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MicroRNAs in idiopathic pulmonary fibrosis, new research progress and their pathophysiological implication

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ABSTRACT

Many studies have shown that microRNAs (miRNAs) play important roles in the development of idiopathic pulmonary fibrosis (IPF). The purpose of this review is to systematically summarize the recent advance of miRNAs in the pathology of IPF, highlighting the new research progress and their pathophysiological implication. Recent studies have shown that miRNAs differentially expressed in blood and lung tissue from IPF patients are closely related to the occurrence of IPF disease, which may be IPF diagnostic markers and prognostic indicators. Furthermore, studies have shown that miRNAs are involved in the pathological mechanisms of IPF, including the lung epithelial repair, epithelial-mesenchymal transition (EMT), fibroblast activation, myofibroblast differentiation, macrophage polarization, alveolar epithelial cells (AEC) senescence and collagen production. In this review, the regulation mechanisms of miRNAs in IPF pathology, such as the long noncoding RNAs (lncRNAs) in miRNA expression, the cross-talk among miRNAs, and the mutual effect of miRNA and DNA methylation, are also systematically reviewed. According to the recent studies of miRNAs in the pathology of IPF, miRNAs play important roles in the pathogenesis of IPF, and miRNAs involved in IPF pathology are helpful to elucidate the pathogenesis of IPF and the treatment of this disease.

Abbreviations

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<td>miRNAs</td>
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<td>IPF</td>
<td>idiopathic pulmonary fibrosis</td>
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<td>EMT</td>
<td>epithelial-mesenchymal transition</td>
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<td>AEC</td>
<td>alveolar epithelial cells</td>
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<td>lncRNAs</td>
<td>long noncoding RNAs</td>
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<td>AE</td>
<td>acute exacerbation</td>
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<td>EV</td>
<td>extracellular vesicle</td>
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<td>TET1</td>
<td>ten-eleven translocation 1</td>
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<td>3′UTR</td>
<td>3′untranslated region</td>
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<td>PMC</td>
<td>pleural mesothelial cell</td>
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<td>TGF-β</td>
<td>transforming growth factor β</td>
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<td>ECM</td>
<td>extracellular matrix</td>
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<td>LOXL2</td>
<td>lysyl oxidase-like 2</td>
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<td>SERPINH1</td>
<td>serpin peptidase inhibitor clade H member 1</td>
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<tr>
<td>IGF-1</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
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<tr>
<td>HAD</td>
<td>histone deacetylase</td>
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LXR liver X receptor
CD99P1 CD99 molecule pseudogene 1

1. Introduction

MicroRNAs (miRNAs) are a class of endogenous, about 20–24 nucleotides of small RNAs that are generated by a Dicer enzyme from single-stranded RNA precursors having hairpin structure of about 70–90 base sizes. They have a variety of important regulatory effects in the biological function of cells.\textsuperscript{1,2} Each miRNA can have multiple targets, and several miRNAs can also regulate the same gene. This complex regulatory network can regulate the expression of multiple genes through a miRNA, or by the combination of several miRNAs.\textsuperscript{3} With the gradual deepening of the study of miRNA regulatory gene expression, it will help us understand the complexity of the genome of higher eukaryotes and the complex gene expression regulation network.\textsuperscript{4}
Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic interstitial lung disease, the lesion site is limited in the lung, and its lung histology and chest high resolution CT characteristics are similar to those of general interstitial pneumonia. The etiology of IPF is unknown and the pathogenesis is not fully elucidated, but there is sufficient evidence that it is associated with immune inflammatory injury. Different specimens showed immune inflammatory response characteristics are not consistent, the immune abnormalities reflected in peripheral blood are more prominent. At present, alveolar epithelial cell (AEC) damage and abnormal repair are the main mechanisms leading to pulmonary fibrosis. After the injury occurs, the repair process can not complete the normal re-epithelialization process, leading to alveolar-capillary injury. Then it induces the cytokine production and the expression of cytokine receptor on the surface of fibroblast, leading to the accumulation of cytokine into the injured site and a pathological damage.

Many studies have shown that miRNAs are involved in the pathogenesis of IPF. For example, the miR-21 is significantly up-regulated in the lungs of IPF model mice and patients with IPF, and miR-21 is involved in the pulmonary fibrosis mechanism. Yang et al. found that the miR-31 was diminished in the lung of model mice and the IPF fibroblasts. Notably, introduction of miR-31 into the lung significantly alleviated the pulmonary fibrosis in model mice, suggesting that miR-31 was an important regulator of the IPF pathogenesis and might be a potential target for treatment of IPF patients (Table 1).

