c-Myc Regulates Proliferation and Fgf10 Expression in Airway Smooth Muscle after Airway Epithelial Injury in Mouse

Thomas Volckaert1,2,3, Alice Campbell1, Stijn De Langhe1,4*

1 Department of Pediatrics, Division of Cell Biology, National Jewish Health, Denver, Colorado, United States of America, 2 Department for Molecular Biomedical Research, Unit of Molecular Signal Transduction in Inflammation, VIB, Ghent, Belgium, 3 Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium, 4 Department of Cellular and Developmental biology, School of Medicine, University of Colorado Denver, Aurora, Colorado, United States of America

Abstract

During lung development, Fibroblast growth factor 10 (Fgf10), which is expressed in the distal mesenchyme and regulated by Wnt signaling, acts on the distal epithelial progenitors to maintain them and prevent them from differentiating into proximal (airway) epithelial cells. Fgf10-expressing cells in the distal mesenchyme are progenitors for parabronchial smooth muscle cells (PSMCs). After naphthalene, ozone or bleomycin-induced airway epithelial injury, surviving epithelial cells secrete Wnt7b which then activates the PSMC niche to induce Fgf10 expression. This Fgf10 secreted by the niche then acts on a subset of Clara stem cells to break quiescence, induce proliferation and initiate epithelial repair. Here we show that conditional deletion of the Wnt target gene c-Myc from the lung mesenchyme during development does not affect proper epithelial or mesenchymal differentiation. However, in the adult lung we show that after naphthalene-mediated airway epithelial injury c-Myc is important for the activation of the PSMC niche and as such induces proliferation and Fgf10 expression in PSMCs. Our data indicate that conditional deletion of c-Myc from PSMCs inhibits airway epithelial repair, whereas c-Myc ablation from Clara cells has no effect on airway epithelial regeneration. These findings may have important implications for understanding the misregulation of lung repair in asthma and COPD.

Introduction

A complex interplay between endodermal and mesodermal cell types defines early developmental competence and cell fate in the lung. As such, proximal-distal patterning of the lung is accompanied by the gradual restricted ability of developmental progenitors to generate the various epithelial lineages in the mature organ [1]. During lung development, Fgf10 (Fibroblast growth factor 10) is expressed in mesenchyme distal to the branching tips where it maintains the multipotent distal epithelial progenitors, but is suppressed proximally and at bifurcation points [2,3,4,5,6,7]. We previously identified the Fgf10-expressing cells in the distal mesenchyme as parabronchial smooth muscle cell (PSMC) progenitors [3,8]. Fgf10 expression as well as the amplification of these PSMC progenitors is regulated by Wnt signaling [3,9,10]. Suppression of Fgf10 expression around the developing airway is crucial to allow for proper maturation of the lung airway epithelium [11,12,13,14,15].

The adult lung is a vital and complex organ that normally turns over very slowly. The epithelial cells that line the airways are constantly exposed to potential toxic agents and pathogens in the environment, and they must therefore be able to respond quickly and effectively to both cellular damage and local production of immune cytokines. Adult stem cells are implicated in both homeostatic tissue maintenance and functional restoration after injury in organs such as skin and gut.

A widely used lung injury model involves the destruction of Clara cells by naphthalene. Only those Clara cells that express cytochrome P4502F2 (encoded by Cyp2f2) are able to convert naphthalene into toxic epoxides leading to cell death. Within a few hours after naphthalene administration nearly all Clara cells have died, except for the few less differentiated variant Clara stem cells that do not express Cyp2f2, making them therefore resistant against naphthalene [16,17,18,19,20]. Ciliated cells quickly spread out, or squamate, under the dying Clara cells in an attempt to cover the basal lamina and maintain the permeability barrier of the epithelium [21].

