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Signaling pathways and their miRNA regulators involved in the etiopathology of idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP)

Abstract

Idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) belong to heterogenic group of interstitial lung diseases (ILD). For the reason that this group of diseases present with complex clinical non-specific features, they represent a diagnostic and therapeutic challenge. In this review we focus on several crucial signaling pathways participating in inflammation, fibrosis and EMT processes, so important in the course of ILD: TNF-α/NF$\kappa\beta$, TGF-β/SMAD, Wnt-β-catenin and PI3K-Akt signaling. Moreover, this review summarizes the role of selected signaling pathways and some miRNAs which are their regulators during development and progression of IPF and HP. Recent advances indicate the potential role of miRNAs as a molecular markers differentiating clinical course of ILD.

Key words: idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, molecular markers, pathogenesis, signaling pathways

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Introduction

Idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) belong to heterogeneous group of interstitial lung diseases (ILD), where profibrotic/antifibrotic as well as proinflammatory/anti-inflamatory imbalance is postulated. However, exact etiology of these diseases is not fully understood, the alveolar epithelial cell injury and dysregulated repair processes are suggested [1–3].

IPF is categorized as one of the most common (17–86%), chronic, progressive lung disease, with low survival rate (median survival 3–5 years after diagnosis), and is currently untreatable [4–6]. Recent studies have confirmed two major risk factors in IPF development: (1) environmental factors (e.g., cigarette smoking, viral infections),(2) genetic factors (e.g., allelic variants of *VEGF, TGF-β, MUC5B*, telomerase complex: *telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC)* [7–9]. As

so far, several important processes involved in IPF are postulated: recruitment of inflammatory cells, deposition of extracellular matrix, accumulation of fibroblasts [2, 10] or activation of epithelial mesenchymal transition (EMT) [11].

HP belongs to a group of immunologically mediated lung diseases. Repeated exposition and inhalation of wide variety of antigens, such as: bacteria (*e.g., Saccharopolyspora rectivirgula*), fungi (*e.g., Trichosporon cutaneum*) [12], animal proteins (mostly avian) [13], chemicals (e.g., di-isocyantes) [14] and incites hypersensitivity reaction of lung with granulomatous inflammation in genetically predisposed subjects [15]. Three key clinical forms of HP are distinguished: acute, subacute and chronic with frequent overlapping of these three forms [3]. Polymorphisms of transporters associated with antigen processing (TAP) genes are found in HP patients [16]. The essential reason of HP is coexistence of genetic/ /environmental factors which are able to stimulate

an exaggerated immune reaction resulting in lung inflammation. Following the immunopathological changes induced in lung cells, the fibroblast expansion and extracellular matrix (ECM) accumulation may occur [2].

Reassuming, chronic inflammation and EMT are recognized as two key pathological processes involved in IPF and HP development where several signaling pathways (e.g., TNF-α/NF$\kappa\beta$, TGF-β//SMAD, Wnt-β-catenin) are activated. Additionally, the role of many different classes of miRNAs, which may regulate the mentioned signaling pathways *via* gene expression at post-transcriptional level has been recognized.

In this review the authors discussed the role of selected signaling pathways and some miRNAs as their regulators in the development and progression of IPF and HP.

Signaling pathways involved in IPF and HP development

Inflammation

Inflammation is postulated as an initial and a critical factor in HP and in IPF is rather a secondary process associated with fibrosis. For a long time it has been assumed that inflammation plays a direct role in pathogenesis of IPF. However, lung cells derived from IPF patients reveal high expression levels of genes involved in proliferation, migration, oxidative stress and remodeling. Several lines of evidence suggests that inflammation may play a key role in acute exacerbation of IPF, but not in chronic disease itself [17]. Additionally, lack of response to long-term anti-inflammatory treatment suggests that epithelial pathway (with cytokine/growth factor releasing and fibroblast migration) may be possible process in pulmonary fibrosis [2]. Selman et al. [2] proposed two different routes for pulmonary fibrosis development: a) the inflammatory pathway and b) the epithelial pathway. Based on the studies on human IPF and animal models of pulmonary fibrosis, five possible hypotheses for the pathogenesis of IPF have been proposed, underlining the controversial role of inflammation:1) direct inflammation hypothesis, 2) matrix hypothesis in which inflammatory mediators are released under distant injury *via* extracellular matrix, 3) growth factor receptor hypothesis, where GFRs release the inflammatory cascade activation, 4) plasticity hypothesis based on EMT and multifarious interactions between inflammatory mediators and GFs and other key factors which facilitate the fibrotic phenotype, 5) vascular hypothesis which suggest that endothelial injury activates the inflammatory cascade leading to fibrosis [17, 18].

