Epicardium-Derived Cardiac Mesenchymal Stem Cells
Expanding the Outer Limit of Heart Repair

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The epicardium is derived from the proepicardial organ, a source of multipotent progenitor cells. Epicardium contribution to the developing coronary vasculature and to cardiac interstitial cells has been established. Studies over the past several years have suggested that epicardium-derived cells can adopt cardiomyocyte and vascular smooth muscle fates and can contribute to cardiac repair when activated by injury.1,2 Recently, Chong et al3 have provided a detailed characterization of a population of epicardium-derived multipotent cardiac progenitor cells (cCFU-Fs). These cells, which do not arise from the bone marrow, neural crest, or myocardium, resemble mesenchymal stem cells (MSCs) and may participate in cardiac development, homeostasis, and repair.3

During early cardiac development, cells derived from the proepicardial organ (a cluster of cells located dorsal and adjacent to the looped heart tube) migrate over the myocardium to form the epicardium. Subsequently, epicardium-derived progenitor cells undergo epithelial-to-mesenchymal transition, invade the underlying myocardium, and differentiate into various cardiac lineages.4,5 Signals and cellular contributions from the epicardium have been shown to be indispensable for the establishment of normal coronary vasculature and myocardial architecture.6 Cardiac interstitial cells arise from epicardium, and recent studies have suggested that reactivation of the epicardium after injury could contribute to scarring or myocardial repair after injury.1,2,6,7 In this context, the recent report from Chong et al3 is particularly relevant, because they used rigorous gene expression, culture, and fate lineage analysis to characterize a population of multipotent MSC-like cells resident in the heart that they called cardiac colony-forming units–fibroblast (cCFU-Fs). These cells derive from the proepicardium, not from cardiac myocytes, neural crest, or bone marrow, and they are able to differentiate into endoderm (eg, colonic epithelium), mesoderm (eg, smooth muscle, cardiac muscle, and adipose tissue), and neuroectoderm derivatives (eg, neurons, glia, and oligodendrocytes). The detailed characterization of this resident cardiac MSC-like population will pave the way for future analysis of the contribution of this cell type to cardiac homeostasis and response to injury.

Chong et al3 identified cCFU-Fs on the basis of their ability to form colonies in culture composed of fibroblast cells and to differentiate into multiple lineages both in vitro and in vivo. Colony-forming units–fibroblast were first identified in the bone marrow.8 These cells were defined as MSCs because of their capacity for clonogenic propagation, long-term in vitro growth, and multilineage differentiation. MSCs have been identified from several adult tissues, and tissue-specific MSCs show biased lineage differentiation potential.9,10 To address whether cardiac tissue also harbors MSC-like populations, these authors used in vitro colony-forming assays, similar to those previously used to characterize bone marrow MSCs.8 The cCFU-Fs isolated from embryonic and adult hearts express MSC markers such as CD44, CD90, CD29, and CD105 and are able to differentiate into a variety of cardiac lineages, including cardiomyocytes. Interestingly, lineage potential is not limited to mesodermal fates, because these cells can also acquire endodermal and neuroectodermal fates, although they are unable to form teratomas in standard assays, which suggests that they are not pluripotent. The cCFU-Fs do not express the pan-hemopoietic marker CD45 or the endothelial markers CD31 and Flk1, but they do express some stem cell markers (Oct4, cMyc, and a low level of Kit4 and Nanog) and hematopoietic stem cell markers (Sca1 and platelet-derived growth factor receptor-α). A previous study suggested that Sca1+CD31− perivascular cells could migrate into injured areas of myocardium and differentiate into cardiomyocytes and endothelial-like cells after acute ischemic injury.11 However, the relationship between Sca1+/CD31− cardiac cells and the cCFU-Fs described by Chong et al3 is not yet clear.

Chong et al3 used a tamoxifen-inducible Wt1-Cre mouse to label embryonic epicardial derivatives and showed that many of these cells express platelet-derived growth factor receptor-α. They subsequently used platelet-derived growth factor receptor-α–GFP+ knock-in mice to isolate and further characterize these cells from embryos and adults. Interestingly, the ability of this population to produce cCFU-Fs appeared to decline in adults. The cCFU-Fs arise from mesodermal precursors, as defined by expression of Mesp1-Cre. Although a recent report has suggested that some cardiac progenitor cells in adult mice may be derived from neural
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crested tissue. Chong et al.13 found that cCFU-Fs are not derived from Wnt1-Cre–expressing neural crest progenitors, although cells with similar characteristics could be isolated from neural crest derivatives in the aorta. Fate mapping with Nkx2.5-Cre did not label substantial numbers of cCFU-Fs, which suggests that they do not arise from Nkx2.5-expressing cardiac progenitors, which are known to give rise to cardiac myocytes.

