ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 2, 2008

DOI: 10.1089/ars.2007.1914

Forum Review

Transforming Growth Factor-β Activation in the Lung: Focus on Fibrosis and Reactive Oxygen Species

KATRI KOLI,[1] MARJUKKA MYLLÄRNIEMI,[2] JORMA KESKI-OJA,[1] and VUOKKO L. KINNULA[2]

ABSTRACT

Transforming growth factor-βs (TGF-β) regulate a wide variety of cellular functions in normal development and are involved in both tissue homeostasis and disease pathogenesis. The regulation of the TGF-β family of growth factors is unique because they are targeted to the extracellular matrix in a biologically inactive form. The release from pericellular matrices and the activation of TGF-β are important mechanisms in several pathophysiologic conditions. Reactive oxygen species (ROS) can activate TGF-β either directly or indirectly *via* the activation of proteases. In addition, TGF-β itself induces ROS production as part of its signal-transduction pathway. The lung is a unique organ, because its structures act as boundaries between gaseous and aqueous phases, allowing the utilization of inhaled oxygen. However, this renders pulmonary tissues vulnerable to the toxic effects of inhaled air. The oxidant pathways are especially relevant in the lung, where TGF-β is known to have a role in tissue repair and connective tissue turnover. In pulmonary fibrosis, TGF-β activation is considered as a hallmark of disease progression. More recently, the oxidative effects of cigarette smoking have been found to activate TGF-β in chronic obstructive pulmonary disease (COPD), a disease consisting of emphysema, airway fibrosis, and focal lung fibrosis. *Antioxid. Redox Signal*. 10, 333–342.

TRANSFORMING growth factor (TGF)-β plays a crucial role in the regulation of lung development and homeostasis. Aberrant TGF-β expression and activation are associated with abnormal lung development and parenchymal lung disorders, including pulmonary fibrosis and other airway diseases. In a large number of studies, alterations in the cellular redox state and increased oxidative stress have been observed to contribute to TGF-β activation. The various mechanisms of TGF-β activation offer numerous alternatives for human lung disease evolution and progression. Elucidation of these mechanisms presents new possibilities for intervention, such as potential antifibrotic drugs being targeted against the TGF-β activation-related processes.

LATENT FORMS OF TGF-β AND THEIR ACTIVATION

Because TGF-β can alter the functions of almost any given cell type, complex machinery has evolved to regulate both the local activation and the cell type specific signaling responses (36, 71). TGF-βs are secreted as latent complexes in which the dimeric mature 25-kDa growth factor is masked by a non-covalent association with the latency-associated protein (LAP). TGF-β and LAP are produced as a single proprotein, which is cleaved by furin-like proteases during secretion. Some cells have been observed to secrete TGF-β in the form of a TGF–β/LAP complex, which is also referred to as the small la-

[1]Departments of Virology and Pathology, Haartman Institute; and [2]Department of Medicine, Division of Pulmonary Medicine, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.

tent complex (71). However, most cells produce TGF-β as a large latent complex, in which latent TGF-β binding protein (LTBP) is associated through its third 8-Cys repeat with the small latent TGF-β. LTBP-1 and -3 can bind all three small latent TGF-β isoforms; in contrast, LTBP-4 seems to bind only TGF-β1 (20, 87). LTBPs target the transport of the large latent TGF-β complex into the extracellular matrix (ECM), and thus LTBPs can regulate the activation process and in that way control the availability of TGF-βs (50). Several mechanisms have been described for the activation of different TGF-β isoforms, and the currently known physiologically important mechanisms are reviewed (see Fig. 1).

Thrombospondin-1-mediated TGF-β activation

Thrombospondin-1 and -2 are trimeric matricellular proteins involved in a variety of biologic processes including tissue remodeling and angiogenesis (16). Thrombospondins function through binding to other macromolecules such as proteoglycans, sulfated glycolipids, and integrins. Thrombospondin-1 can bind to both small and large latent TGF-β complexes and bring about the release of active TGF-β (76). A specific peptide sequence within thrombospondin-1, KRFK, mediates TGF-β activation through its association with a sequence (LSKL) near the amino terminus of LAP. The KRFK peptide, also referred to as the activating peptide, releases mature TGF-β by inducing conformational changes in the protein. The physiologic role of thrombospondin-1 in the activation of TGF-β is well illustrated by the overlapping phenotypes of TGF-β1 and thrombospondin-1 null mice. Treatment of mice with the TGF-β activating peptide of thrombospondin-1 can prevent the lung and pancreatic abnormalities observed in thrombospondin-1 null mice (26). However, the TGF-β1 null phenotype is severe and not fully overlapping with the thrombospondin-1 phenotype, emphasizing the importance for other TGF-β activating mechanisms. Thrombospondin-1 appears to mediate TGF-β activation in a rat model of renal disease, glomerulonephritis (29). Recently, thrombospondin-1 also was linked to angiotensin II-induced TGF-β activation in mesangial, cardiac, and renal cells (77, 114). In addition, angiotensin II receptor antagonism can prevent aortic aneurysms in a mouse model of Marfan syndrome, which is characterized by excessive TGF-β activation (46).

Integrins in TGF-β binding and activation

Many integrins can bind to the RGD sequence present in the LAP of TGF-β1 and -β3, but thus far only two, αvβ6 and αvβ8, have been shown to activate the TGF-β complex (74, 75). TGF-β2 lacks the critical RGD sequence, and therefore, it cannot be activated by integrins. Interestingly, activation by the two αv-containing integrins is carried out differently. The integrin αvβ6 activates large latent TGF-β complexes containing LTBP-1 by a fibronectin-dependent mechanism. Matrix targeting of the complex mediated by fibronectin in addition to LAP-mediated interaction with the cell surface integrin is believed to create the traction necessary for the release of mature TGF-β (2, 38, 56). Under normal conditions, epithelium-specific expression of αvβ6 is present, but after tissue injury, the expression levels increase rapidly (17). Mice lacking β6 integrins develop excessive inflammation but are protected from pulmonary fibrosis because of their decreased TGF-β activation, emphasizing the importance *in vivo* of integrin-mediated TGF-β activation (75). The role of the blood coagulation cascade in the development of acute lung injury in mice may be partially due to the proteinase-activated receptor 1 (PAR1)-mediated enhancement of αvβ6-dependent TGF-β activation (54).

In contrast to αvβ6-mediated TGF-β activation, proteolytic activity is necessary for TGF-β activation by αvβ8 (74). A com-

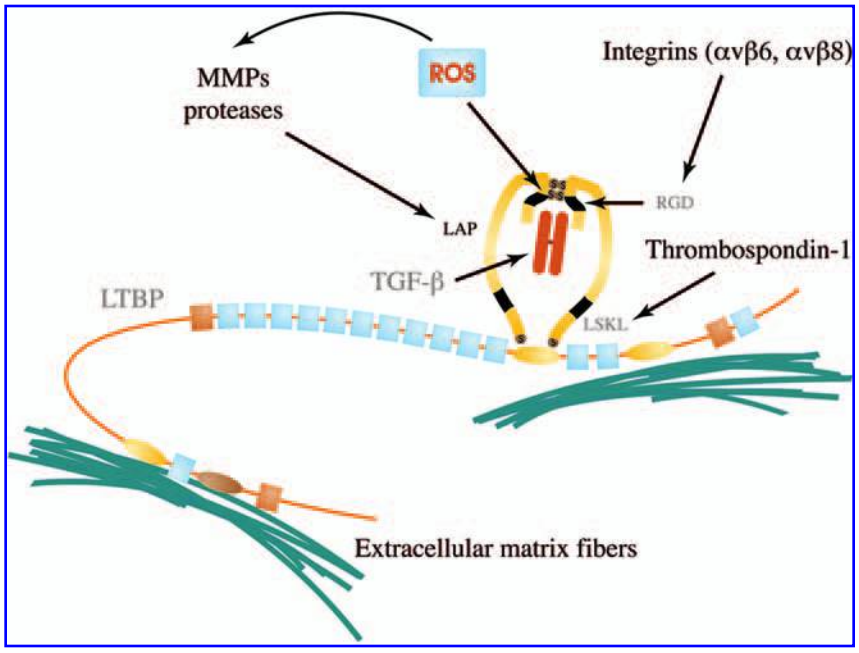

FIG. 1. Activation of latent TGF-β complexes. Large latent TGF-β consists of the mature 25-kDa polypeptide dimer (TGF-β), latency associated protein (LAP), and latent TGF-β binding protein (LTBP). The complex is targeted to the extracellular matrix structures *via* LTBP. Disruption of the non-covalent association between TGF-β and LAP is needed for the release of the active TGF-β, which is then able to bind to the cell-surface receptors. Thrombospondin-1 binds to the LSKL sequence in LAP, which leads to conformational changes and TGF-β activation. Integrins, in turn, bind to the RGD recognition sequence in LAP, leading to release of TGF-β either by tractional force directed against the TGF-β complex (αvβ6) or by MT1-MMP-dependent proteolytic activity (αvβ8). Reactive oxygen species (ROS) can activate TGF-β directly through oxidation induced conformational change in LAP or indirectly though the activation of proteolytic enzymes.

plex made up of the integrin, latent TGF-β, and membrane-type 1 matrix metalloproteinase (MT1-MMP) is formed at the cell surface, and a proteolytic event leads to the release of active TGF-β. This type of activation seems to be important for epithelial homeostasis in the lung, because normal human airways express all of these three proteins. During development, both αvβ6- and αvβ8-mediated TGF-β activation mechanisms appear to play important roles in epithelial mesenchymal interactions of the lung (5). The expression of αvβ8 is often lost during malignant transformation, and re-establishment of αvβ8 expression in lung carcinoma cells reduced tumor growth in nude mice (74). The contribution of RGD-binding integrins to TGF-β activation in the mouse was recently analyzed by using a TGF-β1 gene mutation, which encodes a nonfunctional integrin-binding site (RGE instead of RGD). Mice expressing this mutation displayed severe abnormalities related to the TGF-β1 null phenotype, including abnormal control of inflammation and vasculogenesis (108). These observations further emphasize the importance *in vivo* of integrins in TGF-β activation, although they also clearly point to the existence of multiple activation mechanisms. Other integrins, including αvβ5 and αvβ3, can also contribute to TGF-β activation in fibrotic fibroblasts (7, 8). Whether these integrins act directly to activate TGF-β or have indirect roles in the process is still an open question.

