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Epicardium derived cardiac mesenchymal stem cells: expanding the outer limit of heart repair

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Abstract

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 al have provided a detailed characterization of a population of epicardial–derived multipotent cardiac-progenitor cells (cardiac CFU-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 (EPDCs) undergo epithelial-to-mesenchymal transition (EMT), invade the underlying myocardium and differentiate into various cardiac lineages[4, 5]. Signals and cellular contributions from the epicardium have been shown to be indispensible for the establishment of normal coronary vasculature and myocardial architecture[6]. Cardiac interstitial cells arise from epicardium, and recent studies have suggested that re-activation 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 al.[3] is particularly relevant because they utilize rigorous gene expression, culture, and fate lineage analysis to characterize a population of multi-potent MSC-like cells resident in the heart that they name cardiac-colony-forming units – fibroblast (cCFU-Fs). These cells derive from the pro-epicardium, not from cardiac myocytes, neural crest or bone marrow, and they are able to differentiate into endoderm, mesoderm and neurectoderm derivatives. 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.

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Disclosures

None

Chong et al. identified cCFU-Fs based on their ability to form colonies in culture composed of fibroid cells and to differentiate into multiple lineages both in vitro and in vivo. Colony-forming units – fibroblast were first identified from bone marrow tissue[8]. These cells were defined as MSCs due to their capabilities 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 if cardiac tissue also harbors MSCs-like populations, these authors used in vitro colony-forming assays, similar to those previously used to characterize bone marrow MSCs[8]. cCFU-Fs isolated from embryonic and adult hearts express MSCs 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, as they can also acquire endodermal and neuroectodermal fates, although they are unable to form teratomas in standard assays, suggesting that they are not pluripotent. cCFU-Fs do not express the panhemopoietic marker CD45 or the endothelial markers CD31 and Flk1, but they do express some stem cell markers (Oct4, cMyc and low level of Klf4 and Nanog) and hematopoietic stem cell (HSC) markers (SCA1 and PDGFRα). A previous study suggested that SCA1+/CD31− perivascular cells could migrate into injured areas of myocardium and differentiate into cardiomyocytes and endothelial-like cells following acute ischemic injury[11]. However, the relationship between SCA1+/CD31− cardiac cells and cCFU-Fs described by Chong et al. is not yet clear.

Chong et al. 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 (Pdgfrα). They subsequently used Pdgfrα-GFP^{+} knockin 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. 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 crest tissue[12], Chong et al. 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, suggesting that they do not arise from *Nkx2.5*-expressing cardiac progenitors or 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 finding and further investigation is needed. Chong et al asked whether cCFU-Fs are derived from bone marrow tissue and can rescue the 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 showed that adult bone marrow cells can 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 Chong et al bone marrow derived progenitor cells failed to migrate to the heart to produce cCFU-Fs after either cardiac injury or G-CSF induced HSC mobilization. These authors also examined the relationship between cKIT+ cells and cCFU-Fs and concluded that cCFU-Fs are distinct

from the majority of bone marrow derived cKIT+ cells. These finding are consistent with notion that bone marrow derived cells do not reconstitute injured heart.

During embryonic development epicardium derived cells undergo EMT 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 EPDCs into fibroblast, smooth muscle cells, but not into endothelial cells or cardiomyocytes[7]. A recent report by Smart et al 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[2], although this is controversial[15, 16]. 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 to sham-operated controls, suggesting lack of significant epicardial activation, at least by this assay. The relationship between epicardial progenitor cells described by Smart et al. and cCFU-Fs, which can be induced to activate expression of cardiac markers by co-culture with neonatal rat ventricular myocytes, will need to be established.

In last two decades, the regenerative potential of the heart has been extensively studied[17]. In contrast to the traditional view that the mammalian heart is a post mitotic 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, de-differentiation, and proliferation of mature cardiomyocytes[19]. A recent study demonstrated that neonatal mouse hearts have similar cardiac regeneration potential that is largely lost in early postnatal life[20]. Genetic fate mapping showed that the majority of newly formed cardiomyocytes within the regenerated neonatal murine ventricle are derived from preexisting cardiomyocytes, although a contribution from stem or progenitor cells was not ruled out.

While the generation of new cardiac myocytes from pre-existing 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. 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[23]. Evidence for cardiomyocyte differentiation of MSCs is scant, and paracrine effects have been invoked to explain 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 SCA1+, side-population, cardiosphere-forming and other populations will also be important.

The work of Chong et al. represents an important step towards 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 therapeutic options for myocardial regeneration.

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Non-standard Abbreviations and Acronyms

EPDCs	Epicardium-derived progenitor cells
cCFU-Fs	Cardiac-colony-forming units – fibroblast
EMT	Epithelial-to-mesenchymal transition
MSCs	Mesenchymal stem cells
HSC	Hematopoietic stem cell

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