

TGF- β signalling in COPD: deciphering genetic and cellular susceptibilities for future therapeutic regimens

Melanie Königshoff^a, Nikolaus Kneidinger^b, Oliver Eickelberg^a

^a Comprehensive Pneumology Center, Institute of Lung Biology and Disease (iLBD), Ludwig Maximilians University, Asklepios Hospital, and Helmholtz Zentrum München, Neuherberg / Munich, Germany

^b Department of Medicine, University of Giessen Lung Center, University of Giessen, Giessen, Germany

Summary

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of death in the developed world and associated with a high individual and socioeconomic burden. Despite emerging preventive efforts and ongoing clinical trials, the frequency and mortality of COPD are expected to continue to rise over the next decades.

COPD is defined as an irreversible expiratory airflow limitation, which is caused by various degrees of the following two main features: First, small airway disease (SAD), which includes airway inflammation and remodelling, and second, emphysema, which is characterised by airspace enlargement. The major risk factor for COPD is cigarette smoke exposure; however, the molecular mechanisms linking smoke to different COPD features on the cellular level remain elusive.

The transforming growth factor (TGF)- β superfamily constitutes more than 40 members, which are essential during organ development, a process often recapitulated in chronic diseases. Emerging interest in the role of TGF- β in the pathogenesis of COPD has recently evolved, par-

ticularly since genetic studies have demonstrated an association of gene polymorphisms of the TGF- β superfamily with COPD. In addition, increased expression of TGF- β 1 in COPD lungs and primary cells, such as epithelial cells, macrophages, or fibroblasts isolated from COPD specimens, was reported, suggesting an impact of TGF- β signalling on the development and progression of COPD. Thus, targeted interventions of TGF- β signalling may represent a suitable therapeutic option in COPD.

In this review, we will summarise the current understanding of the impact of TGF- β in COPD pathogenesis. The review is separated into five chapters: 1) an introduction to COPD, 2) an introduction to TGF- β signalling, 3) a summary of TGF- β gene polymorphisms in COPD, 4) a summary of TGF- β signalling in small airway disease, and 5) a summary of TGF- β signalling in emphysema.

Key words: COPD, emphysema; chronic bronchitis; small airway disease; transforming growth factor (TGF)- β

Chronic obstructive pulmonary disease (COPD)

COPD is the fourth leading cause of death in the developed world, with continuously rising prevalence and mortality [1]. COPD is expected to surpass stroke as the third leading cause of death in the next years and is associated with an increasing socioeconomic burden [1, 2]. COPD is characterised by irreversible expiratory airflow limitation due to a range of pathological changes in the lung, as well as extrapulmonary effects [3, 4]. The most important risk factor for COPD is cigarette smoking. Other risk factors include occupational exposures, such as coal dust or air pol-

Abbreviations

| | |
|--------------|---|
| BAL | Bronchoalveolar lavage |
| BODE | Body mass index, airflow obstruction, dyspnoea, exercise capacity index |
| COPD | Chronic obstructive pulmonary disease |
| GOLD | Global Initiative for Chronic Obstructive Lung Disease |
| LTBP | Latent transforming growth factor-beta binding protein |
| NETT | National Emphysema Treatment Trial |
| SAD | Small airway disease |
| SNP | Single nucleotide polymorphism |
| TGF- β | Transforming growth factor-beta |

There is no actual or potential conflict of interest in relation to this article.

lution, as well as airway hyperresponsiveness, asthma, or genetic predisposition [5–9].

Different pulmonary features contribute to the progressive airflow limitation in COPD, which are small airways disease (SAD) and emphysema. SAD, also termed obstructive bronchiolitis, includes airway inflammation with increased mucous production, airway wall remodeling, and peribronchiolar fibrosis [10–12]. Emphysema is defined as destruction of the alveolar architecture due to distal airspace enlargement [4, 13]. The degree of airflow limitation can be easily assessed by spirometry [14–16]. The severity of COPD is spirometrically classified according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria using the ratio of FEV₁ / FVC (table 1) [4]. A second grading system includes the body mass index (B), airflow obstruction (O), dyspnoea (D), and exercise tolerance (E) (BODE) [17, 18].

Importantly, COPD has recently been recognised as a systemic disease with the following manifestations: cardiovascular disease, diabetes,

cancer, skeletal muscle dysfunction, or weight loss [7, 19–22]. These are proposed to result from the systemic effects of smoking and ongoing systemic inflammation [23, 24]. Systemic inflammation in COPD has been clearly demonstrated in past years, assessed by increased oxidative stress [25, 26], activated inflammatory cells [27–30] or increased cytokine levels in the systemic circulation [23, 31–33]. In light of these findings, it was recently proposed to include the term “chronic systemic inflammatory syndrome” to the diagnosis of COPD [34].

Optimal therapeutic management of patients suffering from COPD requires a multidisciplinary approach. Smoking cessation, pharmacological therapy with bronchodilators and glucocorticoids, long term oxygen therapy, pulmonary rehabilitation, and surgery are the corner stones of COPD management, making COPD a preventable and treatable disease [4, 35, 36]. Nevertheless, most therapeutic approaches exhibit only a limited impact on the progression and mortality of this devastating disease [37].

Table 1
GOLD classification.

| Stage | Lung function (post-bronchodilator FEV ₁) | |
|-----------------------|---|--|
| Stage I: mild | FEV ₁ /FVC <0.70 | FEV ₁ ≥80% predicted |
| Stage II: moderate | FEV ₁ /FVC <0.70 | 50% ≤FEV ₁ <80% predicted |
| Stage III: severe | FEV ₁ /FVC <0.70 | 30% ≤FEV ₁ <50% predicted |
| Stage IV: very severe | FEV ₁ /FVC <0.70 | FEV ₁ <30% predicted or FEV ₁ <50% predicted plus chronic respiratory failure* |

* Respiratory failure: arterial partial pressure of oxygen (PaO₂) <8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO₂ (PaCO₂) >6.7 kPa (50 mm Hg) while breathing air at sea level

