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Chapter

The Role of miRNAs in Idiopathic Pulmonary Fibrosis

Koichi Takagi, Munekazu Yamakuchi, Teruto Hashiguchi and Hiromasa Inoue

Abstract

Idiopathic pulmonary fibrosis (IPF) is a genetic heterogeneous disease with high mortality and poor prognosis. IPF is characterized by persistent fibroblasts and relentless accumulation of collagen matrix. Epithelial-mesenchymal transition (EMT) and endothelial-to-mesenchymal transition (EndoMT) contribute to the progression of the fibrotic process. There are some therapeutic drugs that delay this progress, but eradicative medicine does not exist yet. MicroRNAs (miRNAs) are short single-stranded RNAs that regulate posttranscriptional silencing. Recent reports have shown that miRNAs play important roles in the development of IPF, as different expression levels of miRNAs in blood and lung tissue from IPF patients were closely associated with the occurrence of IPF disease. In this chapter, we will discuss the role of miRNAs in the pathogenesis, diagnosis, and treatment of IPF. In particular, we will focus on the regulation of EMT/EndoMT by miRNAs.

Keywords: IPF, miRNA, EndoMT, EMT

1. Introduction

1.1 Overview of idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a genetic heterogeneous disease with high mortality and poor prognosis that is characterized by progressive scarring of the pulmonary parenchyma, which leads to progressive loss of lung function with dyspnea and hypoxemia, and ultimately respiratory failure and death. IPF is a specific form of chronic, progressive, fibrosing interstitial pneumonia of unknown cause. Disease pathogenesis involves airway tissue remodeling through the excess deposition of extracellular matrix (ECM) proteins and the formation of fibroblastic foci, evident by histology [1]. These events are occurred mainly by continuous damage to the lung epithelium, which destroys the basement membrane and activates myofibroblasts. Myofibroblasts may not only come from resident or circulating fibroblasts but also be brought by epithelial cells transdifferentiated through epithelial-mesenchymal transition (EMT) [2]. Signaling through the TGF-β pathway is thought to underlie many of these changes [3].

According to the 2018 joint statement by the American Thoracic Society (ATS), European Respiratory Society (ERS), Latin America Thoracic Association (ALAT), and Japanese Respiratory Society (JRS) [4], the diagnosis of IPF can be secured by exclusion of other known causes of interstitial lung disease (ILD) and either the

presence of high resolution computed tomography (HRCT) pattern of usual interstitial pneumonia (UIP) or specific combinations of HRCT patterns and histopathology patterns in patients subjected to lung tissue sampling. Any plausible cause of secondary interstitial involvement should be excluded by a thorough medical history and other procedures such as laboratory tests or bronchoalveolar lavage (BAL) when necessary. If a specific diagnosis is not made or no potential cause for ILD is identified, then clinical findings and HRCT are considered during multidisciplinary discussion among different experts (including clinicians, radiologists, pathologists, rheumatologists, and thoracic surgeons in selected cases) [4].

1.2 Overview of miRNAs

MicroRNAs (miRNAs) are short single-stranded RNA molecules of 19–25 nucleotides in length that mediate posttranscriptional gene silencing of target genes [5]. MiRNAs have been recognized as a distinct class of small regulatory RNAs in multiple species that regulate a wide variety of functions such as cell proliferation, differentiation, apoptosis, stress response, and immune response [6–8]. MiRNAs regulate the onset and progress of a variety of diseases, such as cancer, infections, and atherosclerosis [9–11]. In lung diseases, many miRNAs contribute to the pathogenesis. For instance, miR-424/503 regulates the progression of pulmonary hypertension (PH) [12, 13]. miR-424/503 expression is reduced in the pulmonary artery of PH patients [13]. One of the targets of miR-424/503 is *Rictor*, and inhibition of miR-424/503 expression increased the expression of *Rictor*, leading to endothelial-to-mesenchymal transition (EndoMT) and migration of pulmonary artery endothelial cells [12]. In allergic asthma, miR-21, miR-126, and miR-145 are upregulated in multiple experimental asthma models [14–16], and miR-221 and miR-485-3p are upregulated in the peripheral blood of pediatric patients with asthma, compared with controls [17].

