Transforming Growth Factor-β Signaling across Ages
From Distorted Lung Development to Chronic Obstructive Pulmonary Disease

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The transforming growth factor (TGF)-β superfamily of secreted growth factors consists of more than 40 members, including the TGF-β isoforms themselves, bone morphogenetic proteins, and activins. Most of these factors have been shown to be essential for proper organ development, a process often recapitulated in chronic diseases. Importantly, TGF-β superfamily members are key regulators of extracellular matrix composition and alveolar epithelial cell and fibroblast function in the lung. Both during lung development and disease, TGF-βs therefore control lung homeostasis by providing the structural requirements and functional micromilieu needed for physiological epithelial cell function and proper gas exchange. Prolonged alterations of TGF-β signaling have been shown to result in structural changes in the lung that compromise gas exchange and lung function, as seen in arrested lung development, a feature of bronchopulmonary dysplasia, lung fibrosis, and chronic obstructive pulmonary disease. All these syndromes share a loss of functional alveolar structures, which ultimately leads to a decreased life expectancy. In this review, we cover our current understanding of the impact of TGF-β signaling on chronic lung disease. We focus on distorted TGF-β signaling in bronchopulmonary dysplasia and chronic obstructive pulmonary disease as prototype diseases of the premature and matured lung, respectively, which are both characterized by functional and structural loss of alveolar units.

Keywords: bronchopulmonary dysplasia; chronic obstructive pulmonary disease; emphysema; small airway disease; transforming growth factor-β

The lung is the key organ of respiration in air-breathing animals, and its principal function is to transport oxygen from the atmosphere into the bloodstream, and to release carbon dioxide from the bloodstream into the atmosphere. In mammals, this exchange of gases takes place in the alveoli, which are hollow spherical outcroppings of the respiratory bronchioles with an average diameter of 200 to 300 μm. These alveoli, which consist of an epithelial layer supported by extracellular matrix, are surrounded by capillaries. It is across this alveolocapillary barrier that gas exchange takes place, and it is clearly advantageous that this barrier should (*1*) be as narrow as possible (to facilitate optimal exchange of gas molecules across the barrier) and (*2*) cover as large a surface area as possible to maximize the area over which gas exchange might take place.

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Disturbances to the alveolar architecture have serious consequences for gas exchange by mammals. In humans, these disturbances are exemplified by diseases such as bronchopulmonary dysplasia (BPD), which limits late lung development and the formation of alveoli, and emphysema, a feature of chronic obstructive pulmonary disease (COPD) that is characterized by progressive destruction of alveoli. Both diseases are characterized by a reduction in total alveolar number and increased airspace size, which leads directly to a reduction in the ratio of gas exchange surface area to lung volume, and respiratory insufficiency.

OVERVIEW OF POSTNATAL LUNG DEVELOPMENT

The process of lung development strives to maximize the surface area available for gas exchange, while minimizing the thickness of the alveolocapillary barrier (the distance between the alveolar epithelium and the capillary endothelium) (1, 2). The branched lung structure is achieved by successive division of the developing airways during early lung development, and then by progressive formation of the developing airspaces—ultimately forming alveoli—during late lung development, by the process of alveolarization (2).

Early lung development starts during the embryonic stage (at 4–7 wk postconception in humans, and Embryonic Day [E] 9.5–E12 in mice) and continues through the pseudoglandular stage (at 5–17 wk in humans, and E12–E16.5 in mice) and canalicular stage (at 16–26 wk in humans, and E16.5–E17.5 in mice) (1). The alveolarization process begins at the end of the canalicular stage. Sections of mouse lungs in the pseudoglandular stage (E15) are illustrated in Figures 1A and 1B, whereas lungs in the canalicular stage (E17) are illustrated in Figures 1C and 1D. The thinning of the interstitial tissue, evident from the open airspaces (compare Figures 1B and 1D), marks the beginning of alveolarization. As late lung development proceeds, distal airways form saccular units in the saccular stage (at 24–38 wk in humans, and E17.5–Postnatal Day [P] 4 in mice), and secondary septae then divide these units (a process called septation) during the alveolar stage (at 36 wk preterm to 36 mo postnatal, or later, in humans, and P4–P28 in mice) (1). A progressive decrease in the size of the alveolar airspaces, together with a concomitant increase in the total number of alveoli, is clearly evident on comparing sections of mouse lungs in the saccular stage (P3; Figures 1E and 1F) and alveolar stage (P28; Figures 1G and 1H) (compare the number of alveoli in the region of fixed area encompassed by the dotted lines). Alveolarization was quantified as the mean linear intercept (MLI), a parameter that relates lung surface area to volume, and is used as a rough indicator of alveolar number, being inversely proportional to the number of alveoli. As illustrated in Figures 1F and 1H, the MLI decreased from 58 ± 8 μm in the saccular stage to 22 ± 5 μm in the alveolar stage, confirming an increase in the number of alveoli. From these images, a dramatic

