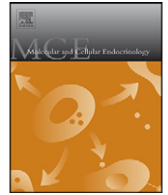
Molecular and Cellular Endocrinology 288 (2008) 86–94

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce



Embryonic stem cells to beta-cells by understanding pancreas development

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ARTICLE INFO

Article history:
Received 8 December 2007
Received in revised form 18 March 2008
Accepted 18 March 2008

Keywords:
Stem cell
Beta-cell
Pancreas
Human
Mouse
Diabetes

ABSTRACT

Insulin injections treat but do not cure Type 1 diabetes (T1DM). The success of islet transplantation suggests cell replacement therapies may offer a curative strategy. However, cadaver islets are of insufficient number for this to become a widespread treatment. To address this deficiency, the production of beta-cells from pluripotent stem cells offers an ambitious far-sighted opportunity. Recent progress in generating insulin-producing cells from embryonic stem cells has shown promise, highlighting the potential of trying to mimic normal developmental pathways. Here, we provide an overview of the current methodology that has been used to differentiate stem cells toward a beta-cell fate. Parallels are drawn with what is known about normal development, especially regarding the human pancreas.

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1. Introduction

The incidence of both Type 1 (T1DM) and Type 2 (T2DM) diabetes is increasing worldwide. Both disorders are characterized by high concentrations of blood glucose (hyperglycaemia), which is avoided by appropriate secretion of insulin by the pancreatic beta-cell. The beta-cells are the major constituent of the islets of Langerhans: a composition of several endocrine cell-types that make up ∼1–2% of the adult pancreas amongst the more prevalent exocrine and ductal components. The shortfall in insulin reflects an absolute loss of beta-cells in T1DM, a deficit that is increasingly recognized as a relative contributor in T2DM. The major goal in treating diabetes is to regain physiological regulation of circulating glucose levels. Currently, this is achieved in T2DM through dietary control and a range of oral medications, such as insulin secretagogues and sensitizers. Where this is inadequate, or in T1DM where the loss of endogenous insulin secretion is absolute, exogenous insulin is injected to replace pancreatic beta-cell function. Although this regime of frequent daily injections and blood glucose monitoring has permitted a relatively normal lifestyle for many individuals, it falls a long way short of the perfect treatment—it is not a cure. It also carries a major psychological impact on patients and their relatives. Thus, the search persists for effective cell replacement therapy to restore normal physiological insulin secretion without the need for repeated injections and invasive monitoring.

To date, the most promising form of beta-cell replacement has been islet transplantation (Shapiro et al., 2000). Although encouraging proof-of-principle, the protocol remains far from ideal, since it requires a large supply of cadaveric material, from which whole islets are isolated, and immunosuppression of the recipient. In tune with all organ transplantation programmes, there is not enough material to cater for the millions of individuals with diabetes currently treated by insulin injection. This imbalance has fostered excitement for alternative 'stem cell therapy'—whereby precursor cells, amenable to expansion, might be directed wholesale to a beta-cell fate, thus providing an unlimited source of material for transplantation. Arguably the most scalable cell-type with a clear potential for beta-cell differentiation is the embryonic stem cell (ESC). Using human ESCs as a starting point also offers the theoretical future of somatic nuclear transfer (SNT; replacing the ESC nucleus with that of a patient's own cell), or inducible pluripotent stem (iPS) cells (reprogramming achieved via the expression of selected transcription factors). Expansion and differentiation of these latter sources would approximate to an autologous cell product.

To achieve effective cell therapy from ESCs, aside from SNT or iPS cells, a number of desirable characteristics can be assembled: first and foremost, for transplantation in patients, the optimal cell

Abbreviations: T1DM, Type 1 diabetes; T2DM, Type 2 diabetes; ESC, embryonic stem cell; SNT, somatic nuclear transfer; iPS, induced pluripotent stem; ICM, inner cell mass; EB, embryoid body; wpc, weeks post-conception; MODY, maturity onset diabetes of the young; DAPT, *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-(*S*)-phenylglycine *t*-butyl ester; MHC, major histocompatibility complex.

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doi:10.1016/j.mce.2008.03.008

source would be human; second, the cell product needs to sense blood glucose and secrete insulin exactly like a normal healthy pancreatic beta-cell; finally, given the quality and longevity of life that can be achieved by insulin injection, cell replacement therapy must be safe. It is appropriate that these criteria make for a long-term research goal. Fully functional beta-cells derived from stem cells have yet to be proven ex vivo. In this review, we focus on current progress placed into the context of understanding normal beta-cell differentiation during gestation, the process whereby beta-cells first arise.

2. A brief history of embryonic stem cells

A stem cell is defined by the ability to self-renew indefinitely, characteristically by asymmetric cell division, coupled to a propensity for differentiation to one or more specialised cell-types (Weissman, 2000). ESCs are notable for pluripotency, the ability to generate all of the body's cell-types that arise from the earliest lineage fates of the inner cell mass (ICM) of the blastocyst (see Section 4.1). This combination offers scalability and the potential for a wide array of therapeutic cell products.

