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Song, Shanshan

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MIF family proteins in lung diseases

Shanshan Song

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MIF family proteins in lung diseases

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and in accordance with

the decision by the College of Deans.

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Monday 14 June 2021 at 9.00 hours

by

Shanshan Song

born on 13 May 1988
in Anhui

Supervisors:

Prof. B.N. Melgert

Prof. G.J. Poelarends

Prof. P. Olinga

Assessment committee:

Prof. H.I. Heijink

Prof. S. Meiners

Prof. J. Bernhagen

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INTRODUCTION AND SCOPE OF THE THESIS

Chronic respiratory diseases are long-lasting diseases of the airways and other structures of the lungs, such as asthma, chronic obstructive pulmonary disease (COPD), occupational lung diseases, and pulmonary fibrosis. According to a study on the global burden of diseases, more than 3 million deaths were caused by COPD in 2015 alone [1]. It was the seventh leading cause of early death in 2017 and projected to be the fourth in 2030 [2]. Another important lung disease that is not considered a chronic lung disease due to its lethality is lung cancer, which adds another 1.4 million deaths annually [3].

The primary cause of both COPD and lung cancer is exposure to cigarette smoke, but also exposure to indoor or outdoor air pollution are important upcoming other causes. Repetitive exposure to these toxic components cause lung injury associated with an increase in inflammation, oxidative stress, cellular senescence and apoptosis. For COPD, abnormal tissue repair is a key pathophysiological feature, which leads to airway abnormalities and chronic bronchitis and/or destruction of parenchymal lung tissue resulting in emphysema, the two key phenotypical pathologies of COPD [4]. The adult healthy lung is a fairly quiescent tissue. However, after injury, stem cells in the respiratory system re-start their cell cycle, proliferate, differentiate into one or more cell populations and restore lung structure [5,6]. Unfortunately, severe damage or ongoing chronic damage cannot be repaired well, resulting in respiratory disease.

The respiratory system comprises of proximal conducting airways and distal alveoli. The proximal airways are covered by a mix of club cells, goblet cells, ciliated cells, basal cells and neuroendocrine cells that together form a pseudostratified epithelium [7]. Club cells and basal cells are progenitor cells, which have been shown to give rise to ciliated cells and goblet cells to maintain the bronchiolar epithelial structural and functional integrity. The distal alveoli are important for gas exchange and consist of flat type 1 alveolar epithelial cells (AEC I) that cover around 90% of the alveoli. Together with the vascular endothelium, these provide the interface between the air and blood compartment and this is essential for successful gas exchange. Within AECI, each alveolus also contains some cuboidal type 2 alveolar epithelial cells (AECII). These are a source of surfactant needed to reduce surface tension in the alveoli and also have the capacity to give rise to AEC I [8]. So far, effective therapies for chronic lung damage are extremely limited and only disease symptoms are treated. Excitingly, a number of recently published studies have suggested the possibility of regenerating lung tissue to ameliorate diseased lung tissue [9–13].

Lung cancer is often caused by ongoing DNA damage by irritants in cigarette smoke resulting in uncontrolled proliferation of and changes in lung epithelial cells. This uncontrolled proliferation of mutated epithelial cells leads to organ failure and death. Lung cancers can be divided into small cell lung cancer (~10-15% of lung cancer cases) and non-small cell lung cancer (~80-85% of lung cancer cases) [14]. Non-small cell lung cancer mainly consists of adenocarcinoma and squamous cell carcinoma [15]. Lung cancer is the most common type of cancer and the survival rate is lower than with other cancers. More than half of the patients with lung cancer die within one year after diagnosis [16].

Considering the lack of treatment options for COPD and lung cancer, a better understanding of the complex pathogeneses and developing appropriate tools for early diagnosis and therapeutic strategies for lung diseases are needed. An intriguing candidate for study is macrophage migration inhibitory factor (MIF) and its family members as this cytokine family is implicated in many chronic lung diseases and lung cancer [17,18].

MIF is a pleiotropic cytokine with multiple properties. In the 1960's MIF was discovered as a factor that could inhibit migration of macrophages and was named accordingly [19,20]. Since then many other functions of MIF have been described, among which an association with inflammation and downregulation of the immunosuppressive actions of glucocorticoids [21]. In addition to this association with inflammation, a growing body of evidence has demonstrated that MIF is involved in the maintenance of cellular homeostasis, i.e. cell survival, angiogenesis, senescence and wound repair. MIF can exert this plethora of biological functions through multiple membrane receptors, such as CD74 (also known as HLA class II histocompatibility antigen gamma chain or HLA-DR antigens-associated invariant chain), CXC chemokine receptor type 2 (CXCR2), CXC chemokine receptor type 4 (CXCR4), atypical chemokine receptor 3 (ACKR3, also known as CXC chemokine receptor type 7, CXCR7) and epidermal growth factor receptor (EGFR), as well as many intracellular interaction partners [22–24].

