MicroRNAs in Signaling Pathways of Idiopathic Pulmonary Fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive disease with high mortality and unclear etiology. Previous evidence supports that the origin of this disease is associated with epigenetic alterations, age, and environmental factors. IPF initiates with chronic epithelial lung injuries, followed by basal membrane destruction, which promotes the activation of myofibroblasts and excessive synthesis of extracellular matrix (ECM) proteins, as well as epithelial-mesenchymal transition (EMT). Due to miRNAs' role as regulators of apoptosis, proliferation, differentiation, and cell-cell interaction processes, some studies have involved miRNAs in the biogenesis and progression of IPF.

miRNAs idiopathic pulmonary fibrosis EMT

1. Idiopathic Pulmonary Fibrosis (IPF)

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic, and devastating interstitial lung disease of unclear etiology with few therapeutic options and an average survival of 3–5 years after diagnosis [1]. Numerous factors have been implicated in the onset and development of this disease. Among them, reactive oxygen species (ROS) are very relevant; since they are associated with the low activity of the antioxidant systems (e.g., superoxide dismutase, catalase, or glutathione system), they support the role of oxidative stress in the physiopathology of IPF [2][3]. An enzymatic system associated especially with oxidative stress in IPF is the NADPH oxidase family [4]. This oxidase family is stimulated by transforming growth factor-beta (TGFβ1), which increases superoxide synthesis and favors oxidative stress, promoting lung fibrosis [5]. It is worth mentioning that Fierro-Fernández et al. [6] studied miRNAs involved in redox regulation in lung fibroblasts of patients with IPF, and found that miR-9-5p had an inhibitory effect on TGFβRII and NADPH4 expression. Moreover, in a bleomycin (BLM) BLM-fibrosis mice model, the over-expression of miR-9-5p reduced the fibrogenesis, and its inhibition abrogated its anti-fibrotic effect in both in vivo and in vitro assays.

The mitochondria play an important role in the generation of ROS and in the apoptosis process; it has also been implicated in IPF disease [7]. Recently, Bueno et al. [8] reported that alveolar epithelial cells type II (AECIIs) from lungs of IPF patients exhibited an accumulation of dysfunctional mitochondria, a fact associated with upregulation of endoplasmic reticulum (ER) stress.

ER stress is another proposed mechanism for the onset and progression of fibrotic diseases, including IPF [3]. Evidence shows that the expression of surfactant protein A2 mutants (SP-A2, SFTPA2) associated with pulmonary

fibrosis induces the production of proteins that cannot be secreted and therefore accumulate in the cytoplasm, leading to protein instability and ER stress [9]. In addition, ER stress and unfolded protein response (UPR) has been associated with IPF through alveolar epithelial cells (AECs) apoptosis, epithelial-mesenchymal transition (EMT), and M2 macrophage polarization [10][11].

Hypoxia is another prominent clinical feature of IPF disease. However, its real role in this pathology is still poorly understood. Mechanistically, hypoxia stimulates the proliferation of fibroblasts through hypoxia-inducible transcription factor-alpha (HIF-1α), and HIF-2α [12]. It has been suggested that, in fibroblasts, HIF-1α targets pyruvate dehydrogenase kinase and switches the glucose metabolism of the cells to glycolysis, inducing the myofibroblast differentiation [13]. Interestingly, in 2014 Bodempudi et al. [14] demonstrated that miR-210 stimulates the proliferation of IPF fibroblasts in response to hypoxia.

