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Commentary

The path and surface marker of SC-β cells

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Islet transplantation has been demonstrated to be able to cure diabetes caused by the loss of functional pancreatic β cells; however, it is severely limited by the shortage of cadaveric islet donor [1]. Human pluripotent stem cell, including embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC), is the most promising source of replaceable functional cells because of the capacity of self-renewal and multiple lineage differentiation, thus β cell differentiation from stem cell attracts much efforts from both academic and industrial communities [2].

Previously in 2014, Rezania and colleagues developed a remarkable multi-step protocol utilizing air-liquid interface system to induce β cell differentiation from human ESCs and iPSCs [3]. The final differentiation product exhibited the capacity of glucose-stimulated insulin secretion, and could reverse diabetes in mouse model within 40 days [3]. Only months later, a group from Harvard University in the US also developed an efficient and scalable protocol using suspension culture based on a combination of 11 compounds through screening more than 150 combinations of 70 compounds [4]. The generated SC-β cells (stem-cell-derived β cells) are functional, not only exhibiting the ability to express key markers of adult pancreatic β cells, but also having mature secretory granules and secreting insulin in response to glucose stimuli in the same way as primary islets. More importantly, the serum glucose level of diabetic immune-deficient mice transplanted with SC-β cells could reduce to normal. Albeit those achievements, however, the SC-β cells are only composed of 30%–40% of the in vitro differentiation product and the identity of other cells remains unclear [3,4], which brings extra unexpected risks and thus hinders the clinical application. Moreover, under the induction of the signaling pathways learned from in vivo development plus large-scale screening of chemicals, whether the generation of β cells indeed follows the developmental path or chooses another alternative path is an interesting question awaiting further dissection.

Recently Melton group at Harvard University reported their revolutionary progress on β cell differentiation (Fig. 1). In the recent article, Veres et al. combined single-cell RNA sequencing technology and computational analysis to look into the cell composition of specific stages and the path of SC-β cell generation [5]. They used a modified pancreatic β cell differentiation protocol which was divided into six stages over a 25-day period, and then sequenced 40,444 cells from stage 3 to stage 6 with apparent heterogeneity to identify major cell types. According to the analysis, cells at different differentiation stages indeed represented various developmental states, from pancreatic progenitor cells to pancreatic endocrine cells to progressively mature α, β cells, along with some non-pancreatic endocrine cells. Noteworthy, those populations were consistently observed in different pluripotent stem cell lines, suggesting the robustness of the protocol and contained differentiation signals.

Very interestingly, they identified a population of endocrine cells in stage 5 and stage 6 called SC-EC cells (stem-cell-derived enterochromaffin cells) which highly expressed enterochromaffin marker genes (LMX1A, ADRA2A, FEV) and serotonin synthesis genes (TPH1, DDC and SLC18A1). More importantly, SC-EC cell-containing aggregates released serotonin upon depolarization using KCl rather than glucose stimulation. Although enterochromaffin cells do not exist in primary human pancreatic islets but in gut, SC-EC cells appear repeatedly even in different protocols they tested. In addition, using the single-cell RNA sequencing technology and computational analysis, they drew a high-resolution map to understand the developmental trajectories of different cell types. For example, poly-hormonal cells that expressed both insulin and glucagon were the early α cell progenitors which further differentiated towards mono-hormonal mature α cells; while mono-hormonal β cells experienced a later transient NGN3 activation followed by endocrine induction [5]. Those valuable information would guide the following studies to make a more efficient protocol to obtain mature bona-fide β cells.

The eventual purpose of the in vitro differentiation is to robustly produce mature β cells which should be at least functionally comparable with primary adult islets. To evaluate the maturity of generated SC-β cells, Veres *et al.* carried out in vitro glucose–stimulated insulin secretion assay for stage 6 cells, and observed that stimulation indices of SC-β cells were similar to human adult islets. Most interestingly, the SC-β cells could be stably cultured in basal medium without any additional compounds and maintained the expression levels of key genes representing β cell maturation for up to 5 weeks. This points out an important issue that continuous culture of SC-β cells in vitro without functional loss would be possible, which is distinct from primary islet culture that would spontaneously dedifferentiate and gradually lose β cell identity. The ability of long-term maintenance of differentiated SC-β cells would greatly advance the SC-β cell manufacture to clinical