In this review, the roles of miRNAs in the pathological mechanisms of IPF and the regulation mechanisms of miRNA expression in IPF pathology will be systematically summarized and this work may be helpful to elucidate the pathogenesis of IPF and the treatment of this disease.

2. MiRNAs may be biomarkers for IPF patients

Detection of miRNA expression in the blood can help us determine the occurrence and prognosis of related diseases. For example, the level of miR-21 in peripheral blood is higher in IPF patients than that in healthy individuals. In miR-21 agomir transfection group, the severity of alveolitis and pulmonary fibrosis is higher than that in the control group, while the alveolitis and pulmonary fibrosis extent is reduced in miR-21 antagonir group compared with control. Evidence shows that miR-21 promotes the progression of IPF and the mechanism is associated with the down-regulation of ADAMTS-1 expression and increasement of pulmonary type 1 collagen and type 3 collagen.

The IPF disease is divided into acute exacerbation (AE) and stable phase, and miRNA expression may be different in both types. These miRNAs in plasma with different expression phenotypes may be potential diagnostic markers for IPF patients. Evidence also shows that 6 miRNAs are differentiated expressed between AE-IPF and stable IPF patients according to a recent study. In these miRNAs, the let-7d-5p expression is decreased in stable IPF and further decreased in AE-IPF, while miR-25-3p is significantly increased in AE-IPF but obviously decreased in stable IPF. The functional prediction of these miRNAs indicates that the cell abnormal proliferation and anti-apoptotic effect may be required in the pathogenesis of AE-IPF.

In serum extracellular vesicle (EV) from the IPF model mice, the miR-21-5p expression is significantly up-regulated in both the acute inflammatory phase and the chronic fibrotic phase. In IPF patients, the miR-21-5p in serum EV is obviously higher than that in healthy individuals. The miR-21-5p level is correlated with the decline rate of vital capacity over 6 months and is independently associated with mortality in the following 30 months, indicating that IPF patients with higher level of serum EV miR-21-5p have a significantly poorer prognosis in clinical practice. To date, there is no definitive prognostic marker for IPF treatment, thus serum EV miR-21-5p may be a potential prognostic biomarker for IPF patients. In serum of IPF patients, miR-21 expression is significantly higher than that in healthy individuals, while serum miR-155 is not significantly associated with IPF pathology. Evidence suggests that the degree of disease damage also correlates with miR-21 and miR-155 expression in serum. Stable expression of miRNAs in serum may be an important indicator of IPF diagnosis and prognosis.

miRNAs may be diagnostic markers of IPF

The miRNAs in lung tissue may be diagnostic markers of IPF patients. Tissue biopsy is an intuitive and effective clinical test method for determining lung lesions and contributes to the diagnosis and treatment of IPF, and abnormal miRNA expression in lung tissue may be directly related to the pathogenesis of this disease.
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Notably, miR-486-5p level is significantly decreased in the lung tissue of patients with IPF. MiRNAs generally affect the pathogenesis of the disease by regulating its target, and the SMAD2, a crucial mediator of pulmonary fibrosis, is confirmed as a direct target of miR-486-5p in IPF pathology. Thus miR-486-5p may have an important role in fibrosis inhibition. We believe that lung tissue biopsy examination will find more abnormal expression of miRNAs, which will contribute to the diagnosis and prognosis evaluation of this disease (Table 1).

3. The miRNAs and lung epithelial repair in IPF
At present, AEC damage and abnormal repair is the main mechanism leading to pulmonary fibrosis. After the injury occurs, the repair process can not complete the normal re-epithelialization process, leading to alveolar-capillary injury. MiR-29c expression is down-regulated in AECs from patients with IPF compared with healthy individuals and the decreased miR-29c in AECs results in higher apoptosis and reduced epithelial renewal in IPF animal model. However, AECs with miR-29c overexpression show higher proliferative rate and viability, leading to less fibrosis and better recovery in vivo. Interestingly, Foxo3a is proved to be a direct target of miR-29c in this pathological mechanism. Thus miR-29c has an ability to prevent IPF by regulating AEC renewal and apoptosis.