On the other hand, the contribution of the endothelial mesenchymal transition (EndMt) process to some pathologies like IPF has been recognized lately. The first evidence showing that lung capillary endothelial cells could originate fibroblast through EndMT in a BLM-fibrosis mice model was performed by Hashimoto et al. in 2010 [15]. It was recently described that an endothelial cell (EC) dysregulation together with aberrant epithelial activation or autoimmune illnesses may evolve from Interstitial Lung Disease (ILD) to progressive pulmonary fibrosis [16]. Additional recent findings have revealed that, while the number of ECs decreases in the lung microenvironment, the number of fibroblasts and myofibroblasts increase during the development of IPF, suggesting a key role of EndMT in this process [17]. It has been suggested that the activation of the TGFβ signaling pathway is common to almost all ailments associated with EndMT, including IPF. Moreover, the ECs that undergo EndMT possess disrupted tight cell-cell junctions while mesenchyme-specific factors like N-cadherin, alpha-smooth muscle actin (α-SMA), fibronectin, and vimentin, among others, are upregulated, contributing to tissue fibrosis progression [3][18]. In this regard, an interesting study in systemic sclerosis demonstrated for the first time that oxidative stress associated with the activity of NADPH-oxidase drives fibrosis and EndMT [19].

2. MicroRNAs (miRNAs)

miRNAs are a group of single-stranded non-coding RNA with a length of 19–25 base pairs. The miRNAs are transcribed in the cell nucleus by RNA polymerase II as primary miRNAs (pri-miRNAs) [20]. Then, under the regulation of RNase III and Drosha, they are processed to form 70–80 nucleotides precursor miRNAs (pre-miRNAs) which are transported from the nucleus into the cytoplasm, via exportin-5 complex and the nuclear protein Ran-GTP. In the cytoplasm, this complex is processed by Dicer into a mature miRNA duplex [21]. A mature miRNA strand forms, and then the miRNA-induced silencing complex (miRISC) combines with the Argonaute protein and pairs with the “seed region” to target mainly the 3’UTR of different mRNAs [22], controlling their expression through its degradation or repressing its translation [23][24]. A large number of these miRNAs conserve target sites that play important roles during cell development and whose absence may lead to death [25][26]. In addition, it is well known that miRNAs are cell, organ, and tissue-specific [27][28], and it has been estimated that more than 60% of human coding genes are regulated by miRNAs [26]. At present, there is overwhelming evidence that miRNAs intervene in the regulation of cellular processes such as apoptosis, proliferation, and cell

differentiation [22][29][30][31]. On the other hand, it is known that the aberrant expression of some miRNA can lead to the development of diseases like fibrosis and cancer [32][33][34].

3. MicroRNAs and IPF

Interestingly, in 2010 differences in the miRNAs expressed in lung tissues of IPF patients were demonstrated for the first time, in comparison with miRNAs found in the lung of healthy subjects [35][36]. A year later it was reported that these deregulated miRNAs were associated with some developmental pathways that involved TGFβ1, Wnt, sonic hedgehog, p53, as well as VEGF and their complex regulatory networks [37]. Today, many studies are focused on evaluating the expression levels of profibrotic and antifibrotic miRNAs, both in the onset and progression of IPF; however, the results obtained still show contradictory expression levels of these miRNAs [38][39][40][41]. It is probable that these variations are related to the complexity of this lung disease since it involves multiple genes and signaling pathways [42][43][44]. In addition, the intricacy of the regulatory process where several miRNAs might regulate a single gene, or numerous genes can be affected by a single miRNA, hinders the better comprehension of the mechanisms involved [45][46]. Currently, IPF is a disease with a poor prognosis because it has a low life expectancy, and it is difficult to diagnose.

