Insights in Stem Cells

Extended Abstract
Vol. 6, Iss. 1
2020

Stem Cell Research 2018-Foxd1-Dependent and Independent Pathways for Reprogramming from Fibroblasts to Induced Pluripotent Stem Cells or Cardiomyocytes- Tasuku Tsukamoto- Ritsumeikan University

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Introduction

Induced Pluripotent Stem Cells (iPSCs) can differentiate into any cell type. Cardiomyogenesis from iPSCs is useful for clinical application in myocardial regeneration. However, the efficiency and duration of producing iPSCs and iPSC-derived cardiomyocytes must be improved. We previously demonstrated that a surface marker profile of Sca1-CD34- or Foxd1+ during the reprogramming process is a predictor of successful iPSC formation. Here, we examine the correlation of feasibility as iPSC predictors between Sca1-CD34- and Foxd1+ cell populations, and their possibility as predictors for cardiomyocyte transdifferentiation. The fate-tracing analysis revealed that most iPSC colonies were formed from GFP-positive cells in which Foxd1 was transactivated in the middle-to-late phase of the reprogramming process. In addition, GFP expression was observed mainly in the Sca1-CD34- cell population. Thus, Foxd1+ could be an indicator of successful reprogramming to iPSCs mainly derived from Sca1-CD34- cells. As for cardiac transdifferentiation, reprogramming cells were sorted based on the expression pattern of Sca1 and CD34, resulting in a higher incidence of beating cell aggregates derived from the Sca1+CD34+ population, which expresses less Foxd1 promoter-driven GFP and contained very few undifferentiated iPSCs. Moreover, the cardiomyocyte marker α-actinin only partially co-localized with GFP expression in the aggregates derived from Sca1+CD34+ or Sca1-CD34- cells. Therefore, Sca1+CD34+ could be a better cell source for Foxd1-independent cardiomyocyte creation despite the failed reprogramming cell population. To establish innovative treatments such as regeneration therapy or personalized medicine,Induced Pluripotent Stem Cells (iPSCs) have extensively been studied throughout the last decade. Somatic cells are reprogrammed to iPSCs by introducing four defined transcription factors (4F: Oct4, Sox2, Klf4, and c-Myc). Reprogrammed iPSCs exhibit unlimited self-renewal and pluripotency in the undi erentiated state like Embryonic Stem Cells (ESCs). Despite considerable research, the e ciency of iPSC induction remains low and there are potential risks of tumorigenicity and immaturity that limit clinical applications. To overcome these barriers, the underlying mechanisms of iPSC induction must be addressed. Some cell surface markers have been studied in the hope of obtaining iPSC progenitors. Stage-Specific Embryonic Antigen-1 (SSEA1) was reported as a positive marker for iPSC progenitors in the late stages of reprogramming. In addition, we found that negative expression of the cell surface markers Stem Cell Antigen-1 (Sca1) and Cluster of 'i erentiation 34 (CD34) represents an excellent early predictor of iPSC reprogramming. Sca1- CD34- reprogramming cells contain abundant iPSC progenitors in the early phase [5]. Previous work also identified the forkhead box transcription factors, Foxd1, and Foxo1 as reprogramming mediators and indicators, based on a comprehensive analysis of the overrepresented transcription factor binding sites in the promoter sequence of hundreds of genes upregulated aier 4F introduction. While Foxo1 was shown to be essential for maintaining pluripotency in ESCs, Foxd1 is known to regulate kidney and retina development. Interestingly, Foxd1 deficiency resulted in decreased iPSC production, with Foxo1 knockdown further reducing it. Since the expression patterns of SSEA1, Sca1/CD34, and Foxd1/Foxo1 have been independently investigated as iPSC predictors for successful reprogramming, it remains unknown whether iPSC progenitors defined by these indicators possess similar or di erent characteristics. iPSCs are expected

This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA

to provide cell sources for regenerative medicine. Cardiomyocytes derived from iPSCs have been targeted for clinical applications in the treatment of end-stage heart failure, but the eciency and duration of iPSC-derived cardiomyocyte production must be improved. iPSC-derived cardiomyocytes are produced through multiple steps, including the collection of somatic cells from the patient, acquiring pluripotency, and diferentiation to cardiomyocytes. He entire reprogramming process can take more than a couple of months. One study successfully shortened the process of cardiomyogenesis using the 4F- or 3F- (4F without c-Myc) transducing reprogramming strategy with some modifications. He induction of 4F without leukemia inhibitory factor (LIF), which is used to maintain pluripotency, partially reprogramed somatic cells and converted the cell fate to other various cell types, including beating cardiomyocyte-like cells. However, it would be of importance to understand how the modified reprogramming process can convert somatic cells to cardiomyocytes. Here, we investigate whether there are shared characteristics between Sca1-CD34- and Foxd1+ reprogramming cells as iPSC predictors and whether other cell populations (e.g., Sca1+CD34+) may preferentially transdiferentiate into cardiomyocyte-like cells . A number of laboratories have been working on the production of cardiomyocytes from other cell types. One prestigious method for direct induction (transdiferentiation) from fibroblasts to cardiomyocytes using gene introduction of the cardiac-specific transcription factors Gata4, Mef2c, and Tbx5 with or without Hand2 has been developed and further improvements have also been reported so far . Although this direct reprogramming enables us to generate induced-cardiomyocytes more rapidly and safely due to no use of undiferentiated cells, the reprogramming eciency by this approach is still limited as induced cardiomyocytes possess the low proliferative capacity. In this study, we focused on more ecient strategies to produce cardiomyocyte from 4F-introduced cells. Various cell culture conditions or protocols for ecient myocardial cell diferentiation have been reported. Cardiogenic stimuli-containing media, such as those containing a low concentration of FBS, N2/B27 supplementation, chemical compounds, bFGF, and EGF, significantly improved the eciency of cardiac diferentiation by modulating TGFβ, bone morphogenetic protein, Wnt or Notch signaling. According to the previous study, 4F-induced cardiac reprogramming is mediated by cardiac progenitor-like cells expressing Mesp1 or Gata4, which are early mesodermal marker genes. Although further studies are needed to explain how cardiomyocytes are induced from Sca1+CD34+ cells, considering the common pathway of cardiac diferentiation and transdiferentiation, the combination of these strategies with Sca1+CD34+ -cell sorting could significantly improve ecient cardiomyogenesis. In conclusion, we revealed that Foxd1-expressed reprogramming cells share the characteristics of Sca1-CD34- iPSC progenitors, suggesting that Foxd1 could be a feasible predictor for successful reprogramming to iPSCs. Moreover, Sca1+CD34+ population, most of which is Foxd1-negative, likely fail to become iPSCs, but exhibits a higher incidence of cardiomyogenesis and less contamination with undiferentiated cells. For terminal cardiac diferentiation, some modifications in the protocol will enable the field to improve the quality and quantity of cardiomyocyte production from 4F-introduced reprogramming cells. Hese improvements will contribute to cardiac regeneration therapy. Our working hypothesis based on the results of the present study.

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This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA

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This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA