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Transcriptional factor RUNX1: A potential therapeutic target for fibrotic pulmonary disease

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Abstract: Runt-related transcription factor-1 (RUNX1), also known as the core-binding factor alpha 2 subunit, is closely related to human leukemia. The functions of RUNX1 in modulating cell proliferation, differentiation, and survival in multiple systems have been gradually discovered with the emergence of transgenic mice. RUNX1 is a powerful transcription factor implicated in diverse signaling pathways and cellular mechanisms that participate in lung development and pulmonary diseases. RUNX1 has recently been identified as a target regulator of fibrotic remodeling diseases, particularly in the kidney. However, the role of RUNX1 in pulmonary fibrosis is unclear. Pulmonary fibrosis is characterized by obscure nosogenesis, limited therapy, and poor prognosis. Moreover, the population of patients with pulmonary fibrosis is gradually increasing. Thus, there is an unmet need for therapeutic targets. In this review, we retrospectively discuss the alteration in *RUNX1* mRNA expression in the RNA sequencing data of human fibrotic lungs and the protein levels in mouse pulmonary fibrosis. Subsequently, we focused on the interaction between RUNX1 and several signaling pathways involved in pulmonary fibrosis. Finally, this review highlights the therapeutic potential of RUNX1 as a target for slowing the progression of fibrotic lung disease.

Introduction

Pulmonary fibrosis, a common lethal lung disease, is characterized by abnormal scarring of lung parenchyma. Pulmonary fibrosis has a poor prognosis, rising morbidity rates, and limited available therapeutic options. Ban *et al.* (2018) reported an increase from 12 to 532 (2000–2012) in the number of patients newly diagnosed with interstitial lung disease (ILD) in their study cohort. Another study revealed that in the United States, the prevalence of idiopathic pulmonary fibrosis (IPF) is twice as high as that reported ten years ago (Lederer and Martinez, 2018). Currently, only two inhibitors have been approved for the treatment of pulmonary fibrosis: (a) nintedanib, a tyrosine kinase inhibitor, and (b) pirfenidone, a cytokine suppressor (Karimi-Shah and Chowdhury, 2015). Large-scale clinical trials have shown that nintedanib and pirfenidone slow down the decline in the forced vital capacity of the lungs (Vancheri *et al.*, 2018; Moor *et al.*, 2020; Flaherty *et al.*, 2018; Richeldi *et al.*, 2011; Richeldi *et al.*, 2014; King *et al.*, 2014; Noble *et al.*, 2011). Lung transplantation is the ultimate treatment option for pulmonary fibrosis. Regrettably, the median survival was barely 4.7 years for 9541 patients who received lung transplantation surgery for their fibrotic lungs (Yusen *et al.*, 2014). Therefore, there is a long-term need for the identification of more therapeutic targets to promote a better prognosis for fibrosis.

The fibrotic process involves a complex series of alterations in protein and transcription levels, signaling pathways, and structural remodeling. Fibrosis is accompanied by extracellular matrix over-deposition, fibroblast proliferation, differentiation, impaired repair ability of alveolar epithelial cells, endothelial dysfunction, cell senescence, mitochondrial injury, and shortened telomerase (Wynn and Ramalingam, 2012). In fibrotic tissues, abnormal activation or inhibition of certain pathways' activities have been observed; these pathways include TGF-β (Ikawa *et al.*, 2008; Kunz *et al.*, 2004), NF-κB (Wang *et al.*, 2021; Peng *et al.*, 2020; Pan *et al.*, 2020), Wnt/β-catenin (Brack *et al.*, 2007; Lee *et al.*, 2020; Gay *et al.*, 2020), and PI3K-Akt signaling (Hsu *et al.*, 2017; Wu *et al.*, 2017), as well as morphogenetic development-related pathways