4. Importance of Epithelial-Mesenchymal Transition (EMT) in the Pathology of IPF

EMT regulates different processes during the early stages of development of most organisms. However, it also plays a role in adult organisms and in the pathology of certain diseases such as organ fibrosis and cancer [47][48]. EMT is a process mainly controlled by three families of transcription factors, namely, Snail1/2, Zeb1/Zeb2, and helix-loop-helix, which intervene in repressing E-cadherin expression and inducing mesenchymal gene expression [49][50][51]. Regarding IPF, it is characterized by presenting fibroblast foci localized in sub-epithelial layers close to areas of alveolar epithelial cell injury and repair, which promote abnormal epithelial-mesenchymal interactions and increase proliferation of fibroblast associated with an excessive synthesis of collagen and ECM [52]. In spite of all this information, the origin and activation process of fibroblasts/myofibroblasts during the pathogenesis of IPF remain undefined and controversial to this day. In 2005, Willis et al. [53], using TGFβ, showed for the first time the transition of alveolar epithelial cells (AECs) to myofibroblast through EMT. A year later, the mechanism involved with fibroblast accumulation during fibrogenesis in IPF was reported. It was revealed that AECs were fibroblasts´ progenitors in vivo through EMT and that ECM was a key regulator of this process [54]. Additionally, it is well known now that EMT is involved in the exacerbated fibroblastic response during epithelial regeneration, a hallmark in the pathology of IPF [55]. The importance of AECs also derived from their role during the epithelial-mesenchymal switch, which induces alterations in their morphology, cellular architecture, and adhesion capacity, conferring on them a higher capacity for migration and resistance to apoptosis.

5. MicroRNAs with Pro-Fibrotic Properties and with Regulatory Activity of Signaling Pathways Linked with IPF

Data obtained from the bleomycin (BLM) mice model or from fibroblasts stimulated with TGFβ showed pro-fibrotic activity of some miRNAs. An example is miR-21, which was the first miRNA studied in the BLM-induced fibrosis mice model in 2010 [36]. However, another novel molecular upstream mechanism evaluated in relation to the function of miR-21 was recently reported, both in a BLM-fibrosis mice model, as well as in human embryonic lung fibroblasts (IMR-90) stimulated with TGFβ. On the other hand, in a pulmonary fibrosis rat model induced by intra-tracheal injection of bleomycin, it was observed that prodigiosin attenuates pulmonary fibrosis by inhibiting miR-410 and leading to the downregulation of the TGFβ1/ADAMTS-1 signaling pathway [56]. Another study identified miR-124 as a regulator of the differentiation from lung resident mesenchymal stem cells (LR-MSCs) to myofibroblasts induced by TGFβ1. Additional evidence showed that changes induced by TGFβ1 such as alterations in cell viability, proliferation, and consequent decreases in cell apoptosis were reversed by the blockage of this miRNA. Moreover, AXIN1 was identified as a new target for miR-124 to activate the Wnt signaling pathway [57]. Another miRNA evaluated in an IPF mouse model induced by bleomycin was miR-9; Previously, miR-301a had been reported as an activator of two major inflammatory pathways in cancer (NF-kB and Stat3) [58]. Recently, Wang et al. [59] showed that miR-301a was over-expressed in the BLM-fibrosis mice model, in lung tissue of patients with IPF, and in normal and fibrotic fibroblasts stimulated with TGFβ. The researchers demonstrated that the genetic deletion of miR-301a reduced the severity of lung fibrosis after bleomycin injection in the fibrotic mice model through a decrement of vimentin, α-SMA, and fibronectin expression. Furthermore, when they blocked miR-301a with an antagomir-301a, they observed a reduction in the proliferation and activation of lung fibroblasts as well as in the structural destruction of lung tissue in its experimental model. They showed that the negative regulation of its target TSC1 by miR-301a promoted the severity of lung fibrosis through the mTOR signaling pathway. Another recent study identified the profibrotic role of miR-424 in human lung fibroblasts (HLFs) stimulated with the TGFβ1, as well as in fibroblasts from IPF patients. The researchers determined that this miRNA was involved in the increment of both α-SMA and CTGF protein expression, as well as in the myofibroblast differentiation. Additionally, it was also established, that the upregulation of miR-424 expression by TGFβ was SMAD3 dependent, acting as a positive regulator of the TGFβ signaling pathway by reducing the expression of Slit2 that exerts anti-fibrotic effects [60]. Given that LR-MSCs play an important role in pulmonary fibrosis, in 2020, Wang et al. [61] analyzed the miRNA and mRNA levels of LR-MSCs with or without TGFβ1 treatment. The microarray analysis made in the cells treated with this cytokine showed overexpression of miR-152-3p, miR-140-3p, miR-148-3p, and miR-7a-5p all of which have as a target the Kruppel-like factor 4 (Klf4). Additionally, it was established that the inhibitor of growth family member 5 (ING5) was the common target for miR-34a-5p, miR-27b-3p, miR323-3p, miR-27a-3p, miR-34c-5p, miR-128-3p, and miR-224-5p, which were also overexpressed. The integrated miRNA/mRNA analysis showed that KLF4 and ING5 could be important targets for IPF treatment, due to their role as regulators in the myofibroblast differentiation and in the EMT process [61].

