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REVIEW ARTICLE

MiRNAs involved in the signaling pathways associated to the pathogenesis of idiopathic pulmonary fibrosis

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Abstract

Idiopathic Pulmonary Fibrosis (IPF) is a progressive and multifactorial interstitial lung disease whose pathophysiology remains unclear. The process initiates by repeated epithelial lung injuries followed by basal membrane destruction occasioning the activation of the epithelial mesenchymal transition (EMT) and myofibroblasts which carry out an excessive synthesis of extracellular matrix (ECM) proteins. Several studies have associated microRNAs (miRNAs) to the biogenesis and development of IPF because miRNAs participate in the processes of apoptosis, proliferation, differentiation and interaction between cells thanks to their role as activators or inhibitors of different receptors in the signaling pathways of TGF-β, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR, which are the main and most studied pathways involved in lung fibrosis. In this context, the knowledge on the altered miRNAs expression and even more their role in the different signaling pathways in IPF will shed light on new therapeutic targets for this lung disease.

Keywords: *miRNAs. Idiopathic pulmonary fibrosis. Epithelial mesenchymal transition. Myofibroblasts.*

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive disease with poor prognosis, and f ew therapeutic options. The incidence and prevalence increase with age, and it is most common seen in male patients older than 65 years. At first, IPF was considered as a chronic inflammatory disorder characterized by progressive fibrosis, however, evidence indicate that this pathology is a product of an epithelial-driven disorder associated with environmental, genetic risk factors, aging-associated processes and a profibrotic epigenetic reprogramming[1]. During the development of IPF, lung parenchyma shows repeated epithelial cell injuries with an aberrant reparation mechanism that cause several lung scars, mainly caused by an excessive fibroblasts activity and their trans differentiation to myofibroblasts. Moreover, the epithelial-mesenchymal transition (EMT) promotes myofibroblasts accumulation, and synthesis of an excessive amount of collagen and extracellular matrix (ECM) proteins by the alveolar epithelial cells (AECs)[2].

MiRNAs are short, non-coding RNAs with an extension of approximately 21-23 nucleotides which play a post-transcriptional regulatory role by targeting specific messenger RNAs for degradation and/or translational repression. To date, there are already some reports about profibrotic and anti-fibrotic miRNAs dysregulation in the pathogenesis of IPF. These mechanisms are carried out since miRNAs have overlapping functions because they can either regulate a single gene or several genes can be affected by a single miRNA. For this reason, is relevant to update the information about altered miRNAs involved in the crucial signaling pathways implicated in IPF to have a better understanding of their pathogenesis mechanisms and to propose possible potential therapeutic targets[3].

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Materials and Methods

This review is focused on a PubMed searching using the following key words: "miRNAs involved in IPF", "miRNAs involved in cellular signaling pathways in IPF" and "miRNAs involved in the TGF-β pathway". The selection was reduced to articles written in the years 2016 to 2020 in English language. The articles found were around fifty; however, the selected papers were the ones that related miRNAs with the TGF-β, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR signaling pathways since these signaling pathways are the most studied ones in pulmonary fibrosis process.

For better understanding, the manuscript is organized according to miRNAs participation in each signaling pathway involved in the pathogenesis in IPF.

First, a general summary about the main signaling pathways involved in the pathogenesis of IPF is explained and then there is a description of the function of each miRNA within them.

EMT process in IPF

EMT is a process in which epithelial cells gradually lose their cell-to-cell junctions, adherens junctions and apical-basal polarity to acquire a mesenchymal cell phenotype. This phenotype gains new invasive properties, and re-synthesizing new extracellular matrix proteins. The EMT process, can be activated by transcription factors and by signaling pathways, being the transforming growth factor beta 1 (TGF-β) its best inductor[4]. This process is controlled by three families of transcription factors: the zinc finger Snail (Snail/Slug) and zinc finger E-Box binding homeobox 1 and 2 (ZEB1/ZEB2) which have, as main function, the repression of E-cadherin expression[5]. Nowadays, it is well stablished that EMT process in IPF is regulated also, by epigenetic modulators like miRNAs which can act by activating or reverting lung fibrosis through the regulation of targets expression involved in EMT signaling pathways such as TGF-β, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR, which induce the activation of resident fibroblasts and their trans differentiation into myofibroblasts, for example, miRNA-326 reduces the expression of profibrotic genes by targetting TGF-β and miRNA-26 directly downregulates CTGF which is the responsable for collagen formation[6].