Maladaptive epithelial repair induced by chronic injury is a common feature of fibrotic diseases, such as IPF, which in turn activates the fibroblasts leading to production of excessive extracellular matrix proteins. Evidence suggests that the miR-323a-3p expression is down-regulated in the epithelium of lungs with IPF and murine bleomycin-induced fibrosis. MiR-323a-3p mimics inhibit the murine lung fibrosis and antagonizes the miR-323a-3p promote that, indicating that this miRNA expression is associated with profibrotic signals during the IPF development. Interestingly, miR-323a-3p attenuates the TGF-α and TGF-β signaling and the caspase-3 expression by directly targeting key adaptors in these important pathological mechanisms. This observation demonstrates that miR-323a-3p has a central role in lung fibrosis of IPF patients.

Evidence shows that the miR-30a expression decreases in AECs during the IPF development, and the up-regulated miR-30a can suppress the AEC apoptosis, inhibits the mitochondrial fission, and reduces the Drp-1 production, indicating that miR-30a inhibits the AEC apoptosis by targeting the Drp-1. Furthermore, miR-30a mimics significantly inhibit the ten-eleven translocation 1 (TET1) expression, while miR-30a inhibitor transfection significantly up-regulates the TET1 expression, and TET1 is further validated as a direct target of miR-30a. In view of the above studies, miR-30a can regulate the Drp-1 promoter hydroxymethylation by inhibiting the TET1 expression through base pairing in the 3’ untranslated region (3’UTR) of TET1 promoters in the pathogenesis of IPF.

The etiology of IPF is unknown and the pathogenesis is not fully elucidated, but there is sufficient evidence that it is associated with the tumor suppressor protein p53. In AECs from IPF lungs or mice with diverse types of lung injuries, the p53 acetylation and the miR-34a expression are increased, while the Sirt1 is down-regulated. However, this observation is significantly reduced after treatment of wild-type mice with CSP, but uPA-deficient mice are unresponsive. Furthermore, CSP-mediated inhibition of miR-34a up-regulates the Sirt1 production, suppresses the p53 acetylation and apoptosis in injured AECs, leading to the prevention of IPF. AEC-specific inhibition of miR-34a suppresses the production of bleomycin-induced p53, while overexpression of miR-34a up-regulates the p53 expression, indicating that the p53-miR-34a feedback can be used as a potential therapeutic target for patients with IPF (Table 1).

4. The miRNAs and epithelial-mesenchymal transition (EMT) in IPF
Evidence shows that the sub-pleural pulmonary fibrosis is a pathological hallmark of IPF, and the EMT contributes to the pleural mesothelial cell (PMC) migration and the sub-pleural pulmonary fibrosis. In PMCs treated with bleomycin, miR-18a-5p expression is down-regulated and the reduced miR-18a-5p contributes to EMT of PMCs by targeting the 3’UTR of transforming growth factor β (TGF-β) receptor II. However, overexpression of miR-18a-5p prevents the bleomycin-induced EMT and inhibits the bleomycin-induced sub-pleural fibrosis in IPF model mice.

The miR-200 family members, miR-200a, miR-200b, and miR-200c, are significantly down-regulated in the lung of IPF model mice, and the expression of miR-200a and miR-200c are also down-regulated in the
lungs of IPF patients. Evidence shows that the miR-200 family members inhibit the TGF-β1 induced EMT of AECs and overexpression of these miRNAs can reverse the IPF pathology, indicating their important role in the pathogenesis of IPF. Yamada et al. reported that the miR-21 expression was up-regulated in lung epithelial cells during the IPF development and that this mechanism promoted the EMT. Liang et al. found that the down-regulation of miR-26a in IPF model mice was related to the lung epithelial cells transforming into myofibroblasts via the target HMGA2, which was a key factor in the process of EMT. This finding deciphers the essential role of miR-26a in the mechanism of EMT in the IPF pathology.

In both human IPF tissue and pulmonary cells, the miR-221 expression is down-regulated and the HMGA2 is up-regulated, and transfection with miR-221 mimics significantly inhibits the HMGA2 expression, suppressed the EMT, and inhibits the cell proliferation. Importantly, HMGA2 is confirmed as a directly target of miR-221 in the pathogenesis of IPF. In bleomycin induced IPF animal model, bleomycin induces thicker alveolar wall and more collagen secretion and deposition, whereas miR-221 treatment reverses the lung fibrosis, leading to thinner alveolar walls and normal lung alveoli. Furthermore, evidence suggests that miR-221 targets the HMGA2 to inhibit the bleomycin induced pulmonary fibrosis by regulating TGF-β1/Smad3-induced EMT.