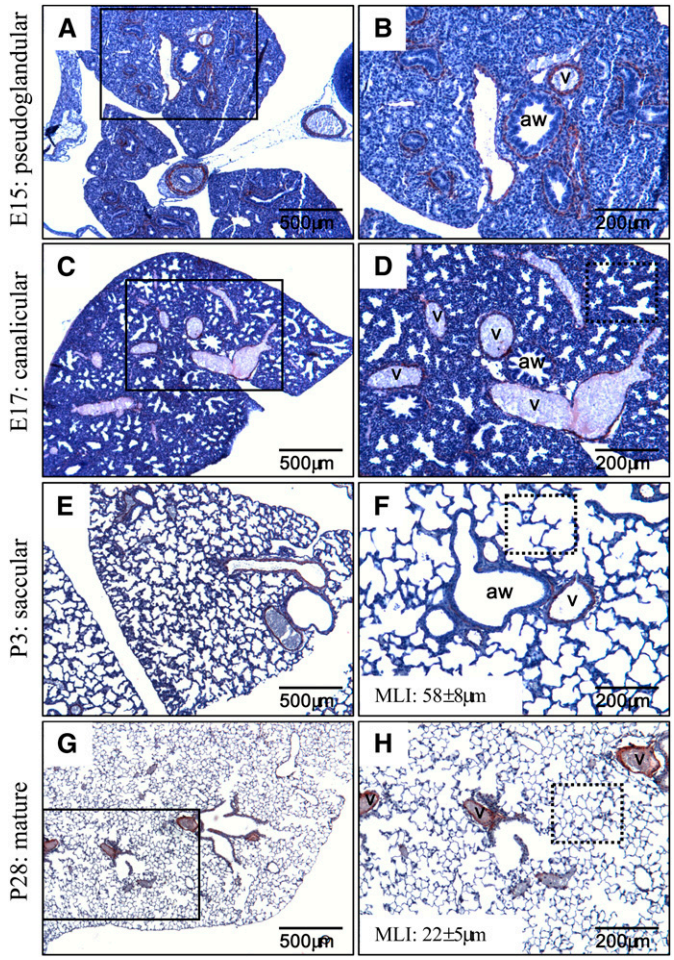

Figure 1. Development of the lung architecture. Paraffin sections (3 μm) of pressure-fixed (20 cm H_2O) lungs at various developmental stages were stained for smooth muscle actin and counterstained with hematoxylin, as described previously (26). Representative sections are illustrated for the (*A* and *B*) pseudoglandular, (*C* and *D*) canalicular, (*E* and *F*) saccular, and (*G* and *H*) late alveolar (mature) stages. Where high-magnification images (*right-hand column*) are derived from low-magnification images (*left-hand column*), the magnified area is demarcated by *solid lines*. *Dotted lines* encompass an area of interstitium. The mean linear intercept (MLI) is specified in (*F*) and (*H*). aw = airways; v = vessels.

increase in the number of alveoli (and, hence, surface area available for gas exchange), during late lung development is clearly evident.

This process of late lung development is carefully controlled by the coordinated action of many different transcription factors, mechanical forces (such as breathing movements), and growth factors (3–5). The diverse and important regulatory roles played by the transforming growth factor (TGF)-β superfamily of growth factors, which control the proliferation, transformation, and apoptosis of several cell types, as well as extracellular matrix (ECM) deposition and remodeling, have led to TGF-β and related superfamily members being accredited with key roles in late lung development (3, 5–7).

TGF-β SIGNALING

The TGF-β superfamily of growth factors encompasses more than 30 members, including the archetypical TGF-β family members, as well as the bone morphogenetic proteins, growth and differentiation factors, activins, and nodal (8, 9). In the case of TGF-β, signaling is initiated by binding of TGF-β ligands to the type II TGF-β receptor (Tgfbr2, also called TβRII), which then forms a complex with a type I TGF-β receptor, which can be either Tgfbr1 (also called activin-like kinase [ALK]-5) or Acvrl1 (also called ALK-1), depending on the cell type (10). The type I receptor then transmits signals within the cell via second-messenger Smad proteins, namely Smad2, Smad3, and Smad4, or by Smad-independent pathways (Figure 2). TGF-β signaling is potentiated by two accessory type III TGF-β receptors, Tgfbr3 (also called betaglycan) and endoglin (CD105, the *eng* gene product) (10). TGF-β signaling is also regulated by Smad6 and Smad7, inhibitory Smads that antagonize TGF-β signaling. Activated (phosphorylated) Smads are translocated into the nucleus, where they regulate gene transcription, and hence cell function (8, 9).

TGF-β SIGNALING IN POSTNATAL LUNG DEVELOPMENT

TGF-β signaling has been widely implicated in early lung development. All three TGF-β ligand isoforms, TGF-β_1, TGF-β_2, and TGF-β_3 (11), as well as the type I and type II TGF-β receptors (6, 12, 13), are expressed in the embryonic rodent lung. The addition of exogenous TGF-β ligands inhibited airway branching *in vitro* (14), and abrogation of TGF-β signaling, either by genetic down-regulation either of Tgfbr2 (15), or of Smad2, Smad3, or Smad4 (16), promoted lung branching *in vitro*. Consistent with these observations, overexpression of the inhibitory Smad, Smad7, which antagonizes TGF-β signaling, promoted lung branching *in vitro* (17).

Fewer studies, however, have examined the role of TGF-β signaling in late lung development. Overexpression of TGF-β_1 ligand in the lung, either by adenovirus-mediated transfer of the *tgfb1* gene to the neonatal rat lung (18) or by conditional overexpression of the *tgfb1* gene in the developing mouse lung between P7 and P14 (19), disrupted late lung development, in particular alveolarization. These studies thus provided strong evidence that TGF-β was a negative regulator of alveolarization. Of interest, and perhaps paradoxically, the blockade of TGF-β signaling by genetic ablation of Smad3 between P7 and P28 generated a similar phenotype in mice, which indicates that TGF-β can also act as a positive regulator of alveolarization (20). Data supporting this idea have also been generated by genetic recombination, in which Smad3 deficiency in mice resulted in progressive airspace enlargement with age, implicating TGF-β in the maintenance of alveolar integrity in the developing, as well as developed, lung (21). Taken together, these data indicate that TGF-β plays a key role in the alveolarization process, as well as in the maintenance of alveolar structure. These studies provided a clear rationale for the hypothesis that the structural changes induced by altered TGF-β signaling in the premature and mature lung may underlie the complex pathophysiology of BPD as well as COPD.

