Editorial

Stem Cell INVESTIGATION

Expansion of cardiac progenitors from reprogrammed fibroblasts as potential novel cardiovascular therapy

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The human heart has an unremitting and laborious job, to continuously provide all the organs in the body with oxygen and nutrients. An insult occurring to the muscular organ in the form of an ischemic injury, such as myocardial infarction (MI) reduces heart function by causing irreversible damage. Severe injuries to the heart can lead to heart failure (HF) and death. Unfortunately, the human heart has very little ability to repair itself upon injury, predominantly due to the inherent quiescent state of the adult cardiomyocytes (CMs), the major "power house" cell type of the heart. Currently, heart transplants remain one of the most successful therapeutic options for patients in end stage HF. However, even if the extensive recipient list could be met with matched available donors, complications arise in the form of graft dysfunction, immune rejection and infection. Therefore there is a pressing need to develop novel cardiac therapies.

Research scientists and physicians alike are exploring new and novel methodologies to repair and sustain function in the damaged adult heart. Current experimental strategies to promote tissue repair/regeneration in the heart involve the administration of cells, or alternatively a cell-free approach (e.g., DNA vectors, modified mRNA and chemical molecules). For an up-to-date review on cell-free approaches to cardiac regeneration, we refer the reader to the following review (1).

Several major caveats are limiting the use of a cell-based cardiac regeneration approach, including a lineage specific source of cells and sizeable cell numbers. A recent finding published in *Cell Stem Cell* by Ding and colleagues (2) may have unraveled a new technique to capture and expand cardiac progenitors, a novel cell source, from fibroblasts. Excitingly, this could provide a renewable source of cardiac specific cells for regenerative therapeutics.

What defines the cardiac progenitor cell (CPCs)?

During developmental cardiogenesis in mammals, the expanding mesoderm expresses multipotent cells which transition into cardiac precursor cell types as they begin to build the heart. Here, two myocardial lineages appear in the early embryonic stages, diverging from a common progenitor, giving rise to the first heart field (FHF) and second heart field (SHF) (3). The FHF consists of CPCs that form the left ventricle and parts of the atria, whereas the SHF contributes proliferating cardiac progenitors that constitute the right ventricle and outflow tract (4). During the cardiac expansion process, the FHF and SHF cells express the genes Nkx2.5 and Isl1, respectively, which encode homeobox transcription factors denoting some of the earliest CPCs (5,6). CPCs have a high potential for cardiovascular therapies due to their ability to generate all three major cell types of the heart—CMs, endothelial cells (ECs) and smooth muscle cells (SMCs).

Previously, many efforts have been put into replacing the damaged areas of the myocardium with the mature CM, derived from pluripotent stem cells and/or direct reprogramming strategies (7-9). Studies have shown functional improvement to the heart upon the delivery of CMs, albeit with varying results. Some believe that the delivered CMs mediated only paracrine effects but did not engraft into the host heart permanently, given their short-term improvement (8-12). On the other hand, scientists have also speculated that the CPC rather than the mature

CM may be the optimal choice for cardiac treatment, in terms of increasing engraftment efficiencies due to its proliferative potential and ability to generate multiple cardiac derivatives. However, it has been a difficult task to capture and expand such a progenitor population, specifically maintaining their self-renewal and multi-lineage differentiation capacities to clinically sizeable levels for regenerative purposes. Recently, several groups have shown expandable CPC populations from differentiating human pluripotent stem cells using Wnt, BMP, and Activin/Nodal inhibition (13), or via the overexpression of a *c-myc* transgene combined with signaling modifiers including IGF1, Hedgehog activators, TGFβ/Activin/BMP inhibitors, and bFGF (14), but a detailed and established method for the generation of an expandable CPC is still highly controversial.

Here, Zhang *et al.* report the novel cellular reprogramming strategy for induction and expansion of CPCs from mouse fibroblasts with combination of transcriptional factors and small molecules (2). First, they induced transient overexpression of four Yamanaka factors (Oct4, Sox2, Klf4, and *c-myc*; OSKM) (15,16) into genetically modified mouse fibroblasts, co-treated with a JAK inhibitor (JI1). Following 6 days of initial induction with OSKM and 2 days of cardiac specification with JI1 and a canonical Wnt activator (CHIR99021), they discovered, a 2-week treatment with a chemically defined media containing BMP4, Activin A, CHIR99021 and SU5402 (a small molecular inhibitor of FGF, VEGF and PDGF signaling), which they referred to as BACS, induced a Pdgfr-α^+/Flk-1^+/Isl1^+/Nkx2.5^+ cell population. These generated cells were tripotent and could differentiate into CMs, ECs and SMCs under each defined condition, thereby referred to as induced expandable CPCs (ieCPCs). Most importantly among their findings, BACS media maintained the ieCPCs for over 18 passages, allowing stable propagations of the ieCPCs with no visual morphological signs of unsolicited differentiation. The BACS treatment was also able to amplify the ieCPC population more than 10^{10}-fold, to desirable cell numbers. However, the article lacks some information regarding detailed machinery analysis including the selection process when choosing these four factors among several signaling modifiers. Furthermore, we are uncertain as to how each of these four factors contributes to CPC commitment and self-renewal, only that removing any one of the four factors results in a significant reduction in the Pdgfr-α^+/Flk-1^+ population.