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(Edeling *et al.*, 2016; Chanda *et al.*, 2019) such as Notch, Hippo, and Hedgehog signaling pathways. Runt-related transcription factor-1 (RUNX1) was discovered to be a novel and important regulator of the kidney (Zhou *et al.*, 2018; Cheng *et al.*, 2020), heart (Kuppe *et al.*, 2020; Li *et al.*, 2021b), and skin fibrosis (Abbasi *et al.*, 2020). RUNX1 is essential for fibroblast proliferation and differentiation (Kim *et al.*, 2014; Dubey *et al.*, 2022). A recent study has suggested that RUNX1 is closely associated with pulmonary fibrosis (O'Hare *et al.*, 2021). Accordingly, we speculated that RUNX1 might be a potential therapeutic target for pulmonary fibrosis.

Targeting transcriptional factors (TFs) to intervene in several aberrant signaling pathways in fibrotic tissue appears to be a powerful method for treating fibrosis (Bradner *et al.*, 2017; Loft *et al.*, 2021). TFs regulate gene expression, and one TF usually mediates the transcription of hundreds of target genes (Mevel *et al.*, 2019). This feature enables them to modulate multiple signaling pathways and biological processes. Targeting promising TFs that influence multiple pathways involved in fibrosis is expected to slow the progression of over-deposition of the extracellular matrix, and structural remodeling. Hence, we reviewed and summarized the connection between the multifunctional TF RUNX1 and fibrotic pulmonary disease to provide a new perspective for clinical work and scientific research.

Structure and Function of RUNX1

RUNX1 belongs to the runt-related family that participates in proliferation, differentiation, and cell survival in various cell lineages (Hsu *et al.*, 2020; Li *et al.*, 2021a; Tang *et al.*, 2021). *RUNX1* encodes the alpha subunit of the core-binding transcription factor. The RUNX1 protein consists of three domains: the runt homology domain (RHD) within the N-terminal region, C-terminal transactivation domain (TAD), and repression domain (RD) (Tang *et al.*, 2018). The RHD shoulders bind DNA, whereas the interaction of RUNX1 protein and nucleic acid requires the β subunit of the core binding transcription factor, which is encoded by CBFβ (Riddell *et al.*, 2020). The RUNX1 protein partners with the β subunit to form a transcriptionally active heterodimer that can activate or repress target gene expression (Mevel *et al.*, 2019).

In Fig. 1, we present the top 15 biological pathways regulated by RUNX1, based on the biological processes and molecular pathways involved in the downstream targets of RUNX1. The hTFtarget online database has curated comprehensive TF-targets regulation from large-scale ChIP-Seq data of human TFs. We obtained a large scale of 22,724 target genes regulated by RUNX1 for enrichment analysis at the biological process and signaling pathway levels. Pathway enrichment analysis of the target genes of RUNX1 has demonstrated that RUNX1 plays a constructive role in the cell cycle, autophagy, ubiquitin-mediated proteolysis, cellular senescence, lysosomes, and apoptosis (Fig. 1A). Gene Ontology (GO) enrichment analysis of the downstream target genes of RUNX1 showed that RUNX1 is an important part of biological processes, such as the proteasomal protein catabolic and RNA catabolic processes, regulation of mitotic cell cycle phase transition, and protein targeting (Yu *et al.*, 2012) (Fig. 1B).

The functions of RUNX1 have been elucidated in knockout mice. Germline knockout of *RUNX1* causes embryonic lethality and a lack of hematopoietic stem cells (Okuda *et al.*, 1996; Wang *et al.*, 1996; Theriault *et al.*, 2004). These animal models initially revealed that *RUNX1* is essential for definitive hematopoiesis. Furthermore, studies using stage- and tissue-specific conditional knockouts have uncovered a myriad of additional roles for *RUNX1* in tissues. Conditional knockout of *RUNX1* in the hematopoietic system can cause reduced hematopoietic stem and progenitor cell growth (Cai *et al.*, 2015), hematopoietic stem cell expansion and subsequent exhaustion (Jacob *et al.*, 2010), impaired megakaryocyte maturation, and platelet production (Ichikawa *et al.*, 2004; Growney *et al.*, 2005). *RUNX1* knockout in the immune system can result in T-cell differentiation blockage and defective B lymphocyte development (Egawa *et al.*, 2007; Seo *et al.*, 2012). The depletion of *RUNX1* in the nervous system results in abnormal pain perception (Chen *et al.*, 2006). Additionally, *RUNX1* deletion in the cardiac system prevents adverse

