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COMMENTARY

Colonizing the heart from the epicardial side

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Abstract

The clinical use of stem cells, such as bone marrow-derived and, more recently, resident cardiac stem cells, offers great promise for treatment of myocardial infarction and heart failure. The epicardium-derived cells have also attracted attention for their angiogenic paracrine actions and ability to differentiate into cardiomyocytes and vascular cells when activated during cardiac injury. In a recent study, Chong and colleagues have described a distinct population of epicardium-derived mesenchymal stem cells that reside in a perivascular niche of the heart and have a broad multilineage potential. Exploring the therapeutic capacity of these cells will be an exciting future endeavor.

Congestive heart failure, the leading cause of morbidity and mortality worldwide, is characterized by the irreversible loss of cardiac cells and function. Implantation of exogenous stem cells or activation of endogenous progenitors holds great potential for the therapeutic regeneration of lost cardiomyocytes and supporting vascular cells [1]. With the progress in the field, it is becoming evident that profound understanding of cardiac developmental biology will be necessary for the discovery of cell types and conditions that can lead to successful cardiac repair.

Recently, epicardium of the heart has received a lot of attention as a potential source of cardiovascular progenitors [2-4]. Traditionally, embryonic epicardium has been known for its paracrine roles in myocardial growth and differentiation and its contribution to cells of cardiac connective tissue and coronary vasculature [5]. Recent lineage tracing studies using epicardial markers Wt1 and Tbx18 have suggested that murine embryonic epicardium-derived cells (EPDCs) can also differentiate into cardiomyocytes [2,3]. Unlike in the embryo, the epicardium in the healthy adult heart remains quiescent; however, upon myocardial infarction, it gives rise to new EPDCs that differentiate into mesenchymal cells expressing fibroblast and smooth muscle markers. Although EPDCs do not adopt cardiomyocyte or endothelial cell fate, they still secrete paracrine factors that strongly stimulate angiogenesis in subepicardial layers [6]. Interestingly, if the murine heart is preconditioned with thymosin β4 (Tβ4) prior to infarction, the adult EPDCs can migrate into myocardium and give rise to new cardiomyocytes [4]. Unfortunately, in a clinically relevant scenario of Tβ4 post-conditioning, the EPDCs remain in the thickened epicardium and do not attain cardiogenic fate [7].

In line with these exciting developments, Chong and colleagues [8] have recently reported a new type of mesenchymal stem cell of epicardial origin found in embryonic and adult mouse heart that, similar to mesenchymal stem cells from bone marrow (BM), can form clonal colonies and undergo a long-term expansion for approximately 40 passages. In the adult heart, these cardiac colony-forming-unit fibroblasts (cCFU-Fs) reside in a perivascular adventitial niche and can be isolated from the non-cardiomyocyte fraction of the heart based on the expression of the surface markers platelet-derived growth factor receptor-α and Sca-1. The cCFU-Fs lack expression of cKit, CD31, Flk1, CD45, Nkx2-5, NG2, and the pluripotency genes *Rex1*, *Sox2*, *Klf4* and *Nanog*. Under optimized conditions, they differentiate *in vitro* and *in vivo* into different mesodermal lineages (for example, bone, cartilage, fat, smooth muscle, endothelium, cardiomyocytes), and when co-transplanted with embryonic stem cells, also attain non-mesodermal fates (for example, liver, neuronal, lung epithelium). Under the 5-aza-2'deoxycytidine treatment or in non-contact co-cultures with neonatal rat cardiomyocytes, 1 to 2% of the cCFU-Fs attain cardiac differentiation markers, but despite some sarcomeric organization, do not spontaneously contract, presumably due to paracrine inhibition by surrounding non-cardiomyocytes [8].

In the elegant Cre-recombinase lineage tracing studies, Chong and colleagues further show that cCFU-Fs originate from mesoderm (expressing Mesp1) but not from neural crest (expressing Wnt1) or cardiomyocytes (expressing Nkx2.5 and Myl2). Importantly, the use of the epicardial-specific Cre lineage tags Wt1 and Gata5 showed that cCFU-Fs derive from proepicardium and

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epicardium, and may be similar or hierarchically related to previously identified EPDCs. In addition, the cCFU-Fs appear to be distinct from previously reported cKIT$^+$ progenitors found in epicardium as these cells also expressed Nkx2-5 [9]. They also appear mostly different from pericytes based on no expression of NG2 or other pericyte markers.

In a previous study [10], the cCFU-Fs were found to be transcriptionally similar, but not identical, to BM CFU-Fs, suggesting their similar early origins, but potentially different tissue-specific functions. To investigate the potential contribution of BM cells to the cCFU-F compartment, Chong and colleagues transplanted green fluorescent protein (GFP)$^+$ BM cells into lethally irradiated mice and examined their contribution to cCFU-F colonies after myocardial infarction and 2 to 8 months of aging. They found that both cCFU-Fs and BM cells were irradiation-sensitive and while GFP$^+$ BM cells were able to reestablish the niche for BM CFU-Fs, they failed under all conditions (including mobilization by granulocyte colony stimulating factor) to contribute to cCFU-Fs. The majority of GFP$^+$ cells that entered the heart post-myocardial infarction were cKit$^+$/CD45$^+$ but none formed cCFU-Fs, suggesting a distinct phenotype of cCFU-Fs from those of BM-derived cKit$^+$ cells. Finally, none of the Wt1-, Gata5-, or Mesp1-tagged cells participated in the formation of BM cCFU-Fs, further suggesting distinct post-gastrulation origins of BM cells and cCFU-Fs.

The cutting-edge studies by Chong and colleagues open multiple avenues for future investigations to answer several important questions: are the cCFU-Fs solely derived from the epicardium, or does the endocardium contribute a fraction of these cells? How different are these cells from other resident stem cells identified in the adult heart [11]? How does aging affect frequency and potency of the cCFU-Fs? Do the endogenous cCFU-Fs differentiate into functional cardiomyocytes *in vivo*? If separated from the non-cardiomyocyte cCFU-F derivatives (perhaps via an α-MHC driven tag), will the *in vitro* differentiated cardiomyocytes become electromechanically active? Could the robust proliferative capacity of cCFU-Fs make them a good candidate for *in vitro* or *in situ* direct reprogramming to the cardiomyocyte fate using transcription factors [12] or microRNAs? Would priming these cells with Tβ4 enhance their cardiogenic potential? And most importantly, to what extent are these findings from mice applicable to a human setting? Answering these questions may bring us a few steps closer to making cardiac regenerative therapies a reality.

Abbreviations
BM, bone marrow; cCFU-F, cardiac colony-forming-unit fibroblast; EPPDC, epicardium-derived cell; Tβ4, thymosin β4.

Competing interests
The author declares that they have no competing interests.

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