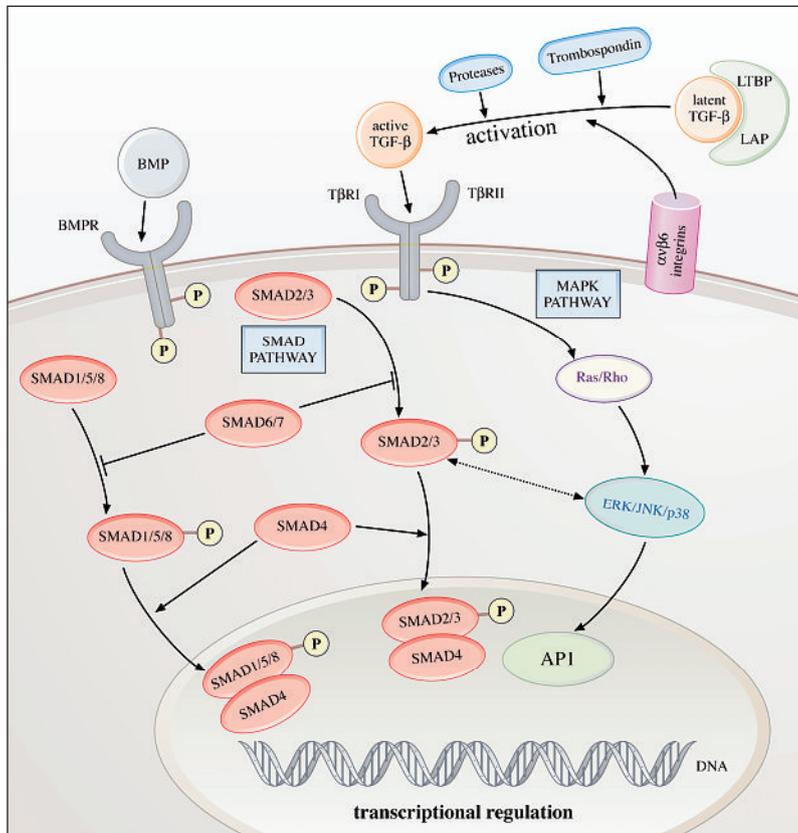
Transforming growth factor-β signalling

The transforming growth factor (TGF)-β superfamily is critically involved in embryonic development, organogenesis, and tissue homeostasis [38, 39]. TGF-β superfamily members act as multifunctional regulators of cell growth and differentiation. The TGF-β superfamily consists of more than 40 members, these include activins, inhibins, bone morphogenetic proteins (BMP), growth and differentiation factors, and TGF-βs themselves. Three different TGF-β isoforms have been characterised thus far: TGF-β1, TGF-β2, and TGF-β3. All three isoforms are expressed in the lung and exert distinct roles in lung development [40, 41]. In this respect, it is most likely that all isoforms are also involved in chronic lung disease however, due to limited availability of appropriate tools, most studies have thus far focused on TGF-β1. TGF-β1 is ubiquitously expressed and secreted by several cell types, such as endothelial, epithelial, and smooth muscle cells, as well as fibroblasts and most cells of the immune system. TGF-β isoforms are synthesised intracellularly in non-covalent association with the latency associated peptide (LAP). This complex is secreted

upon covalent linking to the latent TGF-β binding proteins (LTBP), thus providing a TGF-β reservoir in the extracellular matrix [42]. For active signalling, TGF-β needs to dissociate from the complex, a process which is highly regulated and controlled by different mechanisms [43, 44] (fig. 1). Several proteases, such as plasmin, thrombin, or matrix metalloproteinases, have been described to lead to a release of active TGF-β by degrading LAP. Furthermore, the extracellular matrix protein thrombospondin 1 has been reported to activate TGF-β by inducing conformational changes of LAP. In addition to soluble proteins, integrins have been identified to play an important role in TGF-β activation [43, 44]. Integrins are transmembrane proteins and bind to a specific binding motif of the LAP (RGD sequence, integrin binding motif). In detail, it has been demonstrated that the αvβ6 integrin is able to bind to the complex, directly presenting active TGF-β to TGF-β receptors [44]. Active TGF-β ligands bind to the type II TGF-β receptor, which initiates the formation of a heteromeric complex with type I TGF-β receptors [activin-like kinase 1 or 5], sub-

Figure 1

The TGF- β signalling pathway. TGF- β isoforms are secreted in a complex with the latency associated peptide (LAP) and the latent TGF- β binding proteins (LTBP). TGF- β activation is controlled by proteases e.g. thrombospondin 1 or integrins. Active TGF- β ligands bind to TGF- β receptors, initiating the recruitment and phosphorylation of SMAD2/3. The bone morphogenic protein (BMP) family of the TGF- β superfamily bind to BMP receptors (BMPR), thereby activating SMAD 1/5/8. SMAD-independent pathways, such as mitogen-activated protein kinase (MAPK) pathway may interact with classical TGF- β signalling. An example is shown, whereby the MAPK pathway activates AP-1, which modulates (SMAD-induced) transcriptional activity (see text for further details).



sequently leading to transphosphorylation of the type I receptor by the constitutively active kinase

of the type II receptor. Phosphorylation of the type I receptor results in recruitment of specific intracellular signals mediators, called Smad proteins (fig. 1) [39, 45]. The receptor-regulated Smad2 or Smad3, in combination with the co-Smad Smad4, positively regulate TGF- β -induced effects, while the inhibitory Smads (Smad6 and Smad7) negatively regulate TGF- β signalling. Smad2 and Smad3 have been shown to be phosphorylated by the type I receptor, followed by complex formation with Smad4 and, finally, nuclear translocation and regulation of gene transcription [46].

The bone morphogenic protein (BMP) family of the TGF- β superfamily exhibits similar characteristics. BMP proteins bind to a heteromeric receptor complex of BMP receptors (BMPR), thereby initiating intracellular signalling. BMPR activation leads to activation of Smads 1, 5, and possibly 8 [47]. Furthermore, it has to be pointed out that TGF- β may also signal via Smad-independent pathways. A crosstalk between the mitogen-activated protein kinase (MAPK) pathways and the TGF- β pathway may potentiate, synergise, or even antagonise the canonical TGF- β -Smad pathway [48] (fig. 1).

The purpose of this review is to summarise the current knowledge of the impact of TGF- β signalling on the development and progression of COPD. We focused on the TGF- β signalling pathway for the following reasons: first, recent epidemiological studies have identified TGF- β genes as a promising candidate genes in COPD, and second, increased expression of TGF- β superfamily members, as well as active TGF- β signalling has been demonstrated in animal models of COPD as well as in samples of COPD patients, as outlined in detail in the following chapters.

Transforming growth factor- β gene polymorphisms in COPD

The genetic susceptibility to COPD was first proposed almost 100 years ago [49]. Patients with emphysema were reported to more likely have parents with emphysema compared with unaffected individuals. In addition, only a minor proportion of heavy smokers develop severe airflow obstruction, which further underlines the hereditary contribution on the development of COPD [3, 50]. Smokers who develop COPD seem to be genetically more susceptible to the deleterious effects of cigarette smoke than smokers who do not develop COPD. Further evidence for a hereditary contribution is α -1 antitrypsin deficiency, which is a well-characterised genetic disease inherited in an autosomal recessive pattern [51]. The α -1 antitrypsin acts as a serine protease inhibitor and represents the major inhibitor of neutrophil elastase in the lung. Deficiency of this protein leads to

the development of emphysema at an early age [51, 52].