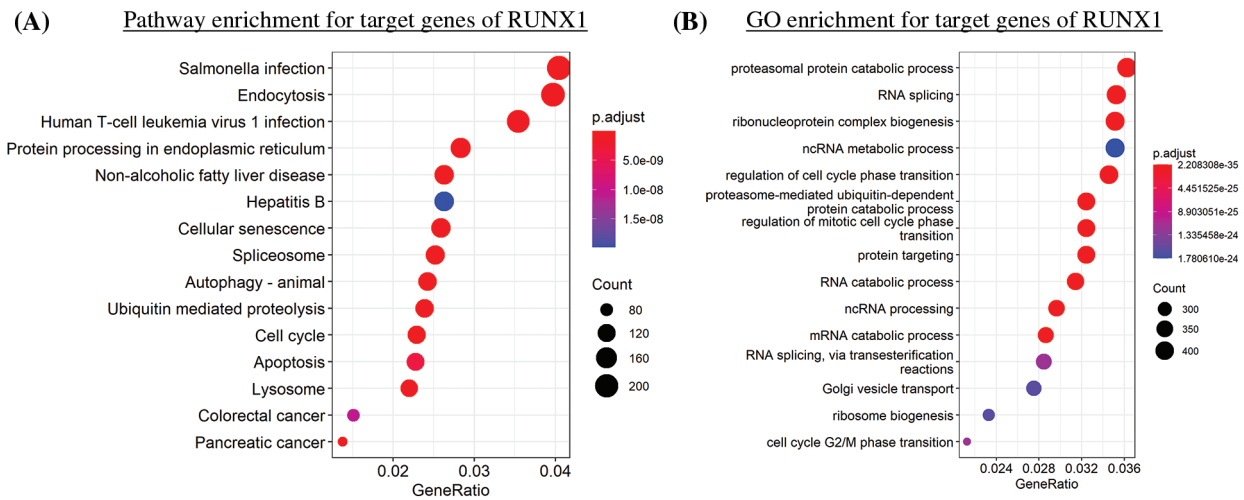

FIGURE 1. Top 15 items of KEGG and GO enrichment analyses for RUNX1 target genes. (A) Summary of pathway enrichment in KEGG. (B) Summary of GO enrichment at the level of biological process.

cardiac remodeling following myocardial infarction and maintains contractile function (McCarroll *et al.*, 2018). A mammary gland without *RUNX1* reduces the number of mature luminal cells (van Bragt *et al.*, 2014). Muscles lacking *RUNX1* can lead to atrophy of denervated myofibers, disorganized myofibrils, and excessive autophagy (Wang *et al.*, 2005; Umansky *et al.*, 2015). However, the effects of *RUNX1* knockout on the respiratory system are nearly absent, and therefore, the role of RUNX1 in the lung is largely unknown.

The Emerging Role of RUNX1 in Pulmonary Development and Diseases

RUNX1 in the developing lung

Levanon *et al.* (2001) described RUNX1 expression in both the epithelium and mesenchyme of mouse lungs at E14.4–E16.5 (Levanon *et al.*, 2001). An earlier study suggested that *RUNX1* deletion from embryonic day (E) 6.5 in alveolar epithelial cells is not congenitally fatal and that the morphological features of fetal and adult lungs are similar to wild-type mouse lungs of the same age (Tang *et al.*, 2017). In addition, Haley *et al.* (2011) reported that RUNX1 was highly expressed in human developing lungs, based on the assessment of RUNX1 expression in human developing lungs obtained from discarded surgical material. Our previous work showed that the RUNX1 protein was highly expressed (4.6-fold change) in adult lungs compared to E18.5 (Tang *et al.*, 2017). Although the reason for the differential expression of RUNX1 in developing and postnatal lungs has not been explored, it may be related to the involvement of RUNX1 in response to stress from the external environment.

