Role of integrin-mediated TGFβ activation in the pathogenesis of pulmonary fibrosis

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Abstract
IPF (idiopathic pulmonary fibrosis) is a chronic progressive disease of unknown aetiology without effective treatment. IPF is characterized by excessive collagen deposition within the lung. Recent evidence suggests that the lung epithelium plays a key role in driving the fibrotic response. The current paradigm suggests that, after epithelial injury, there is impaired epithelial proliferation and enhanced epithelial apoptosis. This in turn promotes lung fibrosis through impaired basement membrane repair and increased epithelial-mesenchymal transition. Furthermore, fibroblasts are recruited to the wounded area and adopt a myofibroblast phenotype, with the up-regulation of matrix-synthesizing genes and down-regulation of matrix-degradation genes.

There is compelling evidence that the cytokine TGFβ (transforming growth factor β) plays a central role in this process. In normal lung, TGFβ is maintained in an inactive state that is tightly regulated temporally and spatially. One of the major TGFβ-activation pathways involves integrins, and the role of the αvβ6 integrin has been particularly well described in the pathogenesis of IPF. Owing to the pleiotropic nature of TGFβ, strategies that inhibit activation of TGFβ in a cell- or disease-specific manner are attractive for the treatment of chronic fibrotic lung conditions. Therefore the molecular pathways that lead to integrin-mediated TGFβ activation must be precisely defined to identify and fully exploit novel therapeutic targets that might ultimately improve the prognosis for patients with IPF.

Introduction
The prognosis of IPF (idiopathic pulmonary fibrosis) is poorer than some cancers, with 5-year survival rates of 43% [1] and a median survival of 2.4 years. Furthermore, the incidence of IPF, currently approx. 4500 new cases annually in the U.K., is rising [1]. The pathogenesis of IPF is incompletely understood, but the lung epithelium is thought to play a key role in orchestrating the fibrotic response [2]. The current paradigm suggests that, after lung injury, there is epithelial damage with subsequent destruction of the alveolar-capillary basement membrane (Figure 1). This permits fibrogenic cell infiltration of the alveolar interstitium, and excess abnormal matrix synthesis characteristic of pulmonary fibrosis. Failure of epithelial repair, through reduced epithelial proliferation and increased apoptosis [3], promotes fibrosis. Furthermore, it has been estimated that up to 30% of fibroblasts in fibrotic lung disease may be derived from epithelial cells [4], hence the epithelium may contribute to fibrosis through EMT (epithelial–mesenchymal transition). The epithelium may also promote fibroblast proliferation, collagen synthesis and myofibroblast transdifferentiation via the paracrine effects of growth factors such as TGFβ (transforming growth factor β) [5]. Indeed, TGFβ may mediate many profibrotic effects in the alveolar epithelium following lung injury.

Given the lack of effective treatment for IPF, there is considerable work focused on identifying novel therapeutic targets through understanding the pathogenesis of IPF. The present review focuses on the pathways involved in integrin-mediated TGFβ activation in the pathogenesis of pulmonary fibrosis.

TGFβ is a central mediator in the development of pulmonary fibrosis
TGFβ is a member of the TGFβ superfamily, a highly conserved group of cytokines including bone morphogenic proteins, activins and inhibins. There are three mammalian isoforms (TGF-β1, -β2 and -β3), encoded by separate genes with distinct and related functions. The effects of TGFβ1 have been best characterized in pulmonary fibrosis, and from this point onwards, references to TGFβ will relate to TGFβ1 unless specified otherwise.

TGFβ is a pleiotropic cytokine that is ubiquitously expressed by all cells and tissues within the body. Studies of knockout mice have highlighted the crucial developmental functions of TGFβ, which also plays a central role in a diverse range of processes, including wound healing, immunity and carcinogenesis. Its pleiotropic effects are notable as it can either inhibit or stimulate the same cellular processes in a cell-type- or tissue-specific manner.

