The atlas of the human hypertrophied heart reveals impaired Ephrin B1-dependent cell communication

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INTRODUCTION: One of the major causes of heart failure is cardiac hypertrophy, a multifactorial process which is accompanied by the dysregulation of various signaling pathways. The hypertrophic response of cardiomyocytes has been extensively studied, however their crosstalk with other cardiac cell types, especially endothelial cells, is less explored. Here, we apply large-scale transcriptomic analysis on single-nuclei level allowing the investigation of the multicellular heterogeneity and communication in the hypertrophic human heart.

RESULTS: Analysis of cardiac location-matched tissue by single nuclei RNA sequencing data of N=5 patients with aortic stenosis (30,079 nuclei) and N=14 healthy controls (58,457 nuclei) revealed significant changes in the transcriptome and a strikingly reduced communication of cardiomyocytes with other cells, especially with endothelial cells. Particularly, the communication of Eph-receptor tyrosine kinases, expressed by cardiomyocytes, with their ephrin ligands, expressed by endothelial

cells, was reduced in the hypertrophic heart. Most prominently, EPH-receptor-B1 (*EPHB1*) was repressed in the hypertrophied heart (0.01±0.001-fold, p<0.0001), which was validated on mRNA and protein level in humans (0.56±0.06-fold, p=0.004) and in a murine pressure overload model (0.49±0.08-fold, p=0.04). This downregulation prevents the activation by its ligand ephrin-B2 (*EFNB2*), which is expressed by endothelial cells, and has inhibited the hypertrophic phenotype of cardiomyocytes in-vitro (cell size for rec-EphrinB2+ phenylephrine (PE) vs. PE: 0.86±0.10-fold, p=0.02) and in a multicellular cardiac organoid model. Furthermore, silencing of endothelial EFNB2 in a co-culture model with endothelial cells and cardiomyocytes was sufficient to induce a hypertrophic and stress responses, measured by increased size (1.33±0.06-fold, p=0.0003), decreased contraction rate (0.78±0.07-fold ,p=0.04), and augmented stress marker expression of cardiomyocytes. Additionally, recombinant-EphrinB2 rescued the hypertrophic response induced by *EFNB2*-knockdown. The functional role of EPHB1 was confirmed by using a AAV6-based cardiomyocyte specific overexpression model, which significantly protected cardiomyocytes from PE-induced hypertrophy in the co-culture system with endothelial cells (cell size for Ephb1-AAV+PE vs. mock-AAV+PE: 0.78±0.01-fold, p=0.0002).

CONCLUSION: Taken together, the human cardiac cell atlas of the hypertrophied heart highlights the importance of the intercellular cross-talk, especially of the EPHB1-EFNB2 axis of cardiomyocytes and endothelial cells, in pathological mechanisms of cardiac hypertrophy and provides a valuable tool for further studies.