The miR-338-5p expression is down-regulated in the lung tissue from bleomycin-induced model mice, and the smoothened is confirmed as a direct target of miR-338-5p. Overexpression of smoothened will lead to the fibrotic phenotype even without TGF-β induction by affecting the EMT procedure, and the smoothened knockdown partially reverses the pulmonary fibrosis phenotype. The fact that miR-338-5p can target SMO to reduce the EMT procedure will help us postpone the development of pulmonary fibrosis. Furthermore, LPA1 is proven to be another downstream target of miR-338-5p, and the miR-338-5p attenuates the pathogenesis of pulmonary fibrosis through targeting LPA1 in animal model.

In a study, Wang et al. found that the expression of miR-375 was significantly down-regulated during AEC trans-differentiation and that miR-375 regulated the alveolar epithelial trans-differentiation through the canonical Wnt pathway and the target FZD8, which was identified as one of the targets of miR-375.

Let-7 participates in a variety of physiological and pathological mechanism, in which play an important regulatory role. Interestingly, inhibition of let-7d results in up-regulation of the mesenchymal markers and down-regulation of epithelial markers both in vitro and in vivo, suggesting that let-7d induced regulatory mechanism is very similar to EMT mechanism might occur in IPF pathology. Pandit also reported the role let-7d in IPF pathogenesis and demonstrated a key regulatory role for this miRNA in preventing lung fibrosis (Table 1).

5. The miRNAs and fibroblast activation in IPF

MiR-27b is confirmed as an anti-fibrotic miRNA that inhibits the fibroblast activation by targeting TGF-β receptor 1 and SMAD2. The miR-27b expression is down-regulated in fibrotic lungs and fibroblasts from mouse model of IPF, and overexpression of miR-27b inhibits the TGF-β1-stimulated expression of collagens and alpha-smooth muscle actin in human pulmonary fibroblasts. Thus miR-27b may be a potential diagnostic marker and therapeutic target for IPF patients.

MiR-29, a down-regulated miRNA in IPF lungs, is also known as a regulator of extracellular matrix (ECM) in the mechanism of fibroblasts resistant to apoptosis. Furthermore, transfection with miR-29c mimics and miR-29c inhibitors reveals that miR-29c regulates the expression of the death receptor Fas, leading to extrinsic apoptosis. Interestingly, evidence suggests that the TGF-β down-regulates the expression of miR-29c as well as Fas receptor, resulting in the resistance to apoptosis. Therefore, up-regulation of miR-29 expression in IPF lungs could not only suppresses the ECM secretion but also restores the sensitivity to apoptosis of lung fibroblasts, which may be an effective new approach to IPF treatment.

Recent study shows that miR-29a is down-regulated in the process of IPF, and restoration of miR-29a suppresses the IPF fibroblast migration. Bioinformatics analysis shows that a total of 24 genes are putative targets of miR-29a, and luciferase reporter assays suggest that the lysyl oxidase-like 2 (LOXL2) and serpin peptidase inhibitor clade H member 1 (SERPINH1) are direct targets of this miRNA. It is confirmed that down-regulation of miR-29a leads to overexpression of LOXL2 and SERPINH1 during the IPF development, indicating that miR-29a and its targets are involved in the pathogenesis of IPF (Table 1).
6. The miRNAs and myofibroblast differentiation in IPF

There is evidence that miRNAs directly regulate the differentiation of myofibroblasts, which are putative effector cells for pathological fibrogenesis in IPF. TGF-β1 induced expression of mir-27a-3p is reduced in lung fibroblasts from IPF patients compared with control, and overexpression of miR-27a-3p inhibits the differentiation of lung fibroblasts into myofibroblasts, whereas knockdown of miR-27a-3p enhanced that through the phenotypic marker of myofibroblasts, α-smooth muscle actin, and the Smad transcription factors, Smad2 and Smad4. This study indicates that miR-27a-3p and its regulatory mechanism is a novel therapeutic approach to treat lung fibrosis, including other fibrotic organ disorders.

Yang et al. found that miR-145 was up-regulated in TGF-β1 induced lung fibroblasts and the lungs isolated from IPF patients. Transfection with miR-145 mimics in lung fibroblasts promoted the SMA-α expression and the formation of focal and fibrillar adhesions. However, miR-145 deficiency protected against the bleomycin-induced lung fibrosis. These data suggests that miR-145 may regulate the myofibroblast differentiation and lung fibrosis and may be a new IPF diagnostic and therapeutic target (Table 1).

