Published in final edited form as:

Cell Mol Life Sci. 2012 July ; 69(14): 2365–2371. doi:10.1007/s00018-012-1012-7.

Targeting NOX enzymes in pulmonary fibrosis

Louise Hecker, Jeff Cheng, and Victor J. Thannickal

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, The University of Alabama at Birmingham, 901 19th Street South, BMR-2 406, Birmingham, AL 35294-0006, USA

Abstract

Oxidative stress has been associated with a number of human fibrotic diseases, including idiopathic pulmonary fibrosis (IPF). Oxidative stress is most often defined as an imbalance between the generation of reactive oxygen species (ROS) in excess of the capacity of cells/tissues to detoxify or scavenge them. Additionally, the regulated production of ROS participates in cellular signaling. Therapeutic strategies to treat IPF have, thus far, focused on augmenting anti-oxidant capacity. Recent studies have demonstrated a critical role for ROS-generating enzymatic systems, specifically, NADPH oxidase (NOX) family oxidoreductases in fibrotic processes. In this review, we examine the evidence for NOX isoforms in the generation and perpetuation of fibrosis, and the potential to target this gene family for the treatment of IPF and related fibrotic disorders.

Keywords

Fibrosis; NADPH oxidase; Oxidative stress

Introduction

Fibrosis of the lung is best characterized as a process by which cellular homeostasis within the terminal gas-exchanging regions—known as alveoli—is altered. There is an expansion in mesenchymal elements, including myofibroblasts, with exuberant extracellular matrix deposition and tissue contraction, while the overlying epithelium is in a state of dysrepair characterized by proliferation, apoptosis, and aberrant differentiation of alveolar type 2 cells. This loss of cellular homeostasis in idiopathic pulmonary fibrosis (IPF) lungs is manifested by accumulating clusters of myofibroblasts (fibroblastic foci) within architecturally remodeled alveolar units; these fibroblastic foci are a key pathological hallmark of IPF. Studies in IPF patients support a link between the abundance of fibroblastic foci in lung biopsies and decreased survival [24]. Lung fibrosis is typically the result of a dysregulated tissue repair response to injury of the alveolar epithelium and/or capillary endothelium; thus, injurious agents that are air-borne or blood-borne, respectively, have the potential to cause lung fibrosis. Fibrosis can result from a variety of acute lung injuries, such as in adult respiratory distress syndrome (ARDS), as well as from chronic inorganic/organic dust exposures, such as in asbestosis or hypersensitivity pneumonitis. A particularly enigmatic form of lung fibrosis is IPF, a fatal lung disease that has not been linked to any single etiological agent. IPF is the most common of interstitial lung diseases, and affects more than 100,000 people in the United States and over 5 million worldwide. The median survival rate for IPF patients is less than 3 years [1], and there are currently no effective therapies that

have been shown to influence survival [16]. There are currently no FDA-approved anti-fibrotic drugs; there is an urgent need to identify effective anti-fibrotic agents for IPF and other fibrotic diseases.

Concepts regarding the pathogenesis of IPF have evolved over the past several years from a process of chronic inflammation, to aberrant wound healing, to a degenerative disease of aging [16]. The pathogenesis of IPF was originally thought to be due to chronic inflammation and reactive oxygen species (ROS)-mediated epithelial cell injury ([8, 14]). However, the role of inflammation in IPF is controversial; Selman et al. [45] has raised the concept that inflammation is a secondary event in the pathogenesis and that IPF may represent an aberrant wound-healing response based on the following observations: (1) limited evidence suggesting that inflammation is prominent in early disease; (2) animal studies have demonstrated that inflammation is not necessary to induce fibrosis; (3) inflammation is not a prominent histopathologic feature and clinical indices typically used to denote inflammation were absent in the lungs of IPF patients; and (4) anti-inflammatory and immunomodulating treatment strategies offered little, if any, benefit to patients with fibrotic disorders. More recent concepts have focused on the possibility that IPF may represent a degenerative disease of aging; studies of familial and sporadic cases of IPF have been associated with telomere shortening [3, 13, 55], supporting the concept that IPF may represent an age-related degenerative disease process [53]. The cause(s) for the shortened telomeres in IPF patients without mutations in telomerase is currently unknown; however, oxidative stress represents one potential mechanism. The role of oxidative stress in fibrosis and aging are well recognized [25], as there is a substantial and growing body of evidence indicating that oxidative stress plays an important role in the pathological development of lung fibrosis as well as fibrosis in multiple organ systems [25]. However, mechanisms of ROS in cellular senescence, impaired epithelial regeneration, lung aging, and fibrosis are not well understood.

