Expression of the Wilms tumor suppressor gene (*Wt1*) in a subpopulation of embryonic cardiomyocytes is required for cardiac development

Carmona, R[1], Cañete, A[1], Ariza, L[1], Torrado, M[2], Mikhailov, A[2] and Muñoz-Chápuli, R[1].

[1] Department of Animal Biology, University of Málaga and Andalusian Center for Nanomedicine and Biotechnology (BIONAND), Málaga, Spain.

[2]Institute of Health Sciences, University of La Coruña, Campus de Oza, Building El Fortin, La Coruña, Spain.

The Wilms' tumour gene, WT1, encodes a zinc-finger transcription factor involved in the development of several organs. WT1 is expressed during mammalian embryonic development in many tissues, including the urogenital system, spleen, spinal cord, diaphragm, coelomic epithelium and epicardium (Armstrong et al., 1993; Moore et al., 1999; Cano et al., 2016; Ariza et al., 2016; Carmona et al., 2016). Post-transcriptional modifications of the Wt1 pre-mRNA lead to the production of up to 24 different isoforms, which seem to serve distinct but overlapping cellular and developmental functions.

We have checked if Wt1 is expressed by the embryonic myocardium. Using transgenic mice lines for lineage tracing (mWt1/IRES/GFP^{Cre};ROSA26R^{EYFP}), we have detected a small population of cardiomyocytes from a Wt1-expressing cell lineage in early developmental stages (E8.5-E9.5). These cardiomyocytes were mainly located in the inflow tract, but some of them were observed in the ventricles. We confirmed Wt1 expression by RT-PCR in hearts before the attachment of proepicardial cells, when the cardiac tube is only constituted of myocardium and endocardium.

We have also studied the mRNA levels of the four main isoforms of Wt1 in a reverse transcriptase-polymerase chain reaction (RT- PCR) assay. We have found differential expression of these Wt1 isoforms in the embryonic heart.

Conditional deletion of Wt1 in cardiac Troponin T expressing cells caused severe damage in the developing heart, particularly muscular defects in the interventricular septum and free ventricular walls, as well as defective sinus venous formation. These embryos did not survive after birth.

Likewise, conditional deletion of GATA4 in Wt1-expressing cells causes a similar phenotype in the myocardium, but also defects in the proepicardium, epicardium and subepicardial space, causing embryonic death around E11.5.

Thus, we conclude that Wt1 is expressed in a subpopulation of early embryonic cardiomyocytes, and this expression seems to be essential for heart development.