VSMCs is induced by platelet-derived growth factor, which is present in human atherosclerotic plaques and functions to recruit VSMCs to the intima. mir-221/mir-222 enhances VSMC proliferation via their target proteins p27(kip1) and p57(kip2). mir-143/mir-145 controls VSMC plasticity by promoting differentiation and repressing proliferation of VSMCs.

4. MICRO RNA IN PLAQUE STABILITY AND RUPTURE

Continuous development of the advanced atherosclerotic lesion leads to the narrowing of the vessel lumens and therefore ischemic symptoms, while plaque rupture and thrombosis result in MI and stroke. The parameters that determine the risk of plaque rupture include the thickness of the fibrous cap, the size of the necrotic core, and the presence of neovascularization and intraplaque haemorrhage (Elmen et al., 2002). A stable, clinically silent plaque has a thick fibrous cap, smaller necrotic core, and little angiogenesis; an unstable plaque has a large necrotic core and a small fibrous cap in addition to (and partially because of) neovascularization. Because of the infancy of miRNA research, direct evidence for miRNAs involvement in plaque stabilization/rupture is still lacking. However, many miRNAs have been shown to regulate cell death, inflammation, VSMC phenotypic switching, and angiogenesis and are therefore potentially implicated in plaque stability/rupture. These mirnAs may also be modulated to keep VSMCs in a quiet state in the fibrous cap, therefore maintaining its stability. Matrix metalloproteinases are strongly implicated in atherosclerotic lesion development and plaque rupture. mir-29b has been shown to be upregulated by oxLDL in VSMCs, which leads to increased MMP-2 and MMP-9 expression through targeting DNA methyltransferase 3b (Krutzfeldt et al., 2005). This miRNA might have implications in plaque rupture. Neoangiogenesis in closely linked to plaque growth, destabilization, and rupture. An anti-angiogenic approach has been proposed as a potential strategy to normalize immature intraplaque vessels and stabilize rupture-prone plaques. An increasing number

of miRNAs have been shown to regulate angiogenesis via modulation of multiple angiogenic pathways.

MICRO RNA THERAPEUTICS IN SKIN FIBROSIS

Several miRNAs are involved in the regulation of key processes that contribute to skin fibrosis, including TGF-beta signaling, ECM deposition, fibroblast proliferation and differentiation, and epithelial to mesenchymal transition or transformation. Some miRNAs are profibrotic and their upregulation favors these processes contributing to fibrosis, while anti-fibrotic miRNAs inhibit these processes and may be reduced in fibrosis (Rayner et al., 2010).

The clinical utility of miRNAs as disease biomarkers has been studied in skin malignancy and has gained the attention of cutaneous fibrosis researchers. MiRNAs regulating skin fibrotic processes, such as let-7a, miRNA-7, miRNA-196a, and miRNA-150, have been detected in decreased levels in the serum of patients with scleroderma and affirm the potential of miRNA as a marker of the disease. Development of miRNA-targeted therapies for the management of skin fibrosis may be channeled into one of two categories: increasing the expression of antifibrotic miRNAs or reducing the expression of profibrotic miRNAs (Esau et al., 2006).

1. MICRO RNA REGULATION OF TRANSFORMING GROWTH FACTOR-BETA SIGNALING

TGF-beta has been implicated in the initiation and maintenance of fibrosis and may interact with miRNAs to elicit these effects. The TGF-beta signaling cascade involves multiple steps (Figure 6): TGF-beta is a soluble mediator and binds to the TGF-beta type II receptor, leading to the recruitment of the TGF-beta type I receptor. The TGF-beta type I receptor conducts the signal through the activation of intracellular Smad2 and Smad3, that bind to Smad4 to form a complex. The Smad complex then regulates the expression of a subset of genes encoding profibrotic factors, such as matrix structural elements (Wang et al., 2010).

MiRNAs are involved at each level of the TGF-beta pathway, targeting TGF-beta protein, receptor, and Smad proteins. MiRNA-29 and miRNA-206 are involved at the beginning of the pathway and lead to reduced expression of TGF-beta protein. However, in scleroderma, these miRNAs are downregulated and as a result sustain the TGF beta signalling cascade with an associated increase in collagen synthesis (Zernecke et al., 2009). MiRNA-29 negatively regulates type I collagen synthesis and miRNA-206 reduces the expressions of types VI and XXIX collagen. Further down the pathway, miRNA-140-5p, let-7g, and miRNA-23b modulate TGF-beta signalling at the level of the receptor, specifically regulating the TGF-beta type II receptor (Figure 6). MiRNA-140-5p downregulates the activity of the TGF-beta type II receptor while let-7g and miRNA-23b upregulate TGF-beta type II receptor activity. Clinically, let-7g, miRNA-23b, and miRNA-140-5p are associated with scleroderma. Furthermore, miRNA-17-5p and miRNA-20 reduce the expression of the TGF-beta type II receptor and are associated with hypertrophic scars.