7. The miRNAs and macrophages in IPF

Macrophage polarization controlled by various transcriptional factors plays an important role in the pathogenesis of the human diseases. For example, STAT1 is responsible for M1 polarization, STAT6 is responsible for M2 activation, and IL-4 and IL-13 activate the STAT6 pathway for M2 polarization. MiRNAs are a class of endogenous, small RNAs of about 20–24 nucleotides in length that have a number of important regulatory effects in the cell, such as the immune cell functions. Li et al. showed that the miR-130b-3p expression was down-regulated in lungs from IPF patients. Insulin-like growth factor (IGF-1) was confirmed as a direct target of miR-130b-3p in the epithelium, and the miR-130b-3p down-regulation led to the activation of fibroblasts and modulated the disordered epithelial-mesenchymal crosstalk by targeting the IGF-1.

Su et al. revealed their important roles in macrophage’s fibrogenesis. According to their report, IL-4 and IL-13 induced the miR-142-5p expression and down-regulated the miR-130a-3p expression in macrophages, leading to persistent fibrosis phenotype. Increased miR-142-5p prolonged the STAT6 phosphorylation by targeting its negative regulator, SOCS1, and down-regulated miR-130a relieved its inhibition of PPARγ, which coordinated the STAT6 signaling. Inhibition of miR-142-5p and increase of miR-130a-3p inhibited the bleomycin-induced lung fibrosis in mice, while macrophages isolated from the tissue samples of patients with IPF displayed increased miR-142-5p and decreased miR-130a-3p expression, indicating that miR-142-5p and miR-130a-3p have ability to regulate the macrophage profibrogenic during the IPF development (Table 1).

8. The miRNAs and cellular senescence in IPF

Studies have shown that cell senescence is involved in the pathogenesis of disease and plays an important role in the development of IPF. The mechanism leading to AEC senescence and the role of AEC senescence in the IPF pathology are poorly understood. There is evidence that miR-34a, miR-34b, and miR-34c may be the senescence-associated miRNAs, and these miRNAs are significantly up-regulated in type II AECs from patients with IPF.

Another report suggests that miR-34a is significantly up-regulated in both human and mouse lung myofibroblasts and that miR-34a knockdown develops more severe pulmonary fibrosis than that in wild-type animals. Interestingly, the increased miR-34a in lung myofibroblasts leads to a senescent phenotype in lung fibroblasts because this miRNA promotes the expression of senescence markers, activates the β-galactosidase activity, and inhibits the cell proliferation. In conclusion, disordered miR-34a promotes the senescence of pulmonary fibroblasts through a negative feedback mechanism to inhibit the pulmonary fibrosis process in the pathogenesis of IPF (Table 1).

9. The miRNAs and TGF-β signaling in IPF

TGF-β is normally synthesized and released in response to tissue injury in order to stimulate the cell differentiation and accelerate the wound healing. High level of TGF-β expression is consistently detected in fibrotic lung diseases, and evidence suggests that
TGF-β has an important role in initiating mechanism of tissue fibrosis during the IPF development. Importantly, TGF-β has an ability to induce the differentiation of fibroblasts into myofibroblasts in the lung, leading to the migration of myofibroblasts to the injured site and the production of ECM. The sustained stimulation of TGF-β is also the main cause of EMT, which mediates the process of alveolar epithelial cells into the migratory fibroblastic cells. Stolzenburg et al. revealed the role of miR-1343 in attenuating TGF-β signaling in lung epithelial cell lines and primary fibroblasts. They found that miR-1343 directly interacted with target, the 3' UTR region of TGF-β receptor genes, which would in turn block the TGF-β signaling in this pathological mechanism, leading to an inhibition of TGF-β induced tissue fibrosis and EMT conversion.

MiR-9-5p is a miRNA related to the phenomenon of lung fibrosis targeting TGFBR2 and NOX4, two key mRNAs involved in fibrogenesis. Data shows that miR-9-5p is operated in an anti-fibrogenic mode in the bleomycin induced animal model by negatively regulating the TGF-β signaling. Furthermore, lungs isolated from IPF patients present increased level of miR-9-5p, indicating its role in human lung fibrosis.

