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Inhibition of the αvβ6 integrin leads to limited alteration of TGF-α-induced pulmonary fibrosis

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PULMONARY FIBROTIC LESIONS are characterized pathologically by mesenchymal cell proliferation, differentiation, and increased deposition of extracellular matrix. A number of growth factors expressed in the lung activate the mesenchymal cell and experimental studies support targeting these fibrogenic growth factors and their receptors as potential therapeutic strategies to inhibit progression of pulmonary fibrosis. Although these profibrotic growth factors have unique receptors, there is a complex interface among their downstream signaling pathways. Many cell signaling pathways ultimately converge onto common pathways that regulate the cellular processes associated with pulmonary fibrosis including proliferation, differentiation, migration, and apoptosis. A more detailed understanding whether and where these fibrotic pathways overlap and interact will be important in designing future therapeutic strategies.

TGF-α, along with epidermal growth factor and amphiregulin, are ligands for the epidermal growth factor receptor (EGFR). Experimental studies in rodents support a role for EGFR activation in fibroproliferative processes following exposure to bleomycin, naphthalene, asbestos, silica, and hyperoxia (1, 38, 41, 53, 54). In addition, lung epithelial-specific overexpression of TGF-α leads to progressive and pronounced pulmonary fibrotic lesions in transgenic mice (21, 31). TGF-α knockout mice are protected from bleomycin-induced fibrosis, and the selective EGFR tyrosine kinase inhibitor gefitinib prevents the development of bleomycin-induced fibrosis and reverses transgenic TGF-α-induced fibrosis (20, 25, 39). EGFR receptor and ligands are elevated in idiopathic pulmonary fibrosis (IPF), cystic fibrosis, bronchopulmonary dysplasia, and the remodeled airways in asthma (8, 19, 40, 45, 46, 50, 51). Together these findings strongly support that EGFR signaling plays a central role in the pathogenesis of pulmonary fibrosis.

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The transforming growth factor-β (TGF-β) family includes multifunctional cytokines that are active in diverse biological processes and are central mediators in the initiation and maintenance of fibrosis in many diseases. TGF-β1 is a potent activator of fibroblast recruitment, extracellular matrix deposition, and myofibroblast differentiation (7). TGF-β1 is a critical mediator of bleomycin-induced fibrosis and transgenic lung epithelial-specific overexpression of TGF-β1 leads to significant fibrotic disease (12, 33). Expression of TGF-β in the airways is increased in fibrotic disease (12, 33). Expression of TGF-β receptors is increased in fibroblasts from patients with scleroderma and increased active TGF-β1 is detected in the lungs of patients with IPF, further supporting antagonism of the TGF-β pathway as a logical strategy for the treatment of fibrosis (28–30).

TGF-β is ubiquitously expressed by all cell types where it is secreted and stored in latent form that is bound mostly to the extracellular matrix through noncovalent associations with its propeptide, the latency-associated peptide (2). Activation of latent TGF-β is increasingly recognized as a critical step for its cellular effects (5). Integrins are transmembrane heterodimers of α and β subunits that bind a wide variety of physically constrained extracellular ligands and regulate cell growth, migration, and survival. A host of studies demonstrates that α6β4 integrin is a primary mediator of TGF-β activation in vivo in the lung (2, 42, 59). Mice with deleted β4 integrin are completely protected from lung fibrosis induced through intratracheal bleomycin or radiation, and blocking β4 with a specific antibody protects mice from lung injury and fibrosis in multiple experimental models (16, 23, 26, 42, 47). Collectively, these data show that inhibition of TGF-β signaling restricted to sites of β4-integrin-mediated TGF-β activation is an effective, selective strategy to treat lung fibrosis and avoid potential off-target effects of total TGF-β inhibition. Further evidence supporting this approach comes from the observation that α6β4 is expressed at low levels in healthy adult tissues but is strongly upregulated on epithelial cells at fibrotic foci in lungs of patients with IPF or pulmonary fibrosis from scleroderma (10, 23, 42). Together these findings have led to a current phase 2 clinical trial of humanized anti-α6β4 antibody in IPF patients (16).

