Biochimica et Biophysica Acta 1832 (2013) 1028–1040

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Oxidative stress and pulmonary fibrosis ☆

Paul Cheresh, Seok-Jo Kim, Sandhya Tulasiram, David W. Kamp *

Department of Medicine, Northwestern University Feinberg School of Medicine and Jesse Brown VA Medical Center, USA

ARTICLE INFO

Article history:
Received 26 September 2012
Received in revised form 26 November 2012
Accepted 28 November 2012
Available online 5 December 2012

Keywords:
Reactive oxygen species
Epithelium
Mitochondria
NADPH oxidase
Apoptosis

ABSTRACT

Oxidative stress is implicated as an important molecular mechanism underlying fibrosis in a variety of organs, including the lungs. However, the causal role of reactive oxygen species (ROS) released from environmental exposures and inflammatory/interstitial cells in mediating fibrosis as well as how best to target an imbalance in ROS production in patients with fibrosis is not firmly established. We focus on the role of ROS in pulmonary fibrosis and, where possible, highlight overlapping molecular pathways in other organs. The key origins of oxidative stress in pulmonary fibrosis (e.g. environmental toxins, mitochondria/NADPH oxidase of inflammatory and lung target cells, and depletion of antioxidant defenses) are reviewed. The role of alveolar epithelial cell (AEC) apoptosis by mitochondria- and p53-regulated death pathways is examined. We emphasize an emerging role for the endoplasmic reticulum (ER) in pulmonary fibrosis. After briefly summarizing how ROS trigger a DNA damage response, we concentrate on recent studies implicating a role for mitochondrial DNA (mtDNA) damage and repair mechanisms focusing on 8-oxoguanine DNA glycosylase (Ogg1) as well as crosstalk between ROS production, mtDNA damage, p53, Ogg1, and mitochondrial aconitase (ACO2). Finally, the association between ROS and TGF-β1-induced fibrosis is discussed. Novel insights into the molecular basis of ROS-induced pulmonary diseases and, in particular, lung epithelial cell death may promote the development of unique therapeutic targets for managing pulmonary fibrosis as well as fibrosis in other organs and tumors, and in aging; diseases for which effective management is lacking. This article is part of a Special Issue entitled: Fibrosis: Translation of basic research to human disease.

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1. Introduction

Fibrosis is characterized by exuberant extracellular matrix (ECM) protein deposition in the basement membrane and interstitial tissue in the setting of an injured overlying epithelium and expansion of activated mesenchymal cells (myofibroblasts). Fibrosis is pathologically evident in numerous diseases involving nearly every organ. Accumulating evidence over the past several decades have identified many of the important pathogenic mechanisms that promote fibrosis, yet the precise molecular mechanisms involved and the crosstalk between implicated pathways are not fully understood. Oxidative stress is one important molecular mechanism underlying fibrosis in a variety of organs, including the lungs. However, the causal role of reactive oxygen species (ROS) released from environmental exposures and inflammatory/interstitial cells in mediating fibrosis as well as how best to target an imbalance in ROS production in patients with fibrosis are not firmly established.

The term "oxidative stress" encompasses all the molecular, cellular and tissue abnormalities resulting from excess ROS production and/or depleted antioxidant defenses [1–3]. As compared to other organs, the lungs are particularly vulnerable to oxidative stress because they are exposed to the highest levels of oxygen; oxygen pressure of inhaled air is 150 mm Hg and that of alveolar air is 100 mm Hg while venous blood oxygen pressures returning from various other organs range from as high as ~45 mm Hg to as low as ~1 mm Hg [2]. As emphasized by others, the traditional concept of fibrosis resulting from an imbalance

