A Twist of Proepicardial Fate

Katherine E. Yutzey

In the developing vertebrate heart, cells that contribute to vascular smooth muscle, cardiac fibroblasts, and coronary endothelial cells are derived from the proepicardium and associated cells originally located at the venous pole of the primitive heart tube. Recently, there has been much interest in the regulatory mechanisms that control epicardium-derived cell (EPDC) lineage development and mobilization with adult cardiac injury. However, less is known of the embryonic origins and inductive mechanisms that control the initial induction of the proepicardium. The study by Schlueter and Brand in the current issue of Circulation Research reports fate mapping of proepicardial progenitors to the somatic mesoderm and demonstrates a critical role for the basic helix-loop-helix transcription factor Twist1 in the earliest stages of proepicardial development.

The heart is the first organ to function in the developing vertebrate embryo, and beating of the primitive heart tube is apparent by embryonic day (E)8 in mouse and by 40 hours of development in chicken embryos. As the primitive heart tube loops, lateral plate mesoderm cells on the right side of the embryo at the cardiac venous pole begin to form the proepicardium. Schlueter and Brand demonstrate through fate mapping studies in avian embryos that at least some cells of the proepicardium are derived from the somatic layer of lateral plate mesoderm. The somatic compartment (somatopleure) of the lateral plate mesoderm is adjacent to the ectoderm and forms the body wall. The new data provide initial evidence that cells of the proepicardium also are derived from somatic mesodermal layer. The proepicardium shares many characteristics with other mesothelia of the major coelomic organs that contribute progenitors of fibroblasts and smooth muscle to the lung, gut, kidney, etc. The embryonic origins of coelomic mesothelia are not well defined, but the initial finding that the proepicardium contains cells from somatic mesoderm is a starting point for future studies of mesothelial cell origins.

Cell lineage markers of the epicardial derivatives are expressed after the formation of a morphologically distinct proepicardium, consisting of an epithelial layer with villous projections, surrounding a mesenchymal core apparent at the inflow segment of the heart. Schlueter and Brand show that Twist1 is expressed before formation of the proepicardium in the somatic mesoderm on the right side of the embryo caudal to the heart. Fate mapping analysis by fluorescent dye labeling or electroporation of reporter plasmids demonstrates that this region later contributes to the proepicardium. Targeted Twist1 gain- and loss-of-function studies demonstrate that Twist1 is necessary and sufficient for formation of the proepicardium and induction of proepicardial transcription factors Tcf21 and Wilm’s tumor (Wt1). Previous studies by this group demonstrated that FGF8 signaling on the right side of the embryo induces expression of the transcription factor Snail1 (Snai1) and subsequent induction of the proepicardium on the right. BMP signaling also has been implicated in proepicardium induction, and Twist1 expression is dependent on BMP2 signaling in endocardial cushion development. Together, these data support a model (Figure) whereby FGF signaling, possibly together with BMP signaling, induces Snail1 and Twist1 upstream of specific regulators of epicardial lineage development, including Tcf21 and Wt1.

Twist1 is a critical regulator of multiple progenitor cell populations in the developing embryo, including those in the endocardial cushions, limb bud, neural crest, and cranial sutures. In the endocardial cushions, Twist1 promotes cell proliferation and migration of mesenchymal valve progenitors. Direct downstream target genes of Twist1 include cell adhesion molecules, notably cadherins, which likely underlie the morphological abnormalities observed with gain or loss of Twist1 in proepicardial progenitors. The direct downstream targets of Twist1 in epicardial progenitors have not yet been identified. It is interesting to note that expression of Tcf21 and Wt1 is affected by gain or loss of Twist1, but it is not known if these genes are directly regulated by Twist1. Likewise, because Twist1 is active in many cell types, epicardial-specific Twist1-mediated regulatory mechanisms that lead to induction of lineage-specific markers are yet to be identified.

