Genetic Analysis of the Role of Procollagen IIA as an Extracellular Regulator of BMP Signaling

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Embryonic development depends on the provision of morphogenetic instruction which are generated by signaling factors such as those of the transforming growth factor-beta (TGFβ) superfamily that cells receive from their neighbours whose activity may be mediated by extracellular matrix (ECM). This instruction will determine lineage differentiation, tissue growth and the tissue patterning in the primary body axes: anterior-posterior, dorsal ventral and left-right. Type IIA procollagen is an isoform of a well-known structural ECM protein with BMP/TGFβ binding capacity. It has been proposed that IIA procollagen may regulate patterning through its role as an antagonist by binding and sequestering growth factors or signaling molecules. Consistent with such a role is the ability of IIA to induce a secondary axis when over-expressed in *Xenopus* embryos. However the essential role of IIA in embryonic patterning and morphogenesis has not been established.

To understand the function of IIA, we have used gene targeting in embryonal stem cells to produce mutant mice that can only produce *Col2a1* mRNA without exon 2. We have obtained compelling evidence that IIA plays an essential role in cardiac morphogenesis by modulating BMP and TGFβ signaling. Mice lacking IIA display multiple heart malformations with many features of some human congenital heart conditions.

In 9.5 dpc IIA-/- embryos the heart remains as a tube with the right and left portions aligned rostro-caudally. At birth the heart defects are consistent with prenatal onset of hypertrophic changes in the myocardium. The spectrum of heart defects include some features of human congenital heart conditions such as the tetralogy of Fallot (ventricular septal defect (VSD), pulmonary valve stenosis, overriding aorta and right ventricular hypertrophy), and the Double Outlet Right Ventricle (where aorta and pulmonary arteries arise wholly or in great part from the right ventricle, with associated ventricular septal defect). We have tested the ability of IIA procollagen to antagonize BMP signaling using BMP responsive vectors in *Xenopus* embryos and can show IIA can antagonize BMP signaling.

Type IIA procollagen may therefore regulate cardiac patterning by binding and sequestering growth factors or signalling molecules. Congenital heart defects many affecting laterality, are the most common birth defect, occurring in 1 per 1000 births. The discovery that IIA procollagen, a "structural" ECM molecule may have a key regulatory role in this process is an important advance leads to a better understanding of the molecular basis of congenital heart malformations.

This work was supported by the Research Grants Council (HKU 7275/00M of HKSAR and the Arthritis & Rheumatism Campaign UK).