Coronary heart disease remains a leading cause of morbidity and mortality, and as such, there is a pressing need to develop efficacious therapies. Any future cell-based therapeutic interventions aimed at revascularization of an ischemic heart, or regeneration of damaged coronary vessels, require a clear understanding of the developmental programs that are in place in the embryo. This knowledge will also be necessary to provide the basis for biologically engineered heart tissues. Despite intensive investigation into the development of the coronary vasculature, the embryonic origin of these cell types has remained unclear. In the 1990s, pioneering experiments by Mikawa and others using the avian embryo demonstrated that coronary vascular smooth muscle cells are derived from the proepicardium,1,2 a transient embryonic structure that emanates from the pericardial serosa adjacent to the sinus venosus3,4 external to the linear heart tube that gives rise to the epicardium.1,5 Subsequently, the proepicardium was found to contribute interstitial cardiac fibroblasts,2 atrioventricular cushion cells,6 cardiac stem cells,7 and, at least in avian species, some coronary endothelial cells (ECs).8 The notion that some cardiac myocytes have an epicardial or proepicardial origin has diminishing support, and was likely to be caused by ectopic or misexpression of transgenic lineage tracing tools used, leading to incorrect conclusions.8

What are the candidates for the origins of coronary ECs? Although there is agreement on a proepicardial origin of most coronary vascular smooth muscle cells and interstitial cardiac fibroblasts, a proepicardial origin for coronary ECs in mammals has been less clear, with controversy in recent years on the origins of coronary ECs. Studies have reached different conclusions, including that the mammalian proepicardium did not provide coronary ECs, and that the situation in avian species was not the same as in mammals, or that the mammalian proepicardium may act as a conduit for already differentiated ECs, possibly from the liver primordium.9 Again, much of the controversy stemmed from studies that, with hindsight, used inappropriate mouse lines, including different Cre lines based on the Wnt promoter.9 Nevertheless, with an increasing suite of genetically engineered mouse lines and more refined lineage tracing strategies, a more coherent picture of coronary vascular development began to emerge: one that included the classic sinus venosus origin of coronary venous ECs, and a ventricular endocardial origin for coronary arteries.10–12

More recently, a sinus venosus origin for the majority of intramural and subepicardial coronary ECs was foreshadowed in a study by Tian et al.13 An inducible Apelin-CreER mouse line was produced to genetically label subepicardial coronary ECs, a population of cells believed to derive from the sinus venosus. They found that the labeled subepicardial coronary ECs gave rise to the majority of intramyocardial coronary ECs in the embryonic heart. This was complemented by data from cultured embryonic heart explants. Genetically labeled or wild-type sinus venosus and atria were combined with labeled or wild-type ventricles. Only when genetically labeled sinus venosus and atria were used were labeled subepicardial ECs observed, providing further evidence against a ventricular endocardial origin for the subepicardial ECs. Furthermore, their single-cell clonal analysis, based on the inducible Apelin-CreER line treated with low doses of tamoxifen to give an infrequent recombination frequency allowed labeling of discrete clones of cells, suggest that coronary arterial and venous ECs have a common origin. Although this study demonstrated that subepicardial ECs invade the ventricular walls to form the endothelium of coronary arteries in addition to the superficial coronary venous system, inconsistencies between previous studies remained. Specifically, it remained necessary to reconcile these findings with the reported ventricular endocardial origins of coronary arterial ECs.

In this issue of Circulation Research, Zhang et al14 report on their study that further clarifies the origins of mammalian coronary ECs. In this elegant study, Zhang et al14 used engineered mouse lines to genetically label ventricular endocardial or sinus venosus cells in the embryonic mouse heart to determine the source of coronary arterial ECs. Using Nfatc1-GFP, Nfatc1-Cre, and Nfatc1-Dre mouse lines, they show that Nfatc1 is expressed in the sinus venosus as well as the ventricular endocardium. Importantly, they also show that Nfatc1 is expressed in coronary ECs at later stages of heart development. To identify genes differentially expressed between endocardial and coronary ECs, fluorescence-activated cell sorting–purified single cells from embryonic hearts were subject to transcriptomic analyses. One gene, natriuretic peptide receptor 3 (Npr3), was expressed in endocardial cells but not in coronary vascular ECs. The authors confirmed...
expression of Npr3 in the endocardium with their newly generated Npr3-GFP-Cre and inducible Npr3-CreER mouse lines. Importantly, the Npr3 lines did not label the sinus venosus. To determine the developmental fate of sinus venosus cells, Zhang et al performed a recombinase-based intersectional lineage tracing strategy. This involved using the Dre-Rox and Cre-Lox systems in parallel with a dual reporter line, Ai66, that requires cells to express both Cre and Dre to be labeled. Nfatc1-Dre; Npr3-CreER; Ai66 embryonic mouse hearts showed few labeled intramyocardial coronary ECs (≈3%). Detecting Nfatc1-Dre-positive cells with a conventional reporter, however, revealed that ≈78% of intramyocardial coronary ECs were labeled. This indicated that the vast majority of these ECs were derived from an embryonic population that is Nfatc1-positive and Npr3-negative, namely the sinus venosus. Thus, the authors provide compelling evidence that the vast majority of coronary ECs in the ventricular free walls—including coronary arterial ECs—derive from the sinus venosus with little contribution from the ventricular endocardium. That the genetic labeling identifies subepicardial ECs and coronary arterial ECs is consistent with the findings of Red-Horse et al. Also consistent with that study was the finding that the sinus venosus contributed a low number of coronary ECs to the interventricular septum. Zhang et al then used the inducible Npr3-CreER line to provide further evidence that the ventricular endocardium contributes significantly to coronary ECs of the interventricular septum, confirming their previous work using genetic lineage tracing with the Mef2c-Cre line,13 Also consistent with that study was the finding that the sinus venosus expressed Npr3 in the endocardium with their newly generated Npr3-CreER mouse lines. Hence, the authors provide compelling evidence that the sinus venosus, including coronary arterial ECs—derive from the sinus venosus and are likely to greatly enhance the feasibility to develop cell-based therapies for revascularization or regenerating coronary vessels. The study by Zhang et al, and other recent studies, go a long way toward resolving seemingly inconsistent theories on coronary vascular development and should help set the stage for the field to further elucidate the molecular, cellular, and bio-physics driving coronary vascular development.

Disclosures

None.

References

Towards Consensus on Coronary Vessel Development: Coronary Arterial Endothelial Cells Derive Primarily From the Sinus venosus During Embryogenesis

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