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

Amanda Goodwin and Gisli Jenkins[1]
Centre for Respiratory Research, Nottingham Biomedical Research Unit, Clinical Sciences Building, City Campus, Hucknall Road, Nottingham NG5 1PB, U.K.

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

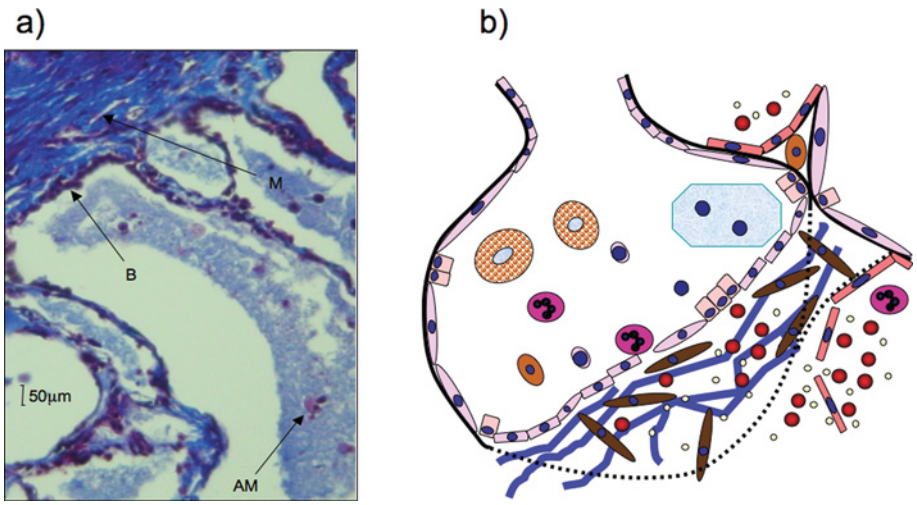
Key words: epithelium, idiopathic pulmonary fibrosis, integrin, transforming growth factor-β (TGFβ).

Abbreviations used: COPD, chronic obstructive pulmonary disease; EMT, epithelial–mesenchymal transition; IPF, idiopathic pulmonary fibrosis; *itgb6*, β6 integrin subunit gene; LAP, latency-associated peptide; LPA, lysophosphatidic acid; LPAR2; 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.

[1]To whom correspondence should be addressed (email gisli.jenkins@nottingham.ac.uk).

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 LTBPs (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 LTBPs, 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.

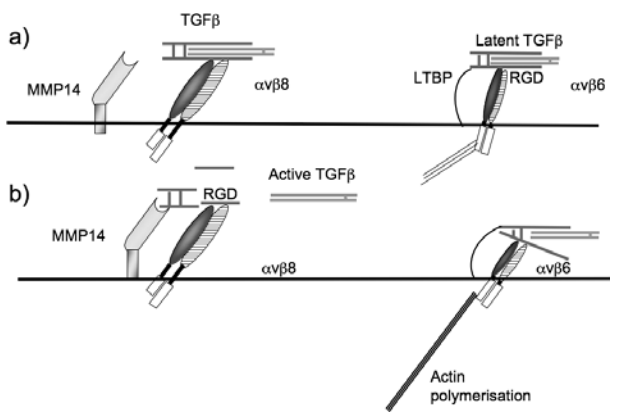
Integrin-mediated activation of TGFβ

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 $\alpha\beta$ 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

Figure 2 | 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 subunit 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 presents 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*$^{-/-}$, 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.

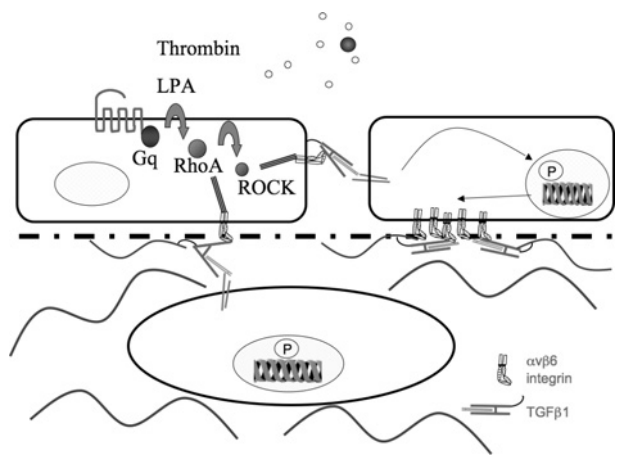
The αvβ6 integrin is constitutively bound to TGFβ, suggesting that the system is primed to detect injurious stimuli. A pathway through which injurious signals are transmitted to the αvβ6 integrin has been described [29,30]. Thrombin and LPA (lysophosphatidic acid) are G-protein-coupled receptor agonists 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 $G_{\alpha q}$, RhoA 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 $G_{\alpha q}$, RhoA and Rho kinase (ROCK), leading to actin polymerization and cellular traction on the cytoplasmic domain of the $\alpha v\beta 6$ 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 myofibroblast-derived matrix proteins (the fibrogenic pathway).



