Pancreatic stellate cells can form new β-like cells

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Regenerative medicine, including cell-replacement strategies, may have an important role in the treatment of Type 1 and Type 2 diabetes, both of which are associated with decreased islet cell mass. To date, significant progress has been made in deriving insulin-secreting β-like cells from human ES (embryonic stem) cells. However, the cells are not fully differentiated, and there is a long way to go before they could be used as a replenishable supply of insulin-secreting β-cells for transplantation. For this reason, adult pancreatic stem cells are seen as an alternative source that could be expanded and differentiated ex vivo, or induced to form new islets in situ. In this issue of the Biochemical Journal, Mato et al. used drug selection to purify a population of stellate cells from explant cultures of pancreas from lactating rats. The selected cells express some stem-cell markers and can be grown for over 2 years as a fibroblast-like monolayer. When plated on extracellular matrix, along with a cocktail of growth factors that included insulin, transferrin, selenium and the GLP-1 (glucagon-like peptide-1) analogue exendin-4, the cells differentiated into cells that expressed many of the phenotypic markers characteristic of a β-cell, and exhibited an insulin-secretory response, albeit weak, to glucose. The ability to purify this cell population opens up the possibility of unravelling the mechanisms that control self-renewal and differentiation of pancreatic cells that share some of the properties of stem cells.

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cells into functional islets [6]. The problem is that, although a high proportion of the differentiated cells express insulin at levels close to those seen in human islets, the cells are not fully differentiated, lacking a secretory response to glucose, and many of the cells co-express more than one hormone. The ES-derived MPCs (generated at an intermediate stage) can, however, be induced to differentiate into functional β-cells following prolonged culture (up to 72 days) under the kidney capsule or fat pads of mice. This suggests that ES- (or iPS-) derived functional β-cells may one day become available for therapeutic trials.

So, where does this leave the elusive adult pancreatic stem cell? There is a perception that such a cell would have important therapeutic implications. If it were present in biopsied material and could be induced to proliferate and differentiate *ex vivo*, then it would provide a patient-specific supply of transplantable cells that would circumvent the requirement for immunosuppression to prevent foreign tissue allograft rejection. In addition, it may be more practical to obtain fully differentiated functional islet cells from such dedicated ‘pancreatic progenitors’ than from ES/iPS cells, and possibly also reduce the chances of teratoma being formed. Finally, a better understanding of the properties of such a cell and how it becomes activated would represent an important breakthrough in the development of strategies to regenerate islets, particularly in Type 2 diabetes. The problem in this case is that, like most solid tissues, the pancreas is terminally differentiated with a very low turnover rate under normal physiological conditions. Recent elegant genetic lineage-tracing studies in mice have shown that β-cell replication is the principal mechanism involved in the maintenance of β-cell mass [7]. This was subsequently confirmed using a DNA-analogue-based lineage-tracing technique [8], whereas autopsy studies in humans provide strong supportive evidence that β-cell replication is the primary mechanism underlying β-cell expansion in childhood [9] and potentially also in obesity/pregnancy.

There is a view that β-cell replication alone may be sufficient to account for maintaining the mass of the pancreas, which appears, at least in rodents, to be set by the size of the progenitor pool in the early embryo [10]. However, there is also strong evidence that new β-cells can be generated by a process of neogenesis from a stem-cell population residing in the pancreatic duct [1]. Thus it was shown over 10 years ago that long-term culture of islet-producing stem cells could be established from ductal epithelial cells or digested pancreatic tissue freshly explanted from human organ donors or prediabetic NOD (non-obese diabetic) mice. There now exists a significant body of data supporting a role for differentiated adult ductal cells as a source of pancreatic progenitors. Increased budding of endocrine cells from the ducts has been observed in rodents undergoing partial pancreatectomy, and in response to treatment with the GLP-1 (glucagon-like peptide-1) analogue exendin-4, and Betacellulin or overexpression of IFN-γ (interferon-γ) or TNFα (tumour necrosis factor α). Lineage tracing of genetically marked ductal cells shows that, in mice, they can give rise to both new islets and acinar tissue after birth and injury [11]. An elegant study in adult mice has shown that new β-cells can be formed from non-β-cells located in the lining of the duct during regeneration of the pancreas in response to duct ligation. Shortly after duct ligation, there was an increased number of cells expressing Ngn3, which is not normally expressed in the adult pancreas [12]. These Ngn3-positive cells were sorted by flow cytometry and implanted into pancreatic buds from Ngn3−/− mice. Under these conditions, the Ngn3-positive cells from the regenerating adult pancreas differentiated into β-cells and other endocrine cell types. As noted above, there is still some controversy over the relative contribution of β-cell replication and neogenesis to the maintenance of β-cell mass under normal physiological conditions, but clearly neogenesis appears to be important in compensatory responses to increased metabolic demands, as seen with increased age, obesity and pregnancy.