Abbreviations: AEC, alveolar epithelial cell; AT2, alveolar epithelial type II; AM, alveolar macrophage; ARE, antioxidant response element; BALF, bronchoalveolar lavage fluid; JNK, c-Jun N-terminal kinase; EGFR, epidermal growth factor receptor; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; ERK, extracellular-signal-regulated kinase; H_2O_2, hydrogen peroxide; IPF, idiopathic pulmonary fibrosis; IRE1α, inositol requiring kinase 1 alpha; IL-1β, interleukin-1 beta; ACO2, mitochondrial aconitase; mtDNA, mitochondrial DNA; mt-hOgg1, mitochondrial human 8-oxoguanine-DNA glycosylase 1; $\Delta\psi_m$, mitochondrial membrane potential; MDM2, mouse double-minute 2 protein; NAC, n-acetyl cysteine; NOX, NADPH oxidase; NOX4, NADPH oxidase-4; NOS, nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; PKCδ, protein kinase C delta; ROS, reactive oxygen species; SERCA, sarcoplasmic ER Ca^{2+} ATPase; αSMA, α smooth muscle actin; O_2^-, superoxide; SOD, superoxide dismutase; SP-C, surfactant protein C; TGF-β, transforming growth factor beta; TNFα, tumor necrosis factor-alpha; UPR, unfolded protein response; XBP1, X-box binding protein 1

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* Corresponding author at: Pulmonary and Critical Care Medicine, Northwestern University Feinberg School of Medicine, McGaw M-330, 240 E. Huron St., Chicago, IL 60611-3010, USA. Tel.: +1 312 908 8163; fax: +1 312 503 0411.
E-mail address: d-kamp@northwestern.edu (D.W. Kamp).

0925-4439/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbadis.2012.11.021

in ROS production and antioxidant defenses is overly simplistic given the diverse pathways that are independently regulated and the ineffectual results from numerous antioxidant trials [1–3].

The purpose of this review is to highlight our current understanding of the causal role of oxidative stress in promoting fibrosis. Given the enormity of the topic and space constraints, we have chosen to restrict our focus to the role of ROS in mediating pulmonary fibrosis. In particular, we emphasize the role of ROS in the pathobiology of asbestosis, which is pulmonary fibrosis resulting from asbestos exposure. Asbestosis shares radiographic and pathologic features with idiopathic pulmonary fibrosis (IPF), a much more common disease that has a worse prognosis, and lacks effective treatment and ideal animal models. We underscore some of the important overlapping oxidative stress-induced molecular pathways resulting in fibrosis in other organs (e.g. liver, heart, etc.) that have recently been reviewed in detail elsewhere [4–6]. We first describe the principal origins of oxidative stress that can lead to pulmonary fibrosis including environmental toxins (e.g. tobacco, occupational exposure such as asbestos or silica, radiation, and drugs, such as bleomycin), mitochondria and NADPH oxidase (NOX), especially NADPH oxidase-4 (NOX4) in inflammatory and lung target cells. Oxidative stress also results from inadequate or deficient antioxidant defenses. Reactive nitrogen species, which are beyond the scope of this review, also contribute to the free radical burden of fibrotic lungs (see for review: [7]). We explore the evidence for alveolar epithelial cell (AEC) apoptosis being important in promoting asbestosis and IPF with an emphasis on ROS derived from the mitochondria- and p53-regulated death pathways as well as recent evidence for an activated endoplasmic reticulum (ER) stress response in patients with IPF. After briefly summarizing how ROS trigger a DNA damage response, we discuss the role for mitochondrial DNA (mtDNA) damage and repair mechanisms focusing on 8-oxoguanine DNA glycosylase (Ogg1) as well as crosstalk between ROS production, mtDNA damage, p53, Ogg1, and mitochondrial aconitase (ACO2), which is a mitochondrial redox-sensor molecule involved in mtDNA maintenance. Finally, the association between oxidative stress and transforming growth factor β (TGF-β)-induced fibrosis is discussed. We examine recent investigations highlighting important crosstalk between ROS, apoptosis, and inflammation. A general hypothetical model depicting the major ROS-driven pathways described in this review is illustrated in Fig. 1. Collectively, these studies are revealing new insights into the molecular basis of ROS-induced pulmonary fibrosis and that may prove useful in the development of novel anti-fibrotic treatment strategies.