In the last decade, two major strategies have been used to identify gene mutations or polymorphisms contributing to the development of COPD. The first approach, genome-wide linkage analysis, involved unbiased scanning of the entire human genome for disease-causing genes. In a second approach, specific individual genes ("candidate genes") were directly investigated for their involvement in COPD. The genes selected for the second approach were chosen because of recently identified functional contributions that may play a role in COPD pathogenesis. Multiple COPD candidate gene associations were recently reported and, among others, TGF- β 1 has been identified as a promising candidate gene related to COPD [6, 53] (table 2).

Table 2

TGF- β 1 gene single nucleotide polymorphisms in COPD.

| SNP ID | Locus | SNP | significant association | | no association | |
|-----------|----------|-----|-------------------------|-------------------------------------|-------------------|---------------------------|
| rs1800469 | promotor | C/T | Celedon et al. | COPD (case-control study) | Van Diemen et al. | COPD (case-control study) |
| | | | Su et al. | COPD (case-control study) | Yoon et al. | COPD (case-control study) |
| | | | Ito et al. | Lung function in emphysema patients | | |
| | | | Hersh et al. | Dyspnoe, BODE index | | |
| rs2241712 | promotor | A/G | Celedon et al. | COPD (family based study) | Yoon et al. | COPD (case-control study) |
| | | | Hersh et al. | Dyspnoe, BODE index | | |
| rs1982073 | exon1 | T/C | Wu et al. | COPD (case-control study) | Van Diemen et al. | COPD (case-control study) |
| | | | Celedon et al. | COPD (case-control study) | Yoon et al. | COPD (case-control study) |
| | | | Ito et al. | Lung function in emphysema patients | | |
| | | | Hersh et al. | Dyspnoe, BODE index | | |
| rs2241718 | 3'UTR | C/T | Celedon et al. | COPD (family based study) | Ito et al. | Emphysema vs smokers |
| rs6957 | 3'UTR | A/G | Celedon et al. | COPD (family based study) | Ito et al. | Emphysema vs smokers |
| | | | Van Diemen et al. | COPD (case-control study) | | |
| rs1982072 | promotor | A/T | | | Ito et al. | Emphysema vs smokers |
| rs2241716 | intron2 | G/A | | | | |
| rs4803455 | intron2 | A/C | | | | |

The first association of a TGF- β 1 genotype with increased susceptibility to COPD was reported in 2004 [54]. The single nucleotide polymorphism (SNP) T+869C (Leu10Pro) was found to occur more frequently in control subjects than in individuals with COPD. The SNP T+869C (Leu10Pro) indicates that a single transition of the nucleotide T to C at position 869 results in an amino acid exchange from leucine to proline in codon 10 of the first exon of the TGF- β 1 gene (reference SNP ID (rs)1982073). The proline allele at codon 10 has been shown to be associated with higher serum TGF- β 1 levels, and increased TGF- β 1 mRNA in peripheral blood mononuclear cells, which altogether suggested that TGF- β 1 protected against the development of COPD. In this study, the authors proposed that TGF- β 1 signalling may prevent emphysema development by inhibiting matrix metalloprotease (MMP)-mediated elastin degradation or promoting elastin synthesis of elastin [54–56].

Subsequently, Celedon et al. reported a significant linkage between the TGF- β 1 gene locus on chromosome 19q and lung function, particularly FEV₁, in former and current smokers in a family-based study (Boston Early-Onset COPD cohort) [57]. In these families, an association between three SNPs in or near the TGF- β 1 gene locus (rs2241712, rs2241718 and rs6957) with low FEV₁ was found. Notably, no association was found with SNP rs1982073, which has been initially reported by Wu et al. [54]. In a case-control study group in the same report, however, the association of SNP rs1982073 with COPD was confirmed. In addition, the T-allele of SNP rs1800469, which has been associated with phenotypes related to asthma, was inversely associated with COPD in the case-control study group [57, 58]. The association of SNP rs1800469 with COPD was further confirmed in a subsequent Chinese COPD cohort. Here, the frequency of the T-allele of SNP rs1800469 was significantly

higher in control subjects than in the COPD patients, along with an increased TGF- β 1 production and higher circulating concentrations of TGF- β 1 [59]. This further supports a protective role of TGF- β 1 in COPD.

The above mentioned SNPs (rs2241712, rs1800469 and rs1982073), however, were not associated with COPD in Koreans, underlining that ethnic diversity has to be considered in COPD susceptibility [60]. Furthermore, in a Dutch COPD study, the SNPs rs1800469 and rs1982073 were not associated with COPD, while rs6957 was confirmed [61]. In 304 white participants in the NETT Genetics Ancillary Study, the association of gene polymorphisms with functional measurements, such as exercise tolerance and symptom severity, was analysed [62]. The three previously reported SNPs (rs1982073, rs1800469, rs2241712) were associated with severe dyspnoea and with an increased BODE index. In a Japanese population, eight different TGF- β 1 SNPs were analysed in COPD patients with CT-documented emphysema [63]. None of them was significantly associated with emphysema; however, the frequency of one significant haplotype of all eight SNPs was significantly higher in emphysema patients. In addition, two previously reported SNPs (rs1800469 and rs1982073) were associated with FEV₁ in patients with emphysema [63].

Importantly, other members of the TGF- β superfamily have also been reported to be associated with COPD. In the NETT Genetics Ancillary Study, latent TGF- β binding protein (LTBP) 4 gene SNPs were significantly associated with an increase in exercise capacity [62].

Most recently, another TGF- β superfamily member was identified in a genome-wide linkage analysis study. An SNP in the type III TGF- β receptor (betaglycan) was found to be associated with lung function in a family-based study. In a case-control cohort, this SNP was associated with low FEV₁ and CT-documented emphysema [64].

In summary, TGF- β 1 clearly represents a suitable candidate gene of COPD pathogenesis, as polymorphisms in genes of TGF- β superfamily members have been identified in different COPD populations. At this point-in-time, however, the results are, at least in part, inconsistent (table 2). This may be due to several reasons: firstly, the frequency of an allele can vary in different ethnic populations and different results can arise if the background of the cases and controls differs; secondly, the aetiology of COPD is complex and multiple genetic and environmental factors con-

tribute to the disease, which may have been overlooked in the previous studies. Third, the heterogeneity and individual severity of COPD features, and their complex diagnosis, may lead to different results. The later studies already considered this heterogeneity of COPD and sought to differentiate between pathological and functional features of COPD. In conclusion, further studies are clearly needed, to elucidate the distinct role of genetic alterations of TGF- β superfamily members in different COPD subtypes in detail.