This idea is further supported by subsequent studies, which have demonstrated that the expression and localization of components of the TGF-β signaling machinery are dynamically regulated during late lung development in both mouse and human lungs (6). Similar changes in the temporal and spatial regulation of the related bone morphogenetic protein family (members of the TGF-β superfamily of growth factors) during late lung development have also been described (7).

Active TGF-β signaling has been reported in the interstitium of the developing lung (6). Alveolar epithelial type II (ATII) cells are proposed to serve as progenitor cells of the alveolar

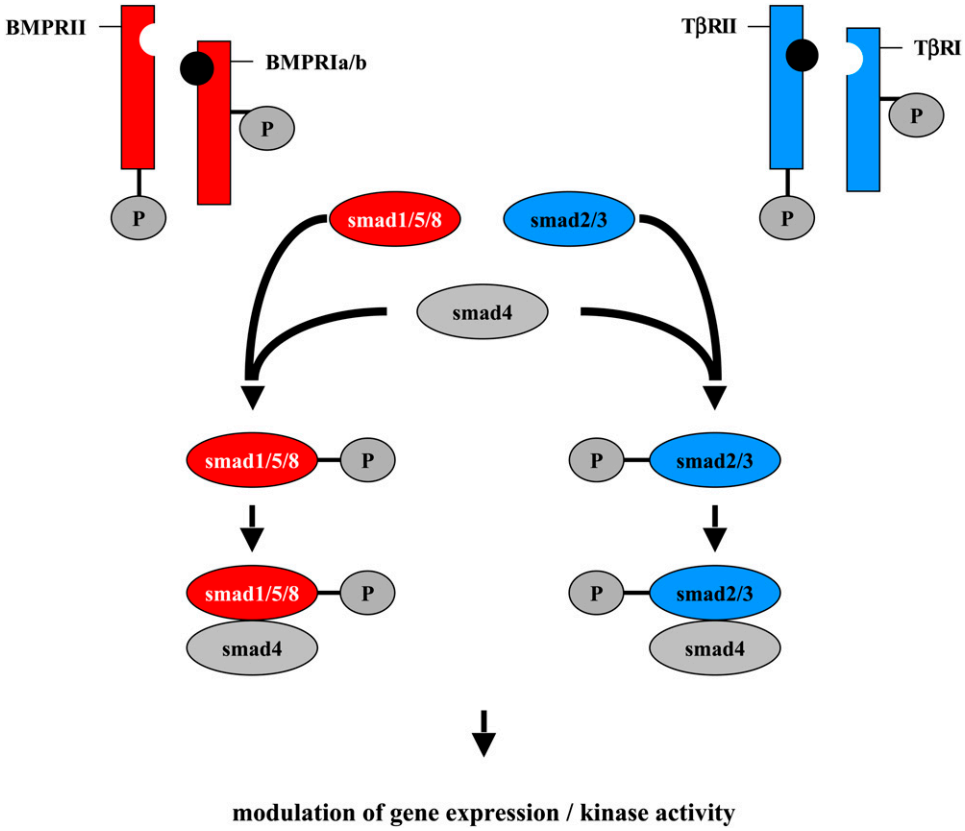

Figure 2. The transforming growth factor (TGF)-β signaling pathway. Ligands of the TGF-β superfamily bind to their respective receptors, initiating the recruitment and phosphorylation (P) of Smad proteins. TGF-β ligands bind to the type II TGF-β receptor (TβRII), which initiates the formation of a heteromeric complex with type I TGF-β receptors (TβRI). Receptor-regulated Smads 2 and 3 are phosphorylated and, in combination with co-Smad 4, translocate to the nucleus, where they regulate gene transcription. The bone morphogenetic protein (BMP) family of the TGF-β superfamily exhibits similar characteristics; BMPs bind to a heteromeric receptor complex of BMP receptors (BMPRII, BMPRIa/b), initiating intracellular signaling. BMPR activation leads to the phosphorylation and activation of Smads 1, 5, and 8.

epithelium, in particular alveolar epithelial type I (ATI) cells, and are an important component of the alveolar unit (22). TGF-β/Smad signaling has been shown to contribute to ATII to ATI transdifferentiation (23) and to epithelial–mesenchymal transition (EMT) (24), an important feature of the alveolarization process (1) and disease development. Thus, it has been proposed that TGF-β signaling in the interstitium during the alveolar phase of late lung development may promote ATII to ATI transdifferentiation. Several studies have indicated that TGF-β signaling plays important regulatory roles in the process of late lung development, in several cell types, including the developing epithelial layer of the immature alveoli, as well as in the maturation of the epithelial and smooth muscle layers of the developing airways.

TGF-β SIGNALING IN DEFECTS OF POSTNATAL LUNG DEVELOPMENT

Although TGF-β signaling is clearly required for normal lung development, this requirement is finely tuned, as described in the preceding section. Clearly, either too much or too little TGF-β signaling leads to impaired alveolar development. Dramatic reductions in TGF-β signaling are rarely encountered naturally, as functional impairment of key components of the TGF-β signaling machinery (such as knockout [KO] animals) are generally lethal. Conditional deletion of Smad3, a key component of the TGF-β signaling cascade, does impact late lung development in mice, causing alveolar enlargement reminiscent of that observed in patients with BPD or emphysema, leading to the proposal that TGF-β is a positive regulator of late lung development (20, 21). When Smad3 KO mice are allowed to age, they spontaneously develop increased airspace enlargement, along with increased matrix metalloproteinase-9 (MMP9) and MMP12 levels in the bronchoalveolar lavage fluid (21). Furthermore, a different Smad3 KO mouse line exhibited altered lung alveolarization, which resulted from reduced peripheral lung cell proliferation during lung development (20). Interestingly, however, artificial up-regulation of TGF-β signaling also severely disrupted late lung development, leading to alveolar enlargement reminiscent of that observed in patients with BPD (18, 19). So dramatic was the effect of TGF-β that conditional overexpression of TGF-β in the developing mouse lung has been proposed as a model for BPD (19).