Historically, ESCs have been derived by taking the ICM of preimplantation blastocysts into in vitro culture (Evans and Kaufman, 1981; Thomson et al., 1998), although it seems that cells from the epiblast offer equivalent potential (Brons et al., 2007). It even appears that pluripotency, akin to that of ESCs, can be imparted by restoring the expression of relatively few transcription factors (Meissner et al., 2007; Okita et al., 2007; Takahashi et al., 2007; Yu et al., 2007). Like the ICM, ESCs retain an ability to differentiate to lineages that are equivalent of gastrulation: the establishment of three germ layers, from which arise all of the body's somatic tissues and organs. Mammalian ESCs were generated first from mouse embryos (Evans and Kaufman, 1981). Seventeen years later, in 1998, human ESCs were reported (Thomson et al., 1998). As a result of these landmarks, many human ESC lines have now been derived and maintained, facilitating investigations of directed differentiation to specific endpoints that include the pancreatic beta-cell.

The optimal strategy for forming beta-cells from ESCs has been keenly debated. In primitive organisms insulin, present in neural cells, acts as a neurotransmitter (Levine, 1981); in mammals, representing millennia of changing evolutionary selection pressures, insulin is restricted to endoderm-derived cells in the pancreas as an endocrine regulator of glucose homeostasis (Murtaugh, 2007). Thus, it seems plausible that arriving at the former cell-type might require fewer, less complex differentiation steps for an ESC compared to the latter navigation of a more circuitous route (Burns et al., 2004). In the laboratory, this raises the idea that human insulin-secreting cells might be generated from ESCs by 'shortcutting' mammalian development and following a relatively direct path. Alternatively, one could attempt mimicry of the normal developmental pathway, more complex, but potentially more reassuring as it builds upon a wealth of developmental biology knowledge and might allow co-differentiation of the other islet cell-types. The following sections describe experiments that can be considered at least partially within these two categories.

3. ESC to insulin-positive cell-types: shortcuts and genetic engineering

Evidence that insulin-secreting cells could be derived from stem cells *in vitro* initially came from studies using mouse ESCs and embryoid body (EB) formation (Soria et al., 2000). Similar results for human ESCs soon followed (Assady et al., 2001). In the latter study, undifferentiated ESCs were allowed to aggregate inducing spontaneous differentiation within the EB—in effect, a haphazard mimic of gastrulation. Not surprisingly, using this approach, the percentage of insulin-positive cells in EBs was low (~1%). In contrast, Soria et al used transfection and cell selection methodology with an insulin-hygromyocin transgene to obtain an insulin-secreting clone. Transplantation of these cells into diabetic mice restored normoglycaemia (Soria et al., 2000). Recognizing the problems inherent in such a low rate of return from EBs, subsequent studies focused on increasing insulin positive cell numbers by other means. In 2001, Lumelsky et al. devised a five step protocol to enrich for insulin-positive cells from mouse ESCs, by selecting cells after EB formation that expressed the intermediate filament protein Nestin (Lumelsky et al., 2001). Nestin, however, is broadly expressed and commonly interpreted as a marker of neuroprogenitors or neural stem cells (Cattaneo and McKay, 1990; Lendahl et al., 1990). Based on the recognition that many transcription factors regulating beta-cell development and insulin gene expression are also required for neuronal differentiation (Rolletschek et al., 2006), Nestin was considered as a potential marker of pancreatic progenitor cells (Cattaneo and McKay, 1990). Lumelsky and colleagues showed structures at the end of their differentiation protocol, strikingly similar to mouse islets. Others have built on this protocol, such as the inclusion of PI3 kinase inhibitors, to enhance the generation and function of the insulin-positive cell-types (Hori et al., 2002). In the latter study, the effectiveness of transplanted cells was proven by restoration of euglycaemia in diabetic mice and subsequent deterioration following transplant removal (Hori et al., 2002). However, the proximity of these cells to true beta-cells remains contentious, especially as a significant proportion of the intracellular insulin is not de novo synthesis but uptake from the culture media by dying cells (Rajagopal et al., 2003; Hansson et al., 2004). Reports of Nestin expression during mouse pancreas development differ (Selander and Edlund, 2002; Bernardo et al., 2006), however, Nestin was not detected during human pancreas development (Piper et al., 2002b). Collectively, these findings have led to suggestions that Nestin expression en route to an insulin-positive cell-type is perhaps more indicative of neuronal differentiation. Either independently or based around Nestin-dependent differentiation protocols, others investigated whether a pancreatic programme could be enforced more rigidly by constitutive (over-) expression of pancreatic transcription factors, such as Pdx1, Ngn3 or Pax4 (Blyszczuk et al., 2003; Miyazaki et al., 2004; Lavon et al., 2006; Treff et al., 2006). Not surprisingly, this strategy induces expression of a number of target genes of these transcription factors. Whereas these experiments have helped our understanding of the pathway from ESC to differentiated beta-cell, it is difficult to assess accurately whether these interventions cause permanent programming of differentiated cell-fate.

In summary, by either accepting random differentiation via EBs or short-cutting normal development by the over-expression of pancreatic progenitor transcription factors, it is possible to generate insulin-positive cells that are capable of restoring euglycaemia in mice. However, these cells are not physiologically normal and whilst they may serve as models for laboratory experiments or, potentially, drug-discovery programmes, it seems unlikely that cell-types for clinical therapy will arise. Thus, we turn to consider the normal developmental pathway from ICM mass to beta-cell and the studies that aimed to mimic it.

4. ESC to beta-cell: the complex pathway of normal development

Given the low frequency of differentiation to desired cell-types by random EB formation, to many researchers, it seemed logical

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