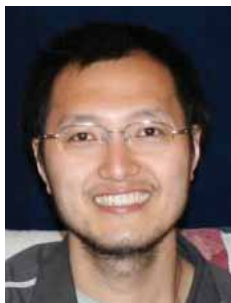




More than a cover: epicardium as a novel source of cardiac progenitor cells



**Bin Zhou¹ &
William T Pu^{2†}**

[†]Author for correspondence
¹Department of Cardiology,
Children's Hospital Boston
and Harvard Stem Cell
Institute, Harvard University,
Boston, MA, USA
Tel.: +1 617 919 4643;
E-mail: bzhou@enders.tch.
harvard.edu
²Department of Cardiology,
Children's Hospital Boston
and Harvard Stem Cell
Institute, Harvard University,
Boston, MA, USA
Tel.: +1 617 919 2091;
Fax: +1 617 730 0140;
E-mail: wpu@enders.tch.
harvard.edu

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Heart failure is a leading cause of morbidity and mortality worldwide, and its prevalence continues to increase [1]. Diverse etiologies lead to heart failure, but these share common features of cardiomyocyte loss, inadequate myocardial perfusion and myocardial fibrosis. Current therapy slows the progression of heart failure and mitigates its symptoms, but does not reverse these underlying problems. Therapeutic strategies intended to reverse disease course in heart failure must overcome the limited capacity of the adult human heart to replace lost cardiomyocytes.

Recently, there has been great interest in progenitor cell-based therapies owing to their potential to generate new cardiomyocytes and supporting vascular cells [2]. A number of progenitor populations with the potential to differentiate into cardiomyocytes have been reported [3]. Thus far, attempts at using these progenitors for functional repair of injured myocardium in human and animal models have been met with mixed results [2,4]. While the promise of this strategy remains, it is clear that success will require learning much more about the underlying biology of cardiac progenitors. An important starting point will be to improve our understanding of how the principle cardiac lineages are established during normal heart development.

The mammalian heart is first recognizable as a linear heart tube, composed of an outer layer of cardiomyocytes and an inner layer of endocardium. The heart tube elongates at both ends by continued differentiation of cardiomyocytes and supporting cell types from progenitors located dorsal and anterior to the heart, in the second heart field [5]. The heart subsequently receives important contributions from two additional extracardiac sources, the neural crest and the proepicardium. Neural crest cells contribute to normal development of the outflow tract and

great vessels [6]. Proepicardial cells migrate onto the surface of the heart, forming an epithelial sheet known as the epicardium. Epicardial cells then undergo an epithelial-to-mesenchymal transition and migrate into the subjacent myocardium. These cells contribute to the development of most coronary smooth muscle, a subset of coronary endothelium and cardiac fibroblasts [7,8]. Additionally, the epicardium and myocardium engage in reciprocal paracrine and cell-cell interactions that are required for the growth and development of each compartment [7].

In vitro, proepicardial explants efficiently differentiate into cardiomyocytes [9]. However, this developmental fate had not been found *in vivo* [7] until recently, when we showed that proepicardial and epicardial cells adopt a cardiomyocyte fate during normal heart development [10]. Cardiac expression of the transcription factor *Wt1* is restricted to the proepicardium and epicardium (Figure 1A) [10,11]. We used the endogenous *Wt1* locus to drive expression of Cre recombinase, which labels Cre-expressing cells by heritably activating Cre-dependent reporters. In addition to previously reported smooth muscle and endothelial fates, *Wt1*-labeled cells differentiated into functional cardiomyocytes. The differentiation of *Wt1*-labeled cells into cardiomyocytes was confirmed by conditional labeling induced at E10.5 (Figure 1B), when the expression of *Wt1* was known to be confined to the epicardium. Independently, a second group reached the same conclusion using a similar Cre lineage-tracing approach based on a different epicardial marker, the transcription factor T-box 18 [12]. These studies, in combination with previous lineage-tracing studies performed in mammalian and avian systems [7], indicate that epicardial progenitors differentiate into cardiomyocyte, smooth muscle, endothelial and fibroblast lineages during normal heart development.

Most cardiomyocytes of the heart are derived from multipotent *Nkx2-5*⁺/*Isl1*⁺ progenitors, which also differentiate into smooth muscle and endothelial lineages [Ma Q, Zhou B, Pu WT, Unpublished Data] [13–15]. We found that *Wt1*⁺ proepicardial cells are likewise descendants of *Nkx2-5*⁺ and *Isl1*⁺ precursors. However, proepicardial cells do

not actively express either of these markers [10], suggesting that *Isl1/Nkx2-5* and *Wt1* are either transiently coexpressed or sequentially expressed earlier in development. Transient coexpression of *Wt1* with *Nkx2-5* was confirmed in cardiac progenitors generated by embryoid body differentiation of embryonic stem cells [10]. These data position the *Wt1*⁺ proepicardial lineage as an early branch from the multipotent *Nkx2-5*⁺/*Isl1*⁺ progenitor lineage.