Although both the EGFR and TGF-β pathways are central in the pathogenesis of pulmonary fibrosis, the relationship or integration between these pathways remains under investigation. There is strong evidence that EGFR ligands and receptors are critical mediators in fibrogenic responses to TGF-β. TGF-β induces EGFR ligand upregulation and receptor expression through the canonical SMAD pathway specifically in fibroblasts (3). Furthermore, EGFR activation by TGF-β is essential for the induction of fibroblast morphological transformation and anchorage-independent growth (4). Recent studies by Zhou et al. (63) demonstrate that TGF-β1 significantly induced the expression of amphiregulin in lung fibroblasts in vitro and antagonism of either amphiregulin or EGFR significantly reduced TGF-β1-induced fibroblast proliferation and expression of smooth muscle actin and extracellular matrix genes. Consistent with these in vitro findings, amphiregulin expression was markedly increased in the lung of TGF-β1 transgenic mice and either siRNA of amphiregulin expression or pharmacological inhibition of EGFR significantly reduced TGF-β1-stimulated collagen accumulation in the lung (63).

Although EGFR activation modulates the fibroproliferative effects involved in the pathogenesis of TGF-β-induced pulmonary fibrosis, potential contributions of TGF-β downstream of EGFR activation have not been reported. To examine the fibroproliferative role of TGF-β activity following EGFR activation in vivo, we measured changes in histology, biochemistry, and physiology in TGF-α transgenic mice where α6β4-mediated TGF-β activation was prevented through both genetic and pharmacological inhibition of the α6β4 integrin.

MATERIALS AND METHODS

Transgenic mice. All mice were derived from the FVB/NJ inbred strain. TGF-α transgenic mice were generated and maintained as described previously (21, 52). Mice homozygous with the rtTA transgene driven by the Clara Cell Specific Protein-rtTA TGF-α/rtTA promoter (abbreviated as CCSP) were mated with mice heterozygous with the human TGF-α transgene driven by a tetracycline-responsive promoter [(TetO)-rtTA TGF-α], to produce bitransgenic (abbreviated as CCSP/TGF-α) and single transgenic pups (abbreviated as CCSP/-). CCSP/- litter-matched controls were used as controls in antibody studies.

Mice functionally deficient in the β4 integrin (abbreviated as β4−/−) have been previously characterized (24). To investigate the role of the β4 integrin subunit in TGF-β-induced fibrosis, β4−/− mice were mated with transgenic CCSP mice and transgenic (TetO)-rtTA TGF-α/rtTA−/− mice. Single-transgenic offspring from each cross were then mated to generate CCSP/TGF-α/β4−/− and CCSP/TGF-α/β4−/− mice. All mice were in a FVB/N background. Mice were genotyped by PCR analysis of tail DNA using primers specific for transgene constructs using conditions as previously described (21, 24).

All mice were housed under specific pathogen-free conditions and protocols were approved by the Institutional Animal Use and Care Committee of the Cincinnati Children’s Hospital Research Foundation. To induce TGF-α expression, doxycycline (Dox) (Sigma, St. Louis, MO) was administered in food (62.5 mg/kg) and water (0.5 mg/kg). Administration of neutralizing antibodies. The inhibitory anti-α6, mAb, 6.3G9, and isotype control antibody, 1E6, have previously been described (57). Antibodies were injected weekly at doses of 3 mg/kg with injection volumes of 200 μl by intraperitoneal injection. This dosing was based on previous dose-response studies administering these antibodies in mouse lung fibrosis models (23, 47). Dosing throughout the study was based on original baseline weights and not adjusted for weight changes.