Interestingly, neonatal rodents chronically exposed to normobaric hypoxia (25) or hyperoxia (26) exhibit arrested alveolarization and develop BPD-like features. Most importantly, both of these animal models of BPD exhibit increased TGF-β signaling (25, 26). In the case of chronic hypoxic exposures, levels of bioactive TGF-β are increased in bronchoalveolar lavage fluids of hypoxia-treated neonatal rodents, and increased expression of at least two TGF-β receptors is observed (25). Similarly, in the case of hyperoxia, a BPD-like alveolar structure developed concomitantly with up-regulated Tgfbr2 and Smad4 expression, and increased TGF-β/Smad2,3 signaling in the developing lung (26). Thus, increased TGF-β signaling is associated with retarded alveolar growth in both models of BPD, underscoring a potentially important role for dysregulated TGF-β activity in BPD.

This idea is strengthened by the observation that dampening of TGF-β signaling in the hyperoxia model of BPD, using neutralizing anti–TGF-β antibodies, largely restored normal alveolar architecture (27), validating a pathological role for TGF-β in hyperoxia-induced arrest of alveolar development. The observation that levels of TGF-β ligands are elevated in lavage fluids from human neonates with BPD undergoing oxygen therapy further indicates that elevated TGF-β levels are indeed associated with the development of BPD in humans (28).

The precise role played by TGF-β in the development of BPD has not been clarified; however, the pivotal effects of TGF-β in cell growth, proliferation, ECM production, and remodeling suggest a multitude of possibilities (Figure 3). For example, TGF-β exhibits potent antiproliferative properties on epithelial cells and some types of smooth muscle cells. Indeed, TGF-β can arrest proliferation of ATII cells (29, 30) and prevent keratinocyte growth factor–stimulated ATII cell proliferation (31). It has also been demonstrated that ATII cells exposed to hyperoxia are significantly more sensitive to the proapoptotic effects of TGF-β than are ATII cells exposed to normoxia (26). These observations suggest that improper TGF-β signaling in BPD might contribute to the alveolar hypoplasia associated with BPD by preventing, at least in part, alveolar epithelial cell proliferation and differentiation.

In addition to the effects of TGF-β on the cellular compartment of the lung, TGF-β also controls the composition of the ECM. The deposition and remodeling of the ECM constitute a key step in the alveolarization process (32), and TGF-β is an important regulator of the synthesis and secretion of collagens, elastin, and other components of the ECM. When primary fibroblasts are cultured in hyperoxia, they are more sensitive to TGF-β signaling, and produce excessive amounts of collagen Iα1, tropoelastin, and tenascin C mRNAs in response to TGF-β stimulation, in comparison with fibroblasts cultured in normoxia (26). TGF-β also regulates the secretion of MMPs and their cognate inhibitors, tissue inhibitors of MMP (TIMPs). Several of these molecules, MMP-1, MMP-2, MMP-9, and TIMP-2, are strongly expressed in the lungs of humans (33) and mice (34) throughout the canalicular, saccular, and alveolar phases of normal lung development. Fibroblasts exposed to hyperoxia also produce more TIMP and less MMP-1 and MMP-2 in response to TGF-β stimulation than do fibroblasts exposed to normoxia (26). Thus, dysregulated TGF-β activity may well impact proper ECM production and remodeling in BPD, and promote excessive ECM production and impair ECM remodeling, leading to the perturbed alveolar structures observed in the lungs of animal models and patients affected with BPD.

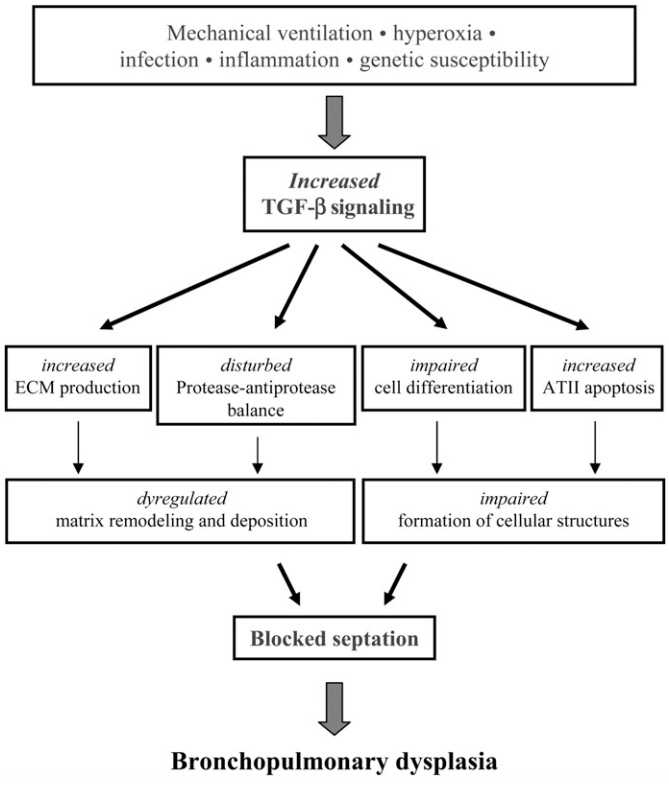

Figure 3. The role of transforming growth factor (TGF)-β signaling in the immature lung. A proposed model depicting the effects of increased TGF-β signaling during the development of bronchopulmonary dysplasia is shown. ATII = alveolar epithelial type II cells; ECM = extracellular matrix.