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

$\alpha v\beta 8$ integrin-mediated TGFβ activation

The $\alpha v\beta 8$ integrin, like $\alpha v\beta 6$, can bind and activate TGFβ. However, the mechanism of TGFβ activation is distinct from that of $\alpha v\beta 6$. Whereas $\alpha v\beta 6$-mediated TGFβ activation relies upon actin polymerization and retains active TGFβ at the cell surface, $\alpha v\beta 8$ 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 $\alpha v\beta 8$ activates TGFβ by acting as a cell-surface shuttle, presenting TGFβ to a cell-surface protease. The $\alpha v\beta 8$ 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 $\alpha v\beta 8$ integrin, which results in the proteolytic cleavage of the LAP and the release of active TGFβ [40].

The $\alpha v\beta 8$ integrin is crucial for vascular and brain development and for suppression of the adaptive immune system [24,41]. In the lung, $\alpha v\beta 8$ integrin-mediated TGFβ activation has been shown to delay epithelial wound closure and inhibit bronchial epithelial proliferation *in vitro* [42]. The importance of $\alpha v\beta 8$-mediated TGFβ activation *in vivo* is supported by the development of autoimmune colitis in mice with conditional loss of the $\alpha v\beta 8$ integrin in dendritic cells [24].

Although there is currently no evidence for the involvement of the $\alpha v\beta 8$ 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 $\alpha v\beta 6$ integrin-mediated TGFβ activation. This activates a paracrine loop involving IL (interleukin)-1β-induced $\alpha v\beta 8$ integrin expression on fibroblasts, resulting in TGFβ activation and subsequent $\alpha v\beta 6$ integrin up-regulation and increased squamous metaplasia [39]. Similar pathways could be active in the pathogenesis of IPF, which requires further study.

$\alpha v\beta 3$ and $\alpha v\beta 5$ integrin-mediated TGFβ activation

Both the $\alpha v\beta 3$ and the $\alpha v\beta 5$ 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 $\alpha v\beta 5$ integrin is not inhibited by protease inhibitors [43], and cellular traction has been proposed to induce TGFβ activation via $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins. Myofibroblasts can liberate and activate TGFβ from extracellular stores by transmitting a contractile force via the $\alpha v\beta 5$ integrin to latent TGFβ [46]. Function-blocking antibodies against $\alpha v\beta 5$ inhibited this contraction-mediated TGFβ activation, which also occurred to a lesser extent with $\beta 1$ and $\alpha v\beta 3$ 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 $\alpha v\beta 3$ and $\alpha v\beta 5$ integrin-mediated TGFβ activation *in vivo*. In mice null for the $\beta 3$ integrin subunit [47], the $\beta 5$ 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 $\alpha v\beta 6$ and $\alpha v\beta 8$ integrins [26]. The role of TGFβ activation by the $\alpha v\beta 3$ and $\alpha v\beta 5$ 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 $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$ and $\alpha v\beta 8$ integrins have all been shown to activate TGFβ *in vitro* and could theoretically influence the pathogenesis of IPF. However, the $\alpha v\beta 6$ 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 homoeostatic 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.

Acknowledgements

We thank Dr Mark Travis (University of Manchester, Manchester, U.K.) and Dr Dean Sheppard (University of California San Francisco, San Francisco, CA, U.S.A.) for their helpful comments during the preparation of the paper.

