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Review

The role of secondary heart field in cardiac development

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ABSTRACT

Although de la Cruz and colleagues showed as early as 1977 that the outflow tract was added after the heart tube formed, the source of these secondarily added cells was not identified for nearly 25 years. In 2001, three pivotal publications described a secondary or anterior heart field that contributed to the developing outflow tract. This review details the history of the heart field, the discovery and continuing elucidation of the secondarily adding myocardial cells, and how the different populations identified in 2001 are related to the more recent lineage tracing studies that defined the first and second myocardial heart fields/lineages. Much recent work has focused on secondary heart field progenitors that give rise to the myocardium and smooth muscle at the definitive arterial pole. These progenitors are the last to be added to the arterial pole and are particularly susceptible to abnormal development, leading to conotruncal malformations in children. The major signaling pathways (Wnt, BMP, FGF8, Notch, and Shh) that control various aspects of secondary heart field progenitor behavior are discussed.

The history of the search for heart fields

The earliest cardiac fate mapping studies date back to the 1940s, when Rawles (1943) identified regions of myocardial developmental potential by grafting fragments of head-process stage chick embryos to determine which regions could generate beating tissue. This early study was the first to define a cardiogenic field, which was noteworthy in that it consisted of two broad fields that were bilateral with respect to the primitive node, and this region was defined as the area of the lateral plate mesoderm that has the potential to form myocardium.

Knowing where the cardiac potential boundaries were allowed more refined experiments that prospectively labeled and observed presumptive heart field cells. Time-lapse cinematography in chick embryos showed that the cardiogenic fields moved as a cohesive unit that retained its spatial relationship during migration between Hamburger Hamilton stage (HH) 6+(1951) to HH9–10 (Dehaan, 1963). Based on observations that no cell mixing occurred when tritium-labeled tissue fragments were grafted into unlabeled host embryos as young as HH5, Rosenquist and DeHaan (1966) hypothesized that the heart field is prepatterned. Using even smaller defined regions of the cardiac field, Stalsberg and DeHaan (1969) mapped subdivisions using radioactively labeled transplants (Fig. 1). These transplants were done in embryos in New culture at HH5, and the embryos could be followed only through HH13, after the heart tube had formed. This study confirmed both Rawles' previous observation of the bilateral heart fields and also the fact that these transplanted regions represented cohesive groups of cells that did not intermingle with unlabeled host cells.

Because all the early mapping studies were performed in New cultures, cells could only be followed for 24–36 h, ending when the heart has only recently closed dorsally to form a tube. de la Cruz and colleagues (1977) used an iron oxide marking technique *in ovo* to allow the embryos to develop longer (Fig. 1). This marking technique allowed the embryos to survive until HH35, when the four-chambered heart with two arterial trunks had formed. If the cranial-most aspect of the outflow pole was labeled at HH12, this region was incorporated into the right ventricular trabeculae by HH22. If the same marking was performed at HH22, the cranial-most outflow region was incorporated into the myocardium beneath the pulmonary semilunar valve cusps. de la Cruz postulated that these regions were formed by a secondary source of myocardium, but she did not look for this additional population.

The source of outflow tract myocardium

The question of where the outflow tract myocardium originated remained open for a number of years, mainly because mapping studies were primarily carried out in explanted embryos. As mentioned previously, developmental failure occurs in explanted embryos before the definitive outflow myocardium has been added to the heart tube. As late as 2001, avian mapping studies carefully analyzed embryos starting from HH4 to 8 (Redkar et al., 2001); however, each stage was mapped for only 20 h, thereby not addressing the source of the outflow tract myocardium (Fig. 1). In

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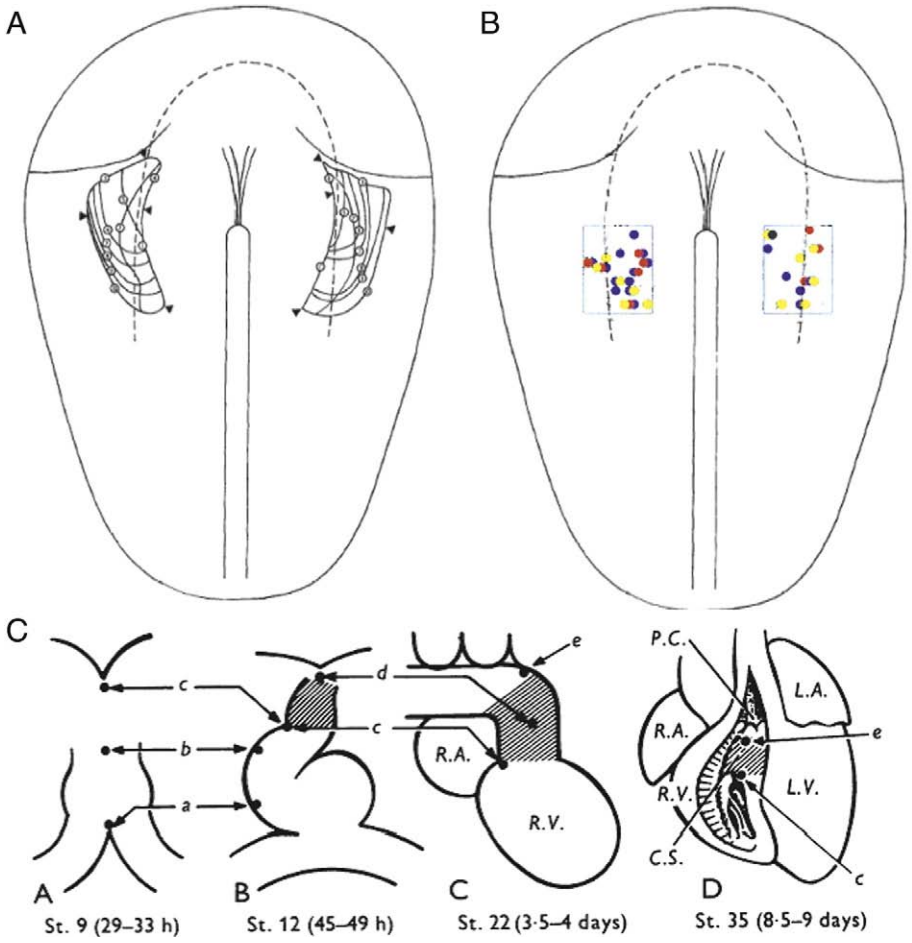