TGF-β activation by proteolysis

The serine protease plasmin was one of the first molecules found to cause activation of TGF-β (69). The proteolytic cleavage of LAP releases mature TGF-β, which is then free to associate with its cell surface receptors. Plasmin also can release the large latent TGF-β complex from the ECM by cleaving LTBP at the amino terminal hinge region (95). Release of the truncated complex is not necessarily associated with increased TGF-β activation, suggesting that ECM release and activation can be regulated independently. Proteolytic release of ECM-bound TGF-β complexes may also enhance activation by other mechanisms, or alternatively, they may block activation because of incorrect targeting of the complexes. The role of plasmin in TGF-β activation has been demonstrated in relation to the dissolution of blood clots during wound healing (44), as well as in smooth muscle cell function (3, 41). Interestingly, mice deficient in plasminogen, which is the inactive precursor of plasmin, display no TGF-β deficiency-related phenotypes, evidence that redundant mechanisms for TGF-β activation must exist (86). In addition to plasmin, many other proteases have been linked to the release or activation or both of TGF-β complexes. These proteases include thrombin, mast cell chymase, leukocyte elastase, and prostate specific antigen (PSA), as well as bone morphogenetic protein (BMP)-1 (28, 40, 96).

Matrix metalloproteinase activity has been implicated in both physiologic and pathologic tissue processes involved in matrix remodeling. The role of MT1-MMP in αvβ8-mediated TGF-β activation was described earlier. However, MMP-2 and -9 have also been implicated in the cleavage of LAP and the release of mature TGF-β (110). Cell surface localization of the complexes and the activation machinery plays a major role in the activation process. CD44, the hyaluronan receptor, was identified as a targeting protein for MMPs, and this is plausibly important in malignant cell growth and tumor angiogenesis (110). MMP activity induced by ROS and RNS in the lung could therefore play a role in the excessive TGF-β activation associated with pathologic conditions such as idiopathic pulmonary fibrosis (IPF) or other fibrotic diseases. TGF-β can also regulate the expression and activation of MMPs, as well as their inhibitors, TIMPs, and in this way, it can contribute to the proteolytic microenvironment in the lung.

ROS-mediated activation of TGF-β

Ionizing radiation was found to induce rapid TGF-β activation *in vivo* (12). ROS produced by ionizing radiation proved to be the direct mediators of the activation process, which in this case does not involve any other proteins. Interestingly, ROS-mediated TGF-β activation is specific for the TGF-β1 isoform, and the unique methionine residue (253) in the TGF-β1/LAP functions as a redox switch center (55). Specifically, elevated levels of HO. were critical for the oxidation of LAP, and these radicals triggered the conformational change leading to release of mature TGF-β. Radiation-induced lung injury, as well as tissue responses to inflammation, can elevate ROS production, which may contribute to TGF-β activation. In addition, ROS produced by solar UV radiation can evoke TGF-β activation and biosynthesis in skin keratinocytes (104). Asbestos fibers carrying iron are known to induce ROS production through Fenton reactions in cellular and cell-free systems. TGF-β activation induced by asbestos is probably mediated through ROS (81). Lung diseases induced by asbestos exposure, such as mesothelioma and asbestosis, are characterized by excessive TGF-β activation (52, 67). Another example of the role of ROS in TGF-β activation is the TGF-β-related lens phenotype of peroxiredoxin 6 (Prx6) null mice (34). Prx6 is one of the cellular enzymes that participate in the defense against oxidative damage. In the absence of this enzyme, mouse lens epithelial cells produce excessive amounts of ROS and active TGF-β, leading to elevated levels of cataractogenic markers.

TGF-β SIGNAL-TRANSDUCTION MECHANISMS

Smad-dependent and -independent signaling

Many different cellular responses are elicited by TGF-β, and these are often cell-type specific (71). We are only beginning to understand the complicated signaling patterns triggered by cellular exposure to TGF-β. The classic response pathway involves Smad-mediated changes in target gene transcription. Other signaling pathways, including MAP kinases, PI3 kinase, and Rho-like GTPases, can modulate Smad-dependent responses as well as elicit Smad-independent responses (32). In addition, c-Abl tyrosine kinase has been identified as one mediator of Smad-independent TGF-β signaling (30). Figure 2 summarizes central TGF-β-induced intracellular signaling pathways.

The cell surface TGF-β receptor complex is composed of type I and II serine/threonine kinases (36) (see Fig. 2). TGF-β binds first to the type II receptor (TGF-βRII), after which the type I receptor (TGF-βRI, ALK-5) is recruited to the complex and activated by a phosphorylation event. The regulatory Smads

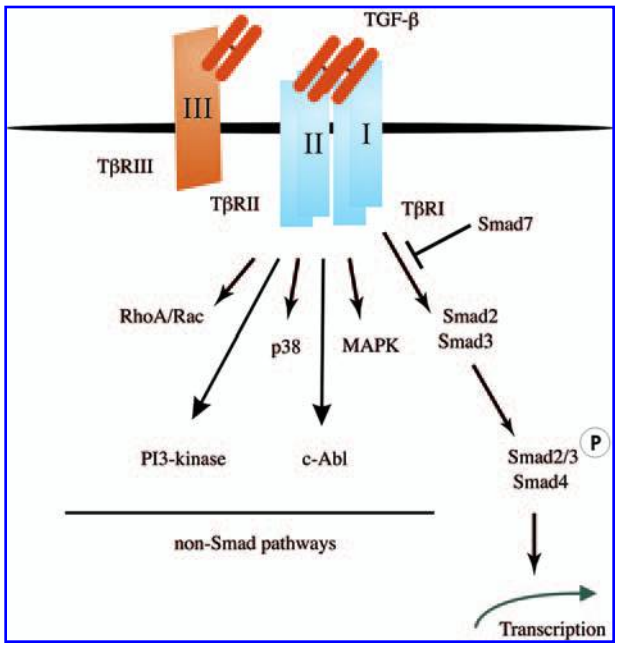

FIG. 2. TGF-β receptor signaling. The TGF-β receptor complex is composed of type I and II serine/threonine kinases. Accessory receptors, such as betaglycan or endoglin (TβRIII) can present TGF-β to the signaling receptors. The constitutively active TGF-β type II receptor (TβRII) phosphorylates the type I receptor (TβRI) on specific serine and threonine residues. Activated TβRI, in turn, directs the signal downstream by directly phosphorylating Smad2 and Smad3. Smad2 and Smad3 form complexes with Smad4, which, in combination with transcription factors, regulate gene transcription. Smad7 is an inhibitor of this pathway. Alternatively, Smad-independent pathways can be activated.

2 and 3 are phosphorylated by TGF-βRI and transported in a complex with Smad4 into the nucleus, where they can cooperate with the transcription machinery and alter gene transcription. The shuttling of Smad proteins between the nucleus and the cytoplasm, as well as their rapid turnover through ubiquitin-mediated degradation, are important as regulators of the TGF-β signaling. Specific Smad-binding elements have been identified in the promoter regions of target genes and, for example, cooperation with the AP-1 transcription factor has been described in the regulation of gene transcription (103). In endothelial cells, the ALK-1 receptor, when present in a complex with TGF-βRII, can mediate TGF-β signaling through the regulatory Smad1 (43).

TGF-β-induced ROS production

Active research on the role of ROS in cellular signaling has produced exciting new signaling paradigms. Many growth factors can induce intracellular production of ROS, which can then regulate redox-sensitive proteins *via* cysteine oxidation (100). Well-known examples of ROS-induced alterations in signaling proteins are the inactivation of protein tyrosine phosphatases (85) and the modulation of AP-1 transcription factor activity (68). TGF-β can induce intracellular ROS production through the NADPH oxidase catalytic subunit Nox4. A biphasic ROS induction has been described, in which the rapid response is believed to be mediated through Nox4 activation and targeting to the cell membranes, whereas the slower and sustained response is associated with increased expression of Nox4 (49, 99). Cellular processes regulated by TGF-β-induced ROS include the proliferation of pulmonary artery smooth muscle cells, cytoskeletal alterations in endothelial cells, and differentiation of cardiac fibroblasts as well as angiogenesis and EMT (epithelial-to-mesenchymal transition)-related processes (27, 49, 93). TGF-β target genes CTGF, PAI-1, and p21 are dependent on ROS induction, because Nox4 knockdown can prevent the induction of these genes by TGF-β. It is also possible that Smad2/3 phosphorylation is affected by Nox4-dependent ROS production (51). Interestingly, TGF-β can induce extracellular ROS production, which points to a role for ROS in intercellular communication and matrix protein modulation (98).

