Functional genomic studies of human heart failure

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Background
Heart failure (HF) is a clinical syndrome characterized by inability of the heart to maintain sufficient output to meet the demands of the body at normal filling pressures. Increased hemodynamic stress on the failing heart results in increased atrial natriuretic peptide (ANP) expression and release, which is now recognized to confer multiple beneficial effects in HF. Although much is known about ANP regulation, few data are available on the role of non-coding RNAs, such as antisense transcripts and miRNAs.

Aims
The aim of this thesis are to improve understanding of transcriptional consequences of strain in human heart cells, with particular emphasis on non-coding RNA regulation of the well-established increase in ANP production and secretion observed in HF.

Methods and results
First, we established a protocol for a controlled strain of cardiac cells and confirmed robust upregulation of ANP in human cardiomyocytes. We then undertook a series of experiments, several of which were based on the strain model, to investigate the effects of the antisense RNA transcript \textit{NPPA-AS1} in regulation of \textit{NPPA} expression (study 1). We describe a regulatory effect in vitro and in vivo that is based not on duplex formation but on interaction with transcription factor binding sites in the \textit{NPPA} promoter. Downregulation of \textit{NPPA-AS1} with antisense oligos in mice resulted in increased ANP expression, nominating \textit{NPPA-AS1} as a putative therapeutic target in HF. In study 2, we screened for miRNA regulators of corin, the enzyme converting proANP to biologically active ANP, and its activity by establishing a serine protease activity assay using a fluorogenic substrate. Based on this assay, we show that miRNA-1 exerts a negative regulatory effect on corin gene expression through binding to the 3’ untranslated region.
In study 3, three miRNAs (miRNA-105, miRNA-155 and miRNA-425) with a negative regulatory effect on ANP was found to be decreased in human cardiomyocytes subjected to strain, and a reverse of the strain-dependent increase of \textit{NPPA} could be seen in cells overexpressing miRNA-155. In study 4, we have systematically evaluated transcriptional changes in human cardiomyocytes and cardiac fibroblasts subjected to strain.

**Conclusion**

The studies in this thesis will provide new information on ANP regulation and other strain-responsive transcripts in the healthy and failing human heart. Such information has potential to result in new therapeutic strategies for heart failure.