TGF- β signalling in small airway disease

By definition, small airways exhibit a diameter of ≤ 2 mm, which includes airways from the fourth to the fourteenth generation of branching. The term "small airway disease (SAD)", also called obstructive bronchiolitis, includes 1) airway inflammation, as evidenced by occlusion of the airways, inflammatory cell influx, and increased mucus production, and 2) increased airway wall thickness due to tissue remodelling and peribronchiolar fibrosis [4, 11, 12, 65]. In general, TGF- β is known to be a potent inducer of a) extracellular matrix target genes, such as collagens, in fibroblasts and b) fibroblast proliferation and activation, both key events in the fibrogenic process [66]. In the last decade, TGF- β has emerged as an important contributor to SAD due to the following observations:

Several studies have reported an increased expression of TGF- β 1 in the airway epithelium of smokers, as well as in patients with chronic bronchitis or COPD [67–70]. TGF- β 1 expression in epithelial cells from patients with chronic bronchitis correlated with basal membrane thickness and the number of peribronchiolar fibroblasts [68, 69]. Additionally, decreased expression of the inhibitory Smad proteins 6 and 7 in bronchial biopsies of COPD patients have been reported, further suggesting increased TGF- β signalling in COPD [71]. Increased TGF- β 1, as well as type I and type II TGF- β receptor, expression in the bronchiolar and alveolar epithelium of COPD patients was correlated with the number of macrophages, and, notably, with clinical features, such as lung function [72]. This was further supported by increased TGF- β 1 expression in airway epithelial cells from COPD patients and smokers, which correlated with peripheral airway obstruction and the burden of cigarette smoking [70]. Enhanced TGF- β signalling was further confirmed by microarray studies. Here, genes involved in extracellular matrix turnover, as well as TGF- β itself were found to be up-regulated in COPD patients [73–75].

Kenyon and colleagues reported that intratracheal administration of recombinant TGF- β 1 to mice resulted in increased airway wall collagen, along with increased type I and III collagen gene

expression as well as total collagen content in distal airways *in vivo*, further supporting the idea that TGF- β 1 signalling leads to peribronchiolar collagen deposition and basal membrane thickening in SAD [76]. Notably, the authors did not observe any detectable inflammation after TGF- β 1 instillation *in vivo* [76]. Increased expression of TGF- β 1 and the type I and type II TGF- β receptors has also been observed in alveolar macrophages of COPD patients [72], however, others have reported decreased TGF- β 1 expression in alveolar macrophages of COPD patients compared with smokers and non-smokers, pointing to a reduced anti-inflammatory capacity, generally mediated by TGF- β 1 [77]. In addition, decreased expression of the type II TGF- β receptor was observed in bronchial glands of COPD patients, which may be responsible for enhanced mucus production in COPD patients [78]. Altogether, these data support the idea that increased TGF- β 1, mainly secreted by airway epithelial cells, contributes to the development of SAD. In this regard, the different susceptibility of specific cell types, such as peribronchiolar fibroblasts, macrophages, or gland cells, which further modulate these processes, needs to be emphasised.

Other studies have focused on the direct effect of cigarette smoke exposure on TGF- β signalling. Interestingly, gene expression studies in human bronchial epithelial cells exposed to cigarette smoke *in vitro* did not reveal a prominent increase in TGF- β signalling, but rather a down-regulation of the TGF- β pathway [79–81]. In contrast, tracheal explants that were exposed to cigarette smoke *in vitro* exhibited enhanced active TGF- β signalling [82]. This was further corroborated in an *in vivo* model, in which mice exposed to cigarette smoke exhibited enhanced pro-fibrotic TGF- β signalling in small airways [83]. In addition, it has been shown that cigarette smoke activates TGF- β 1 in lung fibroblast cultures [84]. In turn, although diverse evidence has been described, these data largely emphasise a role of active TGF- β signalling in tissue remodelling and fibrosis that is possibly induced by cigarette smoke exposure.

TGF- β signalling in emphysema

Emphysema is characterised by rarefaction of alveolar walls, most likely resulting from a reduced capacity of the peripheral lung to repair the cigarette smoke-induced loss of parenchymal tissue [13, 85]. Matrix metalloproteinases (MMP) and their inhibitors, the tissue inhibitors of metalloproteinases (TIMP), which regulate extracellular matrix homeostasis, have been implicated in cigarette smoke-induced pulmonary emphysema [86-88]. While increased TGF- β signalling clearly triggers the development of SAD, the impact of TGF- β signalling on the development of emphysema seems to be opposite of what is observed in SAD. In emphysema, decreased TGF- β signalling may lead to increased MMP expression and subsequent extracellular matrix degradation, which may contribute to existing genetic or acquired susceptibility to emphysema [89, 90].

The expression of MMP2 and MMP9 (gelatinase A and B, respectively), as well as MMP12 (macrophage elastase), is markedly increased in COPD patients and smokers, as well as in several animal models associated with emphysematic changes [91-97]. Importantly, MMP12 knock-out (KO) mice are protected from smoke-induced emphysema [98]. Along these lines, TIMP3 KO mice develop progressive airspace enlargement, emphasising the significance of a balanced MMP/TIMP expression within the local tissue microenvironment of the lung [99, 100]. TGF- β is an important regulator of MMP expression [12]. TGF- β inhibits MMP9 and MMP12 expression in alveolar macrophages and monocytes [101-103]. Mice lacking the $\beta 6$ subunit of the $\beta v\beta 6$ integrin (Itgb6-), which mediates TGF- β activation, exhibited decreased TGF- β signalling along with increased expression of MMP12 in alveolar macrophages [90, 104]. These mice spontaneously developed emphysema over time, which was similar to the course of emphysema commonly observed in humans [90]. This is an important difference to the findings observed in mice overexpressing interferon- γ or tumour necrosis factor- α , which demonstrated more severe and rapidly progressive emphysema [105, 106]. Increased MMP12 expression was inhibited by the expression of constitutively active TGF- β in Itgb6- mice. Overall these findings emphasise an important role of the TGF- β -MMP axis in human emphysema development.

Further evidence that the availability and activation of extracellular TGF- β is crucial in emphysema development, was provided by studies investigating the loss of latent TGF- β binding proteins. Latent TGF- β binding protein (LTBP) 3 KO mice exhibited decreased septation in terminal alveoli along with a transient decrease in TGF- β signalling during lung development postnatally [107]. Mice deleted of LTBP4 expression also developed severe pulmonary emphysema,

cardiomyopathy, and colorectal cancer 4 to 32 weeks postnatally [108]. Similarly to LTBP3 KO mice, LTBP4 KO mice presented reduced TGF- β deposition in the extracellular space. Taken together, both studies underline the role of LTBP as a local regulator of TGF- β signalling and a potential target in emphysema development. Furthermore, posttranslational modifications of TGF- β receptors have been implicated in emphysema development. Mice deficient of $\alpha 1,6$ -fucosyltransferase (Fut8) exhibited altered TGF- β signalling and revealed an emphysema-like phenotype with marked overexpression of MMP12 and MMP13 [109]. With respect to these studies investigating TGF- β superfamily members, it has to be pointed out that the role of other important mediators, such as BMP or activins, as well as the relevance of MAPK and TGF- β pathway interaction, in particular with respect to COPD, are clearly needed to achieve a more comprehensive picture about the distinct role of TGF- β superfamily signalling in this disease.