Immunohistochemistry. TGF-β assay. Immunohistochemical detection of β4 protein was performed as previously described (18). Briefly, paraffin-embedded lung tissue were deparaffinized and hydrated to distilled water. Proteolytic digestion was carried out by applying pepsin (Zymed 00-3009) to tissues for 10 min at 37°C. Tissue was blocked in 4% normal goat serum/TBS-Tween/0.1% BSA as previously described (21, 24).

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Bronchial lavage fluid collection and monitoring TGF-β activation. Bronchoalveolar lavage (BAL) collection was performed on mice as previously described (22) and active TGF-β was determined by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer’s recommendations.

RNA preparation and real-time PCR. Total RNA was extracted from lung tissue and cells by use of the RNeasy Mini Kit from Qiagen (Valencia, CA) as described previously (36). Real-time PCR analysis was performed on a Step One Plus instrument (Applied Biosystems, Foster City, CA). Primer sequences for TGFβ1, IGF1, and MMP10 are provided in Table 1.
Measurements of lung histology, fibrosis score, and pleural thickness. Lung tissue sections were prepared and stained with Masson’s trichrome as described previously (20). Lung tissue sections stained with Masson’s trichrome from mice in both pharmacological and genetic studies were scored for the degree of lung fibrosis by using a novel scoring system developed specifically for the TGF-α transgenic mouse. Each tissue section was assigned a single integer score between zero and five. The number corresponds to the degree of lung fibrosis in the pleural surface, perivascular, and terminal peribronchial adventitial regions of the lung (Table 2). The intervention or genetic designation for each slide was masked to the readers, and the score assigned to each mouse was based on reviewing three separate lung sections.

The pleural thickness was measured by histomorphometric measurement of Masson’s trichrome-stained lung sections by using the measured distance function of MetaMorph as previously described (37). Five random measures per lung section were obtained for each animal by using a Leica DM2700 M bright-field microscope (Leica Microsystems, Buffalo Grove, IL). High-magnification images (×40) were captured with a 3CCD color video camera and analyzed with MetaMorph imaging software (v6.2; Molecular Devices, Sunnyvale, CA).

Total lung collagen assay and pulmonary mechanics. The total lung collagen was quantified by measuring hydroxyproline levels and total soluble collagen (measured by Sircol Collagen Assay, Biocolor) as previously described (35, 37). Lung mechanics were assessed on mice by using a computerized Flexi Vent system (SCIREQ, Montreal, Canada) as previously described (35).

Statistics. All data were analyzed with Prism (Version 5; GraphPad, La Jolla, CA). One-way ANOVA with Tukey’s multiple-comparison posttest was used to compare different experimental groups, and data were considered statistically significant for P values less than 0.05.

RESULTS

Expression of α6β6 integrin during TGF-α-induced pulmonary fibrosis. The α6β6 integrin has been shown to contribute to fibrotic remodeling in mouse models of chemical- and radiation-induced fibrosis. To assess α6β6 integrin expression during TGF-α-induced pulmonary fibrosis, we immunostained the lung sections of control and TGF-α transgenic mice administered Dox for 4 and 8 wk using a monoclonal antibody (clone ch.2A1) that recognizes the β6 subunit. Minimal α6β6 integrin staining is detected in control mice but there is obvious staining throughout the alveolar epithelium in the lungs of TGF-α transgenic mice following 4 wk of Dox administration with a marked increase in epithelial staining intensity in the lungs of TGF-α transgenic mice following 8 wk of Dox (Fig. 1). No expression is detected in the adventitial or pleural fibrotic regions.