A second member of the MIF family has been discovered based on its structural similarities with MIF. This protein is called D-dopachrome tautomerase (D-DT) and it can also bind to MIF receptor CD74. The D-DT gene is located close to the MIF gene (~80 kb apart) on chromosome 22. Despite its structural similarity to MIF, D-DT only shows 34% amino acid sequence similarity to MIF in humans and 27% in mice [25,26].

MIF has been shown to be involved in many chronic lung diseases. Several studies have found differences in MIF levels between COPD patients and (healthy) control individuals [27–31], but some of these showed higher levels while other showed lower levels of MIF in COPD patients compared to controls (Figure 1). Interestingly, MIF-deficient mice were shown to develop age-related spontaneous emphysema and were also shown to be more susceptible to cigarette smoke-induced emphysema than MIF-competent mice [28]. How MIF may contribute or protect against lung damage is yet unclear.

With respect to lung cancer, higher MIF levels were found in lung cancer patients compared to controls and having more MIF was a predictor for poor prognosis [32,33] (Figure 1). Mouse studies have shown that fewer and smaller tumors developed in MIF-deficient mice compared to MIF-competent mice [34]. The biological function of D-DT in chronic lung diseases has not been studied in detail yet, but given the structural similarities between MIF and D-DT and the overlap in receptors, the functions of D-DT have been assumed to be quite similar to those of MIF.

▲ **Figure 1. MIF and D-DT expression in COPD and lung cancer.** BAL: Bronchoalveolar lavage. CS: Cigarette smoke. (Created with BioRender.com)

Scope of this thesis

The aim of the work described in this thesis was to improve our understanding of the expression patterns and functional properties of MIF and D-DT as well as their receptors in lung tissue, specifically in the context of tissue repair, regeneration and proliferation in diseases like COPD and lung cancer.

In **chapter 1**, we aim at obtaining a better insight into the roles of MIF and D-DT in the different stages of tissue injury and review the membrane receptors and intracellular partners of MIF and D-DT as well as their intracellular and extracellular activities. Moreover, we summarize the diverse roles of MIF and D-DT in the pathogenesis of liver, kidney, lung and neuronal diseases. We also outline new concepts that have been introduced in therapeutic strategies for MIF/D-DT-mediated diseases.

In **chapter 2**, we review different stages of tissue repair, and discuss the diversity of myeloid innate immune cells that are involved in lung tissue repair. This chapter demonstrates that myeloid cells are not only leading players in tissue repair, but are also essential in the development of pulmonary fibrosis.

In **chapter 3**, we provide insight into the biological function of D-DT in epithelial repair in COPD. We examined the expression of D-DT in lung tissue and studied the effects of D-DT on lung epithelial cell proliferation and survival. We found that D-DT contributes to alveolar growth, via ACKR3, and promotes proliferation of AECII and/or protects them from apoptosis. Importantly, D-DT was also able to do this in epithelial cells from patients with COPD.

In **chapter 4**, we compare the effects of MIF and D-DT on lung epithelial repair. We first studied the expression of MIF and D-DT in healthy murine and human lung tissue and investigated the effects of MIF and D-DT on outgrowth of alveolar epithelial cells using lung organoid cultures. Our data demonstrate that D-DT contributes to alveolar regeneration by directly promoting proliferation and differentiation of AECII. In contrast to D-DT, MIF inhibits alveolar regeneration indirectly by inhibiting growth support by fibroblasts. In addition, we show that MIF can promote alveolar regeneration when epithelial cells are derived from lung tissue of patients with severe COPD, suggesting changes in MIF signaling in COPD.

In **chapter 5**, we investigate a novel way of inhibiting intracellular MIF levels to study how this affects cell function. We report the generation and characterization of a

MIF-directed proteolysis targeting chimera (PROTAC), which targets MIF molecules for proteasome destruction. In addition, using this PROTAC construct we studied the role of intracellular MIF in proliferation of lung cancer cells. This study illustrates how a PROTAC approach can be a valuable strategy for exploring MIF functions in physiological and pathological conditions.

In **chapter 6**, we further investigated the potential therapeutic options for the treatment of non-small cell lung cancer patients with KRAS mutation. We found ACKR3 overexpression as a bypass and survival mechanism for EGFR knockout in KRAS-driven lung cancer cells. Dual EGFR and ACKR3 inhibition led to significant reduction of ERK phosphorylation and synergistic inhibition of cell growth. Therefore, dual inhibition of EGFR and ACKR3 might be a potential treatment strategy for patients with non-small cell lung cancer.

Finally, we further examine the findings presented in this thesis in the **chapter 7** along with future perspectives. The most important conclusions of each chapter are presented in the **summary chapter**. The work described in this thesis forms a basis for study of the potential use of MIF- and D-DT-directed therapies in COPD and lung cancer.

Declaration of my contributions

Shanshan Song wrote the chapter.

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