Searching for stem cells in the adult pancreas: A futile effort?

Regenerative medicine has been attracting great attention, not only from scientists, but also from the general public. In fact, the Nobel Prize in Physiology or Medicine 2012 was awarded for the discovery that mature cells can be reprogrammed to become pluripotent. With the help of his colleagues, Shinya Yamanaka, one of the 2012 Nobel Prize winners, showed that differentiated adult cells can revert to immature embryonic stem (ES) cell-like cells, which are called induced-pluripotent stem (iPS) cells, by the introduction of defined factors. In principle, iPS cells can differentiate into any type of cells, including pancreatic β-cells, if appropriate culture protocols are applied. Therefore, shouldn’t efforts be made to identify tissue stem cells in the adult pancreas?

It has long been thought that pancreatic β-cells rarely renew once they have terminally differentiated. However, even neuronal cells have been found to regenerate in the adult brain, and an in vitro culture method for neuronal stem cells as ‘neurospheres’ has been established. Besides the brain, adult tissue stem cells have been identified in several other tissues. Therefore, a few investigators have begun to consider that tissue-specific stem cells might also exist in the adult pancreas; thus, many studies have been carried out to identify pancreatic tissue stem cells in order to generate new β-cells. Such studies seem to be promising, because regeneration of pancreatic β-cells could facilitate the development of cell replacement therapies for patients with diabetes. In general, the most straightforward way to generate new β-cells is to isolate tissue stem cells from the pancreas and culture them to induce differentiation into β-cells in vitro. In addition, regeneration of insulin-secreting β-cells is sufficient to cure diabetes, and regeneration of the whole pancreas is not required. Thus, regenerative medicine for diabetes patients should be more feasible than that for patients with other diseases, such as kidney failure, in which functional restoration of complex organs is required.

Isolation of putative pancreatic stem/progenitor cells has been reported from several groups. For examples, progenitor-like cells expressing the neural stem cell marker, nestin, appear after cultivation of human and rat pancreatic islets; spherical cell clusters expressing nestin are formed by floating culture of adult mouse pancreas; and putative progenitor cells with differentiation capacity are isolated from the adult mouse by using fluorescence-activated cell sorting. However, very few following reports regarding these stem/progenitor cells have been published. Therefore, it is not yet clear whether these putative stem/progenitor-like cells have the full potential to differentiate into native pancreatic β-cells and function as stem/progenitors in the pancreas in vivo. Both the existence and the nature of stem/progenitor cells in the adult pancreas are yet to be established.

By using genetic cell lineage tracing in mice, Dor et al. showed that adult pancreatic β-cells are maintained predominantly by self-replication of pre-existing β-cells. They labeled pancreatic β-cells selectively with human alkaline phosphatase by Cre/loxP-based conditional recombination in the adult pancreas, and chased the fate of pre-existing β-cells. From their results, Dor et al. concluded that terminally differentiated β-cells retained proliferative capacity, casting doubt on the role of pancreas-specific tissue stem cells in β-cell replenishment; other studies have supported this notion. Furthermore, studies in which pancreatic acinar or duct cells were specifically labeled by cell lineage tracing have shown that regeneration of β-cells from these exocrine cells can hardly be detected. Thus, it is likely that pancreatic β-cell mass is maintained primarily by self-replication of pre-existing β-cells rather than tissue-specific stem cells or other cells in the adult pancreas (Figure 1).

On the contrary, histological analysis has shown that neogenesis or regeneration of pancreatic β-cells from cells other than pre-existing β-cells occurs in certain conditions. In transgenic mouse models with pancreatic injury, a dramatic proliferation of pancreatic ductal cells, and the appearance of primitive endocrine cells and their subsequent differentiation into mature endocrine cells, including β-cells, has been frequently detected. Unfortunately, these studies are based on snapshot observations of immunohistochemistry, and no direct evidence...
for identifying cell origins of new β-cells has been provided. However, a recent study using genetic cell tracing has shown that unidentified cells located in the ductal lining give rise to Ngn3-expressing cells, which can differentiate into all islet cell types, in partial duct-ligated adult mouse pancreas. Taken together, it is likely that the contribution of non-β-cells to β-cell regeneration is limited, if it occurs at all, under normal physiological conditions in the adult pancreas; however, potential stem/progenitor cells yet to be identified might be activated in the injured pancreas when they are required (Figure 1).