RUNX1 distribution in normal adult lung

Single-cell RNA sequencing technology exquisitely draws the transcriptome profile of the lungs. The human protein atlas is an open online database for mapping human proteins using the integration of various omics technologies, including antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology. Information from the entry of data source GSE130148 in the human protein atlas revealed that mRNA expression of *RUNX1* was most enriched in blood and immune cells, followed by epithelial and mesenchymal cells, while endothelial cells had the lowest expression (Fig. 2) (The Human Protein Atlas, 2022; Karlsson *et al.*, 2021). The fact that *RUNX1* is widely expressed in diverse cells in the lung hints that it might play unknown roles in lung injury and repair.

RUNX1 in pulmonary fibrosis

RUNX1 plays a key role in the progression of pulmonary fibrosis. By comparing different stages of IPF lung tissue, an algorithmic model prediction revealed that *RUNX1* serves as an essential factor affecting the development of fibrosis (McDonough *et al.*, 2019). To date, *RUNX1* mRNA expression in pulmonary fibrosis has been found to be upregulated or downregulated after pulmonary fibrosis. The alteration of RUNX1 protein, which primarily stems from the mouse model, is upregulated in bleomycin-induced pulmonary fibrosis.

For instance, RNA sequencing of human pulmonary fibrosis tissue showed that mRNA expression of *RUNX1* was downregulated in IPF (Log2 fold change −2.99) (Konigsberg *et al.*, 2021). With the analysis of GEO2R, a similar trend of *RUNX1* mRNA expression was observed in other datasets, GSE110147 and GSE68239. Of the differentially expressed genes (DEGs) of GSE110147, we observed that *RUNX1* mRNA expression was mildly downregulated in the IPF lungs compared with normal lungs (Cecchini *et al.*, 2018). Paradoxically, other studies have demonstrated that the mRNA expression of *RUNX1* is increased in pulmonary fibrotic lungs. *RUNX1* was upregulated in a cohort of patients with transplant-stage IPF compared to normal lungs (Log2 fold change 0.661) (Sivakumar *et al.*, 2019). Other studies on sequencing data generated from patients with IFP supported the increased expression of *RUNX1* in fibrotic lungs hypothesis (DePianto *et al.*, 2015; Horimasu *et al.*, 2017). Thus, we can see that the alteration of *RUNX1* mRNA expression in IPF and other pulmonary fibrosis types is debatable. Biological differences and dissimilar cell proportions in tissues may result in the contradictory expression of *RUNX1* mRNA in human fibrotic lung;

