Idiopathic Pulmonary Fibrosis: A Review on Molecular and Cellular Mechanisms

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Idiopathic pulmonary fibrosis is a progressive disease characterized by extracellular matrix accumulation and altered mechanical properties of lung tissue. Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) is attracting considerable attention due to disease acceleration and substantial mortality. The biomechanical properties of tissues are sensed and responded to by mechanotransduction pathways that facilitate sensing of changes in mechanical cues by tissue resident cells and convert the mechanical signals into downstream biochemical signals. In this review pathways such as Wnt/ß-catenin pathway, TGF-ß/Smad signaling pathway and EMT in IPF, VEGF and its relation with PI3K–Akt signalling pathway, PI3-Akt pathway, PDGF Signalling Pathway, Hippo/YAP signalling, JAK/STAT pathway, Rnd3/ p190/Rho-Gap pathway have been discussed. This review also covers current therapeutic strategies in relation to idiopathic pulmonary fibrosis.

Keywords: Idiopathic Pulmonary Fibrosis; IPF; Lung Tissue; Signalling.

Idiopathic pulmonary fibrosis is a progressive, chronic lung disorder, typically known to be fatal. With an unknown etiology and a median survival of 3-4 years, it is one of the most aggressive members and the final pathway of the group of lung disorders known as interstitial lung disorders (ILD0 (Pardo et al., 2002). Also termed cryptogenic fibrosing alveolitis, it starts off as injuries to the lung epithelia and progresses towards chronic alveolar inflammation and finally leads to fibrosis (Wu et al., 2018). Chronic cough and dyspnoea are some of the more common symptoms, impairing the quality of life of the patient. Thoracic CT scans of patients with this disorder are known to show honeycombing zones (Well defined walls on subpleural cystic airspaces) (Martinez et al., 2017) fibroblastic foci, usual interstitial pneumonia (UIP), and patches (Sauleda et al., 2017) The main causes of lung injury that set off the whole cascade of inflammation and damage can be due to continual exposure to toxins like asbestos, silica, cigarette smoke, etc, or even elevated levels of oxygen, leading to ROS mediated damage.

Epidemiology and Occurrence

Having familial and sporadic variants, it is known to affect people worldwide, having no increased predisposition to any particular ethnicity or race. Despite being a rare disease, (<5 per 10000 person-years), in Europe alone, 40,000 new cases are diagnosed annually. IPF accounts for 20-50%
of all ILD cases. Men are more prone to develop this disease and the progression occurs much faster too.

Pathways

Wnt/â-catenin pathway

This pathway is known to determine the fate of epithelial cells during their development. The Wnt family is the constituent of a group of growth factors that are highly conserved (Königshoff et al., 2008). These factors are glycoproteins rich in cysteine and are a vital part of organ development. Absence or defects of these lead to developmental abnormalities in embryonic stages and organ failure in later stages. Of the three different variants of the pathway, the â-catenin dependent Wnt pathway is considered to be canonical (Kim et al., 2008).

The canonical pathway starts with the inhibition of GSK-3â due to phosphorylation by Wnt proteins. This inhibition prevents the ubiquitylation and hence degradation of â- catenin. This causes cytoplasmic accumulation of â-catenin followed by nuclear translocation. In the nucleus, it binds with the lymphoid enhancer-binding factor (LEF) (synonymously-TCF i.e cell-specific transcription factor), consequently leading to transcription and regulation of the target genes of the signaling pathway such as TGF-â, matrix metallo-proteinases (MMPs)-2,7 and 9 (Brabletz et al., 1999; Pardo et al., 2016).

It has been reported that abnormal activation of the pathway occurs in IPF patients, leading to the progression of the disease. Elevated levels of nuclear-translocated â-catenin were found in them, especially in honeycomb modified bronchioles which are considered as abnormal lung architecture (Chilosi et al., 2003).

TGF-â/Smad signaling pathway and EMT in IPF

The pathogenesis of IPF through TGF-â signaling is closely related to the activity of myofibroblasts and this pathway is known as the “master switch” (Chen et al., 2016). These are fibroblasts that have differentiated on stimulation by an inflammatory response and usually express an intracellular contractile protein called alpha-smooth muscle actin (â-SMA). Therefore they are not present in normal lung tissue and are responsible for the repair, restoration of tissue, especially the extracellular matrix and scar formation (Hinz et al., 2016).