In HP, the dispersed antigens provoke a hypersensitivity reaction with granulomatous inflammation in the distal bronchioles and alveoli [13]. Characterized as bronchiolocentric granulomatous lymphocytic alveolitis may develop to fibrosis in advanced stage of HP [3] In HP, the exposed antigens act as specific inducing factors. Immediately after antigen challenge, an immunological response — followed by production of IL-1 and TNF-α by macrophages — takes place [15]. Secretion of IL-1 and TNF-α may lead to adhesion molecule expression on leukocytes and endothelial cells. What is more, macrophages secrete IL-8, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1a, and RANTES that act as chemotactic factors for a variety of cells [19]. The exact mechanism of the disease remains unclear. It is suggested that tolerance may be mediated by regulatory T cells (Treg), a unique population of CD4$^+$ T cells that play a pivotal role in the maintenance of the balance between the tissue-damaging and protective effects of the immune response [20]. The disease chronicity depends on immunopathological processes. Patients with chronic HP show an decrease of CD4+ T cells and exhibit skewing toward Th2 immunological activity [21]. It was confirmed that most patients have usually a low CD4+/CD8+ ratios, typically < 1 in BAL — conversely that in sarcoidosis.

TNF-α/NF$\kappa\beta$ signaling pathway

Tumor necrosis factor (TNF-alpha), which activates NF-κB family of transcription factors, plays an important role in the immune system regulation, influences the expression of cytokines, inducible nitric oxide synthase (iNOS), cyclo-oxygenase 2 (COX-2), growth factors and inhibitors of apoptosis [22]. The activation of NF-κB dimers occurs by two pathways: classical (canonical) and the alternative (non-canonical) pathway [23]. The common regulatory step in both pathways is activation of IκB kinase (IKK) complex encompassing catalytic kinase subunits (IKKα and/or IKKβ) and the regulatory non-enzymatic scaffold protein NEMO (NF-κB essential modulator also known as IKKγ). In the absence of TNF-alpha stimulation, NF-kB is associated with the inhibitor IkB in the cytoplasm. Activation of NF-κB dimers (IKKα and/or IKKβ) is due to IKK-mediated phosphorylation-induced proteasomal degradation of IκB. Therefore, active NF-κB transcription factor subunits may be relocated to the nucleus and

prompt target genes expression [24, 25]. It was recognized that TNF-α after stimulation may be released by macrophages, T-cells, B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells, and keratinocytes [26]. Recently, genetic studies have showed that polymorphism in *TNF-α* gene (TNF-308A) is associated with high *TNF-α* production *in vitro*, and this frequency is significantly increased in HP patients [27]. Another study has demonstrated that high expression of *TNF-α* is associated with a greater risk for developing farmer's lung and pigeon fancier's lung diseases as a form of hypersensitivity pneumonitis [13]. Previous studies in human and on animal model have confirmed that NF-κB plays a vital role in inflammatory process in lung and in the pathogenesis of pulmonary fibrosis [28, 29]. In BLM (bleomycin) mice model lung inflammation and pulmonary fibrosis were significantly relieved by GLP-1 treatment, possibly through inactivation of NF-κB [29].Current data have demonstrated that NF-κB has both pro- and anti-inflammatory functions, permitting NF-κB to play a role both in the initiation and in the resolution of inflammation. Due to the fact that NF-κB takes part in the inflammatory response, the therapeutic strategies to block NF-κB signaling and arrest the disease process may be developed in the future [30].