MicroRNAs with Pro-Fibrotic Properties but Negative Regulators of Smad6/Smad7 Expression

Currently, it is well known that Smad7 blocks the function of Smad2/3, generating a negative effect on TGFβ1/Smad signaling [62]. For example, Wang et al. [63] determined in LR-MSCs treated with TGFβ an overexpression of miR-877-3p with Smad7 as its predictive target. When they developed functional studies with the

mice bleomycin-treated model, miR-877-3p sequestration inhibited the differentiation of LR-MSC to myofibroblasts and attenuated pulmonary fibrosis by its effect on Smad7. Additionally, results obtained from human embryonic lung fibroblasts (HELF) stimulated with TGFβ, and lung tissue of a mice fibrosis model showed that miR-182-5p had profibrotic activities, as a consequence of the lower expression of its target gene Smad7 [64]. An analysis made with different databases showed that two miRNAs were significantly deregulated in IPF samples: miR-31, which was overexpressed, and miR-184, which was down expressed. In order to study the molecular mechanisms involved, A549 epithelial cells were stimulated with TGFβ. Results showed a profibrotic role of miR-31 through downregulation of Smad6 and its failure to impede the phosphorylation of Smad2. In contrast, the antifibrotic role of miR-184 was confirmed when TGFβ abolished its function.

6. MicroRNAs with Anti-Fibrotic Properties and with Regulatory Activity of Signaling Pathways Linked with IPF

Most of the recently reported miRNAs are within this group since almost all are associated with the signaling pathways linked to TGFβ or with the activation of some of their components. A characteristic of this group of miRNAs is that they are important inducers of EMT, and are therefore considered the main contributors to lung fibrosis pathogenesis. In the middle of 2016, Ge et al. [65] showed some evidence pointing out that miR-323a-3p was down expressed in the epithelium of IPF lungs and in the bleomycin-induced fibrosis model. It was demonstrated that antagomirs for miR-323a-3p promoted murine lung fibrosis, and in contrast, when a miR-323a-3p mimic was used, the fibrosis was inhibited. Thus, the researchers concluded that this miRNA attenuated the signaling associated with TGFα and TGFβ, as well as with the caspase-3 expression, by directly targeting the TGFα and Smad2 genes. The miR-29 has been considered an anti-fibrotic miRNA [37][66]. Therefore, the therapeutic effect of a single-stranded RNA miR-29b match type (miR-29 PSh) was evaluated and compared with the effects of a double-stranded miR-29 mimic. In both fibrosis mice models and in cell culture experiments, miR-29b Psh-match showed lesser collagen synthesis than miR-29 mimic, a finding that supports its potential role as an effective therapeutic drug for pulmonary fibrosis [67]. The downregulation of miR-185 had already been associated with rapid IPF progression [68]. However, little is known about the expression of this miRNA in IPF bronchioalveolar lavage (BAL) cells and in alveolar macrophages (THP-1cells). The sub-expression of both miR-185 and miR-29a in this type of sample correlated with the disease severity and with eosinophil infiltration [69]. Furthermore, it was also observed that miR-185 down-regulation was associated with collagen deposition due to AKT pathway activation; while miR-29a downregulation caused the overexpression of the COL1A1 gene. The results obtained highlight the importance of evaluating the expression of these miRNAs in IPF BAL cells [69]. It had already been reported previously that Col5 was overexpressed in the lung tissue of IPF patients [70]; Col5 is regulated by miR-185 and miR-186 by a probable TGFβ signaling pathway activation [71]. On the other hand, the antifibrotic role of miR-130b-3p during the crosstalk between epithelial lung cells and fibroblast, a fact linked with insulin-like growth factor (IGF-1) expression in lung epithelium, was recently demonstrated [72]. Previous studies had already reported that the transcription factor from high mobility group AT-hook 2 (HMGA2) is induced by the TGF-β1/Smad3 signaling pathway during the EMT [35]. Additional evidence demonstrated also an essential role of miR-221 in EMT during liver fibrosis [73]. In this way, the down expression of miR-221 and overexpression of HMGA2 in human IPF tissues

