TRANSATLANTIC CONFERENCE ON LUNG DISEASE

Mitochondrial Dysfunction in Pulmonary Fibrosis

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Abstract

The aging of the human population has resulted in an unprecedented increase in the incidence and prevalence of age-related diseases, including those of the lung. Idiopathic pulmonary fibrosis is a disease of aging, and is characterized by a progressive decline in lung function and high mortality. Recent studies suggest that mitochondrial dysfunction, which can accompany aging phenotypes, may

contribute to the pathogenesis of idiopathic pulmonary fibrosis. In this review, we explore current evidence for mitochondrial dysfunction in alveolar epithelial cells, fibroblasts, and immune cells that participate in the fibrotic process. Further, the fates of these cell populations and the potential to target mitochondrial dysfunction as a therapeutic strategy are discussed.

Keywords: mitochondrial dysfunction; pulmonary fibrosis; aging

(Received in original form May 4, 2017; accepted in final form June 22, 2017)

Supported by National Institutes of Health grants K08 HL135399 (S.R.), P01 HL114470 (V.J.T.), and R01 AG046210 (V.J.T.); and VA Merit Award IO1BX003056 (V.J.T.).

Author Contributions: Conception, design, and analysis: S.R., K.B., and V.J.T. Drafting and revision: S.R. and V.J.T. Final approval: V.J.T.

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Ann Am Thorac Soc Vol 14, Supplement 5, pp S383–S388, Nov 2017

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DOI: 10.1513/AnnalsATS.201705-370AW

Internet address: www.atsjournals.org

The impact of aging on the human population is unprecedented. According to the United Nations, individuals aged 60 years or over will exceed 2.0 billion worldwide, and outnumber younger individuals aged 10–24 years by the year 2050 (1). Thus, age-related diseases are expected to present major social, economic, and biomedical challenges over the next several decades. Idiopathic pulmonary fibrosis (IPF) is one example of an age-related disease. The incidence and prevalence of IPF increase significantly with each decade of age; two-thirds of patients with IPF are older than 60 years at the time of presentation, with a mean age of 66 years at the time of diagnosis (2–4). Despite this strong association, cellular/molecular mechanisms that account for the aging predilection to fibrotic disease are only now

beginning to be explored (5–7). At about 40,000 deaths per year in the United States, the burden of IPF is similar to that of breast cancer (8, 9). The recently approved drugs for IPF, pirfenidone and nintedanib, have modest clinical benefit by decreasing the rate of lung function decline (10, 11). Interestingly, the mechanisms of these drugs are incompletely understood. There is a clear need for studies elucidating mechanisms underlying age-related fibrosis that will lead to the development of more effective therapeutic interventions.

Mitochondrial homeostasis and function is critical for the normal physiology of cells/tissues in diverse organ systems. Mitochondrial dysfunction, on the other hand, may lead to a variety of disease pathologies, ranging from neurodegenerative to metabolic disorders

(12). Importantly, mitochondrial dysfunction is a recognized hallmark of aging (13). Mitochondrial dysfunction is characterized by an increase in the generation of reactive oxygen species (ROS) and altered bioenergetics, which may result in aberrant cellular fates, in particular apoptosis and senescence (14). There is evidence of cellular senescence in both epithelial cells and fibroblasts in the IPF lung (15, 16), whereas apoptosis susceptibility appears to be different in these cell populations (17). In this short review, we explore the evidence for mitochondrial dysfunction in different cell populations, with a focus on epithelial cells, fibroblasts, and immune cells in IPF. The consequences of mitochondrial dysfunction on cell fate that may contribute to or influence the fibrotic process are examined.

Effect of Mitochondrial Dysfunction in Lung Cells

Alveolar Epithelial Cells

Lung alveolar epithelial cells (AECs) are susceptible to injury/apoptosis after exposure to various noxious stimuli. Epithelial injury has long been recognized as the initiating event of fibrosis (18). Persistent epithelial cell injury/apoptosis is crucial for initiation and perpetuation of fibrogenesis in the IPF lung, although the nature of injury is unclear (19–23).

