The role of tyrosine kinases in the pathogenesis of idiopathic pulmonary fibrosis

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ABSTRACT Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease with a median survival time from diagnosis of 2–3 years. Although the pathogenic pathways have not been fully elucidated, IPF is believed to be caused by persistent epithelial injury in genetically susceptible individuals. Tyrosine kinases are involved in a range of signalling pathways that are essential for cellular homeostasis. However, there is substantial evidence from *in vitro* studies and animal models that receptor tyrosine kinases, such as the platelet-derived growth factor receptor, vascular endothelial growth factor receptor and fibroblast growth factor receptor, and non-receptor tyrosine kinases, such as the *Src* family, play critical roles in the pathogenesis of pulmonary fibrosis. For example, the expression and release of tyrosine kinases are altered in patients with IPF, while specific tyrosine kinases stimulate the proliferation of lung fibroblasts *in vitro*. Agents that inhibit tyrosine kinases have shown anti-fibrotic and anti-inflammatory effects in animal models of pulmonary fibrosis. Recently, the tyrosine kinase inhibitor nintedanib has shown positive results in two phase III trials in patients with IPF. Here, we summarise the evidence for involvement of specific tyrosine kinases in the pathogenesis of IPF and the development of tyrosine kinase inhibitors as treatments for IPF.

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Introduction

The diffuse parenchymal lung diseases (DPLD) are a heterogeneous group of diseases in which chronic inflammation and/or epithelial damage causes progressive fibrosis and remodelling of the delicate pulmonary structure [1]. This group of diseases includes the idiopathic interstitial pneumonias, of which idiopathic pulmonary fibrosis (IPF) is the most common [2]. In the USA, the annual incidence of IPF has been estimated to be 0.4–27.1 per 100 000 population using narrow case definitions and 1.2–76.4 per 100 000 population using broad case definitions [3]. However, the incidence of IPF is believed to be increasing [4]. Patients with IPF have a poor prognosis, with a median survival time from diagnosis of 2–3 years [5]. Risk factors for IPF include ageing, smoking and other environmental exposures, and infections, although no definite conclusions about the role of viral infections in IPF have been reached [6, 7].

Usual interstitial pneumonia, the histological counterpart of IPF, is temporally heterogeneous, with areas of mature fibrosis with scarring and honeycomb changes directly adjacent to areas that appear almost normal [5, 8, 9]. Although the pathogenic pathways active in IPF have not been completely elucidated, IPF is believed to be caused by persistent epithelial injury in genetically susceptible individuals [10, 11]. The most convincing evidence supporting this concept stems from familial forms of IPF, in which mutations expressed in the alveolar epithelial cells have been described. These mutations include mutations in surfactant proteins A and C [11] and the genes encoding the components of telomerase (*TERT*/*TERC*), leading to shortened telomeres [12]. As a result of extensive epithelial regeneration and the reactivation of developmental pathways, uncontrolled fibroproliferation due to disturbed epithelial–mesenchymal crosstalk results in progressive scarring of the lung [10, 11, 13]. The disturbed epithelial–mesenchymal crosstalk involves: increased generation of fibrogenic mediators by the epithelium, including the tissue factor/factor VIIa–factor X complex, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF) and connective tissue growth factor [10, 14]; the loss of control of fibroblasts *via* reduced epithelial prostaglandin E2/urokinase plasminogen activator [15]; and the upregulation of developmental pathways such as Wnt and Notch [10, 13, 16], which may act in a paracrine fashion on the parenchyma.

The epithelial–mesenchymal transition, a process whereby differentiated epithelial cells undergo transition to a mesenchymal phenotype giving rise to fibroblasts and myofibroblasts, is one possible, but highly debated, source of the increased number of fibroblasts and myofibroblasts in IPF [10, 17]. Other sources for the increase in fibroblasts include local expansion of the resident population and the entrance of circulating fibrocytes into the lung *via* the gradient formed by the chemokine stromal cell-derived factor-1–CXCR4 axis [10]. Recently, it has also been suggested that transcription factor Foxd1 progenitor-derived pericytes may be an important precursor of lung myofibroblasts [18].

