Redox regulation in pulmonary fibrosis

Valorization

The incidence of age-related lung diseases, including idiopathic pulmonary fibrosis (IPF), lung cancer and chronic obstructive pulmonary disease, is increasing worldwide, warranting more treatment strategies to counteract the high morbidity and mortality associated with these diseases. The work presented in this thesis focused on different redox-modulatory strategies to alleviate features of the age-related disease pulmonary fibrosis.

The next pages will provide an overview of the valorization of the research described in this thesis. Knowledge valorization is "the process of creating value from knowledge by making knowledge suitable and/or societal useful and by translating that knowledge into products, services, processes and entrepreneurial activity" (1). The valorization potential of the work described in this thesis will be discussed with focus on social and economic relevance, the implications for specific target groups outside of academia and possible innovations that can be created from this research as well as their potential implantation.

Social and economic relevance

Since the research performed in this thesis aimed at identifying novel pathways involved in the development of pulmonary fibrosis, the relevance and impact for the society may not be seen immediately but rather in the near future.

During the last years, the average life expectancy increased worldwide. It is estimated that the number of people over 60 years of age will increase by almost 50% between 2015 and 2050, thereby accounting for 22% of the total world population (2). Due to this increase, it is expected that the incidence of age-related lung diseases such as IPF will also increase at a rapid pace. However, the incidence of IPF is also increasing because of better options for diagnosis as well as the increased social awareness. The disease develops asymptomatically for months to years and normally the disease is diagnosed when the patient has already problems breathing which means that there is already significant damage to the lung tissue. Therefore, the survival of patients with IPF after diagnosis is estimated to be only 2-4 years (3). A diagnosis of IPF requires the exclusion of other forms of interstitial lung diseases such asbestos- or bleomycin-induced pulmonary fibrosis since the cause of the disease is not clear, leading to the diagnosis idiopathic pulmonary fibrosis. Therefore, no strategies for disease prevention can be developed.

Currently, there are approximately at least 5 million patient with IPF worldwide but studies have already suggested that the prevalence of IPF is increasing every year by 11% (4). Therefore, the socioeconomic burden related to IPF will also increase in the nearby feature. It has been indicated that once a patient is diagnosed with IPF, the health costs increase from approximately €2.700 to €21.000 per year without the cost of antifibrotic treatment (5). These costs derive from the fact that patients with IPF are often hospitalized also due to comorbidities such as pulmonary arterial hypertension, heart failure, pneumonia and lung cancer. Patients with IPF are admitted twice as often to the

hospital compared to aged matched controls associated with an average cost per IPF-related hospitalization of $16.812 (6). Additionally, patients with IPF need continuous oxygen supply to increase the low levels of oxygen in the blood in a more severe stage due to the significant lung damage.
The impact of IPF is not only limited to a decline in lung function but is also strongly associated with a decrease in health associated quality of life because of the daily limitations in regard to everyday physical and social activities.

Although IPF is such a devastating disease, there are currently only two FDA-approved therapies available for its treatment which will not cure the already induced pulmonary damage. Pirfenidone and nintedanib each cost approximately $100.000 per patient per year in the US (7) and they are not equally effective in all patients. The other costly therapy option is lung transplantation, which is not available for every individual patient. Consequently, there is an urgent need to perform more studies to identify novel leads for the development of new treatment options for pulmonary fibrosis. Our studies have indicated that the oxidant producing enzyme NOX4 oxidizes members of the SRC family kinases, thereby promoting features of pulmonary fibrosis. These observations suggest that targeting redox-sensitive proteins might be a lead for the treatment of pulmonary fibrosis. Moreover, our findings suggest that inhibition of SRC family kinases by their pharmacological inhibitor saracatinib might provide an alternative approach to reduce the progression of IPF.

Every individual is different in terms of their physiological and genetic makeup, which can also be deducted from our studies that show that the responsiveness to different drugs varies in epithelial cells derived from patients with IPF. Furthermore, these results have suggested that not all patient with IPF suffer from an increased oxidant burden, indicating even more the need to implement personalized treatment strategies to maximize the treatment benefits. One way of achieving personalized treatment could include the identification of lung damage biomarkers which would also improve diagnostics as IPF is usually diagnosed when it is already too late, and the lung is already severely damaged. Another way of achieving a more personalized treatment approach, could be testing individual responses to treatments in pulmonary epithelial cells of IPF patients isolated by non-invasive bronchoscopic microsampling directly upon diagnosis. In that way, treatment responses can be predicted on forehand, thereby personalizing and thus optimizing treatment. By using such an approach, costs can also be reduced as the treatment response is evaluated beforehand so that the patient does not have to be switched to a different drug in case of non-responsiveness. Altogether, the studies presented in this thesis may give rise to new therapy strategies to decrease the economic and health care burden associated with IPF.

