Myocardial trabeculation in embryos of *Scyliorhinus canicula* (Elasmobranchii, Chondrichthyans)

Miguel A. López-Unzu[1], Cristina Rodríguez[1,2], Miguel Lorenzale[1], Borja Fernández[1,2], Ana Carmen Durán[1,2]. [1]Animal Biology, University of Málaga, Málaga, Spain, [2]Institute of Biomedical Research in Málaga (IBIMA). University of Málaga, Málaga, Spain

Currently, three types of ventricular myoarchitecture are recognized in vertebrates, namely compact, spongy (trabeculated) and mixed myocardium. Mixed myocardium, which has been recently proposed as the primitive condition in gnathostomes, is composed of two myocardial layers: an inner trabeculated and an outer compact one. The trabeculation process has been studied in teleosts, showing exclusively spongy myocardium, and mammals and birds, characterized by a compact myocardial ventricular wall. In zebrafish, mouse and chicken embryos, the trabeculae develop as luminal myocardial ridges protruding into the lumen. In mammals and birds, further compactation of trabeculae leads to the formation of a compact layer. The potential mechanisms that may contribute to the formation of the ridges are under discussion and include myocardial proliferation, endocardial invagination, and bending of the entire myocardial layer. However, no description of the development of the mixed myocardium is available.

To shed some light on this issue, we have studied the heart development of an elasmobranch species with mixed myocardium, the lesser spotted dogfish (*Scyliorhinus canicula*; Chondrichthyes), by means of histological and immunohistochemical techniques for light microscopy, semithin sections, scanning electron microscopy and transmission electron microscopy.

At the beginning of developmental stage 27 the ventricle has a single layer of cardiomyocytes lined by endocardium. At stage 28, the cardiomyocytes proliferate and stratified in several cell layers. Small spaces can be seen in between cardiomyocytes of the inner layers and the endocardium locally invaginates. At stage 29, bigger spaces lined by endocardial cells are connected with the ventricular lumen. Then, two layers, outer and inner, are recognized in the ventricular wall. The outer layer is the presumptive compact myocardium and the inner one is the presumptive trabeculated myocardium. At stage 30, the trabeculae become more complex, showing a bunch-like branching pattern. At stage 33, the inner trabeculated layer is similar to that of the adult heart, while the outer layer starts to become thicker to form the definitive compact layer.

Our results suggest that in the dogfish the intertrabecular spaces develop by connections between early intramyocardial spaces and the lumen of the ventricle through invaginations of the endocardial line. Chondrichthyans are the earliest diverged lineage of gnathostomes and, consequently, they have the most primitive cardiac design. Although chicken, mouse, and recently zebrafish have been considered powerful vertebrate models to study heart development, we propose that the trabeculation process in the dogfish is representative of the early steps of the ventricular morphogenesis in vertebrates.

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