We have previously shown that surviving ciliated cells after naphthalene, ozone or bleomycin-mediated airway epithelial injury start to secrete Wnt7b, which then activates the PSMC niche to induce Fgf10 expression [22]. We found that Fgf10 secreted by the niche acts on surviving Clara stem cells to break quiescence, induce proliferation and initiate epithelial repair. Here we show that after naphthalene-mediated airway epithelial injury, the Wnt target c-Myc is important for the activation of the PSMC niche and as such induces proliferation and Fgf10 expression in PSMCs. Myc proteins coordinate many interdependent processes, including cell growth (increase in cell mass), cell proliferation...
(DNA replication and cell cycle progression), differentiation and apoptosis [23]. Using an allelic series of mice in which c-Myc expression was incrementally reduced to zero, Trumpf et al. showed that fibroblasts from these mice exhibit reduced proliferation and after complete loss of c-Myc function exit the cell cycle [24]. Our data indicate that conditional deletion of c-Myc from PSMCs prevents activation of the airway epithelial stem cell niche after airway epithelial injury resulting in deficient epithelial repair.

Results

**c-Myc Expression in the Lung Mesenchyme is not Required for Normal Lung Development**

During lung development, Nmyc expression is normally restricted to a distal population of undifferentiated epithelial cells [25], whereas c-Myc is only expressed in the mesenchyme [3]. c-Myc expression is regulated by β-catenin signaling and is lost upon conditional deletion of β-catenin from the lung mesenchyme [3]. In some organs most of the effects of β-catenin signaling are primarily mediated by c-Myc [26]. To test whether during lung development the effects of mesenchymal β-catenin signaling are primarily mediated via c-Myc we conditionally deleted c-Myc from the lung mesenchyme using a Dermo1(Twist2)-Cre line [27]. Interestingly, while ablation of β-catenin from the lung mesenchyme resulted in major differentiation defects and reduced Fgfl expression [3,28], we found that conditional deletion of c-Myc from the lung mesenchyme has no significant effect on either (Fig. 1A–D). At E18.5, Dermo1-Cre;Gscf-Mycf/f [24] conditional knock out lungs appear normal, with normal Fgfl expression (Fig. 1A,B) and with proper differentiation of the airway and vascular smooth muscle cells (Fig. 1C,D), proper differentiation of the distal epithelium in ATII (SftpC) and ATI (Pdpc) cells (Fig. 1C–F) and proper differentiation of the bronchial epithelium into Clara (Sgb1a1) and ciliated cells (ß-Tub) (Fig. 1G,H).

**c-Myc Regulates Activation of the Airway Epithelial Stem Cell Niche after Airway Epithelial Injury**

We recently showed that after airway epithelial injury, surviving epithelial cells secrete Wnt7b, which then activates PSMCs (which constitute a niche for airway epithelial stem cells) to induce proliferation and Fgfl expression [22]. This Fgfl secreted by the PSMC then acts on a subset of Clara stem cells to break quiesence, induce proliferation and initiate epithelial repair [22]. To investigate the requirement of c-Myc in the activation of the PSMC niche and the induction of Fgfl expression in the adult lung after airway epithelial injury we generated Myh11-Cre;Gscf-Mycf/f mice (Myh11: smooth muscle myosin heavy chain) [29], in which we conditionally deleted c-Myc from the PSMCs, shown by in situ hybridization in Fig. 2A,B. Interestingly, we found that the PSMC niche in Myh11-Cre;Gscf-Mycf/f lungs does not get activated after naphthalene-mediated airway epithelial injury. This is manifested by reduced proliferation of the PSMCs, as 9.2% ± 2.1% of PSMCs were BrdU positive in control lungs vs. 2.6% ± 0.23% of PSMCs in Myh11-Cre;Gscf-Mycf/f lungs (P = 0.000005, n≥4) (Fig. 2C,D) [22]. To investigate whether induction of Fgfl expression is also regulated by c-Myc we crossed Myh11-Cre;Gscf-Mycf/f mice with an Fgfl reporter line [3,7,8,22,30]. In contrast to our observations during lung development we found that in the adult lung, 3 days after naphthalene-mediated airway epithelial injury, Fgfl expression in the PSMC niche is regulated by c-Myc, as demonstrated by the lack of induction of Fgfl expression in Myh11-Cre;Gscf-Mycf/f/Fgfllox/lox mice (Fig. 2F) compared to control littermates (Fig. 2E). We previously reported a similar drastic reduction in Fgfl expression and proliferation in PSMCs, after naphthalene-mediated airway epithelial injury, in mice overexpressing Dkk1, a secreted inhibitor of Wnt signaling [22]. To investigate whether epithelial Fgfl signaling is also reduced we investigated in Myh11-Cre;Gscf-Mycf/f mice showing an almost 3 fold decrease in regeneration (Fig. 4I,L,M) compared to control mice (Fig. 4C,F,M). This decrease in regeneration is even more evident at 14 days post injury, with Myh11-Cre;Gscf-Mycf/f mice showing an almost 3 fold decrease in regeneration (Fig. 4I,L,M) compared to control mice (Fig. 4C,F,M).

