A new paradigm in cell therapy for diabetes: Turning pancreatic α-cells into β-cells

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Cell therapy means treating diseases with the body's own cells. One of the cell types most in demand for therapeutic purposes is the pancreatic β-cell. This is because diabetes is one of the major healthcare problems in the world. Diabetes can be treated by islet transplantation but the major limitation is the shortage of organ donors. To overcome the shortfall in donors, alternative sources of pancreatic β-cells must be found. Potential sources include embryonic or adult stem cells or, from existing β-cells. There is now a startling new addition to this list of therapies: the pancreatic α-cell. Thorel and colleagues recently showed that under circumstances of extreme pancreatic β-cell loss, α-cells may serve to replenish the insulin-producing compartment. This conversion of α-cells to β-cells represents an example of transdifferentiation. Understanding the molecular basis for transdifferentiation may help to enhance the generation of β-cells for the treatment of diabetes.

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Abbreviations:
DOX, doxycycline; **DT**, diphtheria toxin A; **DTR**, diphtheria toxin receptor; **HAT**, hypoxanthine-aminopterin-thymidine; **Hprt**, hypoxanthine phosphoribosyltransferase; **RIP**, rat insulin promoter; **rtTA**, reverse tetracycline-responsive transactivator; **TetO**, tetracycline-responsive operator; **YFP**, yellow fluorescent protein.

Introduction

Type I diabetes is a serious metabolic disorder characterized by a loss of functional insulin-producing β-cells. The ultimate objective of therapy for type I diabetes is to replace the functioning β-cell component of the body. This can be achieved by an existing method of cell therapy: islet transplantation, in which islets are isolated from an organ donor and grafted into the liver of the patient *via* the portal vein. Islet transplantation can produce some improvement in diabetic patients but the major limitation to this approach is the shortage of organ donors [1]. Much research is therefore directed towards methods of recreating β-cells in the lab to supplement islets from donors.

Cell therapy means treating diseases with cells, ideally those of the same individual to preserve full immunological compatibility [2]. Four possible sources have been considered for obtaining β-cells: from existing β-cells, by provoking multiplication; from the other pancreatic cell types (acinar, ductal or other endocrine cells), by provoking neogenesis; from human embryonic stem cells, by recapitulating the sequence of developmental events that normally leads to β-cell differentiation; and finally from other endodermal tissue types, particularly those of the liver, by transdifferentiation (or sometimes it is referred to as reprogramming).

Transdifferentiation is defined as the irreversible conversion of one differentiated cell type into another differentiated cell type [3]. It is a subset of a wider class of cell type interconversions called metaplasias, which also includes interconversions between stem cells [3]. These phenomena are important to study for a number of reasons. Firstly, understanding the molecular basis of the conversions will extend our knowledge of the normal developmental mechanism. Secondly, some types of transdifferentiation predispose to neoplasia and are important in human pathology. Thirdly, understanding the molecular rules for cell or tissue-type conversions will improve our ability to reprogramme stem cells for the purpose of therapeutic transplantation.

Transdifferentiation may on occasion occur naturally between embryologically related tissues, and at the molecular

level is stimulated by the altered expression of a master switch gene whose normal function is to distinguish the two cells or tissue types during development. The overexpression of the master switch gene can, under suitable circumstances, trigger an alternate programme of gene expression that demarcates the new cell type.

Regeneration of β-cells

β-cell regeneration is widely thought to rely on self-duplication as demonstrated in various models of partial β-cell ablation [4–6]. The regeneration of β-cells in the pancreas has been described in animal experiments and in human pathology. β-cell regeneration can be induced in response to various experimental treatments, for example following pancreatic duct ligation [7, 8], streptozotocin-induced diabetes [9] or following administration of exendin-4 [10]. The phenomenon has also been observed in diabetic patients following autoimmune suppression [11]. In some of these cases (*e.g.* following pancreatic duct ligation [7]) the source of the regenerating β-cells has been characterised as originating from neurogenin-3-positive cells in the pancreatic ducts. However, in many examples of β-cell regeneration, detailed lineage analyses have not been performed.

Thorel *et al.* set out to examine the role of β-cell regeneration following total or near-total β-cell ablation (as in type I diabetes). The overall aim of the study was to establish whether disparate mechanisms of β-cell regeneration, for example from other pre-existing endocrine cell types such as α-cells, are associated with increased β-cell ablation severity [12]. This approach is unique compared to other examples of β-cell regeneration because previous studies have relied on less severe models of β-cell ablation.

In order to accomplish near-total and specific β-cell ablation, transgenic male mice were generated bearing a transgene containing the rat insulin promoter (RIP) and the diphtheria toxin receptor (DTR) coding sequence. To achieve tightly controlled *in vivo* conditional expression, the RIP-DTR transgene was targeted to the hypoxanthine phosphoribosyltransferase (*Hprt*) locus of the X chromosome. This strategy takes advantage of the potent hypoxanthine-aminopterin-thymidine (HAT) selection strategy in which embryonic stem cells with a partially deleted *Hprt* locus are transfected with a construct containing the 'deleted *Hprt* locus and experimental transgene'. Homologous recombination restores Hprt function, rendering the cells capable of surviving in HAT media. The ability to easily select embryonic stem cells with a single copy insert in a known orientation is beneficial as often randomly inserted constructs can be subjected to positional effects which can impair tissue specificity and expression level [13].

