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Perspective

Mechanisms and techniques of reprogramming – Using PDX-1 homeobox protein as a novel treatment of insulin dependent diabetes mellitus

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ABSTRACT

Homeobox proteins are key regulators of stem cell proliferation and differentiation which function as transcription factors and regulate cell fate decisions. Pancreatic Duodenal Homeobox-1 (PDX-1) is a homeobox protein which acts as a key regulator in the development of b cells in the Islets of Langerhans. It plays an important role in maintaining the identity and function of the Islets of Langerhans, and in the development of the pancreas. There is strong evidence that PDX-1 plays a role in activating the insulin promoter and increasing insulin levels in response to glucose. PDX-1 also binds to sequences within β cells and regulates the promoter activity of a number of islet genes including insulin, glut-2 and neurogenin 3. When fused with the VP16 activation sequence, transfection of the PDX-1 gene has been shown to transform liver cells into insulin producing cells.

Because homeobox proteins are able to passively translocate through cell membranes, due to an intrinsic transduction domain (penetratin), the use of these proteins to reprogram target cells may help overcome the limiting supply of β cells and be a potential future treatment for Type 1 diabetes.

Contents

1. Introduction

Diabetes is a disease of high prevalence [1] with estimates from the World Health Organisation suggesting that in 2011 346 million people worldwide are afflicted [2]. Currently there is no cure. The aim of treatment for diabetes is to restore glucose homeostasis. The traditional method currently being used is via the administration of exogenous insulin. In addition, transplantation and gene/cell therapy are being explored as future therapies. The development of an effective islet transplantation procedure has focused attention on the limiting supply of β cells for the treatment of Type 1 diabetes [3]. Cellular therapy may also provide a potential source of β cells. One procedure, which is clinically relevant, involves reprogramming somatic cells with protein. This method, which avoids non-specific insertion of foreign DNA, involves removing

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autologous cells from the patient and reprogramming these to the required cell line. This procedure removes the need for toxic immunosuppressive drugs, and unlike adult stem cells, which are hard to isolate and in short supply, somatic cells are plentiful in type and number. The use of homeobox proteins for reprogramming is also potentially advantageous due to the endogenous transduction domain ("penetratin") which avoids the difficulties associated with other proteins.

2. Diabetes

There are three forms of diabetes. Type 1 diabetes mellitus, also known as insulin dependent diabetes or juvenile diabetes, accounts for approximately 10% of all diabetes and is the leading cause of renal failure or renal disease [4], loss of vision [1], amputation [5], and a major cause of cardiovascular disease and premature death [6]. These major clinical complications result from long-term hyperglycaemia which occurs because the standard treatment for diabetes cannot maintain blood glucose concentrations within the narrow range that a normally functioning pancreas achieves [4]. Type 2 diabetes mellitus is more commonly known as non-insulin dependent diabetes, or adult onset diabetes, and is the most common of the three. It results from a cellular resistance to insulin action as well as inadequate insulin secretion [1]. The third type of diabetes, gestational diabetes, is hyperglycaemia with onset during pregnancy. The symptoms of gestational diabetes are similar to Type 2 diabetes [2]. Currently, there are approximately 1 056 000 people in Australia with diabetes. Of these there are 1 29 000 people with Type 1 diabetes, 901 000 people with Type 2 diabetes, 21 000 with gestational diabetes and 5000 with their diabetes type not disclosed [7]. The percentage of people with Type 1 diabetes (12.2%) in Australia is higher than the worldwide average (10%).

The causes of Type 1 diabetes include both intrinsic and extrinsic factors involving the patients' genotype, epigenotype, and environment [8]. When Type 1 diabetes is immune mediated, it is caused by the destruction of pancreatic insulin expressing β cells in the Islets of Langerhans. When the immune system is triggered it does not recognise the β cells as "self" and destroys them. This autoimmune reaction, leads to loss of insulin production and secretion and causes hyperglycaemia, which is a characteristic of the disease [1,9]. In the absence of insulin, glucose cannot enter the cells and accumulates in the blood.