**Conditional Deletion of c-Myc from Clara Stem Cells does not Affect Airway Epithelial Regeneration after Injury**

We have previously shown that after naphthalene injury a subset of Clara cells undergo a transient epithelial to mesenchymal transition (EMT) to acquire stem cell-like properties and as such are able to transiently induce the expression of Myb11 [22]. To investigate whether the decrease in airway epithelial regeneration in Myh11-Cre;Gscf-Mycf/f mice is not due to deletion of c-Myc from these Clara cells, transiently expressing Myb11-Cre, we generated Sgb1a1-Cre;Gscf-Mycf/f [33,34] mice in which the c-Myc gene is deleted specifically from all Clara cells. We found that airway epithelial regeneration after naphthalene injury is not affected in Sgb1a1-Cre;Gscf-Mycf/f mice compared to control littermates (Fig. 5A–E), indicating that epithelial c-Myc does not play an important role in airway epithelial regeneration and that the defect in regeneration observed in Myh11-Cre;Gscf-Mycf/f mice can be attributed solely to the loss of c-Myc from the PSMC niche. This is consistent with the fact that during lung development c-Myc expression is restricted to the mesenchyme, whereas Nmyc is expressed solely in the epithelium [3,25].

**Discussion**

The lung has a complex three-dimensional structure that features major differences along its proximal-distal axis in terms of the composition of the endoderm-derived epithelium. The trachea and primary lung buds arise by different morphogenetic processes
Figure 1. Mesenchyme-specific c-Myc ablation does not affect lung development. (A,B) Fgf10 in situ hybridization on E18.5 ctrl (A) and Derma1-Cre;c-Myc<sup>ff</sup> (B) lungs showing that Fgf10 expression is not affected. (C–H) Immunostaining for α-SMA (smooth muscle cells) and Sftpc (ATII cells) (C,D), PDPN (ATI cells) (E,F), and Scgb1a1 (Clara cells) and β-tubulin (ciliated cells) (G,H) on E18.5 ctrl (C,E,G) and Derma1-Cre;c-Myc<sup>ff</sup> (D,F,H) lungs. n=3. Scale bars: 100 μM (A,B and G,H); 200 μM (C–F).

doi:10.1371/journal.pone.0071426.g001
from contiguous regions of the embryonic foregut [35]. A distinguishing feature of the adult mouse cartilaginous airways (i.e. trachea and primary bronchi) is that Fgf10 is expressed in the mesenchyme between the cartilage rings [36,37] and that they contain a discontinuous population of basal stem cells that express p63 and specific keratins (K14 and K5). In addition to basal cells, the luminal epithelium in cartilaginous airways consists of two main columnar epithelial cell types: ciliated cells and Clara cells with a limited number of Clara cell-derived goblet cells. Ciliated cells contain cilia which are involved in the clearance of mucus produced by goblet cells, whereas Clara cells produce secretoglobinins, the most abundant of which is Scgb1a1 (also known as CCSP, CC10 and CCA) [38,39,40].

The more distal airways (small bronchi and bronchioles) have a columnar epithelium surrounded by airway smooth muscle which does not express Fgf10 during normal homeostasis [8]. Clara stem cells predominate over ciliated cells and there are more neuroendocrine cells than in the trachea. More importantly, there is no evidence of basal cells in smaller airways in the mouse during normal homeostasis [41].