Mitochondrial dysfunction may represent an important mechanism for cellular apoptosis, including AECs. Type II AECs, which serve as progenitors of type I AECs, have been shown to undergo apoptosis in association with mitochondrial dysfunction (24). The alveolar epithelium encounters potentially injurious agents that escape upper airway defenses, and these may affect AEC mitochondrial function. Most of these agents, such as cigarette smoke (25), asbestos (26–28), zinc oxide (29), and even hyperoxia (30, 31), result in excessive ROS production, leading to cell injury and/or apoptosis. Apart from ROS production, particulate matter may induce AEC death by mechanisms converging on the interaction of p53 with mitochondrial apoptosis pathways (32, 33). Recent studies have shown that mitochondrial human 8-oxoguanine DNA glycosylase and aconitase-2 are essential for protection against oxidant-induced mitochondrial DNA damage and subsequent AEC apoptosis (34). Novel mechanisms, such as the deficiency of the mitochondrial deacetylase, sirtuin-3, have been demonstrated in IPF lungs; this deficiency contributes to 8-oxoguanine DNA glycosylase acetylation (inactivation), mitochondrial DNA damage and apoptosis of AECs (35). Hypoxia or hypercapnia has been reported to induce mitochondrial dysfunction, which perpetuates AEC injury by mediating epithelial–mesenchymal transition and myofibroblast activation (36, 37).

Dysfunctional mitochondria are normally disposed of through a selective quality-control autophagic pathway, termed “mitophagy” (38). Mitophagy involves the interaction of at least one of several proteins in the mitochondrial membrane with the phagophore; these include FUN14 domain containing 1 (39), BCL2/adenovirus E1B 19 kDa protein–interacting protein 3/NIP3-like protein X (40),

cardiolipin (41), and PTEN-induced putative kinase 1 (PINK1) (42). Defective AEC mitophagy, such as that resulting from PINK1 deficiency, leads to accumulation of dysfunctional mitochondria, promoting fibrosis (43, 44). Interestingly, excessive AEC PINK1 activity (and mitophagy) has been noted in cigarette smoke–induced emphysema (45). The precise mechanisms by which these different mitophagic pathways affect AEC phenotype and fate, including its interactions with fibroblasts and immune cells, remain to be explored.

Fibroblasts

Fibroblasts and myofibroblasts are the key effector cells in tissue fibrosis, by elaboration of extracellular matrix, contraction, and remodeling of injured tissues (46–48). Myofibroblast senescence, accompanied by redox imbalance, contributes to aging-associated persistent fibrosis (15, 16). Putative mechanisms of the activation of fibroblasts in fibrogenesis

include up-regulation of nicotinamide adenine dinucleotide phosphate, reduced form, oxidase 4, which is not only required for transforming growth factor (TGF)- β 1-mediated myofibroblast differentiation (49–51), but also affects mitochondrial biogenesis and bioenergetics in lung fibroblasts (52).

Mitochondrial dysfunction and accelerated senescence are seen in IPF lung fibroblasts, but whether it reflects a distinct endotype of age-associated fibrosis is unclear (53, 54). Chronic exposure to certain environmental triggers, such as cigarette smoke, may result in accumulated damage that leads to mitochondrial dysfunction and accelerated senescence of lung fibroblasts (55). Other putative mechanisms of loss of mitochondrial homeostasis in aging/IPF lung fibroblasts include deficiencies in regulatory mitochondrial proteins, such as sirtuin-3 (35, 56), and impairment in autophagy/mitophagy (43, 57–60).

Figure 1. The role of mitochondrial dysfunction in injury response in the aging lung. Various noxious stimuli induce alveolar epithelial cell injury and apoptosis, leading to efferocytosis by alveolar macrophages. Mitochondrial dysfunction and altered mitochondrial dynamics that accompany aging promote senescence, senescence-associated secretory phenotype (SASP), and other profibrotic cellular phenotypes (see the main text for details). SASP products along with antibodies secreted by lymphocytes may activate profibrogenic activities of myofibroblasts by mechanisms that include the up-regulation of Nox4. Myofibroblast-generated paracrine factors, including reactive oxygen species (ROS), may perpetuate epithelial cell injury. The net result is persistence of apoptosis-resistant senescent myofibroblasts and deposition of excessive matrix, a hallmark of pathologic fibrosis. ECM = extracellular matrix; mt = mitochondrial; Nox4 = nicotinamide adenine dinucleotide phosphate, reduced form, oxidase 4; PDGF = platelet-derived growth factor; TGF- β 1 = transforming growth factor- β 1; SASP = senescence-associated secretory phenotype; SIRT3 = sirtuin 3.