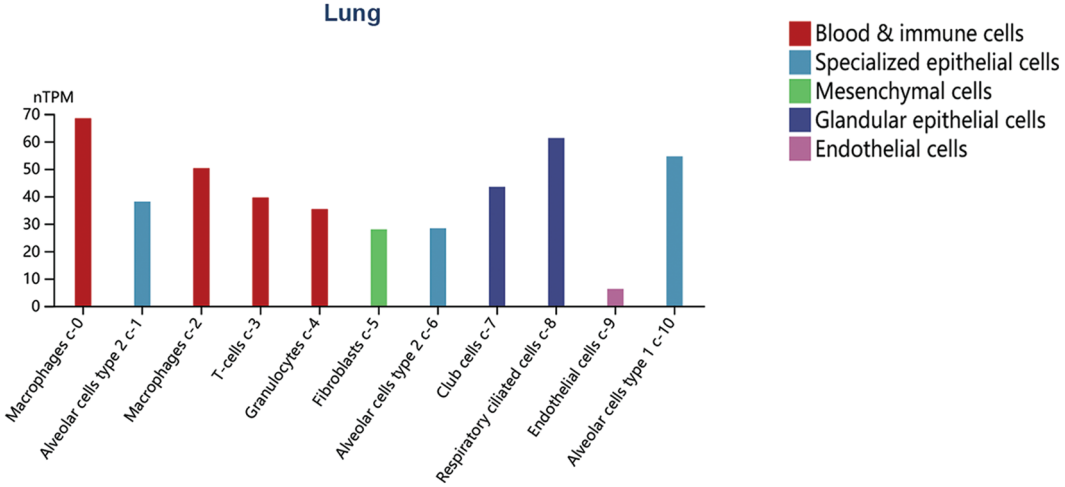

FIGURE 2. Expression of RUNX1 in the lung. Sourced from the human protein atlas (https://www.proteinatlas.org/ENSG00000159216-RUNX1/single+cell+type).

however, a large sample size and more advanced technologies are needed to explore the precise alteration of *RUNX1* expression in pulmonary fibrosis.

Single-cell RNA sequencing technology is becoming a powerful tool to assist in exploring abnormal signaling at the cellular level. Alveolar epithelial cells and fibroblasts are crucial components of the hypothesized mechanism of fibrogenesis. Based on the finite research on human pulmonary fibrosis, *RUNX1* mRNA seems to have a more conspicuous change in fibroblasts/myofibroblast clusters than in alveolar epithelial cells. A study collected lung tissues from patients with IPF, systemic scleroderma-related pulmonary fibrosis, and healthy individuals; the results showed an increased expression of *RUNX1* mRNA mainly in mesenchymal clusters, not in epithelial clusters in fibrotic lung (Tsukui *et al.*, 2020). The distinctly increased expression of *RUNX1* in mesenchymal clusters characterized by fibroblasts/myofibroblasts is consistent with Adams's work (Adams *et al.*, 2020). Additionally, an obvious increase in *RUNX1* mRNA in fibroblasts/myofibroblasts of the human fibrotic lung was observed; however, there was a clear decrease in *RUNX1* expression in type 2 alveolar epithelial cells (Morse *et al.*, 2019). Furthermore, a notable elevation in *RUNX1* mRNA expression in fibroblasts/myofibroblast clusters was demonstrated (Habermann *et al.*, 2020). Notably, in this study, *RUNX1* mRNA expression in type 2 alveolar epithelial cells did not change significantly. Changes in *RUNX1* expression in different cell types in lung fibrosis varied. The question of which cell type is the leading cluster regulated by *RUNX1* in pulmonary fibrosis needs to be answered in the future to steer the precise intervention of RUNX1 in specific cell types.

RUNX1 protein expression has been evaluated in animal models of pulmonary fibrosis (especially bleomycin-induced pulmonary fibrosis). Few studies have reported RUNX1 protein level changes in human fibrotic lung tissue. Increased levels of RUNX1 proteins, including 55 kDa isoform 1 and 37 kDa isoform 2, were found in mouse lungs after bleomycin administration (Dubey *et al.*, 2022; O'Hare *et al.*, 2021; Lin *et al.*, 2020; Ji *et al.*, 2021). However, the molecular mechanism by which the RUNX1 protein exerts its function in pulmonary fibrosis could not be determined and remains unknown. Further research is needed to determine whether RUNX1 directly regulates extracellular matrix molecule-related gene expression or influences the activity of signaling pathways involved in pulmonary fibrosis; nevertheless, it is reasonable to speculate that RUNX1 plays a certain role in pulmonary fibrosis.

