The NADPH oxidase DUOX1 in chronic lung diseases

Impact

Background

The elderly population is growing rapidly worldwide, and will reach previously unprecedented levels, largely through decreased fertility and increased life expectancy (1). Indeed, the number of individuals aged 65 or older is predicted to grow from about 524 million in 2010 to nearly 1.5 billion in 2050. At the beginning of the 20th century, major health threats were infectious and parasitic diseases (e.g. pneumonia, influenza, tuberculosis), also termed communicable diseases (2). However, the advances in modern medicine over the last 200 years have greatly shifted the diseases causing major mortality, and at present, non-communicable diseases (e.g. cancer, heart disease, diabetes, chronic respiratory disease) are the leading cause of mortality. These non-communicable diseases more frequently affect adults and the elderly population, and impose the greatest (economic) burden on global health (3). Thus, instead of healthy aging of the elderly population, the aging population currently experiences many years of living with these disabilities. It is estimated that about 100.000 people die from age-related causes every day (4), of which pulmonary diseases, such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF), account for roughly 7.3 million deaths, which equals 14% of all deaths, worldwide yearly, and largely affects the elderly population (5). In addition, the elderly population is the most at-risk group affected by aforementioned communicable diseases through increased susceptibility to (respiratory) infections, a relevant issue that is made very clear and relevant with the COVID-19 pandemic (6). As the elderly population is increasing, novel therapeutics are required for these non-communicable diseases that improve quality of life and may result in healthy aging (growing old without age-related disabilities), which would also significantly reduce economic and healthcare burden. However, to establish such therapeutics for pulmonary diseases commonly associated with aging, it is critical to understand the fundamental causes and the underlying mechanisms of lung aging and age-related chronic lung diseases.

Research

Oxidants, also termed free radicals or reactive oxygen species (ROS), are compounds generated by metabolism of oxygen, and are found within the environment due to air pollution but are also produced within our bodies due to oxygen metabolism. These ROS may cause harm to cells if their levels become too high (oxidative stress) (7,8). To protect against oxidative stress, our body is equipped with defense mechanisms (antioxidants) that may keep these oxidants at levels where they are not damaging by scavenging them. A

theory regarding ROS and aging was already described in the 1950's, where it was proposed that the lifelong accumulation of damage due to ROS may cause aging and age-related chronic disease development (9). Furthermore, during aging, there is an apparent imbalance between oxidants and antioxidants in favor of oxidants, resulting in oxidative stress, and this has been shown in various age-related lung diseases, such as COPD (7,10). At (relatively low) levels, these oxidants can actually perform important signaling functions, also termed redox signaling (11). One well-recognized function of ROS is the involvement of ROS in host defense, as these species are essential for the removal of pathogens such as bacteria, or cell debris (termed phagocytosis). The phagocyte (cells that perform phagocytosis) NADPH oxidase (NOX), or NOX2, is widely appreciated as a critical component of antimicrobial host defense through the production of ROS (12). Additional NOX enzymes have been discovered in non-phagocytic cells that appear to possess diverse redox signaling functions such as the involvement in cell proliferation, differentiation, and in regulation of gene expression. Several of these NOX enzymes are also expressed in the lungs, where they participate in such redox signaling events following bacterial or viral infection or environmental stress. Specifically, the airway epithelial cells express the NOX enzyme dual oxidase 1, or DUOX1. Upon activation, DUOX1 functions through the production of regulated levels of hydrogen peroxide (H2O2), that has been shown to have important roles for epithelial repair processes following injury (13,14). As such, DUOX1 is be an enzyme that, rather than causing damage to our cells through the production of oxidants, actually has important protective functions. As such, the important contribution of NOX enzymes in e.g. epithelial and inflammatory cell signaling as well as host defense may explain why antioxidant supplementation does not improve lifespan, and is often ineffective during disease presentation (15,16), as scavenging of these ROS species generated by NOX enzymes that are important in various biological functions could be detrimental. In light of this concept, the aim of this thesis was to explore whether DUOX1 function is altered during aging, and whether such alterations may contribute to age-related lung diseases.

