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DIABETES

# A map of β-cell differentiation

**The use of stem-cell-derived β-cells to replace those destroyed in pancreatic islets has the potential to cure diabetes. A new analysis provides a deep mechanistic understanding of islet-cell differentiation from stem cells.** SEE ARTICLE P.368

FABIAN J. THEIS & HEIKO LICKERT

The islets of Langerhans in the pancreas contain insulin-secreting β-cells and glucagon-secreting α-cells. Insulin and glucagon are hormones that cooperate to regulate the levels of glucose in blood. Destruction or dysfunction of β-cells leads to diabetes. Currently, no treatment can stop diabetes progression and its devastating vascular complications. Islet transplantation can often normalize blood glucose levels for several years and prevent the secondary complications of diabetes. However, organ donors are scarce, and alternative sources of islet cells are urgently needed. Stem-cell-derived cells are promising in this respect. On page 368, Veres *et al.*[1] map the molecular steps in the differentiation of stem cells into islet-like cells. The work will inform future efforts to produce islet cells for transplantation.

Human pluripotent stem cells can indefinitely self-renew and generate every cell type in the body. Therefore, immense efforts are ongoing to develop *in vitro* protocols to produce differentiating islet cells from stem cells[2–5]. An ideal protocol would promote the differentiation of stem cells into fully mature α-cells and β-cells, which would then be isolated, purified and reassembled into islet-like structures for transplantation into patients. To achieve such an ambitious goal, the differentiation programs of all islet cells, and the way in which islets are built, need to be fully understood.

Veres *et al.* assayed more than 100,000 cells at different time points during the differentiation of stem cells into pancreatic progenitor cells and then hormone-producing (endocrine) cells. Single-cell RNA sequencing (scRNA-seq) of cells sampled at every step of the differentiation process, followed by computational analyses, made it possible to identify cell types and to track their lineages through time. The authors therefore produced a fine-grained picture of how pancreatic progenitor cells develop into different lineages of differentiated cells (Fig. 1). Current approaches for producing specific differentiated cells from stem cells have variable efficiency, mostly because of cellular heterogeneity and a lack of knowledge about the molecular signalling factors required for the differentiation process. Therefore, the authors' road map of *in vitro* islet-cell differentiation will inform the development of future differentiation protocols.

Veres and colleagues found that pancreatic progenitor cells can be efficiently differentiated. However, progenitor cells treated with slightly different combinations of signalling factors generated different ratios of hormone-expressing and non-endocrine cell types. This suggests that progenitor specification is key to producing the desired terminally differentiated cell types. In the authors' study, the three most abundant hormone-expressing cell types were stem-cell-derived α-cells (SC-α), β-cells (SC-β) and cells resembling enterochromaffin cells (SC-EC). Enterochromaffin cells are normally present in the intestine and produce serotonin, which contributes to the regulation of intestinal movements and digestion.

Interestingly, SC-β that were differentiated and grown for five weeks in the absence of external signalling factors maintained their defining molecular and functional properties. In particular, these cells showed stable glucose-stimulated insulin secretion throughout that period. This observation indicates that glucose responsiveness is a stable trait of SC-β that requires no exogenous factors, which is relevant if SC-β are to be used for antidiabetic-drug screening in the future.

The authors also observed that cells expressing the two islet hormones — insulin and glucagon — are probably immature SC-α, given that their global gene-expression profile matches that of human islet α-cells. These polyhormonal cells became monohormonal, glucagon-expressing cells after five weeks

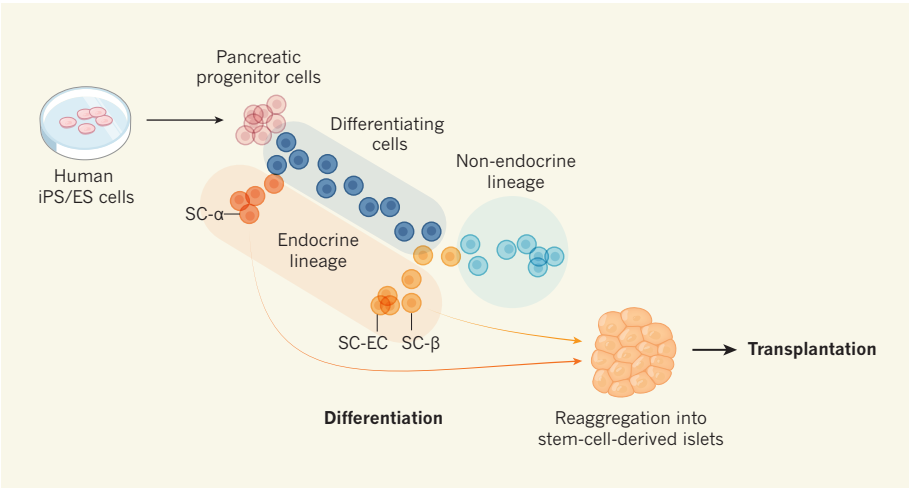


**Figure 1 | Differentiation of human pancreatic islet cells *in vitro*.** Veres *et al.*[1] studied the differentiation of induced pluripotent stem (iPS) or embryonic stem (ES) cells into: pancreatic progenitor cells; hormone-producing, stem-cell-derived α-cells (SC-α), β-cells (SC-β) and cells resembling enterochromaffin cells (SC-EC); and cells that do not produce hormones (non-endocrine cells). The authors performed detailed single-cell RNA sequencing and computational analysis to identify the cell types that emerged over time, to describe their lineage relationships and to characterize their maturation states. The authors used a concept called pseudotime to define how the gene-expression profiles of the various cell types changed over time. They also developed a purification protocol to increase the number of endocrine progenitor cells and β-cells that is a step forward from previously used protocols[2,3]. This study suggests that SC-α and SC-β can be purified and reaggregated to generate stem-cell-derived islets for cell-replacement therapy.

of culture, in agreement with previous observations[6].

