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Endothelial cell lineages of the heart

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SUMMARY

During early gastrulation, vertebrate embryos begin to produce endothelial cells (ECs) from the mesoderm. ECs first form primitive vascular plexi *de novo* and later differentiate into arterial, venous, capillary, and lymphatic endothelial cells. In the heart, there are five distinct EC types: endocardial, coronary arterial, venous, capillary, and lymphatic, which have distinct phenotypes. For example, coronary ECs establish a typical vessel network throughout the myocardium, while endocardial ECs form a large epithelial sheet without angiogenic sprouting into the myocardium. Neither coronary arteries, veins, capillaries, nor lymphatic vessels fuse with the endocardium or open to the heart chamber. It is still unclear during which developmental stage, a specific phenotype of each cardiac EC type is determined. By tracking the migratory patterns and lineage decisions for cardiac EC precursors, the mechanisms involved in EC commitment and diversity are more precisely defined.

Keywords

Coronary Vessel Development; Cardiac Endothelial Cells; Proepicardium; Cell Lineage

ORIGIN OF ENDOCARDIAL ENDOTHELIAL CELLS

A century ago, the embryonic vasculature was believed to form by the invasion of extra-embryonic blood vessels (His, 1868, 1900). However, this model was challenged by the experimental demonstration that blood vessels developed in embryos from which the entire extra-embryonic tissues had been ablated prior to blood vessel formation (Reagan, 1915). Further studies have shown that of three germ layers, only the mesoderm generates ECs independently of extra-embryonic vessels (Coffin and Poole, 1988; Pardanaud and Dieterlen-Lievre, 1999).

Endocardial EC progenitor and cardiomyocyte precursors specifically arise from a rostral portion of the primitive streak (Garcia-Martinez and Schoenwold, 1993). As gastrulation begins, they migrate rostrolaterally, forming bilateral heart fields (Rawles, 1943; Rosenquist and DeHaan, 1966). During embryonic lateral body folding, the bilateral heart fields fuse and form a single tubular heart at the ventral midline of the embryo. During this fusion process, a minor population segregates from the original epithelioid heart field mesoderm, differentiate into ECs, and forms the single endocardial tube, while the majority of mesodermal cells in the heart field enters the cardiomyocyte lineage and forms the presumptive myocardium (Stalsberg and Dehaan, 1969; Linask and Lash, 1993). Thus, both endocardial EC and cardiomyocyte lineages arise from the heart field mesoderm.

The heart field is specified bilaterally at the rostrolateral mesoderm where BMP signaling is present and Wnt signaling is suppressed by signals from the underlying endoderm and

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overlying ectoderm (Sater and Jacobson, 1989; Schultheiss and Lassar, 1997; Yatskievych et al., 1997). The heart field mesoderm is characterized by its unique expression profile of several transcription factors, including *Isl1*, *Nkx2.5*, and *GATA4*.

The majority of cells in the heart field begins to express cardiomyocyte-marker genes in response to endoderm-derived signaling factors, including fibroblast growth factors (FGFs), bone morphogenic proteins, insulin-like growth factor, and activin (Lough et al., 1996; Antin et al., 1996; Schultheiss et al., 1995; 1997; Yatskievych et al., 1997). A minor population is promoted to express EC differentiation-marker genes by angiogenic factors, such as vascular endothelial growth factor (VEGF) and FGFs (Folkman et al., 1987; Flamme and Risau 1992). These studies have raised a question as to how signaling molecules that are broadly expressed in the underlying endoderm induce a small population of the heart field to enter the endocardial EC lineage and the majority to differentiate into cardiomyocytes.