Oxidative stress in pulmonary fibrosis

ROS function in normal physiological cellular signaling and regulation, whereas ROS accumulation can lead to deleterious biological consequences. Oxidative stress is defined as an imbalance of the generation of ROS in excess of the capacity of cells/tissues to detoxify or scavenge them. Such a state of oxidative stress may alter the structure/function of cellular macromolecules that eventually leads to tissue/organ dysfunction. Recent studies implicate oxidative stress as a key mediator in the pathogenesis of IPF. Lung tissues from IPF patients demonstrate “signatures” of chronic oxidative damage [27-29]. Bronchoalveolar lavage fluid (BALF) isolated from IPF patients demonstrate elevated levels of oxidative damaged proteins [32, 35]. Oxidative changes within the lung may provide a positive-feedback mechanism for perpetuating a pro-fibrotic tissue microenvironment by mediating various cellular behaviors that influence tissue homeostasis and the outcome of repair/regeneration [16].

Oxidative stress can influence cellular phenotypes and fates that may favor fibrosis over regeneration. For example, stimulation with varying concentrations of exogenous H₂O₂ determines whether senescence or apoptosis ensues [11]. Exposure to chronic sub-lethal ROS levels results in stress-induced premature senescence of fibroblasts [54]. Treatment with the small molecule antioxidant, *N*-acetylcysteine (NAC) has been demonstrated to reverse p21-induced growth arrest, indicating that elevated ROS may play a causative role in cellular senescence [34]. Fibroblasts acquire an apoptosis-resistant phenotype as a consequence of senescence, whereas senescent endothelial cells appear more susceptible to apoptosis [19, 20]. Extracellular generation of H₂O₂ by lung myofibroblasts may mediate fibrogenic effects in tissues by inducing epithelial cell apoptosis by a paracrine mechanism [57] or by inducing matrix crosslinking reactions in the presence of extracellular heme

peroxidases [31]. Although the role of oxidative stress in fibrosis and aging are recognized [25], the precise cellular sources(s) of ROS/RNS that contribute to the disease pathogenesis remain incompletely understood; likewise, the ability to specifically and effectively target key mediators of this process remains challenging.

NOX enzymes as a major source of oxidative stress in fibrosis

The pathogenesis of IPF has been linked to oxidative stress, and recent evidence has implicated reactive oxygen species (ROS)-generating NADPH oxidase (NOX) enzymes in pulmonary fibrosis. NOX isoforms that have been reported to contribute to tissue fibrosis include NOX1 [2, 37, 48, 59] and NOX2 [33, 37, 41, 48, 60], and NOX4 [4, 9, 21]. In addition to NOX enzymes, another potential source of ROS implicated in fibrosis is mitochondria [39], although the relative contributions and cooperation between mitochondrial ROS and NOX enzymes localized to the mitochondria have not been elucidated [26]. In this review, we focus our discussion on NOX enzymes as emerging therapeutic targets for IPF.

Epithelial and endothelial cells—Epithelial cell death is a prominent feature of the IPF lung [52]. During acute lung injury in mice, NOX1-mediated ROS generation by endothelial and endothelial cells has been shown to induce cell death [10]. In the lungs of IPF patients, expression of NOX4 has been demonstrated in hyperplastic alveolar type II cells [4]. A recent study demonstrates an essential role for NOX4 in mediating fibrogenic effects through alveolar epithelial cell death [9]. NOX4-deficient mice are protected from bleomycin-induced pulmonary fibrosis through modulation of epithelial cell death in vivo; wild-type mice evaluated 1 week following bleomycin injury showed significant epithelial cell apoptosis, whereas NOX4-deficient mice demonstrated significant protection from epithelial apoptosis. Additionally, NOX4-deficient primary alveolar epithelial cells and/or treatment with NOX inhibitors (fulvene-5, a compound that inhibits NOX4/2; and GK136901, a NOX1/4 inhibitor) led to decreased TGF- β 1-mediated ROS generation and protection from apoptosis [9]. Finally, this study demonstrates that NOX4-deficient mice exhibited no significant alterations in inflammatory mediators (MIP1 α , RANTES, MIP-2, TNF, IL-10, and IFN γ) following bleomycin lung injury, suggesting that NOX4 does not contribute to the pathogenesis of fibrosis through modulation of inflammation [9].

