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Expandable progenitors from induced pluripotent stem cells

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We read with interest the Review by Wu *et al.* (Induced pluripotent stem cells: at the heart of cardiovascular precision medicine. *Nat. Rev. Cardiol.* **13**, 333–349; 2016)[1], which provided an insight into precision medicine aimed at successful drug screening and cardiovascular disease modelling. Regarding the differentiation process, we raise two limitations: heterogeneity, and the time-consuming and costly process. Variations exist not only among established clones of human induced pluripotent stem cells (hiPSCs)[2], but also among hiPSC-derived cardiomyocytes (hiPSC-CMs)[3] as final products, suggesting large lot-to-lot variations during differentiation. Given the long duration of the differentiation process, the cytokines required during this process are costly.

Here, we propose that expandable progenitors could be meaningful for resolving these issues (TABLES 1,2). When expandable progenitors are established, these cell lines will be self-renewable and suitable for cryopreservation. Furthermore, differentiation from these cell lines (not from iPSCs) will be possible, indicating that time and cost can be reduced, and less heterogeneous products can be obtained.

Several studies have demonstrated that expandable cardiac progenitor cells (CPCs) have been successfully captured (TABLE 1) without the procedure of immortalization. Lalit *et al.* have shown that five cardiac factors (mesoderm posterior protein 1, T-box transcription factor TBX5, transcription factor GATA-4, homeobox protein Nkx-2.5, and BRG1-associated factor 60C) transdifferentiate mouse fibroblasts into induced CPCs (iCPCs), which can be expanded $>10^{15}$-fold by BIO (6-bromoindirubin-3′-oxime, a canonical Wnt activator) and leukaemia inhibitory factor[4]. iCPCs can differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells. Zhang *et al.* demonstrated that short-term expression of Yamanaka factors with tyrosine-protein kinase JAK inhibitor JI1 and BACS (bone morphogenetic protein 4, activin, CHIR99021, and SU5402) transdifferentiate mouse fibroblasts into induced expandable CPCs (ieCPCs), which can be expanded $>10^{10}$-fold by BACS, and can differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells[5]. A study by Cao *et al.* showed that homogeneous cardiovascular progenitor cells (CVPCs) can be obtained from human pluripotent stem cells (hPSCs) with combined cytokines, and they can self-renew and expand $>10^{7}$-fold[6].

Another potential strategy for obtaining expandable progenitors involves transducing various genes (for example, *MYC*) for immortalization (TABLE 2). Birket *et al.* applied a doxycycline (Dox)-inducible *MYC* expression system on hiPSCs, which enabled robust expansion (>40 population doublings) of CPCs[7]. To induce differentiation, Dox was removed to turn off *MYC* transgene expression. Similar strategies have been reported for production of platelets[8,9] or erythrocytes[10]. Eto and colleagues established immortalized megakaryocyte progenitor cell lines from hPSC-derived haematopoietic progenitors through overexpression of *MYC*, *BMI1*, and *BCL2L1* (REF. 8). Collectively, without immortalization, more effective methods of progenitors expansion will be needed. Alternatively, we can apply the Dox-inducible system using transgenes for immortalization.

Table 1 | **Expandable progenitors without immortalization**

Expandable progenitors	Expansion	Starting cells	Final products
Induced cardiac progenitor cells[4]	$>10^{15}$ fold	Mouse fibroblasts	• Cardiomyocytes • Smooth muscle cells • Endothelial cells
Induced expandable cardiac progenitor cells[5]	$>10^{10}$ fold	Mouse fibroblasts or mouse embryonic stem cells	• Cardiomyocytes • Smooth muscle cells • Endothelial cells
Expandable cardiovascular progenitor cells[6]	$>10^{7}$ fold	Human pluripotent stem cells	• Cardiomyocytes • Smooth muscle cells • Endothelial cells

Table 2 | **Expandable progenitors with immortalization**

Expandable progenitors	Transduction for immortalization	Starting cells	Final products
Expandable cardiac progenitor cells[7]	*MYC* (doxycycline-inducible)	hPSCs	• Cardiomyocytes • Smooth muscle cells • Endothelial cells
Immortalized megakaryocyte progenitor cell lines[8]	*MYC*, *BMI1*, *BCL2L1* (doxycycline-inducible)	hPSCs	Platelets
Immortalized erythrocyte progenitor cells[9]	*MYC*, *BCL2L1* (doxycycline-inducible)	hPSCs	Erythrocytes
hiPSC-derived erythroid progenitor cells[10]	HPV16-E6/E7 (doxycycline-inducible)	hiPSCs	Erythrocytes

hPSC, human pluripotent stem cell; hiPSC, human induced pluripotent stem cell; HPV16, human papilloma virus 16.

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1. Chen, I. Y., Matsa, E. & Wu, J. C. Induced pluripotent stem cells: at the heart of cardiovascular precision medicine. *Nat. Rev. Cardiol.* **13**, 333–349 (2016).
2. Masuda, S. & Hanazono, Y. Induced pluripotent stem cells in long-QT syndrome. *N. Engl. J. Med.* **364**, 181–182 (2011).
3. Miyagawa, S. *et al.* Building a new treatment for heart failure-transplantation of induced pluripotent stem cell-derived cells into the heart. *Curr. Gene Ther.* **16**, 5–13 (2016).
4. Lalit, P. A. *et al.* Lineage reprogramming of fibroblasts into proliferative induced cardiac progenitor cells by defined factors. *Cell Stem Cell* **18**, 354–367 (2016).
5. Zhang, Y. *et al.* Expandable cardiovascular progenitor cells reprogrammed from fibroblasts. *Cell Stem Cell* **18**, 368–381 (2016).
6. Cao, N. *et al.* Highly efficient induction and long-term maintenance of multipotent cardiovascular progenitors from human pluripotent stem cells under defined conditions. *Cell Res.* **23**, 1119–1132 (2013).
7. Birket, M. J. *et al.* Expansion and patterning of cardiovascular progenitors derived from human pluripotent stem cells. *Nat. Biotechnol.* **33**, 970–979 (2015).

8. Nakamura, S. *et al.* Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. *Cell Stem Cell* **14**, 535–548 (2014).
9. Hirose, S. *et al.* Immortalization of erythroblasts by *c-MYC* and *BCL-XL* enables large-scale erythrocyte production from human pluripotent stem cells. *Stem Cell Reports* **1**, 499–508 (2013).
10. Kurita, R. *et al.* Establishment of immortalized human erythroid progenitor cell lines able to produce enucleated red blood cells. *PLoS ONE* **8**, e59890 (2013).

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Competing interests statement
The authors declare no competing interests.