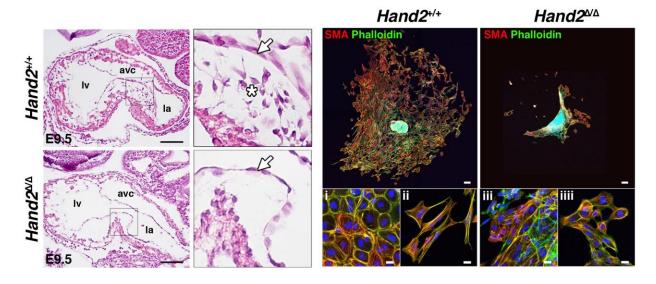
## The HAND2 Cistrome in Mouse Embryonic Hearts Identifies its Target Genes During Endothelial-Mesenchymal Transition in the Atrioventricular Canal

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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-Seg analysis to determine the range of its target seguences (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 reexpress SNAI1 in explant cultures, which results in an increased number of cells undergoing EndMT in vitro. Furthermore, we generated transgenic reporter mouse embryos for cisregulatory 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.



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