New islets can also be formed by a process of transdifferentiation. Acinar cells, for example, when placed in culture...
will spontaneously dedifferentiate and redifferentiate [13]. During the redifferentiation process, the cells acquire characteristics of ductal cells through a process that mimics early stages of pancreaticogenesis. The progenitor cells derived from adult acinar cell cultures can be directed towards an hepatocyte lineage by treatment with the glucocorticoid dexamethasone, while treatment with EGF (epidermal growth factor) and LIF (leukaemia inhibitory factor) can induce formation of β-cells, albeit at low efficiency. Interestingly, liver cells, which incidentally share a common ancestry with the pancreas (both arise from the same region of the ventral foregut; Figure 1), can also undergo transdifferentiation to β-like cells following overexpression of pancreatic transcription factors such as Pdx1 or NeuroD1 [14]. The presence of pancreatic endocrine-hormone-producing cells in the gall bladder and biliary duct emphasizes further the liver as a potential source of new cells for treating diabetes.

It is against this background that, in this issue of the Biochemical Journal, Mato et al. [15] report that stellate cells present within explants of pancreas from lactating rats can be induced to differentiate into insulin-expressing cells. It is not altogether clear why they used lactating rats, although presumably in these animals β-cell mass would be increasing to compensate for the increased metabolic demands during lactation. Their strategy was to select for cells that expressed ABCG2, a member of the ABC (ATP-binding cassette) superfamily of membrane proteins. Expression and activity of these transporters is elevated in haemopoietic and non-haemopoietic stem cells. Their ability to facilitate efflux of lipophilic, fluorescent DNA-intercalating agents such as Hoechst 33342 has led to their identification in fluorescent cell sorting as SP (side population) cells that do not retain the fluorescent dye. Mato et al. [15] generated a pancreatic ABCG2-positive cell line by selecting for cells that were resistance to the anti-cancer drug mitoxantrone. The selected cells could be grown for over 2 years as fibroblast-like cultures that could spontaneously form clusters. The cells were identified as stellate cells on the basis that they expressed vimentin, desmin, α-actin, GFAP (glial fibrillary acidic protein) and exhibited Oil Red O staining of liposoluble material in the cytoplasm, which was identified as vitamin A on the basis of its characteristic fading fluorescence.

This is not the first time that stellate cells have been detected in the pancreas [16]. They have been identified as myofibroblast-like cells that share many features of their hepatocyte counterparts. They can be activated to proliferate and migrate to sites of tissue damage, where they synthesize extracellular matrix to promote tissue repair. Their sustained activation has been associated with the fibrosis that accompanies chronic pancreatitis and with pancreatic cancer. The importance of the study by Mato et al. [15] is that the mitoxantrone-resistant cells could be induced to differentiate into pancreatic endocrine cells. In basal medium the cells expressed Pdx1, as well as the stem cells markers nestin and Thy1.1. The presence of a differentiation cocktail of high glucose, HGF (hepatocyte growth factor), Betacellulin and nicotinamide increased the levels of Ngn3. However, the most dramatic effect was observed with ITS (insulin, transferrin and selenium) and exendin-4 when the cells were cultured on Matrigel1–4 for 2 weeks. Under these conditions the cells expressed Ngn3, NeuroD1, Pax6, Pax4, Pdx-1, GLUT-2, insulin, IAPP PC1/3, PC2, CK19 and glucagon, with reduced levels of the stem cell markers. There was no expression of the exocrine marker amylase or of somatostatin. The differentiated cells exhibited a weak (1.4-fold) insulin-secretory response to glucose (2.8 cf. 20 mM), although it is difficult to assess these data, which were expressed as pg per 100 cellular clusters rather than per μg of DNA, and clearly more detailed studies are required to determine whether this is a robust effect. Differentiation could also be achieved by overexpression of exogenous Ngn3, but the effects were weak, suggesting that Ngn3 alone was not sufficient.

In summary, the study by Mato et al. [15] increases the number of pancreatic and liver cell types that have been shown to (trans)differentiate into β-like cells. One of the strengths of the system is the ability to select a population of cells that can be characterized in detail. It will be of interest to determine whether similar mitoxantrone-sensitive cells can be isolated from mouse pancreas, and thus allow genetic lineage tracing of the ABCG2-positive cells using transgenic technology. Also, given the role of Sonic Hedgehog and Notch [and Wnt (Wingless)] signalling in pancreatic cancer-initiating cells that exhibit some properties of stem cells, this may be an ideal model to investigate the role of these pathways in the maintenance and differentiation of pancreatic stem cells [17]. It will also be important to determine how these cells compare with ABCG2-expressing cells that have previously been identified in cultured islet preparations or with the CD133+ (a stem-cell marker) cells present in the ductal network [18–20]. Finally, it would be of interest to determine whether these cells could be expanded to produce the sufficient quantities (1 billion) that would be required for therapeutic purposes. They might even provide some incisive insights that will finally nail the adult pancreatic stem cell.

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