Over the past decade, bone marrow–derived progenitor cells have been shown to contribute to repair and remodeling of heart tissue in normal and diseased conditions.13,14 However, substantial controversy surrounds these findings, and further investigation is needed. Chong et al.13 investigated whether cCFU-Fs were derived from bone marrow tissue and whether bone marrow–derived cells could rescue cCFU-Fs lost after irradiation. By transplanting bone marrow tissue from a genetically labeled transgenic mice (Actb-eGFP) into lethally irradiated mice, Chong et al.13 showed that adult bone marrow cells could not rescue cCFU-Fs after irradiation injury. Stem cell migration occurs in response to inflammation and injury and is regulated by a number of growth factors, cytokines, and chemokines. However, in experimental conditions used by these investigators, bone marrow–derived progenitor cells failed to migrate to the heart to produce cCFU-Fs after either cardiac injury or granulocyte colony-stimulating factor–induced hematopoietic stem cell mobilization. These authors also examined the relationship between c-kit+ cells and cCFU-Fs and concluded that cCFU-Fs are distinct from the majority of bone marrow–derived c-kit+ cells.

During embryonic development, epicardium-derived cells undergo epithelial-to-mesenchymal transition and differentiate into vascular smooth muscle cells and fibroblasts (and perhaps endothelial cells and cardiomyocytes).5 Myocardial injury reactivates the adult epicardium and contributes to formation of new blood vessels by triggering proliferation and differentiation of epicardium-derived progenitor cells into fibroblasts and smooth muscle cells, but not into endothelial cells or cardiomyocytes.7 A recent report by Smart et al.14 suggests that thymosin-β4 treatment before myocardial infarction may alter the responsiveness and ultimate fate of activated epicardial cells, inducing them to adopt cardiomyocyte fates, although this is controversial.15 It is interesting, and somewhat surprising, to note that Chong et al. did not detect a significant change in the number of cCFU-Fs 5 or 30 days after myocardial infarction compared with sham-operated controls, which suggests a lack of significant epicardial activation, at least by this assay. The relationship between the epicardial progenitor cells described by Smart et al., which are responsive to thymosin β4 and activated by injury, and cCFU-Fs, which express cardiac markers when cocultured with neonatal rat ventricular myocytes but are not activated by injury, will need to be established.

In the past 2 decades, the regenerative potential of the heart has been studied extensively.17 In contrast to the traditional view that the mammalian heart is a postmitotic organ, studies in amphibians, fishes, and rodents have shown a conserved capacity for cardiac regeneration that varies widely with species and with age. In contrast to the limited regenerative potential of the adult mammalian heart, zebrafish can fully regenerate hearts after surgical resection of as much as 20% of the ventricle.18 Cardiac regeneration in zebrafish appears to be unrelated to activation of stem cells. Instead, new myocytes derive from activation, dedifferentiation, and proliferation of preexisting cardiomyocytes.19 A recent study20 demonstrated that neonatal mouse hearts have similar cardiac regeneration potential that is largely lost in the first week of postnatal life. Genetic fate mapping showed that the majority of newly formed cardiomyocytes within the regenerated neonatal murine ventricle are derived from preexisting cardiomyocytes as well, although a contribution from stem or progenitor cells was not ruled out.

Although the generation of new cardiac myocytes from preexisting myocytes accounts for some instances of cardiac regeneration in animal models, the contribution of various progenitor cell populations to functional myocardium in the adult remains controversial.17,21,22 Recent studies in mice and in people suggest that a low level of cardiomyocyte renewal occurs normally, and this process may be enhanced after injury.17,23–25 A wide range of putative cardiac progenitors has been described, and clinical trials have sought to test their effectiveness as therapeutic agents in humans. MSCs derived from bone marrow have been tested in animal models and in patients after myocardial infarction with mixed results.26 Evidence for MSC differentiation into cardiomyocytes is scant, and paracrine effects have been invoked to explain the apparent beneficial actions on myocardial remodeling and function. However, it is conceivable that MSC-like cells such as cCFU-Fs that derive from a specific organ or tissue may exhibit biased differentiation potential. Hence, cCFU-Fs may prove to be therapeutically superior to bone marrow–derived MSCs for cardiac repair or may be more amenable to growth factor–induced cardiomyocyte differentiation. Direct comparisons of cCFU-Fs to other resident stem cell populations that have been described in the heart, including Scal+, sidepopulation, cardiosphere-forming, and other populations, will also be important for understanding the full spectrum of cardiac progenitor cell populations.

The work of Chong et al.13 represents an important step toward rigorously defining cardiac resident stem cell populations. At the same time, it raises numerous questions for further investigation. Is it possible to make functional (beating) cardiomyocytes from cCFU-Fs either in vitro or in vivo? Do cCFU-Fs differentiate into cardiac lineages in vivo and contribute to tissue homeostasis in the adult? Is it possible to activate or to modulate lineage decisions of cCFU-Fs in vivo? Does the activity or potential of cCFU-Fs decline with age? Answers to these questions may provide further insight into new therapeutic options for myocardial regeneration.

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## References


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