RUNX1 in other respiratory diseases

RUNX1 has been shown to play a role in respiratory diseases such as lung cancer, asthma, pulmonary inflammation, and pulmonary arterial hypertension. Our previous study showed that RUNX1 regulates LPS-induced acute lung injury via NF-κB signaling (Tang *et al.*, 2017). RUNX1 was found to play a crucial role in the development and progression of lung neoplasm (Ramsey *et al.*, 2018; He *et al.*, 2019). The loss of RUNX1 has been reported to be associated with aggressive lung adenocarcinomas. Miyamoto *et al.* (2019) found that the absence of RUNX1 impaired the ability of group 2 innate lymphoid cells (ILC2s) to proliferate and produce effector TH2 cytokines and chemokines, and that RUNX1 contributes to airway allergic pathogenesis. In 2021, a study described a novel role for PRMT1 as a coactivator of RUNX1, which might be relevant to epithelial dysfunction in asthma (Zhai *et al.*, 2021). The RUNX1 inhibitor Ro24-7429 exhibited anti-inflammatory and anti-fibrotic effects in a bleomycin-induced pulmonary fibrosis mouse model (O'Hare *et al.*, 2021). A previous study suggested that the administration of RUNX1 inhibitor *in vivo* reduced the severity of pulmonary arterial damage and that targeting RUNX1 could be regarded as a novel therapy for pulmonary arterial hypertension (Jeong *et al.*, 2022). Despite these advances, the role of RUNX1 in lung development and pulmonary disease is not sufficiently understood.

Signaling Crosstalk between RUNX1 and Pathways Involved in Pulmonary Fibrosis and Remodeling

Although the direct effects of RUNX1 in the lungs have not been elucidated, RUNX1 has been shown to interact with some signaling pathways in other tissues. These signaling pathways are also involved in pulmonary remodeling.

RUNX1 interacts with the TGF-β signaling pathway. RUNX1 is activated by TGF-β, and several biological effects of TGF-β stimulation have been shown to involve RUNX1, including myofibroblast differentiation and proliferation (Kim *et al.*, 2014; Dubey *et al.*, 2022; Lin *et al.*, 2020; Ji *et al.*, 2021). For example, inhibition of RUNX1 in fibroblasts decreased the expression of Ki67 and α-SMA, which are proliferation and fibroblast trans-differentiation markers, respectively. RUNX1 overexpression activates the TGF-β signaling pathway and promotes the expression of p15, resulting in cell cycle arrest (Sun *et al.*, 2022). The interactive effect between RUNX1 and TGF-β may create a vicious circle and facilitate the differentiation of fibroblasts and collagen deposition during the development of lung fibrosis.

Cell senescence is an emerging pivotal driving mechanism for pulmonary fibrosis (Yao *et al.*, 2021; Schafer *et al.*, 2017; Waters *et al.*, 2018). RUNX1 has also been associated with senescence (Wolyniec *et al.*, 2009). Senescence-associated β-galactosidase (SA-β-gal) staining of RUNX1 overexpression in human primary foreskin fibroblasts revealed that RUNX1 facilitated G1 arrest and cellular senescence. In addition, Anderson *et al.* (2018) found that RUNX1 mediates growth arrest and senescence of primary cells. The accumulation of senescent cells is associated with age-related diseases, such as pulmonary fibrosis (Yao *et al.*, 2021; Waters *et al.*, 2018; Lin *et al.*, 2020; Parimon *et al.*, 2021; Lin and Xu, 2020). Senescent cells exhibit a senescence-associated secretory phenotype (SASP), which is characterized by an aberrant secretory spectrum of cytokines. Schafer *et al.* (2017) demonstrated an elevated abundance of senescence biomarkers in IPF lungs, where p16 expression increased with disease severity. Moreover, their work showed that the secretome of senescent fibroblasts was fibrogenic. The upregulation of RUNX1 in fibroblasts to mediate fibrogenesis via cell senescence may be a reasonable conjecture of pulmonary fibrosis pathophysiology.