EMT process

It is marked that the disruption of alveolar epithelium integrity with the presence of enhanced migration of fibroblasts into the alveolar spaces may be involved in epithelial pathway of IPF pathomechanism [2]. The presence of subepithelial fibroblast and myofibroblast loci in extracellular matrix followed by the abnormal remodeling of the ECM is considered a prognostic factor in IPF [31]. Type 2 EMTs is associated with wound healing, tissue regeneration, and organ fibrosis. In recent years IPF pathomechanism has been recognized rather as a disease characterized by epithelial injury, abnormal epithelial healing and enhanced fibrotic response than the predominantly inflammatory lung disease. In the pathological background of IPF, epithelial microinjuries, increased inflammatory and/or pro-fibrotic cytokines followed by fibroblast accumulation leads to fibrotic lesion and EMT [32–34]. Therefore, it is postulated that excessive accumulation of fibroblasts generates abnormal epithelial-mesenchymal interactions due to EMT. In addition, the recent study of Yasui *et al.* [35] has shown that EMT is involved in hypersen-

sitivity pneumonitis. Based on animal model, authors have confirmed the correlation between the increased percentage of EMT cells and IL-13 and TGF-β1 mRNA expression, as well as an increased amount of collagen [35].

In human and rat alveolar epithelial cells and in epithelial cell lines it has been confirmed that EMT may be activated by the action of some important pathways, such as TGF-β/SMAD or Wnt/β-catenin signaling [9].

TGF-β/Smad signaling pathway

Many studies have demonstrated that TGF-β//Smad (*transforming growth factor-β/Smad*) is a pleiotropic signaling pathway which play a crucial role in inflammation, wound healing and fibrotic processes [9]. It is also considered as a great inducer of ECM deposition and EMT process in tissue fibrosis *via* Smad-dependent cascade [18, 35, 36]. There are irrefutable evidences that Smad2 and Smad3 play distinct roles in TGF-β /Smad signaling and their expression is different in epithelial cells and fibroblasts. During fibrosis, in adult fibroblast, TGF-β/Smad signaling pathway is working under Smad3 but not Smad2 [37]. It was suggested that TGF-β can suppress Smad 3/4 action via the overexpression of Smad 7 [38]. Smad 7 can act as an inhibitor by suppressing the phosphorylation of Smad 2 and Smad 3 via ubiquitination by Smurf2 ubiquitin-protein ligase. This process contributes to the degradation of the TGF-β R1 and R2 receptor complex, inhibiting TGF-β signaling as a final consequence [39]. It is also suggested that Smad 7 gene expression influences lung airway remodeling and lung injuries, leading to fibrosis through regulating the magnitude of TGF-β signaling [40].

It is documented that during interstitial disease development, the exposure of the airways to allergens results in the inflammatory response with the secretion of TGF-β and other inflammatory mediators. In consequence, the thickening of the lung cell membranes and lung damage may occur. In the next step, lung repair mechanism associated with the secretion of some mediators stimulates migration and proliferation of fibroblasts. Transformation to myofibroblast phenotype is started, which leads to pulmonary fibrosis, impedes gaseous exchange in the lungs and results in the respiratory failure [41].

Therefore, it is accepted that TGF-β is a main cytokine responsible for tissue regeneration, scarring and remodeling. The fibroblasts which are presented in a wound, as specialized myofibroblasts, may activate extracellular matrix *via*

elevated levels of α-smooth muscle actin (SMA), connective tissue growth factor (CTGF), and secretion of matrix proteins, such as collagen and fibronectin [42]. Activation of TGF-β/SMAD signaling pathway may also occur in non-canonical way. TGF-β can activate all three known MAPK pathways: extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK) and c-Jun-N-terminal kinase (JNK). These ways of signaling further regulate Smad-independent TGF-β responses. Moreover, p38 MAPK [43] and JNK [44] usually increase TGF-β/Smad responses. In addition to Smad and MAPK, TGF-β has been shown to activate PI3 kinase/Akt, Rho GTPase pathways and cooperate with Wnt and Notch [45]. The latest data show that pro-fibrotic effects of TGF-β are mediated through upregulation of its downstream effector Connective Tissue Growth Factor (CTGF). TGF-β-induced expression of CTGF stimulates myofibroblast differentiation and collagen synthesis [46]. On the other hand, CTGF enhances the activity of TGF-β leading to the increased binding to TβRI and TβRII [47]. The CTGF expression was related to fibroblast proliferation, cellular adhesion, angiogenesis, and synthesis of ECM [48]. It is expressed in various types of cells, including: epithelial, vascular smooth muscle, and fibroblasts also in lung [48, 49]. It was documented that higher CTGF level contributes to expression of α-smooth muscle actin (α-SMA) and the myofibroblast phenotype in tissue repair or development of connective tissues [50, 51]. What is more, CTGF appears to play a critical role in mediating many important fibroproliferative effects of TGF-β, including the pathogenesis of fibrotic disorders [52–54].On the other hand, other pro-inflammatory cytokines, e.g., TNF-α or IFN-γ, are expressed in macrophages during the wound healing. They suppress matrix genes activities and act as antifibrotic factors [42]. Reassuming, biological effects of TGF-β in lung epithelium include control of airway remodeling, supervision of bronchial fibrosis and wound healing [55].