The senescence-induced growth arrest that accompanies fibroblast mitochondrial dysfunction has been termed “mitochondrial dysfunction–associated senescence,” which is characterized by a distinctive senescence-associated secretory phenotype (SASP) (61). This SASP may potentially contribute to AEC damage, thereby perpetuating further myofibroblast activation (62, 63). The mitochondrial dysfunction–associated senescence phenotype encompasses metabolic/bioenergetic alterations, including low ratio of oxidized to reduced nicotinamide adenine dinucleotide (NAD⁺:NADH), prolonged 5' adenosine monophosphate-activated protein kinase and p53 activation, in addition to partial growth arrest that is reversible by pyruvate supplementation (61, 64). Of note, metabolic reprogramming that induces a “switch” to aerobic glycolysis predisposes fibroblasts to a profibrotic phenotype, a feature of IPF and preclinical models of fibrosis (65, 66).

Immune Cells

Several studies have suggested that both innate and acquired immunity are linked to IPF progression, although mechanisms are unclear (67–71). Lung alveolar macrophages may play a key role in IPF by production of excessive ROS and SASP-associated cytokines, such as IL-1 and IL-8, in addition to platelet-derived growth factor and TGF- β 1 (72–78). In particular, TGF- β 1 secretion is enhanced in IPF macrophages that display apoptosis resistance. This apoptosis resistance is conferred by efficient mitophagic clearance of dysfunctional mitochondria (79). In contrast, deficient mitophagy enhances fibrosis in response to lung injury by mediating myofibroblast differentiation (60) or promoting AEC apoptosis (43). Thus, the mitochondrial quality control system may play varied roles in different cell types in the context of fibrogenesis. Emerging studies provide new insights into the role of macrophage mitochondrial ROS-induced NLR family pyrin domain containing 3 inflammasome signaling that promotes pulmonary fibrosis after

bleomycin injury or exposure to airborne particles, such as silica and asbestos, particularly in the context of aging (80–83).

Aging predisposes to “immunosenescence,” which may increase susceptibility to lung infections (3, 84–90). Mitochondrial dysfunction accompanies and is, at least in part, responsible for immunosenescence in aging (89, 91). Immunosenescence likely exerts effects on host–microbial interactions that determine the composition of the airway microbiome (88). Recent studies propose that host innate immune responses to lung microbiome may influence outcomes in IPF (92, 93). Antimicrobials have been tested in preclinical models, as well as in multicenter, randomized, controlled trials for IPF, with partial success (94, 95).

Age-associated alterations in lymphocytes, both T cells and B cells, have been implicated in IPF progression (67, 96, 97). For example, aging and immunosenescence are associated with an increase in autoantibodies (98, 99). Therapeutic strategies to target autoantibodies during IPF exacerbations are being studied (100). The interplay between various lung cell types, in the context of injury in the aging lung, is summarized in Figure 1.

Therapeutic Approaches

The mitochondrial structural and functional proteome is derived from more than 1,000 genes located in both the nuclear and the mitochondrial genome (101). Inherited mutations or acquired damage in either genome may result in primary mitochondrial disorders with mitochondrial protein defects leading to mitochondrial dysfunction. On the other hand, secondary mitochondrial dysfunction occurs in the setting of environmental insults, such as cigarette smoke (25, 102–104), infections (105, 106), or exposure to drugs (107–109).

Although causation may be multifactorial, mitochondrial dysfunction leads to predictable

primary consequences, such as decreased mitochondrial membrane potential, increased ROS, and altered mitochondrial dynamics (110). These lead to secondary cellular consequences, such as altered bioenergetics, mitochondrial biogenesis, increased apoptosis, and mitochondria–nuclear signaling, which, in turn, form the bases of pathogenesis of disease affecting various organs.

Therapeutic approaches to mitochondrial dysfunction involve broad strategies of genetic engineering, small molecules targeting mitochondria, targeting of metabolic cellular adaptation processes, and, finally, diet and exercise, which have been shown to produce a mitohormetic effect (110). More specifically, the pharmacologic agents that are being explored include those that activate mitochondrial biogenesis (such as resveratrol), modulate autophagy (such as rapamycin or metformin), inhibit apoptosis, and scavenge ROS and toxic metabolites (such as lipoic acid, vitamin C, vitamin E, or coenzyme Q) (111).

Conclusions

There has been increasing recognition of the association of mitochondrial dysfunction with aging and age-associated diseases, such as fibrosis. IPF is strongly associated with aging. The precise nature of the injury in IPF leading to persistent fibrosis is unknown, and may be multifactorial. In this brief review, we have explored one potential mechanism—mitochondrial dysfunction—occurring in various lung cell types, which contributes to the pathogenesis of IPF. Further understanding of these mechanisms would aid discovery of potential biomarkers and much needed newer targeted therapies that not only halt, but also reverse, progression of age-associated fibrotic disorders. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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