References

1 Gribbin, J., Hubbard, R.B., Le Jeune, I., Smith, C.J., West, J. and Tata, L.J. (2006) Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax **61**, 980–985
2 Selman, M. and Pardo, A. (2006) Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. Proc. Am. Thorac. Soc. **3**, 364–372
3 Thannickal, V.J. and Horowitz, J.C. (2006) Evolving concepts of apoptosis in idiopathic pulmonary fibrosis. Proc. Am. Thorac. Soc. **3**, 350–356
4 Willis, B.C. and Borok, Z. (2007) TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am. J. Physiol. Lung Cell. Mol. Physiol. **293**, L525–L534
5 Scotton, C.J. and Chambers, R.C. (2007) Molecular targets in pulmonary fibrosis: the myofibroblast in focus. Chest **132**, 1311–1321
6 Hagimoto, N., Kuwano, K., Inoshima, I., Yoshimi, M., Nakamura, N., Fujita, M., Maeyama, T. and Hara, N. (2002) TGF-β1 as an enhancer of Fas-mediated apoptosis of lung epithelial cells. J. Immunol. **168**, 6470–6478
7 Kim, K.K., Kugler, M.C., Wolters, P.J., Robillard, L., Galvez, M.G., Brumwell, A.N., Sheppard, D. and Chapman, H.A. (2006) Alveolar epithelial cell mesenchymal transition develops *in vivo* during pulmonary fibrosis and is regulated by the extracellular matrix. Proc. Natl. Acad. Sci. U.S.A. **103**, 13180–13185
8 Yu, H., Königshoff, M., Jayachandran, A., Handley, D., Seeger, W., Kaminski, N. and Eickelberg, O. (2008) Transgelin is a direct target of TGF-β/Smad3-dependent epithelial cell migration in lung fibrosis. FASEB J. **22**, 1778–1789
9 Coker, R.K., Laurent, G.J., Shahzeidi, S., Lympany, P.A., du Bois, R.M., Jeffery, P.K. and McAnulty, R.J. (1997) Transforming growth factors β1, β2 and β3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. Am. J. Pathol. **150**, 981–991
10 Coker, R.K., Laurent, G.J., Jeffery, P.K., du Bois, R.M., Black, C.M. and McAnulty, R.J. (2001) Localisation of transforming growth factor β1 and β3 mRNA transcripts in normal and fibrotic human lung. Thorax **56**, 549–556
11 Sime, P.J., Xing, Z., Graham, F.L., Csaky, K.G. and Gauldie, J. (1997) Adenovector-mediated gene transfer of active transforming growth factor β1 induces prolonged severe fibrosis in rat lung. J. Clin. Invest. **100**, 768–776
12 Wang, Q., Wang, Y., Hyde, D.M., Gotwals, P.J., Koteliansky, V.E., Ryan, S.T. and Giri, S.N. (1999) Reduction of bleomycin induced lung fibrosis by transforming growth factor β soluble receptor in hamsters. Thorax **54**, 805–812
13 Bonniaud, P., Margetts, P.J., Kolb, M., Schroeder, J.A., Kapoun, A.M., Damm, D., Murphy, A., Chakravarty, S., Dugar, S., Higgins, L. et al. (2005) Progressive transforming growth factor β1-induced lung fibrosis is blocked by an orally active ALK5 kinase inhibitor. Am. J. Respir. Crit. Care Med. **171**, 889–898
14 Zhao, J., Shi, W., Wang, Y., Chen, H., Bringas, P., Datto, M.B., Frederick, J.P., Wang, X. and Warburton, D. (2002) Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. Am. J. Physiol. Lung Cell. Mol. Physiol. **282**, L585–L593