The direct involvement of decreased TGF- β signalling in emphysema development was also underlined in a recent study demonstrating that Smad3 KO mice spontaneously developed increased airspace enlargement, along with increased MMP9 and MMP12 levels in the bronchoalveolar lavage fluid [110, 111]. Interestingly, Smad3 is required for TGF- β -mediated inhibition of MMP12 expression in alveolar macrophages. Consistently, these findings suggest that Smad3 is also important in TGF- β 1-mediated MMP9 inhibition. Furthermore, a different Smad3 KO mouse line exhibited altered lung alveolarisation, which resulted from reduced peripheral lung cell proliferation during lung development [112, 113].

The impact of decreased TGF- β signalling in emphysema is further substantiated by observations that increased TGF- β signalling in the lung contribute to the development of pulmonary fibrosis [114]. Interestingly, it has been reported that TGF- β overexpression during lung development leads to bronchopulmonary dysplasia, a disease characterised by varying sized areas of interstitial fibrosis as well as areas with enlarged alveolar spaces [111, 115, 116]. Most recently, a distinct phenotype of combined pulmonary fibrosis and emphysema has been also described in humans, mainly in male smokers. This further highlights that the distinct cellular susceptibility and response to an identical injury can vary tremendously [117]. In addition, pathological changes with both emphysema and fibrosis have been reported in a mice overexpressing tumor necrosis factor- α [118]. The role of TGF- β , however, in both the human as well as the experimental model, has not been investigated yet.

In vitro studies, using rat tracheal explants ex-

posed to cigarette smoke, reported ongoing pro-fibrotic mediator expression, such as TGF- β , in small airways, but not in the surrounding parenchyma [119]. Importantly, these findings suggest that the parenchyma fails to repair smoke-induced matrix damage, whereas small airways show a fibrotic response during disease progression. In addition, in an alveolar epithelial cell line, cigarette smoke induced TGF- β expression, which further led to growth inhibition, thus revealing another possible pathomechanism involved in parenchymal tissue destruction in COPD [120, 121].

Recent studies also focused on TGF- β signalling in human emphysema specimen. It has

been shown that interstitial fibroblasts from COPD patients with emphysema released more TGF- β compared with control fibroblasts [84, 122]. Most interestingly, the baseline expression of active intracellular TGF- β mediators, such as phosphorylated Smad3, was reduced, whereas the inhibitory Smads were increased. Consistently, the response of COPD fibroblasts to TGF- β 1 was reduced [122]. These findings further highlight differences in cell-specific susceptibility to TGF- β signalling. Fibroblasts from COPD patients may have reduced capacity to activate repair processes, which subsequently contribute to emphysema development.

Conclusion

As outlined in this review, emerging interest in the role of TGF- β in the pathogenesis of COPD has recently evolved. Genetic association studies have largely provided evidence that TGF- β 1 is a suitable candidate gene in COPD. Future studies that take into account the pathological and functional heterogeneity of COPD will undoubtedly further elucidate the distinct role of genetic alterations within the TGF- β superfamily with respect to different COPD subtypes.

In line with these genetic associations, altered TGF- β signalling has been clearly documented in a number of studies that have investigated pathogenetic mechanisms leading to COPD. The major risk factor for COPD, cigarette smoke, as well as concomitant smoke-induced inflammation has been shown to induce TGF- β production and release. Most importantly, TGF- β acts in a spatiotemporal manner in the lung, in particular relating to the two main features of COPD: small airway disease (SAD), including airway inflammation and remodelling, and emphysema, characterised by airspace enlargement. Intriguingly, the impact of TGF- β signalling on the development of SAD appears to be the opposite of what is observed in emphysema. While increased TGF- β signalling triggers the development of SAD, decreased TGF- β signalling seems to mediate parenchymal tissue destruction in emphysema. These diverse effects of TGF- β signalling observed thus far are critically determined by the cellular susceptibility to TGF- β signalling, which varies in different cellular compartments and types. This spatiotempo-

ral diversity of TGF- β signalling, combined with varying cellular susceptibilities to TGF- β , may well underlie the heterogeneous pathology seen in COPD, and will challenge our innovative potential for targeted therapy of this disease in future studies. In this respect, several thoughts need to be taken into account: first and foremost, we clearly need a more comprehensive analysis of TGF- β signalling in COPD, including in particular the cell-specific expression pattern of signalling receptors and intracellular target genes. This will allow the development of more specific, target-oriented therapies against distinct downstream signalling molecules. Second, the development of novel localised therapeutic delivery options is clearly needed to then facilitate compartment- or cell-type specific therapies against TGF- β signalling components.

Correspondence:

Prof. Dr. Oliver Eickelberg
Comprehensive Pneumology Center
Institute of Lung Biology and Disease (iLBD)
Ludwig Maximilians University Munich and
Helmholtz Zentrum München
Ingolstädter Landstrasse 1
85764 Neuherberg / München
Germany
E-Mail:
oliver.eickelberg@helmholtz-muenchen.de