There may be problems with other ablation models such as pancreatectomy since these models can cause damage to more than one cell type. Therefore, in order to determine whether other remaining cell types contribute to β-cell regeneration, it is critical to cause only specific β-cell injury. By placing the DTR downstream of the RIP, diphtheria toxin A (DT) administration mediated >99% specific β-cell ablation approximately two weeks after the initiation of DT treatment. Following DT treatment, animals exhibited full-blown diabetes, polyuria, polydipsia, polyphagia, ketoacidosis and weight loss; hence throughout the experiment mice required subcutaneous insulin implants to survive. Recovery was a slow process taking approximately five months before insulin implants were no longer necessary, indicating that there were sufficient numbers of regenerating β-cells to control glucose levels (*i.e.* maintain glycaemia below 20 mM). β-cell regeneration was monitored *in toto* for 10 months with core data for the study collected at the one month regeneration time point. At this stage all islets displayed minimal regeneration, and total β-cell mass and pancreatic insulin content had tripled (Fig. 1). The next question to be addressed by Thorel *et al.* was, what is the origin of these nascent β-cells?

β-cells are not regenerated from pre-existing β-cells

To address the cell lineage of the nascent β-cells, ablation following treatment with DT was combined with lineage tracing of insulin-expressing β-cells using the tamoxifen-dependent Cre-loxP system. Upon addition of tamoxifen, roughly all of the β-cells of RIP-CreERT; R26-YFP mice were permanently labelled by yellow fluorescent protein (YFP). This labelling system thus facilitated distinguishing the contribution of differentiated spared β-cells to β-cell regeneration after DT treatment. Observations at one month revealed no increase in β-cell proliferation as well as a tenfold decrease in YFP-positive cells despite β-cell mass tripling, indicating that soon after ablation regeneration occurs from a non β-cell source (Fig. 1).

Regenerating β-cells are derived from α-cells

Immediately after β-cell ablation the islets consisted predominantly of glucagon-expressing α-cells and there was an associated twofold increase in the total pancreatic glucagon content. Also, as β-cell regeneration advanced, bihormonal cells expressing both insulin and glucagon appeared. These observations collectively led to the hypothesis that α-cells may be transdifferentiating into β-cells. This hypothesis was convincingly tested by combining DT-mediated β-cell ablation with lineage tracing of α-cells. Using the glucagon-rtTA (α-cell target); TetO-Cre/R26-YFP (inducible YFP label) mouse model, YFP was conditionally expressed by approximately 90% of α-cells in the presence of the tetracycline analogue doxycycline. Assessment of islets one month after regeneration revealed 65% of insulin-expressing cells were YFP-positive, of which 90% still expressed glucagon, suggesting an α-cell origin for the new β-cells (Fig. 1).

These observations were corroborated by two complimentary experiments showing that: (1) α-cell transdifferentiation to β-cells is reproducible with another transgenic mouse model: GluCre-ROSA26EYFP or GYY mice [14], which express the enhanced YFP specifically in glucagon-expressing α-cells. After extreme β-cell ablation, some insulin-expressing