It was once thought that mesodermal cells of the heart field are a bipotent progenitor that differentiates into both cardiomyocytes and endocardial ECs (Linask and Lash, 1993; Eisenberg and Bader, 1995). The bipotential model was easy to grasp but did not explain how broadly expressed inducing signal would instruct individual cells of the heart field to enter the cardiomyocyte lineage or the endocardial EC lineage. The model also failed to explain either how the heart field mesoderm generates more cardiomyocytes than endocardial ECs. Indeed, cell lineage studies in avian and fish embryos have demonstrated that these two cell lineages of the heart are already segregated at the blastula stage or at early gastrulation, significantly prior to their migration to the heart field. For example, retroviral single cell tagging and tracing studies have detected that individual cells in the rostral half of the primitive streak and those in the heart field give rise to a clone consisting of only one cell type, either endocardial or myocardial (Mikawa et al., 1992; Cohen-Gould and Mikawa, 1996; Wei and Mikawa, 2000). Cell fate studies in zebrafish have also identified a blastomere population that produces only endocardial or myocardial cells (Lee et al., 1994). These studies in both fish and avian embryos indicate that the separation of these two lineages occurs at the blastula stage, prior to formation of mesoderm. Consistent with this model, it has been suggested that EC commitment occurs before and independent of gastrulation, while cardiomyocyte commitment has not yet occurred at the prestreak stage (von Kirschhoffer et al., 1994). It remains to be determined how and when these two cardiac cell lineages are established in the mammalian embryo.

ORIGIN OF CORONARY ECs

Coronary vessels are originally absent from the embryonic heart until the myocardium becomes thickened (Rychter and Ostadal, 1971; Bogers et al., 1989; Wald et al., 1990). The coronary vessel network was once thought to develop through angiogenic sprouting bilaterally from the base of the aorta (Hirakow, 1983; Conte and Pellegrini, 1984; Hutchins et al., 1988). Chick/quail chimera studies have shown that smooth muscle cells of the great vessel arise from a subset of the cardiac neural crest (Jiang et al., 2000). This finding raised the possibility that cells of the coronary arteries were derived from neural crest. Another possibility was that they arose from the heart field mesoderm, as the endocardial EC and cardiomyocyte lineages do. However, retroviral labeling of cardiac neural crest or the heart tube never gave rise to tagged cells within the coronary system (Mikawa et al., 1992; Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996). There is another cell population that migrates to the heart and forms the epicardium. This third cardiac cell population arises from an extracardiac mesoderm, called the proepicardium (PE), that develops as a cauliflower-like protrusion from the ventral lip of the anterior intestinal portal immediately posterior to the presumptive atrium (Ho and Shimada, 1978; Viragh and Challice, 1981; Himura and Hirakow, 1989; Manner et al., 2001; Nahirney et al., 2003).

The PE consists of two morphologically distinct cell types: a superficial mesothelial epithelium and an internal mesenchymal core (Nahirney et al., 2003). The surface mesothelial component of the PE undergoes a unique morphogenetic process, forming multiple epithelial villi that protrude toward the myocardium of the looping stage heart, while the core mesenchymal component fills the inner space of the PE (Figs. 2 and 3). As soon as attached to the myocardium, the growing PE begins to envelop the myocardium, forming the epicardium. While cells of the PE were originally thought to be the anlagen only for the epicardium, retroviral tagging of PE cells produces either virally tagged cardiac fibroblasts or coronary vessels containing tagged endothelial or smooth muscle cells (Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996). These studies have provided the first direct proof for the PE origin of coronary vasculature, including ECs, smooth muscle cells, and perivascular fibroblast cells of coronary arteries. Following retroviral cell lineage studies, both adenoviral tagging (Dettman et al., 1998) and chick/quail chimera analysis of the proepicardium (Gittenberger-de-Groot *et al.*, 1998; Manner, 1999; Vrancken Peeters et al., 1999) have reached the same conclusion (reviewed in Manner et al., 2001; Bernanke and Velkey, 2002; Reese et al., 2002; Majesky, 2004; Manner, 2006; Olivey et al., 2004; Wessels and Perez-Pomares, 2004; Tomanek, 2005). Importantly, individual virally labeled clones contain only one cell type, either ECs, smooth muscle, or fibroblasts (Mikawa and Fischman, 1992; Mikawa and Gourdie, 1996; Dettmann et al., 1998). Recent studies have further shown that PE-derived cells transiently form blood islands in the subepicardial space (Tomanek et al., 2006). Consistent with the above results of retroviral cell lineage studies, our immunohistochemical analysis has identified differentiated ECs in the PE, many in the core mesenchyme and few in the superficial mesothelium (Fig. 3A–C). To address the differentiation potential of individual PE cells into coronary vascular cell types including ECs, we have cultured isolated PE cells at clonal density and examined if they are differentiated into ECs or other vascular cells (Fig. 3D–G). Interestingly, the clonal density culture generates a cluster of cells that express either an EC marker or a smooth muscle marker. Cell clusters containing two cell types are rarely found. Taken together it is likely that these cell lineages can be segregated in the PE before its arrival at the heart. However, it remains to be determined how the fate of individual PE cells is diversified into epicardial, coronary EC, smooth muscle or fibroblast cells.