and in bronchial epithelial cells treated with TGFβ were detected for the first time. Additionally, the researchers observed EMT suppression when they transfected a miR-221 mimic in a mouse bleomycin-pulmonary fibrosis model, a fact derived from directly targeting HMAG2. This last fact supports the role of the TGFβ1/Smad3 signaling pathway and its possible importance as a therapeutic target for the treatment of lung fibrosis [74]. Another miRNA that may have therapeutic applications in IPF is miR-1343, which is capable of reducing the expression of both TGFβRI and TGFβRII by directly targeting their 3′-UTRs in A549 epithelial cells and in normal lung fibroblasts stimulated with pro-fibrotic cytokine TGFβ [75]. Additionally, after TGFβ exposure, a significant increase in the levels of miR-1343 was observed and this phenomenon has been associated with an important decrease in pSmad2 and pSmad3 levels in the epithelial cells and fibroblasts, and with a reduction of fibrotic makers and repression of EMT [75]. Another miRNA of relevance is miR-27a-3p whose expression is downregulated in lung fibroblasts from IPF patients in comparison to fibroblasts from control subjects' lungs. When this miRNA was over-expressed, pulmonary fibrosis induced in the bleomycin-mice model was mitigated by targeting α-SMA, and Smad2/4 transcription factors. These results support the role of miR-27a-3p as a negative regulator of lung fibrosis since it inhibits myofibroblast differentiation [76]. The expression of miR-27b was decreased in lung tissue of bleomycin-treated mice when compared to control mice, and in turn, the treatment of lung fibroblasts with bleomycin reduced the expression of this miRNA. Interestingly, its overexpression using a lentiviral vector in the LL29 human pulmonary fibroblasts stimulated with TGFβ1 inhibited the mRNA expression of collagen 3a1 and αSMA proteins, identifying TGF-βRI and Smad2 as their direct target genes. This result pointed out that miR-27b acts as an anti-fibrotic miRNA in pulmonary fibrotic fibroblasts [77]. On the other hand, Zhang et al. [78], reported that miR-18a-5p inhibits sub-pleural pulmonary fibrosis by targeting TGFβRII and in consequence inhibits the TGFβ-Smad2/3 signaling pathway. Additional results reported by Huang et al. [79] showed that when a lentivirus expressing miR-18a-5p is injected into bleomycin-treated mice, pulmonary fibrosis and sub-pleural fibrosis were attenuated. An analysis made to evaluate the expression of miR-101 in lung tissues of IPF patients, from the Lung Tissue Research Consortium (LTRC), showed that this was one of the most downregulated miRNAs. In this way, the regulation of this miRNA and its cellular signaling was investigated. When miR-101was overexpressed in a fibrotic cell line (HLL29), the TGFβ-induced protein expression of α-SMA, COL1A1, and COL3A1 was inhibited. In contrast, with the transfer of anti-miR-101 to a normal lung, fibroblasts' cell line increased the protein expression of these collagens and α-SMA. The results suggested that miR-101 inhibits the differentiation of fibroblasts to myofibroblasts stimulated by TGFβ via the SMAD2/3 signaling pathway. Additionally, the effect of miR-101 overexpression through gene transfer on the bleomycin mice model was examined. The results revealed a better lung function, with a reduction of COL1A1 and COL3A1, and of NFATc2, FZD6, and TGFβRI mRNA levels. Thus, the conclusion was that miR-101 is an anti-fibrotic miRNA with potential therapeutic properties to treat IPF [79]. In 2009, it was reported that miR-155 was induced by proinflammatory stimuli such as IL-1 and TNF-α in macrophages and dendritic cells [80][81]. Afterward, Pottier et al. [82] demonstrated, by in vitro functional assays, keratinocyte growth factor (KGF) as a new target for miR-155 in lung fibroblasts. Moreover, results obtained from a bleomycin-treated mice model showed that miR-155 expression levels in the lung fibroblasts correlated with the degree of lung fibrosis and depicted it as a potential key player during tissue injury. In contrast, in a recent study, it was reported that miR-155 was down-expressed in human pulmonary fibroblasts stimulated with TGFβ. And when this miRNA was overexpressed to investigate its regulatory role in this cellular model, they observed attenuation of