Interestingly, we observed that DUOX1 expression and activity was dramatically lost with age in the lungs (or more specifically, airway epithelial cells) of mice as well as in humans, and that this resulted in impaired immune responses and decreased repair capacity of lung epithelial cells in response to injurious challenges often encountered in the environment. Furthermore, we observed that lifelong absence of DUOX1 in mice enhanced some aspects of lung aging, as these mice developed accelerated age-related degenerative changes in the lungs, with enlargement of the alveolar walls, also termed senile emphysema. Furthermore,

we observed that in the age-related chronic lung disease COPD, DUOX1 protein levels are also suppressed, which was observed in both human lung tissues from COPD patients and in mouse models of COPD. The level of lung DUOX1 positively correlated with lung function in these COPD patients, indicating that low levels of DUOX1 are associated with worse lung function. Furthermore, we observed that lifelong absence of DUOX1 in mice worsened experimental emphysema development in a mouse model, which is characterized by alveolar enlargement through alveolar wall destruction, a major hallmark of COPD. Additionally, we found that DUOX1 suppression in COPD may be in part explained by smoking, which is a major risk factor for chronic lung disease development. Because of our observation of decreased DUOX1 during aging, we also sought to explore how aging and DUOX1 impact on other age-associated chronic lung diseases such as asthma in the elderly and idiopathic pulmonary fibrosis. Although results from these studies are preliminary, we observed that aging does not significantly affect the pathogenesis of experimentally-induced pulmonary fibrosis using administration of the chemotherapeutic agent bleomycin. Moreover, we found that DUOX1 is actually enhanced during experimental pulmonary fibrosis, and that lifelong absence of DUOX1 appeared to minimize some critical features of IPF pathogenesis. We also observed that aging leads to decreased features of experimentally-induced allergic asthma in mice induced by repeated exposure to house dust mite allergen, with less inflammation, remodeling of the airways, and mucous overproduction. While DUOX1 strongly contributed to these features of allergic asthma in young mice, it contributed less strongly to allergic inflammation in the elderly mice and appeared to alter the type of inflammation observed.

The findings obtained from the research conducted in this thesis are of scientific relevance, as knowledge gained from the current studies may contribute to the scientific field of lung aging and age-related chronic lung disease. Furthermore, they may also contribute to social sectors such as healthcare and pharmaceutical companies, and may support potential solutions to social challenges such as the worldwide aging population. First of all, our findings challenge the the overall perception that ROS contribute to aging and age-related lung disease, and may additionally warrant caution for antioxidant supplementation, which is thought to be beneficial during aging and in age-related diseases. As previously mentioned, people should be informed about the potential detrimental effects of antioxidants (as they may prevent beneficial signaling functions of NOX enzymes such as DUOX1 as described in this thesis) during aging and in chronic diseases, and may even be discouraged from such supplementation strategies. Moreover, our research may open up avenues to try

and unravel how modulation of DUOX1 during aging may limit and/or delay lung aging and age-related lung disease development. Future studies could explore if restoring DUOX1 (meaning restoring DUOX1 levels/activity) within airway epithelium of elderly mice (or human subjects) could potentially protect against the loss of repair responses and senile emphysema as a result of aging, or whether this may protect against the development of features of emphysema/COPD in mouse models of COPD. Although this thesis focused on the importance of the NOX enzyme DUOX1 during aging, our work may also provoke research to further address the importance of how age-related changes in the redox balance may contribute to (lung) aging and age-related disease development. For example, the question of how aging may affect other NOX enzymes, or other cellular sources of ROS in the cell, may be addressed in future studies. Vice versa, whether these ROS-generating sources may be involved in aging, and age-related pathology, either by progressing or slowing aging, are questions that are just as important. To this end, we have prepared a concise literature review/perspective, that will focus on these sources of ROS and will highlight the scientific evidence for these sources in lung aging and age-related lung pathologies. Additionally, this perspective will also aim to re-emphasize the importance of addressing these critical questions mentioned above. Our results may also be of relevance to healthcare, pharmaceutical companies, and to the critical issue of a worldwide aging population with reduced quality of life due to disabling conditions. In the future, lung DUOX1 levels (or hypermethylation status of the DUOX1 promoter) could be envisioned as a screening tool in individuals at middle age (~45-65 years of age) to monitor for susceptibility to age-related chronic lung disease development. In individuals where relatively low levels of airway epithelial DUOX1 would be observed (in healthy individuals of middle age, or e.g. COPD patients), strategies that would restore lung DUOX1 function/expression would be of interest to tailor more personalized treatment. These strategies may ultimately increase quality of life in the elderly by concomitantly reducing disabling conditions and improving lung health, thereby contributing to healthy aging. Ultimately, the goal is to improve lung health in the elderly, as lung health is associated with good health (such as increased metabolic rate, activity, and physical performance), which will also significantly reduce the healthcare costs (17). However, treatment options targeting DUOX1 in the elderly currently do not exist and additionally, these strategies would have to be non-invasive. As such, to fully realize such strategies, therapeutics have to be developed to allow for such non-invasive treatment, and would likely involve inhalation strategies.

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