Signaling pathways involved in EMT process

EMT is regulated by extracellular ligands such as TGF-β, interleukin-1 (IL-1), connective tissue growth factor (CTGF) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), which is activated by tumor necrosis factor alpha (TNF-α), insulin-like growth factor-1 (IGF-1), secreted glycoproteins of the wingless/integrate-1 (Wnt)[5] and ADAM metallopeptidase with thrombospondin type motif 1 (ADAMTS1)[7].

All of them, have the property of initiating the intracellular signaling cascades through the activation of one or more EMT-driving transcription factors such as SNAIL1/2 and ZEB1/2 and downregulating the expression of adhesion molecules like E-cadherin, being the high-mobility group AT-hook2 (HMGA2) the one that facilitates the transcription of SNAIL1[5].

It has also been proved that MiRNAs have a stimulant role in extracellular matrix protein synthesis through collagen genes such as COL1A1, COL1A2, COL3A1, COL5A2, COL4A2, LOXL and matrix metallopeptidase (MMP) like MMP2, MMP3 and MMP10, among others[8].

TGF-ß signaling pathway

In lung diseases like IPF, there is an increasing evidence that proves the activation of TGF-β pathway by lung cells to maintain homeostasis in response to tissue injury[9]. This cytokine is secreted, in a latent form, bound to latency-associated peptide (LAP), but when it is released from this peptide, TFG-β dimers conform a complex with the type I TGF-β receptor (TGF-BRI) and type II receptor (TGF-BRII). This complex initiates a signal transduction by a Smad-dependent or non-Smad-dependent pathway[8]. In the Smad-dependent pathway, activated TGF-BRI phosphorylates cytoplasmic Smad2/3 transcription factors and translocate them into the nucleus. Smad4 facilitate this process and together with ZEB1/2 complex modulate the pro-fibrotic genes expression associated with EMT process, fibroblasts differentiation, proliferation migration and deposition of collagen[10]. Moreover, the Smad 2/3 complex represses the expression of E-cadherin through SNAIL1 and SNAIL2 which induce the expression of mesenchymal proteins such as N-cadherin, fibronectin, and metalloproteinases[11]. On the other hand, Smad6 and Smad7 inhibits the phosphorylation of Smad 2/3 complex by competing with Smad4 or TGF-βRI which is also regulated by Smurf2 E3 ubiquitin ligase. For this way, TGF-β signaling pathway is downregulated by Smad6, Smad7, and Smurf2[10].

In the non-Smad mediated pathway TGF-β can activate other signaling cascades, like mitogen-activated protein kinase (MAPK) pathway which can mediate Smad phosphorylation and activation through the

activation of extracellular signal-regulated kinase (Erk) and c-Jun N-terminal kinase (JNK) pathways. Also, TGF-β can activate other signaling mediators such as p38 MAPK kinase kinases family (MAPKKKS) and TGF-β activated kinase 1 (TAK1) which has the function of activating NF-kB signaling pathway. This dual ability of TGF-β to activate Smad and non Smad signaling pathways has an important role in the EMT process[12].

There are many others signaling pathways involved in the pathogenesis of IPF, however, the Wnt/β-catenin and phosphoinositide 3 kinase (PI3K)/protein kinase B (Akt) (PI3K/Akt) signaling pathways are the most relevant because they are related to cell proliferation, differentiation, and extracellular matrix production.

Wnt/ß catenin signaling pathway

In presence of injury Wnt/β-catenin signaling pathway promotes alveolar repair and remodeling, fibroblast migration and proliferation and extracellular matrix production through the stimulation of β-catenin by Wnt[13].

The activation of this pathway begins with the stimulation of TGF-β which generates the release of Wnt from the axis inhibition protein 1 (AXIN1)- glycogen synthase kinase 3 beta (GSK3β) - adenomatous polyposis coli (APC) (AXIN1/GSK3β/APC) complex, which, in a steady state, leads to ubiquitin-mediated proteolysis of β-catenin. When Wnt is released from this complex, there is a nuclear translocation of β-catenin via the phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) and suppression of GSK-3b and AXIN1 activity, this causes the accumulation of β-catenin and then suffers a nuclear translocation, by this way it can regulate the fibronectin gene 1 (FN1), the metalloproteinase 7 (Mmp 7) and cyclin D1 with the consequent production of extracellular matrix[14].