References

- Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970–2002. *JAMA*. 2005;294:1255–9.
- Sullivan SD, Ramsey SD, Lee TA. The economic burden of COPD. *Chest*. 2000;117:5S–9S.
- Macnee W. Pathogenesis of chronic obstructive pulmonary disease. *Clin Chest Med*. 2007;28:479–513, v.
- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, 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;176:532–55.
- Eisner MD, Balmes J, Katz PP, Trupin L, Yelin EH, Blanc PD. Lifetime environmental tobacco smoke exposure and the risk of chronic obstructive pulmonary disease. *Environ Health*. 2005;4:7.
- Hersh CP, DeMeo DL, Silverman EK. National Emphysema Treatment Trial state of the art: genetics of emphysema. *Proc Am Thorac Soc*. 2008;5:486–93.
- Luppi F, Franco F, Beghe B, Fabbri LM. Treatment of chronic obstructive pulmonary disease and its comorbidities. *Proc Am Thorac Soc*. 2008;5:848–56.
- Viegi G, Pistelli F, Sherrill DL, Maio S, Baldacci S, Carrozzi L. Definition, epidemiology and natural history of COPD. *Eur Respir J*. 2007;30:993–1013.
- Blanc PD, Iribarren C, Trupin L, Earnest G, Katz PP, Balmes J, et al. Occupational exposures and the risk of COPD: dusty trades revisited. *Thorax*. 2009;64:6–12.
- Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350:2645–53.
- Sturton G, Persson C, Barnes PJ. Small airways: an important but neglected target in the treatment of obstructive airway diseases. *Trends Pharmacol Sci*. 2008;29:340–5.
- Chung KF. Cytokines as targets in chronic obstructive pulmonary disease. *Curr Drug Targets*. 2006;7:675–81.
- Minai OA, Benditt J, Martinez FJ. Natural history of emphysema. *Proc Am Thorac Soc*. 2008;5:468–74.
- Matsuba K, Wright JL, Wiggs BR, Pare PD, Hogg JC. The changes in airways structure associated with reduced forced expiratory volume in one second. *Eur Respir J*. 1989;2:834–9.
- Cosio M, Ghezzi H, Hogg JC, Corbin R, Loveland M, Dorman J, Macklem PT. The relations between structural changes in small airways and pulmonary-function tests. *N Engl J Med*. 1978;298:1277–81.
- Lamb D, McLean A, Gillooly M, Warren PM, Gould GA, MacNee W. Relation between distal airspace size, bronchiolar attachments, and lung function. *Thorax*. 1993;48:1012–7.
- Martinez FJ, Han MK, Andrei AC, Wise R, Murray S, Curtis JL, et al. Longitudinal change in the BODE index predicts mortality in severe emphysema. *Am J Respir Crit Care Med*. 2008;178:491–9.
- Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350:1005–12.
- Chatila WM, Thomashow BM, Minai OA, Criner GJ, Make BJ. Comorbidities in chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2008;5:549–55.
- King DA, Cordova F, Scharf SM. Nutritional aspects of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2008;5:519–23.
- Agusti A, Soriano JB. COPD as a systemic disease. *Copd*. 2008;5:133–8.
- MacNee W. Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2005;2:50–60.
- Agusti AG, Noguera A, Sauleda J, Sala E, Pons J, Busquets X. Systemic effects of chronic obstructive pulmonary disease. *Eur Respir J*. 2003;21:347–60.
- Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest*. 2007;131:1557–66.
- Rahman I, Morrison D, Donaldson K, MacNee W. Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med*. 1996;154:1055–60.
- Pratico D, Basili S, Vieri M, Cordova C, Violi F, Fitzgerald GA. Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F2alpha-III, an index of oxidant stress. *Am J Respir Crit Care Med*. 1998;158:1709–14.
- Noguera A, Busquets X, Sauleda J, Villaverde JM, MacNee W, Agusti AG. Expression of adhesion molecules and G proteins in circulating neutrophils in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 1998;158:1664–8.
- Barcelo B, Pons J, Ferrer JM, Sauleda J, Fuster A, Agusti AG. Phenotypic characterisation of T-lymphocytes in COPD: abnormal CD4+CD25+ regulatory T-lymphocyte response to tobacco smoking. *Eur Respir J*. 2008;31:55–562.
- Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti A, MacNee W. Oxidative stress and airway inflammation in severe exacerbations of COPD. *Thorax*. 2005;60:293–300.
- Tetley TD. Inflammatory cells and chronic obstructive pulmonary disease. *Curr Drug Targets Inflamm Allergy*. 2005;4:607–18.
- Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM. Raised CRP levels mark metabolic and functional impairment in advanced COPD. *Thorax*. 2006;61:17–22.
- Noguera A, Batle S, Miralles C, Iglesias J, Busquets X, MacNee W, Agusti AG. Enhanced neutrophil response in chronic obstructive pulmonary disease. *Thorax*. 2001;56:432–7.
- van Durme YM, Verhamme KM, Aarnoudse AJ, Van Pottelberge GR, Hofman A, Witterman JC, et al. C-Reactive Protein Levels, Haplotypes, and the Risk of Incident Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2008.
- Fabbri LM, Rabe KF. From COPD to chronic systemic inflammatory syndrome? *Lancet*. 2007;370:797–9.
- Barnes PJ. Future treatments for chronic obstructive pulmonary disease and its comorbidities. *Proc Am Thorac Soc*. 2008;5:857–64.
- Fabbri LM, Boschetto P, Mapp CE. COPD guidelines: the important thing is not to stop questioning. *Am J Respir Crit Care Med*. 2007;176:527–8.
- Barnes PJ, Hansel TT. Prospects for new drugs for chronic obstructive pulmonary disease. *Lancet*. 2004;364:985–96.
- Kim IY, Kim MM, Kim SJ. Transforming growth factor-beta: biology and clinical relevance. *J Biochem Mol Biol*. 2005;38:1–8.
- ten Dijke P, Hill CS. New insights into TGF-beta-Smad signalling. *Trends Biochem Sci*. 2004;29:265–73.
- Coker RK, Laurent GJ, Shahzeidi S, Hernandez-Rodriguez NA, Pantelidis P, du Bois RM, et al. Diverse cellular TGF-beta 1 and TGF-beta 3 gene expression in normal human and murine lung. *Eur Respir J*. 1996;9:2501–7.
- Gatherer D, Ten Dijke P, Baird DT, Akhurst RJ. Expression of TGF-beta isoforms during first trimester human embryogenesis. *Development*. 1990;110:445–60.
- Annes JP, Munger JS, Rifkin DB. Making sense of latent TGF-beta activation. *J Cell Sci*. 2003;116:217–24.
- Munger JS, Harpel JG, Gleizes PE, Mazzieri R, Nunes I, Rifkin DB. Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int*. 1997;51:1376–82.
- Sheppard D. Integrin-mediated activation of latent transforming growth factor beta. *Cancer Metastasis Rev*. 2005;24:395–402.
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003;113 685–700.
- Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev*. 2005;19:2783–810.
- Miyazono K, Maeda S, Imamura T. BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev*. 2005;16:251–63.
- Javelaud D, Mauviel A. Crosstalk mechanisms between the mitogen-activated protein kinase pathways and Smad signaling downstream of TGF-beta: implications for carcinogenesis. *Oncogene*. 2005;24:5742–50.
- Knudson RJ, James Jackson Jr. the young pulmonologist who described familial emphysema. An historical footnote. *Chest*. 1985;87:673–6.
- Walter R, Gottlieb DJ, O'Connor GT. Environmental and genetic risk factors and gene-environment interactions in the pathogenesis of chronic obstructive lung disease. *Environ Health Perspect*. 2000;108(Suppl 4):733–42.