The importance of protein phosphorylation and tyrosine kinases in cellular homeostasis

Protein phosphorylation by kinase enzymes is a fundamentally important mechanism of signal transduction in eukaryotic cells. Protein kinases control cellular processes including proliferation, cell cycle progression, metabolic homeostasis, transcriptional activation, differentiation and development, and apoptosis [19, 20]. The human protein kinase genome (kinome) encodes 90 protein tyrosine kinases, comprising 58 receptor tyrosine kinases (RTKs) and the cytoplasmic or non-RTKs [20].

RTKs are membrane receptors that activate intracellular signalling pathways upon binding of growth factors to their extracellular domains. This process usually involves the oligomerisation (typically dimerisation) of tyrosine kinase monomers, followed by autophosphorylation of the intracellular kinase domain to increase catalytic activity. A host of signalling molecules (enzymes that are tyrosine-phosphorylated or adaptor proteins) are recruited and activated, and link RTK activation to downstream signalling pathways [21, 22]. RTKs can also become activated as a consequence of the primary activation of another type of receptor, a G-protein coupled receptor (GPCR), *i.e.* the RTK acts as an effector molecule for the GPCR, in a process known as transactivation. This process can be ligand dependent or independent. Some RTKs can transactivate one another, for example, PDGF receptor (PDGFR) can transactivate epidermal growth factor receptor (EGFR). Transactivation of RTKs has been closely linked to inflammation and tissue healing [23]. Non-RTKs lack extracellular and transmembrane domains and modulate signalling pathways within the cytoplasm. As with RTKs, phosphorylation and autophosphorylation lead to the activation of non-RTKs [24].

Tyrosine kinases have low basal activity and are activated transiently in response to specific stimuli [24]. An auto-inhibitory "molecular brake" in their structure has been shown to regulate the activity of many RTKs [25]. The cellular consequences of RTK activation are complex and depend on the cell type and the signal transduction pathway that is activated [22]. Key signalling pathways activated by RTKs include mitogen-activated protein kinase (MAPK) pathways comprising Ras–extracellular signal-regulated kinase (ERK), p38 and c-Jun Nh2-terminal kinase (JNK) (also known as stress-activated protein kinase),

phosphatidylinositol 3-kinase (PI3K)–Akt and Janus kinase (JAK)–signal transducer of activated transcription (STAT) pathways [26].

PDGFRα and β are RTKs that bind members of the PDGF family of growth factors (PDGF-A, -B, -C and -D). PDGF signalling is activated in response to tissue injury to promote wound closure and scar formation [27]. PDGF-B/PDGFRβ-signalling is prominent in vascular remodelling [28], while PDGF-A/PDGFRα signalling has a broader role in tissue homeostasis and repair, including in the lungs [27, 29].

Vascular endothelial growth factor receptors (VEGFR)1, 2 and 3 bind vascular endothelial growth factor (VEGF) ligands (VEGF-A, -B, -C, and -D) and placental growth factor [30], and regulate angiogenesis [31]. VEGF is a mitogen, and survival and differentiation factor for endothelial cells in the lung [32], with VEGF levels >500-fold higher in the lung than in the plasma [33]. It is believed that VEGF plays a role in maintenance of the lung and the repair of the pulmonary circulation [33]. In addition, it has been shown that the alveolar epithelium is a rich source of VEGF and that epithelial VEGF influences mesenchymal cells during lung development. VEGF is critical for lung development and may enhance epithelial proliferation and resistance to apoptosis in an autocrine fashion [34, 35]. Moreover, VEGF has also been shown to be a critical regulator of surfactant production by alveolar type II cells [36].

Fibroblast growth factors (FGFs) are a family of 22 growth factors that bind to four RTKs: fibroblast growth factor receptor (FGFR)1–4. FGFs are involved in the regulation of processes including cell proliferation, differentiation and survival, angiogenesis, homeostasis, wound healing, and the regulation of fibroblast proliferation and the production of collagen [37, 38].