Interestingly, recent studies have suggested the occurrence of interconversion among endocrine cells, especially between α- and β-cells. Under extremely severe conditions leading to almost total β-cell loss, transdifferentiation of α-cells into β-cells has been observed. In addition, many investigators have noticed that increased proportions of α-cells are often found in gene-modified mice with β-cell damage. In general, the reason for this phenomenon is thought to be an increase in the relative number of α-cells as a result of β-cell death rather than an increase in the absolute number of α-cells. Very recently, however, cell lineage tracing experiments showed that the loss of β-cells is a result of dedifferentiation, but not death, of β-cells, at least in mice with specific disruption of FoxO1 in pancreatic β-cells, mice lacking insulin receptors in GLUT4-expressing cells (GIRKO) and db/db mice. Intriguingly, a subset of dedifferentiated β-cells was found to convert into glucagon-producing α-cells. The process of embryonic differentiation of α- and β-cells has been investigated extensively, and it was found that ectopic expression of certain transcription factors can change the fate of differentiation. The pancreas is the organ in which metaplasia, the replacement of one differentiated cell type with another differentiated cell type, is often observed. Tumorigenesis is also frequent in the exocrine pancreas. Together, these findings suggest that differentiated pancreatic endocrine and exocrine cells maintain high plasticity in their differentiation capacity. Indeed, experimental conversion of pancreatic acinar cells into duct or liver cells has also been observed.

Taking advantage of such high plasticity of mature pancreatic cells, attempts have been made to generate insulin-producing β-like cells in vitro. In particular, pancreatic ductal cells have attracted attention as a cell source of new β-cells, as pancreatic endocrine cells are budded from ductal structures during organogenesis. Thus, some investigators have suggested that ductal cells represent facultative progenitors in the adult pancreas. Indeed, in vitro cultivation has been found to induce insulin-producing cells from human and mouse pancreatic ductal cells. In addition, in vitro generation of insulin-producing cells from pancreatic acinar cells of rodents or humans has been reported by several groups. As a large number of pancreatic exocrine cells can be obtained as a byproduct of islet transplantation, the acinar cell is an intriguing source for the generation of transplantable surrogate β-cells. These results have shown that pancreatic exocrine cells (duct and acinar cells) might have the potential to serve as progenitor cells for β-cells.

Newts are capable of regenerating several anatomical structures and organs, including their limbs. However, their adult limbs have no pre-existing immature stem cells. Therefore, how do they regenerate their lost limbs? Limb regeneration in newts occurs in two major steps: dedifferentiation of adult differentiated cells into a stem cell state similar to embryonic cells, and development of these cells into new tissue. In mammals, such dramatic phenotypic changes do not occur in adult cells. We have thus lost the ability to regenerate complex organs. Nevertheless, we cannot exclude the possibility that plasticity in differentiation capacity is still retained in adult mammalian cells. Indeed, the fact that the introduction of just three or four factors is sufficient for reprogramming of differentiated cells into pluripotent stem cells (i.e., iPS cells) might indicate this potential plasticity. Experimental evidence for direct reprogramming, in which one differentiated cell type is directly converted into another differentiated cell type by gene transfer without mediating the pluripotent state, has also been reported in mammalian cells by several investigators. Thus, theoretically, we might be able to generate fully differentiated pancreatic β-cells from other differentiated cells by direct reprogramming, without the use of tissue stem cells in the adult pancreas.

In these situations, is there a significant reason to search for tissue stem cells in the adult pancreas? The answer is ‘yes’. We still do not know how native pancreatic β-cells acquire the sophisticated function of well-regulated insulin secretion during differentiation. Still, we have not developed the procedure to generate fully functional β-cells from ES cells or iPS cells, or adult cells. Understanding the detailed process of differentiation of native β-cells in the adult pancreas is a critical step for the establishment of a procedure to generate new β-cells that can be used in cell replacement therapy for patients with diabetes. For this purpose, it is necessary to identify cells that give rise to functional β-cells in the adult pancreas, irrespective of whether they are immature stem cells or mature differentiated cells (Figure 1).

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REFERENCES
1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663–676.
2. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992; 255: 1707–1710.
3. Minami K, Seino S. Pancreatic acinar-to-beta cell transdifferentiation in vitro. Front Biosci 2007; 13: 5824–5837.
4. Dor Y, Brown J, Martinez OI, et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004; 429: 41–46.

5. Xu X, D’Hoker J, Stangé G, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 2008; 132: 197–207.

6. Thorel F, Népote V, Avril I, et al. Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature* 2010; 464: 1149–1154.

7. Talchai C, Xuan S, Lin HV, et al. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 2012; 150: 1223–1234.