In primary lung fibroblasts, TGF-β1 induces the WISP1 expression, miR-92a reverses the TGF-β1 induced WISP1 expression, and miR-92a knockdown increases the WISP1 production. An inverse relationship for WISP1 and miR-92a is also found in a TGF-β1 dependent lung fibrosis model in vivo. The finding that miR-92a regulates the TGF-β1 induced WISP1 expression in pulmonary fibrosis will help us find new IPF diagnostic and therapeutic targets.

In lung and multiple cell lines from IPF patients, miR-326 regulates the TGF-β1 expression and the miR-326 level is inversely correlated to the TGF-β1 expression level. Transfection of miR-326 mimics is sufficient to inhibit the TGF-β1 expression and attenuate the pulmonary Fibrosis, suggesting this mRNA on the regulation of fibrosis during the IPF development. Furthermore, it is confirmed that miR-326 can directly down-regulate the profibrotic genes, Ets1, Smad3, and matrix metalloproteinase (MMP) 9, whereas it up-regulates the antifibrotic genes, such as Smad7 (Table 1).

10. The miRNAs and collagen production in IPF

Type I collagen has low antigenicity, biodegradability, and promotes the cell growth and proliferation. IPF is characterized by the abnormal hyperplasia of fibroblasts depositing type I collagen within the alveolar wall affecting the ventilation function of lung cells, leading to serious breathing problem. In view of the decreased expression of miR-29c and the increased expression of type I collagen in IPF, miR-29c may affect the pathogenesis of IPF by affecting type 1 collagen. Khalil et al. demonstrated that the mechanism of miR-29 inhibition in IPF fibroblast involved the inappropriately low protein phosphatase (PP) 2A function, which led to histone deacetylase (HDA) C4 phosphorylation. The fact that overexpression of HDAC4 in IPF fibroblasts restored the miR-29c level and inhibited the type I collagen production confirmed this observation.

In bleomycin-induced lung fibrosis in miR-155-/-mice, miR-155 knockdown develops the exacerbated lung fibrosis, increases the TGF-β secretion, the collagen 1 and 3 production and the collagen deposition, accompanied by deregulation of the liver X receptor (LXR) α, a miR-155 target gene, in lung fibroblasts and macrophages. Overexpression of miR-155 reduces the fibrosis phenotype of IPF and inhibition of LXRα also reduces that in experimental lung fibrosis and in IPF lung fibroblasts. Thus miR-155/LXR pathway may be a new IPF diagnostic and therapeutic target.

The expression of miR-26a is down-regulated in the lungs of model mice and patients with IPF, and this change leads to a posttranscriptional derepression of connective tissue growth factor (CTGF), and promotes the collagen production. Interestingly, miR-26a down-regulation is due to the TGF-β1 mediated phosphorylation of Smad3, whereas overexpression of miR-26a will inhibit the nuclear translocation of p-Smad3 by directly targeting Smad4. This finding suggests a new strategy for the prevention and treatment of IPF.

Type collagen V is a minor collagen that intercalates within type I collagen, a major collagen in the lung tissue, and evidence suggests that collagen V overexpression and collagen V mediated immune response play important roles in the pathogenesis of IPF. Luciferase reporter assay shows that the collagen V is directly regulated by miR-185 and miR-186. In the lungs of IPF patients, the expression of miR-185 and miR-186 are down-regulated, and the miR-185 and miR-186 are not correlated with disease severity of IPF. However, overexpression of miR-185 and miR-186 will block the TGF-β induced collagen V production and alleviates the TGF-β induced EMT. Thus miR-185 and miR-186 may be the blocking targets for collagen synthesis in the pathology of IPF.
11. The regulation mechanisms of miRNA expression in IPF

Long noncoding RNAs (lncRNAs) affect the miRNA expression. Most of the lncRNAs are catalyzed by RNA polymerase II, but their sequences are less conserved and have strong specificity in tissues and cells. Recent studies have shown that lncRNAs are involved in many important regulatory processes, such as X chromosome silencing, genomic imprinting, chromatin modification, transcriptional activation, transcriptional interference, and nuclear transport, and these regulatory effects of lncRNAs have also attracted widespread attention. Evidence suggests that lncRNAs are involved in cell differentiation and individual developmental regulation at multiple levels and are closely related to human diseases, however, it is unknown whether lncRNAs are involved in IPF.

