General abstract

Fibrosis is an important component of many lethal diseases. It is characterized by excessive extracellular matrix production, affecting tissue architecture and function. In the lung, the most common form of fibrosis is idiopathic pulmonary fibrosis (IPF), a disease with a five-year survival of about 20% and thus a higher mortality rate than many cancers. During the development of lung fibrosis, the extracellular matrix changes in composition and structure. The cells responsible for the production and maintenance of extracellular matrix are fibroblasts that differentiate under pro-fibrotic conditions towards a myofibroblastic phenotype, characterized by increased expression of α-smooth muscle actin. This process of myofibroblast differentiation is, in part, regulated by the chemical and mechanical properties of the extracellular matrix, resulting in a reciprocal relationship between cells and matrix, where both players affect each other.

So far, no effective treatment has been developed against IPF. We hypothesize that this is due to the classic focus on soluble factors involved in the process of fibrosis. Considering the emerging evidence for a regulatory role of the extracellular matrix in the development of fibrosis, it may be interesting to approach the extracellular matrix proteins as a target for therapeutic intervention. Therefore, in this thesis we set out to investigate several aspects of this intricate relationship between matrix and cells in the context of the development of lung fibrosis.

In **chapter 2** we studied the development of lung fibrosis in the murine model of bleomycin-induced lung fibrosis. On the matrix side, we focused on the most reported change in the fibrotic extracellular matrix, the deposition of collagen, measured by incorporation of deuterated water in hydroxyproline. On the cell side, we analyzed gene expression in the lungs of the same mice. This allowed us to correlate the changes in the matrix and at the cellular level in order to identify changes in gene expression that are related to changes at the matrix level. In this pool of genes that is regulated during fibrosis we continued to search for cellular processes in which these genes are involved, in order to identify pathways relevant in fibrosis. This way, we were able to create a gene expression signature of fibrosis within the lung. Several genes related to the extracellular matrix were highly correlated with collagen deposition, such as thrombospondin 2, playing a role in fibril formation, lysyl oxidase, important for the crosslinking of collagen, and the collagen degrading enzyme matrix metallopeptidase 14. Furthermore, collagen deposition also highly correlated with some genes not thought to be directly involved in extracellular matrix remodelling. *In vitro* upregulation of many of these genes after $TGF\beta_1$-induced myofibroblast differentiation provides further evidence for the involvement of these correlating genes in the process of lung fibrosis. This qualifies these genes as interesting targets for future research into pathways relevant for fibrosis.

Within the gene expression signature of fibrosis, several extracellular matrix proteins other than type I collagen were upregulated in lungs of mice with active fibrosis. Elastin was the most highly correlated gene. Furthermore, also type V collagen and tenascin C were strongly correlated with new collagen formation. In **chapter 3**, we zoomed in on these three changes on the matrix side of the fibrotic story and their position within the positive feedback loop of lung fibrosis. We first confirmed the presence of elastin, type V collagen and tenascin C protein in histological sections of the lungs from mice with bleomycin-induced lung fibrosis. Surprisingly, while elastin staining continued to increase at least five weeks after fibrosis induction, tenascin C and type V collagen staining initially increased, but started to decrease after 3 weeks. We could confirm the contribution of fibroblastic cells to the increased expression of these matrix proteins *in vitro* by culturing human lung fibroblasts under fibrotic conditions, i.e. in the presence of $TGF\beta_1$. Finally, we identified a pro-fibrotic effect of extracellular elastin by culturing human lung fibroblasts on elastin-coated culture plates and observing an increased expression of the myofibroblastic markers α-smooth muscle actin and type I collagen.

During the development of fibrosis in the lungs the mechanical properties of the lung tissue change. Due to the deposition of large amounts of unstructured extracellular matrix proteins, the stiffness of the lung tissue increases. Indirectly, increased stiffness will influence the mechanical loading resident lung cells are exposed to during the breathing cycle: stiffer sections of the lung will deform less, thereby reducing the exposure of fibroblasts to cyclic mechanical loading. In **chapter 4** we show that this reduction in cyclic mechanical loading increases gene expression of myofibroblast markers, such as α-smooth muscle actin and type I collagen, in human lung fibroblasts. Cyclic mechanical loading did not change the expression of the fibronectin ED-A splice variant, but did decrease the paracrine expression of $TGF\beta_1$, thereby suggesting a possible regulation mechanism for the observed effects. The data suggest that cyclic loading experienced by healthy lung cells during breathing may prevent fibroblasts from differentiating towards myofibroblasts.

In a review of the literature (**chapter 5**) we focused on the involvement of caveolin in fibrotic diseases. Caveolin levels have been shown to be reduced in tissues before the onset of fibrosis, indicating involvement in the development of fibrosis. It is an integral membrane protein necessary for the formation of caveolae – invaginations in the plasma membrane rich in cholesterol and signaling molecules, such as integrins and the TGFβ-receptor. In this position, caveolin can influence TGFβ signaling, cell matrix interaction, and the sensitivity of cells to chemical and mechanical cues, thereby taking a central spot in many fibrosis-relevant cellular processes.

From the above and information in the literature, we conclude that there are many different ways in which the properties of the fibrotic extracellular matrix can influence fibroblast behavior and thus disease development. The resulting positive feedback loops between cells and matrix could play an important role in the progressive nature of lung fibrosis. This suggests that targeting lung fibrosis at the level of this reciprocal cell matrix interaction could be more effective than traditional treatment approaches.