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Review

The developmental origins and lineage contributions of endocardial endothelium☆

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ABSTRACT

Endocardial development involves a complex orchestration of cell fate decisions that coordinate with endoderm formation and other mesodermal cell lineages. Historically, investigations into the contribution of endocardium in the developing embryo was constrained to the heart where these cells give rise to the inner lining of the myocardium and are a major contributor to valve formation. In recent years, studies have continued to elucidate the complexities of endocardial fate commitment revealing a much broader scope of lineage potential from developing endocardium. These studies cover a wide range of species and model systems and show direct contribution or fate potential of endocardium giving rise to cardiac vasculature, blood, fibroblast, and cardiomyocyte lineages. This review focuses on the marked expansion of knowledge in the area of endocardial fate potential. This article is part of a Special Issue entitled: Cardiomyocyte Biology: Integration of Developmental and Environmental Cues in the Heart edited by Marcus Schaub and Hughes Abriel.

1. Introduction

The development of endocardial cells during embryogenesis is a complex process that involves various stages of cell fate specification and transformation. Studies in recent years using mouse lineage tracing and assays in chick and zebrafish development have continued to elucidate important mechanisms by which these fate choices are made. Furthermore, new advances in embryonic stem cell-directed differentiation into endocardium have provided a new avenue by which to understand this cell population.

Extensive lineage tracing experiments have been performed to help elucidate the origins and functions of different mesodermal lineages. In addition to discussion of these studies, we also summarize the molecules that mark different mesodermal lineages with a particular focus on those that are related to endocardial/hematoendothelial development (Table 1).

As an overall summary, this review begins by evaluating the state of knowledge about the initial fate choices during germ layer specification and mesoderm development that contribute to endocardial lineage decisions. This is followed by evaluating studies during late embryogenesis and early post-natal life that have shown a much broader lineage contribution of endocardium, with evidence that these cells give rise to the cardiac vasculature and blood. The last section focuses primarily on studies in embryonic stem cell differentiation that have been critical for understanding developmental fate choices into the endocardial lineage. These studies have also revealed a unique potential for endocardium to give rise to the cardiac lineage by Notch and Wnt-dependent mechanisms that has been corroborated in mouse fate mapping studies.

2. Origins and specification of endocardial cells

The developing heart is broadly considered to originate from two distinct cellular sources: the first heart field (FHF), which contributes the left ventricle and the majority of the atria, and second heart field (SHF), giving rise to the right ventricle and outflow tract. Although it is still controversial whether they represent two distinct 'organ field', these two fields are incorporated into the heart in rapid succession, resulting in both anatomically and molecularly distinct contributions. Arising from both of these fields are the two layers of the early primitive heart: the endocardium and the myocardium. Whilst much focus has been placed on mapping the origins of the myocardium and the molecular cues involved in its development, little attention has been paid to the accompanying endocardium.

The endocardium develops within the cardiogenic mesoderm via a process of *de novo* vasculogenesis. Beginning as paired endothelial tubes, these two structures fuse to form a single endocardial tube within the cardiac field [1,2]. Whilst the differentiation of the endocardium is spatially and temporally distinct from the developing vasculature and hematopoietic regions of the developing embryo [3], these three cell

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Table 1
Molecular markers associated with hemato-endothelial cell types.

Molecule	Marker	Endocardium	Endothelium	Blood
TF	SCL/Tal1	+	+	+
TF	ETV2	+	+	+
TF	NFATC1	+	−	−
TF	ISL1	+	+	−
TF	HAND1	−	+	−
TF	NKX2-5	+	+	+
TF	MESP1	+	+	+
TF	EOMES	+	+	−
TF	T	+	+	+
TF	TBX18	−	+	−
TF	RUNX1	+	+	+
TF	GATA1	nd	+	+
R	TIE2	+	+	+
R	Flk1/KDR	+	+	+
J	VE-Cadherin	+	+	+
CS, J	CD31	+	+	+
CS	CD34	+	+	+
CS	CD43	−	−	+
CS	CD235a	−	−	+
CS	CD41	+	+	+
CS	CD45	−	−	+

Markers that distinguish hemato-endothelial lineages. Abbreviations: transcription factor (TF), cell surface (CS), junction (J), receptor (R). Not determined (nd). Molecules are indicated as being expressed, even if transiently, as a marker of that lineage at any stage of development/differentiation.

types have much in common. The endocardium develops adjacent to and contiguous with the dorsal aorta, and the dorsal aorta is a site of hematopoiesis [4]. In addition to similar anatomical location, these three lineages share a range of molecular markers in common, such as Scl/Tal1, Flk1/Kdr and Fli1 [5–7] (see Table 1). Lineage tracing experiments have definitively shown divergence of these cell types at or prior to gastrulation [8,9], however the potency of precardiac mesoderm to form endothelium and blood (elaborated below) is further evidence of their close molecular nature and suggests a common antecedent, prior to lineage restriction.

The cardiac field has been identified in the early vertebrate embryo just prior to the onset of gastrulation. Using classical embryological cell labeling and transplantation techniques, Tam and colleagues mapped cardiac progenitor cells (CPCs) in the mouse embryo to the lateral posterior epiblast of the E6.5 (early primitive streak stage) embryo. While most of the cells traced in these experiments differentiated into myocardium, a subset of cells did contribute to endocardium, demonstrating that at least a proportion of the endocardium derives from cells either in close proximity to cardiomyocytes or a common progenitor exists [8]. Unfortunately, the nature of these experiments involves tracing several cells at a time, precluding examination of the common progenitor theory.