Epithelial-to-mesenchymal transition (EMT) is a process by which cells lose epithelial markers/phenotype and acquire mesenchymal characteristics. Studies in animal models support a role for EMT in contributing to the accumulation of fibroblasts in lung fibrosis [23, 51]. However, the role and significance of EMT in the pathogenesis of human IPF remain unclear. The molecular mechanisms and cellular phenotypes/fates of epithelial cells with apparent expanded cellular plasticity during fibrogenesis warrants further investigation. Although studies support a role for ROS-mediated EMT [7, 44], the role of NOX enzymes in mediating EMT or affecting epithelial cell plasticity has not been determined.

Mesenchymal and smooth muscle cells—In IPF, there is a loss of cellular homeostasis within the alveolar wall, and there is clearly an expansion of mesenchymal cells within the interstitium with more discrete areas of assembled bundles of muscle-like cells within so-called fibroblast foci, a typical feature of usual interstitial pneumonia (UIP), the histopathological hallmark of IPF. These muscle-like cells have been characterized as myofibroblasts, and their contractile activities contribute to alveolar collapse and the associated restrictive physiology, reduced lung compliance, and gas-exchange abnormalities characteristic of IPF. Adjacent to fibroblastic foci, alveolar epithelial cells appear to be in a state of dysrepair with spatially disorganized areas of rapid proliferation and apoptosis, suggesting a possible defect in differentiation of the alveolar epithelium. Whether the

ineffective epithelial regeneration drives myofibroblast activation or whether it is the unrestrained mesenchymal activation that impairs re-epithelialization is not entirely clear. Persistent myofibroblast differentiation/survival appears to play a central role in the pathogenesis of IPF. Thus, targeting the activated/contractile myofibroblast phenotype represents a promising new strategy for developing therapeutic interventions for IPF.

The NOX4 isoform has been implicated in fibrogenesis by studies in the kidney [5, 41, 50, 60], in vascular remodeling/fibrosis associated with chronic hypertension [2], in the heart [22, 48], and in the pancreas [38]. More recently, a specific role for NOX4 in mediating myofibroblast functions in lung fibrosis has been identified; mRNA expression of NOX4 was found to be induced by the pro-fibrotic cytokine, transforming growth factor- β 1 (TGF- β 1), while other NOX/DUOX isoforms were unaffected [21]. NOX4-dependent generation of ROS (specifically, H₂O₂) was found to be required for TGF- β 1-induced myofibroblast differentiation, extracellular matrix generation, and contractility of lung myofibroblasts. Genetic or pharmacologic targeting of NOX4 attenuated lung fibrogenesis in two different murine models of lung injury [21]. Other studies demonstrate that NOX4 is expressed in lung fibroblasts from patients diagnosed with IPF [4].

Patients diagnosed with IPF frequently acquire secondary pulmonary hypertension and subsequent vascular lesions [12, 43]. Although treatment for pulmonary hypertension could improve functional outcomes and survival of IPF patients, available treatments including longterm supplemental oxygen and targeted vascular therapy remain unproven. It was recently demonstrated that NOX4 is expressed in thickened pulmonary arteries of IPF patients [42]. Therapeutic targeting of NOX4 could potentially inhibit hypoxia-induced vascular remodeling that leads to pulmonary hypertension [40]. Together, these studies suggest that NOX4 and related isoforms may be expressed and activated in different cells of the alveolar and vascular wall and contribute in different ways to fibrogenic processes (Fig. 1).

There is evidence for significant crosstalk between the renin-angiotensin-aldosterone system and TGF- β 1 in organ fibrosis [56, 61]. This effect is, at least in part, mediated by induction/activation of NOX1, NOX2, and/or NOX4 [2, 5, 48, 50, 59, 60]. Therefore, strategies aimed at specifically blocking the source of ROS (i.e., “targeted” anti-oxidative therapy) through inhibition of NOX family NADPH oxidases, may prove to be more effective as anti-fibrotic therapies.