2. IPF and miRNAs

Profiling of miRNAs in the lung tissue from IPF patients was performed by different groups [18–22]. In IPF lungs, the expression of miR-21 and miR-155 was increased, whereas the expression of let-7a, miR-29, miR-30, and miR-101 was decreased [23–25]. MiR-21 expression was increased in the serum of IPF patients, and its levels correlated with a decrease in lung function [26]. In the miRNA expression profiling of sputum-derived exosomes from IPF patients, 21 miRNAs were differentially expressed, among which seven (e.g., miR-142-3p and miR-33a-5p) were upregulated and 14 (e.g., Let-7d-5p) were downregulated [27]. In bronchoalveolar lavage fluid (BALF), miR-29a and miR-185 are downregulated in IPF patients [28, 29]. However, little is known about miRNA functions in IPF; much of the functional data has been inferred from chemically induced mouse IPF models. In the lungs of mouse IPF models, miR-21, miR-9, and miR-155 were increased [19, 21, 30], and miR-26, miR-101, miR-139, miR-200, miR-326, miR-489, miR-503, and miR-708 were decreased [24, 31–37]. Furthermore, miR-21 expression was increased in myofibroblasts from IPF lungs, and its inhibition in mouse IPF models attenuated the disease severity [19]. The expression of miR-200 family members was reduced in mouse IPF models and restoration of miR-200c inhibited fibrosis [33]. Similarly, miR-26a was decreased in mouse IPF models and its inhibition caused pulmonary fibrosis, while overexpression repressed fibrotic disease [38]. Finally, miR-326 was decreased in mouse IPF models, and its administration mimicked inhibited TGF-β expression and attenuated fibrosis [34].

3. The role of miRNAs in IPF-contributing cells

Epithelial cells communicate each other via adhesion molecules under physiological condition. Among many adhesion molecules, E-cadherin plays an important role in maintaining tight junctions of epithelial cells. Recurrent and nonresolving injury to lung epithelial cells appears to be a key driver of pulmonary fibrosis. Recent studies have supported the concept that altered alveolar epithelial cell phenotypes, including those produced by increased endoplasmic reticulum stress, predispose to further epithelial cell injury and abnormal repair, facilitating the development of pulmonary fibrosis [39, 40], and thus suggested that the epithelial abnormalities/dysfunction underlying fibrosis should be referred to as reprogramming. The ability of epithelial cells to change into mesenchymal cells through EMT plays an important role in the development of IPF.

Myofibroblasts and related mesenchymal cells are generally accepted as the cells predominantly responsible for fibrotic destruction/distortion of the lung in IPF [41]. Proliferation of fibroblasts and their differentiation into myofibroblasts are important in the development of IPF. The stage of mesenchymal cell differentiation at which IPF myofibroblasts acquire hallmark pathological properties, the mechanisms underlying their pathological differentiation, and the roles of signals from epithelial cells, immune cells, and the matrix in this process has not been fully elucidated and requires further research.

The lung extracellular matrix (ECM) is comprised of collagens, elastin, glycoproteins, and proteoglycans, which serve as structural scaffolding for cells and provide the mechanical stability and elastic recoil necessary for proper lung function. In normal wound healing, myofibroblasts that produce ECM proteins are recruited during the active phase of repair, but are quickly removed via apoptosis once the repair process is underway. Most of the scar matrix subsequently resolves and is replaced by a more permanent tissue. In IPF, myofibroblasts are relatively resistant to apoptosis, and their persistence leads to excessive scarring [42]. A hallmark of IPF is the accumulation of myofibroblasts in clusters called fibroblastic foci and extensive ECM deposition within the interstitium, resulting in destruction of alveolar architecture.

Vascular muscularization driven by repeated small injury is the main pathogenesis of PH, leading to the sustained fibrotic process of intima [43]. Different cellular processes have been described during pulmonary artery remodeling of PH-associated IPF, including endothelial dysfunction [44], EndoMT as a source of myofibroblasts [45], and pulmonary artery smooth muscle proliferation [46].