fibroblast proliferation, migration, and collagen synthesis. Furthermore, the researchers observed a decrease in Smad1gene expression after miR-155 inhibition in this cellular model, by which they suggested an indirect miR-155-SMAD interaction linked to the TGFβ signaling pathway [83]. It had already been confirmed previously that miR-200a, miR-200b, and miR-200c were downregulated in mice with lung fibrosis induced by bleomycin [84]. However, during the early stages of acute respiratory distress syndrome (ARDS) induced in a mouse model by lipopolysaccharides (LPS), it was also shown that miR200b/c was downregulated, which was associated with an increment of their protein targets ZEB1 and ZEB2. Likewise, when a lentiviral packaged miR200b/c cDNA or ZEB1/sh RNA vectors were intratracheally administered, the pulmonary inflammation and fibrosis were reduced. Moreover, an increment of E-cadherin protein levels and a suppression of vimentin and αSMA protein expression were observed. These effects were associated with the inhibition of p38 MAPK and TGFβ/Smad3 signaling pathways [85]. To elucidate the role of some miRNAs, the miss-expressed miRNAs were firstly investigated by microarray expression, and afterward, their functions in activated lung fibroblasts were investigated. The researchers found a miR-19a-19b-20a sub-cluster with the ability to suppress the activation in vitro of fibroblasts stimulated with TGFβ. Moreover, when this miRNA sub-cluster was intratracheally transferred into bleomycin-treated lungs, their results revealed not only the down expression of pro-fibrotic genes such as ACTA2, COL1A1, or CTGF and Serpin, but also the over-expression of anti-fibrotic genes such as Dcn, Igfbp5, and MMP3 [86]. Another interesting example is miR-133a, which, in spite of being overexpressed in human lung fibroblasts (HLF) stimulated with TGFβ1, exhibited antifibrotic properties. To investigate whether this miRNA induced by TGFB modulated the fibroblasts' differentiation into myofibroblasts, a miR-133a mimic was transfected into HLF. Surprisingly, the fibroblast differentiation was attenuated by a reduction of α-SMA expression. In contrast, the miR-133a inhibitor improved TGFβ-induced myofibroblast differentiation. Additionally, target analysis and luciferase reporter assays showed TGFβRI, CTGF, and COL1A1 as direct targets of miR-133a. Moreover, in functional analysis in a BLM fibrosis mice model, it was demonstrated that the overexpression of miR-133a ameliorated lung fibrosis by a negative feedback regulator of TGFβ profibrogenic pathways [87]. In an interesting study, where the expression profile of miRNAs in exosomes obtained from BAL fluid (BALf) of elderly patients with IPF was analyzed, the downregulation of miR-30a was evidenced. In functional assays, miR-30a overexpression attenuated the expression of TGFβ activated kinase1/MAP3K7 binding protein3 (TAB3), α-SMA, and fibronectin in both 293T and A549 cells stimulated with TGFβ, in comparison with control culture. The results showed that the downregulation of miR-30a in BALf exosome may be a biomarker for IPF diagnostic, or its overexpression may be an optional treatment for IPF [88]. Meanwhile, Wu et al., in 2020 [89], reported that miR-30a not only regulated the expression of α-SMA but also regulated the expression of fibroblast activation protein alpha (FAP-α) and COL1A1 in fibroblasts stimulated with TGFβ1. Additionally, their results demonstrated that miR-30a had a role in cell proliferation with or without TGFβ1 treatment, via regulating FAP-α expression. A down-expression of miR-340-5p in various fibrosis-related diseases had already been reported. However, its role in IPF is unknown. It had been described previously that the miR-340-5p/activating transcription factor 1 (ATF1) axis regulates the proliferation and invasion of lung cancer cells [90].