Cellular alterations in gene transcription induced by TGF-β are affected by signaling cross-talk between other pathways [(53), Fig. 3]. As described earlier, in addition to the classic Smad-signaling pathway, TGF-β itself can elicit changes in ROS production and MAPK activation. This creates flexibility for cell type–specific responses to TGF-β. In addition, growth-factor or stress-activated responses leading to alterations in ROS production and MAPK activation can alter cellular TGF-β responsiveness. For example, TGF-β stimulated expression of p21 is dependent on the Smad pathway as well as ROS-medi-

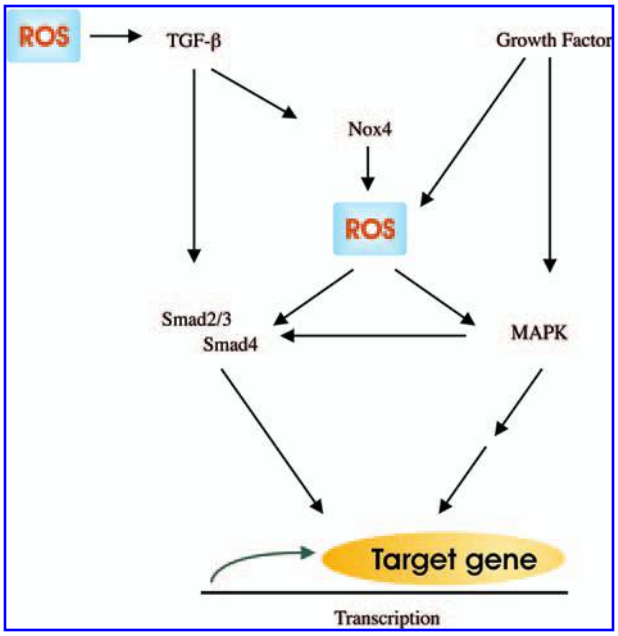

FIG. 3. TGF-β signaling cross-talk. In addition to being involved in TGF-β activation, reactive oxygen species (ROS) are part of the signal-transduction pathway leading to target gene transcription. TGF-β induces cellular ROS production through the NADPH oxidase catalytic subunit Nox4. ROS can alter the activation of Smads as well as mitogen-activated protein kinases (MAPKs). Cross-talk with other growth-factor pathways can also modify TGF-β target gene transcription.

ated ERK activation, indicative of a cooperative model for p21 induction in human keratinocytes (58).

REGULATION OF ANTIOXIDANT ENZYMES BY TGF-β

The lung possesses a cell-specific antioxidant battery, which is regulated by many different mechanisms, including cytokines and the cellular redox state (21, 22, 59, 60, 62). Only a few studies have examined the role of TGF-β in the regulation of antioxidant/detoxification enzymes; most of them point to no changes or a decrease in expression levels of antioxidant enzymes by TGF-β. This contrasts with the effects of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, which generally induce the expression of these enzymes both *in vitro* and *in vivo* (21, 59, 83, 84). Manganese superoxide dismutase (MnSOD) is induced by TNF-α, whereas some other cytokines do not seem to alter the expression of this enzyme (37). Moreover, severe hyperoxia results in decreased expression or inactivation of MnSOD in association with cell injury or lung fibrosis or both (18, 24, 63, 70). A possible downregulation of extracellular SOD (ECSOD) by TGF-β may occur in human alveolar epithelial cells (61). In addition, similar changes have been observed with epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and granulocyte macrophage colony stimulating factor (GM-CSF) in vascular smooth muscle cells (91).

Glutamate cysteine ligase (GCL), the rate-limiting enzyme in glutathione (GSH) synthesis, is induced by Nrf2-dependent transcriptional regulation through ARE (antioxidant response element) (21). The level of GCL, or at least the heavy subunit of this enzyme, is induced by oxidants and cytokines, but again, TGF-β1 downregulates the levels of GCL mRNA and immunoreactive protein (6, 102). The TGF-β induced decrease in the levels of the catalytic subunit of GCL correlates with a decrease in cellular GSH and an increase in cellular ROS (11). Interestingly, the TGF-β-dependent downregulation in ARE-dependent GCL expression is mediated by Smad3-ATF3. The expression of glutaredoxins (Grxs), another family of enzymes associated with the GSH homeostasis, is also decreased by TGF-β (80), further suggesting that TGF-β, in general, downregulates the expression of antioxidant enzymes. These *in vitro* results are in agreement with the low/absent immunoreactivities of MnSOD, ECSOD, GCL, thioredoxin, and Grx1 in the fibrotic lung lesions of patients with IPF (histopathology of usual interstitial pneumonia, UIP) (61, 65, 80, 101, 102) and the decline/proteolysis of ECSOD in experimental lung fibrosis (35). In addition to endogenous antioxidant enzymes, exogenously administered antioxidants such as SOD-mimetics evoke a significant reduction of TGF-β (51). Overall, the effects of TGF-β in combination with oxidative stress *in vivo* may have multiple consequences in the defense system of human lung.

TGF-β IN HUMAN FIBROTIC LUNG DISEASES

The lung tissue can respond to external factors (such as tobacco smoke) in various ways, leading either to parenchymal fibrosis (formation of excess connective tissue) or emphysema (loss of parenchymal tissue). The current conception of fibrosis and emphysema pathogenesis is summarized in Fig. 4.

Pulmonary fibrosis

Although the initial pathogenic factors and mechanisms behind IPF and other idiopathic parenchymal disorders are poorly understood, enhanced expression and activation of latent TGF-β is considered a hallmark of fibrosis progression. Genotypic variances that enhance the cellular TGF-β secretion might act as susceptibility factors for IPF and subsequent posttransplant pulmonary fibrosis (chronic rejection) in the same patient (9, 33). Active TGF-β has been detected from patients with fibrotic pulmonary disease, whereas in healthy individuals, TGF-β exists mostly in a latent form (14, 107). Figure 5 shows phosphorylated Smad2-positive cells in the alveolar epithelium

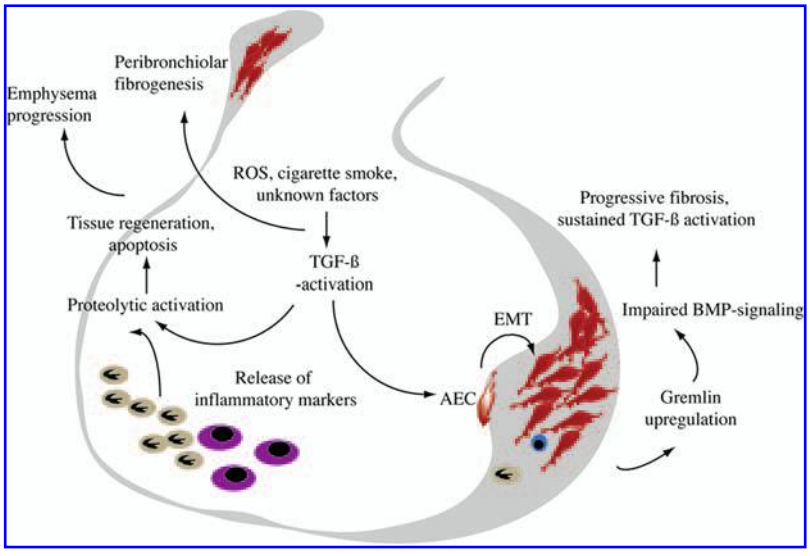

FIG. 4. Activation of TGF-β in the lung. Extracellular matrix–associated LTBP/TGF complexes are susceptible to activation by environmental factors. TGF-β activation can result in the chemotactic accumulation of inflammatory cells and fibroblasts, disturb the pericellular proteolytic balance, and induce epithelial-to-mesenchymal transition (EMT). Genetic, environmental, and unknown factors determine the outcome of TGF-β activation and subsequent events: either the accumulation of connective tissue to the alveolar wall (fibrosis) or the disappearance of alveolar structure (emphysema).

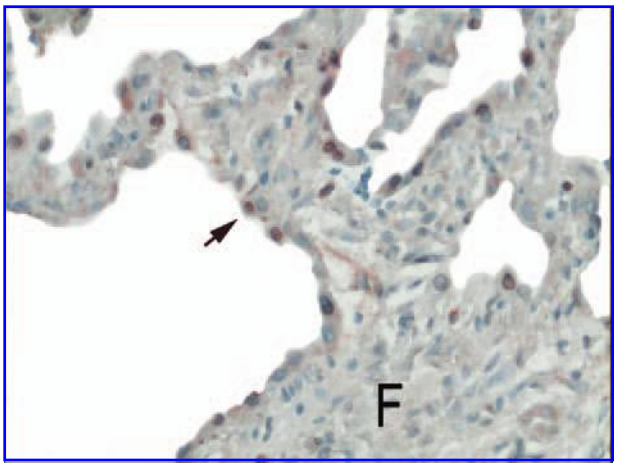

FIG. 5. Photomicrograph of an advanced lesion of a patient with IPF/UIP, stained with phosphorylated Smad2 antibody. An epithelial cell showing nuclear Smad2 positivity (red-brown) is pointed with an *arrow*. Activated, hyperplastic epithelial cells around the thickened lung parenchyma and a fibroblast focus (F) are seen, and most of these cells are Smad2 positive. Magnification ×400.

around a fibroblast focus (our unpublished results). In experimental animal models of pulmonary interstitial disease, such as the mouse bleomycin model, enhanced levels of TGF-β expression, activation, and downstream signaling have been observed (57, 88, 112). Several approaches have been used to interfere with ROS production and TGF-β activation. Animal studies on direct or indirect inhibition of TGF-β or its receptor have yielded numerous promising compounds, but these have not been tested in the treatment of human IPF. Knockout studies have shown that Smad3 is a central mediator of experimental fibrosis (113) and that overexpression of the inhibitory Smad7 can protect from fibrosis (78). TGF-β can also alter other growth-factor pathways, including BMP signaling during fibrosis development (64). Receptor tyrosine kinase inhibitors, developed mainly for cancer treatment, have been used to block TGF-β action directly [ALK 5 inhibitor (48)] or indirectly [imatinib, Abl tyrosine kinase inhibition (1, 30)]. Bearing in mind the connection between thrombospondin-1 induced and angiotensin II–induced TGF-β activation (77, 114), recent results obtained in mice provide interesting evidence that the angiotensin II type 1 receptor inhibitor losartan may have an antifibrotic effect (109). Although a large body of knowledge from vascular biology suggests that angiotensin II mediates smooth muscle cell and cardiac muscle structure alterations toward fibrotic remodeling, less attention has been paid to the potential role of angiotensin II in pulmonary fibrosis. In human IPF tissue, angiotensin peptides (angiotensin II precursors) are generated locally within the fibroblasts and alveolar type II epithelial cells, and they colocalize with apoptotic alveolar epithelium (66), indicating that they may have extravascular functions in the lung. The future/ongoing clinical studies on all of the previously mentioned drugs (tyrosine kinase inhibitors, AT/ACE-inhibitors) will be among the first human studies in which an antifibrotic approach instead of an antiinflammatory treatment strategy is used.