RUNX1 has also been linked to Akt signaling, and the PI3k-Akt signaling pathway is also involved in fibrosis

(Qin *et al.*, 2021; Wang *et al.*, 2022; Zhang *et al.*, 2021b; Kiszałkiewicz *et al.*, 2017). The PI3K-Akt pathway is an intracellular signal transduction pathway that promotes protein synthesis, glucose metabolism, cell survival, cell cycle progression, and angiogenesis in response to extracellular signals. Epithelial-mesenchymal transition (EMT) induced by TGF-β in renal epithelial cells requires RUNX1 and p110δ-mediated Akt activation (Zhou *et al.*, 2018). A similar connection between RUNX1 and Akt signaling was established in renal fibroblasts, indicating that RUNX1 may play an important role in fibrotic lesions by regulating the PI3k-Akt signaling pathway (Cheng *et al.*, 2020). This evidence for the connection between RUNX1 and the PI3k-Akt signaling pathway, suggests that RUNX1 potentially plays a role in pulmonary fibrosis with the assistance of the PI3k-Akt signaling pathway.

In addition, arising shreds of evidence have shown that sustained reactivation of the Wnt/β-catenin pathway is related to the pathogenesis of fibrotic disorders (Chanda *et al.*, 2019; Guo *et al.*, 2012; Hu *et al.*, 2020; Tao *et al.*, 2016; Distler *et al.*, 2019). Previous studies have illustrated the correlation between RUNX1 and Wnt/β-catenin signaling pathways. Hair follicle stem cells originate from RUNX1-expressing embryonic cells, and RUNX1 causes activation and proliferation of mouse hair follicle stem cells to ensure adult skin integrity by regulating the Wnt signaling pathway (Osorio *et al.*, 2011). The microarray database revealed that RUNX1 expression was positively related to multiple molecules participating in the Wnt/β-catenin signaling pathway, such as LEF1, CD44, WNT5A, and CTNNB1 (Li *et al.*, 2019). These data indicate that RUNX1 mediates the activity of the Wnt/β-catenin signaling pathway and serves as a potential mechanism of fibrogenesis and progression.

RUNX1 is involved in the complex network of non-coding RNA (microRNAs [miRNAs] and long non-coding RNAs [lncRNAs]) and tissue fibrosis (Cheng *et al.*, 2020; Lin *et al.*, 2020; Zhang *et al.*, 2021a). Noncoding RNAs are the focus and frontiers of fibrotic lesions. miRNAs are small endogenous RNAs that post-transcriptionally mediate gene expression by binding to the 3′UTR of target mRNAs. lncRNAs are defined as transcripts >200 nucleotides in length that lack protein-coding potential and play a vital role at transcriptional and translational levels. Specifically, miRNA-194 targets RUNX1 to promote renal fibrosis via the PI3K-Akt signaling pathway (Cheng *et al.*, 2020). In addition, miRNA-30b ameliorates diabetic nephropathy by suppressing RUNX1 and negatively regulating the PI3K pathway (Zhang *et al.*, 2021a). Furthermore, lncRNA Hoxaas3, which responds to the TGF-β/Smad pathway, promotes pulmonary fibrogenesis via the miR-450b-5p-Runx1 axis (Lin *et al.*, 2020).

Further studies are warranted to determine whether RUNX1 is linked to pulmonary fibrosis by regulating these signaling pathways (Fig. 3).

RUNX1 as a Novel Target in Pulmonary Fibrosis

Pulmonary fibrosis is an irreversible pathological process that poses a huge global health and economic burden. There is a persistent unmet need for more therapeutic methods, as patients with pulmonary fibrosis have poor long-term outcomes and a low quality of life. Considering the close interactions between the TF RUNX1 and pathological processes involved in pulmonary fibrosis, novel therapies aimed at targeting RUNX1 may achieve a more efficacious therapeutic response by impacting multiple downstream signaling pathways to slow down fibrotic progression or reverse early-stage fibrosis and prevent the destruction of the alveolar structure.