From gene expression analysis, the earliest (although not restricted) marker of cardiac progenitor cells is the T-box transcription factor, *Eomesodermin* (*Eomes*). *Eomes* expression begins in the early epiblast stage (E5.75) and is restricted to the prospective posterior region of the epiblast [10]. Lineage tracing studies using the ROSA26^{R} reporter demonstrated that a substantial proportion of endocardial and myocardial cells were LacZ positive at E9.5, as well as definitive endoderm [11]. Unfortunately, it was not possible to determine whether the emergence of the cardiac field occurs earlier than E6.5 from this study, as this *Eomes*^{Cre} line is expressed through to E7.5. In the same study, the bHLH transcription factor, *Mesp1*, was found to be a direct target of *Eomes*. *Mesp1* expression coincides with the earliest cell tracing of the cardiac field at E6.5 and is also considered one of the early markers of cardiac progenitor cells. Like *Eomes*, *Mesp1* expression is broader than the cardiac region giving rise to hemogenic lineages and skeletal myogenic precursors as well [12]; however this expression is exclusive to mesoderm. *Mesp1* is expressed transiently (E6.25–E7.5) in the lateral posterior region of the early primitive streak stage embryo, and labels mesodermal derivatives, including CPCs [13]. Early lineage tracing studies utilizing Mesp1^{Cre} and persistence of an impermanent LacZ reporter failed to detect expression in endocardial cells [14]. However, more recent work utilizing permanently activated markers has shown *Mesp1* expressing cells contributing to endocardium [15,16] and *Mesp1*-positive cells populate the entire endocardium as well as myocardium at E14.5 [16]. This conclusively demonstrates that endocardial precursors derive from *Mesp1* expressing cells in the gastrulating embryo.

Consistent with a co-located origin for both endocardial and myocardial cells in mouse, lineage tracing in zebrafish embryos has shown early regionalization of CPCs, including endocardium. Single cell labeling has mapped CPCs to the lateral margins of the zebrafish blastula, with cardiac potential dissipating towards the dorsal region [17]. Interestingly, endocardial cells were restricted to the most ventral region of the cardiac field. If this restricted region of endocardial progenitors also occurs in the mouse embryo, it may account for the low frequency of endocardial labeling observed in mouse lineage tracing experiments [8]; however such a region in the mouse embryo has yet to be described. By late blastula stages in the zebrafish, endocardial precursors have relocated more dorsally to reside at the lateral regions of the embryo and are intermixed with myocardial progenitors [18].

Several lines of evidence from differentiated embryonic stem cell experiments and Cre-mediated lineage tracing have provided compelling evidence toward the common progenitor discussion. Seminal work by Misfeldt et al. showed that NFATc1 marks cardiac progenitor cells with multilineage potential, giving rise to myocardium and endocardium [19]. Furthermore, clonal assays of embryonic cells directed to mesodermal identity and expressing the vascular endothelial growth factor receptor *Flk1* (*Kdr*) were shown to be capable of generating cardiomyocytes and endothelial cells (as well as vascular smooth muscle) from single cell isolates, demonstrating the multipotent capability of these cells [20]. In the embryo, *Flk1* is expressed in mesodermal cells exiting the primitive streak (E7.0–7.5) and lineage tracing shows they colonize both myocardium and endocardium [21]. Together, these data are suggestive of an uncommitted, multipotent progenitor at late primitive streak stages.

In conjunction with this, multipotent progenitors have also been described in populations of the SHF. Lineage tracing using Cre lines labeling the SHF (*Isl1*^{Cre}, *Isl1*$^{mER\text{-}Cre\text{-}mER}$ or *Mef2*-AHF^{Cre}), demonstrate that both myocardial and endocardial cells are derived from this population [22,23]. Moretti et al. went further to show that single *Isl1*$^{+}$ progenitors isolated and cultured from the mouse embryo could give rise to three lineages in the early heart, namely endocardium, myocardium and smooth muscle [24]. Interestingly, the greater propensity for these cells to generate endothelium, including endocardium, appeared to occur more frequently when the homeodomain transcription factor, NKX2-5, was not expressed.

More recently, permanent cell labeling techniques have addressed the common progenitor question directly in the mouse embryo. Using *Mesp1*^{Cre} lines, two independent groups have demonstrated that rare recombination events irreversibly labeling low numbers of cells will give rise to either myocardial or endocardial clones but never both in the FHF whereas clones residing in the SHF comprise of both myocardium and endocardium [15,16]. Lescroart and colleagues followed these events using a Doxycycline inducible *Mesp1*^{rtTA} line, followed by expression profiling of cells from the early (E6.5) or late (E7.25) *Mesp1*-expressing population. Consistent with their lineage tracing experiments, cells of the early Mesp1 lineage were found to be heterogeneous and express markers of either the myocardial or endocardial identity whereas cell populations of the late Mesp1-expressing lineage were found to express markers of both myocardium and endocardium. This supports a model for restricted lineage commitment of early CPCs and multi-lineage commitment of late CPCs in the *Mesp1* expressing mesoderm.

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