Monocytes and macrophages have a key role in regulating tissue repair and fibrosis, and several molecular pathways regulating their activity are being investigated as potential treatments for IPF. Macrophages possess many functions that would be expected to promote fibrosis, including fibroblast proliferation [47], regulation of ECM components [48], and secretion of profibrotic cytokines and growth factors. However, macrophages may also possess antifibrotic properties [47, 49]. Several studies have attributed these opposing functions to different macrophage populations distinguished along the lines of classical (M1) and alternative (M2) macrophage activation [50–52], with M1 macrophages being antifibrotic and M2 macrophages being either profibrotic or regulatory.

Adaptive immune responses are involved in the pathological process in IPF patients. Infiltration of activated CD4+ T-cells into IPF lung is often observed before symptoms are developed [53]. Mature dendritic cells also infiltrate in the pulmonary parenchyma of IPF patients [54]. FoxP3 positive regulatory T-cells are diminished in both the circulation and BALF of IPF patients [55].

3.1 Alveolar epithelial cells

miRNA microarray analysis of lung tissue from healthy controls and IPF patients showed that the expression of several let-7 family members was lower in IPF patients [18]. The human let-7 family includes 12 members (let-7-a1, a2, a3, b, c, d, e, f1, f2, g, 1, and miR-98), located on eight different homologous chromosomes [56]. *In vitro* inhibition of let-7d induced an increase in mesenchymal markers in lung epithelial cell lines. The High Mobility Group A2 (*HMGA2*) 3′ UTR has seven conserved sites complementary to the let-7 miRNA [57], and HMGA2 was significantly overexpressed in IPF lungs compared with controls [58]. In lung epithelial cell lines, let-7d regulated *HMGA2* expression, and the increase in *HMGA2* after TGF-β stimulation depended on inhibition of let-7 [18]. HMGA2 facilitates the transcription of *SNAI1* and *TWIST*, the transcriptional repressors of adherens, which are tight and desmosomal junction components, leading to reduced intracellular adhesion. Moreover, it is a mediator of TGF-β-induced EMT. Downregulation of let-7d also led to upregulation of other fibrosis-relevant targets, such as RAS, insulin-like growth factor-1 (IGF-1), and IGF-1 receptor, and thus to the profound and sustained changes in cellular phenotype that are indeed observed in IPF [18] (**Figure 1**).

The expression of miR-26a was significantly decreased in the lungs of mice following the administration of bleomycin [59]. Alignment of miR-26a with the *HMGA2* 3′UTR sequence revealed one potential conserved seed site. Transient introduction of miR-26a into A549 cells, a pulmonary epithelial cell line, led to a significant decrease in HMGA2. However, the reduction in miR-26a expression induced an obvious EMT phenotype in A549 cells together with a clear alteration in certain related proteins, including E-cadherin, vimentin, and α-SMA [59]. Loss of function of miR-26a facilitated lung epithelial cells to transform into myofibroblasts and induce pulmonary fibrosis in mice. Lin28D is also one of the conserved putative target genes of miR-26a. Inhibition of miR-26a induced Lin28D expression and caused an obvious EMT phenotype in A549 cells [31].