The activity of signaling pathways induced by TGF-β depends on cell type, cell differentiation, interaction with other signaling pathways (PI3K/Akt or Wnt/β-catenin) and individual genetic predispositions.

In relation to therapy glucocorticoids (GCs) are among the first-line therapeutics in inflammatory diseases. They are responsible among others for inhibition of phagocytosis and lysosomal breakdown. They can also reduce the number of lymphocytes, eosinophils, and monocytes [56].

It has been documented that the SNP polymorphisms in NR3C1 gene (glucocorticoid receptor) don't influence the secretion of many factors, such as: TGF-β, GM-CSF, VEGF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-12, IL-13, IL-16, IL-17. On the other hand, in contrary, the study carried out by Panek *et al.* [56], has documented that SNPs in NR3C1 gene intensify non-sensitivity to GCs in asthmatic patients. It should be emphasized that Tth111I and N363S polymorphisms also significantly modulate the level of TGF-β1 in patients with asthma, who have been treated with GCs.

PI3K-Akt signaling pathway

It is commonly known that PI3K/Akt (*phosphatidyl inositol 3-kinase/ v-akt murine thymoma viral oncogene homolog 1*) signaling pathway regulates cell growth, proliferation and apoptosis. In lung fibrosis in IPF patients, the PI3K/Akt *via* Akt is abnormally activated under mechanisms modulating the activity of the PI3K-AKT-FOXO3a axis, while PTEN – the main inhibitor of PI3K/Akt signaling — is downregulated [9]. The mechanism of fibrosis regulation *via* PI3K/Akt is not fully understood but it has been recognized that during tissue repair, overgrowth of fibroblasts and type I collagen — as a complex with α2β1 integrin — are eliminated by apoptosis, in response to collagen matrix contraction. It has been recognized that PTEN which is activated during collagen matrix contraction, stimulates fibroblast apoptosis. Enhanced Akt activation decreases PTEN level and alters fibroblast survival. It was confirmed on *in vitro* model, where PTEN null fibroblasts presented enhanced level of Akt phosphorylation and therefore resistance to collagenmatrix contraction-induced apoptosis [57]. Moreover, FOXO3a — one of the target gene for Akt — is recognized as a strong inhibitor of cell cycle and apoptosis. Therefore, FOXO3a deficiency could protect IPF fibroblasts from apoptosis and enhanced fibrosis. What is more, in regulation of fibrosis process many interactions with other signaling pathways, such as TGF-β/Smad, MEK/ERK or VEGF, Jak/STAT, have been documented. It should be pointed that this complex regulation of fibrosis with many interacting signaling pathways involved, suggests that single molecules of the mentioned paths may not be accepted as good molecular targets for therapy.