On a further note was the use of a JAK inhibitor (Jl1), in early stages of reprogramming and mesodermal differentiation to acquire the ieCPCs. In the same issue of *Cell Stem Cell*, another report from Lalit *et al.* demonstrated direct reprogramming of adult mouse fibroblasts into a Nkx2.5^+/CXCR4^+ CPC population using 5 cardiac genes together with a Wnt activator (BIO) and a JAK/STAT activator (LIF) (17), which is somewhat opposed to the report of Zhang *et al.* More details with regard to the molecular and cellular signatures explaining the similarities and discrepancies in these papers should be addressed with more developed genomic and epigenomic studies, which could help justify the BACS treatment to engender the purified CPC population more reasonably.

A novel cell source for *in vivo* cardiac regeneration

An expandable, multipotent progenitor cell-type that is pre-committed to the cardiac lineages has great potential as a therapy. From this viewpoint, the ieCPCs produced by Zhang *et al.* also marks an interesting candidate cell type for *in vivo* cardiovascular therapies. Indeed, the ieCPCs transplanted into a mouse MI model differentiated into CMs, ECs and SMCs when assessed 2 weeks following intramyocardial delivery, and improved cardiac function after MI. More intriguingly the group identified the presence of blood vessels in the grafted region, possibly insuring long-term engraftment survival of the transplanted ieCPCs within the host myocardium, which was not addressed in the paper. It could also be of importance to highlight the machinery mechanism by which the ieCPCs are mobilized and integrated into the host myocardium and if the benefits seen are paracrine mediated and/or a result of direct cardiac regeneration by and engraftment of the ieCPCs donor cells into the host hearts.

Furthermore, although the team compared the effects of intramyocardially delivered ieCPCs with mouse fibroblasts in a mouse MI model, for the viewpoint to determine a more favorable cell source for cardiovascular regeneration therapies, a direct comparison between the ieCPC and mature CM could potentially help brand the therapeutic values of the ieCPC. A study utilizing this direct comparison was previously reported from the Murray lab. In their report (18), the administration of human embryonic stem cell-derived Pdgfr-α^+/KDR$^+$ cardiovascular progenitor cells (hESC-CVPs) and mature cardiomyocytes (hESC-CMs) both improved cardiac function one month following reperfusion injury in a nude rat MI model more efficiently

than human bone marrow mononuclear cells. Interestingly, hESC-CVPs did not form larger grafts or more significant numbers of human vessels in the infarcted heart than hESC-CMs. Thus, it is extremely intriguing to evaluate whether this would also be the case for the ieCPCs generated by Zhang *et al.*

Moving forward: how far are we from a successful cell based technology to regenerate the diseased heart?

In the last decade stem cell therapeutics have begun to rapidly evolve, yet many technical issues ensue including the viability of the transplanted cells that may lead to irrepressible tumor formation, coupling of exogenous cell types to the host myocardium and overcoming immune rejection (19). Zhang *et al.* show that morphologically, the delivery of the ieCPCs can reduce major architectural remodeling, depicted by decreased scar sizes three months post injury and implantation. Furthermore, the group was able to report enhanced cardiac function in ieCPC treated animals following the onset of MI. Additional functional analysis with MRI would have been useful to produce images of cardiovascular structures with limited artifacts, including the size of the grafted region.

In the study by Zhang *et al.*, the ieCPCs were delivered immediately with the onset of the coronary artery ligation during the acute phase of MI. Many papers often focus on cell-based therapies in the acute MI phase, however there is a major clinical need for patients suffering from chronic ischemic HF. Perhaps such an investigation outweighs the scope of this paper, however it would be of extreme interest to study the behavior of the ieCPC in a more rigid environment, and if these cells are also effective in the chronic ischemic HF setting by replacing damaged heart tissues into new functional CMs and vascular cells.

Nevertheless the findings by Zhang *et al.* have direct implications that an induced and expandable cardiac progenitor may be clinically relevant for patients with severe ischemic cardiac injury. In order to facilitate successful cell therapies directed at the ischemic heart, it is vital for cell-based grafts to endure long-term survival; the life of the patient if possible. Of equal importance is the risk of teratoma formation of long-term grafts, which can interfere with normal cardiac function. Zhang *et al.* reported the absence of any teratoma formation in the ieCPC-transplanted mice up to 8 weeks after the transplantation. However, it would be mandatory to investigate the long-term efficacy and safety of the ieCPCs *in vivo* before proceeding forward to clinical application, as injections of pluripotent stem cell-derived cell types are known to be at high risk for teratoma formation (20).

In conclusion accumulating evidence is now shedding light on the therapeutic potential of the CPC, as shown in these papers (2,17). The continuation of such studies, including novel methods to generate and maintain large scale numbers of CPCs along with the cellular and molecular machineries with more global transcriptomic and epigenetic analyses will undoubtedly help advance our understanding of heart regeneration and may one day unlock the full potential of cell-based therapies in cardiac regenerative medicine.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Comment on: Zhang Y, Cao N, Huang Y, *et al.* Expandable cardiovascular progenitor cells reprogrammed from fibroblasts. Cell Stem Cell 2016;18:368-81.

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