So far, positive preliminary results have been obtained in human studies with the antioxidant glutathione precursor *N*-acetylcysteine (NAC). Glutathione is a small-molecular-weight antioxidant abundantly available in the epithelial lining fluid of alveoli. It was initially thought that NAC might be able to restore the antioxidant capacity of the fibrotic lung, but more recently, other antifibrotic mechanisms of action have been proposed. *In vitro*, NAC induces TGF-β type II receptor and PDGF receptor proteolysis and TGF-β and PDGF ligand inactivation *via* monomerization (72, 79). Furthermore, NAC abrogates TGF-β induced Smad2/3 phosphorylation and induces Smad7 mRNA expression. Both mechanisms are mediated by the displacement of TGF-β from endoglin and ligand inactivation by monomerization in hepatic profibrogeneic cells (72). Another intensively studied repressor of profibrotic signaling, γ-interferon, although an indisputable TGF-β repressor in experimental studies (45), has been found to be disappointing in the clinic (4, 10).

Chronic obstructive pulmonary disease (COPD)

IPF is considered the prototype of human interstitial pneumonias that lead to a progressive increase in lung parenchymal ECM components. In addition to IPF, several other interstitial lung diseases exhibit areas of lung fibrosis that may have developed *via* TGF-β-mediated mechanisms. The lungs of patients with COPD, in contrast, display another type of lung parenchymal injury, in which cigarette smoke has induced the loss of tissue architecture and volume. Although major differences are found in the histopathology of human COPD and UIP/IPF, COPD offers some illuminating perspectives for research into lung fibrosis [reviewed in (39)]. In addition to the destruction of lung parenchyma (pulmonary emphysema), airway narrowing (obstruction) with peripheral airway fibrosis is observed. Airway fibrosis and peripheral/basal fibrotic lesions can also be detected in the parenchyma of the COPD patient's lungs. The emphysematous areas of COPD that consist of enlarged/destructed airspaces express several fibrosis associated matrix genes and proteases. This suggests that both their absolute levels and balance dictate in which direction the lung damage will proceed (either emphysema or fibrosis). In simplistic terms, when considering the outcome of TGF-β signaling and its regulation, it can be hypothesized that the regulatory pathways that are induced in fibrosis appear to be downregulated in the emphysematous lung.

Genetic association analyses have revealed a significant association in the single-nucleotide polymorphisms of the promoter and genomic regions of TGF-β and COPD (19). The latent TGF-β binding protein-4 (LTBP-4) and TGF-β1 are associated with variable COPD phenotypes (47). The emphysematous lung undergoes a significant decline in the TGF-β mRNA levels (42). Recent studies on the lung tissues of COPD patients have suggested lower TGF-β and TGF-β receptor expression in COPD lung, as assessed by semiquantitative immunohistochemical scoring (111), and decreased release of TGF-β from the macrophages of COPD patients (82). However, the situation is not necessarily the same in the distinctly fibrotic areas of the same COPD lung. Local areas of the lungs reveal increased expression of TGF-β (RT-PCR) in the bronchial airways of patients compared with healthy smokers

and upregulation of TGF-β type II receptor in the pulmonary arteries of severe COPD (13) pointing to a role for TGF-β, at least in the development of airway fibrosis and pulmonary arterial hypertension in COPD (31, 97). These findings from human lungs are in line with the observations of increased TGF-β in the airways of smoke-exposed mice (23), and the release of active TGF-β and nuclear immunostaining of phosphorylated Smad2 after the exposure of rat tracheal explants to cigarette smoke (106). Importantly, those changes could be prevented by the oxidant scavenger tetramethylthiourea. Relatively minor, if any, changes in the expression of Smads 2-4 have been found in COPD, whereas a significant downregulation of Smad 6/7 is found, suggesting that also intracellular feedback mechanisms in TGF-β signaling are disturbed in COPD (90).

The effects of cigarette smoke have been widely investigated *in vitro*. These studies have revealed variable effects on the release/activation of TGF-β, depending on the duration and dose of the exposure, as well as the density of the cultured cells (105). Observations of experimental pulmonary emphysema may also be difficult to extrapolate directly to human COPD, because many studies describe developmental changes/septal formation and the development of emphysema-like lesions without cigarette-smoke exposure. However, these studies have revealed important associations between TGF-β homeostasis and alveolar formation/emphysema. TGF-β null mice die of inflammation within 1 month after birth, which has limited their use for the investigation of emphysema (89). Mice homozygous for the disrupted allele for LTBP-4 develop severe emphysema, profound defects in the structure of their elastic fibers, and reduced deposition of TGF-β and phosphorylated Smad2 proteins in their epithelial cells (92). Interestingly, LTBP-3 deficiency, with a temporary decrease in TGF-β signaling, correlates with the inhibition of septation and the development of emphysema (25). These findings are in line with the loss of integrin-mediated activation of TGF-β and age-dependent pulmonary emphysema through alterations in the expression of matrix metalloproteinase-12 [metalloelastase, a proteinase associated with smoking-related emphysema (73)]. Overall, these and related studies (15) suggest that a balance in the activities of the TGF-β and Smad3 is required to maintain the alveolar integrity so that excessive signaling results in fibrosis (excessive repair) or defective signaling in destruction (lack of repair). The glutathione precursor *N*-acetylcysteine (NAC) has been investigated in relation to COPD. This compound induces GSH synthesis, decreases TGF-β induced Smad2/3 phosphorylation, and induces Smad7 mRNA expression. The results with NAC have been equivocal, but in systematic analysis, it did prove to be beneficial in a subset of patients with COPD (94).

CONCLUSIONS

Although TGF-β activation is considered to be a central mechanism in the pathogenesis of many diseases, the exact mechanisms of TGF-β expression and activation, its tissue responses, as well as the regulation of ROS in the human lung are still incompletely understood. Experimental and clinical studies have revealed complex oxidative pathways involved in TGF-β activation and signal transduction, which may be related to the initiation or progression of pulmonary disorders such as IPF and COPD. Clearly, an understanding of such pathways and their reciprocal regulation is crucial before one can attain specific drug development for these intractable diseases. Simplified mouse models of pulmonary fibrosis and COPD provide novel mechanistic insights into lung enzyme and growth factor networks, whereas novel methods for the analysis of human tissues generate an increasing amount of information on the molecular background of the pathogenesis of fibrosis. When results from animal models and clinical studies are summarized, it seems that TGF-β-mediated and ROS-mediated profibrotic signals can be considered putative targets for the treatment of pulmonary fibrosis.

ACKNOWLEDGMENTS

This work was supported by the Academy of Finland, Finnish Cancer Foundation, Finnish Cultural Foundation, Sigrid Juselius Foundation, Biocentrum Helsinki, Helsinki University Hospital Fund, the University of Helsinki, the Finnish Antituberculosis Association Foundation, the Finnish Medical Foundation, the Jalmari and Rauha Ahokas Foundation, and the Yrjo Jahnsson Foundation.

ABBREVIATIONS

ARE, antioxidant response element; BMP, bone morphogenetic protein; COPD, chronic obstructive pulmonary disease; ECSOD, extracellular superoxide dismutase; EGF, epidermal growth factor; GCL, glutamate cysteine ligase; GM-CSF, granulocyte–macrophage colony-stimulating factor; GSH, glutathione; Grx, glutaredoxins; IPF, idiopathic pulmonary fibrosis; LAP, latency-associated protein; LTBP, latent TGF-β–binding protein; MT1-MMP, membrane-type 1 matrix metalloproteinase; MMP, matrix metalloproteinase; NAC, *N*-acetylcysteine; PAR1, proteinase-activated receptor 1; PSA, prostate-specific antigen; PDGF, platelet-derived growth factor; Prx, peroxiredoxin; RNS, reactive nitrogen species; ROS, reactive oxygen species; MnSOD, manganese superoxide dismutase; TGF-β, transforming growth factor β; TIMP, tissue inhibitor of matrix metalloproteinase; UIP, usual interstitial pneumonia.