3. Current treatment

The current treatment for Type 1 diabetes is via the administration of exogenous insulin. The use of insulin delays the development of disease complications [6] and prolongs survival but is far from ideal [10]. There are many different types of insulin on the market and each patient needs an individualised regimen. The non-physiological time–action profiles of conventional insulin remain a significant obstacle. To overcome this, rapid insulins are used before meals and long-acting insulins used to provide basal insulin levels, simulating non-diabetic insulin profiles more closely [11]. Intensive insulin therapy by way of an insulin infusion pump is another mode in which insulin can be delivered. The use of a subcutaneous insulin delivery system, such as a pump, was found to delay diabetic complications in the 'Diabetes Control and Complication Trial' conducted in 2004 [12]. Some of the advantages of an insulin pump over multiple daily insulin injections, include better glycaemic control and stability with lower daily doses of insulin and reduction of hypoglycaemic episodes [13]. The use of intraperitoneal insulin infusion offers a more physiologic route of insulin delivery. In addition to insulin treatment, patients must also monitor their blood glucose levels and follow a strict low-sugar diet and exercise schedule [10].

3.1. Transplantation

Pancreatic islet cell transplantation has been suggested as a possible cure for Type 1 diabetes. Islet transplantation can restore endogenous β cell function [14], and insulin independence can be achieved following an islet cell transplant in conjunction with the use of non-steroidal immunosuppression [15]. However, patients required up to three transplants to restore glucose homeostasis [14]. This approach has the potential to restore the regulatory unit of the pancreas but may not be a general solution because of the limitations inherent in the use of toxic immunosuppressive agents, as well as the limited number of donors available [10]. More work needs to be done on preserving islet function, as five years post-transplant most patients had reverted to using some exogenous insulin. Also the use of toxic immunosuppressive drugs needs to be monitored closely as complications of these medications included mouth ulcers, anaemia, ovarian cysts, hypertensive disorders and high cholesterol levels [14].

Another transplant option is xenotransplantation which is the transfer of cells or organs from another species. This is a controversial option due to the potential risks of infection and disease transmission from the grafted tissue, and as with all transplants, the risks associated with the use of immunosuppressive drugs must be considered [16,17]. It is currently being trialled in New Zealand by Living Cell Technologies where live porcine islet cells are being transplanted into humans. It works by secreting insulin and glucagon in response to the patient's changing glucose levels. The cells are protected from the patient's immune system by a microcapsule [17].

3.2. Gene/cell therapy

Stem cell therapy may also represent a potential source of treatment for Type 1 diabetes. Because the pathology of this disease is caused by the autoimmune destruction or malfunction of pancreatic β cells, and consequently, a lack of insulin [18], the development of an effective islet transplantation procedure has focused attention on the limiting supply of β cells [3]. Various sources for new β cells are therefore being sourced, including embryonic stem cells (ESCs), adult stem cells (ASCs) and transdifferentiation (see below) of certain types of differentiated cells such as hepatocytes from the liver [3]. Although, each of these approaches has its own limitations. ESCs are harvested from the inner cell mass of the pre-implantation blastocyst [19], and are considered to be pluripotent, meaning they are able to differentiate into most cells that arise from the three germ layers [20]. ESCs can be genetically modified using a number of methods, for example, inserting immunosuppressive molecules such as Fas ligand, or removing immunoactive proteins such as B7 antigens or insertion of genes coding for the recipients major histocompatibility complex to make them completely compatible with the patient's own tissues, thus reducing the need to use toxic immunosuppressive drugs [19]. They therefore have the potential to create cell populations, tissues and organs for implantation [19]. However, in ESC transplantation there is the danger of uncontrolled growth of the transplanted stem cells, and ESCs have previously been shown to form tumours when transplanted [21].

ASCs or tissue stem cells, are found within otherwise differentiated tissues in the adult and foetus. They are thought to provide the basis of tissue regeneration, replacement and repair, and many are multipotent in that they demonstrate the capacity to differentiate into multiple cell types not associated with the tissue from which they are derived [21]. ASCs are accessible in most patients,