PI3K-Akt signaling pathway

This pathway has the function of regulating fibroblasts growth, proliferation, apoptosis, and collagen production; however, this pathway is down regulated in IPF which leads to Akt up regulation under mechanisms that modulate the activity of the phosphatidylinositol 3-kinase (PI3K)-protein kinase B (Akt)- forkhead box O3 (FoxO3a) and mammalian target of rapamycin (mTOR) (PI3K-Akt-FOXO3a-mTOR) axis. PI3K stimulates the synthesis of phosphatidylinositol-3,4,5-triphosphate (PIP3) which causes the activation of Akt. One of Akt targets is FoxO3a which is a powerful inhibitor of cell cycle due to the activation of the cyclin-dependent kinases (CDK) inhibitor protein p27 which promotes G1 arrest, however, FoxO3a activity is abnormally low in IPF fibroblasts because phosphatase and tensin homolog (PTEN), the main regulator of this pathway, is poorly expressed. This leads to fibroblasts autophagy inhibition thus promoting fibroblasts proliferation, increases the expression of alpha-smooth muscle actin (α-SMA) and fibronectin and reduces collagen degradation creating a collagen-rich environment[15].

MTOR has a role in cell growth, proliferation and apoptosis, it forms two distinct complexes which are mammalian target of rapamycin complex 1 (mTORC1) and mammalian target of rapamycin complex 2 (mTORC2), the first one controls cell growth and the second one regulates cell proliferation and survival: being the tuberous Sclerosis complex (TSC) the negative regulator of mTORC1 and PI3K the negative regulator of mTORC2[16].

MiRNAs have an important role in these signaling pathways because they can act as pro fibrotic or antifibrotic mediators through the stimulation or inactivation of different receptors in the signaling cascade, further examples of this will be seen below.

Figure 1 summarizes the different signaling pathways mentioned above, such as TGF-β, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR as well as their cross-linking. Also, several receptors involved in these pathways are illustrated, which can be stimulated or inhibited by the action of different miRNAs which can either stimulate or inhibit the trans differentiation of fibroblasts into myofibroblasts, pro fibrotic genes activation and extracellular matrix proteins synthesis.

MiRNAs involved in the pathogenesis of IPF

The compilation of miRNAs implicated in IPF was grouped depending on the signaling route and the process they regulate. They are better illustrated in Table 1.

MiRNAs involved in EMT in a Smad-dependent way

There are some miRNAs that have been studied in different samples like in a bleomycin murine model, in human A549 cells, bronchial epithelial cells and mice fibroblasts respectively for their antifibrotic role in IPF due to their up regulation, such as miRNA-877-3p, miRNA-1343 and miRNA-133.