Inflammatory cells—Studies in human subjects with IPF have demonstrated enhanced generation of ROS from alveolar inflammatory cells, primarily neutrophils and macrophages, which may promote alveolar epithelial cell injury [8, 49]. The evidence for NOX2 in lung fibrosis comes primarily from animal models that show protection from lung injury induced by bleomycin [36] or carbon nanotubes [47] in mice with a genetic deficiency of NOX2. A p47^{phox}-dependent NOX isoform is required for the development of pulmonary fibrosis in a murine lung injury model; the protection in p47^{phox}^{-/-} mice was accompanied by enhanced neutrophilic inflammation and matrix metalloproteinase (MMP)-9 activity [36]. Interestingly, neutrophils from BALF isolated from IPF patients exhibit elevated expression of p47^{phox} and p67^{phox} [58], supporting a potential role for the NOX2 isoform in this disease.

Targeting NOX enzymes in anti-fibrotic strategies

Although accumulating data support the concept that NOX enzymes play critical roles in fibrogenesis, no specific NOX inhibitors have been identified or tested in animal models. However, anti-oxidant strategies with NAC have shown some potential benefits [15]. NAC is thought to exert its function as an antioxidant via its main metabolite, cysteine, a precursor

of glutathione (GSH) biosynthesis. The anti-fibrotic effects of NAC have been documented in animal models of lung fibrosis; administration of NAC in rodents led to increased levels of lung GSH in control animals and following bleomycin-induced lung injury [17]. Preventative therapy with NAC enhanced lung GSH content and decreased collagen accumulation following bleomycin administration [18, 46]. These preclinical efficacy studies led to clinical development of NAC, in which NAC therapy demonstrated clinical efficacy in IPF patients, and is one of the few phase III trials to meet the pre-determined primary end-point of the study [15]. In this randomized, placebo-controlled clinical trial (IFIGENIA), patients were treated with a high dose of the anti-oxidant NAC (600 mg three times daily) in addition to the standard treatment regimen (prednisone, azathioprine). The study suggested that the addition of NAC to the conventional therapy significantly slowed lung function decline after 1 year, as demonstrated by preservation of forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) in the NAC-treated group. However, the study showed no significant effect on survival as compared to conventional therapy alone [15]. Another study sponsored by the US National Institutes of Health (www.clinicaltrials.gov; NCT00650091: PANTHER), is currently active and will evaluate the effectiveness of NAC vs. placebo. Despite limitations of the IFIGENIA clinical trial, the development of therapeutic strategies targeting oxidative stress pathways remains promising.

Therapeutic strategies that more directly target the source(s) of ROS generation, including pharmacological inhibitors of specific NOX isoforms, may prove to be more specific and effective in comparison to antioxidant interventions for IPF. Which isoforms are most optimal drug targets for the treatment of this disease remains uncertain; it is likely that specific NOX isoforms are regulated in a spatio-temporal and cell-specific manner during the course of the inflammatory and repair response to lung injury. Several groups have utilized high-throughput screening approaches to discover small-molecule inhibitors targeting NOX enzymes [6, 30]. GenKyoTex (Geneva, Switzerland) has recently identified a candidate drug for pulmonary fibrosis (GKT137831); a small-molecule NOX4/NOX1 dual inhibitor. GKT137831 demonstrates strong antifibrotic activity at low dose with much better efficacy than pirfenidone in curative model of bleomycin-induced pulmonary fibrosis in mice, as reported by the company's Web site (<http://www.genkyotex.com/index.php?rubID=39>; accessed 2011-08-28). In 2010, GKT137831 was granted orphan drug status for the treatment of IPF by the European Commission; GenKyoTex expects to initiate phase I clinical trials in 2011. Academic drug discovery efforts are also ongoing at the University of Alabama at Birmingham. With the concerted efforts of academia, industry, funding agencies, and patient advocacy groups, more effective therapies for IPF and other fibrotic diseases can be realized.

Acknowledgments

This work was supported by U.S. National Institutes of Health grants, R01 HL067967 (VJT), R01 HL094230 (VJT), and R01 HL086836 (JC).