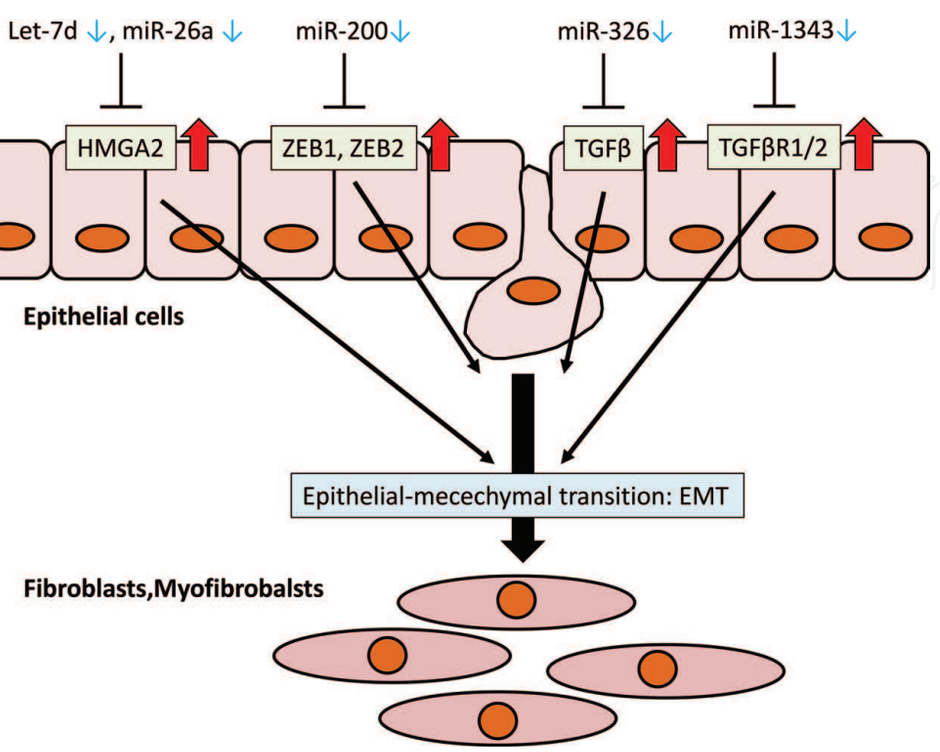

Figure 1.
The role of miRNAs in alveolar epithelial cells for IPF.

The miR-200 family has been suggested to regulate the progression of pulmonary fibrosis by suppressing the EMT of alveolar epithelial cells (AECs). MiR-200 family members correlated with E-cadherin expression in epithelial cells [60, 61] and negatively correlated with ZEB family expression, which has been implicated in EMT [62]. Furthermore, the miR-200 family members have been identified as EMT suppressors through direct targeting of ZEB1 and ZEB2 [63]. MiR-200 is downregulated in the lungs of IPF patients and mouse IPF models [33]. In AECs, overexpression of miR-200, particularly miR-200b and miR-200c, attenuated TGF-β1-induced mesenchymal cell morphological characteristics and TGF-β1-repressed E-cadherin expression [33].

In fibrotic lungs, TGF-β1 is overexpressed in a broad range of cells, including epithelial cells, leading to the dysregulation of normal lung homeostasis by increasing the synthesis and altering the balance of matrix metalloproteinases and their inhibitors [64]. MiR-326 targets the *TGF-β1* 3′UTR, and miR-326 levels inversely correlate with TGF-β1 protein levels in multiple human cell lines and in the lungs of mouse IPF models [34]. Restoration of miR-326 levels by intranasal delivery of miR-326 mimics was sufficient to inhibit TGF-β1 expression and attenuate the fibrotic response in the lungs of mouse IPF models [34]. Furthermore, miR-1343 targets the 3′UTRs of *TGFBR1* and *TGFBR2* and attenuates EMT and fibrogenesis by inhibiting the TGF-β1 pathway in alveolar epithelial cell lines [65].

3.2 Fibroblasts and myofibroblasts

Several studies have shown that some miRNAs including miR-21, miR-29, miR-101, miR-153, miR-154, miR-155, and miR-210 regulate the function of fibroblasts in the lungs (**Figure 2**).

The expression of miR-21 is increased in the lungs of mouse IPF models and of patients with IPF [19]. In mouse IPF models, miR-21 inhibition by lentiviruses encoding anti-miR-21 suppressed morphological markers of pulmonary fibrosis compared with controls [23]. TGF-β1 is the most important profibrogenic cytokine, which increases the expression of miR-21 through SMAD2/3/4 in lung fibroblasts [19] [66]. MiR-21 inhibition inversely regulates TGF-β1-induced ECM protein expression in human pulmonary fibroblast cell lines [23]. Smad7, which inhibits the proliferation of interstitial fibroblasts, is a direct target of miR-21 and negatively correlates with miR-21 in pulmonary fibroblasts [19].