- 51 Lomas DA. The selective advantage of alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med.* 2006;173:1072–7.
- 52 Petrache A, Fijalkowska I, Zhen L, Medler TR, Brown E, Cruz P, et al. A novel antiapoptotic role for alpha1-antitrypsin in the prevention of pulmonary emphysema. *Am J Respir Crit Care Med.* 2006;173:1222–8.
- 53 Silverman EK, Speizer FE, Weiss ST, Chapman HA Jr, Schuette A, Campbell EJ, et al. Familial aggregation of severe, early-onset COPD: candidate gene approaches. *Chest.* 2000;117:273S–4S.
- 54 Wu L, Chau J, Young RP, Pokorny V, Mills GD, Hopkins R, et al. Transforming growth factor-beta1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax.* 2004;59:126–9.
- 55 Fang KC, Wolters PJ, Steinhoff M, Bidgol A, Blount JL, Caughey GH. Mast cell expression of gelatinases A and B is regulated by kit ligand and TGF-beta. *J Immunol.* 1999;162:5528–35.
- 56 McGowan SE, Jackson SK, Olson PJ, Parekh T, Gold LI. Exogenous and endogenous transforming growth factors-beta influence elastin gene expression in cultured lung fibroblasts. *Am J Respir Cell Mol Biol.* 1997;17:25–35.
- 57 Celedon JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, et al. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet.* 2004;13:1649–56.
- 58 Silverman ES, Palmer LJ, Subramaniam V, Hallock A, Mathew S, Vallone J, et al. Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med.* 2004;169:214–9.
- 59 Su ZG, Wen FQ, Feng YL, Xiao M, Wu XL. Transforming growth factor-beta1 gene polymorphisms associated with chronic obstructive pulmonary disease in Chinese population. *Acta Pharmacol Sin.* 2005;26:714–20.
- 60 Yoon HI, Silverman EK, Lee HW, Yoo CG, Lee CT, Chung HS, et al. Lack of association between COPD and transforming growth factor-beta1 (TGFB1) genetic polymorphisms in Koreans. *Int J Tuberc Lung Dis.* 2006;10:504–9.
- 61 van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Nolte IM, Boezen HM. Decorin and TGF-beta1 polymorphisms and development of COPD in a general population. *Respir Res.* 2006;7:89.
- 62 Hersh CP, Demeo DL, Lazarus R, Celedon JC, Raby BA, Bidditt JO, et al. Genetic association analysis of functional impairment in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2006;173:977–84.
- 63 Ito M, Hanaoka M, Droma Y, Hatayama O, Sato E, Katsuyama Y, et al. The association of transforming growth factor beta 1 gene polymorphisms with the emphysema phenotype of COPD in Japanese. *Intern Med.* 2008;47:1387–94.
- 64 Hersh CP, Hansel NN, Barnes KC, Lomas DA, Pillai SG, Coxson HO, et al. Transforming Growth Factor Beta Receptor-3 is Associated with Pulmonary Emphysema. *Am J Respir Cell Mol Biol.* 2009.
- 65 Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet.* 2004;364:709–21.
- 66 Gharaee-Kermani M, Hu B, Phan SH, Gyetko MR. Recent advances in molecular targets and treatment of idiopathic pulmonary fibrosis: focus on TGFbeta signaling and the myofibroblast. *Curr Med Chem.* 2009;16:1400–17.
- 67 Aubert JD, Dalal BI, Bai TR, Roberts CR, Hayashi S, Hogg JC. Transforming growth factor beta 1 gene expression in human airways. *Thorax.* 1994;49:225–32.
- 68 Vignola AM, Chanez P, Chiappara G, Merendino A, Zinnanti E, Bousquet J, et al. Release of transforming growth factor-beta (TGF-beta) and fibronectin by alveolar macrophages in airway diseases. *Clin Exp Immunol.* 1996;106:114–9.
- 69 Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, et al. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med.* 1997;156:591–9.
- 70 Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, et al. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med.* 2001;163:1476–83.
- 71 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;385:649–53.
- 72 de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 1998;158:1951–7.
- 73 Wang IM, Stepaniants S, Boie Y, Mortimer JR, Kennedy B, Elliott M, et al. Gene expression profiling in patients with chronic obstructive pulmonary disease and lung cancer. *Am J Respir Crit Care Med.* 2008;177: 402–11.
- 74 Ning W, Lee J, Kaminski N, Feghali-Bostwick CA, Watkins SC, Pilewski JM, et al. Comprehensive analysis of gene expression on GOLD-2 Versus GOLD-0 smokers reveals novel genes important in the pathogenesis of COPD. *Proc Am Thorac Soc.* 2006;3:466.
- 75 Ning W, Li CJ, Kaminski N, Feghali-Bostwick CA, Alber SM, Di YP, et al. Comprehensive gene expression profiles reveal pathways related to the pathogenesis of chronic obstructive pulmonary disease. *Proc Natl Acad Sci. U S A* 2004;101: 14895–900.
- 76 Kenyon NJ, Ward RW, McGrew G, Last JA. TGF-beta1 causes airway fibrosis and increased collagen I and III mRNA in mice. *Thorax.* 2003;58:772–7.
- 77 Pons AR, Sauleda J, Noguera A, Pons J, Barcelo B, Fuster A, Agusti AG. Decreased macrophage release of TGF-beta and TIMP-1 in chronic obstructive pulmonary disease. *Eur Respir J.* 2005;26:60–6.
- 78 Baraldo S, Bazzan E, Turato G, Calabrese F, Beghe B, Papi A, et al. Decreased expression of TGF-beta type II receptor in bronchial glands of smokers with COPD. *Thorax.* 2005;60: 998–1002.
- 79 Yoneda K, Peck K, Chang MM, Chmiel K, Sher YP, Chen J, et al. 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:S85–9.
- 80 Spira A, Beane J, Shah V, Liu G, Schembri F, Yang X, et al. Effects of cigarette smoke on the human airway epithelial cell transcriptome. *Proc Natl Acad Sci. U S A* 2004;101:10143–8.
- 81 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 Lung Cell Mol Physiol.* 2007; 292:L1248–56.
- 82 Wang RD, Wright JL, Churg A. Transforming growth factor-beta1 drives airway remodeling in cigarette smoke-exposed tracheal explants. *Am J Respir Cell Mol Biol.* 2005;33:387–93.
- 83 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; 174:1327–34.
- 84 Wang H, Liu X, Umino T, Kohyama T, Zhu YK, Wen FQ, et al. Effect of cigarette smoke on fibroblast-mediated gel contraction is dependent on cell density. *Am J Physiol Lung Cell Mol Physiol.* 2003;284:L205–13.
- 85 Thorley AJ, Tetley TD. Pulmonary epithelium, cigarette smoke, and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis.* 2007;2:409–28.
- 86 Srivastava PK, Dastidar SG, Ray A. Chronic obstructive pulmonary disease: role of matrix metalloproteases and future challenges of drug therapy. *Expert Opin Investig Drugs.* 2007; 16:1069–78.
- 87 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;3:494–8.
- 88 Churg A, Wang RD, Tai H, Wang X, Xie C, Dai J, et al. Macrophage metalloelastase mediates acute cigarette smoke-induced inflammation via tumor necrosis factor-alpha release. *Am J Respir Crit Care Med.* 2003;167:1083–9.
- 89 Roberts AB. Medicine: Smoke signals for lung disease. *Nature.* 2003;422:130–1.
- 90 Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, et al. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. *Nature.* 2003;422:169–73.
- 91 Baraldo S, Bazzan E, Zanin ME, Turato G, Garbisa S, Maestrelli P, et al. Matrix metalloproteinase-2 protein in lung periphery is related to COPD progression. *Chest.* 2007;132: 1733–40.
- 92 Demedts IK, Morel-Montero A, Lebecque S, Pacheco Y, Cataldo D, Joos GF, et al. Elevated MMP-12 protein levels in induced sputum from patients with COPD. *Thorax.* 2006;61: 196–201.