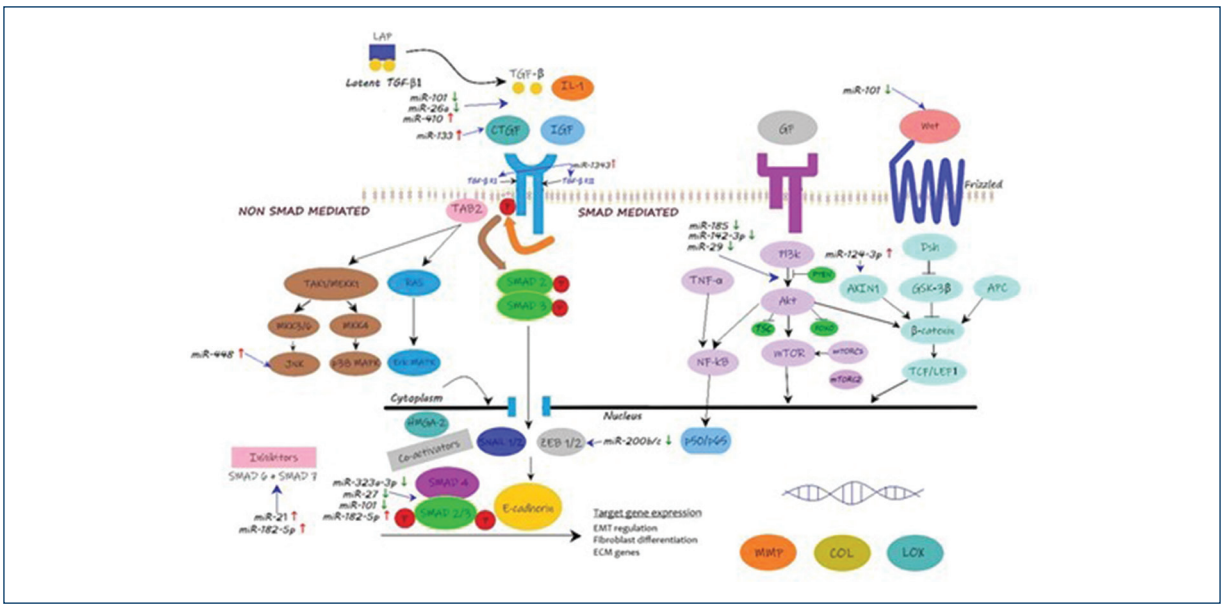

Figure 1. Proposal crosstalk between TGF-β with Smad, non-Smad dependent pathway, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR signaling pathways involved in the EMT process and some examples of miRNAs mentioned in this review involved in the fibrotic process. (Image inspired in the work of Derynck, R., & Zhang, Y.E. [2003], Rajasekaran, S., Rajaguru, P., & Sudhakar Gandhi, P. S. [2015] and Razali, R. A., Lokanathan, Y., Yazid, M. D., Ansari, A. S., Saim, A. B., & Hj Idrus, R. B. [2019]).

MiRNA-877-3p can inhibit TGF-β-induced myofibroblast differentiation through the up regulation of Smad7[17], miRNA-1343 represses both TGF-β I and II receptor thus reducing the expression of fibrosis markers such as α-SMA and COL1A[18] and miRNA-133 inhibits myofibroblast differentiation through the down regulation of profibrotic genes such as COL1A1 and CTGF[19].

On the other hand, there are miRNAs that even though have an antifibrotic role in health, they favor the development of IPF due to their downregulation status in this disease such as, miRNA-184, miRNA-155 miRNA-27a-3p, miRNA-19a- 19b-20a sub-cluster, miRNA-29b, miRNA-18a-5p, miRNA-200b/c, miRNA-27b, miRNA-185, miRNA-186, miRNA-130b-3p, miRNA-101, miRNA-323a-3p and miRNA-221.

MiRNA-184, studied in A549 cells, is not able to regulate Akt and Smad2[20], miRNA-155 studied in human lung fibroblasts, is not able to regulate Smad1[21] and miRNA-27a-3p studied in a pulmonary fibroblasts line can not inhibit, the phenotypic marker of myofibroblasts, α -SMA, Smad2 and Smad4[22]. These three miRNAs generate an induction of EMT transition, fibroblast proliferation and migration and collagen synthesis.

The next miRNAs have been studied in a bleomycin murine model: MiRNA-19a- 19b-20a sub-cluster is not able to suppress pro-fibrotic genes, including COL1A1 and CTGF[23], miRNA-29b is not able to suppress the expression COL1A1 and COL3A[24] and miRNA-18a-5p contributes with EMT because it is not able to regulate TGF-β type II receptor which might therefore provide a novel approach to the treatment of IPF[25]. MiRNA-200b/c is a possible therapeutic target too because this miRNA attenuates early pulmonary fibrosis by inhibiting ZEB1/2 via Smad-mediated way with the consequent inhibition of the expression of E-cadherin, however its expression is diminished in a fibrosis status[26], and MiRNA-27b role is affected because it cannot inhibit the expression of collagens genes such as COL1A1, COL3A1, COL4A1 and α-SMA through the regulation of Smad2, that is the reason why it has been proposed as a possible therapeutic target as well[27].

MiRNA-185, miRNA-186 and miRNA-130b-3p have been studied on human lung fibrotic samples and it has been seen that both miRNA-185 and miRNA-186 fail on the regulation of COL5A1 gene[28] and miRNA-130b-3p is unable to regulate the action of IGF-1 on EMT process[29]. MiRNA-101 and miRNA-323a-3p have been studied human lung fibrotic samples but also in a

Table 1. MiRNAs involved in the crucial signaling pathways of Idiopathic Pulmonary Fibrosis *(Continued)*