Increased expression of α6β6 integrin has been shown to contribute to fibrotic remodeling via activation of TGF-β. In TGF-α transgenic mice we previously reported no evidence of active TGF-β in the lung homogenates following 1 and 4 days of the administration of Dox (21). To assess whether TGF-β activation evolves later during TGF-α-induced fibrosis, we measured active TGF-β in the BAL fluid (BALF) of TGF-α transgenic mice after fibrosis was already established. We observed a significant increase in active TGF-β levels in the BALF of TGF-α mice administered Dox for 6 wk compared with controls or TGF-α transgenic mice following 2 wk of Dox administration (Fig. 2A). We also assessed α6β6/TGF-β pathway specific gene transcripts in lung homogenates of TGF-α transgenic mice administered Dox at early and late time points. As shown in Fig. 2B, transforming growth factor β-induced (TGFβi), insulin-like growth factor 1 (IGF1), and matrix metalloproteinase-10 (MMP10) transcript levels are unchanged in TGF-α transgenic mice 4 days after Dox induction compared with control but are significantly upregulated in the lungs at 6 wk on Dox. TGFβi is known to be directly induced by TGF-β, serves as a ligand recognition sequence for several integrins, and has been demonstrated to directly increase primary fibroblast production of collagen (43). In sum, the β6 integrin and active TGF-β are expressed at later time points following the induction of TGF-α after fibrosis is already established and progressing.

Therapeutic neutralization of β6 integrin attenuates TGF-α-induced fibrosis. To determine the contribution of the α6β6/TGF-β pathway on the progression of established fibrotic disease, TGF-α transgenic mice already administered Dox for 4 wk were treated with function-blocking anti-α6β6 antibody (6.3G9) while remaining on Dox for an additional 4 wk (8 wk

Table 2. Fibrosis score for TGF-α transgenic mice

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<thead>
<tr>
<th>Score</th>
<th>Pleural Surface</th>
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<tr>
<td>0</td>
<td>Normal pleura</td>
<td>Normal adventitia</td>
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<tr>
<td>1</td>
<td>Pleural thickening scattered covering &lt;50% of lung circumference</td>
<td>OR Fibrosis covers &lt;50% of vascular and terminal peribronchial adventitial circumference</td>
</tr>
<tr>
<td>2</td>
<td>Pleural thickening scattered covering &lt;50% of lung circumference</td>
<td>AND Fibrosis covers &lt;50% of vascular and terminal peribronchial adventitial circumference</td>
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<tr>
<td>3</td>
<td>Pleural thickening covering &gt;50% of lung circumference but thickness is inconsistent</td>
<td>AND Fibrosis covers &lt;50% of vascular and terminal peribronchial adventitial circumference</td>
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<tr>
<td>4</td>
<td>Pleural thickening covering entire lung circumference with near uniform thickness</td>
<td>AND Fibrosis covers &gt;50% of perivascular adventitial circumference but &lt;50% of terminal peribronchial adventitial circumference</td>
</tr>
<tr>
<td>5</td>
<td>Pleural thickening covering entire lung circumference with near uniform thickness</td>
<td>AND Fibrosis covers &gt;50% of vascular and terminal peribronchial adventitial circumference</td>
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Controls included single transgenic mice (CCSP/-) and TGF-α transgenic mice treated with isotype control antibody (1E6) following 4 wk of administration while also continuing on Dox an additional 4 wk. Body weights in TGF-α transgenic mice treated with isotype control antibody decreased 25% from baseline following 8 wk of Dox administration (Fig. 3A). Body weight loss was attenuated in mice treated with anti-αvβ6 antibody, with an average weight loss of 13% from baseline at 8 wk on Dox; however, body weights remained lower than CCSP/- controls (Fig. 3A). There were no differences in the total lung hydroxyproline levels between TGF-α transgenic mice treated with control or anti-αvβ6 antibodies (Fig. 3B).