Other miRNAs are involved in TGF-beta pathway regulation at the level of Smad proteins: MiRNA-145 is hypothesized to target Smad3 mRNA while miRNA-18 and miRNA-146a limit the expression of Smad4 protein. Downregulating the expressions of these Smad proteins inhibits the downstream profibrotic effects of the TGF-beta pathway, including ECM protein synthesis. The downregulation of miRNA-18 and miRNA-145 are associated with hypertrophic scars and scleroderma, respectively and may represent therapeutic treatment targets for these conditions. Unlike other described Smad proteins, Smad7 is a recognized antagonist of the TGF-beta profibrotic effects. Some miRNAs exploit this avenue in the regulation of skin fibrosis: TGF-beta signaling upregulates miRNA-21 transcription and miRNA-21 in turn reduces the expression of Smad7 releasing the inhibition on the TGF-beta pathway to create a feedforward loop. MiRNA-503 is

hypothesized to function similarly to miRNA-21 by reducing Smad7 expression. We hypothesize that clarifying differences in the roles and mechanisms of miRNA-21 and miRNA-503 in modulating TGF-beta signaling may reveal how these pathways can be harnessed therapeutically in the management of skin fibrosis (Hergenreider et al., 2012).

2. MICRO RNA REGULATION OF EXTRACELLULAR MATRIX SYNTHESIS AND DEGRADATION

Excessive ECM protein deposition is a key pathogenic feature of skin fibrosis. ECM is composed predominantly of collagen, and other proteins including fibrillin, fibronectin, and elastin. Increased ECM deposition is a result of increased ECM protein synthesis and/or decreased degradation. ECM degradation occurs predominantly from the actions of matrix metalloproteinases. When homeostasis between matrix synthesis and degradation is lost, skin fibrosis may result. Various miRNAs modulate skin fibrosis by regulating ECM protein synthesis and degradation. MiRNA-29 is a key negative regulator of ECM protein synthesis. MiRNA-29 has been studied in scleroderma patients and was found to be consistently downregulated in patient-derived skin fibroblasts. MiRNA-29 directly targets type I collagen mRNA and is proposed to be a downstream mediator of the following profibrotic molecules: TGF-beta, PDGF-beta, and IL-4. One study found that treatment of normal skin fibroblasts with TGF-beta, PDGF beta and IL-4 induced a scleroderma-like phenotype and downregulated miRNA-29 to an extent similar to that observed in scleroderma fibroblasts. These results suggest TGF-beta, PDGF-beta and IL-4 mediate skin fibrosis in part by downregulating miRNA-29 expression. Apart from type I collagen, miRNA-29 participates in the regulation of other ECM proteins (including fibrillin and elastin) by repressing the TGF-beta, nuclear factor-kappa B and mitogen activated protein kinase profibrotic signaling pathways. Considering that miRNA-29 is a critical suppressor of ECM proteins, the reintroduction of miRNA29 to fibrotic skin using mimics or viral vectors could be a potent anti-fibrotic strategy (Yang et al., 2011).

In addition to miRNA-29, several other miRNAs negatively regulate collagen expression and are downregulated in fibrotic skin diseases: let-7a, miRNA-133a, and miRNA-150 in scleroderma; and miRNA-7, miRNA-26a, miRNA-129-5p, miRNA-133b, and miRNA-196a in scleroderma and keloids (Figure 6). These miRNAs target collagen directly with the exception of miRNA-150. Downregulation of miRNA-150 leads to the overexpression of integrin beta-3 and can activate TGF-beta signaling; activated TGF-beta signaling stimulates Smad3 phosphorylation inducing the transcription of collagen and the development and maintenance of skin fibrosis. Evidence suggests that miRNAs may have important clinical applications and may modify disease management in practice (Torella et al., 2011).

3. MICRO RNA REGULATION OF FIBROBLAST PROLIFERATION, DIFFERENTIATION AND DERIVATION FROM EPITHELIAL CELLS

In the skin, fibroblasts play a crucial role in ECM protein synthesis and differentiate into myofibroblasts with high proliferative capacity and ability to synthesize large numbers of ECM molecules. Although fibroblasts are considered to be derived from mesenchymal cells, they may have non-mesenchymal precursors as epithelial cells can differentiate into fibroblasts in a process termed “epithelial to mesenchymal transition or transformation”. Our understanding of the role of EMT in skin fibrosis is evolving and EMT is suggested to be involved in the pathogenesis of scleroderma. MiRNAs modulate skin fibrosis by regulating the following key fibroblastic processes: proliferation, differentiation, and EMT (Xin et al., 2009). Fibroblast proliferation is a target of both miRNA-21 and miRNA-31. MiRNA-21 elicits this effect by inhibiting Sprouty1, a recognized antagonist of fibroblast proliferation. Sprouty1 achieves its inhibitory role on fibroblasts by blocking

extracellular receptor kinase and MAPK, two pathways implicated in fibroblast proliferation. Therefore, miRNA-21 indirectly releases the inhibition on the ERK and MAPK pathways resulting in increased fibroblasts. The mechanism via miRNA-31 promotes fibroblast proliferation has not been elucidated and presents opportunity for further research.