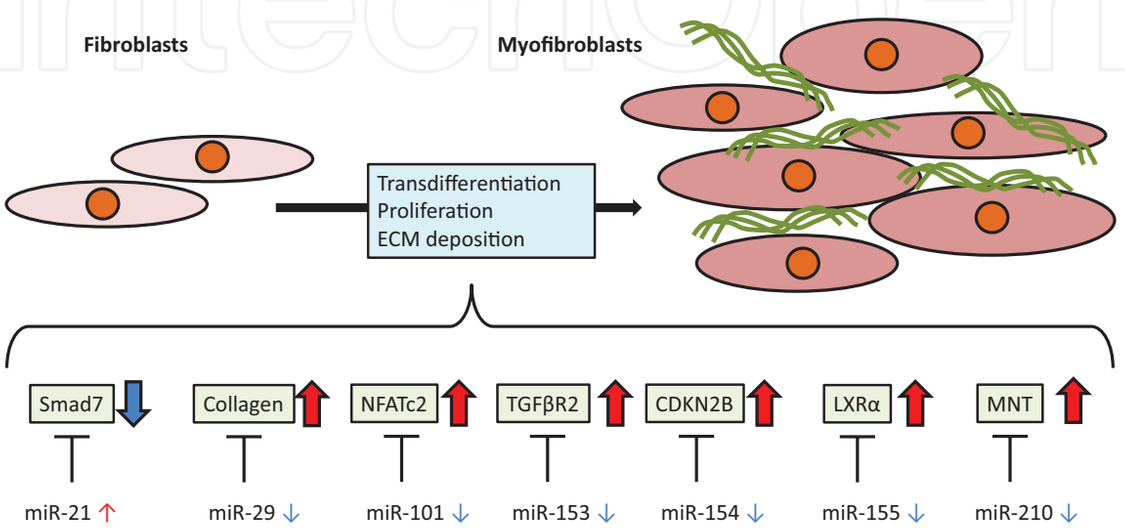

Figure 2.
The role of miRNAs in fibroblasts for IPF.

Members of the miR-29 family, which consists of three mature members, miR-29a, miR-29b, and miR-29c, are among the most downregulated miRNAs in IPF lungs [66]. The expression of miR-29c is also decreased in the lungs of mouse IPF models [67]. MiR-29 has many target genes involved in the composition and regulation of ECM, thus downregulation of miR-29 is capable of contributing to excessive ECM deposition [20, 68]. In human fetal lung fibroblasts, miR-29 suppression upregulated a series of genes, such as TGF-β1 regulated genes and adhesion molecules associated with the fibrotic phenotype [18].

MiR-101 is also one of the most downregulated miRNAs in fibrotic lungs from IPF patients and mouse IPF models [24]. Hypoxia-inducible factors (HIFs) contribute to the pathogenesis of pulmonary fibrosis through Nuclear Factor-Activated T-cell (NFAT) c2 [69]. MiR-101 suppressed WNT5a-induced proliferation of lung fibroblasts via inhibition of NFATc2 signaling by targeting Frizzled receptor 4/6 and attenuated bleomycin-induced pulmonary fibrosis in mouse IPF models [24].

MiR-153 is an intragenic miRNA that is embedded in genes encoding islet-associated protein (IA)-2 and 2β. It has been shown to participate in the pathogenesis of neurodegenerative diseases [70] and inhibit the proliferation and invasion of many types of malignant cells [71]. Furthermore, TGF-β1 downregulates miR-153 expression in lung fibrosis, and miR-153 was downregulated in the fibrotic phase, which was characterized by excessive proliferation of fibroblasts and deposition of ECM in mouse IPF models [72]. However, miR-153 reduced the contractile and migratory activities of fibroblasts. TGFBR2, a transmembrane serine/threonine kinase receptor for TGF-β, has been identified as a direct target of miR-153; moreover, miR-375 regulates SMAD2/3 phosphorylation and the fibrogenic activity of lung fibroblasts through TGFBR2 inhibition [72].

The majority of increased miRNAs in IPF localizes to chromosome 14q32 and is enriched with members of the miR-154 family. TGF-β1 regulates the expression of miRNAs in the 14q32 cluster and induces the expression changes similar to those seen in IPF. TGF-β1 induces cell proliferation through CDK4 and CDKN2B, a CDK4 inhibitor, which is a miR-154 target. Furthermore, TGF-β1-induced increases in proliferation were significantly reduced in the presence of miR-154 inhibitor in fibroblasts, and miR-154-induced changes in proliferation were mediated through the activation of the WNT/β-catenin pathway in fibroblasts [73].

