CELL REPROGRAMMING FOR TREATMENT OF TYPE I DIABETES

by

HUA SHI

(Under the Direction of Dexi Liu)

ABSTRACT

Type 1 diabetes (T1D) is a worldwide disease that is caused by T cell-mediated elimination of pancreatic β cells responsible for producing insulin, a peptide hormone that decreases blood glucose level. Lack of insulin in T1D patients leads to too much of glucose in the blood (hyperglycemia) and not sufficient glucose in the cells, resulting in life-threatening diseases. The most effective treatment option currently available for T1D is insulin therapy. However, insulin overdose easily happens and this could lead to hypoglycemia, loss of consciousness and seizure among other detrimental effects. Therefore, research has been conducted to develop a safer and more effective alternative, and cell reprogramming to convert non-insulin producing cells into β or β-like cells has attracted most attention. The goal of this thesis is to provide an overview and recent progress made in the use of cell reprogramming as a potential new treatment for T1D.

INDEX WORDS: Type 1 Diabetes; Insulin; Insulin Producing Cells; Cell-reprogramming

CELL REPROGRAMMING FOR TREATMENT OF TYPE I DIABETES

by

HUA SHI

B.S., Central South University, China, 2018

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2020

CELL REPROGRAMMING FOR TREATMENT OF TYPE I DIABETES

by

HUA SHI

Major Professor:	Dexi Liu
Committee:	Arthur Roberts Houjian Cai

Electronic Version Approved:

Ron Walcott
Interim Dean of the Graduate School
The University of Georgia
August 2020

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Dexi Liu of the Department of Pharmaceutical and Biomedical Science at the University of Georgia. Dr. Liu gave me very detailed suggestions whenever I had a question about course work, research, or writing. He spent a lot of time with patience steering me through my graduate study. I am very grateful to Dr. Liu for his willingness to motivate and encourage me and appreciate his academic rigor and high standard that help me never stop improving myself. Besides, I would like to thank my thesis committee members, Dr. Arthur Roberts and Dr. Houjian Cai for their encouragement, insightful comments and advice.

I am grateful to my fellow lab mates, Guisheng Zhang and Huan Liu for their care and help in my graduate study. I enjoyed the time we spent together in the past two years.

Finally, I must say thanks to my parents, my partner, Meina Bian, and my friends. They gave me financial and emotional support throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without their support.

LIST OF ABBREVIATIONS

AD-MSCs	Adipose-derived MSCs
ATP	Adenosine triphosphate
$CD4^+$	Cluster of differentiation 4
$CD8^+$	Cluster of differentiation 8
ESCs	Embryonic stem cells
GLP-1	Glucagon like peptide 1
Hes1	H-loop-helix 1
hESCs	Human embryonic stem cells
HLA	Human leukocyte antigen
htESLCs	Human testis-derived embryonic stem-like cells
iPSCs	Induced pluripotent stem cells
K_{ATP} channel	ATP-sensitive K^+ channel
MafA	MAF BZIP transcription factor A
MafB	MAF BZIP transcription factor B
MHC	Major histocompatibility complex
MSCs	Mesenchymal stem cells
Ngn3	Neurogenin3
Pax4	Paired box 4
Pdx1	Pancreatic and duodenal homeobox 1
Ptf1A	Pancreas transcription factor 1 subunit alpha

RA	Retinoic acid
SSC	Spermatogonium stem cells
STZ	Streptozotocin
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TGF-β	Transforming growth factor-β
Th	T helper

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Chaper. 1 Introduction

Diabetes is a condition in people who have an impaired function to regulate blood glucose level. In the US, the estimated number of diabetic patients over 18 years of age is 30.3 million, representing about 10% of total population (Maahs et al., 2010). Approximately 1.5 million Americans are diagnosed with diabetes each year. Diabetes can be the cause of heart disease, stroke, neurological disease, kidney disease, foot disease, eye disease, gum and dental disease, and sexual and bladder diseases. Diabetes was the 7th leading cause of death in the US with 83,564 diabetes-caused deaths and 270,702 deaths linked to diabetes in 2017.

