ImmunoTools *special* Award 2021

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Cardiac fibroblasts in homeostasis and disease

Background

Cardiac fibrosis, characterised by an excessive accumulation of extracellular matrix (ECM) in myocardial tissue, represents an increasing global health problem associated with nearly all types of heart diseases. Cardiac fibroblasts, a cell type for a long time ignored, have been recently recognised as fundamental contributors to maladaptive changes in myocardial tissue in response to pathophysiological stimuli of different aetiology. The activation and differentiation of cardiac fibroblasts represent a critical step in the cardiac fibrosis onset and progression. Besides their function in ECM synthesis and remodelling, cardiac fibroblasts contribute to the inflammatory process in the affected myocardium through the secretion of pro-inflammatory cytokines and inflammatory cells recruitment. Fibrosis progression is associated with increased myocardium stiffness and impaired conduction, leading to heart failure. There is still much ambiguity regarding the fibroblasts specific role and signaling pathways involved in the pathology. Further comprehensive studies are needed to identify the potential molecular targets for targeted and personalised antifibrotic therapy.

The Aim of the Project

This project aims to identify the candidate genes and proteins implicated in pathological fibroblast activation following myocardial injury. We will investigate the particular molecular mechanisms and signalling pathways by which the candidate genes contribute to profibrotic changes in the affected myocardium. Finally, we will explore how the chosen genes and proteins modulate fibroblasts secretory profile and interaction with the other cell types residing in myocardial tissue.

Experimental Strategy

The project starts with the proteome and transcriptome profiling of human cardiac fibroblasts isolated from the myocardium of patients undergoing heart transplantation due to heart failure and the unaffected myocardium of donors. Based on these analyses, the candidate genes/proteins implicated in profibrotic phenotype

development will be chosen for further studies. Validation of transcriptomics and proteomics data will also be performed in other cohorts of adult cardiac fibroblasts from healthy donors hearts and patients with inflammatory dilated cardiomyopathy / ischemic cardiopathy.

The mechanistic studies will be conducted on foetal human cardiac fibroblasts to investigate the role of chosen genes/proteins in fibroblasts activation and their involvement in specific signaling pathways. The chosen genes knockdown will be achieved using siRNA transfection. Next, fibroblasts activation will be induced with a single profibrotic cytokine (TGF-β or IL-11- ImmunoTools) or with the cytokine cocktail validated for the cell differentiation into myofibroblast phenotype (TGF-β, IL-4, IL-10, IL-13 - ImmunoTools). After the stimulation, fibroblasts differentiation, profibrotic markers expression, and inflammatory cytokines expression will be assessed.

To perform a comprehensive evaluation of candidate genes/proteins role in fibroblasts activation and cell-cell communication, different *in vitro* models will be used in the study. Two-dimensional (2D) *in vitro* model will include fibroblasts monoculture and co-culture with macrophages, both direct and indirect. Human macrophages will be derived from circulating $CD14^+$ monocytes isolated from healthy donors and patients with systemic sclerosis (fibrotic phenotype), as recently described by Rudnik et al., 2021.

The effects of the chosen genes silencing in cardiac fibroblasts will be further investigated using 3D co-culture model. Fabrication and culture of human cardiac microtissues will be performed as described previously (Błyszczuk et al., 2020). We will generate two types of microtissues containing: (1) iPSC-derived cardiomyocytes and fibroblasts, (2) iPSC-derived cardiomyocytes, fibroblasts and macrophages. Contractile properties and changes in action potential profile will be measured to assess the effects of siRNA transfection against the chosen genes followed by profibrotic cytokines stimulation.

The evaluation of human cardiac fibroblasts secretory profile represents an important part of our research. The conditioned medium from fibroblasts culture will be collected and analysed in terms of different cytokines concentrations. The medium from the co-culture models and 3D cardiac microtissues will be used to determine the total secretory activity of co-cultured cells. With ImmunoTools ELISA sets, we will assess whether the chosen candidate genes silencing modulates the expression of particular cytokines in untreated and stimulated with profibrotic stimuli cells. This analysis will help us identify the genes/proteins contributing to inflammatory phenotype in fibrosis and determine the molecular targets for the potential antifibrotic therapy.