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EDITORIAL

How do you make new β-cells in humans? An attempt to create human β-cells from α-cells of brain-dead donors

A century has passed since the discovery of insulin, and one of the longstanding desires of medical researchers of diabetes is to establish a cure for diabetes by regenerating the pancreatic β-cells of patients. To achieve this goal, it is true that basic research toward elucidating the mechanism of pancreatic development has progressed substantially over the past 30 years. For example, the key transcription factors involved in pancreatic β-cell differentiation have been elucidated. Furthermore, owing to advancements in induced pluripotent stem cell technology, patient-derived somatic cells can be reprogrammed into stem cells with pluripotency. Therefore, it has become possible to differentiate patient-derived cells with lower immunogenicity *in vitro* into functional cells and transplant them into the patient's body. Under these circumstances, there are mainly two methods for regenerating β-cells. One is a method of driving induced pluripotent stem cells toward functional β-cells *in vitro*, and research in this direction is being carried out around the world. The other method is to convert associated cell types other than β-cells of a diabetes patient into β-cells using some reprogramming factors.

Attempts to reprogram non-β-cells into functional β-cells begun >20 years ago. For example, it was reported that insulin-producing cells can be induced by overexpressing pancreatic duodenal homeobox gene 1 (Pdx1) in αTC1 cells of the α-cell line, and then stimulating them with growth factors[1]. It has also been reported that ectopic expression of the Pdx1-VP16 fusion protein together with NeuroD1 or neurogenin 3 in hepatocytes can induce insulin-positive cells and improve diabetes in streptozotocin-induced diabetic mice[2]. Furthermore, it has been reported that ectopic expression of the transcription factors Pdx1, musculoaponeurotic fibrosarcoma oncogene family A (MafA) and neurogenin 3 in pancreatic acinar cells using an adenovirus vector or transgenes can induce functional β-cells[3,4]. Thus, it has become clear that depending on the cell type, even differentiated cells can be reprogrammed into β-cells using a combination of transcription factors, including Pdx1.

An important issue in recent studies is that when demonstrating the reprogramming of cells, the lineage of the starting cells must be clearly shown. For example, to illustrate reprogramming from pancreatic acinar cells, it is necessary to show that the starting cells are indeed pancreatic acinar cells using specific markers, such as elastase[4]. As mentioned previously, several attempts have been made to reprogram non-β-cells into β-cells, but all of these studies have used mice. Previous studies have not shown whether human β-cells can be reprogrammed for actual clinical applications. Practically, it is necessary to show that mouse α-cells can be reprogrammed into β-cells by carrying out cell lineage tracing experiments, which depend on mouse genetics. In humans, even the promoter of the glucagon gene that regulates α-cell specificity is not clearly defined, so it is still impossible to carry out cell lineage experiments with human cells.

A breakthrough study showing that it is possible to reprogram pure α-cells isolated from human brain death donors into functional β-cells, avoiding such difficulties of lineage tracing, was recently published in the journal, *Nature*. Furuyama *et al.*[5] isolated individual cells from human pancreatic islets of brain death donors, and then carried out cell sorting using antibodies against surface markers specific to α-cells, to obtain α-cells with a purity of ≥99%. Based on the technology to purify human α-cells and the adenovirus vector expression system, they analyzed the combination of transcription factors that need to be expressed to enable reprogramming of the α-cells into β-cells. They found that by simultaneously expressing Pdx1 and MafA in human α-cells, and then planting the cells under the kidney capsule of a diabetes mouse model, the cells can be reprogrammed into functional β-cells that also have glucose-stimulated insulin secretion ability (Figure 1)[5]. Therefore, this study showed that it is possible to treat hyperglycemia with reprogrammed β-cells in mice.

For some time, the team of Grompe *et al.*[6] has been collecting monoclonal antibodies that recognize cell type-specific surface antigens of pancreatic endocrine cells. In collaboration with this team, Furuyama *et al.*[5] developed a method to purify human endocrine cells with high purity. Furthermore, Furuyama's experiments demonstrated the interesting fact that the reprogramming of α-cells into β-cells is significantly promoted by culturing cells in three dimensions rather than in two dimensions. After infecting human donor-derived α-cells with reprogramming factors using an adenoviral vector, they made cell aggregates on a low-adhesion plate and cultured the cells to create pseudo-islets. In these pseudo-islets, α-cell-specific gene expression was downregulated over time, and at the same time, β-cell-specific gene expression was acquired.

In addition, even if cells do not reach a sufficient maturity level *in vitro*, if cells are actually planted under the kidney

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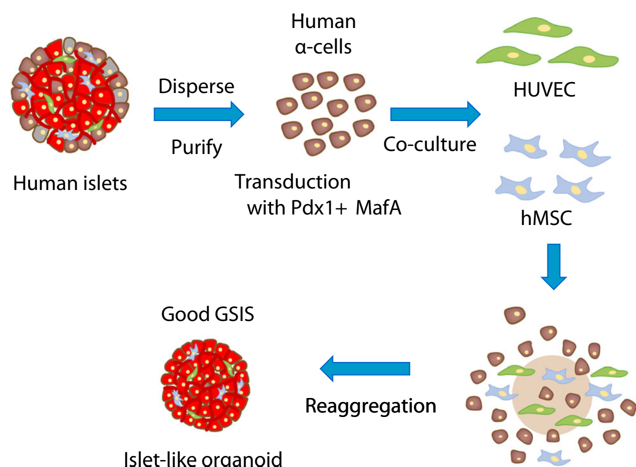