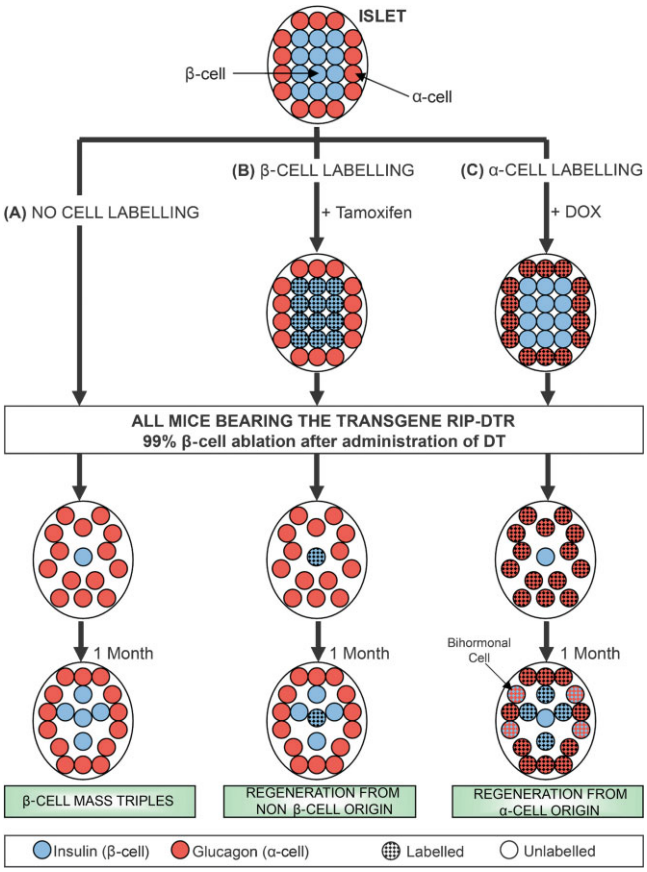

Figure 1. Regenerating β-cells are formed from α-cells. **A:** Ablation of β-cells. RIP-DTR male mice express DTR on 100% of β-cells. Administration of diphtheria toxin A (DT) causes ablation of 99.6% of the β-cell mass, but after 1 month of regeneration β-cell mass had tripled. **B:** β-cell labelling. β-cells of RIP-CreERT mice express CreERT recombinase under the control of the RIP. Upon addition of tamoxifen, the enzyme transiently translocates to the nucleus, where it removes a translational stop sequence that prevents transcription of YFP. YFP becomes expressed, and 95% β-cells are labelled by permanent, heritable expression of the protein. During regeneration, dilution of labelled β-cells (80–7.6%) occurs while β-cell mass increases, consistent with a model of regeneration from non β-cell origins. **C:** α-cell labelling. α-cells of glucagon-rtTA mice express reverse tetracycline-responsive transactivator (rtTA) under the control of glucagon promoter. Upon addition of the tetracycline analogue doxycycline (DOX), rtTA activation stimulates expression of Cre recombinase under the control of the tetracycline-responsive operator (TetO). The enzyme transiently translocates to the nucleus, where it removes a translational stop sequence that prevents transcription of YFP. YFP becomes expressed, and 90% of α-cells are labelled by permanent, heritable expression of the protein. During regeneration there is evidence for α- to β-cell reprogramming due to the appearance of bihormonal (glucagon+/insulin+) cells and insulin cells which are YFP-positive.

cells co-expressed YFP. (2) α-cells are absolutely required for bihormonal cell formation. Using an approach to co-ablate both α- and β-cells (administration of DT to RIP-DTR; glucagon-DTR mice) resulted in no bihormonal cells being formed [12]. The results clearly showed that the bihormonal cells arise from α-cells.

Molecular basis for converting α-cells to β-cells

The question arises, what is the molecular basis for the conversion of α-cells to β-cells? A number of transcription factors may play a critical role in the molecular mechanisms responsible for the reprogramming following extreme β-cell ablation including:

1. **Pax4**. Ectopic expression of Pax4 in embryonic α-cells induces their conversion into β-cells [15]. Pax4 presumably induces the conversion from α-cells to β-cells by repressing Arx. Indeed a previous study has shown that deleting Arx in mouse pancreas also induces a switch from α-cells to β-cells, suggesting a reciprocal relationship exists between Arx and Pax4 transcription factors in pancreas development [16].
2. **Pdx1**. A number of investigations have demonstrated the utility of Pdx1 (either alone or in combination) to induce the conversion of pancreatic acinar cells [17] or hepatocytes to β-cells [18, 19]. Furthermore, Pdx1 suppresses the α-cell phenotype and enhances the β-cell phenotype by binding directly to the glucagon and insulin promoters, respectively [20, 21].
3. **Nkx6.1.** The homeodomain transcription factor Nkx6.1 may also be involved in the switch from α- to β-cells since it suppresses glucagon gene expression and can induce insulin transcription [22].

While all three transcription factors are upregulated during β-cell regeneration following ablation [12], it is not known whether all three transcription factors are required for transdifferentiation to occur. Presumably the molecular mechanism is a combination of the repression of the α-cell phenotype and activation of β-cell specific genes. It is also intriguing that both phenotypes appear to co-exist in the same cells, suggesting that loss of the α-cell phenotype is not a prerequisite to gain of the β-cell phenotype.

Summary and future perspectives

While the work presented by Thorel and colleagues provides robust evidence for the transdifferentiation of α-cells to β-cells under conditions of extreme β-cell loss, several challenges remain to be addressed. The first challenge concerns the identification of the factor(s) that drive transdifferentiation and whether these could be used to induce transdifferentiation directly. The second concerns the intermediate (bihormonal) nature of many of the cells following regeneration. The challenge here is to understand the molecular and extracellular cues required to enhance β-cell maturation so that populations of insulin-positive/glucagon-negative β-cells are produced. This challenge could more easily be addressed through the development of an *in vitro* model for the transdifferentiation of α-cells to β-cells. The third challenge concerns the identification of the 'master gene' distinguishing α- and β-cells. While there is evidence for a role for Pax4 in converting embryonic α-cells to β-cells [15] it is not known

whether this is solely responsible for the induction of the α-cell phenotype in the present study. Alternative players exist in the form of Pdx1 and Nkx6.1 and these remain to be tested in the appropriate mouse models. Identifying the molecular basis for the transdifferentiation of α-cells to β-cells will be an important step in harnessing the therapeutic potential of transdifferentiated β-cells for diabetes therapy. Indeed, showing the same cell type conversion in human pancreatic α-cells would be an essential step in the translation to a cure for diabetes.

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