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Connecting the coronaries: How the coronary plexus develops and is functionalized

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ABSTRACT

The establishment of the coronary circulation is one of the final critical steps during heart development. Despite decades of research, our understanding of how the coronary vasculature develops and connects to the aorta remains limited. This review serves two specific purposes: it addresses recent advances in understanding the origin of the coronary endothelium, and it then focuses on the last crucial step of coronary vasculature development, the connection of the coronary plexus to the aorta. The chick and quail animal models have yielded most of the information for how these connections form, starting with a fine network of vessels that penetrate the aorta and coalesce to form two distinct ostia. Studies in mouse and rat confirm that at least some of these steps are conserved in mammals, but gaps still exist in our understanding of mammalian coronary ostia formation. The signaling cues necessary to guide the coronary plexus to the aorta are also incompletely understood. Hypoxia-inducible transcription factor-1 and its downstream targets are among the few identified genes that promote the formation of the coronary stems. Together, this review summarizes our current knowledge of coronary vascular formation and highlights the significant gaps that remain. In addition, it highlights some of the coronary artery anomalies known to affect human health, demonstrating that even seemingly subtle defects arising from incorrect coronary plexus formation can result in significant health crises.

Introduction

As the heart begins forming, the myocardial and endocardial layers of the heart are each composed of a single layer, and oxygen and nutrients are passaged via simple diffusion. With the growth of the ventricles, endothelial-lined trabeculae in the ventricles maintain sufficient surface area such that a vasculature is not required to supply oxygen and nutrients until as late as embryonic day (E) 15.5 in the mouse (Olivey and Svensson, 2010). However, a functional vasculature is required for the heart to undergo successful compaction of the ventricular myocardium. The coronary vasculature develops in two stages: first, a vascular plexus forms and encompasses the heart, and then, this plexus remodels into a mature vasculature that connects to the aorta. In the mature heart, the connections that bridge the plexus and the aorta occur through two ostia or openings through which the left and right coronary arteries connect to the aorta (Fig. 1). The specific segments of the coronary arteries that connect to the aorta are referred to as the coronary stems, and this term will be used to distinguish the specific segment of the main coronary arteries that penetrates the aorta from the general coronary vasculature. These main coronary arteries subsequently branch off the base of the aorta, with the left coronary artery further dividing into the circumflex artery, which curves around the base of the left ventricle, and the left anterior descending artery, which reaches toward the apex of the left ventricle. Together, these arteries supply the mature ventricles with oxygen and nutrients.

Origin of coronary endothelium

The coronary plexus first forms as a series of discontinuous endothelial patches that spread from the sinus venosus around the ventricles to form a complete plexus. The origin of these endothelial cells is highly controversial, having been attributed to the proepicardium, the sinus venosus, and the endocardium. The original studies detailing the origins of the coronary endothelium relied on avian species due to their amenability to *in ovo* manipulation, have been thoroughly discussed by Riley and Smart (2011), and will be briefly summarized. These initial studies used retroviral labeling, either *in ovo* or in an *in vitro* proepicardial

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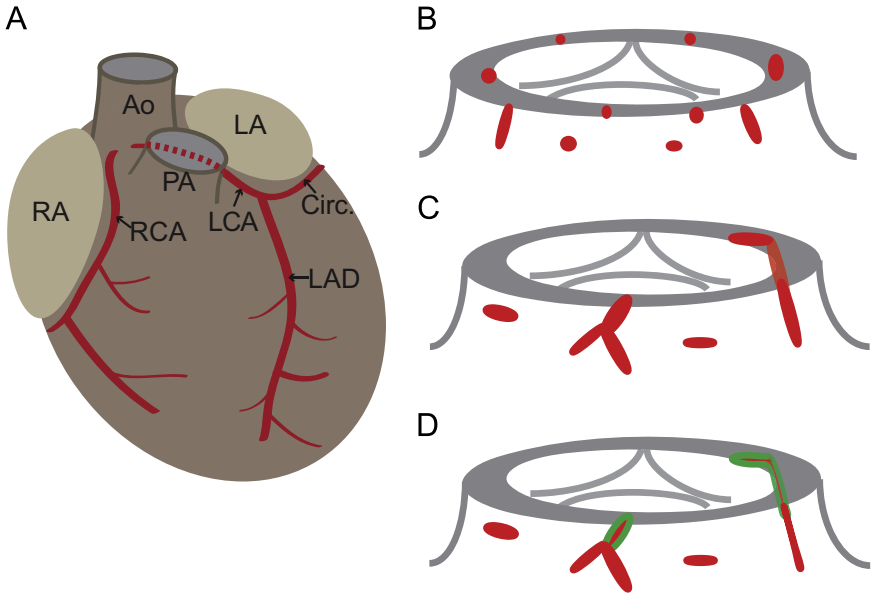