Masson’s trichrome staining of lung sections of TGF-α transgenic mice treated with isotype control antibody demonstrate marked pleural thickening with advanced perivascular and peribronchial fibrosis affecting both large and small vessels and airways (Fig. 4A). TGF-α mice treated with anti-αvβ6 antibody displayed reduced pleural thickening compared with isotype control-treated TGF-α transgenic mice. To quantify the overall changes in the fibrotic lesions of the lungs, we established a scoring system based on the distribution and severity of fibrotic lesions in perivascular, peribronchial, alveolar, and pleural areas of the lung. Compared with CCSP/- control mice, overexpression of TGF-α for 8 wk caused a significant increase in the fibrosis score that was not significantly reduced in TGF-α mice treated with anti-αvβ6 antibody (Fig. 4B). To quantify the fibrotic changes specifically in the pleura, the pleural thickening of lung sections was measured. Mice treated with anti-αvβ6 antibody demonstrate significantly reduced pleural thickening compared with isotype control antibody-treated TGF-α transgenic mice (Fig. 4C). Changes in lung mechanic measurements of airway resistance, tissue elastance, and lung compliance were significantly attenuated in TGF-α transgenic mice treated with anti-αvβ6 antibody compared with TGF-α transgenic mice treated with isotype control antibody but values remained altered compared with nonfibrotic CCSP/-

**Fig. 1.** αvβ6 expression in the lung following TGF-α overexpression. The lungs of TGF-α mice were administered doxycycline (Dox) to induce TGF-α overexpression in the lung epithelium and immunostained with an antibody (ch.2A1) specific for the β6 subunit. αvβ6 expression is increased from controls after 4 wk of Dox and is highly expressed in the epithelium after 8 wk of Dox. No αvβ6 expression is detected in the pleural or adventitial fibrotic regions.

**Fig. 2.** Activation of the TGF-β pathway after TGF-α-induced fibrosis is established. A: active TGF-β levels in the bronchoalveolar lavage fluid (BALF) from TGF-α mice are significantly elevated from control mice following 6 wk of Dox (n = 4–7 for each group). B: αvβ6/TGF-β pathway specific gene transcripts transforming growth factor β-induced (TGFβi), insulin-like growth factor 1 (IGF1), and matrix metalloproteinase-10 (MMP10) are unchanged in TGF-α transgenic mice 4 days after Dox induction compared with control but are significantly upregulated in the lungs at 6 wk on Dox. Real-time PCR analysis was performed on total lung RNA isolated from the lungs.
controls (Fig. 5). Together, increases in pleural thickening and alterations in lung mechanics in TGF-α/H9251 mice were significantly attenuated when αvβ6 was inhibited after disease was already established, supporting a role for the αvβ6 pathway in mediating a component of EGFR/TGF-α-induced pulmonary/pleural fibrosis.

Lack of β6 integrin attenuates TGF-α-induced fibrosis. Genetic ablation of the β6 integrin has been shown to impair...
TGF-β signaling in the lung and protect mice from developing pulmonary fibrosis (23, 42). To test the effects of genetic β6 integrin inhibition at the initiation of lung fibrosis, CCSP and TGF-α transgenic mice were mated with β6-null mice to generate CCSP/TGF-α/β6+/+ and CCSP/TGF-α/β6−/− mice and the degree of fibrosis compared in adult mice 8 wk following administration of Dox. Active TGF-β levels, as assessed by ELISA from the BAL, were significantly increased in CCSP/TGF-α/β6+/+ compared with CCSP/-/β6−/− controls, whereas levels were unchanged between CCSP/TGF-α/β6−/− and CCSP/-/β6−/− controls (data not shown, n = 5–7 mice in each group). These findings support that the β6 integrin mice prevented the TGF-α-induced increase of active TGF-β. Masson’s trichrome staining of lung sections of CCSP/TGF-α/β6−/− mice demonstrated mild attenuation of adventitial and pleural fibrosis compared with CCSP/TGF-α/β6+/+ (Fig. 6). The lung fibrosis score was significantly reduced in CCSP/TGF-α/β6−/− mice compared with CCSP/TGF-α/β6+/+ (Fig. 7A), and there was a trend toward reduced pleural thickening in CCSP/TGF-α/β6−/− mice compared with CCSP/TGF-α/β6+/+ mice, but differences were not statistically significant (Fig. 7B). There were no differences in soluble lung collagen levels between CCSP/TGF-α/β6+/+ and CCSP/TGF-α/β6−/− mice (Fig. 7C). Lung mechanic measurements of airway resistance and tissue elastance were significantly reduced in CCSP/TGF-α/β6+/− mice compared with CCSP/TGF-α/β6+/+ mice with no differences in lung compliance (Fig. 8). In sum, ablation of the β6 integrin modestly attenuated histological and physiological changes in the lungs of TGF-α transgenic mice following 8 wk of Dox.