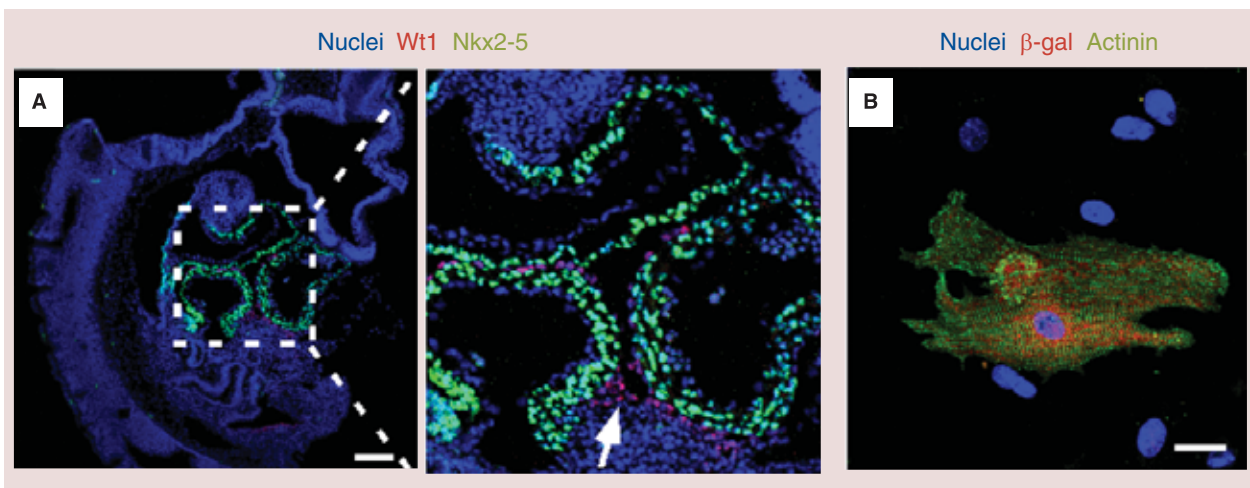
While the epicardium has been largely ignored in clinical cardiology, the rich and essential contributions of the epicardium to multiple lineages of the developing heart suggest that it likely has important functions in the adult heart. This hypothesis is supported by several indirect lines of evidence. First, studies in zebrafish also support a key role of epicardium in myocardial homeostasis and wound healing. After amputation of the heart apex, zebrafish repair the wound through myocardial regeneration. This process involved reactivation of fetal epicardial genes [16]. Epicardial cells contributed to the regenerated myocardium, although the specific lineages derived from epicardium were not determined. Activation of fetal epicardial genes and the cellular contribution to myocardium was also observed during homeostatic growth of the myocardium [17]. Second, selective labeling of postnatal epicardium by retroviral injection into the pericardial space labeled coronary vascular cells and occasional cardiomyocytes after myocardial infarction [18].

Third, epicardial progenitors isolated from adult human and murine heart differentiate into coronary vascular cells *in vitro*, suggesting that epicardial progenitors persist into adult life [19,20]. Indeed, a subset of cells within the postnatal epicardium express the stem cell marker *c-kit* [18].

Collectively, the evidence suggests that the epicardium is much more than a cover over the outside of the adult heart. It is enticing to envision how the rich developmental repertoire of the epicardium might be manipulated to reverse disease course in heart failure. However, a number of critical questions must be answered to evaluate the therapeutic potential of progenitors within the postnatal epicardium. What is the contribution of epicardial progenitors to myocardial wound healing? Does the adult epicardium differentiate into fibroblasts, thereby contributing to myocardial fibrosis? Do epicardial progenitors with cardiomyocyte differentiation potential persist into adulthood in human and murine epicardia? Are epicardial progenitors multipotent? If so, what regulates their lineage decisions? Can these lineage decisions be manipulated to promote a cardiomyocyte and coronary vascular fate, and to inhibit a fibroblast fate?

Although current trials of cell-based therapy in heart failure have had limited success, the overall strategy remains promising owing to its potential to reverse the underlying disease process. A detailed understanding of the

Figure 1. Proepicardial progenitors differentiate into cardiomyocytes during normal heart development.



(A) Mid-gestation mouse heart stained for proepicardial and epicardial marker *Wt1* and cardiomyocyte marker *Nkx2-5*. In the heart, *Wt1* expression was confined to proepicardium (arrow) and epicardium. (B) *Wt1*-derived cells were pulse labeled at E10.5. At E14.5, a subset of cells coexpressed the genetic lineage tracer β -gal and the cardiomyocyte marker α -actinin.

developmental biology and hierarchy of cardiac progenitors will lead us to the optimal progenitor cells and teach us how to best expand, mobilize, deliver and direct the differentiation of these progenitors. The finding that epicardial progenitors differentiate into cardiomyocytes indicates that epicardial progenitors are a novel cardiac progenitor population that should be evaluated in regenerative approaches to heart failure.

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