REFERENCES

1. Abdollahi A, Li M, Ping G, Plathow C, Domhan S, Kiessling F, Lee LB, McMahon G, Grone HJ, Lipson KE, and Huber PE. Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis. *J Exp Med* 201: 925–935, 2005.
2. Annes JP, Chen Y, Munger JS, and Rifkin DB. Integrin αvβ6-mediated activation of latent TGF-β requires the latent TGF-β binding protein-1. *J Cell Biol* 165: 723–734, 2004.
3. Antonelli-Orlidge A, Saunders K, Smith S, and D'Amore P. An activated form of transforming growth factor-β is produced by cocultures of endothelial cells and pericytes. *Proc Natl Acad Sci U S A* 86: 4544–4548, 1989.
4. Antoniou KM, Nicholson AG, Dimadi M, Malagari K, Latsi P, Rapti A, Tzanakis N, Trigidou R, Polychronopoulos V, and Bouros D. Long-term clinical effects of interferon gamma-1β and

colchicine in idiopathic pulmonary fibrosis. *Eur Respir J* 28: 496–504, 2006.
5. Araya J, Cambier S, Morris A, Finkbeiner W, and Nishimura SL. Integrin-mediated transforming growth factor-β activation regulates homeostasis of the pulmonary epithelial-mesenchymal trophic unit. *Am J Pathol* 169: 405–415, 2006.
6. Arsalane K, Dubois CM, Muanza T, Begin R, Boudreau F, Asselin C, and Cantin AM. Transforming growth factor-β1 is a potent inhibitor of glutathione synthesis in the lung epithelial cell line A549: transcriptional effect on the GSH rate-limiting enzyme gamma-glutamylcysteine synthetase. *Am J Respir Cell Mol Biol* 17: 599–607, 1997.
7. Asano Y, Ihn H, Yamane K, Jinnin M, Mimura Y, and Tamaki K. Involvement of $\alpha v\beta 5$ integrin-mediated activation of latent transforming growth factor-β1 in autocrine transforming growth factor-β signaling in systemic sclerosis fibroblasts. *Arthritis Rheum* 52: 2897–2905, 2005.
8. Asano Y, Ihn H, Yamane K, Jinnin M, Mimura Y, and Tamaki K. Increased expression of integrin $\alpha v\beta 3$ contributes to the establishment of autocrine TGF-β signaling in scleroderma fibroblasts. *J Immunol* 175: 7708–7718, 2005.
9. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, and Hutchinson IV. Genotypic variation in the transforming growth factor-β1 gene: association with transforming growth factor-β1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation* 66: 1014–1020, 1998.
10. Bajwa EK, Ayas NT, Schulzer M, Mak E, Ryu JH, and Malhotra A. Interferon-gamma1b therapy in idiopathic pulmonary fibrosis: a meta-analysis. *Chest* 128: 203–206, 2005.
11. Bakin AV, Stourman NV, Sekhar KR, Rinehart C, Yan X, Meredith MJ, Arteaga CL, and Freeman ML. Smad3-ATF3 signaling mediates TGF-β suppression of genes encoding Phase II detoxifying proteins. *Free Radic Biol Med* 38: 375–387, 2005.
12. Barcellos-Hoff M and Dix T. Redox-mediated activation of latent transforming growth factor-β1. *Mol Endocrinol* 10: 1077–1083, 1996.
13. Beghe B, Bazzan E, Baraldo S, Calabrese F, Rea F, Loy M, Maestrelli P, Zuin R, Fabbri LM, and Saetta M. Transforming growth factor-β type II receptor in pulmonary arteries of patients with very severe COPD. *Eur Respir J* 28: 556–562, 2006.
14. Bergeron A, Soler P, Kambouchner M, Loiseau P, Milleron B, Valeyre D, Hance AJ, and Tazi A. Cytokine profiles in idiopathic pulmonary fibrosis suggest an important role for TGF-β and IL-10. *Eur Respir J* 22: 69–76, 2003.
15. Bonniaud P, Kolb M, Galt T, Robertson J, Robbins C, Stampfli M, Lavery C, Margetts PJ, Roberts AB, and Gauldie J. Smad3 null mice develop airspace enlargement and are resistant to TGF-β-mediated pulmonary fibrosis. *J Immunol* 173: 2099–2108, 2004.
16. Bornstein P. Thrombospondins as matricellular modulators of cell function. *J Clin Invest* 107: 929–934, 2001.
17. Breuss JM, Gillett N, Lu L, Sheppard D, and Pytela R. Restricted distribution of integrin β6 mRNA in primate epithelial tissues. *J Histochem Cytochem* 41: 1521–1527, 1993.
18. Briehl MM, Cotgreave IA, and Powis G. Downregulation of the antioxidant defence during glucocorticoid-mediated apoptosis. *Cell Death Differ* 2: 41–46, 1995.
19. Celedon JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, Reilly JJ, Kwiatkowski DJ, Chapman HA, Laird N, Sylvia JS, Hernandez M, Speizer FE, Weiss ST, and Silverman EK. The transforming growth factor-β1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet* 13: 1649–1656, 2004.
20. Chen Y, Ali T, Todorovic V, O'Leary JM, Downing AK, and Rifkin DB. Amino acid requirements for formation of the TGF-β-latent TGF-β binding protein complexes. *J Mol Biol* 345: 175–186, 2005.
21. Cho HY, Reddy SP, and Kleeberger SR. Nrf2 defends the lung from oxidative stress. *Antioxid Redox Signal* 8: 76–87, 2006.
22. Cho HY, Reddy SP, Yamamoto M, and Kleeberger SR. The transcription factor NRF2 protects against pulmonary fibrosis. *FASEB J* 18: 1258–1260, 2004.
23. Churg A, Tai H, Coulthard T, Wang R, and Wright JL. Cigarette smoke drives small airway remodeling by induction of growth factors in the airway wall. *Am J Respir Crit Care Med* 174: 1327–1334, 2006.
24. Clerch LB, Massaro D, and Berkovich A. Molecular mechanisms of antioxidant enzyme expression in lung during exposure to and recovery from hyperoxia. *Am J Physiol* 274: L313–L319, 1998.
25. Colarossi C, Chen Y, Obata H, Jurukovski V, Fontana L, Dabovic B, and Rifkin DB. Lung alveolar septation defects in Ltbp-3-null mice. *Am J Pathol* 167: 419–428, 2005.
26. Crawford SE, Stellmach V, Murphy-Ullrich JE, Ribeiro SM, Lawler J, Hynes RO, Boivin GP, and Bouck N. Thrombospondin-1 is a major activator of TGF-β1 in vivo. *Cell* 93: 1159–1170, 1998.
27. Cucoranu I, Clempus R, Dikalova A, Phelan PJ, Ariyan S, Dikalov S, and Sorescu D. NAD(P)H oxidase 4 mediates transforming growth factor-β1-induced differentiation of cardiac fibroblasts into myofibroblasts. *Circ Res* 97: 900–907, 2005.
28. Dallas SL, Zhao S, Cramer SD, Chen Z, Peehl DM, and Bonewald LF. Preferential production of latent transforming growth factor-β2 by primary prostatic epithelial cells and its activation by prostate-specific antigen. *J Cell Physiol* 202: 361–370, 2005.
29. Daniel C, Wiede J, Krutzsch HC, Ribeiro SM, Roberts DD, Murphy-Ullrich J, and Hugo C. Thrombospondin-1 is a major activator of TGF-β in fibrotic renal disease in the rat in vivo. *Kidney Int* 65: 459–468, 2004.
30. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, and Leof EB. Imatinib mesylate inhibits the profibrogenic activity of TGF-β and prevents bleomycin-mediated lung fibrosis. *J Clin Invest* 114: 1308–1316, 2004.
31. De Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, and 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* 158: 1951–1957, 1998.
32. Derynck R and Zhang YE. Smad-dependent and Smad-independent pathways in TGF-β family signalling. *Nature* 425: 577–584, 2003.
33. El-Gamel A, Awad MR, Hasleton PS, Yonan NA, Hutchinson JA, Campbell CS, Rahman AH, Deiraniya AK, Sinnott PJ, and Hutchinson IV. Transforming growth factor-β (TGF-β1) genotype and lung allograft fibrosis. *J Heart Lung Transplant* 18: 517–523, 1999.
34. Fatma N, Kubo E, Sharma P, Beier DR, and Singh DP. Impaired homeostasis and phenotypic abnormalities in Prdx6-/- mice lens epithelial cells by reactive oxygen species: increased expression and activation of TGF-β. *Cell Death Differ* 12: 734–750, 2005.
35. Fattman CL, Chu CT, Kulich SM, Enghild JJ, and Oury TD. Altered expression of extracellular superoxide dismutase in mouse lung after bleomycin treatment. *Free Radic Biol Med* 31: 1198–1207, 2001.
36. Feng XH and Derynck R. Specificity and versatility in TGF-β signaling through Smads. *Annu Rev Cell Dev Biol* 21: 659–693, 2005.
37. Flanders KC, Bhandiwad AR, and Winokur TS. Transforming growth factor-βs block cytokine induction of catalase and xanthine oxidase mRNA levels in cultured rat cardiac cells. *J Mol Cell Cardiol* 29: 273–280, 1997.
38. Fontana L, Chen Y, Prijatelj P, Sakai T, Fässler R, Sakai LY, and Rifkin DB. Fibronectin is required for integrin $\alpha v\beta 6$-mediated activation of latent TGF-β complexes containing LTBP-1. *FASEB J* 19: 1798–1808, 2005.
39. Gauldie J, Kolb M, Ask K, Martin G, Bonniaud P, and Warburton D. Smad3 signaling involved in pulmonary fibrosis and emphysema. *Proc Am Thorac Soc* 3: 696–702, 2006.
40. Ge G and Greenspan DS. BMP1 controls TGF-β1 activation via cleavage of latent TGF-β-binding protein. *J Cell Biol* 175: 111–120, 2006.
41. George SJ, Johnson JL, Smith MA, Angelini GD, and Jackson CL. Transforming growth factor-β is activated by plasmin and inhibits smooth muscle cell death in human saphenous vein. *J Vasc Res* 42: 247–254, 2005.
42. Golpon HA, Coldren CD, Zamora MR, Cosgrove GP, Moore MD, Tuder RM, Geraci MW, and Voelkel NF. Emphysema lung tissue gene expression profiling. *Am J Respir Cell Mol Biol* 31: 595–600, 2004.