**DISCUSSION**

TGF-β is a multifunctional cytokine prominently involved in the pathogenesis of fibrosis through its potent effects on fibroblast differentiation, extracellular matrix formation, and epithelial mesenchymal transformation (14). Increased TGF-β activation is demonstrated in multiple mouse models of injury-induced pulmonary fibrosis and progressive fibrotic disease develops in adenoviral or transgenic TGF-β overexpression models (33, 49). Here we demonstrated upregulation of active TGF-β in the BAL of TGF-α transgenic mice along with increased mRNA levels of the TGF-β target genes, TGFβi, IGF1, and MMP10, but only after TGF-α-induced fibrosis is already established. Minimal activation of TGF-β proximately following TGF-α induction is consistent with our earlier findings in which we did not detect activity of TGF-β in lung homogenates 1 or 4 days following TGF-α induction (21). Together these findings support TGF-β activation as a late,
indirect, and partial contributor to fibrogenesis in the TGF-α transgenic model.

One of the primary mechanisms of activation of TGF-β in the lung is through the αvβ6 integrin (2). To specifically determine the role for TGF-β in the TGF-α fibrosis model we inhibited the αvβ6 integrin after fibrosis was established by administering mice a function blocking anti-αvβ6 antibody. TGF-α transgenic mice administered αvβ6 integrin inhibitor displayed modest attenuation in the progression of physiological and histological end points of lung fibrosis compared with controls. One of the prominent pathological features of pulmonary fibrosis in the TGF-α transgenic model is the progressive thickening of the lung pleura. This feature is also found in patients with several human fibrotic diseases. Previous reports demonstrate that transient transfer of the active TGF-β1 gene by adenoviral vectors into the pleural cavity and mesothelium induces homogenous, prolonged, and progressive pleural fibrosis associated with severe impairment of pulmonary function (13). In the present study, pharmacological neutralization of the αvβ6 integrin reduced the pleural thickening by almost 50%, showing that pleural fibrosis observed in TGF-α transgenic mice is partly dependent on the TGF-β pathway.

To further validate the anti-αvβ6 antibody data and to examine the efficacy of inhibiting the αvβ6 integrin at the initiation of fibrosis, TGF-α transgenic mice were mated with β6-null mice and fibrosis end points were compared between wild-type and knockout mice. In agreement with the pharmacological studies, genetic ablation of the β6 integrin mildly attenuated selected physiological and histological end points, yet a significant degree of fibrosis remained evident. Since the increases in TGF-β following TGF-α overexpression were prevented in β6 integrin knockout mice, these findings support a limited role for the TGF-β pathway in the progression of lung fibrosis in the TGF-α model. However, our assessment of TGF-β was limited to only the BAL and does not conclusively

Fig. 7. Genetic loss of β6 integrin attenuates progression of lung fibrosis. A: lung fibrosis score is significantly attenuated in TGF-α transgenic mice in the β6 knockout mice. B: mean pleural thickness was not significantly attenuated in TGF-α transgenic mice in the β6-nulls. C: total lung soluble collagen levels were elevated in TGF-α transgenic mice and unchanged in TGF-α transgenic mice in the β6 knockout mice. Data are means ± SE (n = 4–8 for each group).

Fig. 8. Genetic loss of β6 integrin attenuates progression of TGF-α-dependent changes in lung mechanics. Increases in airway resistance and tissue elastance were significantly attenuated in TGF-α transgenic mice in the β6 knockout mice compared with wild-type TGF-α transgenic mice; there were no differences lung compliance. Data are means ± SE (n = 6–8 for each group).
demonstrate fibrosis is independent of the TGF-β pathway at a cellular or basal level.