They are influenced by and secrete profibrotic growth factors called TGFâ (Transforming growth factor). TGFâ1 is an isoform of the multifunctional family of cytokines, primarily associated with IPF (Ghararee-Kermani et al., 2009). It stimulates differentiation of fibroblasts to myofibroblasts, which causes deposition of ECM proteins like fibrillin, fibronectin, and collagen at the site of injury and causes Epithelial to Mesenchymal transition (EMT) in alveolar type II epithelial cells (AECs) (marmai et al., 2011). These mesenchymal stem cells cause proliferation and hence with the combined effect of collagen deposition, accelerate the fibrotic process.

TGF- â1 levels, Smad 2/3, and â-SMA levels were found to be increased in rats with Bleomycin-induced EMT (Chen et al., 2016). TGF-â1 signaling starts when it binds to its serine/threonine kinase transmembrane receptor TGFâRII which phosphorylates serine/threonine residues in the transmembrane domain of the TGFâRI receptor. This activates the TGFâRI kinase that causes phosphorylative activation of Smad-2 and Smad-3, which later downstream, get attached to Smad-4, forming a heterotrimeric complex. This complex gets translocated to the nucleus where it interacts with transcription binding factors and regulates target genes that cause EMT (Gu et al., 2007).

Moreover, TGF-â further promotes fibrosis by signaling cells to produce profibrotic inflammatory molecules like PDGF, ILs (1B and 13), etc. and inhibits collagen and ECM degrading proteins called Matrix metalloproteases (MMPs) (Gyetko et al., 2009).

VEGF and its relation with PI3K–Akt signalling pathway

Vascular endothelial growth factor (VEGF), is a glycoprotein that promotes angiogenesis and hence vascularity. In the lungs, it is present in the alveolar epithelium (AEC) Normally, VEGF binds to tyrosine kinase receptors VEGFR1 and VEGFR2 which, by signal transduction, activate the PI3K-AKT pathway as well as the FAK (Focal adhesion kinase) pathway. These pathways promote fibrosis by recruiting pro-fibrotic factors like TGFâ.

VEGF also has a direct contribution to fibrosis by association with angiogenic inflammatory chemokines like Monocyte Attractant protein (MCP-1) and IL-8 and hence promoting
excessive ECM synthesis (Hosseinzadeh et al., 2018). Persons with IPF have been reported to have reduced levels of VEGF in the Bronchoalveolar lavage fluid but increased levels in the plasma. VEGF increase in the plasma has been associated with poor gas exchange and a higher difference in alveolar-arterial oxygen. (AaDO2) (Hambly et al., 2015)

The PI3-Akt pathway

TGFB plays an important role here, in the activation of this pathway by stimulating autocrine secretions of growth factors, that in turn activate the PI3-Akt pathway. This pathway is an anti-apoptotic/pro-survival pathway. Phosphoinositide kinase 3 (PI3), at the membrane receptor gets activated by cytokines or growth factors like VEGF, and in turn activates Phosphatidylinositol trisphosphate (PIP3), which, further downstream, activates Akt. This molecule is associated with inhibition of pro-Apoptotic signaling molecules and constituents like Caspase-9, Bax, and FOXO genes. It directly activates Bcl-XL and stimulates Mdm2, which is a negative regulator of p53. Phosphatase and tensin homolog (PTEN) is an inhibitor of PIP3 and hence prevents stimulation of the multi-target pro-survival protein Akt. In IPF individuals, PTEN levels were found to be low, and hence a strong anti-apoptotic response is seen. Moreover, it was found that the Akt suppressed the powerful cell cycle inhibiting gene FOXO3a in the nucleus (Yan et al., 2014). Therefore, lower levels of PTEN and FOXO3a deficiency protects the fibroblasts from Apoptosis

PDGF Signalling Pathway

The mitogen PDGF is produced by lung epithelium during injuries, usually chronic. This includes smoke from tobacco/nicotine, tar, or work hazards like coal dust or asbestos. PDGF produced by this damaged epithelium may, by paracrine stimulation, cause unrestrained connective tissue fibroblast proliferation and collagen deposition (Antoniades et al., 1992). Alveolar macrophages are known to secrete PDGF-B, which is completely absent in healthy lung tissue. They also secrete PDGF-A. These chemoattracting factors bring more lung myofibroblasts, which secrete PDGF-AA, which results in the formation and deposition of more collagen, contributing to the fibrosis (Bonner et al., 2010).

