CHAPTER ONE

GENERAL INTRODUCTION

Fibrosis is defined by abundant production and deposition of collagen and other extracellular matrix proteins in tissue resulting in permanent scarring which leads to destructive remodeling of damaged organs. Naturally, extracellular matrix protein production is a part of a normal repair response to restore tissue architecture after injury. However, persistent and continuous injury may lead to imbalanced regulation of extracellular matrix metabolism in the affected organ and often leads to organ malfunction (1, 2).

Fibrosis can occur in many organs including liver (3), lung (4), kidney (5), intestine (6), heart (7) and bone marrow (8). Among those organs, liver and lung fibrosis are most prevalent (9, 10). Liver fibrosis contributed to almost 2.4% of global deaths in 2017 (11) and the incidence rate of idiopathic pulmonary fibrosis (IPF), the most severe form of lung fibrosis ranges from 2 to 30 cases per 100.000 person-years, with a median survival of approximately 3-5 years from the time of diagnosis (12). In most patients, fibrosis is only diagnosed when the organ is already severely injured and not able to function normally anymore. To date, limited treatment options exist for fibrosis patients and organ transplantation is the only curative option for patients with advanced disease (13, 14). Therefore, researchers and clinicians are trying to find novel therapeutic modalities to cure or stop fibrosis and a way to detect or predict the progression of disease and provide better clinical management for patients.

In fibrosis, (myo)fibroblasts have been shown to become senescent, characterized by increases in the levels of senescence-associated β-galactosidase (SA-β-gal), P16, P21, P53, and a senescence-associated secretory phenotype (15). These (myo)fibroblasts produce aberrant amounts of extracellular matrix proteins and several studies have found that this extracellular matrix is not only a consequence but also an important driver of fibrosis development and progression (16-18). Extracellular matrix and associated proteins may therefore be candidates for use as biomarkers or as therapeutic targets in fibrosis (19, 20). One of these candidates is osteoprotegerin (OPG), which has many matrix-related functions. It is an important regulator of bone matrix metabolism by being the soluble receptor for receptor activator of nuclear factor kappa B ligand (RANKL) and it has been shown to interact with many other extracellular matrix-related proteins (21, 22). OPG has also been shown to be associated with fibrosis development and may have potential as a biomarker or as a target for therapy in fibrotic disease (23). The role of RANKL in fibrosis is more of a mystery. Hence, further in-depth clinical and mechanistic studies are needed to validate the clinical relevance of OPG as a biomarker and therapeutic target, along with its interactions with RANKL and other extracellular matrix proteins, in fibrosis.

The aim of this thesis was to increase our knowledge of the role of OPG and RANKL and their interactions with extracellular matrix proteins in fibrotic disease development, particularly in liver and pulmonary fibrosis. In **chapter 2**, we summarized the current knowledge of OPG/RANKL in fibrotic diseases and critically discussed the potential use of OPG as a biomarker and/or novel therapeutic target for fibrosis. We concluded that OPG may represent a novel biomarker and therapeutic target for fibrosis. However, larger

cohorts of patient data along with further in-depth mechanistic studies were warranted to reveal the role of OPG in the fibrosis machinery to validate the clinical relevance of OPG. To that end, in **chapter 3** we elucidated the expression, production and role of OPG in liver fibrosis development using samples from patients with liver cirrhosis, mice with CCL4-induced liver fibrosis, murine precision-cut liver slices, and several relevant cell lines. In **chapter 4**, we investigated the expression of OPG in pulmonary fibrosis and whether OPG expression could be used as a biomarker to diagnose or predict the progression of IPF using samples from healthy controls, patients with IPF, mouse precision-cut lung slices, and primary human lung fibroblasts. In **chapter 5**, we examined the role of RANKL in lung tissue repair and particularly its effect on proliferation of alveolar type II epithelial cells using a murine model of silica-induced pulmonary fibrosis and human/murine lung organoids. To investigate the interaction between OPG and other extracellular matrix proteins, in **chapter 6** we assessed the possible association between OPG with four other important fibrosis-related growth factors and extracellular matrix proteins, i.e. connective tissue growth factor (CTGF), vascular endothelial growth factor (VEGF), fibulin-1, and collagen 1α1, using lung tissue of patients with IPF, primary lung fibroblasts, and a murine model of bleomycin-induced pulmonary fibrosis. To gain deeper understanding of the interactions between OPG/RANKL and extracellular matrix proteins, in **chapter 7** we assessed the impact of pathological stiffness on OPG/RANKL expression by primary lung fibroblasts. We cultured fibroblasts on methacrylated-gelatine hydrogels mimicking healthy and fibrotic stiffnesses and analyzed markers of senescence and fibrosis-associated gene expression including RANKL and OPG. In **chapter 8,** we investigated the impact of extracellular matrix deposited by IPF-derived fibroblasts on senescence development in primary lung fibroblasts grown on this matrix. We analyzed OPG/RANKL, along with other markers of senescence, characterized senescence-associated secretory phenotype secretion and assessed the expression of fibrotic response-associated genes. Finally, in the **General Discussion,** we summarized key findings, critically evaluated them and presented directions for future study.

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