Fig. 1. The heart field at HH 5, as defined by (A) Stalsberg and DeHaan (1969) and (B) Redkar et al (2001; adapted to the Stalsberg and DeHaan schematic). While Stalsberg and DeHaan observed non-overlapping regions that gave rise to distinct components of the heart, this same organization is not observed at HH5 in the Redkar study. In (B), red dots indicated regions that gave rise to atria, yellow dots indicated regions that gave rise to the sinus venosus, blue dots correlated with ventricles, and black dots indicated regions that gave rise to bulbus arteriosus. (C) The existence of cells that contribute to the outflow tract later in development was suggested by the work of de la Cruz et al (1977). Lowercase letters indicate specific positions that were labeled and where these points end up as the heart continues to develop. Note the fact that cells labeled at the distal outflow tract as late as HH22 are below the pulmonary outflow valves at HH35. Abbreviations: R.A., right atrium; R.V., right ventricle; P.C., pulmonary semilunar valve cusps; C.S., crista supraventricularis; L.A., left atrium; L.V., left ventricle.

addition, this study concluded that there was no identifiable organization within the heart field, which remains somewhat puzzling in light of earlier and later studies.

The same year, three different groups described a population of cells that contributed to the heart after the initial heart tube had formed. Kelly et al (2001) created a fibroblast growth factor (FGF)10-nlacZ reporter mouse that showed expression in the myocardium of the right ventricle and outflow tract and in the pharyngeal mesoderm at E9.5 (Fig. 2A). DiI labeling in this reporter mouse determined that the right ventricle and the outflow tract myocardium are added from both the pharyngeal arch core and splanchnic mesoderm from E8.25 to E10.5 (Kelly et al., 2001). Both FGF10 and the nlacZ transcripts are down-regulated as these secondarily added myocardial cells are added to the heart tube, whereas β-galactosidase (β-gal) protein encoded by nlacZ is still present in these cells, supporting their origin as the FGF10-positive cells in the pharynx.

Two additional studies identified sources of the myocardial cells that contributed to the lengthening outflow tract, using chick as the model. Mjaatvedt et al (2001) labeled myocardial progenitor cells using either Mitotracker or a replication-deficient adenovirus that expressed β-gal. After labeling cells cranial to the heart tube and observing labeled cells in the outflow tract, Mjaatvedt et al defined this progenitor population as the anterior heart field. When Mjaatvedt et al ablated the bilateral heart fields as defined by Rosenquist and DeHaan (1966), the embryos only formed a rudimentary heart tube, leading to the assumption that the outflow tract progenitors were a separate population from the bilateral heart fields (Fig. 2B).

Waldo et al (2001) also used cell labeling to determine the origin of the outflow tract myocardial progenitors. After observing that heart field markers Nxk2.5 and Gata4 were expressed in the pharyngeal mesoderm caudal to the outflow tract at HH14, this region was labeled with Mitotracker. Embryos that developed to HH22 showed robust labeling in the proximal outflow tract. Interestingly, HNK1, an antibody commonly used to identify migrating cardiac neural crest cells in the chick, also labeled this population of splanchnic mesoderm, but only near the outflow tract. HNK1 was found to colocalize with the myocardial marker MF20 at the junction of the splanchnic mesoderm with the outflow tract. More discrete than either the Kelly or the Mjaatvedt study, this population was termed the secondary heart field (Fig. 2C).

Relationship of the second, anterior, and secondary heart fields

Since their initial description, the relationships between these three novel regions of myocardial progenitors and the bilateral heart fields have been extensively refined. One of the key steps in this refinement was the identification of Islet (Isl)1 as a heart field marker (Yuan and Schoenwolf, 2000). Isl1 expression begins as asymmetric