- 93 Molet S, Belleguic C, Lena H, Germain N, Bertrand CP, Shapiro SD, et al. Increase in macrophage elastase (MMP-12) in lungs from patients with chronic obstructive pulmonary disease. *Inflamm Res*. 2005;54:31-6.
- 94 Foronjy R, Nkyimbeng T, Wallace A, Thankachen J, Okada Y, Lemaître V, D'Armiento J. Transgenic expression of matrix metalloproteinase-9 causes adult-onset emphysema in mice associated with the loss of alveolar elastin. *Am J Physiol Lung Cell Mol Physiol*. 2008;294:L1149-57.
- 95 Churg A, Wang R, Wang X, Onnervik PO, Thim K, Wright JL. Effect of an MMP-9/MMP-12 inhibitor on smoke-induced emphysema and airway remodelling in guinea pigs. *Thorax*. 2007;62:706-13.
- 96 D'Armiento J, Dalal SS, Okada Y, Berg RA, Chada K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell*. 1992;71:955-61.
- 97 Finlay GA, O'Driscoll LR, Russell KJ, D'Arcy EM, Masteron JB, FitzGerald MX, O'Connor CM. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am J Respir Crit Care Med*. 1997;156:240-7.
- 98 Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science*. 1997;277:2002-4.
- 99 Leco KJ, Waterhouse P, Sanchez OH, Gowing KL, Poole AR, Wakeham A, et al. Spontaneous air space enlargement in the lungs of mice lacking tissue inhibitor of metalloproteinases-3 (TIMP-3). *J Clin Invest*. 2001;108:817-29.
- 100 Martin EL, Moyer BZ, Pape MC, Starcher B, Leco KJ, Veldhuizen RA. Negative impact of tissue inhibitor of metalloproteinase-3 null mutation on lung structure and function in response to sepsis. *Am J Physiol Lung Cell Mol Physiol*. 2003;285:L1222-32.
- 101 Feinberg MW, Jain MK, Werner F, Sibinga NE, Wiesel P, Wang H, et al. Transforming growth factor-beta 1 inhibits cytokine-mediated induction of human metalloelastase in macrophages. *J Biol Chem*. 2000;275:25766-73.
- 102 Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, et al. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. *J Biol Chem*. 2000;275:36653-8.
- 103 Tajirian T, Dennis JW, Swallow CJ. Regulation of human monocyte proMMP-9 production by fetuin, an endogenous TGF-beta antagonist. *J Cell Physiol*. 2000;185:174-83.
- 104 Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, et al. The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell*. 1999;96:319-28.
- 105 Wang Z, Zheng T, Zhu Z, Homer RJ, Riese RJ, Chapman HA Jr, et al. Interferon gamma induction of pulmonary emphysema in the adult murine lung. *J Exp Med*. 2000;192:1587-600.
- 106 Churg A, Wang RD, Tai H, Wang X, Xie C, Wright JL. Tumor necrosis factor-alpha drives 70% of cigarette smoke-induced emphysema in the mouse. *Am J Respir Crit Care Med*. 2004;170:492-8.
- 107 Colarossi C, Chen Y, Obata H, Jurukovski V, Fontana L, Dabovic B, Rifkin DB. Lung alveolar septation defects in Ltbp-3-null mice. *Am J Pathol*. 2005;167:419-28.
- 108 Sterner-Kock A, Thorey IS, Koli K, Wempe F, Otte J, Bangsow T, et al. Disruption of the gene encoding the latent transforming growth factor-beta binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Dev*. 2002;16:2264-73.
- 109 Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, et al. Dysregulation of TGF-beta1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. *Proc Natl Acad Sci USA*. 2005;102:15791-6.
- 110 Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, et al. Smad3 null mice develop airspace enlargement and are resistant to TGF-beta-mediated pulmonary fibrosis. *J Immunol*. 2004;173:2099-108.
- 111 Warburton D, Gaudie J, Bellusci S, Shi W. Lung development and susceptibility to chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 2006;3:668-72.
- 112 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 Lung Cell Mol Physiol*. 2005;288:L683-691.
- 113 Shi W, Bellusci S, Warburton D. Lung development and adult lung diseases. *Chest*. 2007;132:651-6.
- 114 Sime PJ, Xing Z, Graham FL, Csaky KG, Gaudie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest*. 1997;100:768-76.
- 115 Vicencio AG, Lee CG, Cho SJ, Eickelberg O, Chuu Y, Haddad GG, Elias JA. Conditional overexpression of bioactive transforming growth factor-beta1 in neonatal mouse lung: a new model for bronchopulmonary dysplasia? *Am J Respir Cell Mol Biol*. 2004;31:650-6.
- 116 Gaudie J, Galt T, Bonniaud P, Robbins C, Kelly M, Warburton D. Transfer of the active form of transforming growth factor-beta 1 gene to newborn rat lung induces changes consistent with bronchopulmonary dysplasia. *Am J Pathol*. 2003;163:2575-84.
- 117 Cottin V, Nunes H, Brillet PY, Delaval P, Devouassoux G, Tillie-Leblond I, et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. *Eur Respir J*. 2005;26:586-93.
- 118 Lundblad LK, Thompson-Figueroa J, Leclair T, Sullivan MJ, Poynter ME, Irvin CG, Bates JH. Tumor necrosis factor-alpha overexpression in lung disease: a single cause behind a complex phenotype. *Am J Respir Crit Care Med*. 2005;171:1363-70.
- 119 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;40:268-76.
- 120 Marwick JA, Kirkham P, Gilmour PS, Donaldson K, Mac NW, Rahman I. Cigarette smoke-induced oxidative stress and TGF-beta1 increase p21waf1/cip1 expression in alveolar epithelial cells. *Ann N Y Acad Sci*. 2002;973:278-83.
- 121 Rennard SI, Togo S, Holz O. Cigarette smoke inhibits alveolar repair: a mechanism for the development of emphysema. *Proc Am Thorac Soc*. 2006;3:703-8.
- 122 Togo S, Holz O, Liu X, Sugiura H, Kamio K, Wang X, 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;178:248-60.