The HAND2 Cistrome in Mouse Embryonic Hearts Identifies its Target Genes During Endothelial-Mesenchymal Transition in the Atrioventricular Canal

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During the embryonic development of the mammalian heart, the formation of the cardiac valves is a critical step towards the establishment of an unidirectional blood flow. Valves formation takes place in the outflow tract (OFT) and in the atrioventricular canal (AVC), where the cells of the endothelial lining of the heart, the endocardium, undergo endothelial-mesenchymal transition (EndMT) and proliferate to form cardiac cushions that will be remodeled into cardiac valves. Among the transcription factors that direct the proliferation and fates of cardiac progenitor cells, the basic helix-loop-helix protein HAND2 plays a critical role during the differentiation of second heart field (SHF) derived structures (OFT and right ventricle). Indeed, *Hand2*-deficient mouse embryos display severe right ventricle hypoplasia and die prior to embryonic day E10.5. The direct targets and gene regulatory networks controlled by HAND2 during heart morphogenesis have remained elusive thus far. Using mice expressing a 3xFLAG epitope-tagged HAND2 protein, we studied the spatio-temporal distribution of HAND2 and performed ChIP-Seq analysis to determine the range of its target sequences (cistrome) in E10.5 embryonic hearts. In addition to the identification of HAND2 target genes in the SHF, we have established that HAND2 directly controls a network of genes that regulate EndMT in the AVC. Indeed, the endocardial cells of *Hand2*-deficient embryos fail to undergo EndMT both *in vivo* and in an *in vitro* AVC explant culture system. As the expression of *Snai1*, a key regulator of EndMT, is absent from *Hand2*-deficient endocardial cells, adenoviruses were used to re-express SNAI1 in explant cultures, which results in an increased number of cells undergoing EndMT *in vitro*. Furthermore, we generated transgenic reporter mouse embryos for *cis*-regulatory modules (CRMs) directly bound by endogenous HAND2 chromatin complexes in the *Snai1* genomic landscape. We found that one of these CRMs is active in the cardiac cushions in both the OFT and the AVC. In addition, this CRM recapitulates most of the endogenous *Snai1* embryonic expression at E10.5. Altogether, our study establishes that HAND2 is an important regulator of *Snai1* and other EndMT genes in the endocardium of the atrioventricular canal.