15 Denton, C.P., Merkel, P.A., Furst, D.E., Khanna, D., Emery, P., Hsu, V.M., Silliman, N., Streisand, J., Powell, J., Akesson, A. et al. (2007) Recombinant human anti-transforming growth factor β1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. **56**, 323–333
16 Prud'homme, G.J. (2007) Pathobiology of transforming growth factor β in cancer, fibrosis and immunologic disease, and therapeutic considerations. Lab. Invest. **87**, 1077–1091
17 Chen, Y., Ali, T.M., Todorovic, V., O'Leary, J.M., Downing, A.K. and Rifkin, D.B. (2005) Amino acid requirements for formation of the TGF-β-latent TGF-β binding protein complexes. J. Mol. Biol. **345**, 175–186
18 Karsdal, M.A., Larsen, L., Engsig, M.T., Lou, H., Ferreras, M., Lochter, A., Delaissé, J.M. and Foged, N.T. (2002) Matrix metalloproteinase-dependent activation of latent transforming growth factor-β controls the conversion of osteoblasts into osteocytes by blocking osteoblast apoptosis. J. Biol. Chem. **277**, 44061–44067
19 Taipale, J., Koli, K. and Keski-Oja, J. (1992) Release of transforming growth factor-β1 from the pericellular matrix of cultured fibroblasts and fibrosarcoma cells by plasmin and thrombin. J. Biol. Chem. **267**, 25378–25384
20 Yu, Q. and Stamenkovic, I. (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev. **14**, 163–176
21 Tatler, A.L., Porte, J., Knox, A., Jenkins, G. and Pang, L. (2008) Tryptase activates TGFβ in human airway smooth muscle cells via direct proteolysis. Biochem. Biophys. Res. Commun. **370**, 239–242
22 Humphries, J.D., Byron, A. and Humphries, M.J. (2006) Integrin ligands at a glance. J. Cell Sci. **119**, 3901–3903
23 Munger, J.S., Huang, X., Kawakatsu, H., Griffiths, M.J., Dalton, S.L., Wu, J., Pittet, J.F., Kaminski, N., Garat, C., Matthay, M.A. et al. (1999) The integrin αvβ6 binds and activates latent TGFβ1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell **96**, 319–328
24 Travis, M.A., Reizis, B., Melton, A.C., Masteller, E., Tang, Q., Proctor, J.M., Wang, Y., Bernstein, X., Huang, X., Reichardt, L.F. et al. (2007) Loss of integrin αvβ8 on dendritic cells causes autoimmunity and colitis in mice. Nature **449**, 361–365
25 Yang, Z., Mu, Z., Dabovic, B., Jurukovski, V., Yu, D., Sung, J., Xiong, X. and Munger, J.S. (2007) Absence of integrin-mediated TGFβ1 activation *in vivo* recapitulates the phenotype of TGFβ1-null mice. J. Cell Biol. **176**, 787–793
26 Aluwihare, P., Mu, Z., Zhao, Z., Yu, D., Weinreb, P.H., Horan, G.S., Violette, S.M. and Munger, J.S. (2009) Mice that lack activity of αvβ6- and αvβ8-integrins reproduce the abnormalities of *Tgfb1*- and *Tgfb3*-null mice. J. Cell Sci. **122**, 227–232
27 Huang, X., Wu, J., Zhu, W., Pytela, R. and Sheppard, D. (1998) Expression of the human integrin β6 subunit in the alveolar type II cells and bronchiolar epithelial cells reverses lung inflammation in β6 knockout mice. Am. J. Respir. Cell Mol. Biol. **19**, 636–642