TGFβ has profound effects on epithelial cells and fibroblasts, which are central to the pathogenesis of pulmonary fibrosis. TGFβ promotes epithelial cell apoptosis [6], EMT

Key words: epithelium, idiopathic pulmonary fibrosis, integrin, transforming growth factor β (TGFβ).
Abbreviations used: COPD, chronic obstructive pulmonary disease; EMT, epithelial-mesenchymal transition; IPF, idiopathic pulmonary fibrosis; αvβ6, integrin subunit gene; LAP, latency-associated peptide; LPA, lysophosphatidic acid; LPA receptor 2; LTBP, latent transforming growth factor β-binding protein; MMP, matrix metalloproteinase; PAR-1, protease-activated receptor-1; TGFβ, transforming growth factor β.

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Figure 1 | Pathology of IPF

(a) Histological section of lung from patient with IPF stained with Masson’s trichrome. Shows increased matrix (M) with bronchiolization of type 1 cells (B) and traction of alveoli. Alveoli contain proteinacious exudate and inflammatory cells including activated macrophages (AM). (b) Cartoon depicting histological features of IPF. Alveolar basement membrane is disrupted with type 2 cell hypertrophy and bronchiolization of type 1 cells. There is exudation of intravascular fluid with inflammatory and apoptotic cells within the alveoli. Beneath the epithelial cell layer there is provisional matrix deposition containing increased matrix, fibroblasts and myofibroblasts and platelets.

[7], epithelial cell migration [8], collagen synthesis, fibroblast proliferation and transdifferentiation into myofibroblasts [5]. Furthermore, the role of TGFβ is well described in IPF. TGFβ is increased in tissue samples from both animal models of IPF [9] and IPF patients [10]. Overexpression of an adenovirus encoding active TGFβ leads to persistent pulmonary fibrosis [11], and inhibiting TGFβ with soluble TGFβ receptor [12], or a TGFβ receptor 1 (ALK5) inhibitor [13], ameliorates pulmonary fibrosis. Furthermore, mice null for the TGFβ signalling molecule Smad3 are protected from pulmonary fibrosis [14].

The crucial role of TGFβ in the pathogenesis of IPF makes it an attractive therapeutic target. Unfortunately, its pleiotropic nature and roles in normal tissue homoeostasis may make global inhibition of TGFβ problematic in the treatment of chronic diseases such as IPF. Pre-clinical and clinical studies to date have not found convincing evidence of toxicity associated with TGFβ blockade, although there are concerns that long-term toxicity may be a serious potential pitfall of global TGFβ inhibition [15,16]. Thus identifying and targeting tissue- or disease-specific mechanisms of TGFβ activation are attractive alternatives for the treatment of IPF.

TGFβ must be activated in order to have a biological effect

TGFβ is secreted by most cells in association with the LTBP’s (latent TGFβ-binding proteins) as the large latent complex, which is sequestered in the matrix. TGFβ itself is synthesized as the small latent complex consisting of active TGFβ non-covalently associated with the LAP (latency-associated peptide). The tissue specificity of TGFβ may be partially determined by the LTBP’s, which bind TGFβ in an isoform-specific fashion [17]. Furthermore, TGFβ associated with the latent complex is quiescent while stored in the matrix. Therefore, for TGFβ to exert any biological effects, it must be activated by dissociating from, or altering its interaction with, the LAP. Several processes can cause this, including physical processes such as temperature extremes, low pH and oxidation. TGFβ can also be activated by a number of proteases, including plasmin, tryptase, thrombin, elastase, MMP (matrix metalloproteinase)-2 and MMP-9 [18–21]. However, the importance of these mechanisms in vivo has yet to be defined. The best characterized mechanisms of in vivo TGFβ activation are mediated by interactions with thrombospondin or integrins.

Integrin-mediated activation of TGFβ

Integrins are heterodimeric transmembrane proteins consisting of α and β subunits. They are capable of binding to extracellular matrix proteins as well as a range of other molecules, including cell-surface ligands, transmembrane proteins, soluble proteases and growth factors [22].