	Sample	miRNA	Function in health	Target of action	Effect	Status in IPF	Reference
MiRNAs involved in the EMT in a Smad-dependent pathway							
I	Bleomycin murine model	miRNA-877-3p	Anti-fibrotic	Smad7	Regulation of fibroblasts differentiation into myofibroblasts	Up regulated	Wang et al., 2016
II	Human A549 and bronchial epithelial cells	miRNA-1343	Anti-fibrotic	TGFBRI and TGFBRII	Inhibits Smad2/3 phosphorylation, nuclear translocation, and reduces expression of markers of fibrosis such as α-SMA and COL1A1	Up regulated	Stolzenburg et al., 2016
III	Mice fibroblasts NIH3T3	miRNA-133a	Anti-fibrotic	COL1A1 gene and CTGF	Regulation of TGF-β1induced myofibroblast differentiation	Up regulated	Wei et al., 2019
IV	A549 cells	miRNA-184	Anti-fibrotic	AKT and Smad2	Increase of TGF-β and PI3K-AKT-NF-κB pathway	Down regulated	Wang et al, 2020
V	Human lung fibroblasts	miRNA-155	Anti-fibrotic	Smad1	Regulation of fibroblast proliferation, migration, and collagen synthesis	Down regulated	Chi et al., 2019
VI	Human HEK-293T and pulmonary fibroblast line	miRNA-27a-3p	Anti-fibrotic	Smad2 and Smad4	Regulation of myofibroblast differentiation and expression of type 1 collagen, fibronectin and α -SMA	Down regulated	Cui et al., 2016
VII	Bleomycin murine model	miRNA-19a-19b-20a sub-cluster	Anti-fibrotic	TGF-BRI and TGF-BRII	Regulation of pro-fibrotic genes, including COL1A1 and CTGF	Down regulated	Souma et al., 2018
VIII	Bleomycin murine model and NIH/3T3 cells	miRNA-29b	Anti-fibrotic	COL1A1 and COL3A1	Collagen expression Regulation	Down regulated	Yamada et al., 2017
IX	Bleomycin murine model	miRNA-18a-5p	Anti-fibrotic	TGF-BRII	EMT regulation	Down regulated	Zhang et al., 2017
X	Bleomycin murine model	miRNA-200b/c	Anti-fibrotic	ZEB1 and ZEB2	Regulation of E-cadherin expression and EMT	Down regulated	Cao et al., 2018
XI	Bleomycin murine model	miRNA-27b	Anti-fibrotic	TGFBR1 and Smad2	Regulation of fibroblasts activation and expression of COL1A1, COL3A1, COL4A1 and α- SMA	Down regulated	Zeng et al., 2017
XII	Human lung fibrotic samples	miRNA-185 and miRNA-186	Anti-fibrotic	COL5A1	Regulation of collagen V expression and EMT	Down regulated	Lei et al., 2016
XIII	Human lung fibrotic samples	miRNA-130b-3p	Anti-fibrotic	IGF-1	Regulation of fibroblasts activation and EMT	Down regulated	Li et al., 2016

(Continues)

Table 1. MiRNAs involved in the crucial signaling pathways of Idiopathic Pulmonary Fibrosis (*Continued*)

