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*^{gt}). We also utilized a loss-of-function model for PCP membrane component VANGL2, (*Vangl2*$^{+/-}$). 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*$^{+/gt}$*;Vangl2*$^{+/-}$ embryos had a three fold increase in heart defects compared to singly heterozygous *Shroom3*$^{+/gt}$*;Vangl2*$^{+/+}$ or *Shroom3*$^{+/+}$*;Vangl2*$^{+/-}$ 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.