Hippo/YAP signalling

This pathway is known for its role in organ size regulation and apoptosis. This is carried out by gene products regulated by the transcriptional activator YAP (yes association protein). The presence of YAP leads to transcription, which then leads to the growth of the organ. The physiological importance of this was seen in studies on knockout mice (Brooks et al., 2007: Zheng et al., 2019) where overexpression of YAP led to a drastic increase in liver size, finally causing hepatocellular carcinoma.

YAP is suppressed by a kinase cascade, consisting of conserved protein kinases Mst1, Mst2 (mammalian Ste20-like kinases, which are Hippo homologs in mammals), Salvador (Sav1), Lats1, Lats2 (Large tumor suppressor, homologs of Wts), and Mob1 (Mats homologs) (Zheng et al., 2019) Mst-Sav1 complexes phosphorylate and activate the Lats, which further downstream cause phosphorylative inactivation of YAP.

In IPF individuals, nuclear YAP was found to be high (can also be verified by checking levels of Ajuba, the transcriptional target of YAP), while Sav and Mst1/2 are found to be suppressed. This can be compared with normal alveolar and bronchial cells where Sav and Mst1/2 are found in abundance (Gokey et al., 2018).

The JAK/STAT pathway

Janus kinase (JAK) is a family of tyrosine kinases with four members- JAK1, JAK2, JAK3, and Tyk2. Downstream effects of activation of JAK is proliferation, differentiation, and migration of cells (Rawlings et al., 2004). JAK gets activated and autophosphorylated on cytokine/growth factor binding (Valentino et al., 2006). These stimulants include VEGF, TGF-α, Angiotensins, and IL-6, 13. JAK further activates the Signal transducer and activator of transcription (STAT) proteins by phosphorylation. This causes dimerization and nuclear translocation where target genes are transcribed.

As found frequently in IPF patients, JAK2 phosphorylation by TGF-α and other growth factors caused STAT3 activation and was found to promote fibrosis by conversion of fibroblasts to myofibroblasts. STAT3 activation by IL-6 is associated with Epithelial to Mesenchymal transition (EMT) (Milara et al., 2018).

Rnd3/p190/Rho-Gap pathway

RhoA is a GTPase that regulates cell
Table 1. Current therapies

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Target</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>Smad pathway</td>
<td>Inhibits Smad/non-Smad signaling cascades. Autophagy is also enhanced (Chitra 2015)</td>
</tr>
<tr>
<td>Tralokinumab</td>
<td>IL-13 pathway</td>
<td>Tralokinumab inhibits the IL-13 pathway and hence prevents fibrotic inflammation (Murray et al., 2014)</td>
</tr>
<tr>
<td>Nintedanib</td>
<td>PDGF, VEGF, FGF pathways, ECM proteins, mRNA</td>
<td>Binds to the PDGF, VEGF, FGF receptors and prevents progression of signaling. Inhibits collagen deposition induced by TGF-α by downregulation of ECM protein mRNAs (Clarke et al., 2017)</td>
</tr>
<tr>
<td>Anti-CTGF antibodies</td>
<td>CTGF</td>
<td>Inhibits Connective tissue growth factor (CTGF) which is activated by TGF-α and promotes collagen deposition (Rafii et al., 2013)</td>
</tr>
<tr>
<td>Monoclonal antibody GS-6624</td>
<td>enzyme lysyl oxidase-like 2 (LOXL2)</td>
<td>LOXL2 cross-links collagen fibre to form scaffolds on which fibroblasts can proliferate. These antibodies inhibit it and hence prevent fibroblast growth</td>
</tr>
<tr>
<td>BIBF 1120</td>
<td>FGF, VEGF and PDGF receptors</td>
<td>BIBF 1120 is a tyrosine kinase inhibitor that targets the receptors of FGF, PDGF, and VEGF and hence prevents angiogenic signalling and proliferation.</td>
</tr>
<tr>
<td>Tetrahiomolybdate and minocycline</td>
<td>Angiogenesis</td>
<td>These chemicals are angiostatic and hence prevent angiogenesis and fibrosis.</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>MMPs, TGF-α, collagen 1, CTGF</td>
<td>MMPs like Matrylsin (MMP 7) regulates TGF-α and other inflammatory cytokines. Doxycycline inhibits the MMPs as well TGF-α and CTGF signalling</td>
</tr>
<tr>
<td>Carbon monoxide</td>
<td>Fibronectin</td>
<td>CO inhale in small amounts is known to inhibit TGF pathway components like collagen-I and fibronectin. Quercetin induces Heme oxygenase, whose activity produces CO and hence indirectly attenuates TGF-α pathway.</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Heme Oxygenase</td>
<td></td>
</tr>
</tbody>
</table>

