Novel Cardiac Progenitors for All Components of the Heart Except for the Right Ventricle

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Abstract

Mammalian heart is first recognized as the cardiac crescent at the egg cylinder stage, which had been thought to form all components of the heart. Recently, several lineage-tracing experiments using dye-labeling and mouse genetic technique have demonstrated that another cardiac progenitor population, called the second heart field, contributes to the outflow tract, the right ventricle, and a part of the atria. This finding has provided a great impetus for the understanding of cardiac morphogenesis, but it has also raised additional questions such as the potential existence of unique cardiac progenitors that give rise to the left ventricle and the conduction system. Here, we show that a novel cardiac progenitor population is identified by expression and lineage tracing analysis of the *Sfrp5* gene. We found that *Sfrp5* gene expression was constantly seen in the venous pole of the forming heart, which is later differentiated into the myocardium of the sinus venosus, a precursor of the sinoatrial node. Descendants of *Sfrp5*-expressing cells were found to contribute not only to the sinus venosus, but also to the left ventricle, atria, and the outflow tract by lineage-tracing analysis using *Sfrp5-Cre* and *-Ert2Cre* mice. These results indicate that *Sfrp5*-expressing cells could include progenitors for the sinus venosus, atria, the left ventricle, and the outflow tract, but not in the right ventricle, implying that the origin of the right ventricle essential for pulmonary circulation could be unique from progenitors for other components in mammalian cardiac development. In this session, we would like to discuss a new understanding of cardiac progenitor distribution.

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Mammalian cardiac development starts with the cardiac crescent, which forms the primary heart tube and then the four-chambered heart along with the progressive differentiation of progenitors into cardiomyocytes [1–3]. In this process, two independent pulmonary and systemic circulatory systems are established. For the establishment of these systems, remodeling of the ancestral heart into these two separate systems might occur with the production and rearrangement of progenitor cells. Recent findings pertaining to another cardiac lineage called the secondary heart field (SHF) modified the classical view of cardiac development that the myocardium was derived from a single source. The SHF emerges in the splanchnic mesoderm to supply cells at both the arterial and venous poles of the heart tube, including the right ventricle (RV), most of the atria and outflow tract (OFT). However, the progenitor population that forms the first heart field (FHF), which gives rise to the left ventricle (LV) and the rest of the atria and the sinus venosus (SV) are still ambiguous. Since Wnt signalling is one of the most important regulatory pathways in vertebrate cardiogenesis [4–8], we focused on the Secreted frizzled-related protein (Sfrp) sub-family, known to encode secreted decoy receptors for Wnt signalling, and analyze its expression and lineage during cardiac development [9].

We first observed expression of *Sfrp5*. At E7.5, *Sfrp5* was detected in the lateral and caudal region of the *Mlc2a* and *Nkx2-5* expressing region in the cardiac crescent. This region was distinct from the *Isl1* and *Hcn4*-expressing region, but mostly overlapped with the region marked by *Tbx5*. These observations suggest that *Sfrp5* expression seems to be restricted in undifferentiated FHF progenitors existing in the lateral sides of the cardiac crescent. During the heart tube looping stage (E8.0–E8.5), *Sfrp5* was continuously expressed in the venous pole of the heart. This *Sfrp5*-expressing area was distinct from the expression area of *Mlc2a* and *Nkx2-5*, indicating that *Sfrp5* is exclusively expressed in the undifferentiated cardiomyocytes. At E9.5, *Sfrp5* expression was restricted to the *Tbx18* and *WT1* expressing the ventral venous pole, suggesting that *Sfrp5* is expressed in progenitors of such SV and epicardium.

Since *Sfrp5* expression was found in progenitors of the FHF at E7.5, and also detected continuously in the progenitors of the SV and the epicardium, we hypothesize that *Sfrp5*-expressing cells include progenitors for the LV, the atrium, the SV and the epicardium. To test this possibility, we performed lineage analysis of *Sfrp5*-expressing cells by crossing the *Sfrp5*-Cre line with Rosa26R or CAG-floxed-CAT-eGFP reporter mice [10]. We found that β -galactosidase (LacZ) labeled cells were detected in the OFT, LV, atria, and venous pole, but not in the RV

at E9.5. Labeled cells overlapped with TnT in the OFT, LV, and atria, suggesting that descendants of *Sfrp5*-expressing cells differentiated into the chamber myocardium in addition to the SV. At E13.5, LacZ-stained cells were mostly found in the LV and also in the WT1-expressing pericardium and the CD31-expressing endocardium. Labelled cells were found in part of the interventricular septum but rarely in the RV. These observations suggest that *Sfrp5* is expressed in progenitors of the SV, pericardium, epicardium, endocardium, and the OFT, as well as in all chamber myocardium except for the RV.

Since *Sfrp5* expression is restricted in the venous pole of the heart at E8.5, which does not include the chamber myocardium, we speculated that cells expressing *Sfrp5* prior to E8.5 might include progenitors of the FHF. To evaluate the fate of *Sfrp5*-expressing cells at specific developmental stages, we crossed a tamoxifen inducible *Sfrp5*-ERT2 Cre mouse line with reporter mice and found that LacZ staining revealed that cells labelled at E7.5 were found in the OFT, LV, atria, SV, and venous pole but not in the RV. Moreover, labelled cells at E8.5 and E9.5 were still found in the atria, indicating that *Sfrp5*-expressing cells at the venous pole may supply cardiomyocytes for the inflow tract. These data suggest that *Sfrp5*-expressing cells at E7.5 include common progenitors for the SV, OFT, and all chamber myocardium except for the RV.

Although the expression pattern of *Sfrp5* was distinct from that of *Isl1*, an SHF marker at E7.5, descendants of *Sfrp5*-expressing cells were found in the OFT, which was thought to be derived from the SHF. This observation gave rise to a hypothesis that the expression of *Sfrp5* precedes that of *Isl1* in the splanchnic mesoderm. To test this possibility, we performed lineage-tracing analysis using *Isl1*-Cre and CAG-floxed-CAT-mRFP reporter/*Sfrp5*-venusYFP mice and found that the YFP signal in the ventral venous pole rarely overlapped with derivatives of *Isl1*-expressing cells. Instead, in *Sfrp5*-Cre/CAG-floxed-CAT-eGFP-reporter mice, GFP-labelled cells expressed *Isl1* in the dorsal splanchnic mesoderm and part of the OFT. These findings indicate that part of the SHF lineage is derived from *Sfrp5*-expressing cells to contribute to the OFT and atria.

Based on our findings, we propose a model for the contribution of *Sfrp5*-expressing cardiac precursors to heart development (Fig. 50.1). We found that *Sfrp5*-expressing cells at E7.5 contributed to the OFT, the LV, the atria, and the SV, but not to the RV, by fate mapping experiments using *Sfrp5*-Cre and -ERT2Cre mice. This observation indicates the possibility that the FHF lineage and the SV might share a common origin. Given that *Isl1* lineages contribute to all myocardium except for the LV [11, 12], the fate of *Isl1* lineages to the RV or *Sfrp5* lineages to the LV may be exclusively determined during the earliest stages of cardiogenesis.

Fig. 50.1 A new model for heart development: Crescent shaped heart primordium is divided into two layers along the mediolateral axis. The medial side of heart crescent, expressing *Islet1*, forms splanchnic mesoderm and its decedents contribute to all cardiac components except the LV. On the other hand, the lateral side of heart crescent, expressing *Sfrp5*, localizes in the mesenchymal cells in the inflow tract to form the SV, and its decedents contribute to all cardiac components except the RV

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