References

1. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med*. 2000; 161:646–664. [PubMed: 10673212]
2. Akasaki T, Ohya Y, Kuroda J, Eto K, Abe I, Sumimoto H, Iida M. Increased expression of gp91phox homologues of NAD(P)H oxidase in the aortic media during chronic hypertension: involvement of the renin-angiotensin system. *Hypertens Res*. 2006; 29:813–820. [PubMed: 17283869]

3. Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tudor RM, Phillips JA 3rd, Lansdorp PM, Loyd JE, Armanios MY. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. *Proc Natl Acad Sci USA*. 2008; 105:13051–13056. [PubMed: 18753630]
4. Amara N, Goven D, Prost F, Muloway R, Crestani B, Boczkowski J. NOX4/NADPH oxidase expression is increased in pulmonary fibroblasts from patients with idiopathic pulmonary fibrosis and mediates TGFbeta1-induced fibroblast differentiation into myofibroblasts. *Thorax*. 2010; 65:733–738. [PubMed: 20685750]
5. Block K, Eid A, Griendling KK, Lee DY, Wittrant Y, Gorin Y. Nox4 NAD(P)H oxidase mediates Src-dependent tyrosine phosphorylation of PDK-1 in response to angiotensin II: role in mesangial cell hypertrophy and fibronectin expression. *J Biol Chem*. 2008; 283:24061–24076. [PubMed: 18559349]
6. Borbely G, Szabadkai I, Horvath Z, Marko P, Varga Z, Breza N, Baska F, Vantus T, Huszar M, Geiszt M, Hunyady L, Buday L, Orfi L, Keri G. Small-molecule inhibitors of NADPH oxidase 4. *J Med Chem*. 2010; 53:6758–6762. [PubMed: 20731357]
7. Cannito S, Novo E, di Bonzo LV, Busletta C, Colombatto S, Parola M. Epithelial-mesenchymal transition: from molecular mechanisms, redox regulation to implications in human health and disease. *Antioxid Redox Signal*. 2010; 12:1383–1430. [PubMed: 19903090]
8. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest*. 1987; 79:1665–1673. [PubMed: 3034979]
9. Carnesecchi S, Deffert C, Donati Y, Basset O, Hinz B, Preynat-Seauve O, Guichard C, Arbiser JL, Banfi B, Pache JC, Barazzzone-Argiroffo C, Krause KH. A Key Role for NOX4 in Epithelial Cell Death During Development of Lung Fibrosis. *Antioxid Redox Signal*. 2011; 15:607–619. [PubMed: 21391892]
10. Carnesecchi S, Deffert C, Pagano A, Garrido-Urbani S, Metrailler-Ruchonnet I, Schappi M, Donati Y, Matthay MA, Krause KH, Barazzzone Argiroffo C. NADPH oxidase-1 plays a crucial role in hyperoxia-induced acute lung injury in mice. *Am J Respir Crit Care Med*. 2009; 180:972–981. [PubMed: 19661248]
11. Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN. Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. *Biochem J*. 1998; 332(Pt 1):43–50. [PubMed: 9576849]
12. Colombat M, Mal H, Groussard O, Capron F, Thabut G, Jebrak G, Brugiere O, Dauriat G, Castier Y, Leseche G, Fournier M. Pulmonary vascular lesions in end-stage idiopathic pulmonary fibrosis: histopathologic study on lung explant specimens and correlations with pulmonary hemodynamics. *Hum Pathol*. 2007; 38:60–65. [PubMed: 16949908]
13. Cronkhite JT, Xing C, Raghu G, Chin KM, Torres F, Rosenblatt RL, Garcia CK. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008; 178:729–737. [PubMed: 18635888]
14. Crystal RG. Oxidants and respiratory tract epithelial injury: pathogenesis and strategies for therapeutic intervention. *Am J Med*. 1991; 91:39S–44S. [PubMed: 1928210]
15. Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, MacNee W, Thomeer M, Wallaert B, Laurent F, Nicholson AG, Verbeken EK, Verschakelen J, Flower CD, Capron F, Petruzzelli S, De Vuyst P, van den Bosch JM, Rodriguez-Becerra E, Corvasce G, Lankhorst I, Sardina M, Montanari M. Highdose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med*. 2005; 353:2229–2242. [PubMed: 16306520]
16. Ding Q, Luckhardt T, Hecker L, Zhou Y, Liu G, Antony VB, de Andrade J, Thannickal VJ. New insights into the pathogenesis and treatment of idiopathic pulmonary fibrosis. *Drugs*. 2011; 71:981–1001. [PubMed: 21668038]
17. Giri SN, Hyde DM, Schiedt MJ. Effects of repeated administration of N-acetyl-L-cysteine on sulfhydryl levels of different tissues and bleomycin-induced lung fibrosis in hamsters. *J Lab Clin Med*. 1988; 111:715–724. [PubMed: 2453601]
18. Hagiwara SI, Ishii Y, Kitamura S. Aerosolized administration of N-acetylcysteine attenuates lung fibrosis induced by bleomycin in mice. *Am J Respir Crit Care Med*. 2000; 162:225–231. [PubMed: 10903246]