Huang et al. determined the interaction of lncRNAs and miRNAs by motif search and manual comparison. The dysregulated miRNAs in IPF pathology were used in this study, including let-7d, miR-21, miR-29, miR-31, miR-101, and miR-199, and a total of 34 lncRNAs with potential binding sites to these miRNAs were identified. Interestingly, four of these lncRNAs were inversely correlated to these disordered miRNAs, and the lncRNA CD99 molecule pseudogene 1 (CD99P1) promoted the proliferation and α-smooth muscle actin production in lung fibroblasts, while the lncRNA n341773 has an ability to inhibit the collagen expression in lung fibroblasts. It is suggest that CD99P1 and n341773 are involved in the regulation of IPF pathogenesis and that the interaction of lncRNAs and miRNAs plays an important role in the pathology of IPF and is a new research direction that will contribute to the elucidation of the IPF pathogenesis.

The potential cross-talks among miRNAs affect the miRNA expression. Each miRNA can have multiple target genes, and several miRNAs can also regulate the same gene. This complex regulatory network can be a combination of several miRNAs to fine regulate the expression of a gene, but also through the mutual regulation of miRNA and miRNA to achieve the impact of disease pathology. Liang et al. found that Lin28B promoted the EMT by inhibiting the let-7d, whereas inhibition of Lin28B suppressed the TGF-β1 induced lung fibrogenesis and attenuated the EMT in IPF pathology. Interestingly, Lin28B was one of the direct targets of miR-26a and increased miR-26a could enhance the expression of let-7d through the Lin28B inhibition, indicating that Lin28B mediated the regulatory effect of miR-26a on the biogenesis of let-7d. This study presents a new approach to miRNA regulation, that is, a miRNA interacts with another miRNA to affect the pathogenesis of IPF.

The mutual effects of miRNAs and DNA methylation affect the miRNA expression. MiRNA promoter may be present in the DNA methylation sites, and miRNAs may also be involved in DNA methylation mechanism by inhibition of DNA methyltransferase (DNMT). In lung biopsies and lung fibroblasts from patients with IPF, miR-17~92 expression is significantly down-regulated compared with control, whereas the DNMT 1 expression and the DNA methylation degree of the miR-17~92 promoter are significantly increased. Combined with other data, it is can be sure that there is a novel epigenetic feedback loop between miR-17~92 and DNMT 1 in lung fibrosis.

TGF-β affects the miRNA expression in IPF. TGF-β belongs to a TGF-β superfamily that regulates cell growth and differentiation, and evidence suggests that TGF-β plays an important role in IPF pathogenesis. Study of the TGF-β role in the pathology of IPF and the relationship between TGF-β and miRNAs will help us elucidate the pathogenesis of IPF and the treatment of diseases. In the present study, Liang et al. found that miR-153 expression was dysregulated in the lungs of IPF model mice and TGF-β1 decreased the miR-153 expression. Interestingly, increased miR-153 inhibited, whereas the miR-153 knockdown promoted the profibrogenic activity of TGF-β1 by targeting the TGFBR2, a transmembrane serine/threonine kinase receptor for TGF-β. This finding suggests that TGF-β can affect the expression of specific miRNA, and specific miRNA can also affect the effect of TGF-β through its targets in the pathogenesis of IPF. The expression of miR-199a-5p is also significantly up-regulated in IPF patients compared with control. The level of miR-199a-5p is also selectively increased in myofibroblasts from injured mouse lungs. Evidence suggests that increased miR-199a-5p expression is due to the induction of TGF-β and that miR-199a-5p is a key effector of TGF-β signaling in lung fibroblasts by targeting the caveolin-1, a critical mediator of IPF pathology.

Collagen affects the miRNA expression in IPF. Nho et al. found that miR-96 inhibited the FoxO3a function in IPF fibroblasts on type I collagen matrix. When the IPF fibroblasts cultured in collagen, miR-96
expression was significantly up-regulated compared with control, accompanied by a decrease in FoxO3a mRNA level. However, when the miR-96 function was inhibited, the expression of FoxO3a and its target proteins p21, p27, and Bim was increased, leading to an inhibition of IPF fibroblast proliferation. These data suggest that collagen-rich matrix can pathologically alter the miR-96 expression, inhibits the FoxO3a function, leading to maintenance of IPF fibroblasts in the pathological phenotype.