43. Goumans MJ, Valdimarsdottir G, Itoh S, Lebrin F, Larsson J, Mummery C, Karlsson S, and ten Dijke P. Activin receptor-like kinase (ALK)1 is an antagonistic mediator of lateral TGF-β/ALK5 signaling. *Mol Cell* 12: 817–828, 2003.
44. Grainger DJ, Wakefield L, Bethell HW, Farndale RW, and Metcalfe JC. Release and activation of platelet latent TGF-β in blood clots during dissolution with plasmin. *Nat Med* 1: 932–937, 1995.
45. Gurujeyalakshmi G and Giri SN. Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-β and procollagen I and III gene expression. *Exp Lung Res* 21: 791–808, 1995.
46. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi C, Podowski M, Neptune ER, Halushka MK, Bedja D, Gabrielson K, Rifkin DB, Carta L, Ramirez F, Huso DL, and Dietz HC. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 312: 117–121, 2006.
47. Hersh CP, Demeo DL, Lazarus R, Celedon JC, Raby BA, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, and Silverman EK. Genetic association analysis of functional impairment in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 173: 977–984, 2006.
48. Higashiyama H, Yoshimoto D, Kaise T, Matsubara S, Fujiwara M, Kikkawa H, Asano S, and Kinoshita M. Inhibition of activin receptor-like kinase 5 attenuates bleomycin-induced pulmonary fibrosis. *Exp Mol Pathol* 83: 39–46, 2006.
49. Hu T, Ramachandrarao SP, Siva S, Valancius C, Zhu Y, Mahadev K, Toh I, Goldstein BJ, Woolkalis M, and Sharma K. Reactive oxygen species production via NADPH oxidase mediates TGF-β-induced cytoskeletal alterations in endothelial cells. *Am J Physiol Renal Physiol* 289: F816–F825, 2005.
50. Hyytiäinen M, Penttinen C, and Keski-Oja J. Latent TGF-β binding proteins: extracellular matrix association and roles in TGF-β activation. *Crit Rev Clin Lab Sci* 41: 233–264, 2004.
51. Jackson IL, Chen L, Batinic-Haberle I, and Vujaskovic Z. Superoxide dismutase mimetic reduces hypoxia-induced O2*-, TGF-β, and VEGF production by macrophages. *Free Radic Res* 41: 8–14, 2007.
52. Jagirdar J, Lee TC, Reibman J, Gold LI, Aston C, Begin R, and Rom WN. Immunohistochemical localization of transforming growth factor-β isoforms in asbestos-related diseases. *Environ Health Perspect* 105(suppl 5): 1197–1203, 1997.
53. Javelaud D and Mauviel A. Crosstalk mechanisms between the mitogen-activated protein kinase pathways and Smad signaling downstream of TGF-β: implications for carcinogenesis. *Oncogene* 24: 5742–5750, 2005.
54. Jenkins RG, Su X, Su G, Scotton CJ, Camerer E, Laurent GJ, Davis GE, Chambers RC, Matthay MA, and Sheppard D. Ligation of protease-activated receptor 1 enhances αvβ6 integrin-dependent TGF-β activation and promotes acute lung injury. *J Clin Invest* 116: 1606–1614, 2006.
55. Jobling MF, Mott JD, Finnegan MT, Jurukovski V, Erickson AC, Walian PJ, Taylor SE, Ledbetter S, Lawrence CM, Rifkin DB, and Barcellos-Hoff MH. Isoform-specific activation of latent transforming growth factor-β (LTGF-β) by reactive oxygen species. *Radiat Res* 166: 839–848, 2006.
56. Keski-Oja J, Koli K, and von Melchner H. TGF-β activation by traction? *Trends Cell Biol* 14: 657–659, 2004.
57. Khalil N, Parekh TV, O'Connor RN, and Gold LI. Differential expression of transforming growth factor-β type I and II receptors by pulmonary cells in bleomycin-induced lung injury: correlation with repair and fibrosis. *Exp Lung Res* 28: 233–250, 2002.
58. Kim YK, Bae GU, Kang JK, Park JW, Lee EK, Lee HY, Choi WS, Lee HW, and Han JW. Cooperation of H2O2-mediated ERK activation with Smad pathway in TGF-β1 induction of p21WAF1/Cip1. *Cell Signal* 18: 236–243, 2006.
59. Kinnula VL and Crapo JD. Superoxide dismutases in the lung and human lung diseases. *Am J Respir Crit Care Med* 167: 1600–1619, 2003.
60. Kinnula VL, Crapo JD, and Raivio KO. Generation and disposal of reactive oxygen metabolites in the lung. *Lab Invest* 73: 3–19, 1995.
61. Kinnula VL, Hodgson UA, Lakari EK, Tan RJ, Sormunen RT, Soini YM, Kakko SJ, Laitinen TH, Oury TD, and Pääkkö PK. Extracellular superoxide dismutase has a highly specific localization in idiopathic pulmonary fibrosis/usual interstitial pneumonia. *Histopathology* 49: 66–74, 2006.
62. Kinnula VL, Vuorinen K, Ilumets H, Rytilä P, and Myllärniemi M. Thiol proteins, redox modulation and parenchymal lung disease. *Curr Med Chem* 14: 213–222, 2007.
63. Knirsch L and Clerch LB. Tyrosine phosphorylation regulates manganese superoxide dismutase (MnSOD) RNA-binding protein activity and MnSOD protein expression. *Biochemistry* 40: 7890–7895, 2001.
64. Koli K, Myllärniemi M, Vuorinen K, Salmenkivi K, Ryynänen MJ, Kinnula VL, and Keski-Oja J. Bone morphogenetic protein-4 inhibitor gremlin is overexpressed in idiopathic pulmonary fibrosis. *Am J Pathol* 169: 61–71, 2006.
65. Lakari E, Pääkkö P, and Kinnula VL. Manganese superoxide dismutase, but not CuZn superoxide dismutase, is highly expressed in the granulomas of pulmonary sarcoidosis and extrinsic allergic alveolitis. *Am J Respir Crit Care Med* 158: 589–596, 1998.
66. Li X, Molina-Molina M, Abdul-Hafez A, Ramirez J, Serrano-Mollar A, Xaubet A, and Uhal BD. Extravascular sources of lung angiotensin peptide synthesis in idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 291: L887–L895, 2006.
67. Liu JY and Brody AR. Increased TGF-β1 in the lungs of asbestos-exposed rats and mice: reduced expression in TNF-α receptor knockout mice. *J Environ Pathol Toxicol Oncol* 20: 97–108, 2001.
68. Lo YY and Cruz TF. Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. *J Biol Chem* 270: 11727–11730, 1995.
69. Lyons R, Keski-Oja J, and Moses H. Proteolytic activation of latent transforming growth factor-β from fibroblast-conditioned medium. *J Cell Biol* 106: 1659–1665, 1988.
70. MacMillan-Crow LA, Crow JP, and Thompson JA. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. *Biochemistry* 37: 1613–1622, 1998.
71. Massagué J. How cells read TGF-β signals. *Nat Rev Mol Cell Biol* 1: 169–178, 2000.
72. Meurer SK, Lahme B, Tihaa L, Weiskirchen R, and Gressner AM. N-acetyl-L-cysteine suppresses TGF-β signaling at distinct molecular steps: the biochemical and biological efficacy of a multifunctional, antifibrotic drug. *Biochem Pharmacol* 70: 1026–1034, 2005.
73. Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, and Sheppard D. Loss of integrin αvβ6-mediated TGF-β activation causes Mmp12-dependent emphysema. *Nature* 422: 169–173, 2003.
74. Mu D, Cambier S, Fjellbirkeland L, Baron JL, Munger JS, Kawakatsu H, Sheppard D, Broaddus VC, and Nishimura SL. The integrin αvβ8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-β1. *J Cell Biol* 157: 493–507, 2002.
75. Munger J, Huang X, Kawakatsu H, Griffiths M, Dalton S, Wu J, Pittet J-F, Kaminski N, Garat C, Matthay M, Rifkin D, and Sheppard D. The integrin αvβ6 binds and activates latent TGF-β1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 96: 319–328, 1999.
76. Murphy-Ullrich JE, and Poczatek M. Activation of latent TGF-β by thrombospondin-1: mechanisms and physiology. *Cytokine Growth Factor Rev* 11: 59–69, 2000.
77. Naito T, Masaki T, Nikolic-Paterson DJ, Tanji C, Yorioka N, and Kohno N. Angiotensin II induces thrombospondin-1 production in human mesangial cells via p38 MAPK and JNK: a mechanism for activation of latent TGF-β1. *Am J Physiol Renal Physiol* 286: F278–F287, 2004.
78. Nakao A, Fujii M, Matsumura R, Kumano K, Saito Y, Miyazono K, and Iwamoto I. Transient gene transfer and expression of Smad7 prevents bleomycin-induced lung fibrosis in mice. *J Clin Invest* 104: 5–11, 1999.
79. Okuyama H, Shimahara Y, Kawada N, Seki S, Kristensen DB, Yoshizato K, Uyama N, and Yamaoka Y. Regulation of cell growth by redox-mediated extracellular proteolysis of platelet-derived growth factor receptor-β. *J Biol Chem* 276: 28274–28280, 2001.