The EGFR or ErbB family comprises four RTKs: EGFR (ErbB1) and ErbB2–4 [39]. EGFR has numerous ligands, including epidermal growth factor (EGF) and TGF-α [39]. The EGFR/ErbB family is involved in development and cell proliferation, differentiation, survival, adhesion and migration [40].

Tyrosine kinases in pulmonary fibrosis

A number of protein tyrosine kinases have been implicated in the development and progression of fibrosis [27], including PDGF, FGF, VEGF and EGF. Much of the evidence for the role of tyrosine kinases in the pathobiology of lung fibrosis is based on data from animals treated with bleomycin, which induces an inflammatory response and pulmonary fibrosis in both rodents and humans [41].

PDGF

Evidence suggests that abnormal expression of PDGF plays an important role in the development of pulmonary fibrosis [42]. PDGF-A and PDGF-B mRNA were increased in bronchoalveolar lavage (BAL) cells from the lungs of bleomycin-treated hamsters, compared with saline-treated control animals [42], while in bleomycin-treated mice, PDGF-C mRNA was increased and localised to the area of lung injury [41]. Furthermore, blocking PDGFR has been shown to attenuate the progression of pulmonary fibrosis in a rat model of vanadium-induced fibrosis [43].

In patients with pulmonary fibrosis, alveolar macrophages are the principal source of PDGF-B, while myofibroblasts are the main source of PDGF-A [44]. Myofibroblasts also express elevated levels of PDGFRα, suggesting that both paracrine and autocrine modes of signalling are in operation [45]. Alveolar macrophages from the lungs of patients with IPF spontaneously release PDGF at concentrations four times higher than that in alveolar macrophages from healthy individuals [45]. PDGF has been shown to stimulate the proliferation of lung fibroblasts [46], and fibroblasts taken from patients with IPF show higher PDGFR expression than fibroblasts taken from non-fibrotic controls [47].

FGF

TGF-β, primarily TGF-β1, is a critical mediator of lung fibrosis, inducing connective tissue synthesis and fibroblast proliferation in the lung and representing a paracrine signal for the development of pulmonary fibrosis [48]. The effects of TGF-β in lung fibrosis are mediated in part by FGF-2 release and upregulation of FGFR1 and FGFR2 expression [48, 49]. FGF-2 has been shown to stimulate the proliferation of lung fibroblasts from patients with IPF and enhance TGF-β1-induced proliferation synergistically [46]. In bleomycin-treated mice, blocking FGF-2 signalling alleviated fibrosis by inhibiting the epithelial–mesenchymal transition induced by TGF-β1 [49].

Mast cells are a major source of FGF-2 in patients with IPF. The number of FGF-2-containing mast cells is increased in patients with IPF and they accumulate preferentially in areas of extracellular matrix deposition [50, 51]. Increased FGF-2 levels were found in the BAL fluid of patients with IPF, and correlated positively with alveolar–arterial oxygen gradient measured at maximal exercise and negatively with diffusing capacity of the lung for carbon monoxide in patients with IPF [51].

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By contrast with the effects of FGF-2, FGF-7 has been shown to have epithelium-protective effects [52], while FGF-10, *via* its receptor FGFR2b, stimulates lung progenitor cells during repair and has been shown to attenuate lung fibrosis in bleomycin-treated mice [53]. Patients with IPF have higher FGFR expression in lung fibroblasts than nonfibrotic controls [47]. Furthermore, the FGF-signalling axis is dysregulated in patients with IPF, with FGF-9, a potent inducer of FGF-10, and FGFR2b downregulated in patients with IPF and in bleomycin-treated mice [54].

