

Are there pancreatic progenitor cells from which new islets form after birth?

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Diabetes occurs when there is an inadequate functional mass of insulin-producing pancreatic β cells. Replacement of β cells by islet transplantation is a novel therapy for diabetes, although a major obstacle has been the limited amount of islet tissue available. A rigorous demonstration of the existence of postnatal pancreatic progenitor cells would clearly give focus to the development of innovative therapeutic techniques, including possibly stimulating formation of new endogenous β cells.

Throughout life, β -cell mass is dynamic with compensatory changes to meet demand and replenish the slow turnover of cells,¹ suggesting either a capacity of β cells to regulate their mass and/or the presence of stem cells or progenitor cells that can differentiate into insulin-producing cells by the process of NEOGENESIS. In mice, rats, and humans the β -cell mass remains linear with body weight or BMI.^{2,3} The mass of β -cells increases by replication of existing β cells and increases in cell volume (hypertrophy). Additionally, islet neogenesis has long been assumed to contribute throughout much of life,¹⁻³ however, a recent study by Dor *et al.*⁴ has challenged this assumption. The authors used lineage tracing of genetically marked β cells in mice, and concluded that no new β cells are formed from non-insulin-expressing stem cells or progenitor cells, either after birth or following 70% pancreatectomy. They suggest: "there is a time during embryonic development, or early postnatal life, at which the number of islets is set." After this time, all new β cells are generated by replication of pre-existing β cells.⁴ The conclusions of this study have, nevertheless, been controversial and we have previously raised a number of criticisms,⁵ including the difficulty of definitively proving that something does not happen. Additionally, inherent problems of leakiness over time of CRE RECOMBINASE and inadequate sampling after birth and throughout the pancreas—particularly in newly regenerated lobes after partial pancreatectomy—might have biased the results.

Although ongoing lineage-tracing studies might prove conclusively whether neogenesis actually happens, the current, overwhelming evidence is that it does. In rats, we have found two waves of neogenesis: one immediately after birth and the second before weaning (24 days). We have estimated that between days 20 and 31, the β -cell number triples and that at least 30% of the new β cells are not derived from replication of pre-existing β cells.⁵ After 90% pancreatectomy, adult rats show substantial pancreatic regeneration that is achieved by both replication of pre-existing endocrine or exocrine cells and proliferation of ducts and their subsequent differentiation into new pancreatic lobes. Judged by the increased presence of hormone-producing cells in the ductal epithelium, increased neogenesis has also been reported in a number of other experimental conditions, including treatment with glucagon-like peptide 1 or exendin 4.⁶ In these models, transient ductal expression of the transcription factor Pdx1 precedes any increase in the number of hormone-positive cells. In human pancreas, neogenesis could potentially have a more important role than increased replication in the compensation of β -cell mass seen with obesity. Replication is very low in human β cells;⁷ in individuals with obesity, pancreatic neogenic regions with many ductal cross-sections containing hormone-positive cells are seen more commonly than the enlarged islets that would result from enhanced replication. Both mechanisms for adding new β cells (replication and neogenesis) are likely to be functional after birth in all species, but they might make different contributions in different species.

If neogenesis occurs, it implies the existence of stem cells or progenitor cells. Although there have been recent reports of multipotential clonogenic stem cells or progenitor cells isolated from pancreas,⁸ estimating the number of progenitors needed to make a new islet could support the role of a particular cell type. As the nature of the progenitor cells is unknown, we

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can only estimate their doubling time from the data for rodent cell-cycle length (≥ 10 or 12 h). In the adult rat pancreatectomy model, we find islets in newly forming lobes at 3 days post-surgery—48 h after increased replication is seen in the pancreatic remnant. For each typical islet (150 μm diameter, composed of $\sim 1,500$ endocrine cells) formed within this 48 h window, at least 32 (5 doublings of 10 h each) or 64 (4 doublings of 12 h each) progenitor cells would be required. Hence, we expect that significant numbers of progenitor cells must replicate to give rise to the multiple new islets formed by 72 h. Bromodeoxyuridine incorporation after pancreatectomy is first seen in the epithelial cells of the common pancreatic ducts. Additionally, hormone-producing cells are observed within the basement membrane of ducts, which are composed of ductal epithelial cells with occasional undifferentiated basal cells. We therefore consider that ductal epithelial cells are likely to be the progenitors. As ductal cells replicate they transiently express Pdx1, which is also widely expressed in the embryonic pancreatic progenitors but restricted to β and δ cells by the time of birth. We hypothesize that a rapidly replicating, mature duct cell transiently assumes a less-differentiated and less-restricted phenotype that can redifferentiate into any of the pancreatic cell types.^{5,9} Such plasticity would provide abundant multipotent progenitors in adult pancreatic ducts for the normal renewal process. Additional support for this dedifferentiation concept comes from *ex vivo* studies with human islet-depleted pancreatic tissue, which, after expansion and manipulation of the culture conditions, forms glucose-responsive, insulin-containing islet tissue budding from ductal cysts.¹⁰ Similar *in vitro* plasticity has been suggested for acinar cells and even pancreatic β cells.⁸

The concept of a ductal origin of new β cells has been supported by the identification of markers expressed in the β cells of newly formed islets after partial pancreatectomy. Gene expression profiles of β cells excised by laser capture microdissection from islets in both old and new lobes of the same adult pancreas were compared using oligonucleotide microarrays.⁵ Six differentially expressed genes have been confirmed by reverse transcription polymerase chain reaction

and immunostaining to have high expression in β cells of new islets and very low expression in older islets. Additionally, these genes were highly expressed in adult ductal cells and β cells of neonatal rats. The transient expression of these multiple markers in both newly regenerated and neonatal β cells, but not mature adult β cells, and their sustained expression in pancreatic ducts strongly support a ductal origin of the β cell.

One implication of the pancreatic ducts as a progenitor pool is that they might be unlimited *in vivo* and, if triggered to differentiate, could meet the demand for insulin secretion caused by obesity or insulin resistance. Unlimited islet formation, however, could result in life-threatening hypoglycemia, so there must be regulatory mechanisms to control ductal proliferation and differentiation, thus limiting neogenesis. Consequently, understanding the regulation of neogenesis following stimulation could lead to islet replacement therapies *in vitro*, as well as *in vivo*.

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GLOSSARY

NEOGENESIS

New islet formation is seen as the budding of hormone-positive cells from ductal epithelium; these cells are presumed to differentiate from pancreatic stem cells or progenitor cells

CRE RECOMBINASE

An enzyme used to catalyze the site-specific recombination of DNA molecules *in vitro*

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Competing interests

The authors declared they have no competing interests.