REFERENCES

Asakura, T., & Karino, T. (1990). Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *66*(4), 1045-66.

Ambros, V. (2004). The functions of animal microRNAs. *Nature*. *431*(7006), 350–5.

Arroyo, J.D., Chevillet, J.R., Kroh, E.M. (2020). Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci USA *108*(12), 5003-5008.

Bahadori, M. (2008). New Advances in RNAs. *ArchIran Med. 11*, 435–43.

Bentwich, I., Avniel, A., Karov, Y. (2007). Identification of hundreds of conserved and nonconserved human microRNAs. *37*(7), 766–770.

Chen, X., Liang, H., & Zhang, J., (2009). Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol. 22*, 125–132.

Chen, H., Lan, H.Y., Roukos, D.H., Cho, W.C. (2014). Application of microRNAs in diabetes mellitus. *1233*, 176–184.

Chendrimada, T.P., Gregory, R.I., & Kumaraswamy, E. (2005). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*. *436*, 740-744.

Condorelli G, & Dimmeler S. (2008). MicroRNAs: Components of an integrated system controlling cardiac development, physiology, and disease pathogenesis. *79*, 551–552.

Elmen, J., Lindow, M., & Schutz, S., (2002). LnA-mediated microrn A silencing in nonhuman primates. *Nature*. *452*(7189), 896–9.

Esau, C., Davis, S., & Murray, S.F. (2006). mir122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab.* 3(2), 87–98.

Fasanaro, P., Greco, S., Ivan, M., Capogrossi, M.C., & Martelli, F. (2010). microRNA: emerging therapeutic targetsin acute ischemic diseases. *Pharmacology & therapeutics. 125*(1), 92–104.

Glass, C.K., & Witztum, J.L. (2001). Atherosclerosis. The road ahead. *Cell*. *104*(4), 503–16.

Han, J., Lee, Y., Yeom, K.H. (2006). Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell. 125*, 887-901.

Hatfield, S., & Ruohola-Baker, H. (2008). microRNA and stem cell function. *Cell Tissue Res. 331*, 57–66.

Hergenreider, E., Heydt, S., Treguer, K., (2012). Atheroprotective communication between endothelial cells and smooth muscle cells through mirn As. *Nat Cell Biol. 14*(3), 249–56.

Hutvagner, G., & Zamore, P.D. (2002). A microRNA in a multiple-turnover RNAi enzyme complex. *Science. 297*, 2056-2060.

Ji, R.R., Kohno, T., Moore, K.A., Woolf, C.J. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci 26*, 696-705.

Krutzfeldt, J., Rajewsky, N., Braich, R, (2005). (2005). Silencing of micrornAs in vivo with 'antagomirs'. *Nature*. *438*(7068), 685–9.

Oral, J. (2014). Maxillofac Pathol. *18*(2), 229–234.

Overview of atherosclerosis. (2010). *Elsevier B.V. P* 2(1), 1.

Rayner, K.J., Suarez, Y., Davalos, A., (2010). mir-33 contributes to the regulation of cholesterol homeostasis. *Science*. *328*(5985), 1570–3.

Torella, D., Iaconetti, C., Catalucci, D., (2011). microrn A-133 controls vascular smooth muscle cell phenotypic switch in vitro and vascular remodeling in vivo. *Circ Res. 109*(8), 880–93.

Wang, K.C., Garmire, L.X., Young, A, (2010). role of microrn A-23b in flow-regulation of rb phosphorylation and endothelial cell growth. *Proc Natl Acad Sci* U S A *107*(7), 3234–9.

Wightman, B., Ha, I., & Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell. 75*(5), 855–62.

Xin, M., Small, E.M., Sutherland, L.B. (2009). microrn As mir-143 and mir-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev. 23*(18), 2166–78.

Yang, B., Lin, H., Xiao, J., Lu, Y., Luo, X., & Li, B. (2007). The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med. 13*, 486–491.

Yang, K., He, Y.S., Wang, X.Q., (2011). Mir146a inhibits oxidized low-density lipoproteininduced lipid accumulation and inflammatory response via targeting toll-like receptor 4. *FEBS Lett. 585*(6), 854–60.

Zhang, C. (2008). MicroRNomics: A newly emerging approach for disease biology. *PhysiolGenomics. 33*, 139–47.

Zernecke, A., Bidzhekov, K., Noels, H, (2009). Delivery of microrn A-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal. 2*(100), 81.