The mammalian genome encodes 18 α subunits and eight β subunits that heterodimerize to form 24 αβ integrin combinations. Eight integrins, including all five αv-containing integrins, bind ligands through an RGD (Arg-Gly-Asp) sequence. An RGD sequence is found in the LAP of TGFβ1 and TGFβ3, which facilitates the activation of TGFβ by at least four αv-containing integrins (αvβ3, αvβ5, αvβ6 and αvβ8) in vitro. The LAP of TGFβ2 does not contain an
Two known mechanisms of integrin-mediated TGFβ activation

(a) Latent TGFβ binds the αvβ6 and αvβ8 integrins at the interface of the α and β subunits and the RGD motif of the TGFβ LAP. The LAP is tethered to the matrix, or cell surface, through LTBP. (b) Activation of TGFβ by the αvβ8 integrin does not require association of the cytoplasmic domain and the cytoskeleton. The αvβ8 integrin brings TGFβ to MMP-14 which proteolytically digests the LAP and permits the active TGFβ to diffuse freely. However, αvβ6 integrin-mediated TGFβ activation leads to structural changes within the latent TGFβ complex mediated via traction induced on the tethered αvβ6 integrin-LAP complex via the cytoskeleton. There is no release of free TGFβ from the complex and therefore there is an absolute requirement for cell-cell contact between the αvβ6 integrin-expressing cell and the TGFβ-responsive cell.

RGD motif and no integrin-mediated activation of TGFβ2 has been described.

In vivo, activation of TGFβ by integrins appears to play a major role during development and in various disease models. Integrins can activate TGFβ via two main mechanisms (Figure 2): a protease-dependent mechanism (αvβ8) and a mechanism involving cell traction (αvβ3, αvβ5 and αvβ6).

Studies using genetically modified mice have demonstrated that integrins are important in the non-proteolytic activation of TGFβ in the lung [23] and on dendritic cells [24]. The replacement of aspartic acid in the RGD motif with glutamic acid renders it unable to bind integrins. Mice bearing this mutation have a phenotype almost identical with TGFβ1-null mice [25], suggesting that integrins contribute significantly to the developmental effects of TGFβ activation in vivo. Recent data using αvβ6 (itgb6−/− mice), where itgb6 is β6 integrin subunit gene) and αvβ8 (itgb8−/−) -null mice have suggested that the phenotype associated with TGFβ1- and TGFβ3-null mice is due primarily to loss of these two integrins [26].

αvβ6 integrin-mediated TGFβ activation

The αvβ6 integrin was the first integrin to be implicated in the activation of TGFβ [23]. Expression of αvβ6 is significantly increased in injured epithelia, but overexpression is not sufficient to promote fibrosis [27]. The αvβ6 integrin itself must be activated before TGFβ activation can occur, and the evidence suggests that this occurs in response to cytoskeletal changes.

The cytoplasmic tail of the β6 subunit binds to the actin cytoskeleton, and mutation of this domain or treatment of cells with cytochalasin D, an inhibitor of actin polymerization, abolishes αvβ6 integrin-mediated TGFβ activation [23]. Additionally, in cells lacking LTBP-1, αvβ6 cannot activate TGFβ, therefore αvβ6 integrin-mediated TGFβ activation is critically dependent on the association of latent TGFβ with LTBP-1 within the large latent complex [28]. TGFβ-activating activity can be restored by inducing the expression of a short fusion protein with regions mimicking the LAP-binding and extracellular matrix-binding domains of LTBP-1 [28]. Overall, these studies suggest that αvβ6-mediated TGFβ activation is dependent on tethering the αvβ6 integrin–TGFβ complex to the cell surface.

The αvβ6 integrin is constitutively bound to TGFβ, suggesting that the system is primed to detect injurious stimuli. A pathway through which injurious signals are transmitted to the αvβ6 integrin has been described [29,30]. Thrombin and LPA (lysophosphatidic acid) are G-protein-coupled receptor agonists released from platelets following injury. Both of these substances have been shown to promote αvβ6 integrin-mediated TGFβ activation and are implicated in the development of IPF [31,32]. Thrombin and LPA induce αvβ6 integrin-mediated TGFβ activation via PAR1 (protease-activated receptor-1) and LPAR2 (LPA receptor 2) respectively, which in turn induce cytoskeletal changes via the G-protein Gαq, RhO and Rho kinase [30]. Therefore inflammatory mediators released in response to tissue injury activate TGFβ, by binding to epithelial cell-surface receptors and inducing cytoskeletal contraction, resulting in a conformational change in the αvβ6 integrin–latent TGFβ complex.