TGF-β IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Although BPD is a prototypical disease with a functional loss of alveolar structures in the newborn, these features are recapitulated in the lungs of patients with COPD, in particular emphysema. COPD is the fourth leading cause of death in the developed world, with continuously rising prevalence and mortality (35–37). COPD is characterized by irreversible expiratory airflow limitation due to two main intrapulmonary features: small airway disease and emphysema. Small airway disease includes airway inflammation with increased mucus production, airway wall remodeling, and peribronchiolar fibrosis, whereas emphysema is defined as destruction of the alveolar architecture due to distal airspace enlargement (38–41). Emerging interest in the role of TGF-β in the pathogenesis of COPD has evolved, particularly because genetic studies have identified TGF-β as a promising candidate gene related to COPD (42, 43). Studies of various COPD populations identified several single-nucleotide polymorphisms (SNPs) in patients with COPD. These polymorphisms have also been linked to functional measurements, such as airflow obstruction, dyspnea, as well as computerized tomography–based emphysema status. Some of these SNPs, however, have not been confirmed in various populations or cohorts. Given the complex etiology, as well as the heterogeneity and individual severity of COPD features, this is not entirely surprising, and underlines the need for further studies deciphering the relevance of TGF-β gene polymorphisms in COPD.

Evidence of impaired functional TGF-β_1 signaling has emerged from studies investigating tissue from patients with COPD. Several studies reported increased expression of TGF-β_1 in the airway epithelium of smokers, as well as in patients with chronic bronchitis or COPD (44–48). Furthermore, decreased expression of the inhibitory Smads 6 and 7 in bronchial biopsies of patients with COPD has been reported, further suggesting increased TGF-β signaling in COPD (49). Increased TGF-β_1 signaling has been associated with enhanced fibrotic airway remodeling, as well as with clinical features, such as lung function (50). The intratracheal administration of recombinant TGF-β_1 to mice resulted in increased collagen in the distal airways without noticeable inflammatory processes *in vivo*, further underlining that TGF-β_1 signaling leads to airway wall remodeling and peribronchiolar fibrosis (51). Notably, decreased TGF-β_1 and TGF-β receptor expression has also been reported for various cell types, such as macrophages and bronchial glands, highlighting once more the finely tuned TGF-β signaling system, with the various susceptibilities of distinct cell types (52, 53) (Figure 4).

The most important risk factor for COPD is cigarette smoke. Increased TGF-β_1 expression in airway epithelial cells from patients with COPD and smokers correlated with the burden of cigarette smoking (48). It is therefore reasonable to investigate TGF-β signaling in response to cigarette smoke exposures. Several studies have focused on the direct effect of cigarette smoke exposure on TGF-β signaling. Surprisingly, whole genome expression studies in human bronchial epithelial cells exposed

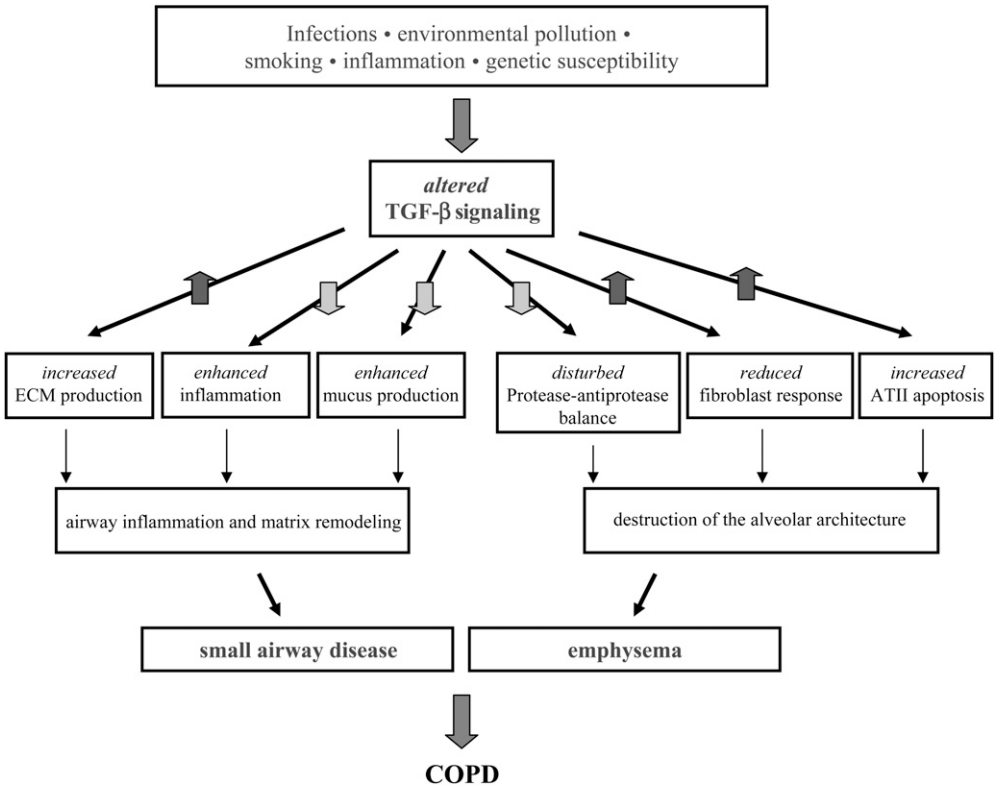

Figure 4. The role of transforming growth factor (TGF)-β signaling in the mature lung. A proposed model depicting the effects of altered TGF-β signaling during the development of chronic obstructive pulmonary disease is shown. ATII = alveolar epithelial type II cells; COPD = chronic obstructive pulmonary disease; ECM = extracellular matrix.

to cigarette smoke *in vitro* have revealed a down-regulation of the TGF-β pathway (54–56), whereas tracheal explants that were exposed to cigarette smoke *in vitro* exhibited enhanced active TGF-β signaling (57). Importantly, mice exposed to cigarette smoke *in vivo* exhibited enhanced profibrotic TGF-β signaling in the small airways (58). In addition, it has been shown that cigarette smoke activates TGF-β_1 in lung fibroblast cultures (59), altogether suggesting that TGF-β effects in airway remodeling and fibrosis may be provoked by cigarette smoke.