MiR-155 is required for normal immune function [74]. Its overexpression is associated with inflammation, autoimmunity, and cancer [75], whereas miR-155-deficient mice develop age-related airway fibrosis [76]. Liver X receptor alpha (LXRα), which is an oxysterol-activated transcription factor, has a conserved 3′UTR seed-region sequence complementary to miR-155, and inhibition of LXRα ameliorated lung fibrosis in miR-155-deficient mice [77]. However, enforced expression of miR-155 reduced the profibrotic phenotype of IPF and miR-155-deficient fibroblasts [77]. Thus, miR-155 attenuates lung fibrosis through inhibition of LXRα in lung fibroblasts.

The expression of miR-210 has been found to be increased in patients with rapidly progressive IPF, who typically have experienced hypoxia [22]. Consistently, miR-210 was upregulated in IPF fibroblasts in response to hypoxia, and its knockdown suppressed the hypoxia-induced proliferation of IPF fibroblasts. Furthermore, HIF-2α regulated miR-210 expression and miR-210-mediated proliferation of IPF fibroblasts in response to hypoxia. Finally, miR-210 promoted IPF fibroblast proliferation through repression of its downstream target, MNT [78].

3.3 Endothelial cells

PH is frequently seen in IPF patients and is commonly attributed to hypoxic vasoconstriction and capillary destruction. Pathology findings include endothelial

proliferation and medial hypertrophy that exceed those expected in the setting of hypoxia [79] (**Figure 3**). HIF-1 activates the transcription of genes encoding angiogenic growth factors, including vascular endothelial growth factor (VEGF), angiopoietin (ANGPT), placental growth factor (PGF), and platelet-derived growth factor (PDGF), which are secreted by hypoxic cells and stimulate endothelial cells, leading to angiogenesis. Furthermore, HIF-1 mediates hypoxia-induced phenotypic changes typical of EndoMT in endothelial cells [80].

ANGPT1 is a ligand for receptor tyrosine kinase Tie2 [81], which is expressed in endothelial cells [82]. ANGPT1-Tie2 signaling has been shown to be involved mainly in angiogenic activity and promoting maturation of blood vessels, and it is regulated by Akt and MAPK signaling [83, 84]. The activation of ANGPT1-Tie2 signaling correlates with the severity of PH [85]. Mir-34a is downregulated in rat pulmonary arterial hypertension models [86], and ANGPT1 and Tie2 are potential targets of miR-34a, as they have a conserved miR-34a seed sequence in their 3′UTR [87]. Overexpression of miR-34a also downregulated the expression of PDGFRA, one of therapeutic targets of IPF, in pulmonary artery smooth muscle cells [86].

EndoMT has been proposed to be another potential source of myofibroblasts. In EndoMT, endothelial cells lose their own markers and obtain mesenchymal phenotype. Silencing of miR-155 directly increased RhoA expression and activity in endothelial cells and affected phosphorylation of the downstream LMK. A selective Rho kinase inhibitor partly suppressed EndoMT, strengthening the notion that RhoA plays a central role in EndoMT. Overexpression of miR-155 suppressed EndoMT, hence miR-155 functions as a negative regulator of RhoA signaling in TGF-β-induced EndoMT [88]. EndoMT has been considered as a source of myofibroblasts in PH [45], and miR-155 is downregulated in IPF lungs. Thus, miR-155 has been suggested to affect PH-associated IPF through inducing EndoMT.

3.4 Inflammatory cells

Macrophages possess many functions that would be expected to promote fibrosis, including regulation of fibroblast proliferation, recruitment, and activation, direct regulation of ECM components, and secretion of profibrotic cytokines and growth factors. There are two opposite characteristic types of functional macrophages; pro-inflammatory (M1) macrophages induced by toll-like receptor (TLR) and T helper type 1 (Th1) signals and anti-inflammatory (M2) macrophages

