Outflow tract banding in the chick causes aberrant alterations in the ECM and coronary vasculature

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In embryonic development, the epicardium is essential to form a normal functioning heart. Epicardial derived cells contribute to the heart as it develops, including fibroblasts and vascular smooth muscle cells. Previous studies have shown that a heartbeat is required for epicardium formation. Further, preliminary studies from our laboratory have shown that the development of the epicardium is aberrant when the haemodynamics are altered.

This study aims to investigate how the epicardium and some of its derived cell lineages respond to altered haemodynamics in the developing embryo. Since the aetiology of many congenital heart defects is unknown, we suggest that an alteration in the heart’s haemodynamics might provide an explanatory basis for some of them.

In order to change the heart’s haemodynamics, outflow tract banding (OTB) using a double overhang knot was performed on HH21 chick embryos, with harvesting at HH29 or HH35. After external phenotypic characterisation of OTB and control hearts, the RNA levels for a number of genes involved with ECM production and coronary vascular development were analysed by qPCR. Further, immunoblotting has been performed on cadherin proteins and the late fibroblast marker TCF21. In addition, immunohistochemistry was performed against smooth muscle actin in order to visualise the number, wall thickness and lumen area of the coronary vessels.

Upon phenotypic characterisation, OTB hearts (n=10) showed external bleeding between the myocardial wall and the epicardium. By qPCR analysis, the HH29 (n=3) OTB hearts showed higher DDR2 and collagen XII expression, whereas at HH35 (n=3) collagen I was also downregulated and there was differential expression in a number of genes that are involved with angiogenesis and blood vessel maturation. Immunoblots at HH35 (n=6) showed a downregulation in the late fibroblast marker TCF21 (p=0.0001) but no change in cadherin expression. The immunohistochemistry results (n=3) have showed fewer vessels in the interventricular septum (p=0.011) and a higher myocardial wall to lumen ratio in HH35 OTB hearts (p=0.005). In conclusion, these studies suggest that changes in haemodynamics can cause aberrant coronary vascular formation and changes in collagen deposition. Future studies will involve immunohistochemistry on the TCF21 positive cells and collagen I, and in situ hybridisation on collagen XII expression.