In the cartilaginous airways basal cells are considered to be on top of the stem cell hierarchy and are able to self renew and give rise to both Clara cells, goblet cells and ciliated cells [42]. Clara
cells themselves are also considered stem Clara cells and during normal homeostasis can give rise to new Clara cells and terminally differentiated ciliated cells [43,44]. Cellular plasticity (including but not limited to differentiation, dedifferentiation, and transdifferentiation) is a frequently encountered cell behavior during injury repair [45,46,47,48,49,50,51]. Interestingly, p63 is a master regulator required for the development of basal cells [52] and induces a basal cell phenotype and squamous metaplasia when ectopically expressed in Clara cells [53]. This form of Clara cell reprogramming may happen to some extent after airway epithelial injury, as under such conditions Clara cells have been shown to be able to give rise to basal cells [44].

Interestingly, our unpublished data suggest that Fgf10 plays a role in the differentiation of airway epithelial cells into basal stem cells during lung development (Volckaert et al., manuscript submitted).

Our data presented here indicate that c-Myc plays an important role in regulating the activity of the PSMC niche in the adult lung. We found a role for c-Myc in regulating proliferation of PSMCs as well as the induction of Fgf10 expression within PSMCs cells after airway epithelial injury. Interestingly, we found no important role for c-Myc in the mesenchyme during lung development indicating that the function of c-Myc during lung development is redundant and that other not yet identified factors may compensate for the loss of c-Myc during lung development. The lack of defective smooth muscle cell differentiation or maintenance in Myh-Cre; c-Myc<sup>f/f</sup> lungs suggests that c-Myc may play a specific role in activation of the PSMC niche after injury. Together with the finding that epithelial c-Myc does not play an important role in lung epithelial homeostasis or repair after injury we conclude that targeting c-Myc may be a great way to treat lung diseases characterized by abnormal proliferation of smooth muscle cells, such as asthma and pulmonary arterial hypertension in which Wnt signaling plays a role [54]. In addition, we have previously shown that Fgf10 secreted by the PSMCs modulates the differentiation of Clara cells into goblet cells [22], which is a hallmark of the asthmatic airway. Future experiments will be needed to determine if loss of mesenchymal c-Myc may also reduce proliferation of (myo)fibroblasts in the bleomycin model of pulmonary fibrosis, in which Wnt signaling plays an important role [55,56,57,58,59,60,61]. If so, targeting c-Myc might be an effective and selective way to treat fibroproliferative lung diseases in general.

**Materials and Methods**

**Study Approval**

All experiments were conducted in strict accordance with the recommendations in the guide for the care and use of laboratory animals. The protocol was approved by the National Jewish Health institutional animal care and use committee #AS2774.

**Mouse Strains**

Myh11-Cre (Tg[Myh11-cre-EGFP]2Mik/J) mice were obtained from Jackson Laboratories. Dermo1-Cre mice were a kind gift from Dr. David Ornitz [27]. Scgb1a1-Cre were a kind gift from Dr. Thomas Mariani [33,34]. c-Myc<sup>f/f</sup> mice were a kind gift from Dr. Andreas Trumpp [24]. Adult mice were 8 weeks old at time of...
Figure 4. Conditional c-Myc deletion from the epithelial stem cell niche impairs epithelial regeneration after injury. (A–L) Immunostaining for Scgb1a1 (Clara stem cells) and β-tubulin (ciliated cells) (A–C,G–I) or Scgb1a1 (Clara stem cells) and CGRP (neuroendocrine bodies) (D–F,J–L) on ctrl (A–F) and Myh11-cre;Myc<sup>fl/fl</sup> (G–L) lungs 3 days (A,D,G,J), 7 days (B,E,H,K) and 14 days (C,F,I,L) after naphthalene injury. (M) qPCR analysis of relative Scgb1a1 mRNA abundance in lungs from ctrl and Myh11-cre;Myc<sup>fl/fl</sup> mice 3, 7 and 14 days after naphthalene treatment. **P<0.01, *P<0.05 vs. respective control. n=3. Scale bars: 200 μM (A–C and G–I); 50 μM (D–F and J–L).

doi:10.1371/journal.pone.0071426.g004
naphthalene administration. Animals were maintained in a pathogen-free environment.

\[ \text{\(\beta\)-gal Staining} \]