80. Peltoniemi M, Kaarteenaho-Wiik R, Saily M, Sormunen R, Pääkkö P, Holmgren A, Soini Y, and Kinnula VL. Expression of glutaredoxin is highly cell specific in human lung and is decreased by transforming growth factor-β in vitro and in interstitial lung diseases in vivo. *Hum Pathol* 35: 1000–1007, 2004.
81. Pociask DA, Sime PJ, and Brody AR. Asbestos-derived reactive oxygen species activate TGF-β1. *Lab Invest* 84: 1013–1023, 2004.
82. Pons AR, Sauleda J, Noguera A, Pons J, Barcelo B, Fuster A, and Agusti AG. Decreased macrophage release of TGF-β and TIMP-1 in chronic obstructive pulmonary disease. *Eur Respir J* 26: 60–66, 2005.
83. Rahman I, Biswas SK, Jimenez LA, Torres M, and Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. *Antioxid Redox Signal* 7: 42–59, 2005.
84. Rahman I, Yang SR, and Biswas SK. Current concepts of redox signaling in the lungs. *Antioxid Redox Signal* 8: 681–689, 2006.
85. Rhee SG, Bae YS, Lee SR, and Kwon J. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. *Sci STKE* 2000: PET, 2000.
86. Romer J, Bugge TH, Pyke C, Lund LR, Flick MJ, Degen JL, and Dano K. Impaired wound healing in mice with a disrupted plasminogen gene. *Nat Med* 2: 287–292, 1996.
87. Saharinen J and Keski-Oja J. Specific sequence motif of 8-Cys repeats of TGF-β binding proteins, LTBPs, creates a hydrophobic interaction surface for binding of small latent TGF-β. *Mol Biol Cell* 11: 2691–2704, 2000.
88. Santana A, Saxena B, Noble NA, Gold LI, and Marshall BC. Increased expression of transforming growth factor beta isoforms (β1, β2, β3) in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 13: 34–44, 1995.
89. Shapiro SD. Transgenic and gene-targeted mice as models for chronic obstructive pulmonary disease. *Eur Respir J* 29: 375–378, 2007.
90. Springer J, Scholz FR, Peiser C, Groneberg DA, and Fischer A. SMAD-signaling in chronic obstructive pulmonary disease: transcriptional down-regulation of inhibitory SMAD 6 and 7 by cigarette smoke. *Biol Chem* 385: 649–653, 2004.
91. Stralin P and Marklund SL. Vasoactive factors and growth factors alter vascular smooth muscle cell EC-SOD expression. *Am J Physiol Heart Circ Physiol* 281: H1621–H1629, 2001.
92. Sterner-Kock A, Thorey IS, Koli K, Wempe F, Otte J, Bangsow T, Kuhlmeier K, Kirchner T, Jin S, Keski-Oja J, and von Melchner H. Disruption of the gene encoding the latent transforming growth factor-β binding protein 4 (LTBP-4) causes abnormal lung development, cardiomyopathy, and colorectal cancer. *Genes Dev* 16: 2264–2273, 2002.
93. Sturrock A, Cahill B, Norman K, Huecksteadt TP, Hill K, Sanders K, Karwande SV, Stringham JC, Bull DA, Gleich M, Kennedy TP, and Hoidal JR. Transforming growth factor-β1 induces Nox4 NAD(P)H oxidase and reactive oxygen species-dependent proliferation in human pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 290: L661–L673, 2006.
94. Sutherland ER, Crapo JD, and Bowler RP. N-acetylcysteine and exacerbations of chronic obstructive pulmonary disease. *COPD* 3: 195–202, 2006.
95. Taipale J, Koli K, and Keski-Oja J. Release of transforming growth factor-β1 from the pericellular matrix of cultured fibroblasts and fibrosarcoma cells by plasmin and thrombin. *J Biol Chem* 267: 25378–25384, 1992.
96. Taipale J, Lohi J, Saarinen J, Kovanen PT, and Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-β1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 270: 4689–4696, 1995.
97. Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, and Umeda A. Increased expression of transforming growth factor-β1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 163: 1476–1483, 2001.
98. Thannickal VJ, Day RM, Klinz SG, Bastien MC, Larios JM, and Fanburg BL. Ras-dependent and -independent regulation of reactive oxygen species by mitogenic growth factors and TGF-β1. *FASEB J* 14: 1741–1748, 2000.
99. Thannickal VJ and Fanburg BL. Activation of an H2O2-generating NADH oxidase in human lung fibroblasts by transforming growth factor-β1. *J Biol Chem* 270: 30334–30338, 1995.
100. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279: L1005–L1028, 2000.
101. Tiitto L, Kaarteenaho-Wiik R, Sormunen R, Holmgren A, Pääkkö P, Soini Y, and Kinnula VL. Expression of the thioredoxin system in interstitial lung disease. *J Pathol* 201: 363–370, 2003.
102. Tiitto LH, Peltoniemi MJ, Kaarteenaho-Wiik RL, Soini YM, Pääkkö PK, Sormunen RT, and Kinnula VL. Cell-specific regulation of gamma-glutamylcysteine synthetase in human interstitial lung diseases. *Hum Pathol* 35: 832–839, 2004.
103. Verrecchia F, Vindevoghel L, Lechleider RJ, Uitto J, Roberts AB, and Mauviel A. Smad3/AP-1 interactions control transcriptional responses to TGF-β in a promoter-specific manner. *Oncogene* 20: 3332–3340, 2001.
104. Wang H and Kochevar IE. Involvement of UVB-induced reactive oxygen species in TGF-β biosynthesis and activation in keratinocytes. *Free Radic Biol Med* 38: 890–897, 2005.
105. Wang H, Liu X, Umino T, Kohyama T, Zhu YK, Wen FQ, Spurzem JR, Romberger DJ, Kim HJ, and Rennard SI. Effect of cigarette smoke on fibroblast-mediated gel contraction is dependent on cell density. *Am J Physiol Lung Cell Mol Physiol* 284: L205–L213, 2003.
106. Wang RD, Wright JL, and Churg A. Transforming growth factor-β1 drives airway remodeling in cigarette smoke-exposed tracheal explants. *Am J Respir Cell Mol Biol* 33: 387–393, 2005.
107. Xu YD, Hua J, Mui A, O'Connor R, Grotendorst G, and Khalil N. Release of biologically active TGF-β1 by alveolar epithelial cells results in pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 285: L527–L539, 2003.
108. Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, and Munger JS. Absence of integrin-mediated TGF-β1 activation in vivo recapitulates the phenotype of TGF-β1-null mice. *J Cell Biol* 176: 787–793, 2007.
109. Yao HW, Zhu JP, Zhao MH, and Lu Y. Losartan attenuates bleomycin-induced pulmonary fibrosis in rats. *Respiration* 73: 236–242, 2006.
110. Yu Q and Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. *Genes Dev* 14: 163–176, 2000.
111. Zandvoort A, Postma DS, Jonker MR, Noordhoek JA, Vos JT, van der Geld YM, and Timens W. Altered expression of the Smad signalling pathway: implications for COPD pathogenesis. *Eur Respir J* 28: 533–541, 2006.
112. Zhang K, Flanders KC, and Phan SH. Cellular localization of transforming growth factor-β expression in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 147: 352–361, 1995.
113. Zhao J, Shi W, Wang YL, Chen H, Bringas P Jr, Datto MB, Frederick JP, Wang XF, and Warburton D. Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. *Am J Physiol Lung Cell Mol Physiol* 282: L585–L593, 2002.
114. Zhou Y, Poczatek MH, Berecek KH, and Murphy-Ullrich JE. Thrombospondin 1 mediates angiotensin II induction of TGF-β activation by cardiac and renal cells under both high and low glucose conditions. *Biochem Biophys Res Commun* 339: 633–641, 2006.

Address reprint requests to:
Vuokko Kinnula
Department of Medicine
Division of Pulmonary Medicine
University of Helsinki
P.O. Box 22 (Haartmaninkatu 4)
FI-00014 University of Helsinki
Helsinki, Finland

E-mail: vuokko.kinnula@helsinki.fi

Date of first submission to ARS Central, September 8, 2007; date of acceptance, September 10, 2007.

This article has been cited by:

