Rnf20 fine-tunes the endothelial cell–cardiomyocyte crosstalk to safeguard heart development

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Signals from endothelial cells are known to orchestrate cardiac morphogenesis and function, however the precise molecular mechanisms are largely unexplored. In a screen for epigenetic factors with critical function in heart development, we identified Rnf20 as an important contributor. Rnf20 is the major E3 ubiquitin ligase responsible for monoubiquitination of histone H2B at lysine 120 (H2Bub1), which plays a key role in transcriptional control. The important function of Rnf20 for physiological heart development is further supported by exome sequencing data, which identified mutations in Rnf20 to be associated with congenital heart defects. However, its function in the different cardiac cell types for correct cardiogenesis is largely unexplored.

Here, we investigated the role of Rnf20 in the vascular control of heart development *in vivo* and *in vitro* using inducible deletion of Rnf20 in the distinct cardiac cells. *Rnf20* ablation in Isl1+ cardiovascular progenitor cells resulted in severe cardiac and vascular abnormalities in mice. The *Rnf20* depleted hearts exhibited a small right ventricle, hypoplastic ventricular wall, abnormal trabeculation and unique accumulation of cardiac jelly. Moreover, endothelial cell alignment and density was affected, being accompanied with the downregulation of angiocrine signals. One major group of genes regulated by Rnf20 were signal dependent genes, which prompted us to further study the function of Rnf20 in the different cardiac cell types. Deletion of *Rnf20* in Cdh5+ endothelial cells phenocopied the ablation in cardiovascular progenitors. Its loss resulted in ventricular septal defects, myocardial thinning and extrasystolic arrhythmias, suggesting a key role of Rnf20 in endothelial cells for cardiac morphogenesis and rhythmicity. RNAseq and ATACseq analysis of both endothelial cells and cardiomyocytes isolated from the endothelial specific knockout hearts identified deregulated signaling molecules in cardiac endothelial cells and upstream regulators of the transcriptional response of cardiomyocytes to endothelium-derived signals. Interestingly many typical markers of endothelial to mesenchymal transition (EndMT), e.g. fibronectin, vimentin, Icam1, Mmp2, a large set of collagens as well as TFs driving EndMT in the heart such as Hand2, Foxc1, Notch, were highly upregulated upon Rnf20 loss suggesting that Rnf20 safeguards endothelial cell identity by preventing EndMT. Furthermore, we detected significant transcriptional changes in cardiomyocytes upon *Rnf20* deletion in endothelial cells. Upregulated genes were enriched in GO terms linked to cell-cell signaling, regulation of Ca^{2+} concentration and heart contraction, consistent with the arrhythmic phenotype observed in $Rnf20^{iEC\text{-}KO}$ embryos. Further, TOBIAS footprinting analysis identified many TFs involved in EndMT (Sox9, Hif1a, Tbx2/3, Smads) highly bound at Rnf20 target genes in endothelial cells and tumor suppressors restricting cell proliferation, e.g. Tp53 and Tp73 in cardiomyocytes.

Taken together, our study revealed a key role of the E3 ligase Rnf20 in inhibiting EndMT and vicious signaling promoting cardiomyocyte cell cycle withdrawal.