The Role of SHROOM3 in Congenital Heart Disease

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Background and Hypothesis: Congenital heart defects (CHD) are the most common, and most frequently fatal birth defects, but most etiology remains unknown. We identified a patient with CHD and implicated a gene called SHROOM3. SHROOM3 binds Dishevelled2 which is the central cytoplasmic component of both canonical and noncanonical Wnt/planar cell polarity (PCP) signaling pathways. PCP drives cell movement and is important to embryogenesis and disruption causes CHD. We hypothesize CHD can result from SHROOM3-loss-of-function due to PCP disruption.

Project Methods: To interrogate SHROOM3’s role in CHD and PCP we utilized an established in vivo SHROOM3-loss-of-function model, Shroom3 gene trap mice (Shroom3<sup>gt</sup>). We also utilized a loss-of-function model for PCP membrane component VANGL2, (Vangl2<sup>+-/-</sup>). We assayed genetic interaction between Shroom3 and Vangl2 during cardiac development by crossing singly heterozygous null mice to produce compound heterozygous embryos, harvested embryos, and performed histologic analysis for cardiac defects. We also utilized a human in vitro SHROOM3-loss-of-function model, a CRISPR-Cas9 edited SHROOM3 knockout HEla cell line. We assayed cell movement using a scratch assay.

Results: Compound heterozygous Shroom3<sup>+/gt</sup>;Vangl2<sup>+-/-</sup> embryos had a three fold increase in heart defects compared to singly heterozygous Shroom3<sup>+/gt</sup>;Vangl2<sup>++/+</sup> or Shroom3<sup>+/+</sup>;Vangl2<sup>+-/-</sup> embryos (3 of 19 or 15.7%, versus 1 of 17 or 5.2%, and 1 of 19 or 4.8%, respectively), demonstrating a trend towards genetic interaction between SHROOM3 and VANGL2/PCP during cardiac development. The scratch assays demonstrated cell movement defects due to SHROOM3-loss-of-function consistent with increased cell movement.

Conclusion and Potential Impact: We demonstrate SHROOM3 interacts with Wnt/PCP during cardiac development. Further interrogation of SHROOM3’s role in Wnt signaling will provide insight into the mechanisms by which a novel CHD candidate participates in cardiogenesis and will improve CHD diagnosis, management, and therapeutic development.