The role of TGF-β signaling in emphysema is less defined and investigated. Emphysema is characterized by the rarefaction of alveolar walls with loss of parenchymal tissue (60). MMP and TIMP, which regulate ECM homeostasis, have been implicated in cigarette smoke–induced pulmonary emphysema, much like their suggested involvement in BPD and animal models thereof (61). Several studies identified TGF-β is an important regulator of MMP expression. Most of these studies indeed suggested that too little or inhibited TGF-β signaling may lead to increased MMP expression and subsequent ECM degradation, which may contribute to emphysema development (62, 63). In detail, it has been shown that TGF-β inhibits MMP9 and MMP12 expression in alveolar macrophages and monocytes (64, 65), and that mice lacking the β_6 subunit of the $\alpha_v\beta_6$ integrin exhibited decreased TGF-β signaling along with increased expression of MMP12 in alveolar macrophages (63). These mice spontaneously developed progressive alveolar enlargement over time, which was similar to the course of human emphysema. Evidence of decreased TGF-β signaling in emphysema was further provided by *in vitro* studies investigating rat tracheal explants exposed to cigarette smoke. Here, ongoing TGF-β expression has been observed in small airways, but not in the surrounded parenchyma (66).

Importantly, too much TGF-β signaling may also contribute to emphysema development. It has been shown that cigarette smoke–induced TGF-β signaling further potentiated the growth-inhibitory effects in alveolar epithelial cells, thereby providing another potential pathomechanism responsible for parenchymal tissue destruction in COPD (67, 68). Furthermore, it has been demonstrated that interstitial fibroblasts from patients with COPD with emphysema exhibited reduced baseline expression of active intracellular TGF-β mediators, such as phosphorylated Smad3, whereas inhibitory Smads were increased (69). Most interestingly, the response of COPD fibroblasts to TGF-β_1, although releasing increasing amounts of TGF- β_1, was reduced (69).

CONCLUSIONS

BPD is a significant complication of premature birth, affecting up to 10,000 newborns annually in the United States alone, and has long-term respiratory consequences that reach beyond childhood (70, 71). The severity and socioeconomic impact of BPD is superseded by COPD, a disease with similar characteristics in terms of functional alveolar loss. The data reviewed herein provide compelling evidence of the involvement of the TGF-β signaling pathway in the pathogenesis of BPD and COPD, and highlight similar mechanisms between these diseases.