Since the PE develops adjacent to the sinus myocardium, it is highly likely that it arises from the lateral margins of the heart fields (Redkar et al., 2002; Moorman et al., 2007). The exact mechanism that induces and specifies the PE fate in the mesoderm at the specific region of the embryo is currently unknown. However, it is known that the PE develops from mesodermal cells that overlay the liver bud endoderm (Manner, 1992; Viragh et al., 1993; Nahirney et al., 2003), suggesting an inductive interaction between the liver-bud endoderm and the PE-progenitor mesoderm. This possibility has recently been tested using a chick/quail chimera approach (Ishii et al., 2007). When a donor quail liver bud is implanted in the posterior-lateral regions of host chick embryo, PE marker genes (Robb et al., 1998; Reese et al., 1999; Landerholm et al., 1999; Hatcher et al., 2004; Watt et al., 2004; Schlueter et al., 2006) are induced in host mesodermal cells ectopically at the implantation site. The work suggests the potential role of a liver bud endoderm-derived paracrine cue(s) in PE induction. Currently, a molecule(s) that mediates the PE-inducing activity of the liver bud is undefined. Induction of the heart field mesoderm depends upon paracrine signals from the underlying foregut endoderm (Schultheiss *et al.*, 1995; 1997; Lough *et al.*, 1996). Signals from the resulting heart field mesoderm in turn initiate hepatic cell differentiation and liver bud formation in the ventral foregut endoderm (Fukuda-Taira, 1981; Gualdi *et al.*, 1996; Duncan, 2003). In addition to these two reciprocal mesoderm-endoderm interactions in the induction/differentiation of cardiomyocytes and hepatocytes, a tissue-tissue interaction between the liver endoderm and overlaying mesoderm may play a role in induction and/or specification of the PE fate within the mesoderm.

LYMPHATIC ECS OF THE HEART

In contrast to the extensive studies on the origin of endocardial and vascular ECs, little is known about the ontogeny of the lymphatic ECs of the heart. It was once thought that the lymphatic system of the forelimbs develops through the sprouting from pre-existing lymph sacs (Sabin, 1909). However, recent studies using markers for the lymphatic ECs have detected lymphangioblasts one day prior to the formation of the lymph sacs (Schneider et al., 1999). A recent model predicts that a local budding from the existing capillary or venous network is the key event in initiating lymphatic vessel formation. This model, however, does not appear to be applicable to lymph vessel formation of the heart.

In the heart, lymph vessels form alongside the coronary vessels, but they do not appear to not originate from the PE (Wilting et al., 2007). In chick/quail chimera studies, a donor quail PE produce many ECs that are integrated into host coronary vessels, including arteries, veins and capillaries. However, in no case are quail ECs intermingled in the lymphatic's of the host chick heart, except for a large lymphatic trunk at the base of the chick heart. In murine embryos, scattered lymphatic EC marker-positive cells have been detected in the subepicardium of the embryonic heart. Taken together, it can be postulated that immigrating lymphangioblasts contribute to the cardiac lymphatic system. The data also suggest that coronary vessels and lymph vessels are derived from different sources, but grow in close association with each other.