19. Hampel B, Malisan F, Niederegger H, Testi R, Jansen-Durr P. Differential regulation of apoptotic cell death in senescent human cells. *Exp Gerontol.* 2004; 39:1713–1721. [PubMed: 15582287]
20. Hampel B, Wagner M, Teis D, Zwerschke W, Huber LA, Jansen-Durr P. Apoptosis resistance of senescent human fibroblasts is correlated with the absence of nuclear IGFBP-3. *Aging Cell.* 2005; 4:325–330. [PubMed: 16300485]
21. Hecker L, Vittal R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, Pennathur S, Martinez FJ, Thannickal VJ. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. *Nat Med.* 2009; 15:1077–1081. [PubMed: 19701206]
22. Henderson BC, Sen U, Reynolds C, Moshal KS, Ovechkin A, Tyagi N, Kartha GK, Rodriguez WE, Tyagi SC. Reversal of systemic hypertension-associated cardiac remodeling in chronic pressure overload myocardium by ciglitazone. *Int J Biol Sci.* 2007; 3:385–392. [PubMed: 17848984]
23. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA.* 2006; 103:13180–13185. [PubMed: 16924102]
24. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, Flint A, Thurlbeck W, Cherniack RM. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am J Respir Crit Care Med.* 2001; 164:1025–1032. [PubMed: 11587991]
25. Kinnula VL, Fattman CL, Tan RJ, Oury TD. Oxidative stress in pulmonary fibrosis: a possible role for redox modulatory therapy. *Am J Respir Crit Care Med.* 2005; 172:417–422. [PubMed: 15894605]
26. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proc Natl Acad Sci USA.* 2010; 107:15565–15570. [PubMed: 20713697]
27. Kuwano K, Hagimoto N, Maeyama T, Fujita M, Yoshimi M, Inoshima I, Nakashima N, Hamada N, Watanabe K, Hara N. Mitochondria-mediated apoptosis of lung epithelial cells in idiopathic interstitial pneumonias. *Lab Invest.* 2002; 82:1695–1706. [PubMed: 12480919]
28. Kuwano K, Kunitake R, Kawasaki M, Nomoto Y, Hagimoto N, Nakanishi Y, Hara N. P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 1996; 154:477–483. [PubMed: 8756825]
29. Kuwano K, Nakashima N, Inoshima I, Hagimoto N, Fujita M, Yoshimi M, Maeyama T, Hamada N, Watanabe K, Hara N. Oxidative stress in lung epithelial cells from patients with idiopathic interstitial pneumonias. *Eur Respir J.* 2003; 21:232–240. [PubMed: 12608435]
30. Laleu B, Gaggini F, Orchard M, Fioraso-Cartier L, Cagnon L, Houngninou-Molango S, Gradia A, Duboux G, Merlot C, Heitz F, Szyndralewicz C, Page P. First in class, potent, and orally bioavailable NADPH oxidase isoform 4 (Nox4) inhibitors for the treatment of idiopathic pulmonary fibrosis. *J Med Chem.* 2010; 53:7715–7730. [PubMed: 20942471]
31. Larios JM, Budhiraja R, Fanburg BL, Thannickal VJ. Oxidative protein cross-linking reactions involving l-tyrosine in transforming growth factor-beta1-stimulated fibroblasts. *J Biol Chem.* 2001; 276:17437–17441. [PubMed: 11279068]
32. Lenz AG, Costabel U, Maier KL. Oxidized BAL fluid proteins in patients with interstitial lung diseases. *Eur Respir J.* 1996; 9:307–312. [PubMed: 8777969]
33. Looi YH, Grieve DJ, Siva A, Walker SJ, Anilkumar N, Cave AC, Marber M, Monaghan MJ, Shah AM. Involvement of Nox2 NADPH oxidase in adverse cardiac remodeling after myocardial infarction. *Hypertension.* 2008; 51:319–325. [PubMed: 18180403]
34. Macip S, Igarashi M, Fang L, Chen A, Pan ZQ, Lee SW, Aaronson SA. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. *EMBO J.* 2002; 21:2180–2188. [PubMed: 11980715]
35. Maier K, Leuschel L, Costabel U. Increased levels of oxidized methionine residues in bronchoalveolar lavage fluid proteins from patients with idiopathic pulmonary fibrosis. *Am Rev Respir Dis.* 1991; 143:271–274. [PubMed: 1990939]

