Dedifferentiation for Replication of Human β-Cells

A Division Between Mice and Men?

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Human β-cells, generated in ample quantities, are a prerequisite in order to realize a wider application of β-cell replacement therapy for diabetes. Theoretically, there are several potential sources of new β-cells, including embryonic or adult stem/progenitor cells, transdifferentiation of nonislet tissues such as liver, or the proliferation of fully differentiated β-cells (1). Because it has been demonstrated that all β-cells could theoretically be induced to proliferate (2,3), a significant increase in β-cell number might be achieved by simply stimulating the proliferation of existing β-cells through application of specific growth factors, as has been achieved for cultured rat and mouse β-cells (4). Unfortunately, stimulation of human β-cell proliferation in culture has proven to be much more difficult than for rodent cells. In a recent comparison, the conditions leading to efficient proliferation of rat β-cells were not found to have an effect on human β-cells (5). However, it should be emphasized that this does not preclude the possibility that unidentified culture conditions for human β-cell expansion could exist.

Another potential source of β-cells that has recently received much attention is the fibroblast-like cells that become evident following the gradual loss of endocrine cells in long-term cultures of human islets (6,7). The nature and origin of these fibroblast-like cells emerging from islets have been closely examined because of their proposed capacity to function as endocrine precursors (7–9). These cells are not only able to proliferate and expand, but are also able to retain their ability to produce at least small amounts of insulin following induction of differentiation (7). Some studies have suggested that these rapidly proliferating fibroblastoid cells are derived from mature β-cells by epithelial-mesenchymal transition (EMT) (8). In other studies, these cells were characterized by their expression of nestin (7,10), an intermediate filament protein that has been mainly associated with neuronal precursors. The proliferating nestin-positive islet precursor cells, which have mixed epithelial-mesenchymal phenotypes, may thus represent a phenomenon specific for the culture conditions. Thus, the true precursor capacity of these cells remains uncertain due to the uncertainty of their cellular origin and very limited evidence of endocrine differentiation after expansion.

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DsRed, red fluorescent protein; EMT, epithelial-mesenchymal transition; GFP, green fluorescent protein.

DOI: 10.2337/db08-0364

See accompanying original article, p. 1575.

The hypothesis that mature β-cells are able to undergo EMT followed by proliferation and subsequent redifferentiation was considered practically buried after the appearance of four articles in 2007 that addressed this issue by using lineage tracing of β-cells in islets from transgenic and knockout mice (11–14). All four articles describe experiments where β-cells are labeled using insulin or pancreatic-duodenal homeobox factor-1 promoter–driven expression of the Cre recombinase in combination with various reporter genes that are dependent upon the expression of Cre to generate an irreversible β-cell–specific label. The overall conclusion from these articles was that the proliferating fibroblast-like cells derived from cultured islets were not generated from β-cells, because no β-cell–specific lineage label was expressed in these cells. In one study, a β-cell–specific label was found in cells that did not express insulin or pancreatic-duodenal homeobox factor-1, suggesting that dedifferentiation of β-cells could be a possibility (14). However, proliferation of these cells was negligible and the authors thus concluded that β-cells did not contribute to the expandable pool of cells found in the cultures.

Considering the important differences in the capacity of human and rodent β-cells to proliferate, it might be speculated that differences also exist in their ability to undergo EMT. However, this has been difficult to study because reliable cell lineage studies are only possible with efficient methods for tracing of the cells that change their phenotype. In this issue of *Diabetes*, Russ et al. (15) describe the successful development of lineage-tracing technology for cultured human β-cells. The authors used a dual viral system for tracing the fate of β-cells in vitro. β-cell–specific expression of Cre recombinase was achieved using a lentivirus expressing Cre under the control of the rat insulin II promoter and a reporter lentivirus expressing a reporter cassette consisting of a "floxed " red fluorescent protein (DsRed) gene followed by the green fluorescent protein (GFP) gene. In the absence of Cre, the reporter expresses DsRed, thus labeling non–β-cells red. In contrast, in β-cells expressing both the Cre and the reporter viruses, the DsRed encoding sequence is excised by the Cre recombinase and the reporter generates GFP-labeled β-cells. Because the lentiviral reporter is integrated into the islet cell chromosomal DNA, the reporter will be propagated into all cells originating from the initial infected cells, thus providing an efficient method for lineage tracing.

To test the specificity of the system, the authors used noninsulin as well as insulin-expressing cell lines and found that GFP was expressed only in the insulinoma cells and that non–β-cells expressed only DsRed. When human islet cells were infected with both viruses, both GFP-expressing cells and DsRed-expressing cells were observed, whereas cells infected with only the reporter virus

were red. The frequency of GFP-expressing cells was in accordance with the efficiency of viral infection in these cultures being ~70%. Furthermore, specificity was confirmed by showing expression of C-peptide in 98% of Cre-expressing cells. In long-term cultures of infected cells, it was observed that the frequency of cells expressing both GFP and C-peptide gradually decreased, indicating dedifferentiation of β-cells. However, the surprising finding of this study was that the fraction of GFP-expressing cells was constant for at least 14 passages, representing ~14 cell doublings. Accordingly, direct assessment of cell proliferation showed that GFP-positive cells proliferated to the same extent as other cell types present in these cultures. These data suggest that human β-cells in culture can dedifferentiate into a vimentin-expressing mesenchymal-like cell type and that these cells have significant potential for proliferation.

Similar experiments were also conducted with mouse islets. In accordance with previous in vivo studies, the proliferation of GFP-positive mouse cells was low and, following 20 days in culture, there was a marked decrease in the frequency of GFP-expressing cells, whereas the frequency was constant for human cells. These findings suggest a major species difference between mouse and human β-cell plasticity. Unlike rodent β-cells, human β-cells appear not to proliferate in the highly differentiated insulin-expressing state, but they have the capacity to undergo dedifferentiation and continue to proliferate long-term. Whether this represents true EMT remains unresolved in the present study. Likewise, the phenotypic characterization of the proliferating cells is not sufficient to judge whether these β-cell–derived cells represent a mesenchymal stem cell population, as suggested by some studies (16).

The double viral lineage–tracing approach described in this study shows a high degree of specificity; however, there are several differences and limitations when compared with the lineage tracing used in transgenic mice. Because the infectivity of a single virus is 70%, it can be expected that <50% of β-cells are labeled with both viruses. This would mean that less than one-half of the β-cells can be traced with this method, and whether these cells represent a subpopulation of β-cells is not known at present. Furthermore, it cannot be excluded that the viral infection itself has some undesired effects on differentiation or proliferation. Finally, using this lineage tracing technology, it is not possible to characterize events taking place during the initial period after infection because Cre-mediated activation of the reporter and expression of GFP to detectable levels takes 4–5 days.

The convincing demonstration of human β-cell dedifferentiation by Russ et al. is an important observation, particularly because it raises the intriguing possibility of fundamental differences between mouse and human β-cell biology. From a therapeutic standpoint, the essential question remains whether the expanded cells can be redifferentiated into an endocrine phenotype. Much confusion continues to exist in this field, primarily due to the uncertainty of the true nature and potential of islet precursors to the islet-derived cell populations described in various studies. Russ et al. may have taken us one step ahead by demonstrating the unexpected plasticity of human β-cells. However, we are still lacking the final steps that will bring us to the ultimate goal of effective generation of physiologically functioning human insulin-producing cells.

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