There are two types of diabetes, type 1 (T1D) and type 2 (T2D). T1D, once known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which patients exhibit an elevated glucose concentration in blood due to loss of pancreatic β cells that are responsible for producing insulin, a peptide hormone that plays a critical role in maintaining glucose homeostasis. T1D is an autoimmune disease mediated by T cells. Approximately 10% of the diabetic population belongs to T1D. The first line treatment for T1D is insulin therapy where patients are injected with insulin when their blood glucose level is high.

T2D, a more common type, also called adult diabetes, is caused by the loss of insulin sensitivity in which cells are no longer responsive to insulin when blood glucose level is high. T2D patients are known to have higher insulin level, commonly called hyperinsulinemia. The mechanism of T2D is not currently understood but is believed to be linked to family history, lack of physical activity, overweight or obesity. While dietary and

life-style changes are mostly recommended for prevention of T2D. The treatment for T2D includes oral administration of drugs (e.g. metformin), healthy diet, and exercise.

Significant efforts have been made in recent years for improved treatment of diabetes. Although most research focuses on T2D because of its larger patient population, research on development of an improved method for T1D has also been actively conducted. For insulin therapy, alternative methods such as insulin pen, insulin pumps and oral insulin have been developed in order to improve the insulin accuracy and patient compliance. Although improvements have been reported, all those new approaches cannot mimic the endogenous insulin secretion pattern and have the risk of hypoglycemia. As lack of pancreatic β cells is the direct cause for T1D, strategies have been developed to transplant the whole pancreas organ, pancreatic islets, and β cells into T1D patients or convert other types of cell to insulin producing β-like cells. Various approaches taken to reprogramming cells into insulin producing cells are the primary focus of this thesis.

The materials summarized in this thesis are organized to sequentially present the information on glucose homeostasis, T cell-mediated elimination of pancreatic β cells, the pros and cons of current insulin therapy, the strategies for β cell substitution for T1D treatment, and the current progress in cell reprogramming-based therapy for treatment of T1D. Perspectives and future direction toward clinical applications of currently developed cell reprogram approaches are meant to frame a hopeful and bright future for a new and improved method to help T1D patients live a healthy life.

Chaper. 2 Glucose homeostasis and type 1 diabetes

2.1. Glucose homeostasis

The blood glucose level is maintained within a narrow range in the body. The mechanism regulating glucose concentration is commonly called glucose homeostasis (Röder et al., 2016) which is primarily maintained by two peptide hormones, insulin and glucagon. Insulin lowers and glucagon raises glucose concentration. The fasting glucose in healthy humans is at 80 - 100 mg/dL level, 101-125 mg/dL is considered pre-diabetes, and >126 mg/dL is diabetes (American Diabetes Association Position Statement, 2017). Glucose concentration after a meal fluctuates depending on the type and amount of food one consumes.

When the level of blood sugar rises after a meal, insulin is released by pancreatic β cells. Insulin in blood binds to insulin receptor and forces about 2/3 of body cells (primarily cells in muscle, liver and adipose tissue) to take up glucose from the blood through glucose transporter type 4, thus decreasing blood glucose concentration. Insulin can also bind to liver cells that can convert more glucose into glycogen for storage (this process is called glycogenesis) and inhibit gluconeogenesis to decrease the blood glucose level. Different from muscle cells, pancreatic β cells express glucose transporter type 2 which has high capacity and low affinity (Efrat, 1997). The low affinity makes it a glucose sensor that can only transport glucose when glucose concentration is high. The high capacity makes these transporters work with high efficiency. Therefore, when blood

glucose level is beyond the normal range or higher, the glucose enters into β cells triggering cellular respiration to produce adenosine triphosphate (ATP), generating the complexes of ATP and ATP-sensitive K_+ (K_{ATP}) channel protein. ATP binding to Kir6.2 site of K_{ATP} channel proteins shuts down the influx of K_+, and depolarizes the β cell membrane. Membrane depolarization activates the voltage-dependent calcium channel, increases the influx of calcium ions, activates the process of exocytosis of insulin containing vesicles to release insulin. The process of glucose-induced insulin secretion (Aoyagi et al., 2011) is shown in Fig. 1.