VEGF

While neovascularisation is fundamental to tissue repair after injury, the role of angiogenesis in IPF is unclear [10]. Both increased and decreased angiogenesis have been observed, demonstrating the extensive regional and temporal heterogeneity of angiogenesis in IPF [55]. Although limited, there is growing evidence that VEGF may have mitogenic and profibrotic effects on fibroblasts [27]. VEGF levels have been shown to be increased in bleomycin-treated mice and anti-VEGF gene therapy attenuated inflammation and fibrosis in this model [56]. Overexpression of VEGF in the lungs of a transgenic mouse model stimulated inflammation and remodelling with sub-epithelial fibrosis [57].

VEGF is a potent inducer of vascular permeability and acts, at least in part, by increasing expression of matrix metalloproteinases, which are essential for extracellular matrix remodelling, wound healing and angiogenesis, and have been implicated in the pathogenesis of IPF [58]. In the BAL fluid of 20 patients with IPF, VEGF levels correlated with the protein permeability index, a ratio of BAL fluid to plasma protein that indicates the permeability of alveolar epithelial cells, as well as with levels of matrix metalloproteinases 3, 7 and 9 [58].

There is some evidence that VEGF levels may reflect disease severity and predict disease progression in patients with IPF [59, 60]. In a study of 41 patients with IPF, serum VEGF correlated strongly with an "interstitial score" determined using high-resolution computed tomography [59]. In addition, data from 28 patients suggested that there was an inverse correlation between baseline serum VEGF and change per month in vital capacity over the next 12 months [59]. When patients were split into two groups based on median serum VEGF at presentation, 5-year survival was 42.9% in the patients with high VEGF levels and 80.0% in patients with low VEGF levels, although the difference was not statistically significant [59]. Similarly, in a study investigating relationships between biomarkers in the BAL fluid and clinical outcomes in 20 patients with IPF, baseline VEGF levels were significantly higher in patients who had a rapid decline in lung function over 1 year than in non-progressors, and in patients who died compared with those who survived [57].

EGF

Evidence suggests that TGF-α, *via* activation of EGFR, contributes significantly to pulmonary fibrosis [39]. ErbB ligand induction requires PDGFR mediation and engages a positive autocrine/paracrine feedback loop *via* ErbB receptors [60]. In addition, PDGFRs are essential for TGF-β-stimulated ErbB ligand upregulation and TGF-β-specific signals are required for ErbB receptor activation. Thus, profibrotic responses involve the cooperative action of PDGF and ErbB signalling [60].

In rats, bleomycin-induced lung injury increased the expression of EGFR and its ligand TGF-α in the lungs [61], while chronic epithelial expression of TGF-α in transgenic mice caused progressive pulmonary fibrosis [62]. Blocking EGFR inhibited the progression of pulmonary fibrosis in a rat model [43] and inhibition of ErbB2 and 3 signalling protected against pulmonary fibrosis in bleomycin-treated mice [63]. TGF-α and EGFR expression are increased in the lung tissue of patients with IPF compared with control lung tissue [64].

Other tyrosine kinases

Other tyrosine kinases may also contribute to pulmonary fibrosis. Non-RTKs of the *Src* family, which include Fyn, Yes, Fgr, Lyn, Hck, Lck and Blk [65], are required for the epithelial–mesenchymal transition after TGF-β1 signalling in alveolar epithelial cells [66]. Both the RTK JAK and the non-RTK c-Abl have also been shown to play a role in fibrosis [27].

Current tyrosine kinase inhibitor landscape

In patients with pulmonary arterial hypertension (PAH), another hyperproliferative, nonmalignant disease primarily of the pulmonary vasculature, vascular remodelling has been shown to be mediated in part through PDGF, and also *via* c-KIT signalling. Accordingly, tyrosine kinase inhibitors have proven beneficial in animal models of PAH [67]. In patients with PAH with extensive pulmonary vascular remodelling (pulmonary vascular resistance (PVR) >800 dyn·s·cm^{-5}), despite dual or triple PAH therapy, the tyrosine kinase inhibitor imatinib was shown to result in a reduction in PVR and an increase in 6-min walking distance [68].