In vivo data have demonstrated the importance of αvβ6 integrin-mediated TGFβ activation in the pathogenesis of IPF. Mice that do not express the β6 subunit (itgb6−/− mice) cannot form the αvβ6 integrin. These mice develop mild inflammation in the lungs and skin, but are protected from bleomycin-, LPS (lipopolysaccharide)- and ventilator-associated lung injury, as well as bleomycin-induced pulmonary fibrosis and renal fibrosis [23,29,33]. Additionally, an anti-αvβ6 monoclonal antibody has been shown to prevent pulmonary fibrosis, renal fibrosis and acute lung injury in vivo [33–36].

Expression of itgb6 is increased at the mRNA level in bleomycin-induced lung fibrosis [30] and at the protein level in IPF patients [30,35]. This suggests that, although increased αvβ6 integrin expression is insufficient to increase TGFβ activation, abnormal regulation of the αvβ6 subunit may participate in the pathogenesis of IPF. Indeed, TGFβ itself up-regulates itgb6 expression [37], possibly mediated via the Ets1 transcription factor [38]. This process can be inhibited by blocking TGFβ or the αvβ6 integrin, suggesting a self-amplifying paracrine loop [39]. Thus it is possible that lung injury promotes αvβ6 integrin-mediated TGFβ activation, which in turn amplifies the signal through increasing itgb6
Figure 3 | Possible pathogenic mechanism of IPF

Initial alveolar injury leads to release of platelet-derived mediators, including thrombin and LPA. These activate their respective G-protein-coupled receptors, PAR1 and LPAR2, and induce a signalling cascade involving Gq or RhoA and Rho kinase (ROCK), leading to actin polymerization and cell traction on the cytoplasmic domain of the αvβ6 integrin, permitting activation of matrix- or cell-associated TGFβ (the initiating pathway). Active TGFβ acts in a paracrine fashion on neighbouring cells, leading to receptor Smad phosphorylation and translocation and increased synthesis of the itgb6 gene in epithelial cells (the amplification pathway). Owing to the disruption of the basement membrane, cell-cell contact is possible between epithelial cells and fibroblasts enabling epithelial cell induction of fibroblast- and basement membrane, cell–cell contact is possible between epithelial cells and fibroblasts enabling epithelial cell induction of fibroblast- and myofibroblast-derived matrix proteins (the fibrogenic pathway).

Gene expression (Figure 3). Understanding this process could identify new therapeutic targets for IPF.

αvβ8 integrin-mediated TGFβ activation

The αvβ8 integrin, like αvβ6, can bind and activate TGFβ. However, the mechanism of TGFβ activation is distinct from that of αvβ6. Whereas αvβ6-mediated TGFβ activation relies upon actin polymerization and retains active TGFβ at the cell surface, αvβ8 integrin-mediated TGFβ activation does not require the cytoplasmic domain of the β subunit, and is therefore not influenced by cytoskeletal contraction.

It seems that αvβ8 activates TGFβ by acting as a cell-surface shuttle, presenting TGFβ to a cell-surface protease. The αvβ8 integrin co-localizes at the cell surface with MMP-14, also known as MT1-MMP (membrane-type 1 matrix metalloproteinase). Consequently, latent TGFβ is presented to MMP-14 by the αvβ8 integrin, which results in the proteolytic cleavage of the LAP and the release of active TGFβ [40].

The αvβ8 integrin is crucial for vascular and brain development and for suppression of the adaptive immune system [24,41]. In the lung, αvβ8 integrin-mediated TGFβ activation has been shown to delay epithelial wound closure and inhibit bronchial epithelial proliferation in vitro [42]. The importance of αvβ8-mediated TGFβ activation in vitro is supported by the development of autoimmune colitis in mice with conditional loss of the αvβ8 integrin in dendritic cells [24].