1. Y. C. Yang, N. Zhang, K. Van Crombruggen, G. H. Hu, S. L. Hong, C. Bachert. 2012. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. *Allergy* **67**:10, 1193-1202. [CrossRef]
2. Behzad Yeganeh, Subhendu Mukherjee, Lyn M. Moir, Kuldeep Kumawat, Hessam H. Kashani, Rushita A. Bagchi, Hoeke A. Baarsma, Reinoud Gosens, Saeid Ghavami. 2012. Novel non-canonical TGF-# signaling networks: Emerging roles in airway smooth muscle phenotype and function. *Pulmonary Pharmacology & Therapeutics* . [CrossRef]
3. Sheena D. Brown, Lou Ann S. Brown. 2012. Ethanol (EtOH)-Induced TGF-#1 and Reactive Oxygen Species Production Are Necessary for EtOH-Induced Alveolar Macrophage Dysfunction and Induction of Alternative Activation. *Alcoholism: Clinical and Experimental Research* n/a-n/a. [CrossRef]
4. Irfan Rahman, Vuokko L Kinnula. 2012. Strategies to decrease ongoing oxidant burden in chronic obstructive pulmonary disease. *Expert Review of Clinical Pharmacology* **5**:3, 293-309. [CrossRef]
5. Ali A Sovari, Adhir Shroff, Abraham G Kocheril. 2012. Postinfarct cardiac remodeling and the substrate for sudden cardiac death: role of oxidative stress and myocardial fibrosis. *Expert Review of Cardiovascular Therapy* **10**:3, 267-270. [CrossRef]
6. Mohamed Kodiha, Ursula Stochaj. 2012. Nuclear Transport: A Switch for the Oxidative Stress—Signaling Circuit?. *Journal of Signal Transduction* **2012**, 1-18. [CrossRef]
7. Marta R. Gorowiec, Lee A. Borthwick, Sean M. Parker, John A. Kirby, Gabriele C. Saretzki, Andrew J. Fisher. 2012. Free Radical Generation Induces Epithelial-to-Mesenchymal Transition in Lung Epithelium via a TGF-#1-dependent Mechanism. *Free Radical Biology and Medicine* . [CrossRef]
8. Jeroen De Jonge, Kim Marie OlthoffLiver regeneration 87-101. [CrossRef]
9. Ying Chen, Peter T Dawes, Jonathan C Packham, Derek L Mattey. 2012. Interaction between smoking and functional polymorphism in the TGFB1 gene is associated with ischaemic heart disease and myocardial infarction in patients with rheumatoid arthritis: a cross-sectional study. *Arthritis Research & Therapy* **14**:2, R81. [CrossRef]
10. Bert Callewaert, Chi-Ting Su, Tim Van Damme, Philip Vlummens, Fransiska Malfait, Olivier Vanakker, Bianca Schulz, Meghan Mac Neal, Elaine C. Davis, Joseph G.H. Lee, Aicha Salhi, Sheila Unger, Ketil Heimdal, Salome De Almeida, Uwe Kornak, Harald Gaspar, Jean-Luc Bresson, Katrina Prescott, Maria E. Gosendi, Sahar Mansour, Gérald E. Piérard, Suneeta Madan-Khetarpal, Frank C. Sciurba, Sofie Symoens, Paul J Coucke, Lionel Van Maldergem, Zsolt Urban, Anne De Paepe. 2012. Comprehensive clinical and molecular analysis of 12 families with type 1 recessive cutis laxa. *Human Mutation* n/a-n/a. [CrossRef]
11. Hideko Sone, Hiromi AkanumaOxidative Stress-Mediated Signaling Pathways by Environmental Stressors 175-194. [CrossRef]
12. Adam Chodobski, Brian J. Zink, Joanna Szmydynger-Chodobska. 2011. Blood–Brain Barrier Pathophysiology in Traumatic Brain Injury. *Translational Stroke Research* . [CrossRef]
13. Loïc Guillot, Olivier Tabary, Nadia Nathan, Harriet Corvol, Annick Clement. 2011. Macrolides: New therapeutic perspectives in lung diseases. *The International Journal of Biochemistry & Cell Biology* **43**:9, 1241-1246. [CrossRef]
14. Stephanie Carnesecchi , Christine Deffert , Yves Donati , Olivier Basset , Boris Hinz , Olivier Preynat-Seauve , Cecile Guichard , Jack L. Arbiser , Botond Banfi , Jean-Claude Pache , Constance Barazzone-Argiroffo , Karl-Heinz Krause . 2011. A Key Role for NOX4 in Epithelial Cell Death During Development of Lung Fibrosis. *Antioxidants & Redox Signaling* **15**:3, 607-619. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
15. Bryan N. Brown, Ian M. Price, Franklin R. Toapanta, Dilhari R. DeAlmeida, Clayton A. Wiley, Ted M. Ross, Tim D. Oury, Yoram Vodovotz. 2011. An agent-based model of inflammation and fibrosis following particulate exposure in the lung. *Mathematical Biosciences* **231**:2, 186-196. [CrossRef]
16. Hyun Soon Lee. 2011. Mechanisms and consequences of TGF-ß overexpression by podocytes in progressive podocyte disease. *Cell and Tissue Research* . [CrossRef]
17. Tonny W. Naranjo, Damaris E. Lopera, Lucy R. Diaz-Granados, Jhon J. Duque, Angela Restrepo, Luz E. Cano. 2010. Histopathologic and immunologic effects of the itraconazole treatment in a murine model of chronic pulmonary paracoccidioidomycosis. *Microbes and Infection* **12**:14-15, 1153-1162. [CrossRef]
18. WITOLD MAZUR, PAMELA LINDHOLM, KIRSI VUORINEN, MARJUKKA MYLLÄRNIEMI, KAISA SALMENKIVI, VUOKKO L. KINNULA. 2010. Cell-specific elevation of NRF2 and sulfiredoxin-1 as markers of oxidative stress in the lungs of idiopathic pulmonary fibrosis and non-specific interstitial pneumonia. *APMIS* **118**:9, 703-712. [CrossRef]

19. Sarah Park, Ji-Yeon Ahn, Min-Jin Lim, Mi-Hyoung Kim, Sae-lo-oom Lee, Yeon-Sook Yun, Gajin Jeong, Jie-Young Song. 2010. IM-412 inhibits transforming growth factor #-induced fibroblast differentiation in human lung fibroblast cells. *Biochemical and Biophysical Research Communications* **399**:2, 268-273. [CrossRef]
20. H. Sone, H. Akanuma, T. Fukuda. 2010. Oxygenomics in environmental stress. *Redox Report* **15**:3, 98-114. [CrossRef]
21. João F Passos, Glyn Nelson, Chunfang Wang, Torsten Richter, Cedric Simillion, Carole J Proctor, Satomi Miwa, Sharon Olijslagers, Jennifer Hallinan, Anil Wipat, Gabriele Saretzki, Karl Lenhard Rudolph, Tom B L Kirkwood, Thomas von Zglinicki. 2010. Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Molecular Systems Biology* **6**. . [CrossRef]
22. Zhi Zhong, Shigeki Tsukada, Hasibur Rehman, Christopher J. Parsons, Tom P. Theruvath, Richard A. Rippe, David A. Brenner, John J. Lemasters. 2010. Inhibition of transforming growth factor-#/Smad signaling improves regeneration of small-for-size rat liver grafts. *Liver Transplantation* **16**:2, 181-190. [CrossRef]
23. Bashier Osman, Anke Doller, El-Sayed Akool, Martin Holdener, Edith Hintermann, Josef Pfeilschifter, Wolfgang Eberhardt. 2009. Rapamycin induces the TGF#1/Smad signaling cascade in renal mesangial cells upstream of mTOR. *Cellular Signalling* **21**:12, 1806-1817. [CrossRef]
24. T. Allen Merritt, Douglas D. Deming, Bruce R. Boynton. 2009. The ‘new’ bronchopulmonary dysplasia: challenges and commentary. *Seminars in Fetal and Neonatal Medicine* **14**:6, 345-357. [CrossRef]
25. Vuokko L Kinnula, Nobuhisa Ishikawa, Ulrich Bergmann, Steffen Ohlmeier. 2009. Proteomic approaches for studying human parenchymal lung diseases. *Expert Review of Proteomics* **6**:6, 619-629. [CrossRef]
26. Amarjit S Naura, Chetan P Hans, Mourad Zerfaoui, Youssef Errami, Jihang Ju, Hogyoung Kim, Khalid Matrougui, Jong G Kim, A Hamid Boulares. 2009. High-fat diet induces lung remodeling in ApoE-deficient mice: an association with an increase in circulatory and lung inflammatory factors. *Laboratory Investigation* **89**:11, 1243-1251. [CrossRef]
27. Peter J Barnes. 2009. Novel mechanisms and new therapies for chronic obstructive pulmonary disease. *Therapy* **6**:6, 795-804. [CrossRef]
28. Harriet Corvol, Florence Flamein, Ralph Epaud, Annick Clement, Loic Guillot. 2009. Lung alveolar epithelium and interstitial lung disease. *The International Journal of Biochemistry & Cell Biology* **41**:8-9, 1643-1651. [CrossRef]
29. Philipp Markart , Thomas Luboeinski , Martina Korfei , Reinhold Schmidt , Malgorzata Wygrecka , Poornima Mahavadi , Konstantin Mayer , Jochen Wilhelm , Werner Seeger , Andreas Guenther , Clemens Ruppert . 2009. Alveolar Oxidative Stress is Associated with Elevated Levels of Nonenzymatic Low-Molecular-Weight Antioxidants in Patients with Different Forms of Chronic Fibrosing Interstitial Lung Diseases. *Antioxidants & Redox Signaling* **11**:2, 227-240. [Abstract] [Full Text PDF] [Full Text PDF with Links]
30. Hee Sun PARK, So Ri KIM, Yong Chul LEE. 2009. Impact of oxidative stress on lung diseases. *Respirology* **14**:1, 27-38. [CrossRef]
31. Christopher S. Stevenson. 2009. Chronic obstructive pulmonary disease susceptibility: Is it due to poor oxygen metabolism?. *Journal of Organ Dysfunction* **5**:4, 218-223. [CrossRef]
32. Hye-Ryun Kang, Jae-Young Lee, Chun Geun Lee. 2008. TGF-#1 as a therapeutic target for pulmonary fibrosis and COPD. *Expert Review of Clinical Pharmacology* **1**:4, 547-558. [CrossRef]
33. Catharina Bertram, Ralf Hass. 2008. Cellular responses to reactive oxygen species-induced DNA damage and aging. *Biological Chemistry* **389**:3, 211-220. [CrossRef]
34. Vuokko L. Kinnula . 2008. Redox Imbalance and Lung Fibrosis. *Antioxidants & Redox Signaling* **10**:2, 249-252. [Citation] [Full Text PDF] [Full Text PDF with Links]
35. Masuko Ushio-Fukai, Randall S. Frey, Tohru Fukai, Asrar B. MalikChapter 8 Reactive Oxygen Species and Endothelial Permeability **61**, 147-189. [CrossRef]