The study by Schlueter and Brand takes advantage of the accessibility of the chicken embryo to examine the embryonic origins of the proepicardium. Fate mapping and cell lineage tracing in avian embryos were used in the 1990s in the first studies of epicardial lineage development and in the discovery of EPDCs. More recently, Cre-based cell lineage tracing has been used to examine the embryonic origins of the epicardium and the fates of epicardial-derived cells in mice. These studies have led to controversial results in some cases and are limited by the inability to control the timing of Cre activation and lack of epicardial specificity in some of the genetically manipulated mouse lines. The chicken embryo offers an alternative system in which the timing and location of introduction of a lineage marker is under greater control and the fate of cells can be monitored longitudinally. A disadvantage is that the introduction of a fluorescent tracer...
dye or electroporation of a reporter plasmid may not be completely restricted to a single cell type. Schlueter and Brand\(^1\) have used these techniques to define a region of the embryo, including somatic mesoderm near the axial junction with splanchnic mesoderm, that contributes cells to the epithelial proepicardium. However, not all of the proepicardial cells or mesenchymal cells within the proepicardium are labeled. Thus, it is likely that cells of multiple embryonic origins contribute to the proepicardium and associated cell types, which ultimately generate the connective tissue and coronary vessels of the mature heart.

There is increasing evidence for heterogeneity of cells in the proepicardium region that contribute to multiple lineages in the mature heart. Fate-mapping of Twist1-positive cells in chicken embryos provides evidence for contributions to smooth muscle and possibly fibroblast cell lineages at later stages of heart formation.\(^1\) Endothelial derivatives of Twist1-expressing cells were not observed. However, more extensive analysis is necessary to fully determine the derivatives of the Twist1-expressing proepicardial lineage. The predominant labeling of smooth muscle and potentially fibroblast lineages as Twist1 derivatives is similar to that observed for Tbx18, Tcf21, and Wt1 Cre-based epithelial lineages in mice.\(^5\,\,15,16\) There is increasing evidence that coronary endothelial cells arise from an alternative source in proximity to the proepicardium labeled by Semaphorin 3D (Sema3d) and Scleraxis Cre-expressing lines, although this is like the sole source of vascular endothelial cells in the heart.\(^15,17\) The responsiveness of Tcf21 and Wt1 to Twist1 in avian embryos, taken together with the similar cell types arising from these lineages, supports a mechanism whereby Twist1 functions upstream of Tcf21 and Wt1 in cardiac vascular smooth muscle and fibroblast progenitor development in the proepicardium.

**Twist1** expression is downregulated in the epithelial proepicardium and epicardial cells as they migrate over the surface of the heart.\(^1\) However, at later stages, **Twist1** is expressed in EPDCs present in atrioventricular canal along with other markers of epithelial-to-mesenchymal transition (EMT), including Snai1.\(^18\) The initial formation of the proepicardium involves a mesenchymal-to-epithelial transition, and **Twist1** is subsequently downregulated in the epithelial epicardium. At later stages, EPDCs are generated by EMT of the epithelial epicardial cell layer, and **Twist1** expression is induced.\(^19\) **Twist1**, together with Snai1, functions in delamination and migration/invasion of mesenchymal cells in multiple embryonic structures and also in metastatic cancers.\(^10\) Therefore, **Twist1** is likely to have important roles in early morphogenesis of the proepicardium and also in delamination of EPDCs later in development. Further studies are necessary to define the specific roles for **Twist1** in these transitions in proepicardium and EPDC progenitors in the developing heart.

In the adult mouse heart, epicardial cells are activated and new EPDCs are generated after cardiac ischemic injury.\(^20\) This discovery has generated significant interest as a potential mechanism in cardiac regeneration or repair. Regulatory mechanisms that promote the generation and lineage development of EPDCs in the embryonic heart also are induced with epicardial activation after injury. One of the earliest events of epicardial activation is the induction of EMT of the epicardial epithelial cell layer to generate new populations of subepicardial mesenchymal cells.\(^20,21\) Multiple genes involved in EMT, including Snai1 and **Twist1**, are induced with epicardial activation. Expression of the EPDC transcription factors Tcf21 and Wt1 also is induced in newly generated subepicardial cells after ischemic injury. It seems reasonable that expression of Tcf21 and Wt1 is dependent on **Twist1** function in activated EPDCs based on embryonic studies. However, the specific functions of **Twist1** and its potential role in EPDC lineage development after cardiac injury are yet to be determined.

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**Disclosures**

None.

**References**


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**Figure.** Model for **Twist1** regulation of proepicardial development.


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