36. Manoury B, Nenau S, Leclerc O, Guenon I, Boichot E, Planquois JM, Bertrand CP, Lagente V. The absence of reactive oxygen species production protects mice against bleomycin-induced pulmonary fibrosis. *Respir Res.* 2005; 6:11. [PubMed: 15663794]
37. Masamune A, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. *Am J Physiol Gastrointest Liver Physiol.* 2008; 294:G99–G108. [PubMed: 17962358]
38. Meng QR, Gideon KM, Harbo SJ, Renne RA, Lee MK, Brys AM, Jones R. Gene expression profiling in lung tissues from mice exposed to cigarette smoke, lipopolysaccharide, or smoke plus lipopolysaccharide by inhalation. *Inhal Toxicol.* 2006; 18:555–568. [PubMed: 16717027]
39. Mitchell C, Robin MA, Mayeuf A, Mahrouf-Yorgov M, Mansouri A, Hamard M, Couton D, Fromenty B, Gilgenkrantz H. Protection against hepatocyte mitochondrial dysfunction delays fibrosis progression in mice. *Am J Pathol.* 2009; 175:1929–1937. [PubMed: 19808650]
40. Mittal M, Roth M, Konig P, Hofmann S, Dony E, Goyal P, Selbitz AC, Schermuly RT, Ghofrani HA, Kwapiszewska G, Kummer W, Klepetko W, Hoda MA, Fink L, Hanze J, Seeger W, Grimminger F, Schmidt HH, Weissmann N. Hypoxia-dependent regulation of nonphagocytic NADPH oxidase subunit NOX4 in the pulmonary vasculature. *Circ Res.* 2007; 101:258–267. [PubMed: 17585072]
41. Ohshiro Y, Ma RC, Yasuda Y, Hiraoka-Yamamoto J, Clermont AC, Isshiki K, Yagi K, Arikawa E, Kern TS, King GL. Reduction of diabetes-induced oxidative stress, fibrotic cytokine expression, and renal dysfunction in protein kinase C β -null mice. *Diabetes.* 2006; 55:3112–3120. [PubMed: 17065350]
42. Pache JC, Carnesecchi S, Deffert C, Donati Y, Herrmann FR, Barazzzone-Argiroffo C, Krause KH. NOX-4 is expressed in thickened pulmonary arteries in idiopathic pulmonary fibrosis. *Nat Med.* 2011; 17:31–32. author reply 32–33. [PubMed: 21217672]
43. Patel NM, Lederer DJ, Borczuk AC, Kawut SM. Pulmonary hypertension in idiopathic pulmonary fibrosis. *Chest.* 2007; 132:998–1006. [PubMed: 17873194]
44. Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature.* 2005; 436:123–127. [PubMed: 16001073]
45. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med.* 2001; 134:136–151. [PubMed: 11177318]
46. Shahzeidi S, Sarnstrand B, Jeffery PK, McAnulty RJ, Laurent GJ. Oral N-acetylcysteine reduces bleomycin-induced collagen deposition in the lungs of mice. *Eur Respir J.* 1991; 4:845–852. [PubMed: 1720100]
47. Shvedova AA, Kisin ER, Murray AR, Kommineni C, Castranova V, Fadeel B, Kagan VE. Increased accumulation of neutrophils and decreased fibrosis in the lung of NADPH oxidase-deficient C57BL/6 mice exposed to carbon nanotubes. *Toxicol Appl Pharmacol.* 2008; 231:235–240. [PubMed: 18534653]
48. Stas S, Whaley-Connell A, Habibi J, Appesh L, Hayden MR, Karuparthi PR, Qazi M, Morris EM, Cooper SA, Link CD, Stump C, Hay M, Ferrario C, Sowers JR. Mineralocorticoid receptor blockade attenuates chronic overexpression of the renin-angiotensin-aldosterone system stimulation of reduced nicotinamide adenine dinucleotide phosphate oxidase and cardiac remodeling. *Endocrinology.* 2007; 148:3773–3780. [PubMed: 17494996]
49. Strausz J, Muller-Quernheim J, Stepling H, Ferlinz R. Oxygen radical production by alveolar inflammatory cells in idiopathic pulmonary fibrosis. *Am Rev Respir Dis.* 1990; 141:124–128. [PubMed: 2297170]
50. Taira M, Toba H, Murakami M, Iga I, Serizawa R, Murata S, Kobara M, Nakata T. Spironolactone exhibits direct renoprotective effects and inhibits renal renin-angiotensin-aldosterone system in diabetic rats. *Eur J Pharmacol.* 2008; 589:264–271. [PubMed: 18582458]
51. Tanjore H, Xu XC, Polosukhin VV, Degryse AL, Li B, Han W, Sherrill TP, Plith D, Neilson EG, Blackwell TS, Lawson WE. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med.* 2009; 180:657–665. [PubMed: 19556518]