2. Origins of oxidative stress in pulmonary fibrosis

Biological systems are continually exposed to both extrinsic sources of reactive oxidants (e.g. tobacco, asbestos/silica, radiation, bleomycin and other drugs, etc.) and those that arise endogenously in inflammatory cells as well as epithelial, mesenchymal and endothelial cells within tissues [8]. Several enzymatic systems contribute to ROS production including NOXs, xanthine oxidase, nitric oxide synthase (NOS), and the mitochondrial electron transport chain [8,9]. They are also produced from the metabolism of a wide spectrum of drugs and xenobiotics [8]. ROS, including superoxide (O_2^-) and hydrogen peroxide (H_2O_2), play a central role in host defense by killing microbes in phagocytic cells. Although excess amounts of ROS are toxic and can promote fibrosis, lower levels of ROS are 'physiologic' by functioning as signaling molecules that mediate various cellular responses including proliferation, migration, differentiation, and gene expression [7–9]. The highly reactive HO• can be generated from O_2^- and H_2O_2 in the presence of small amounts of redox-activate ferrous iron complexed with toxins such as asbestos fiber from outside of cell, via the Fenton-catalyzed Haber–Weiss reaction shown in Eq. (1) [9,10]. Alkoxyl radicals are also formed by iron catalysis of organic hydroperoxides as shown in Eq. (2).

$$O_2^- + H_2O_2 \xrightarrow{\text{iron}} HO^- + HO\bullet + O_2 \quad (1)$$

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO\bullet + HO^- \quad (2)$$

Redox cycling is a common mechanism by which quinones and related species are reduced by a flavoenzyme (e.g. cytochrome P450 reductase) to a free radical that then reacts with oxygen to generate O_2^- [9]. Some fibrogenic agents implicated in redox cycling include the herbicide paraquat, the anticancer drug doxorubicin and the diabetogenic compound alloxan [8]. Free radicals also arise through the oxidation of phenols, aromatic amines and hydrazines by heme proteins, and some compounds undergo autoxidation catalyzed by transition metal ions [9,11]. Because these ROS-producing reactions are associated with fibrosis, they have been the focus of investigative interest [10]. Finally, as reviewed in this section, oxidative stress can also be caused by depletion of antioxidant defense mechanisms, resulting in increased levels of ROS. In general, low levels of ROS production can result in cell proliferation and activation of antioxidant defenses, but higher ROS levels trigger DNA damage, p53 activation, cell cycle blockade, and cell death via apoptosis and/or necrosis; all of these may be important in a culminating fibrotic response.

At least 4 lines of evidence convincingly show that ROS generated internally or induced by environmental exposure play a vital role in the development of pulmonary fibrotic diseases as reviewed elsewhere [1,7,12–14]: (1) Oxidized proteins and lipid products like 8-isoprostrane and carbonylated proteins have been identified in exhaled air, bronchoalveolar lavage fluid, and lung tissue from patients with fibrotic lung diseases. (2) Bleomycin-induced pulmonary fibrosis, the most commonly utilized experimental model, is associated with marked increases in the levels of ROS, oxidized proteins, DNA and lipids. (3) Increased oxidative DNA damage is detected in patients with IPF as well as workers with silicosis and asbestosis and animal models with silica or asbestos induced lung fibrosis. (4) Antioxidants and iron chelators can attenuate bleomycin- and asbestos-induced pulmonary fibrosis in rodent models.

2.1. Mitochondria-derived ROS

ROS generated from the mitochondria of key target cells appear important in mediating pulmonary fibrosis. Mitochondrial dysfunction results in the generation of ROS (e.g. H_2O_2 and O_2^-) as the electron transport chain is uncoupled from proton pumping and ROS are released into the cytosol [15]. Carter and associates [16–19] have established a key role for H_2O_2 production by the mitochondria of alveolar macrophages (AM) in causing asbestosis. They reported that AM exposed to asbestos fibers produces H_2O_2, which is blocked by catalase or mitigation of AM mitochondrial stress. Further, Rac1, a Rho GTP binding protein family member, increases AM mitochondrial H_2O_2 production while asbestos-induced AM H_2O_2 production is reduced by knockdown of the iron–sulfur protein of complex III in the mitochondrial electron transport chain, a major site or ROS production. Increased levels of Rac1 are localized in the mitochondria of AM of asbestosis patients, and asbestos exposed mice with a conditional deletion of Rac1 have less oxidative stress and pulmonary fibrosis [16–19] (Fig. 2). Taken together, these studies demonstrate that asbestos triggers AM H_2O_2 production by transferring electrons from complex III to Rac1, which then drive down-stream signaling pathways, inflammation and cellular injury that result in asbestosis in mice. Carter et.al have proposed that Rac1 is a novel biomarker for pulmonary fibrosis. An important role for H_2O_2 mediating pulmonary fibrosis is also supported by the protective effects of catalase in a rodent model of asbestosis [20]. As reviewed elsewhere, exogenous