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Induction of multiple cycles of pancreatic β-cell replacement

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The pancreas is an organ that can be subdivided into 2 functionally distinct compartments: (1) the exocrine tissue, composed of acinar and ductal cells, which are, respectively, implicated in the synthesis and transport of digestive enzymes and ions; (2) the endocrine tissue organized into cell clusters, termed islets of Langerhans. These contain 5 distinct cell subtypes α-, β-, δ-, ε-, and PP-cells secreting the glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide (PP) hormones, respectively.[1] Insulin acts to lower blood glucose levels following a rise in glycemia, whereas glucagon plays the opposite function in the case of low glycemic levels.

Type 1 diabetes corresponds to an autoimmune disease ultimately resulting in pancreatic insulin-producing β-cell loss and, therefore, chronic hyperglycemia. While current therapies provide a measure of control of blood glucose levels, treated diabetic patients may still develop severe vascular complications.[2] Finding alternative therapies is, therefore, a major goal for current research. Using the mouse as a model, we generated animals allowing the inducible and reversible expression of a single gene, *Pax4*.[3-5] Importantly, the ectopic expression of *Pax4,* triggered in adult α-cells at different ages, was found to induce their conversion into cells displaying most features of true β-cells, suggesting that age is not a limiting factor in these conversion processes.[6] Of equal interest was the observation that converted α-cells were continuously replaced. Combining proliferation assays and several lineage-tracing approaches, the source of such neo-formed cells was traced back to duct-lining cells. Taken together, our results support the notion that, upon Pax4-mediated α-to-β-like cell conversion, duct-lining cells are mobilized, proliferate, and re-activate processes classically observed solely during the course of embryonic development. Specifically, we demonstrate that duct-lining cells re-express the pro-endocrine gene *Ngn3* and undergo an epithelial-to-mesenchymal transition (EMT) prior to acquiring a glucagon$^+$ cell identity, such cells being further converted into β-like cells upon *Pax4* misexpression.

Aiming to determine whether the newly formed β-like cells were functional, we treated *Pax4*-misexpressing animals with a high dose of streptozotocin, a compound inducing the specific ablation of endogenous β-cells.[7] While all control mice died from the consequences of extreme hyperglycemia, all transgenic animals survived. Importantly, the continued monitoring of the glycemia of streptozotocin-treated *Pax4*-misexpressing animals showed a peak in glycemic levels, but, unlike controls, a subsequent and progressive return to normal levels was noted. In-depth examination of the pancreas of these animals outlined an initial loss of β-cells upon streptozotocin treatment, followed by a continuous β-like cell neogenesis allowing islet reconstitution. Interestingly, using lineage tracing of α-cells, a vast majority of neo-formed β-like cells was found to be derived from glucagon-producing cells. Lastly, we investigated whether the whole β-cell mass could be re-regenerated: to this end, the "surviving" animals were re-treated with streptozotocin. The assessment of β-cell mass and glycemic levels showed a clear neo-genesis of functional β-like cells. Taking advantage of this approach, we demonstrate that the complete β-cell mass can be regenerated at least 3 times, indicating that the pancreas has an inherent capability to regenerate multiple times the whole content of insulin-producing cells.

Thus, these data provide evidence that, within the pancreas, a source of "facultative" precursor cells does exist and can be mobilized to replace lost endocrine cells. Clearly, both the nature of this specific population and the signals involved remain to be characterized. While ducts seemingly contain precursor cells, one cannot exclude an alternative original source, such as acinar cells, as was recently proposed.[8] Nevertheless, finding ways to control the expression of *Pax4*, *Ngn3*, EMT inducers, or of their molecular targets/co-factors, will most certainly be of great interest in the context type 1 diabetes research.

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