

Getting to the core of fibrosis: targeting redox imbalance in aging

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There has been recent progress in the development of anti-fibrotic drugs for the treatment of idiopathic pulmonary fibrosis (IPF) (1,2). Interestingly, the precise mechanisms of action of these drugs are not known (3). Importantly, although both drugs have been shown to slow the progression of disease, neither has been shown to definitively improve quality of life or survival. Clearly, improved therapies for the treatment of IPF and other fibrotic diseases are needed. It has been suggested that core pathways that mediate fibrosis in multiple organ systems may serve as better targets for anti-fibrotic drug development (4). Liepelt and Tacke (5) recently highlight redox imbalance in the context of aging as one of these core pathways.

As the average life expectancy continues to increase, the elderly population is growing at a rapid pace. Between 2015 and 2050, the proportion of the world's population over 60 years will nearly double from 12% to 22%. By 2020, the number of people >60 years will outnumber children younger than 5 years (World Health Organization, <http://www.who.int/mediacentre/factsheets/fs404/en/>; accessed December 4, 2015). With this shift in the elderly demographic, it has become increasingly important to understand the contribution of aging to disease pathogenesis.

Aging has been defined as the accumulation of diverse deleterious changes in cells and tissues that increase the risk of disease and death (6). Aging results in decreased resistance to multiple forms of stress, as well as increased susceptibility to numerous diseases. Progressive fibrosis is a hallmark of aging in various organ systems, including

the liver (7), kidney (8), pancreas (9) and lung (10). IPF, the most fatal and progressive fibrotic lung disease, disproportionately affects the elderly population and is now widely regarded as a disease of aging (11-13). The incidence and prevalence of IPF increase with age; two-thirds of IPF patients are older than 60 years at the time of presentation with a mean age of 66 years at the time of diagnosis (14). Further, the survival rate for IPF patients markedly decreases with age (11). Although the roles of specific aging hallmarks in the pathogenesis of IPF have not been fully elucidated, numerous studies implicate age-related alterations in cellular function in the pathogenesis of IPF (15,16).

Aging and fibrotic disease are both associated with cumulative oxidant burden, and lung tissue from IPF patients demonstrate "signatures" of chronic oxidative damage (17,18). The "oxidative stress theory" posits that a progressive and irreversible accumulation of oxidative damage caused by reactive oxygen species (ROS) impacts critical aspects of the aging process by contributing to impaired physiological function, increased incidence of disease, and a reduction in life span. Oxidative stress can lead to extensive modifications or damage to macromolecules including DNA, lipids and proteins and can also lead to increased production of cytokines. The lungs are particularly prone to insult and injury by oxygen free radicals given their direct exposure to the environment via inspired air (19). Further, environmental insults to lung may serve as a "second hit" which accelerate the aging process by promoting persistently elevated oxidative stress levels leading to increased susceptibility to disease (20).

Oxidative stress may represent a core pathway by which other “damage” theories of aging are based. Examples include genomic instability as a result of DNA damage, and accumulation of glycated crosslinks during protein damage that can result in pathogenesis associated with cardiovascular and neurodegenerative disease (21). Recent studies of familial and sporadic cases of IPF have been associated with telomere shortening (22,23), further supporting the concept that IPF may represent an age-related degenerative disease process (24). The cause(s) for the shortened telomeres in IPF patients without mutations in telomerase is currently unknown; however, oxidative stress represents one potential mechanism. A better understanding of the mechanisms that mediate oxidant-antioxidant imbalance in aging may be critical to the development of more effective therapeutic strategies.

Despite the well-recognized role of oxidative stress in fibrosis and aging (25), the ability to precisely target key mediators of this process has proved difficult. By definition, oxidative stress occurs when cellular ROS levels overwhelm the cellular antioxidant capacities, thus therapeutic strategies have been directed inhibiting oxidant generation as well as stimulating antioxidant capacity. A number of antioxidant therapeutic strategies have shown promise in various preclinical models, however, they have failed to demonstrate efficacy in the clinic. In the PANTHER trial, the antioxidant NAC was evaluated versus placebo in IPF patients, however it was recently reported to have no effect on the primary end-point of a change in forced vital capacity (FVC) (26). Although there may be several potential reasons for this observed lack of efficacy of anti-oxidants, one important consideration is the potential for ROS to function as redox signaling molecules for physiologic cell signaling (27). In fact, ROS may be viewed as “antagonistically pleiotropic” by mediating detrimental effects in the context of aging or an age-related disease (28-30). Based on its pleiotropic functions, it can be argued that targeting the primary enzymatic source of ROS (rather than anti-oxidant approaches) may offer a more promising strategy.

The NADPH-oxidase (Nox) enzymes are an evolutionarily conserved gene family that is most consistently linked to host defense mechanisms, including lung fibrosis (20,31). Among the seven members of the Nox family, Nox4 has been implicated in a variety of fibrotic diseases, including the liver (32-34), skin (35), kidney (36,37), heart (38,39), and lung (15,40,41). Although Nox4 is considered to be among the most promising targets for fibrotic disease (42,43), currently no selective Nox4

inhibitors are clinically available. A dual Nox inhibitor, GKT137831, reported to target Nox1 and Nox4 has recently undergone a phase II clinical trial for diabetic nephropathy (44) (www.clinicaltrials.gov; NCT02010242), although results have not been published. A number of other groups are currently developing Nox4 inhibitors; however, Nox4 drug discovery/development has proved to be particularly formidable for several reasons. To date, Nox inhibitors lack specificity for a single isoform; identification of small-molecule inhibitors that specifically target Nox4 is a major challenge. The crystal structure of Nox4 is not known; the absence of crystallization data precludes traditional rational drug design approaches. Screening methods for Nox inhibitors typically utilize ROS detection-based screening assays that have limited specificity. Further, it may be difficult to discern whether a putative inhibitor is acting directly on Nox *vs.* inhibition of a signaling pathway(s) leading to Nox induction/activation. One study reported that of >350 Nox inhibitors described, a majority of these did not directly block enzymatic activity, but rather they showed interference with upstream signaling pathways or were ROS scavengers (45). Finally, although Nox4 has unique functional properties compared to its family members, the Nox homologs share several common structural features. It will be important to identify and compare the structural features of compounds with different affinities for each of the Nox's in order to gain insights into the properties that yield specificity for particular Nox isoforms. Given the accumulating data on Nox4 as a “core pathway” in diverse fibrotic disorders, the search for safe, specific and effective Nox4 inhibitors continues.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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