	Sample	miRNA	Function in health	Target of action	Effect	Status in IPF	Reference
XIV	Human lung fibrotic samples and bleomycin murine model	miRNA-101	Anti-fibrotic	Wnt and Smad 2/3	Regulation of fibroblasts proliferation	Down regulated	Huang et al., 2017
XV	Human lung fibrotic tissue and bleomycine murine model	miRNA-323a-3p	Anti-fibrotic	Smad2	Regulation of fibroblasts proliferation	Down regulated	Ge at al., 2016
XVI	Human lung fibrotic samples and A549 cells	miRNA-221	Anti-fibrotic	HMGA2	EMT regulation	Down regulated	Wang et al., 2016
XVII	Bleomycin murine model and TGF-β treated human embryonic lung fibroblasts	miRNA-182-5p	Pro-fibrotic	Smad2 and Smad7	Increases the expression of profibrotic proteins such as fibronectin, α-SMA, Smad2 and Smad3 Inhibition of Smad7	Up regulated	Chen et al, 2019
XVIII	Bleomycin murine model and human lung fibroblast	miRNA-21	Pro-fibrotic	ADAMTS-1	Promotes interstitial fibroblasts proliferation and increase deposition of ECM	Up regulated	Zhou et al., 2018
XIX	Bleomycin murine model	miRNA-410	Pro-fibrotic	TGF-β/ ADAMTS1 pathway	Increases extracellular matrix proteins deposition and fibroblasts proliferation	Up regulated	Liu et al., 2018
XX	Human lung fibroblasts	miRNA-424	Pro-fibrotic	Slit2	Positive feed-back regulation of TGF-ß-induced expression of myofibroblast differentiation markers including α -SMA and CTGF	Up regulated	Huang 2020
XXI	A549 cells	miRNA-31	Pro-fibrotic	Smad2, Smad4 and Smad6	Promotes profibrotic factors expression like MMP7 Inhibits the expression of Smad6	Up regulated	Wang et al, 2019
MiRNAs involved in the EMT in a non Smad-dependent pathway							
XXII	TGF-β treated human lung fibroblasts	miRNA-340-5p	Anti-fibrotic	p38/ ATF1 signaling axis	Reduces extracellular matrix deposition, fibroblasts markers such as α-SMA and COL1 and reduces fibroblasts proliferation, and viability	Up regulated	Wei et al., 2020
XXIII	Bleomycin murine model	miRNA-448	Anti-fibrotic	JNK	Increased cellular apoptosis, decrease of cell viability and reduced collagen synthesis by fibroblasts	Up regulated	Xu et al., 2020
XXIV	Lipopolysaccharide fibrosis murine model	miRNA-506	Anti-fibrotic	P65 (NF-KB subunit)	Decreased fibroblasts proliferation and EMT	Down regulated	Zhu et al., 2019

(Continues)

Table 1. MiRNAs involved in the crucial signaling pathways of Idiopathic Pulmonary Fibrosis (*Continued*)

	Sample	miRNA	Function in health	Target of action	Effect	Status in IPF	Reference
MiRNAs involved in the EMT involved in other signaling pathways							
XXV	Bleomycin murine model	miRNA-152–3p, miRNA-140–3p, miRNA-148 b-3p, and miRNA-7a-5p _____ miRNA-34a-5p, miRNA-27 b-3p, miRNA-323–3p, miRNA-27a-3p, miRNA-34c-5p, miRNA-128–3p, and miRNA-224–5p	Anti-fibrotic _____ Anti-fibrotic	Klf4 gene _____ Ing5 gene	EMT induction by downregulation of TGF- β and Wnt/β-catenin pathways _____ EMT induction Through activation of PI3K/AKT and Wnt/β-catenin pathways	Down regulated _____ Down regulated	Wang et al., 2020
XXVI	Human BAL and THP-1 cells	miRNA-185	Anti-fibrotic	AKT and COL1A1 activation through the down regulation of miR-29	Decreased collagen expression and extracellular matrix deposition	Down regulated	Tsitoura et al., 2016
XXVII	Bleomycin in vitro model (MLE-12 cell line)	miRNA-142-3p	Anti-fibrotic	PI3K/AKT/mTOR	Decreased cellular apoptosis and increase of of IL-1 and TNF-α levels	Down regulated	Guo et al., 2017
XXVIII	Mice and human lung tissue samples	miRNA-29c	Anti-fibrotic	Foxo3a	Decreased cellular apoptosis	Down regulated	Xie et al., 201
XXIX	Bleomycin murine model	miRNA-124-3p	Pro fibrotic	AXIN1	TGF-β1 induced fibroblasts differentiation and increased Wnt pathway	Up regulated	Lu et al., 2019
XXX	Bleomycin murine model and human fibrotic lung sample	miRNA-301	Pro-fibrotic	TSC1/mTOR pathway	Promotes fibroblasts activation, proliferation and trans differentiation into myofibroblasts and collagen deposition	Up regulated	Wang et al., 2020

miRNA: microRNA; TGFBRI: type I TGF-β receptor; TGFBRII: type II TGF-β receptor; α-SMA: alpha-smooth muscle actin ; TGF-β1: transforming growth factor beta 1; CTGF: connective tissue growth factor; PI3K-AKT-FOXO3a-mTOR: phosphatidylinositol 3-kinase, protein kinase B, forkhead box O3, mammalian target of rapamycin; NF-kb: nuclear factor kappa-light-chain-enhancer of activated B cells; ZEB 1 / 2: zinc finger E-Box binding homeobox 1 and 2; EMT: epithelial mesenchymal transition; IGF-1: insulin-like growth factor-1; Wnt: wingless and int-1; HMGA2: high-mobility group AT-hook2; ADAMTS-1: metallopeptidase with thrombospondin type motif 1; ECM: extracellular matrix; MMP: matrix metallopeptidase; JNK: c-Jun N-terminal kinase; Klf4: kruppel-like factor 4; Ing5: growth family member5; BAL: bronchoalveolar lavage; IL1: interleukin 1; TNF-α: tumor necrosis factor alpha; AXIN: axis inhibition protein 1; TSC1: tuberous sclerosis complex.