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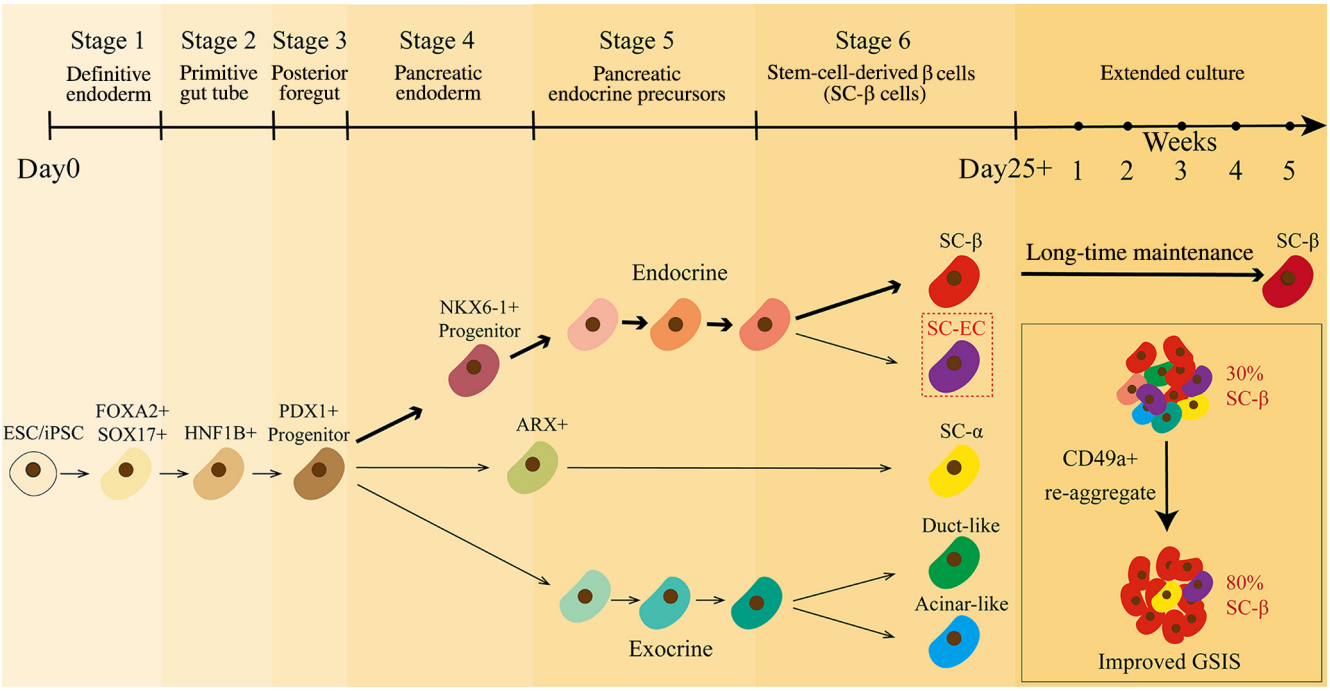

Fig. 1. Single-cell RNA-sequencing to dissect the path of SC-β cells. Single-cell RNA-sequencing has identified major cells population derived from human pluripotent stem cells at different stages, which resemble various developmental states in vivo, from pancreatic progenitor cells to pancreatic endocrine cells to progressively mature α, β cells, along with some non-pancreatic endocrine cells, including newly identified SC-EC cells (stem-cell-derived enterochromaffin cells). Moreover, SC-β cells can maintain cell identity during 5 weeks' extended culture. Re-aggregation of sorted CD49a-positive cell in SC-β stages could enrich SC-β cells (from 30% to up to 80%) and improve glucose-stimulated insulin secretion.

application, although why only SC-β cells but not primary islets can maintain for long time remains to be understood. In addition, the architecture of islets cannot be simply replaced by single SC-β cells, thus engineered islet-like structure or islet organoid containing major cell types would be emergently required [6].

Cell purity is another concern in cell therapy. On one hand, residual undifferentiated pluripotent stem cells may cause tumorigenesis, hindering clinical trial of β cell replacement therapy. Scientists have developed some strategies to selectively eradicate pluripotent stem cells, based on the metabolic or proliferative difference between these two distinct cell types. For example, Im and colleagues reported that BET inhibitor could selectively eliminate undifferentiated pluripotent cells [7]. On the other hand, non-target cells may also cause some unexpected effects to hurdle cell therapy, therefore, discovery of surface markers of target cells or lineages could help to solve this problem by cell sorting technique. In pancreatic differentiation field, CD24 was reported as a marker for differentiated PDX1-positive pancreatic progenitor cells [8]; in another study, CD142 was found to be able to enrich pancreatic endoderm cells and CD200 and CD318 for endocrine cells [9]. In this recent research, Veres et al. focused on the expression pattern of SC-β cells using the single cell RNA-sequencing dataset. Eventually they identified CD49a that could greatly enrich β cells. They sorted and re-aggregated CD49a-positive cells and found that 80% of cells within the clusters were β cells expressing mature markers, and more importantly, exhibited better response to glucose stimulation [5].

There are still some remaining concerns regarding cell differentiation and further clinical application. First, why enterochromaffin cells that do not exist in native pancreas appear in such differentiation process? What signal has contributed to such ectopic patterning? Similar to this issue, although CD49a is showed with great potential to enrich SC-β cells and sorted CD49a-positive cells followed by re-aggregation technique could improve β cell function, CD49a is not specific for β cells in adult islets. Those observations raise a long-standing but non-ignorable question: how faithful should the in vitro differentiation be to in vivo development to reach the best output of generating functional cell types? Second, although the final cell aggregates contain up to 80% insulin-positive SC-β cells, safety risk still cannot be excluded due to the undesired differentiated cell types and residual undifferentiated pluripotent cells. Devices with the capacity to keep the SC-β cells physically isolated but to be able to exchange nutrient and oxygen with in vivo environment would be a right choice, such as polymer-capsules [10].

Conflict of interest

The authors declare that they have no conflict of interest.

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Yinglei Li is a Ph.D. candidate at Wuhan University. She obtained B.S. degree from Zhengzhou University. Her current research aims to generate functional beta cells from human pluripotent stem cells, and use this system to dissect the underlying mechanisms to understand how some specific risk SNPs contribute to diabetes susceptibility.

Wei Jiang is a professor at Medical Research Institute at Wuhan University. His research interest is to apply the basic knowledge from developmental biology and genetics/epigenetics as well as long noncoding RNAs, to cell lineage differentiation aiming to uncover the molecule mechanism underlying endodermal disease such as diabetes. His team is focusing on human pluripotent stem cell, beta cell differentiation and disease modeling.