Wnt/βcatenin signaling pathway

The Wnt-catenin signaling pathway regulates many biological processes, such as: fate determination, motility, polarity, primary axis formation
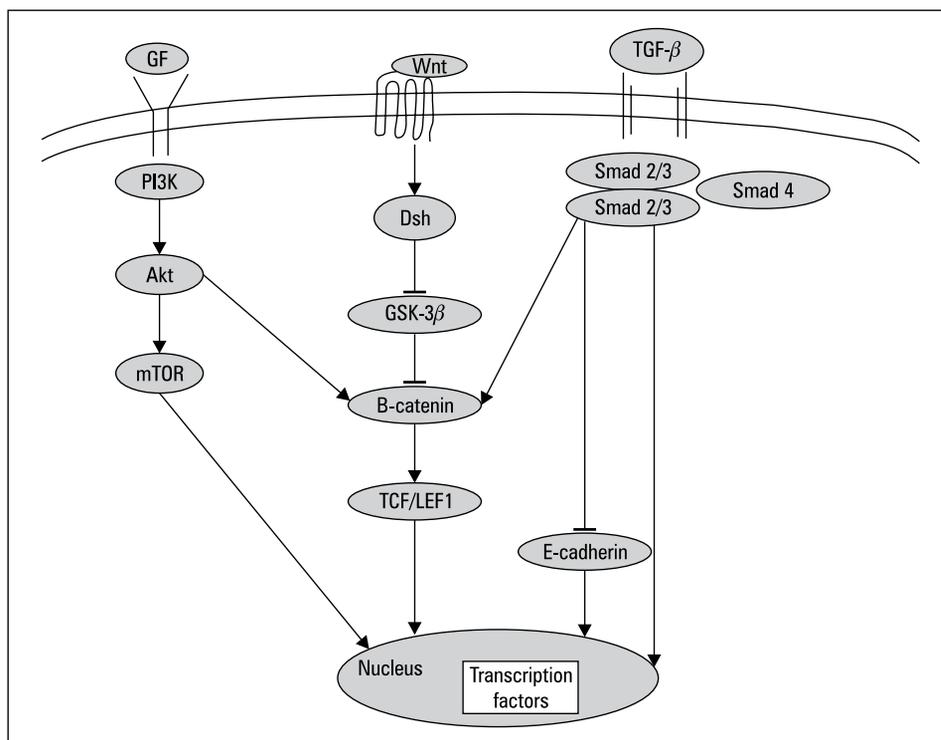
Figure 1. The proposal of a crosstalk between signaling pathways involved in EMT process. Abbreviations;TGF-β — transforming growth factor-β; Smad 2 — mothers against decapentaplegic homolog 2 protein; Smad 3 — mothers against decapentaplegic homolog 3 protein; Smad 4 — mothers against decapentaplegic homolog 4 protein; Wnt — wingless/integration signaling; Dsh — phosphoprotein Dishevelled; GSK3 — glycogen synthase kinase 3; TCF/LEF-T — cell factor/lymphoid enhancing factor; GF — growth factors; PI3K — Phosphoinositide 3-kinase; Akt — Protein kinase B mTOR — mechanistic target of rapamycin

and carcinogenesis. Recent reports have shown that deregulated Wnt-catenin signaling has important negative consequences in embryonic development [58].

In respiratory epithelial cells, the Wnt/β-catenin signaling pathway is necessary for lung branching and distal airway cell specification [59, 60]. Lately, it has been confirmed that in lung biopsies from IPF patients, high activation of the canonical Wnt/β-catenin signaling pathway is associated with tissue repair and fibroblast activation [61, 62].

Based on the above mentioned data, it is thought that Wnt/β-catenin signaling plays an important role in many pathological processes in lung, such as: inflammation, remodeling and fibrosis. Recently, it has been also documented that Wnt/β-catenin signaling is involved in the induction of EMT, an important step in fibrosis development [63]. The study of Königshoff *et al.* [64] confirmed significantly increased expression of Wnt1,3a, 7b and 10b, Fzd2, Fzd3, β-catenin, and LEF1 in IPF patients. What is more, authors have demonstrated that Wnt1, Wnt3a, β-catenin, and GSK-3β are localized in alveolar and bronchial epithelium [64]. Especially Wn3a is

recognized as a promotor of lung epithelial cell proliferation and myofibroblast activation [64]. Interestingly, the increased level of β-catenin and Wnt/β-catenin signaling pathway promotes fibroblast migration and proliferation and is associated with ventilator-induced pulmonary fibrosis in previously healthy persons [65]. These data indicate that activation of β-catenin may be a common feature of lung fibrosis, including pathological process in IPF.