bleomycin murine model, miRNA-101 acts via the Wnt and TGF-β/Smad2/3 pathways but its downregulated status generates an increase in fibroblasts proliferation, collagen synthesis and elevation of α-SMA[30] and miRNA-323a-3p is not able to regulate Smad2/3 complex therefore it cannot suppress fibroblast differentiation and expression of matrix proteins.[31] MiRNA-221 is another one studied on human lung fibrotic samples

but also in A549 cells and it was seen that it cannot regulate EMT process because it not able to regulate HMGA2[32].

Finally, there are some miRNAs which have a pro fibrotic role due to their up regulation such as miRNA-182-5p, miRNA-21, miRNA-410, miRNA-424 and miRNA-31.

MiRNA-182-5p and miRNA-21 have been studied in a bleomycin murine model but also in human lung fibroblasts and it was found that miRNA-182-5p has a direct effect stimulating on Smad2/3 complex and inhibiting Smad7[33] and miRNA-21 causes an abnormal deposition of ECM through the increase of TGF-β/ADAMTS-1 signal pathway and inhibition of Smad7 too[34]. MiRNA-410 was studied in a bleomycin model and is involved in the activation of TGF-β1/ADAMTS-1 signaling pathway as well and it has a promising therapeutic future because through the inhibition of this miRNA there would be a regulation of extracellular matrix proteins like COL1 and COL3 and fibroblasts proliferation[35].

In some recent studies, there has been found that miRNA-424 has an up regulated status in human lung fibroblasts due to Slit2 inhibition which generates a positive feed-back regulation of the TGF-ß-induced expression of myofibroblast differentiation markers including α-SMA and CTGF[36]. and miRNA-31 promotes Smad2 and Smad4 heterodimers formation and inhibits Smad6 in A 549 cells[20].

miRNAs involved in EMT in a non Smad-dependent way

In recent studies it has been demonstrated that there are some miRNAs that act on non-Smad dependent pathways with an antifibrotic role like miRNA-340-5p, miRNA-448 and miRNA-506.

MiRNA-340-5p which is upregulated in TGF-β treated human lung fibroblasts regulates the ATF1/p38 signaling axis that reduces extracellular matrix, fibroblasts markers such as α-SMA and COL-1and reduces fibroblasts proliferation[37].

MiRNA-448 studied in mice fibrotic tissue, was found to be upregulated and its function is to suppress the JNK signaling pathway that can inhibit IPF progression by downregulating cell proliferation and collagen synthesis by promoting apoptosis in fibroblasts[38].

On the other hand, miRNA-506 has an antifibrotic role through the inhibition of p65 which is a subunit of NF-κB, however its downregulated status in a fibrosis murine model promotes fibroblasts recruitment, EMT and decreases fibroblast apoptosis[39].

MiRNAs involved in EMT through other signaling pathways

There are some several miRNAs that have an antifibrotic role, however, due to their down regulated status in lung fibrosis they are not able to fulfill their role.

For example, miRNA-152–3p, miRNA-140–3p, miRNA-148 b-3p, and miRNA-7a-5p studied in bleomycin murine model, are not able to downregulate Kruppel-like factor 4 (Klf4), a negative regulator of α-SMA which has a direct effect on SNAI2, therefore suppressing TGFβ1-induced EMT and they are not able to regulate the Wnt/β- catenin pathway either. Also, miRNA-34a-5p, miRNA-27 b-3p, miRNA-323–3p, miRNA-27a-3p, miRNA-34c-5p, miRNA-128–3p, and miRNA-224–5p cannot regulate the growth family member5 (Ing5) which regulates PI3K/AKT and Wnt/β-catenin pathway therefore there is an increment in the fibrosis process[40].