Motility and contractility using the actin-myosin bundle regulation and cytoskeleton (Monaghan-Benson et al., 2018). It is activated by either mechanical signals or the growth factor TGF-α. Inhibition of ROCK (Rho-associated coiled-coil containing protein kinase), a downstream protein regulated by RhoA, was found to decrease fibrosis. Rho activity is enhanced in fibroblast, as compared to the normal level in healthy lung tissue and along with ROCK, is known to cause acute inflammation and fibrotic progression. This was confirmed by studies on RhoA miRNA silenced mice in which the collagen and fibronectin was reduced (Zhou et al., 2018).

Rnd3 is antagonistic to RhoA activity by activation of fp190RhoGAP (p190). Levels of Rnd3 were found to be low in IPF individuals and studies on the Rnd3 gene silenced with small-interfering RNA (siRNA) caused an increase in fibronectin, collagen, and SMA, hence confirming that loss on Rnd3 promotes fibrosis.

**Immune system in IPF**

The immune system is constantly active due to chronic injury and inflammation and can have the dual role of protection or promotion of fibrosis. However, in IPF, it was found that the contribution of the immune system to the progression of fibrosis outweighed the protective functions.

**Innate immunity**

The innate immunity mainly involves the alveolar macrophages, its Toll-like receptors (TLRs), and defensins (from neutrophils). TLR3, specifically, was found to be responsible for downregulating fibrotic proliferation. Studies on TLR3 genes with an SNP showed reduced cytokine production and hence an increase in fibrotic activity. TLR3 null mice had increased collagen
deposition and profibrotic cytokine secretion (Ley et al., 2014).

Levels of other cytokines like IL-8, MCP-1/CCL2, and macrophage inflammatory protein MIP-1a were found to be higher in the lungs of IPF individuals. These cause chemotaxis and activation of monocytes, neutrophils and lymphocytes (Bringardner et al., 2008)

Adaptive immunity

These neutrophils are found to be key components in alveolitis due to the release of the proteolytic enzyme neutrophil elastase (NE). Heat shock protein (HSP70) was found to cause CD4 T cell activation, which produces profibrotic cytokines like IL-4. CD 28 null cells were found to be elevated in IPF patients and contributed to fibrosis. Th1 cells play a protective role by secreting IL-12, which induces pro-inflammatory cytokine IFNα (Jakubsick et al., 2004). IFNα is known to reduce mRNA levels of TGF-B, procollagen 1, and 2. Studies show that the levels of IFNα reduce in the BALF and circulation of IPF patients (Desai et al., 2018). In contrast, Th2 derived cytokines like IL13, IL4, etc. excessively stimulate proliferation of alveolar macrophages that secrete CCL18/ PARC that stimulate collagen secretion and fibroblast-myofibroblast differentiation (Prasse et al., 2007).

Th-17 cells are known to secrete IL-17, which was found to induce fibrotic lesions and collage accumulation in murine models. They also secrete IL-22, which in contrast to IL-17, has a protective role. However, the levels of Th-17 did not significantly differ in IPF and control and hence have to be studied further. Similarly, Innate Lymphoid Cells like NK cells have been identified in IPF, but their contributions remain unknown, as is the case with B cells (Desai et al., 2018).

CONCLUSION

The pathogenesis of IPF through TGF-α signaling is closely related to the activity of myofibroblasts and this pathway. This review covers other transcription factors and pathways. Current therapeutic options slow the progression of IPF, but do not halt or reverse the scarring in the lung. The matrix is bioactive and plays a role the progression of IPF as a regulator of cellular phenotype and behavior. Mechanical cues that are first sensed via receptors on the cell membrane are converted to chemical signals via mechanotransduction pathways that regulate many aspects of cell behavior including motility, proliferation, morphology, and survival. Opportunities in targeting pathways to treat IPF have the potential, when combined with other therapeutic interventions, to halt the progression of fibrosis.

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Conflict of interest

None.

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