TGF-β induces fibrogenesis through both SMAD-mediated canonical and noncanonical pathways. The SMAD-independent pathways include the mitogen-activated protein kinase (MAPK) cascade and the phosphatidylinositol 3'-kinase (PI3K) pathways (48). The relative contribution from both the canonical and noncanonical TGF-β pathways in mediating pulmonary fibrosis remains under investigation; however, recent studies in both fibroblasts and in the TGF-β transgenic mouse model demonstrate that TGF-β induces fibrogenesis extensively through MAPK and PI3K activation independent of Smad activity (3, 63). The EGFR also activates multiple downstream effector pathways, and previous data from our laboratory demonstrate that TGF-α-induced pulmonary fibrosis is directly mediated through activation of the MAPK and PI3K pathways (32, 37). These reports thus suggest that both the EGFR and SMAD-independent TGF-β pathways overlap significantly through the MAPK and PI3K in mediating fibrogenesis in vivo. In the present study, the attenuation of fibrosis from β6 inhibition likely reflects the TGF-β canonical pathways contribution to TGF-α fibrosis, since MAPK and PI3K pathways would remain activated through the intact EGFR signaling.

A number of reports in animal models of lung fibrosis demonstrate that strategies to inhibit TGF-β signaling markedly prevent pulmonary fibrosis (9, 11, 12, 34). However, incomplete resolution of lung fibrosis progression by targeting the TGF-β pathway is not unique to the TGF-α model (27). Several studies also report that antagonizing the TGF-β pathway in bleomycin-induced pulmonary fibrosis does not completely prevent fibrosis (17, 44, 55), and inhibition of TGF-β had no effect on airway remodeling in the mouse model of house dust mite-induced chronic allergic airway disease (15). In addition to the lung, models of renal fibrosis also demonstrate fibrosis progression despite inhibition of the TGF-β pathway (56, 62). And liver fibrosis in mice caused by Schistosoma mansoni infection is completely independent of the TGF-β pathway but is mediated through interleukin-13 (27). The variability in the effects of targeting TGF-β likely reflect differences in the relative contribution of non-TGF-β fibrogenic proteins and pathways in mediating fibrogenesis among the fibrosis models and strains of mice studied.

In summary, β6 integrin inhibition attenuated pulmonary fibrosis in the TGF-α transgenic fibrosis model, with the most prominent effects noted in the pleural regions of the lung. These findings support that bidirectional cross talk exists between EGFR and TGF-β in vivo in the propagation of lung/pleural fibrosis. However, the results from the present study suggest that TGF-β pathway inhibition alone is not effective to sufficiently reverse ongoing fibrosis following EGFR activation. These findings are also consistent with our previous work in the TGF-α model in which specific pharmacological inhibitors to the MAPK and PI3K pathways were only modestly effective in reversing fibrosis when administered after extensive fibrotic disease was already present (32, 35). Together these data implicate heterogeneity of multiple active pathways in the maintenance of pulmonary fibrotic lesions. The translational implications from these findings suggest that effective pharmacological therapy to reverse disease or prevent progression may require simultaneous inhibition of multiple pathways.

In IPF, upregulation of the signaling intermediates from several fibrogenic pathways have been identified including TGF-β, EGFR, MAPK, and PI3K (6, 8, 29, 58, 60, 61). Many cell signaling pathways ultimately converge onto common pathways that regulate the cellular processes associated with pulmonary fibrosis, including fibroblast proliferation, differentiation, migration, and reduced apoptosis. Continued studies in preclinical models such as the TGF-α transgenic model will identify the mechanistic role of these pathways involved in the initiation, maintenance, and distribution of fibrotic lesions in the pleura and adventitia and will be important in designing future therapeutic strategies.

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DISCLOSURES

S. M. Violette and P. H. Weinreb are employed by Biogen, the maker of the inhibitor we tested in this manuscript.

AUTHOR CONTRIBUTIONS


REFERENCES

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