MiRNA-185 downregulation in human bronchoalveolar lavage (BAL) contributes to collagen deposition due to AKT overactivation and miRNA-29a downregulation which causes the overexpression of COL1A1 gene, therefore this miRNA has been proposed as possible diagnosis biomarkers for patients with IPF[41]. Moreover, with miRNA-142-3p mimic transfection, in bleomycin-induced injuries in an *in vitro* IPF model it was possible to alleviate cell injury and production of pro-inflammatory factors through the regulation of PI3K/AKT, which indicated the protective role of this miRNA, however, even though it has a anti fibrotic role it is diminished in a fibrosis state[42]. Another example is miRNA-29c which have an impact on cell apoptosis of AECS2 because its function is to regulate FoxO3a, however, this miRNA is negatively regulated in human lung tissue resulting in a resistance to apoptosis and lack of regulation of ECM-related target genes such as COL3A[43].

Finally, there are some miRNAs which have a pro fibrotic role due to their up regulation such as miRNA-124-3p and miRNA-301a, both have been studied on a bleomycin model and miRNA-301a has also been studied on human lung fibrotic tissue samples.

MiRNA-124-3p plays an important role in TGF-β1 induced fibrogenic cell differentiation through the regulation of AXIN1 protein, which is involved in the activation of Wnt pathway[44] and miRNA-301a promotes the up regulation of TSC1 gene which acts through the mTOR signaling pathway promoting fibroblasts activation and has been proposed as a possible therapeutic target[45].

Results

After reviewing fifty original articles about MiRNAs involved in idiopathic pulmonary fibrosis, it was decided to include only those that related miRNAs to the main signaling pathways involved in the fibrotic process of lung parenchyma, such as TGF-β, Wnt/β-catenin and PI3K-Akt-FOXO3a-mTOR pathways.

MiRNAs are short, non-coding RNAs with an extension of approximately 21-23 nucleotides which play an important role in the genesis and development of IPF thanks to their supra regulated state by favoring epithelial mesenchymal transition and fibroblasts stimulation with the consequent synthesis of extracellular matrix proteins such as collagen; however, there is another pro fibrotic mechanism in which miRNAs cannot exert their antifibrotic effect because they are in an infra regulated state, which generates an inadequate control of intracellular receptors of the signaling pathways which will favor lung fibrosis.

Among these signaling pathways there is the TGF-β pathway, which exert its function through a Smad and non Smad dependent way. It is the main pathway involved in the regulation of the expression of profibrotic genes as well as the differentiation and proliferation of fibroblasts and the consequent deposition of collagen. There are also other alternative pathways such as the Wnt/β-catenin pathway and the PI3K-Akt-FOXO3a-mTOR pathway, which are also stimulated by miRNAs, and they related to alveolar repair through fibroblasts proliferation and extracellular matrix proteins production which generates pulmonary fibrosis.

Discussion

IPF is a progressive and degrading interstitial lung disease with poor prognosis and few therapeutic options. Nowadays it has been proposed that several miRNAs play an important role in the pathogenesis of this disease, both in human and murine fibrosis models, because they are able to regulate cellular processes such as cellular apoptosis, proliferation, and differentiation through the stimulation of different receptors within signaling pathways which causes a remodeling effect of lung parenchyma.

In this review the current knowledge about these miRNAs was grouped and synthesized to have a better understanding about their different mechanisms of action within the main signaling pathways which have as common end the trans differentiation of fibroblasts into myofibroblasts and the synthesis of extracellular matrix proteins that contribute with the fibrotic process.

Conclusions

MiRNAs have been studied in IPF, to expand the knowledge about the pathogenesis of this disease but also, they have been postulated as a new therapeutic option because nowadays the treatment of this disease is not very effective and is not curative either, so if it is possible to modify or block the action of a specific miRNA it could generate a change in the mechanism of the disease. Although it is not yet known whether this therapy will have adverse effects, it is believed that therapy based on miRNAs could be the basis of an effective treatment for pulmonary fibrosis since there is a precedent regarding the possible utility of miRNAs as therapeutic agents. This has been studied in different types of cancer where in which it is used miRNA mimic therapy and viral vectors containing the miRNA target to restore the down regulated status of anti fibrotic miRNAs and anti-miRNAs to block the pro fibrotic role of supra regulated miRNAs. However, it is suggested to expand the studies about miRNAs involved in IPF to continue updating information about different mechanisms of action altered in this pathology so that in the future, being able to help patients suffering from this chronic and devasting disease by proposing a new therapeutic agent more effective than those existing today and to provide a better quality of life.

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The authors declare that they have no conflict of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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