Although there is currently no evidence for the involvement of the αvβ8 integrin in the pathogenesis of pulmonary fibrosis, it may play an important role in the airway fibrosis seen in COPD (chronic obstructive pulmonary disease) [39]. Squamous metaplasia, part of the pathological process associated with COPD, is driven by αvβ6 integrin-mediated TGFβ activation. This activates a paracrine loop involving IL (interleukin)-1β-induced αvβ8 integrin expression on fibroblasts, resulting in TGFβ activation and subsequent αvβ6 integrin up-regulation and increased squamous metaplasia [39]. Similar pathways could be active in the pathogenesis of IPF, which requires further study.

αvβ3 and αvβ5 integrin-mediated TGFβ activation

Both the αvβ3 and αvβ5 integrins are up-regulated in the dermal epithelium in systemic sclerosis. Both can activate TGFβ in scleroderma fibroblasts and this is associated with the transition of fibroblasts into fibrogenic myofibroblasts [43–45]. Activation of TGFβ by the αvβ5 integrin is not inhibited by protease inhibitors [43], and cellular traction has been proposed to induce TGFβ activation via αvβ3 and αvβ5 integrins. Myofibroblasts can liberate and activate TGFβ from extracellular stores by transmitting a contractile force via the αvβ5 integrin to latent TGFβ [46]. Function-blocking antibodies against αvβ5 inhibited this contraction-mediated TGFβ activation, which also occurred to a lesser extent with β1 and αvβ3 integrins [46]. This integrin-mediated TGFβ activation was limited to culture substrates with stiffness comparable with fibrotic tissue, suggesting that this mechanism maintains TGFβ activation after fibrosis has begun. This is the first direct evidence that mechanical stress can activate an extracellular matrix-bound cytokine [46].

However, there is currently no direct evidence for αvβ3 and αvβ5 integrin-mediated TGFβ activation in vivo. In mice null for the β3 integrin subunit [47], the β5 subunit [48] or both [49], there is no apparent loss of TGFβ activity. Furthermore, recent data have demonstrated that many of the developmental and immune effects of TGFβ are mediated via the αvβ6 and αvβ8 integrins [26]. The role of TGFβ activation by the αvβ3 and αvβ5 integrins thus needs to be clarified in disease models.

Conclusions

TGFβ is a key cytokine in the pathogenesis of IPF. It is stored as an inactive molecule in the extracellular matrix and is primed for activation following injury. Our understanding of the mechanisms of integrin-mediated TGFβ activation has increased dramatically in recent years. The αvβ3, αvβ5, αvβ6 and αvβ8 integrins have all been shown to activate TGFβ in vitro and could theoretically influence the pathogenesis of IPF. However, the αvβ6 integrin has been studied the
most extensively, and is the most highly implicated in the pathogenesis of IPF.

As integrins can activate TGFβ, and thus promote IPF, they are attractive therapeutic targets, not least because they are spatially and temporally restricted. The αvβ6 integrin is particularly attractive in this regard, because it is restricted to epithelial tissues, and inhibition of αvβ6 integrins would minimize disruption to the crucial homeostatic functions of TGFβ. Because IPF is a disease characterized by the presence of activated fibroblasts and myofibroblasts, which do not express the αvβ6 integrin, it has been suggested that therapies targeting multiple integrins simultaneously may be superior [50]. Additionally, given that proteases can act with integrins in TGFβ activation, protease inhibitors themselves are worthy of further investigation. Ultimately, given the complex nature and the unpredictable course of IPF, combinations of these therapeutics could be necessary.

Finally, the molecular pathways leading to integrin-mediated TGFβ activation and the self-amplifying pathways that increase integrin gene expression are still incompletely understood. Dissecting these molecular pathways, and identifying where dysregulation occurs during the pathogenesis of IPF should help us to better understand the disease as well as identify novel therapeutic strategies for the treatment of IPF, which may hopefully improve the prognosis of this devastating disease.

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References


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