

# **A developmental model for the pathogenesis of cardiac arterio-ventricular fistulae**

Paul Palmquist-Gomes<sup>1,2</sup>, José C. Martín-Robles<sup>1,2</sup>, Sara Cano-Ballesteros<sup>1,2</sup>, Juan A. Guadix<sup>1,2</sup>, José M. Pérez-Pomares<sup>1,2</sup>

<sup>1</sup>Department of Animal Biology, Institute of Biomedicine of Málaga (IBIMA), Faculty of Sciences, University of Málaga (UMA), Málaga, Spain.

<sup>2</sup>Area of Biotechnology, Andalusian Centre for Nanomedicine and Biotechnology (BIONAND), Campanillas (Málaga), Spain.

Coronary Artery Fistulae (CAF) are congenital coronary artery (CA) anomalies consisting of an abnormal communication of a coronary artery with either a cardiac chamber or a large cardiac vessel. Although their incidence in the Western population is low, CAF can lead to complications such as myocardial hypertrophy, endocarditis, heart dilatation and cardiac failure. CAFs can appear as an isolated anomaly or linked to some other forms of congenital heart disease like Left Ventricular Non-Compaction (LVNC) and intrinsic CA anatomy anomalies, but their etiology remains unknown. In this work we have used two different experimental models (transgenic mice and avian embryos) to investigate on the developmental mechanics of CAF formation. In order to tackle this goal, we have manipulated epicardial development and ventricular wall compaction, two inextricably related developmental events during coronary embryogenesis. Conditional integrin  $\alpha 4$  gene deletion in the septum transversum/proepicardial (ST/PE) region (G2-Gata4+) disrupts early epicardium development and reduces cardiomyocyte proliferation, leading to the thinning of the ventricular compact myocardial layer. Reduction in compact myocardium thickness associates to the presence of multiple ventricular myocardial discontinuities and focal endocardial extrusion. This same phenotype can be experimentally reproduced in chick embryos using a cryocauterization method (Palmquist-Gomes et al., 2016). Our results suggest that the partial absence of epicardium in  $\alpha 4$ integrin;G2-Gata4Cre mouse embryos and the cryoinjury in avian embryos generate myocardial discontinuities in the embryonic ventricular wall, which promote endocardial extrusion towards the pericardial cavity and the early contact of the endocardium with coronary progenitors at the epicardial surface of the heart. In the case of avian embryos, this phenomenon leads to precocious smooth muscle differentiation from epicardial mesenchymal cells, and the formation of pouch-like structures that closely resemble CAF. We conclude that anomalous compact myocardial embryonic growth can originate CAF.