

Lineage analysis of ventricular trabeculae to decipher the role of Nkx2-5 in conduction system development

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During cardiac development, transient invaginations of the myocardium, termed trabeculae, appear at the endocardial surface of the ventricles. Trabecular compaction during fetal development is an essential step in generating a functionally competent ventricular wall. Trabeculae also contain progenitor cells of the ventricular conduction system (VCS), a complex network of Purkinje fibers that controls the rapid propagation of electrical activity in the ventricles. Defects in ventricular compaction and conduction have been observed in patients and mutant mice carrying mutations in *NKX2-5*, encoding a key transcriptional regulator of heart development. In order to analyze the link between trabecular fate and VCS differentiation, we carried out a genetic lineage analysis of trabecular fate using *Cx40-CreERT2* mice expressing a tamoxifen-inducible Cre recombinase in ventricular trabeculae and later in the definitive ventricular conduction system. These mice were crossed with *Rosa26-Confetti* mice for a prospective clonal analysis of trabeculae. Our results show that the peripheral VCS segregates progressively during embryonic development. Cells exclusively fated to give rise to the VCS are present in the trabecular compartment as early as E9.5. Specification of new conductive myocytes within the trabecular compartment during subsequent development contributes to the formation of a complex Purkinje fiber network at birth. We performed the same lineage tracing experiments in *Nkx2-5* heterozygous mice with severe hypoplasia of the Purkinje fiber network at adult stages. The number of Purkinje fibers originating from early *Nkx2-5* haploinsufficient progenitors is identical to control. In contrast, we observed a progressive increase of trabecular cells that do not give rise to the VCS at later stage of development. This suggests that *Nkx2-5* plays a role in the progressive recruitment of trabecular cells into the VCS as well as later in maintenance of the conductive phenotype. Overall this study highlights the early segregation of the ventricular conduction system lineage at the onset of trabeculation and the role of *Nkx2-5* at later stages of the formation of the Purkinje fiber network.