Target groups

The findings presented in this thesis are especially of interest to the academic community as well as the pharmaceutical industry. The results described in this thesis have been presented at various national and international meetings to discuss them with peers inside the research field. For instance, the work presented in this thesis has been presented at the annual meetings of the European Respiratory Society and the ERS Lung Science Conference as well as at the NOX NADPH Oxidases Gordon meetings. Moreover, the results described in this thesis are published or will be published in peer-reviewed journals to make it available to the community and to increase knowledge transfer.

However, it is also of great importance to present scientific work outside the field to the general public to increase social awareness of IPF. Scientific research is the basis for all progress and without research, treatment developments stand still. Research gives lung patients the prospect of improvement of their health status. Within the scientific community, we have teamed up with a specialized lung hospital to work with primary human lung cells directly derived from IPF patients. Part of this work was sponsored by the Longfonds which is a Dutch organization that promotes the communication form the lab bench to the general community and in specific to patients with a lung disease. Consequently, some results presented in chapter 7 and 8 have also been published in the newspaper and the homepage of this organization, making them accessible for everyone.

During the last decade, the pharmaceutical industry has become very interested in studying rare diseases and as a result, various new rare-disease treatments have entered the market. Since there are only two drugs available for IPF treatment and novel therapeutic interventions are necessary, it is a highly profitable industry. It has been suggested that the IPF drug market will increase from $900 million in 2015 to $3,2 billion by 2025 (8). Additionally, drugs that have the possibility to attain orphan drug status (when only a small percentage of the population is affected by the disease) are very attractive for the pharmaceutical industry as the status implies support for clinical trials, reduced fees as well as a 10-year patent protection to motivate the pharmaceutical companied to invest in these drugs. Currently, various IPF drugs are being evaluated in clinical trials (9) and our data also suggest that the SFK inhibition by saractinib would be a possible treatment option for IPF patients and would therefore be of interest to the pharmaceutical industry. Intriguingly, saracatinib has just received orphan drug status by the FDA in the US (March 2019) (10).

Innovation

Our understanding regarding the role of ROS in IPF is still very limited mainly due to the fact that detection of oxidant pathways in clinical specimens is difficult and methods to analyze them are limited. Especially reversible modifications of redox-sensitive proteins

may represent an important mechanism in dysregulated cell signaling and might be more important than stable protein oxidation products. However, studies are very limited. Another problem with studies of ROS in disease models such as IPF is that they typically address the importance of a specific NOX enzyme or antioxidant system throughout the disease model, which may not necessarily translate into clinical practice where different pathways are involved. Consequently, it might be more important to address specific modifications of proteins as we did in chapter 5, where we focused on one specific cysteine oxidation which might be a novel mechanism of SRC kinase activation.

Addressing the causality of specific ROS pathways requires the use of appropriate models, and currently used models of pulmonary fibrosis do not adequately model the pathophysiology of human IPF and its progression. In our studies, we have used different models (cell lines, bleomycin mouse model and primary cells derived from IPF patients) to study the effects of oxidant regulation in IPF development. Moreover, we have also utilized different cell types, i.e. epithelial cells and fibroblasts, to study the effects of SRC family kinase inhibition. It is of great importance to not only address treatment responses in fibroblasts, the effector cells, but also to investigate them in epithelial cells, which initiate and mediate fibrotic and inflammatory responses. The lung epithelium plays a crucial role in the development of IPF which makes it pivotal to investigate the effects of fibrotic drugs on the lung epithelium as well. Also, while IPF is well-known to be age-associated, most studies are still performed only in cell lines which does not recapitulate the contribution of age-associated alterations. Therefore, we have also investigated the effects of SRC family kinase inhibition on primary epithelial cells isolated from patients with IPF. Although our knowledge about IPF is growing, we still know relatively little with respect to the precise molecular mechanisms. The results in this thesis contribute to our understanding regarding the effects of oxidant regulation and targeting of redox-sensitive proteins in the pathophysiology of IPF. However, to effectively treat every individual patient, better treatment options need to be developed and if possible individualized, also called personalized medicine.

Planning and implantation

Before personalized medicine with respect to IPF can be implemented into the clinic, more studies need to be performed on the use of bronchial epithelial cells isolated by bronchoscopic microsampling to predict therapy outcome. Based on our preliminary pilot data, it can be suggested that patients could be divided in subgroups possibly based on their redox or inflammatory status or the activity of SRC kinases. In the beginning, dividing patients into specific subgroups will increase the associated treatment costs but, in the future, this will decrease costs associated with false treatments in addition to increasing treatment effectiveness. This thesis provides a first lead to study the importance of the redox-sensitive proteins SRC kinases and their modifications by the oxidant producing

enzyme NOX4 and subsequently investigated the effects of their inhibition. Additionally, this thesis provides first ideas to implement a more personalized approach in the treatment of pulmonary fibrosis.

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