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References

1. Copland I, Post M. Lung development and fetal lung growth. *Paediatr Respir Rev* 2004;5(Suppl A):S259–S264.

2. Roth-Kleiner M, Post M. Genetic control of lung development. *Biol Neonate* 2003;84:83–88.
3. Cardoso WV. Molecular regulation of lung development. *Annu Rev Physiol* 2001;63:471–494.
4. Jankov RP, Keith Tanswell A. Growth factors, postnatal lung growth and bronchopulmonary dysplasia. *Paediatr Respir Rev* 2004;5(Suppl A):S265– S275.
5. Warburton D, Schwarz M, Tefft D, Flores-Delgado G, Anderson KD, Cardoso WV. The molecular basis of lung morphogenesis. *Mech Dev* 2000;92:55–81.
6. Alejandre-Alcazar MA, Michiels-Corsten M, Vicencio AG, Reiss I, Ryu J, de Krijger RR, Haddad GG, Tibboel D, Seeger W, Eickelberg O, *et al.* TGF-β signaling is dynamically regulated during the alveolarization of rodent and human lungs. *Dev Dyn* 2008;237:259–269.
7. Alejandre-Alcazar MA, Shalamanov PD, Amarie OV, Sevilla-Perez J, Seeger W, Eickelberg O, Morty RE. Temporal and spatial regulation of bone morphogenetic protein signaling in late lung development. *Dev Dyn* 2007;236:2825–2835.
8. Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev* 2005;19:2783–2810.
9. Feng XH, Derynck R. Specificity and versatility in TGF-β signaling through Smads. *Annu Rev Cell Dev Biol* 2005;21:659–693.
10. Eickelberg O, Morty RE. Transforming growth factor β/bone morphogenic protein signaling in pulmonary arterial hypertension: remodeling revisited. *Trends Cardiovasc Med* 2007;17:263–269.
11. Pelton RW, Johnson MD, Perkett EA, Gold LI, Moses HL. Expression of transforming growth factor-β_1, -β_2, and -β_3 mRNA and protein in the murine lung. *Am J Respir Cell Mol Biol* 1991;5:522–530.
12. Zhao Y, Young SL. Expression of transforming growth factor-β type II receptor in rat lung is regulated during development. *Am J Physiol* 1995;269:L419–L426.
13. Zhao Y, Young SL, McIntosh JC, Steele MP, Silbajoris R. Ontogeny and localization of TGF-β type I receptor expression during lung development. *Am J Physiol* 2000;278:L1231–L1239.
14. Liu J, Tseu I, Wang J, Tanswell K, Post M. Transforming growth factor β_2, but not β_1 and β_3, is critical for early rat lung branching. *Dev Dyn* 2000;217:343–360.
15. Zhao J, Bu D, Lee M, Slavkin HC, Hall FL, Warburton D. Abrogation of transforming growth factor-β type II receptor stimulates embryonic mouse lung branching morphogenesis in culture. *Dev Biol* 1996;180:242–257.
16. Zhao J, Lee M, Smith S, Warburton D. Abrogation of Smad3 and Smad2 or of Smad4 gene expression positively regulates murine embryonic lung branching morphogenesis in culture. *Dev Biol* 1998;194:182–195.
17. Zhao J, Shi W, Chen H, Warburton D. Smad7 and Smad6 differentially modulate transforming growth factor β–induced inhibition of embryonic lung morphogenesis. *J Biol Chem* 2000;275:23992–23997.
18. Gauldie J, Galt T, Bonniaud P, Robbins C, Kelly M, Warburton D. Transfer of the active form of transforming growth factor-β_1 gene to newborn rat lung induces changes consistent with bronchopulmonary dysplasia. *Am J Pathol* 2003;163:2575–2584.
19. Vicencio AG, Lee CG, Cho SJ, Eickelberg O, Chuu Y, Haddad GG, Elias JA. Conditional overexpression of bioactive transforming growth factor-β_1 in neonatal mouse lung: a new model for bronchopulmonary dysplasia? *Am J Respir Cell Mol Biol* 2004;31:650–656.
20. Chen H, Sun J, Buckley S, Chen C, Warburton D, Wang XF, Shi W. Abnormal mouse lung alveolarization caused by Smad3 deficiency is a developmental antecedent of centrilobular emphysema. *Am J Physiol* 2005;288:L683–L691.
21. Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, Lavery C, Margetts PJ, Roberts AB, Gauldie J. Smad3 null mice develop airspace enlargement and are resistant to TGF-β–mediated pulmonary fibrosis. *J Immunol* 2004;173:2099–2108.
22. Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. *Respir Res* 2001;2:33–46.
23. Bhaskaran M, Kolliputi N, Wang Y, Gou D, Chintagari NR, Liu L. Trans-differentiation of alveolar epithelial type II cells to type I cells involves autocrine signaling by transforming growth factor β_1 through the Smad pathway. *J Biol Chem* 2007;282:3968–3976.
24. Willis BC, Borok Z. TGF-β–induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol* 2007;293:L525–L534.
25. Vicencio AG, Eickelberg O, Stankewich MC, Kashgarian M, Haddad GG. Regulation of TGF-β ligand and receptor expression in neonatal rat lungs exposed to chronic hypoxia. *J Appl Physiol* 2002;93:1123–1130.
26. Alejandre-Alcazar MA, Kwapiszewska G, Reiss I, Amarie OV, Marsh LM, Sevilla-Perez J, Wygrecka M, Eul B, Kobrich S, Hesse M, *et al.* Hyperoxia modulates TGF-β/BMP signaling in a mouse model of bronchopulmonary dysplasia. *Am J Physiol* 2007;292:L537–L549.
27. Nakanishi H, Sugiura T, Streisand JB, Lonning SM, Roberts JD Jr. TGF-β–neutralizing antibodies improve pulmonary alveologenesis and vasculogenesis in the injured newborn lung. *Am J Physiol* 2007;293:L151–L161.
28. Lecart C, Cayabyab R, Buckley S, Morrison J, Kwong KY, Warburton D, Ramanathan R, Jones CA, Minoo P. Bioactive transforming growth factor-β in the lungs of extremely low birthweight neonates predicts the need for home oxygen supplementation. *Biol Neonate* 2000;77:217–223.
29. Konigshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, Jahn A, Rose F, Fink L, Seeger W, Schaefer L, *et al.* Wnt1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. *J Clin Invest* 2009;119:772–787.
30. Konigshoff M, Wilhelm A, Jahn A, Sedding D, Amarie OV, Eul B, Seeger W, Fink L, Gunther A, Eickelberg O, *et al.* The angiotensin II receptor 2 is expressed and mediates angiotensin II signaling in lung fibrosis. *Am J Respir Cell Mol Biol* 2007;37:640–650.
31. Zhang F, Nielsen LD, Lucas JJ, Mason RJ. Transforming growth factor-β antagonizes alveolar type II cell proliferation induced by keratinocyte growth factor. *Am J Respir Cell Mol Biol* 2004;31:679–686.
32. Roth-Kleiner M, Post M. Similarities and dissimilarities of branching and septation during lung development. *Pediatr Pulmonol* 2005;40:113–134.
33. Masumoto K, de Rooij JD, Suita S, Rottier R, Tibboel D, de Krijger RR. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases during normal human pulmonary development. *Histopathology* 2005;47:410–419.
34. Ryu J, Vicencio AG, Yeager ME, Kashgarian M, Haddad GG, Eickelberg O. Differential expression of matrix metalloproteinases and their inhibitors in human and mouse lung development. *Thromb Haemost* 2005;94:175–183.
35. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970–2002. *JAMA* 2005;294:1255–1259.
36. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;176:532–555.
37. Rennard SI. Chronic obstructive pulmonary disease: linking outcomes and pathobiology of disease modification. *Proc Am Thorac Soc* 2006;3:276–280.
38. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;364:709–721.
39. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, *et al.* The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:2645–2653.
40. Barnes PJ. Future treatments for chronic obstructive pulmonary disease and its comorbidities. *Proc Am Thorac Soc* 2008;5:857–864.
41. Macnee W. Pathogenesis of chronic obstructive pulmonary disease. *Clin Chest Med* 2007;28:479–513, v.
42. Hersh CP, DeMeo DL, Silverman EK. National emphysema treatment trial state of the art: genetics of emphysema. *Proc Am Thorac Soc* 2008;5:486–493.
43. Silverman EK. Progress in chronic obstructive pulmonary disease genetics. *Proc Am Thorac Soc* 2006;3:405–408.
44. Aubert JD, Dalal BI, Bai TR, Roberts CR, Hayashi S, Hogg JC. Transforming growth factor β_1 gene expression in human airways. *Thorax* 1994;49:225–232.
45. Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, la Rocca AM, Bellia V, Bonsignore G, Bousquet J. Transforming growth factor-β expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:591–599.
46. Vignola AM, Chanez P, Chiappara G, Merendino A, Zinnanti E, Bousquet J, Bellia V, Bonsignore G. Release of transforming growth factor-beta (TGF-β) and fibronectin by alveolar macrophages in airway diseases. *Clin Exp Immunol* 1996;106:114–119.
47. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 2001;34:50s–59s.
48. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-β_1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2001;163:1476–1483.