Generally, there are two effective strategies for suppressing the expression of RUNX1. One is a small molecule inhibitor that interrupts the combination of RUNX1 and CBFB to form functional dimers, and the other is to silence RUNX1 RNA to stop DNA transcription. Two types of RUNX1 inhibitors that interfere with the binding of RUNX1 and the target sequence (Ro5-3335 and Ro24-7429, respectively), were used *in vivo* and *in vitro*, consequently blocking possible cellular signal transduction (Cunningham *et al.*, 2012). Ro5-3335 served an anti-fibrotic role, reducing N-cadherin expression induced by TGF-β stimulation in the A549 cell lineage (O'Hare *et al.*, 2021). Furthermore, Ro24-7429, a small molecule inhibitor, was initially invented for the human immunodeficiency virus type 1 (HIV-1) protein and was proven to be safe in a phase 2 trial of AIDS (Haubrich *et al.*, 1995). Ro24-7429 robustly ameliorated lung fibrosis and inflammation in the bleomycin-induced acute fibrosis stage (O'Hare *et al.*, 2021). With the intervention of RNA silencing of RUNX1, TGF-β failed to activate major components, such as fibronectin and alpha-SMA, in human lung fibroblasts (Dubey *et al.*, 2022). A well-designed carrier with a surface carrying an antibody

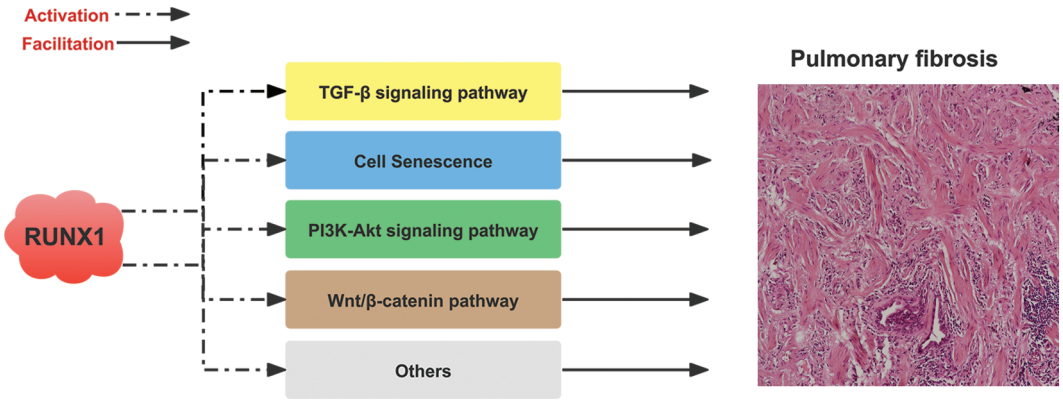

FIGURE 3. A schematic representation of the possible pathways mediated by RUNX1 that are involved in the fibrotic lung.

fragment to combine with mesenchymal cells in the lungs was administered in a bleomycin-induced pulmonary fibrosis model. The carrier managed to silence RUNX1 expression in the lung, leading to the reduction in collagen deposition and triumphing over the effects of pulmonary fibrosis (Ji *et al.*, 2021). Accordingly, pharmacological targeting of RUNX1 could be a potential strategy to tackle uncontrolled fibrotic tissue remodeling.

Conclusion

Accumulating evidence has indicated that RUNX1 plays a role in pulmonary fibrosis. RUNX1 modification in the fibrotic lungs at the mRNA and protein levels is in its infancy, while inhibition or deletion of RUNX1 *in vivo* has an anti-fibrotic effect in mice. The therapeutic effect of the inhibition or deletion of RUNX1 in an animal model has limited value. For pulmonary fibrosis, implementing targeted therapy through the intervention of RUNX1 in primate or clinical trials would be more persuasive in the future. Considering the powerful function of RUNX1 in multiple tissues and organs, systemic administration may result in fatal or unexpected adverse effects, and the safety and efficacy of influencing RUNX1 expression should be considered. New signs of progress have helped us determine which delivery method is more effective. Administration of RUNX1 small interfering RNA by inhalation presented a promising effect of reducing collagen deposition in fibrotic mouse lungs, which indicated that inhaled administration directly delivered to the lung could be a better option than oral administration. Research on RUNX1 should extend to the possible molecular mechanisms and signaling pathways of RUNX1 in fibrosis. Research on the role of RUNX1 in pulmonary fibrosis is an urgent requirement with translational potential.

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