Hypoxia affects the miRNA expression in IPF. In the pathology of IPF, the differential expression of miRNAs may be related to local hypoxia in lung tissue. For example, Bodempudi et al. revealed that the miR-210 expression markedly increased in IPF fibroblasts induced by hypoxia and that knockdown of miR-210 significantly decreased the proliferation of hypoxia induced IPF fibroblast. Data indicated that hypoxia affected the miR-210 expression through its target HIF-2α, and increased miR-210 promoted the IPF fibroblast proliferation by repressing the c-myc inhibitor, MNT. These data suggest that pathologic factors can affect the miRNA expression, and disorderly expression of miRNAs in turn will increase the degree of IPF pathology.

Component of Chinese herb affects the miRNA expression in IPF. Emodin is a component of Chinese herb that has been reported to used as an anticancer drug, mainly for leukemia, gastric cancer and others, and Emodin is the most commonly used for antibacterial. A lot of bacteria, such as staphylococci, hemolytic streptococcus, are inhibited. In bleomycin-induced EMT of AECs, and Emodin ingredient H19 affects the pathological mechanisms through miR-29b regulation. Data suggests that H19 interacts with miR-29b through directly binding to its 3’UTR and the H19 expression is positively correlated with COL1A1 and Acta2 expression. The regulatory mechanisms of H19/miR-29b participate into the IPF pathogenesis and also are the potential diagnostic and therapeutic targets for IPF patients.

Tectorigenin, a natural plant active ingredient, inhibits the abnormal proliferation of pulmonary fibroblasts in vitro and enhances the miR-338* expression that might in turn inhibits the LPA1. Thus tectorigenin may be a potential IPF therapeutic drug, worthy of further clinical research.

Paclitaxel has been found in the best natural anticancer drug, has been widely used in clinical breast cancer, ovarian cancer and some head and neck cancer and lung cancer treatment. Paclitaxel, as a diterpenoid alkaloid compound with anticancer activity, is highly favored by botanists, chemists, pharmacologists, molecular biologists. Paclitaxel attenuates the bleomycin-induced pulmonary fibrosis in animal models, with reduction of the collagen deposition and the wet lung weight to body weight ratio, and paclitaxel inhibits the effect of TGF-β1 on the expression of Smad3 and phosphorylated Smad3 (p-Smad3), up-regulates the levels of E-cadherin, and vimentin. Interestingly, miR-140 mediates the anti-pulmonary fibrosis effect of low dose of paclitaxel, low-dose PTX prevents the pulmonary fibrosis by suppressing the TGF-β1/Smad3 pathway by up-regulating miR-140 in IPF pathology (Table 1).

12. Conclusion and perspective

MiRNAs play important roles not only in the cell differentiation and biological development process but also in the development of IPF pathogenesis. Many studies have shown the therapeutic efficacy of miRNA mimics or inhibitors in a variety of human diseases. However, evidence shows that some nucleic acid drugs can not been employed yet in practical therapeutic applications due to specific molecular mechanisms, such as the activation of an innate immune response, since the double-stranded RNAs are easily modified by enzymes such as RNase and lack in vivo stability. Recently, Yamada et al. developed novel type of miR-29b mimics that resemble “Pnk-RNA” and “Psh-RNA”, and this novel platform structure of RNA can be adapted not only to miR-29b but also to all miRNAs. This novel mimic RNA “miR-29b Psh-match” shows an enhanced therapeutic effect in bleomycin-induced IPF in model mice. Administration by inhalation of the miR-29b Psh-match may be useful for treatment of IPF patients, and this novel type of miRNA may be a new breakthrough for in vivo treatment of IPF. With further study on the mechanisms of miRNAs, the use of in vivo injection of miRNA analogs or antagonists to study the therapeutic effect of miRNAs in vivo will raise awareness and use of miRNAs to a new level.

Studies of a portion of miRNAs show that miRNAs participate in a series of important processes in life processes, including cell proliferation, apoptosis, cell...
death, fat metabolism and cell differentiation. According to our review, miRNAs are differentially expressed in blood and lung tissue of IPF patients and are closely related to the pathogenesis of IPF. Furthermore, miRNAs are confirmed as important regulators involved in the pathological mechanisms of IPF, such as the lung epithelial repair, EMT, fibroblast activation, myofibroblast differentiation, macrophage polarization, AEC senescence and collagen production. In addition, many factors are identified as modulators of miRNA abnormal expression in the IPF pathology. These regulatory factors can influence the pathogenesis of IPF by affecting the expression of specific miRNAs. These findings suggest that miRNAs may be the diagnostic and therapeutic targets for IPF and are also indicators of IPF disease prognosis. MiRNAs may represent a way of treatment of IPF disease at a newly discovered level.

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