POTENTIAL ROLES OF CARDIAC STEM CELLS

It has been reported that bone marrow-derived cells differentiate into cardiomyocytes and coronary endothelial and smooth muscle cells when injected into the infarcted heart (Orlic et al., 2001; Kajstura et al., 2005; Silva et al., 2005). More recently, resident multipotential cardiac stem/progenitor cells have been suggested in adult myocardium in rodents and human (Beltrami et al., 2003; Messina et al., 2004; Oh et al., 2003; Matsuura et al., 2004; Mohri et al., 2006). Whether these cells originate from embryonic cardiac mesoderm or whether they migrate late in development from other tissues such as the bone marrow (Quaini et al., 2002; Simper et al., 2003; Bayes-Genis et al., 2004; Thiele et al., 2004) remains an issue of debate (reviewed in Anversa and Nadal-Ginard, 2002; Leri et al., 2005; Ellison et al., 2007; Trella et al., 2007). As discussed above, current evidence suggests that, during cardiogenesis, different cardiac cell types originate from spatially- and temporally-distinct progenitor cells, favoring the latter possibility. Recent *in vitro* and *in vivo* lineage studies suggest that the PE is capable of producing cardiomyocytes and coronary vessel cell types (Kruihof et al., 2006; Schlueter et al., 2006; Cai et al., 2008) but that at least endothelial cell lineages are distinct from those that contribute to cardiomyocytes (Cai et al., 2008). It remains to be determined whether endothelial cell differentiation from stem cell population is regulated by the same or distinct mechanism between organogenesis and regeneration.

CONCLUDING REMARKS

Cell fate mapping and lineage studies have identified distinct origins of individual cardiac EC types. The data provide clues for defining cell-cell and molecular interactions that regulate cardiac EC differentiation and diversity. Questions that remain to be determined include: 1) How are endocardial EC precursors specified within the heart field mesoderm, while exposed to the same signals as cardiomyocyte precursors? 2) What mechanisms induce the EC fate within the PE? 4) What cell-cell and molecular interactions are responsible for specifying early ECs to their arterial, venous, capillary or lymphatic identities in the embryonic heart? 5) How plastic are early ECs? Understanding the mechanisms that govern these processes will substantially further our understanding of vascular system development and patterning during embryogenesis.

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Fig. 1.

Schematic illustration of the heart field mesoderm (cm, black), paraxial mesoderm (pam, light gray) and lateral plate mesoderm (lpm, dark gray) in zebrafish (left panel) and chick (middle panel) gastrula stage embryos. an-ve: the animal-vegetal axis, v-d: the ventral-dorsal axis, and a-p: the anterior-posterior axis, ps: the primitive streak. Right panel: Lineage relationships between multiple endothelial cell types generated from mesoderm. As three germ layers form via gastrulation, endocardial ECs differentiate from the heart field mesoderm, while coronary vasculature, including ECs, differentiate from a distinct mesodermal tissue, the proepicardium. Non-cardiac arterial, venous and capillary ECs are generated from the lateral plate and somatic mesoderm. Lymphatic ECs appear to differentiate from venous ECs during budding off.

Fig 2.

Expression pattern of PE and liver-bud markers in chick embryos. (A) Single whole-mount *in situ* hybridization for a PE marker *Wt1* (purple). (B) Double *in situ* hybridization for *Wt1* (purple) and a liver-bud marker *Hex* (green). (C) Section from an embryo double hybridized for a PE marker *Cfc* (purple) and a liver bud marker *Hex* (green). Experimental procedures as described elsewhere (Ishii et al., 2007).

Fig. 3. Detection of EC differentiation in the developing PE (A–C) and in clone density culture of PE cells (D–G)

(A) A transverse section through the PE, immunostained for an EC marker QH1 (red) and a mesothelial PE cell marker Wt1 (green). Dorsal is up. DAPI was used to visualize nuclei (blue). (B,C) High magnification of a boxed region in A. While many ECs are found in the mesenchymal core (asterisk) of the PE, some ECs are also detected in the Wt1-positive mesothelial component of the PE (arrowheads). (D) Single PE cell cultured for 6hr at the clonal density. The quail PE cells were dissociated by pipetting after treatment with collagenase and with trypsin/EDTA. The cells were cultured in a growth medium (M199 with 0.1 mg/ml heparin, 0.05 mg/ml endothelial cell growth supplement and 10% fetal bovine serum) on a plastic dish. (E) Daughter cells of (D) after 36 hr of plating. (F,G) Cluster of PE cells triple stained for EC marker (QH1; red), smooth muscle marker (smooth muscle alpha-actin; green) and DAPI for nuclei (blue).