28 Annes, J., Chen, Y., Munger, J. and Rifkin, D (2004) Integrin αVβ6-mediated activation of latent TGF-β requires the latent TGF-β binding protein-1. J. Cell Biol. **165**, 723–734
29 Jenkins, R.G., Su, X., Su, G., Scotton, C.J., Camerer, E., Laurent, G.J., Davis, G.E., Chambers, R.C., Matthay, M.A. and Sheppard, D. (2006) Ligation of protease-activated receptor 1 enhances αvβ6 integrin-dependent TGF-β activation and promotes acute lung injury. J. Clin. Invest. **116**, 1606–1614
30 Xu, M.Y., Porte, J., Knox, A.J., Weinreb, P., Maher, M.T., Violette, S.M., McAnulty, R.J., Sheppard, D. and Jenkins, R.G. (2009) Lysophosphatidic acid induces αvβ6 integrin mediated TGF-β activation via the LPA2 receptor and the small G protein $G_{\alpha q}$, and this pathway is involved in lung fibrosis. Am. J. Pathol. **174**, 1264–1279
31 Howell, D.C., Laurent, G.J. and Chambers, R.C. (2002) Role of thrombin and its major cellular receptor, protease activated receptor-1, in pulmonary fibrosis. Biochem. Soc. Trans. **30**, 211–216
32 Tager, A.M., LaCamera, P., Shea, B.S., Campanella, G.S., Selman, M., Zhao, Z., Polosukhin, V., Wain, J., Karimi-Shah, B.A., Kim, N.D. et al. (2008) The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. Nat. Med. **14**, 45–54
33 Ma, L.J., Yang, H., Gaspert, A, Carlesso, G., Barty, M.M., Davidson, J.M., Sheppard, D. and Fogo, A.B. (2003) Transforming growth factor-β-dependent and -independent pathways of induction of tubulointerstitial fibrosis in $\beta6^{-/-}$ mice. Am. J. Pathol. **163**, 1261–1273
34 Hahm, K., Lukashev, M.E., Luo, Y., Yang, W.J., Dolinski, B.M., Weinreb, P.H., Simon, K.J., Wang, L.C., Leone, D.R., Lobb, R.R. et al. (2007) αvβ6 integrin regulates renal fibrosis and inflammation in Alport mouse. Am. J. Pathol. **170**, 110–112
35 Horan, G.S., Wood, S., Ona, V., Li, D.J., Lukashev, M.E., Weinreb, P.H., Simon, K.J., Hahm, K., Allaire, N.E., Rinaldi, N.J. et al. (2008) Partial inhibition of integrin αvβ6 prevents pulmonary fibrosis without exacerbating inflammation. Am. J. Respir. Crit. Care Med. **177**, 56–65
36 Puthawala, K., Hadjiangelis, N., Jacoby, S.C., Bayongan, E., Zhao, Z., Yang, Z., Devitt, M.L., Horan, G.S., Weinreb, P.H., Lukashev, M.E. et al. (2008) Inhibition of integrin αvβ6, an activator of latent transforming growth factor-β, prevents radiation-induced lung fibrosis. Am. J. Respir. Crit. Care Med. **177**, 82–90
37 Wang, A., Yokosaki, Y., Ferrando, R., Balmes, J. and Sheppard, D. (1996) Differential regulation of airway epithelial integrins by growth factors. Am. J. Respir. Cell Mol. Biol. **15**, 664–672
38 Bates, R.C., Bellovin, D.I., Brown, C., Maynard, E., Wu, B., Kawakatsu, H., Sheppard, D., Oettgen, P. and Mercurio, A.M. (2005) Transcriptional activation of integrin β6 during the epithelial–mesenchymal transition defines a novel prognostic indicator of aggressive colon carcinoma. J. Clin. Invest. **115**, 339–347
39 Araya, J., Cambier, S., Markovics, J.A., Wolters, P., Jablons, D., Hill, A., Finkbeiner, W., Jones, K., Broaddus, V.C., Sheppard, D. et al. (2007) Squamous metaplasia amplifies pathologic epithelial–mesenchymal interactions in COPD patients. J. Clin. Invest. **117**, 3551–3562
40 Mu, D., Cambier, S., Fjellbirkeland, L., Baron, J.L., Munger, J.S., Kawakatsu, H., Sheppard, D., Broaddus, V.C. and Nishimura, S.L. (2002) The integrin αvβ8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-β1. J. Cell Biol. **157**, 493–507
41 Proctor, J.M., Zang, K., Wang, D., Wang, R. and Reichardt, L.F. (2005) Vascular development of the brain requires β8 integrin expression in the neuroepithelium. J. Neurosci. **25**, 9940–9948
42 Neurohr, C., Nishimura, S.L. and Sheppard, D. (2006) Activation of transforming growth factor-β by the integrin αvβ8 delays epithelial wound closure. Am. J. Respir. Cell Mol. Biol. **35**, 252–259
43 Asano, Y., Ihn, H., Yamane, K., Jinnin, M., Mimura, Y. and Tamaki, K. (2005) Increased expression of integrin αvβ3 contributes to the establishment of autocrine TGF-β signaling in scleroderma fibroblasts. J. Immunol. **175**, 7708–7718
44 Asano, Y., Ihn, H., Jinnin, M., Mimura, Y.M. and Tamaki, K. (2006) Involvement of αvβ5 integrin in the establishment of autocrine TGF-β signaling in dermal fibroblasts derived from localized scleroderma. J. Invest. Dermatol. **126**, 1761–1769
45 Asano, Y., Ihn, H., Yamae, K., Jinnin, M. and Tamaki, K. (2006) Increased expression of integrin αvβ5 induced the myofibroblastic differentiation of dermal fibroblasts. Am. J. Pathol. **168**, 499–510
46 Wipff, P., Rifkin, D.B., Meister, J. and Hinz, B. (2007) Myofibroblast contraction activates latent TGF-β1 from the extracellular matrix. J. Cell Biol. **197**, 1311–1323
47 Hodivala-Dilke, K.M., McHugh, K.P., Tsakiris, D.A., Rayburn, H., Crowley, D., Ullman-Culleré, M., Ross, F.P., Coller, B.S., Teitelbaum, S. and Hynes, R.O. (1999) β3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J. Clin. Invest. **103**, 229–238
48 Huang, X., Griffiths, M., Wu, J., Farese, Jr, R.V. and Sheppard, D. (2000) Normal development, wound healing, and adenovirus susceptibility in β5-deficient mice. Mol. Cell. Biol. **20**, 755–759
49 Reynolds, L.E., Wyder, L., Lively, J.C., Taverna, D., Robinson, S.D., Huang, X., Sheppard, D., Hynes, R.O. and Hodivala-Dilke, K.M. (2002) Enhanced pathological angiogenesis in mice lacking β3 integrin or β3 and β5 integrins. Nat. Med. **8**, 27–34
50 Hinz, B. (2009) Tissue stiffness, latent TGF-β1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. Curr. Rheumatol. Rep. **11**, 120–126

Received 28 April 2009
doi:10.1042/BST0370849