Tissues containing \( Fg\text{f}1\text{d}^{\text{c}}\) allele were dissected, and \(\beta\)-gal staining was performed at 3 days after naphthalene injury. Lungs were dissected and fixed in 4% PFA in PBS at room temperature for 5 minutes, rinsed in PBS, injected with freshly prepared X-gal solution, transferred into a vial of X-gal solution, and stained at 37\(^\circ\)C overnight. After rinsing with PBS, lungs were postfixed in 4% PFA in PBS at room temperature overnight. For microtome sections, after 4% PFA fixation, lungs were washed in PBS, dehydrated, and paraffin embedded.

\[ \text{Immunofluorescence} \]

All staining was done on paraffin sections of formalin-fixed lungs. Immunofluorescent staining was performed with the following primary antibodies: mouse anti-\(\beta\)-tubulin (3F3-G2; Seven Hills Bioreagents), goat anti-Scbg1a1 (T-18; Santa Cruz Biotechnology Inc.), rabbit anti-Scbg1a1 (Seven Hills Bioreagents), rabbit anti-CGRP (Sigma-Aldrich), rabbit anti-Fgfr2 (Bek) (C-17; Santa Cruz Biotechnology Inc.), mouse anti-\(\alpha\)-SMA cy3 conjugate and unconjugated (14A; Sigma-Aldrich), rabbit anti-Sftpc (Seven Hills Bioreagents), mouse anti-PDPN (Iowa hybridoma bank). All fluorescent staining was performed with secondary antibodies from Jackson Immunoresearch (except the Cy3-conjugated \(\alpha\)-SMA) and mounted using Vectashield with DAPI.

Figure 5. Epithelial \(c\text{-Myc}\) deletion does not affect airway epithelial regeneration after injury. (A–D) Immunostaining for Scgb1a1 (Clara stem cells) and \(\beta\)-tubulin (ciliated cells) (A,B) or Scgb1a1 (Clara stem cells) and CGRP (neuroendocrine bodies) (C,D) on ctrl (A,C) and Scgb1a1-Cre;\(c\text{-Myc}^{\text{f/f}}\) (B,D) lungs 7 days after naphthalene injury. (E) qPCR analysis of relative Scgb1a1 mRNA abundance in lungs from ctrl and Scgb1a1-Cre;\(c\text{-Myc}^{\text{f/f}}\) mice 7 days after naphthalene treatment. \(P>0.05\), \(n\geq3\). Scale bars: 200 \(\mu\)M (A,B); 50 \(\mu\)M (C,D).

doi:10.1371/journal.pone.0071426.g005
(Vector Labs). Photographs were taken with a Zeiss AxiosImager and Axiosvision software.

qPCR

RNA was isolated from lung accessory lobes using RNeAlter (Ambion) and Total RNA Kit I (Omega Biotek) according to the manufacturer’s instructions. RNA concentration was determined by spectrophotometry. cDNA was generated using SuperScript III First-Strand Synthesis System (Invitrogen) according to the manufacturer’s instructions. Comparative real-time PCR was performed for $\beta$-glucuronidase (Mm00446953_m1) and Scgb1a1 (Mm00447102_m1) Tissue Specific in Expression Assays (Applied Biosystems) using a StepOne Plus system (Applied Biosystems). $\beta$-glucuronidase was used as a reference control to normalize equal loading of template cDNA.

Naphthalene Treatment

Naphthalene (Sigma-Aldrich) was dissolved in corn oil at 30 mg/ml and administered intraperitoneally at 8 weeks of age, with doses adjusted according to strain to achieve a 95% decrease in the abundance of $\Delta$-glucuronidase (Mm00446953_m1) and Scgb1a1 mRNA in total lung RNA of WT mice at 3 days after injection. Control mice for regeneration studies were WT littermates.

Proliferation

Mice were given intraperitoneal injections of 10 $\mu$l BrdU (GE Healthcare) per gram body weight 4 hours before sacrifice. Lungs were fixed in 4% paraformaldehyde, dehydrated and embedded. Sections were stained with monoclonal anti-BrdU (clone BU-1; GE Healthcare) according to the manufacturer’s instructions. FITC-labeled anti-mouse secondary antibodies were used (Jackson Immunoresearch). All slides were mounted using Vectashield with DAPI.