Fig. 1. Coronary artery anatomy and stem formation. (A) In the mature heart, two main coronary arteries are present. The left coronary artery (LCA) runs between the pulmonary artery (PA) and the left atrium (LA) and branches into the circumflex (Circ.) artery and the left anterior descending (LAD) artery. The right coronary artery (RCA) branches off the right side of the aorta (Ao) and follows the right atrium (RA) as it travels to the apex of the heart. (B)–(D) A schematic overview of coronary ostia formation in avians. B) Thin blood vessels (denoted in red) from the coronary plexus invade the peritruncal region and penetrate the aortic wall. C) These small vessels coalesce to form two distinct ostia. D) Smooth muscle cells (denoted in green) encompass the coronary stems, beginning at the ostia themselves and over time encompassing the rest of the coronary arteries. All three of these steps have been documented in chick and quail; whether the thin vessels (B) are initially present before coalescing into two distinct ostia in mammals is unknown.

explant that was subsequently transplanted, and quail–chick chimeras in which a quail proepicardial explant was placed in a chick host (Mikawa and Fischman, 1992; Poelmann et al., 1993; Perez-Pomares et al., 1998; see also Greulich and Kispert, 2013 for a detailed description of these studies). In these experiments, labeled proepicardial cells contributed to the coronary vessels and transplanted quail cells expressed the quail endothelial marker QH-1. The inclusion of liver primordium in some of the proepicardial explants has led to the hypothesis that the liver is also a source of coronary endothelial cells (Poelmann et al., 1993; Lie-Venema et al., 2005). However, subsequent studies have not supported this hypothesis.

More recent proepicardial lineage-tracing studies in mouse (summarized, along with additional mouse-based experiments, in Table 1) have challenged the long-held assumption that the proepicardium gives rise to coronary endothelium. Lineage-tracing studies using the standard proepicardial marker Tbx18 showed that Tbx18-lineage cells give rise not only to the epicardium but also to myocardium, cardiac fibroblasts, pericytes, and coronary smooth muscle (Cai et al., 2008). However, these experiments did not identify Tbx18 lineage-traced cells within the coronary endothelial population, even with the use of sensitive detection methods such as fluorescence activated cell sorting (Cai et al., 2008). Zhou et al. (2008) simultaneously performed similar lineage analyses using the WT-1 promoter, with similar results. Zhou et al. also found that WT-1-derived proepicardial cells gave rise to myocardium and coronary smooth muscle. In rare cases, though, WT-1-derived cells also contributed to the coronary endothelial population, but this event was too infrequent to support WT-1-positive proepicardial cells as the major source of coronary endothelial cells (Zhou et al., 2008). However, just as the avian transplantation studies can be compromised by dissection integrity, Cre-mediated lineage tracing has its own unique pitfalls. In the case of the WT-1-Cre lines, these shortcomings include potential low levels of recombination beyond the targeted tissue (Zhou and Pu, 2012) and expression of WT-1 in the coronary endothelium and even some cardiomyocytes (Greulich and Kispert, 2013). In the Tbx18-lineage study, the myocardial "progeny" is more likely a second population of Tbx18-expressing cells within the myocardium rather than descendants of the earlier Tbx18-expression proepicardium (Christoffels et al., 2009). Although species-specific differences may explain, at least in part, the apparent inconsistencies between the chick and mouse data, one alternative hypothesis is that some coronary endothelial cells derive from a source other than the Tbx18-/WT-1-positive proepicardium.

Thus, in an attempt to identify other sources of coronary endothelium, recent studies in the mouse have employed clonal analyses to begin elucidating the contributions of the sinus venosus and the endocardium to the coronary plexus. Because the mouse embryo is less amenable to *in vivo* manipulation than the avian embryo, a key step for addressing the origin of the coronary endothelium is identifying a coronary endothelium-specific gene. Red-Horse et al. (2010) took advantage of the apelin-lacZ knock-in mouse, which shows expression in adult coronary endothelium but not endocardium (Sheikh et al., 2008). An analysis of apelin expression during coronary plexus formation confirmed that apelin is expressed in the developing coronary endothelium and also showed that apelin expression is continuous with the sinus venosus (Red-Horse et al., 2010). This expression pattern, in combination with clonal analyses based on an inducible VE-cadherin Cre mouse line, suggested that VE-cadherin-positive endothelial cells from the sinus venosus migrate into the heart to generate the majority of the coronary plexus (Red-Horse et al., 2010). Further, subsequent clonal analyses using the apelin promoter suggest that arterial and venous coronary endothelial cells share a common progenitor (Tian et al., 2013b).

As a potential resolution of the apelin expression pattern, the historical avian experiments, and the proepicardial lineage-tracing studies in mice, Katz et al. (2012) identified a population of WT1-negative, Tbx18-negative, semaphorin 3D-positive proepicardial cells. Lineage traces of these semaphorin 3D-expressing cells show that they migrate from the proepicardium into the heart and give rise to a portion of the coronary smooth muscle and endothelium,