Figure 1 | *Ex vivo* reprogramming of human α-cells toward functional β-cells by adenoviral transduction of key transcription factors. Human islets isolated from human brain-dead donors were dispersed into individual cells. These cells were purified into α-cells with >99% purity by fluorescent activated cell sorting using α-cell-specific cell-surface markers. Purified human α-cells were adenovirally transduced with pancreatic duodenal homeobox gene 1 (Pdx1) and musculoaponeurotic fibrosarcoma oncogene family A (MafA). These cells were then coaggregated with human mesenchymal stem cells (hMSC) and human umbilical vein endothelial cells (HUVEC) to make islet-like organoids. The resulting organoids showed highly efficient glucose-responsive insulin secretion in culture.

capsule of a diabetes model, differentiation and maturation of β-cells can be achieved owing to the cell niche and growth factors present *in vivo*. Producing fully differentiated β-cells in real-time *in vitro* is not necessary. In fact, Furuyama *et al.*[5] found that as much as 68% of the original α-cells were reprogrammed into insulin-positive, glucagon-negative β-cells.

The true nature of type 1 diabetes is an autoimmune response to the patient's own pancreatic β-cells. Therefore, even if the remaining cells can be converted into β-cells, the reprogrammed β-cells will eventually be targeted by the immune system, so there is also the idea that this strategy will not solve the fundamental problem. Interestingly, however, β-cells reprogrammed from α-cells have previously been reported to escape attack from the autoimmune system[7]. Therefore, Furuyama *et al.*[5] also decided to study the immunogenicity of their α-cell-derived β-cells. Cytotoxic T lymphocytes established from patients with type 1 diabetes and α-cell-derived β-cells were cocultured. The resulting reprogrammed cells were attacked by the T lymphocytes targeting proinsulin, but not by clones recognizing pathological β-cells of patients with type 1 diabetes. Therefore, β-cells reprogrammed from non-β-cells might be less immunogenic.

To date, many protocols for inducing the differentiation of β-cells from induced pluripotent stem cells and embryonic stem cells have been devised, but the problem of where these induced cells should be transplanted and the problem of inducing undifferentiated insulin-producing cells that produce multiple hormones, such as glucagon and insulin, have not been solved. Furthermore, as already mentioned, many studies have been carried out using α-cells, which are developmentally close to β-cells, as a suitable target for β-cell induction. However, when β-cells are induced at the expense of the remaining α-cells of diabetes patients, as α-cells have their own roles, such as promoting glucose production during hypoglycemia and controlling amino acid degradation, some adverse effects associated with the loss of α-cells might occur.

According to a recent analysis by Furuyama *et al.*[5], the ectopic expression of Pdx1 and MafA can reprogram not only α-cells, but also pancreatic polypeptide cells into β-cells in humans, and δ-cells are also reprogrammable into β-cells, at least in mice. This means that many pancreatic endocrine cells are equipped with the plasticity to become β-cells in response to Pdx1 and MafA. Hence, when considering transdifferentiation into β-cells, it is not always necessary to target only α-cells, and in the future, identifying endocrine cells that result in fewer adverse effects in a patient when converted into β-cells is also important.

This study by Furuyama *et al.*[5] clarified the plasticity of human pancreatic endocrine cells skillfully without using genetic lineage tracing methods. From the viewpoint of clinical application, reprogramming into pancreatic β-cells might be possible by endoscopically approaching the pancreatic islets from the pancreatic duct and expressing Pdx1 and MafA in the remaining pancreatic α-, PP, and δ-cells. By utilizing the human islet cell purification and culture methods developed in this study, human islet cell research is expected to achieve great advancements. For example, the meaning of the coexistence of various cell types in a pancreatic islet might be clarified. In addition, if the details of the reprogramming process from human non-β-cells toward β-cells can be clarified and mimicked, inducing β-cells from the cells remaining in the patient might become a practical approach in the near future.

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DISCLOSURE

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REFERENCES

1. Watada H, Kajimoto Y, Miyagawa J, *et al*. PDX-1induces insulin and glucokinase gene expressions in alphaTC1 clone 6 cells in the presence of betacellulin. *Diabetes* 1996; 45: 1826–1831.
2. Kaneto H, Nakatani Y, Miyatsuka T, *et al*. PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. *Diabetes* 2005; 54: 1009–1022.
3. Zhou Q, Brown J, Kanarek A, *et al*. *In vivo* reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* 2008; 455: 627–632.
4. Miura M, Miyatsuka T, Katahira T, *et al*. Suppression of STAT3 signaling promotes cellular reprogramming into insulin-producing cells induced by defined transcription factors. *EBioMedicine*. 2018; 36: 358–366.
5. Furuyama K, Chera S, van Gurp L, *et al*. Diabetes relief in mice by glucose-sensing insulin-secreting human α-cells. *Nature* 2019; 567: 43–48.
6. Dorrell C, Schug J, Canaday PS, *et al*. Human islets contain four distinct subtypes of β cells. *Nat Commun* 2016; 7: 11756.
7. Xiao X, Guo P, Shiota C, *et al*. Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. *Cell Stem Cell* 2018; 22: 78–90.

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