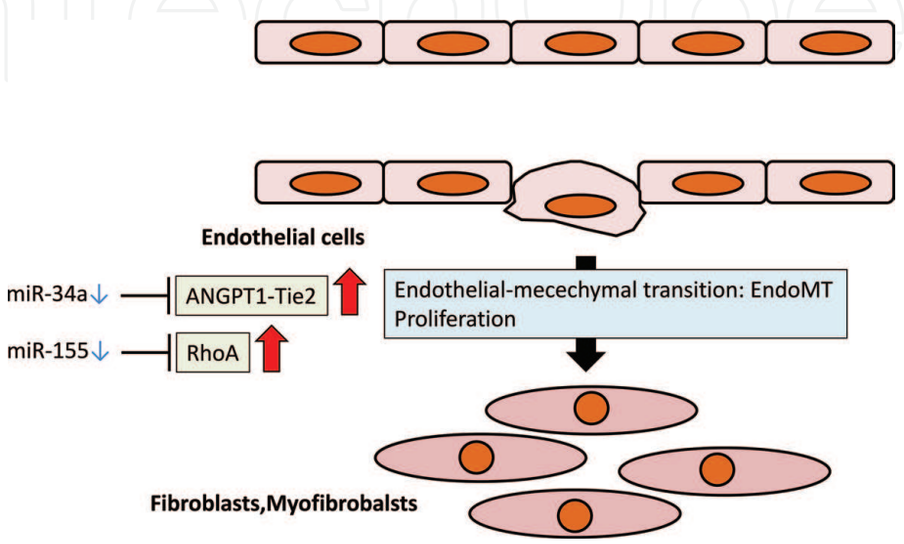

Figure 3.
The role of miRNAs in pulmonary endothelial cells for IPF.

activated by Th2 signals [89] [90]. Th2 cytokines, IL-4 and IL-13 (IL-4/IL-13), contribute to extensive tissue fibrosis in mouse models [91], and IL-4/IL-13-induced macrophages activate fibroblasts by secreting pro-fibrogenic cytokines. IL-4/IL-13 induced CCL18 in lung macrophages of IPF patients, and secreted CCL18 increased the production of collagen from fibroblasts [92]. Many transcriptional factors, such as STAT (signal transducers and activator of transcription) family, regulate macrophage polarization. M1 macrophage polarization is controlled via STAT1, and M2 macrophages activation is mediated by STAT6 [93]. To maintain STAT6 phosphorylation, M2 macrophages overcome strong expression of suppressor of cytokine signaling 1 (SOCS1), resulting in the prolongation of fibrogenesis (**Figure 4**).

IL-4/IL-13 induced miR-142-5p expression and suppressed miR-130a-3p expression in macrophages [94]. Transfection of macrophages with either miR-142-5p anti-sense oligonucleotides or miR-130-3p mimics significantly reduced the expression of M2 markers induced by IL-4 and regulated macrophage pro-fibrogenesis [94]. SOCS1 3′UTR has seven conserved sites complementary to miR-142-5p RNA, and PPARγ 3′UTR has seven conserved sites complementary to miR-130a-3p RNA. The expression of SOCS1 is reduced in IPF lungs, and bleomycin-induced lung fibrosis and inflammation were enhanced in SOCS1-deficient mice compared with WT mice [95]. Thus, miR-142-5p and miR-130a-3p regulate pulmonary fibrosis through SOCS1 and PPARγ in macrophages.

Downregulation of miR-185 has been demonstrated in IPF lung tissue and alveolar macrophages [29, 96]. MiR-185 inhibited cell growth and proliferation by directly targeting AKT1 [97]. Downregulation of miR-185 resulted in AKT activation via increased DNMT1 expression, leading to promoter hypermethylation and silencing of *PTEN* [77]. Moreover, miR-185 directly bound and degraded the 3′UTR of *AKT1* mRNA [98]. Finally, TGF-β1 inhibited the expression of miR-185 in macrophages, and thus TGF-β1 signaling has been suggested to induce proliferation and activation of macrophages through inhibition of miR-185 expression [98].