Currently, the only product licensed (in some countries) for the treatment of IPF is pirfenidone. The mechanism of action of pirfenidone in IPF has not been fully established. However, pirfenidone has been shown to exert anti-fibrotic and anti-inflammatory properties in a variety of *in vitro* systems and animal models of pulmonary fibrosis [69].

Several tyrosine kinase inhibitors have been shown to reduce pulmonary fibrosis in animal models, *e.g.* imatinib, nilotinib, gefitinib, SU5918 and SU11657 [70–74]. Imatinib, an inhibitor of PDGFRα and β, discoidal domain receptors 1 and 2, c-kit and c-Abl, has been shown to have anti-fibrotic activity, not only in the lung, by interfering with a number of tyrosine kinase cascades. Combined inhibition of c-Abl and PDGFR might be critical for the treatment of fibrosis in systemic sclerosis [75, 76]; while a prominent role for the stem cell factor–c-Kit axis has been demonstrated in pulmonary fibrosis [77]. However, in a 96-week, phase II placebo-controlled trial in 119 patients with IPF, imatinib failed to meet the primary end-point of time to disease progression (defined as >10% decline from baseline in forced vital capacity (FVC) % predicted) or death [78]. Whether the inhibitory spectrum of imatinib or the design of the clinical trial was responsible for the failure is not clear.

Nintedanib (formerly known as BIBF 1120) is a potent intracellular inhibitor of FGFR1, 2 and 3, PDGFRα and β, and VEGFR1, 2 and 3 [79]. It also inhibits the *Src* family tyrosine kinases Lck, Lyn and Flt-3 [79]. In bleomycin-treated mice and in a mouse model of silica-induced pulmonary inflammation and fibrosis, nintedanib reduced lung inflammation and fibrosis, as demonstrated by reduced lung collagen and *via* histology [80]. In primary human lung fibroblasts from patients with IPF, nintedanib inhibited FGF-, PDGF- and VEGF-induced profibrotic effects and cell migration, and reduced TGF-β-induced collagen deposition [47]. Nintedanib has also been shown to inhibit TGF-β-induced fibroblast to myofibroblast differentiation in primary human lung fibroblasts [80], a model in which the activity of imatinib is questionable [81]. Inhibition of c-Abl by imatinib has been shown to prevent TGF-β-induced morphological transformation in murine fibroblasts [82], but no activity was demonstrated in human lung fibroblasts [81].

A phase II, randomised controlled trial (the TOMORROW trial) evaluated the efficacy and safety of nintedanib *versus* placebo in 432 patients with IPF [83]. The results of this study suggested that 12 months' treatment with nintedanib 150 mg twice daily slows the decline in lung function, reduces acute exacerbations and preserves health-related quality of life (as measured using the St George's Respiratory Questionnaire (SGRQ) score) in patients with IPF [83]. The results of two replicate 12-month phase III, randomised, placebo-controlled trials of nintedanib 150 mg twice daily in 1066 patients with IPF (the INPULSIS trials) have recently been published [84]. The results of these trials showed that nintedanib slowed disease progression by significantly reducing the annual rate of decline in FVC, and was associated with side-effects (most commonly diarrhoea) that were manageable in most patients. There were significant differences, in favour of nintedanib, on the time to first acute exacerbation and change from baseline in SGRQ score in INPULSIS-2, but not in INPULSIS-1. An analysis of pooled data on the time to first confirmed or suspected exacerbation, as categorised by an adjudication committee, found a significant benefit of nintedanib *versus* placebo [84].

Outlook

Tyrosine kinase signalling plays a critical role in a wide variety of cellular processes. There is substantial evidence from *in vitro* studies and animal models that specific tyrosine kinases are involved in the pathogenesis of pulmonary fibrosis. Clinical trials of agents that inhibit tyrosine kinase signalling pathways in patients with IPF suggest that such agents have potential value in slowing disease progression. Further research is underway to elucidate how specific kinases contribute to the pathogenic processes in lung fibrosis, which may identify additional targets for therapeutic interventions.

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