Editorial

Stem Cell INVESTIGATION

Novel direct reprogramming technique for the generation of culture-expandable cardiac progenitor cells from fibroblasts

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Since the tremendous discovery of induce-pluripotent stem cells (iPSCs) in 2006 (1,2), investigators have been trying to induce somatic cell reprogramming into certain lineage cells following the basic method, in which lineage-related critical genes are initially screened out of numerous candidates and several master genes are then selected by transduction of multiple genes with appropriate culture conditions in somatic cells confirming transdifferentiation of the transduced cells. The technique is called as "direct reprogramming" which can avoid generating iPSCs and previous studies have successfully demonstrated lineage reprogramming into a variety of differentiated cell types such as neuronal cells (3), hepatocytes (4), and cardiomyocytes (5). Despite of the achievement of direct lineage reprogramming, investigators next challenged the generation of progenitor cells rather than terminally differentiated cells by direct reprogramming, because progenitor cells are generally proliferative and have advantage of large scale expansion as a tool for regeneration therapy. Indeed, recent reports exhibited that reprogrammed progenitors of neuronal cells (3), hepatocytes (6), and cardiomyocytes (7) were capable of proliferation.

Cardiac progenitor cells (CPCs) have been identified with a variety of markers (*Table 1*) including: (I) transcription factors of Mesp1, Ist1, and Nkx2.5 in embryonic stem cells, (II) cell surface proteins of CXCR4, PDGFR-a, Flk-1/KDR, and SIRPA in pluripotent stem cells, and (III) cell surface proteins of Sca-1 and c-Kit in mammalian somatic stem cells. On the other hand, induced-CPCs including cardiomyocyte-like cells have also been attempted to generate from fibroblasts by direct reprogramming methods (*Table 2*). In the very recent study, Lalit *et al*. (7) reported that a combination of cardiac factors and signaling molecules reprogramed adult mouse fibroblasts into expandable induced cardiac progenitor cells (iCPCs). The iCPCs were multipotent and could differentiate into not only cardiomyocytes but also smooth muscle cells and endothelial cells that also could be sources of myocardium. Moreover, iCPCs generate myocardium when injected into the embryonic and adult post-MI mouse heart.

First, the authors focused on 22 genes including 18 candidate genes that are critical during embryonic cardiovascular development and 4 iPSC factors, and finally selected 5 genes (Gata4, Mesp1, Tbx5, Nkx2.5, and Baf60c) for generating CPCs utilizing an Nkx2.5 cardiac reporter mouse model expressing enhanced yellow fluorescent protein (EYFP). The source of fibroblasts was chosen from adult but not neonatal fibroblasts for reprogramming in terms of clinical applicability. They next worked out optimizing culture conditions to let the generated CPCs to proliferate/expand with activating Wnt and JAK/STAT signaling by chemical compounds. Intriguingly, the proliferating iCPCs exhibited neither protein expression for pluripotency of Oct4 nor cardiovascular lineage differentiation markers of α-actinin, smooth muscle-myosin

Table 1 CPC source and isolation with markers

Species/origin	Isolation methods	Markers	Differentiation	References
Human				
ESC/PSC	–	SIRPA	CM	(8)
Myocardium	ED/TE	c-Kit	CM, SMC, EC	(9)
Mouse				
Embryonic heart	ED	Nkx2.5	CM	(10)
ESC	–	Isl1, Nkx2.5, Flk1	CM, SMC, EC	(11)
ESC	–	Mesp-1	CM, SMC, EC	(12)
Embryonic heart	ED	c-Kit	CM	(13)
Adult heart	ED	c-Kit	CM, EC	(14)
Adult heart	ED/TE	c-Kit	CM, SMC, EC	(9)
Adult heart	ED + MACS	Sca-1	CM	(15)
Adult heart	ED + CFC	Sca-1	CM, EC	(16)
Adult heart with MI	ED + FACS	Sca-1$^+$/CD45$^-$	CM, SMC, EC	(17)
Adult heart	ED + FACS + LC	Sca-1$^+$/Lin$^-$	CM, SMC, EC	(18,19)

CPC, cardiac progenitor cell; ESC, embryonic stem cell; PSC, pluripotent stem cell; SIRPA, signal-regulatory protein alpha; CM, cardiomyocyte; ED, enzymatic digestion; TE, tissue explant; SMC, smooth muscle cell; EC, endothelial cell; MACS, magnetic-activated cell sorting; CFC, colony forming culture; MI, myocardial infarction; FACS, fluorescence-activated cell sorting; LC, long culture.