MicroRNAs Down-Regulated and Associated with Apoptosis

Although the accumulation of apoptosis-resistant fibroblasts is a hallmark of IPF lungs, the precise mechanisms by which these cells acquire this characteristic have not been fully elucidated yet [91]. For instance, in 2009 it was demonstrated that the miR-29 downregulation in tumoral cells increased the antiapoptotic protein expression and cellular resistance to apoptosis [92]. To date, the contribution of miRNAs in the mechanisms regulating apoptosis susceptibility in IPF is not fully understood. In this context, how apoptosis intervenes in IPF remains a controversial topic. The miR-29 family has been reported as one of the miRNAs downregulated in fibrotic diseases [37]. The consequence of this downregulation has been linked with an increase in the expression of profibrotic genes associated with excessive ECM deposition [93][94]. In a study performed in lung fibroblasts of BLM-mice, and in a human fetal lung fibroblast cell line (HFL1), the role of miR-29c as a regulator of lung fibroblasts was evaluated. To attain this objective both miR-29c inhibitor and miR-29c mimic were used. The researchers found that the miR-29c mimic inhibits Fas repression induced by TGFβ, restoring the apoptosis sensitivity, a fact that may become an effective strategy for the treatment of IPF [95]. A year later in 2017, Xie et al. [96] also analyzed the miR-29c downregulation function in lung epithelial cells. The researchers observed a decrease in the apoptotic response with higher viability and proliferation in a mouse lung epithelial cell line (MLE) treated with bleomycin and transfected with the miR-29c mimic, in comparison to MLE control cells. The molecular studies demonstrated that miR-29c suppressed the lung epithelial cells' apoptosis by targeting Foxo3, a transcription factor that upregulates cell death and downregulates anti-apoptotic proteins. On the other hand, miR-142-3p is a miRNA that has been previously implicated in the cell apoptosis and inflammatory response in fibrosis of cardiomyocytes [97]. However, its role in apoptosis and inflammation in the IPF disease is unknown. In this regard, Guo et al. [98] studied the effects of miR-142-3p mimic or miR-142-3p inhibitor transfection on MLE-12 cells exposed to bleomycin. After the miR-142-3p mimic transfection, an improvement in the cell viability and a reduction in the levels of IL-1 and TNF-α through down-activation of Cox-2 and the P13K/AKT/mTOR signaling pathway were observed. The functional effects of the miR-142-3p inhibitor were completely opposite. Previously, it had been reported that miR-506 holds the ability to inhibit EMT targeting Snai2, the E-cadherin transcriptional suppressor [99]. Therefore, the aim was to evaluate whether miR-506 serves as a regulator of pro-fibrotic factors in early-stage lung fibrosis; the study used an LPS lung fibrosis mice model. Moreover, the researchers specifically focused on target genes of miR-506 involved with the apoptotic and inflammatory responses. The overexpression of this miRNA in the mice model led to the attenuation of LPS-mediated pulmonary fibrosis. The experimental results demonstrated that miR-506 induced apoptosis directly targeting p65, an essential unit of NF-κB that has a crucial role in the transcriptional responses including cell division, cell survival, differentiation, immunity, and inflammation [100]. It was suggested therefore that miR-506 is a key regulator in pulmonary-fibrosis progression [101]. Another miRNA considered important in fibrosis is MiR-448, which had been pointed out as a tumor suppressor in several types of cancer [102].

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