49. Springer J, Scholz FR, Peiser C, Groneberg DA, Fischer A. Smad-signaling in chronic obstructive pulmonary disease: transcriptional down-regulation of inhibitory Smad 6 and 7 by cigarette smoke. *Biol Chem* 2004;385:649–653.
50. de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. Transforming growth factor β_1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:1951–1957.
51. Kenyon NJ, Gohil K, Last JA. Susceptibility to ovalbumin-induced airway inflammation and fibrosis in inducible nitric oxide synthetase–deficient mice: mechanisms and consequences. *Toxicol Appl Pharmacol* 2003;191:2–11.
52. Pons AR, Sauleda J, Noguera A, Pons J, Barcelo B, Fuster A, Agusti AG. Decreased macrophage release of TGF-β and TIMP-1 in chronic obstructive pulmonary disease. *Eur Respir J* 2005;26:60–66.
53. Baraldo S, Bazzan E, Turato G, Calabrese F, Beghe B, Papi A, Maestrelli P, Fabbri LM, Zuin R, Saetta M. Decreased expression of TGF-β type II receptor in bronchial glands of smokers with COPD. *Thorax* 2005;60:998–1002.
54. Yoneda K, Peck K, Chang MM, Chmiel K, Sher YP, Chen J, Yang PC, Chen Y, Wu R. Development of high-density DNA microarray membrane for profiling smoke- and hydrogen peroxide–induced genes in a human bronchial epithelial cell line. *Am J Respir Crit Care Med* 2001;164:S85–S89.
55. Spira A, Beane J, Pinto-Plata V, Kadar A, Liu G, Shah V, Celli B, Brody JS. Gene expression profiling of human lung tissue from smokers with severe emphysema. *Am J Respir Cell Mol Biol* 2004;31:601–610.
56. Maunders H, Patwardhan S, Phillips J, Clack A, Richter A. Human bronchial epithelial cell transcriptome: gene expression changes following acute exposure to whole cigarette smoke *in vitro*. *Am J Physiol* 2007;292:L1248–L1256.
57. Wang RD, Wright JL, Churg A. Transforming growth factor-β_1 drives airway remodeling in cigarette smoke–exposed tracheal explants. *Am J Respir Cell Mol Biol* 2005;33:387–393.
58. Churg A, Tai H, Coulthard T, Wang R, Wright JL. Cigarette smoke drives small airway remodeling by induction of growth factors in the airway wall. *Am J Respir Crit Care Med* 2006;174:1327–1334.
59. Wang H, Liu X, Umino T, Kohyama T, Zhu YK, Wen FQ, Spurzem JR, Romberger DJ, Kim HJ, Rennard SI. Effect of cigarette smoke on fibroblast-mediated gel contraction is dependent on cell density. *Am J Physiol* 2003;284:L205–L213.
60. Thorley AJ, Tetley TD. Pulmonary epithelium, cigarette smoke, and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2007;2:409–428.
61. Elias JA, Kang MJ, Crothers K, Homer R, Lee CG. State of the art: Mechanistic heterogeneity in chronic obstructive pulmonary disease: insights from transgenic mice. *Proc Am Thorac Soc* 2006;3:494–498.
62. Roberts AB. Medicine: smoke signals for lung disease. *Nature* 2003;422:130–131.
63. Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, Sheppard D. Loss of integrin $\alpha_V\beta_6$-mediated TGF-β activation causes MMP12-dependent emphysema. *Nature* 2003;422:169–173.
64. Feinberg MW, Jain MK, Werner F, Sibinga NE, Wiesel P, Wang H, Topper JN, Perrella MA, Lee ME. Transforming growth factor-β_1 inhibits cytokine-mediated induction of human metalloelastase in macrophages. *J Biol Chem* 2000;275:25766–25773.
65. Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME. Transforming growth factor-β_1 inhibition of macrophage activation is mediated via Smad3. *J Biol Chem* 2000;275:36653–36658.
66. Churg A, Zhou S, Preobrazhenska O, Tai H, Wang R, Wright JL. Expression of profibrotic mediators in small airways versus parenchyma after cigarette smoke exposure. *Am J Respir Cell Mol Biol* 2009;40:268–276.
67. Marwick JA, Kirkham P, Gilmour PS, Donaldson K, Mac NW, Rahman I. Cigarette smoke–induced oxidative stress and TGF-β_1 increase $p21^{waf1/cip1}$ expression in alveolar epithelial cells. *Ann N Y Acad Sci* 2002;973:278–283.
68. Rennard SI, Togo S, Holz O. Cigarette smoke inhibits alveolar repair: a mechanism for the development of emphysema. *Proc Am Thorac Soc* 2006;3:703–708.
69. Togo S, Holz O, Liu X, Sugiura H, Kamio K, Wang X, Kawasaki S, Ahn Y, Fredriksson K, Skold CM, *et al.* Lung fibroblast repair functions in patients with chronic obstructive pulmonary disease are altered by multiple mechanisms. *Am J Respir Crit Care Med* 2008;178:248–260.
70. Thebaud B, Abman SH. Bronchopulmonary dysplasia: where have all the vessels gone? Roles of angiogenic growth factors in chronic lung disease. *Am J Respir Crit Care Med* 2007;175:978–985.
71. Thebaud B, Ladha F, Michelakis ED, Sawicka M, Thurston G, Eaton F, Hashimoto K, Harry G, Haromy A, Korbutt G, *et al.* Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization. *Circulation* 2005;112:2477–2486.