Table 2 Direct reprogramming of fibroblast into CM or CPC

Species/origin (fibroblast)	Defined factors for reprogramming	Efficiency of initial reprogramming (%)	Differentiation	References
Human				
H9F	Gata4, Mef2c, Tbx5, Esrrg, Mesp1, Zefpm2, Myocard	7–18	CM	(20)
AC, AD	Gata4, Mef2c, Tbx5, Mesp1, Myocard	5–40	CM	(21)
ND, AD	Gata4, Hand2, Mef2c, Tbx5, miR-1, miR-133	5–20	CM	(22)
Mouse				
AC, AD	Gata4, Mef2c, Tbx5	0.01–0.1	CM	(5)
AC, AD	Gata4, Hand2, Mef2c, Tbx5	15–55	CM, SMC, EC	(23)
AC, AD, AL	Mesp1, Tbx5, Gata4, Nkx2.5, Baf60c	0.008–0.015	CM, SMC, EC	(7)

CM, cardiomyocyte; CPC, cardiac progenitor cell; H9F, transgenic H9 embryonic stem cell-derived fibroblast; AC, adult cardiac fibroblast; AD, adult dermal fibroblast; AL, adult lung fibroblast; ND, neonatal dermal fibroblast; SMC, smooth muscle cell; EC, endothelial cell.

heavy chain, and CD31 even after extensive passaging. It was suggested that the genetically engineered iCPCs under specific culture conditions could be expanded keeping their characteristics as progenitor cells. iCPCs were successfully induced from not only cardiac fibroblasts but also adult lung and adult tail tip (dermal) fibroblasts.

For iCPC differentiation into cardiovascular lineages, iCPCs exited progenitor state losing Nkx2.5 gene expression and differentiated into CM marker-positive cells including MLC-2v-/MLC-2a-positive ventricle-/atrium-like cells (80–90%), SMC marker-positive cells (5–10%), and EC marker-positive cells (1–5%) over 20 days after plating with

cardiac differentiation medium in culture. Although the iCPC-derived CMs were not spontaneously contracting, co-culture with ES-derived CMs allowed them contract expressing calcium transients. These findings indicate that the iCPCs spontaneously differentiated into mainly cardiomyocytes rather than vascular cells under cardiac differentiation condition, however, culture under vascular differentiation conditions may increase the differentiation frequency into SMCs and ECs in iCPCs. When the iCPCs were transplanted in the cardiac crescent of mouse embryos, iCPC-derived cells integrated with host cells within the heart tube and iCPC-derived CMs were observed in developing both atria and ventricles as well as outflow track, demonstrating no spatial preference within the heart tube. In contrast, iCPC-derived CD31 positive ECs or SMCs failed to detect *in vivo* even though endothelial and smooth muscle differentiation from iCPCs could be observed *in vitro*. This discrepancy might be due to the complexity of cardiac developmental regulations in embryos. Finally, the iCPCs were transplanted in adult mouse heart after myocardial infarction (MI) to examine if the transplanted iCPCs differentiate into cardiovascular cells in ischemic myocardium. One and half million of iCPCs were injected to ischemic myocardium (ischemic border zone) 2 days following MI induction. Some of the injected iCPCs could be detected as cardiovascular cell marker-positive cells in histological sections of ischemic myocardium 28 days after cell injection exhibiting significantly increased survival rate compared with the control. Although the remained iCPCs are thought to be differentiated into cardiovascular cells, thinking about $1.0–1.5\times10^6$ of iCPC cardiac injection, the remained cell number would not be enough to contribute to improve cardiac function. The favorable effect of the large number of cardiac iCPC injection appears to be due to the indirect/paracrine effect, i.e., secreted growth factor/cytokine/anti-apoptosis factor, rather than direct contribution to tissue regeneration. Also, if the iCPCs have a homing capacity to ischemic myocardium, the intravenous drip infusion or direct infusion via coronary arteries of iCPCs but not cardiac local injection with open chest surgery would be less invasive and easy/useful way of cell transplantation as a treatment.

In conclusion, the present study demonstrated that CPCs were successfully induced from murine fibroblasts derived from 3 different organ sources by direct reprogramming method with new 5 gene combinations and JAK/STAT signaling activation. In terms of clinical application for cardiovascular diseases, the iCPCs have advantages because of its expansion capacity in culture compared with the previously reported differentiated reprogrammed cardiomyocytes or cardiomyocyte-like cells. The next issue to be tested would be the generation of directly reprogrammed iCPCs from human fibroblasts or other cell types, i.e., lymphocyte which is easy to harvest from peripheral blood avoiding abnormal karyotype after genetic modifications. Ideally speaking, higher frequency of the transplanted iCPC recruitment/differentiation into cardiovascular lineages in postnatal ischemic myocardium will be required for practical cardiovascular regeneration therapy. Nevertheless, this study gave rise to a great progress in the research filed of direct reprogramming with somatic cells for the generation of cardiovascular cells.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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