Stretch, stiffness, sensing, signaling & speed

Summary

Cardiac tissue consists of different cell types, like cardiac fibroblasts (CFs) and cardiomyocytes (CMs). These cells reside within a complex structure called the extracellular matrix (ECM). CFs control the ECM turnover, by producing proteins that break down and build up the ECM. Pathological conditions, like cardiac overload and myocardial infarction, induce cardiac fibrosis: the production of excess ECM, in particular collagen. The fibrotic process is initiated when CFs become activated and differentiate into myofibroblasts. Cardiac fibrosis has a functional purpose of healing necrotic myocardium, replacing dead cells and clearing ECM debris, thereby preventing further damage. However, fibrosis also has adverse effects like disturbing impulse conduction and increasing stiffness of the myocardium, hampering cardiac pump function. This thesis investigates the role of mechanical load on the function of CFs. CFs can sense mechanical forces, referred to as mechanosensing. Mechanical forces are then converted into changes in cell function via mechanotransduction.

In response to stretch, cardiac tissue produces brain natriuretic peptide (BNP), which has been suggested to have beneficial effects in heart failure patients. Cartilage intermediate layer protein 1 (CILP1) is a matricellular protein expressed by CFs which recently gained interest as a marker and pathogenic factor in cardiac disease. In this thesis, we explored the mechanism of stretch-induced changes in the genes regulating these proteins (Nppb and Cilp1, respectively).

In chapter 2 we showed that CFs subjected to cyclic stretch (1 Hz, 10%) induces a significant increase in Nppb and BNP expression, as well as induction of genes related to myofibroblast differentiation like Acta2. Myofibroblasts are characterized by the synthesis of α-smooth muscle actin (α-SMA), transcribed from the Acta2 gene. Moreover, recombinant BNP inhibites transforming growth factor (TGFβ1)-induced Acta2 expression. In a next step we were able to find strong indications for a role of Piezo, a recently discovered stretch sensitive ion channel. The effects of stretch were reproduced by stimulation with the Piezo1 agonist Yoda1. Silencing of Piezo1 reduced the stretch-induced Nppb and Tgfβ1 expression in CFs.

Further studies in chapter 3 indicated that cyclic stretch induces a significant reduction in Cilp1 gene expression as did stimulation with Yoda1. Silencing of Piezo1 caused an increased Cilp1 gene expression in both stretch and non-stretch conditions. In conclusion, our study identifies Piezo1 as mediator of stretch-induced Nppb and Tgfβ1 in CFs (chapter 2). Moreover, Piezo1 is involved in the stretch-induced downregulation of Cilp1 in CFs (chapter 3).

Increased cardiac mechanical loading caused by volume or pressure overload commonly exist over a long period of time (years). However, daily activities such as exercise create shorter lasting changes in mechanical loading (hours). The influence of such relatively short-term loading changes on CF is incompletely understood. In chapter 4 we investigated the effect of shorter periods of cyclic stretch on CF activation. Results from these studies

implicate that 1h cyclic stretch is a stimulus causing CF activation as indicated by temporary induction of early response genes: Nppb, Tenascin C, Tgfβ1 and Heat Shock Protein 70 at 4h. However, this is effect has disappeared after 24h and therefore, 1h cyclic stretch is not sufficient for sustained CF activation or initiation of myofibroblast differentiation (chapter 4). Also, a 1 hour change in stretch amplitude to 20%, during a 24 hour 10% stretch protocol did not change gene expression.

Isolation and culturing of CFs induces rapid activation of cell proliferation and differentiation toward a myofibroblast phenotype. Myofibroblast differentiation in cell culture is partly mediated by the non-physiologically high stiffness of the culture plates. In chapter 5 we have developed engineered heart matrix (EHM) by culturing CFs within a natural collagen-1 hydrogel with a physiological stiffness (Youngs modulus of ~15 kPa). Results showed that CFs were evenly distributed throughout the gels and maintain a quiescent phenotype up to 13 days of culturing. Baseline gene expression levels of markers for myofibroblast differentiation, Acta2 and Connective Tissue Growth Factor (Ctgf) were significantly lower in EHM-fibers compared to CFs cultured in 2D monolayers on silicon and hard plastic culture plates. CF baseline gene expression of Tgfβ1 and Nppb were higher in EHM-fibers compared to the monolayers. Importantly the CFs response to stimuli like stretch and TGFβ1 was maintained in EHM.

In chapter 6 we investigated the effect of longer lasting myocardial stretch in an in vivo situation, by studying the change in left atrial (LA) structure and function in a dog model of chronic mitral regurgitation (MR). This chapter was aimed at identifying potential underlying myocardial disease mechanisms using echocardiography, histology and gene expression analysis of the LA as well as computational modeling. Histology and gene expression analysis showed increased fibrosis and increased mRNA expression of collagen type 1 (Col1a1) after chronic MR, while no effect was found on genes related to CF activation and stretch, Acta2, Ctgf and Nppb. The combination of histological and echocardiographic findings along with results from computational modeling implicate that the changes in LA reservoir and contractile function are the result of a combination of eccentric hypertrophy and fibrosis.

Overall, results from this thesis have contributed to understanding the role of Piezo1 in stretch-induced changes in expression of Nppb, Tgfβ1 and Cilp1 in CF. The development of EHM showed that culturing CFs in a physiological environment keeps them quiescent, indicating stiffness is an important factor determining CF function and differentiation. The quiescent state of CFs in our EHM makes it a better model for studying the role of CFs for future research. In the bigger picture of chronic atrial volume overload, the in vivo studies showed the complexity of structural remodeling, involving a combination of eccentric hypertrophy (involving myocytes) and fibrosis (involving CFs).