The identification of SC-EC was surprising, because enterochromaffin cells are not normally present in the pancreas. Notably, these cells remained stable for up to eight weeks after transplantation into mice. The ratio of SC-EC to pancreatic endocrine cells (SC-α and SC-β) varied substantially when the authors changed the cocktail of signalling factors given to the cells at the differentiation stage that matches the induction and patterning of the gut and pancreas *in vivo*. This observation suggests that the specification of pancreatic and gut progenitor cells is essential for the generation of organ-specific endocrine cells. Different combinations of signalling factors also produced different ratios of non-proliferative endocrine cell types and proliferative non-endocrine cell types (acinar and ductal pancreatic cells).

To engineer safe and functionally mature stem-cell-derived islet equivalents, the desired endocrine-cell subtypes must be isolated and purified, and then reaggregated into pseudoislets[4]. The authors found that a simple dissociation and reaggregation procedure can remove most of the proliferative non-endocrine cells from the culture. Furthermore, they identified integrin α1 as a surface molecule expressed by SC-β. Magnetic cell sorting using an integrin α1 antibody enabled the authors to obtain cultures containing 80% of SC-β.

Endocrine induction — the formation of hormone-producing cell types in the pancreas — is a key step in the differentiation of islet cells from stem cells. The authors' gene-expression analysis of approximately 50,000 single cells allowed them to reconstruct the lineage relationships between human pancreatic progenitor cells and differentiated endocrine-cell types using a previously developed computational approach called pseudotemporal ordering[7] (Fig. 1). It also revealed the order in which these cell types emerge, and the dynamic molecular changes that occur along each differentiation trajectory. Other studies have used scRNA-seq and computational analysis to produce time series (rather than single temporal snapshots) and to model the population dynamics of cells involved in embryonic developmental processes[8,9]. This combined approach could be used to optimize the timing of the various steps in differentiation protocols.

How close are we to a therapeutic product for β-cell replacement? A clinical trial is already ongoing to test the safety and efficacy of transplanting stem-cell-derived pancreatic progenitor cells into people with type 1 diabetes, to restore control of glucose levels in the blood (see go.nature.com/2feyyud). However, transplanted pancreatic progenitor cells still need to differentiate and mature into β-cells to achieve effective glucose-stimulated insulin secretion. Given that we cannot control the signalling factors to which the cells are exposed *in vivo*, it is desirable to develop a product that secretes insulin in response to glucose when the cells are still in culture, and that becomes effective as soon as they are transplanted[5]. Moreover, transplanting only SC-β might not be sufficient to treat type 1 diabetes, because α-cells are also essential for tight control of hormone secretion from human islets, and for overall regulation of glucose levels.

Veres *et al.* have made great progress in addressing several of these problems. They provide a detailed scRNA-seq data set that covers the major steps of *in vitro* differentiation of islet cells, which will be useful for characterizing the cells produced when signalling factors are changed in different protocols. One key finding of their study is that SC-α and SC-β can be generated together, but that gut endocrine SC-EC and proliferative non-endocrine cells also emerge. This finding will inform the production of a defined and safe therapeutic product, which will require SC-α and SC-β to be isolated with a high level of purity, and proliferative progenitor cells removed to avoid the risk of cancer. Finally, the authors' detailed analysis of the scRNA-seq data has led to the identification of novel surface markers and signalling pathways that will further improve islet-cell differentiation and purification procedures. Overall, this study gets us a step closer to β-cell replacement in the clinic. ■

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CANCER

# Manipulating a tumour suppressor

**Tumours often become resistant to treatment, but how this occurs is poorly understood. An analysis of how the protein Rb affects tumour growth and the response to therapy might cast light on the problem.** SEE LETTER P.424

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The development of cancer invariably involves cellular proteins that have unwanted effects or that become inactive. Therapeutically targeting proteins that are more active than normal is conceptually straightforward — a drug needs to be found that, like a wrench thrown into a machine, halts the malicious activity. By contrast, remedying the loss of a type of protein called a tumour suppressor, which normally opposes cancer growth, presents a conundrum. How can a protein be targeted by drugs if it is no longer present? One way of getting around this problem and finding new drug targets is to identify other effects, such as molecular changes, that occur when a tumour suppressor is lost or does not function. On page 424, Walter *et al.*[1] report their analysis of the role of the tumour-suppressor protein retinoblastoma (Rb), using an experimental system that allowed them to engineer the loss and reactivation of this protein in lung cancer in mice.

Since the discovery of Rb's tumour-suppressor activity from studies of children with a type of eye cancer, and the subsequent identification of Rb loss or inactivation as a characteristic of many human tumours, this protein has served as a model for studying tumour suppressors in cancer[2]. Rb can act as a key brake on cell-cycle progression, and it promotes cellular differentiation into specific cell types, which slows the development of cancer. Many cancers block Rb function by increasing the activity of enzymes called cyclin-dependent kinases (Cdks), which inactivate Rb through phosphorylation[3] (the addition of phosphate groups to it; Fig. 1a). Anticancer treatments involving inhibitor drugs that target Cdk4 and Cdk6 to prevent them from inactivating Rb have shown some