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Shox2 in Pacemaker and Epicardial Development

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Background – The heart generates from two cardiogenic fields in the dorsal mesocardium: the first and second heart field. The first heart field gives rise to the primary heart tube and the second heart field adds cardiomyocytes to the venous as well as the arterial pole of this tube. At the venous pole these cardiomyocytes are derived from a subgroup of the second heart field, the Posterior Heart Field (PHF). Shox2 has an important role in formation of the PHF that contributes to major parts of the cardiac inflow tract including the specialized conduction system and the epicardium. We hypothesize that mutation of Shox2 leads to abnormal sinoatrial node (SAN) / pacemaking function as well as abnormal epicardial development during cardiogenesis.
Methods – For the assessment of embryonic heart rate and atrioventricular conduction time, electrophysiological recordings were performed in isolated hearts of wildtype and Shox2-/- embryos of 12.5 days post conception (dpc). Furthermore, immunohistochemical analysis was performed with antibodies specifically against MLC-2a, Nkx2.5, HCN4 and Wt1, and 3D-reconstructions were made.
Results – Compared to wildtype, Shox2-/- embryos showed a significant lower heart rate (105±36 bpm vs 74±15 bpm; P=0.032), no differences were observed in atrioventricular conduction time (76±24 ms vs 80±14 ms; P=ns). Immunohistochemical analysis and 3D-reconstructions showed hypoplasia and aberrant differentiation of the PHF derived sinus venosus myocardium in Shox2-/- embryos of 12.5 dpc. In both wildtype and Shox2-/- embryos HCN4 was widely expressed throughout the complete sinus venosus myocardium including the SAN. The expression of Wt1 and 3D-reconstructions of the pro-epicardial organ (PEO) in Shox2-/- embryos showed a decreased PEO size at 9.5 dpc with normal epicardial spreading at 12.5 dpc. At latter stages the ventricles showed decreased numbers of epicardium derived cells and abnormal ventricular wall morphology.
Conclusions – Shox2 has a crucial role in venous pole development of the heart including the SAN and its pacemaking function. Furthermore, we demonstrate that Shox2 is essential for proper epicardial lineage development.