For BrdU labeling and qPCR analysis, each experiment was repeated with samples obtained from at least 3 different lungs preparations. All results are expressed as mean ± SEM. The significance of differences between 2 sample means was determined by the Student’s $t$ test. $P$ values less than 0.05 were considered statistically significant.

Author Contributions

Conceived and designed the experiments: TV SDL. Performed the experiments: TV AC SDL. Analyzed the data: TV SDL. Wrote the paper: TV SDL.
44. Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, et al. (2009) The role of
43. Evans MJ, Johnson LV, Stephens RJ, Freeman G (1976) Renewal of the
42. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, et al. (2009) Basal cells as
41. Pack RJ, Al-Ugaily LH, Morris G (1981) The cells of the tracheobronchial
40. Rock JR, Randell SH, Hogan BL (2010) Airway basal stem cells: a perspective
39. Rock JR, Hogan BL (2011) Epithelial progenitor cells in lung development,
38. Rawlins EL, Hogan BL (2006) Epithelial stem cells of the lung: privileged few or
37. Tiozzo C, De Langhe S, Carraro G, Alam DA, Nagy A, et al. (2009) Fibroblast
36. Sala FG, Del Moral PM, Tiozzo C, Alam DA, Warburton D, et al. (2011)
35. Cardoso WV, Lu J (2006) Regulation of early lung morphogenesis: questions,
34. Simon DM, Arikan MC, Srisuma S, Bhattacharya S, Andalcio T, et al. (2006)
33. Ji H, Houghton AM, Mariani TJ, Perera S, Kim CB, et al. (2006) K-ras
32. Chen H, Matsumoto K, Brockway BL, Rackley CR, Liang J, et al. (2012)
31. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, et al. (2005)
30. Kelly RG, Brown NA, Buckingham ME (2001) The arterial pole of the mouse
29. Zhou Q, Wei Y, Szekeres C, Kugler MC, Wolters PJ, et al. (2009) Epithelial cell
28. Xi Y, Wei Y, Sennino B, Ulsamer A, Kwan I, et al. (2009) Identification of pY654-
27. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, et al. (2008)
26. Konigshoff M, Eickelberg O (2009) WNT signaling in lung disease: a failure or a
25. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amane OV, et al. (2009)
24. Stocum DL (2004) Tissue restoration through regenerative biology and
23. Kai T, Spradling A (2004) Differentiating germ cells can revert into functional
22. Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, et al. (2009)
21. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 453: 627–632.
20. Daniel Y, Liao G, Dixon D, Linsolle RI, Lori A, et al. (2004) Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. Am J Physiol Cell Physiol 287: C171–181.
19. Koster MI, Kim S, Mills AA, DeMayo FJ, Roop DR (2004) p63 is the molecular switch for initiation of an epithelial stratification program. Genes Dev 18: 129–131.
18. Cohen ED, Ihida-Stansbury K, Lu MM, Panettieri RA, Jones PL, et al. (2009) Wnt signaling regulates smooth muscle precursor development in the mouse lung via a tenascin C/PDGFR pathway. J Clin Invest 119: 2538–2549.
17. Konigshoff M, Balsara N, Pfaff EM, Kramer M, Chrobak I, et al. (2008) Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. PLoS One 3: e2142.
16. Xi Y, Wei Y, Sennino B, Ulsamer A, Kwan I, et al. Identification of pY654-beta-catenin as a critical co-factor in hypoxia-inducible factor-1alpha signaling and tumor responses to hypoxia. Oncogene.
15. Ulsamer A, Wei Y, Kim KK, Tan K, Wheeler S, et al. Axin pathway activity regulates in vivo pY654-beta-catenin accumulation and pulmonary fibrosis. J Biol Chem 287: 5164–5172.
14. Kim Y, Kugler MC, Wei Y, Kim KK, Li X, et al. (2009) Integrin alpha3beta1-dependent beta-catenin phosphorylation links epithelial Smad signaling to cell contacts. J Cell Biol 184: 509–522.
13. Kim KK, Wei Y, Szekeres C, Kugler MC, Wolters PJ, et al. (2009) Epithelial cell alpha3beta1 integrin links beta-catenin and Smad signaling to promote myofibroblast formation and pulmonary fibrosis. J Clin Invest 119: 213–224.