It is also known that Wnt/β-catenin signaling pathway may link the IPF progression with other signaling pathways as a special cross-talk between them. Mainly TGF-β could trigger Wnt/β-catenin signaling pathway and initiate the cellular matrix accumulation. In Wnt/β-catenin signaling, the regulation of fibrosis, the nuclear translocation of β-catenin *via* the phosphorylation of ERK1/2 and suppression GSK-3b activity seem to be crucial [66]. The proposal crosstalk between signaling pathways involved in EMT process is shown in Figure 1.

miRNA a potential gene expression regulators

It is well identified that miRNAs are able to post-transcriptionally regulate gene expression

Table 1. Important miRNAs regulating the selected signaling pathways in inflammation process in IPF and HP

miRNA	Target	Function	Disease	Process	Reference
miR-146	IL-8	In human lung alveolar epithelial tumor A549 cells miR-146a negatively regulates the release of IL-8 and RANTES	HP?	inflammation	[71]
miR-155	NF-κB IFN-γ	In human mesangial cells, IFN-γ and TNF-α induce miR-155 expression and regulate inflammatory and immune responses,	HP?	inflammation	[74]

in almost all aspects of physiological conditions, such as: cell differentiation, cell growth, mobility and apoptosis. Therefore, miRNAs are noted as important factors involved in the development of many human diseases. Many classes of miRNAs exhibiting abnormal expression have been recognized as therapeutic targets.

It is noteworthy that miRNAs can be detected in various biological materials, such as: tissue, blood, serum, plasma, body fluids. Current studies have confirmed that exosomes are "bioactive vesicles" that promote intercellular communication and immunoregulatory processes by transporting molecules between cells [67, 68]. What is more, exosomal miRNAs (by being packaged in lipid vesicles) are protected against degradation and can be functionally delivered to target cells.

miRNAs have been revealed to be expressed in a tissue-specific and developmental stage-specific manner [69]. Also the lung has been shown to have a very specific miRNA expression profile. It has been shown that some miRNAs have characteristic lung specific expression profile. For example, these that participate in biological processes in the lungs, i.e., miR-155, miR-26a, let-7, miR-29, miR-15/miR-16, miR-223, miR-146a/b and the miR-17-92 cluster are involved in homeostasis and in the lung development [70]. Other, like miR-146a/146b [71], miR-155 [72] are important in pulmonary inflammation. All mentioned miRNAs may show up/down regulation in lung. Moreover, it is documented that smoking also influences miRNA expression levels. Expression profiling study in the rats exposed to environmental cigarette smoke revealed 24 downregulated miRNAs (especially let-7 family, miR-10, -26, -30, -34, -99, -122, -123, -124, -125, -140, -145, -146, -191, -192, -219, -222, and -223) when compared to control group [73]. In the respiratory system, miRNAs are important in normal pulmonary development and lung homeostasis. Recent studies have also documented that altered miRNAs expression profiles may be related to pathological processes within the lung, such as inflammatory diseases or lung fibrosis.

Many data have demonstrated that miRNAs are involved in the regulation of adaptive immune system: development, differentiation of B and T cells, proliferation of monocytes and neutrophils, antibody production or release of inflammatory mediators. They may work in the negative feedback regulation of inflammation following activation by the innate immune system. In IPF and HP diseases, miRNAs have been recognized as negative regulators of many signaling pathways involved in inflammation process (Table 1).

Also, miRNA dysregulation has been recognized in fibrotic disorders, as regulators of tissue injury and fibrosis, and especially as EMT controlling factors in IPF and HP (Table 2).

Interestingly, the special attention has focused on significance of miR-21 in pulmonary fibrosis. The study on animal model has confirmed that miR-21 is upregulated in the lungs of mice with bleomycin-induced fibrosis. Moreover, miR-21 has been shown to be upregulated also in the lungs of patients with idiopathic pulmonary fibrosis, under pro-fibrogenic activity of TGF-β [81]. Additionally, let-7d has been recognized as significantly decreased in idiopathic pulmonary fibrosis, which correlates with increased collagen deposition during EMT in mouse lung [76]. Also miR-155 is involved in both inflammatory and pulmonary fibrosis [81]. Functions of the mentioned miRNAs are summarized in Tables 2 and 3.