52. Thannickal VJ, Horowitz JC. Evolving concepts of apoptosis in idiopathic pulmonary fibrosis. *Proc Am Thorac Soc.* 2006; 3:350–356. [PubMed: 16738200]
53. Thannickal VJ, Loyd JE. Idiopathic pulmonary fibrosis: a disorder of lung regeneration? *Am J Respir Crit Care Med.* 2008; 178:663–665. [PubMed: 18796651]
54. Toussaint O, Medrano EE, von Zglinicki T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. *Exp Gerontol.* 2000; 35:927–945. [PubMed: 11121681]
55. Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci U S A.* 2007; 104:7552–7557. [PubMed: 17460043]
56. Uhal BD, Kim JK, Li X, Molina–Molina M. Angiotensin-TGF-beta 1 crosstalk in human idiopathic pulmonary fibrosis: autocrine mechanisms in myofibroblasts and macrophages. *Curr Pharm Des.* 2007; 13:1247–1256. [PubMed: 17504233]
57. Waghay M, Cui Z, Horowitz JC, Subramanian IM, Martinez FJ, Toews GB, Thannickal VJ. Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. *FASEB J.* 2005; 19:854–856. [PubMed: 15857893]
58. Wang CL, Kang J, Li ZH. Increased expression of NADPH oxidase p47-PHOX and p67-PHOX factor in idiopathic pulmonary fibrosis. *Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chin. J Tuberc Respir Dis.* 2007; 30:265–268.
59. Wang P, Tang F, Li R, Zhang H, Chen S, Liu P, Huang H. Contribution of different Nox homologues to cardiac remodeling in two-kidney two-clip renovascular hypertensive rats: effect of valsartan. *Pharmacol Res.* 2007; 55:408–417. [PubMed: 17324585]
60. Whaley-Connell A, Habibi J, Nistala R, Cooper SA, Karuparthi PR, Hayden MR, Rehmer N, DeMarco VG, Andresen BT, Wei Y, Ferrario C, Sowers JR. Attenuation of NADPH oxidase activation and glomerular filtration barrier remodeling with statin treatment. *Hypertension.* 2008; 51:474–480. [PubMed: 18172055]
61. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008; 214:199–210. [PubMed: 18161745]

Fig. 1.

Sources of NOX/DUOX-dependent generation of ROS in the lung. A number of different cell types of the alveolar-airway and vascular wall have been shown to express specific isoforms of NOX family enzymes. Their activation may contribute to the overall tissue oxidative stress, or function as signaling proteins in determining the phenotype and fates of these cells