Novel Pancreatic Precursor Cell Lines for Studying β-Cell Differentiation

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Dianects results from inadequate mass of functional pancreatic β-cells. Type 1 diabetes is linked to selective autoimmune destruction of pancreatic β-cells, and type 2 diabetes is attributable to an inability to compensate for the extra demand of obesity or insulin resistance. Therefore, knowledge of how to stimulate growth and differentiation of islet cells is critical for developing new therapies. Results from in vivo studies indicate that adult ductal epithelium retains the ability to differentiate and to form new acini and islets of Langerhans. The inability to repeat this differentiation in vitro partly results from a lack of rapid assay to monitor the process of differentiation. Here, we describe isolation and characterization of precursor cell lines from mouse duct and islets that permit easy monitoring of pancreatic differentiation.

Evidence has been accumulating that the adult ductal epithelium has the potential to differentiate into exocrine and endocrine cells. In the adult pancreas of many species, all four islet cell types could be immunolocalized in the pancreatic ducts as occasional single cells or as small budding islets. The occurrence of these cells/islets can be increased by various experimental conditions, such as overexpression of γ-interferon in the β-cells of transgenic mice, after partial pancreatectomy, and after recent onset of type 1 diabetes. We have extensively studied ductal differentiation after partial pancreatectomy (1). Most if not all cells in the common pancreatic duct rapidly respond to a partial pancreatectomy by replication that is followed by a transient increase in the formation of new terminal ductules, which then rapidly differentiate into new acini, islets, and mature ducts. These observations suggest that the PDX-1/IDX-1-expressing ductal cells can be considered as facultative stem cells. This role for most duct cells is further supported by studies giving colchicine 4 h at the onset (18–24 h) of replication in the common pancreatic duct. All of the cells arrested in mitosis in the common pancreatic duct within the 4 h from onset of replication are the columnar ductal epithelial cells, not the rare (~1 in 100) cells that are small and ovoid with fairly undifferentiated cytoplasm. These latter cells may be true pancreatic stem cells, but they do not make a major contribution to regeneration after pancreatectomy. Based on these studies, we suggest that most if not all cells in the adult ductal epithelium retain the potential to differentiate into islet and exocrine cells.

Pancreatic precursor cell lines were derived from isolated common pancreatic ducts and islets from transgenic mice carrying a temperature-sensitive SV40 T antigen. “Immortomice” have been successfully used for obtaining cell lines of several tissue origins (3). When cells from the Immortomice are cultured at 33°C, SV 40 T antigen is expressed, and cells become conditionally immortalized; however, at 37 or 39°C, the T antigen expression is shut off, and cells differentiate appropriately. Isolated common pancreatic ducts and islets were handpicked and cultured at 33°C, which resulted in conditionally immortalized epithelial cell lines IM D-1 and IM I-1. When grown at 33°C, neither cell line has islet/neuroendocrine granules, immunostain for islet markers, or expresses detectable islet markers by reverse transcriptase-polymerase chain reaction analysis. Thus, there are no appreciable differences in the cell lines derived from ducts and islets. Ultrastructural analysis of cells grown at 33°C shows unremarkable cells with few organelles; however, after 1 week of culture at 37°C, the cells have increased rough endoplasmic reticulum (RER), mitochondria, and interdigitations with neighboring cells. After 2 weeks at 37°C, >50% of the cells have died, but most of the remaining cells further differentiated into well-developed RER and Golgi, numerous mitochondria and crinophagic vacuoles (secondary lysosomes), and numerous granules. We observed various phenotypes of granules, but granules within a cell were homogeneous. Although some granules resembled insulin granules and glucagon granules, none were typical of the granules seen in primary islets. We also transplanted cells grown at either temperature under the kidney capsule of nude mice. Approximately 1–2 weeks after transplantation, grafts of cells from ducts and islet lines appeared as scattered single cells or as clumps with both duct-like and islet-like phenotypes. Many cells had neuroendocrine granules, but we did not observe any cell with characteristic insulin granules. These observations indicate that pancreatic precursor cell lines respond to differentiation signals generated by culturing at 37°C and when transplanted in vivo under kidney capsule, by differentiating toward neuroendocrine cell type.

To develop a sensitive and rapid assay to analyze differentiation in these IM D-1 and IM I-1 cell lines, we used the unique ability of the insulin promoter to selectively activate gene expression in β-cells. A reporter plasmid containing rat insulin I promoter (−410 to +1 bp) regulating expression of...
Clontech’s enhanced green fluorescent protein (GFP) was constructed. Selective expression from rINS1:GFP and control CMV:GFP constructs was confirmed by transfecting these plasmids into IM D-1, IM I-1, βTC-3, and HeLa cell lines. Proper expression of our constructs was verified: CMV:GFP was expressed in each of the four cell lines, and rINS1:GFP was expressed only in βTC-3 cells. IM D-1 and IM I-1 cells were transfected with rINS1:GFP plasmid, and cell lines with stable integration of this plasmid were selected using G418. In parallel, we also derived cell lines with stable integration of CMV:GFP plasmid. We have found no ultrastructural differences (in terms of morphology) in these stable transfectants and the parent cell lines in culture before or after transplantation. To test our GFP cell lines functionally, we adopted the protocol of Mashima et al. (4) and cultured the cells at 33°C in the presence of 2 nmol/l activin A, 1 nmol/l betacellulin, or the combination of the two. With this combination of factors, our precursor cell lines expressed GFP with only a few cells fluorescent at 48 h, but increasing numbers were seen by 5 days; no GFP+ cells were seen with cells cultured with only one of the factors. This observation provides proof of principle for our rINS1:GFP reporter cell lines as a rapid detection system for differentiation of precursor cells into β-cells.

With these transfected precursor cell lines, we have very powerful tools. These transfected cell lines uniquely provide a sensitive and quick assay for reporting even low levels of stimulation of the expression of β-cell–specific genes as assayed by GFP fluorescence in the live cells. This will permit mapping various steps involved in the differentiation of pancreatic precursor cells into β-cells. These cell lines also provide a simple in vitro system to identify and analyze the role of intracellular and extracellular factors in the differentiation of the ductal epithelium. Thus, these stable cell lines will be excellent models of the differentiation of β-cells, and their ease of rapid read-out makes them an incredible tool.

REFERENCES