Some lymphocytic cytokines are considered profibrotic with direct effects on fibroblast and myofibroblast activity in IPF. Th1, Th2, and Th17 have been linked to IPF pathogenesis. The Th1 subset produces IL-1α, TNF-α, PDGF, and TGF-β1. The Th2 subset typical interleukins are IL-4 and IL-13, which are directly involved in fibroblast activation and ECM production. The Th17 subset indirectly promotes fibrosis by increasing TGF-β1 levels, and it is positively regulated by TGF-β1,

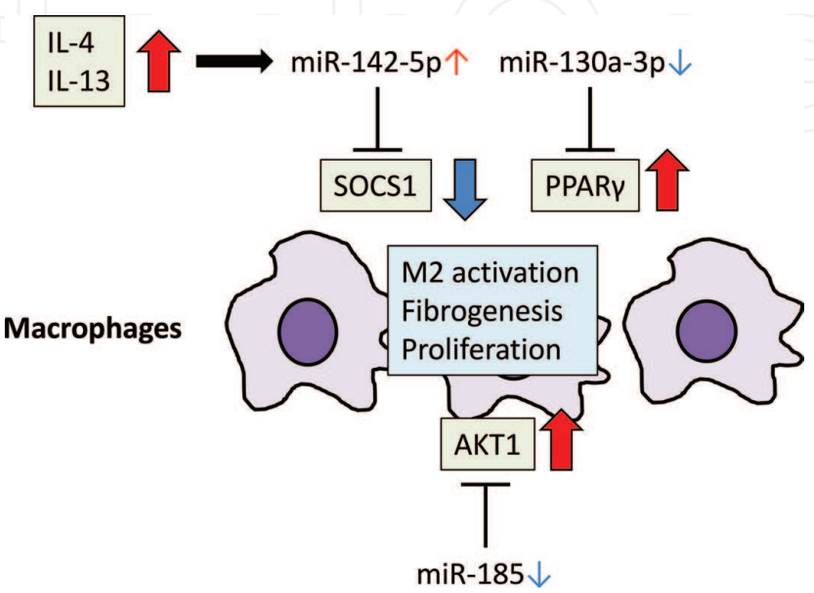

Figure 4.
The role of miRNAs in macrophages for IPF.

creating a positive feedback loop [99]. In both BALF and the peripheral blood of IPF patients, the number of CD4 + CD25 + FOXP3+ regulatory T-cells (Tregs) is decreased compared with healthy people. Thus, Tregs' numerical and functional deficiency may play a central role in the initial phases of IPF pathogenesis [55].

In CD4+ T cells, the level of miR-155 expression is positively associated with the expression of the Th2 cytokines, IL-5 and IL-13, and miR-155 suppresses inhibitors of cytokine production including SOCS1 [100]. However, Foxp3 inhibition significantly decreased miR-146a expression in Tregs. The target gene of miR-146a is TNF receptor-associated factor 6 (TRAF6). Foxp3 and miR-146a have been suggested to regulate colonic inflammation and fibrosis by negatively regulating TRAF6 [101].

4. Conclusions

IPF is a heterogeneous disease that is affected and developed by various genetic backgrounds and environments. The recent development and approval of antifibrotic agents such as nintedanib and pirfenidone, both of which reduced the lung function decline rate in IPF patients in clinical trials, offer hope that it may be possible to alter the increased mortality associated with IPF; however, these therapeutic effects are not sufficient for curing the disease. To reveal new therapeutic IPF targets, genetic screening of IPF patients, including miRNAs, is an effective option. Over the past decade, the role of miRNAs in both promoting and alleviating chronic respiratory disease has become increasingly apparent. In IPF, some miRNAs have been indicated to be associated with EMT, EndoMT, fibrocyte differentiation, or ECM deposition. Investigation of miRNAs and their target genes associated with IPF may lead to new therapy. However, there have been some problems with applying miRNAs in the clinical scene: (1) multiple groups performing studies with similar experimental conditions have reported different and often contrasting results. Hence, collection methods and specimen parts need to be standardized, and better assays are needed for high-confidence miRNA measurements. (2) It is not clear whether miRNA misregulation is a direct cause of disease or an indirect result of gene regulatory processes. Most studies did not attempt to establish the necessity and sufficiency of the miRNA under investigation. (3) It is still difficult to interfere with miRNA expression in the human body, and it is possible that such interference will cause unexpected adverse reactions. (4) It has not been established how to introduce miRNAs into target cells safely and effectively, and the cost of this treatment will probably be high. Thus, miRNAs are still insufficient to be used as therapeutic targets in the clinical scene. Nevertheless, miRNAs may be important tools as IPF biomarkers. To use miRNAs clinically, further studies are necessary to elucidate the association between miRNAs and IPF.

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