Conclusions

Our review summarizes the current knowledge on important signaling pathways involved in the pathogenesis of IPF and HP, with an emphasis on two important processes, i.e., inflammation and EMT. We underline a fundamental role of some miRNAs which have the ability to modulate multiple genes and pathways. In recent years there has also been an explosion of reports on miRNA involvement in lung diseases, including interstitial lung diseases (ILD), which may have diagnostic and future therapeutic value. We focus

Table 2. Important miRNAs regulating the selected signaling pathways in EMT process in IPF and HP

miRNA	Target	Function	Disease	Process	Reference
miR-424	Smurf2	Enhances a-SMA expression, regulates the myofibroblast differentiation during EMT by increasing the activity of TGF-β pathway	IPF	EMT	[75]
let-7d	HMGA2 TGF-β1	Antifibrotic agent Increases the collagen deposition during EMT	IPF	EMT	[76]
miR-326	TGF-β Smad 7	Fibrotic agent	IPF	EMT	[77]
miR-154	FZD4	Promotes proliferation and migration in lung fibroblast	IPF	EMT	[78]
miR-375	FZD8	regulates AEC trans-differentiation through the Wnt/β-catenin pathway	IPF	EMT	[79]
miR-199a	TGF-β1	Pathogenic activation of fibroblast	IPF	EMT	[80]

Table 3. Important miRNAs regulating the selected signaling pathways in both inflammation and EMT processes in IPF and HP

miRNA	Target	Function	Disease	Process	Reference
miR-21	Smad7	Inhibits pro-fibrogenic activity of TGF-β pathway in fibroblasts	IPF	EMT	[82]
	IL1-β	miR-21 acts as an anti-inflammatory agent within a negative regulatory loop: NF-κB activity is necessary for miR-21 induction, but by targeting PDCD4, miR-21 inhibits NF-κB and its pro-inflammatory transcriptional targets.	HP?	inflammation	[83]
miR-17~92 cluster	TGF-β	Regulates lung epithelial cell development	IPF	EMT inflammation	[84]
miR-200 family	E-cadherin ZEB1 ZEB2 TGF-β	These miRNAs are regulators/inhibitors of EMT and act to maintain the epithelial phenotype by targeting the expression of the E-cadherin transcriptional repressors, ZEB1 and ZEB2	IPF HP?	Inflammation EMT	[85, 86]
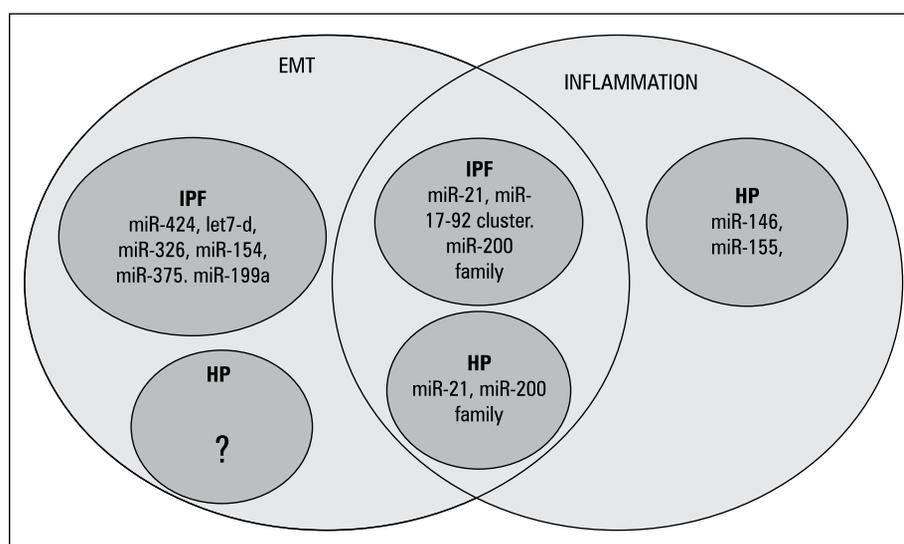
Figure 2. MiRNAs that regulate inflammation, EMT or both processes in ILD pathogenesis

on some miRNAs which control the processes of inflammation (miR-146, miR-155) and EMT (miR-424, let-7d, miR-326, miR-154, miR-375, miR-199) in lung disorders; especially IPF and HP. MiRNAs that regulate inflammation, EMT or both processes in ILD are shown in Figure 2.

Based on the progress in molecular biology and in the area of understanding of inflammatory and fibrosis processes in lung and other organs, it seems highly likely that studies focused on miR-NA role in the pathogenesis of ILD are extremely important and necessary.

Conflict of interest

The authors declare no conflict of interest.

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