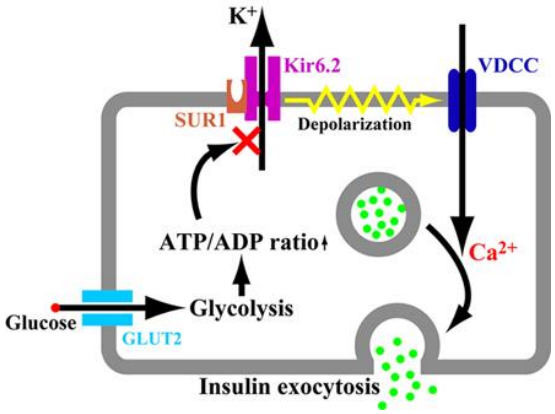

Fig. 1. Mechanism of glucose induced insulin release by pancreatic β cells

If the blood glucose level is low due to heavy exercise or lack of food for extended periods, glucagon is released by pancreatic α cells to increase the blood glucose level. The blood glucose is taken up by glucose transporter type 1 on the membrane of α cells. The type 1 glucose transporter is a uniporter responsible for regulating the basic glucose uptake for the cellular respiration. After glucose enters α cells, it drives synthesis of ATP in mitochondria. Intracellular ATP level in α cells reflects the plasma glucose level. The hypoglycemia results in low intracellular ATP

level that inhibits the K^{+}_{ATP} channel in α cells to shut down the efflux of K^{+}, which results in the depolarization of α cell membrane. The depolarization then activates the voltage-dependent calcium channel and increases the influx of calcium ion that facilitates the exocytosis of glucagon containing vesicles. Once in blood circulation, glucagon travels to the liver and binds to glucagon receptor on the surface of liver cells and stimulates the breakdown of glycogen stored in hepatocytes into glucose. The produced glucose is released into the blood and increases blood glucose level. The process of glucagon secretion (Müller et al., 2017) is shown in Fig. 2.

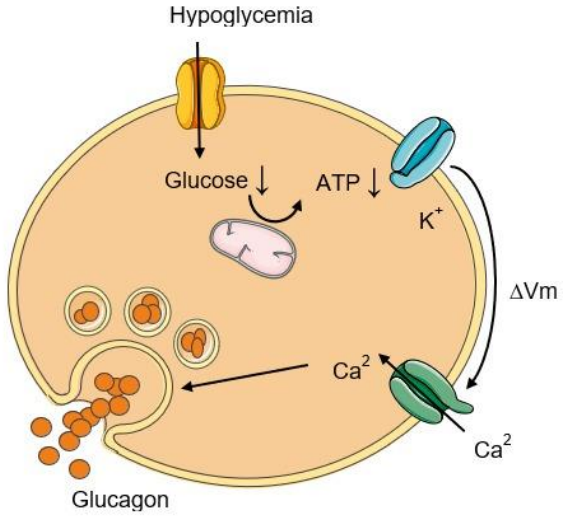

Fig. 2. Mechanism of glucagon release by pancreatic α cells

Glucose homeostasis is therefore maintained by a balance between the activity of α cells and β cells in pancreas in releasing glucagon or insulin in response to blood glucose concentration.

2.2. The cause of T1D

T1D is considered as an autoimmune disease characterized by the presence of antibodies and cytotoxic T cells against β cells in T1D patients, causing the destruction of β cells and reduction of insulin production (Bottazzo et al., 1974; Palmer et al., 1983). With the decrease of β cell mass, the insulin secreted by β cells cannot maintain the glucose homeostasis, therefore, the hyperglycemia occurs. There are a few different underlying pathophysiological mechanisms that have been studied.

Firstly, the autoimmunity is believed as the direct cause of the pathophysiology of T1D with genetic elements playing an important role. T1D patients appear born with a genetic predisposition to the disease. For example, it was reported that human leukocyte antigen (HLA) gene encoding major histocompatibility complex (MHC) class II molecules contributes up to 40% of T1D risk (Pugliese, 2004). Hyperexpression of MHC class I molecules was demonstrated in the insulin-containing islets and MHC class II molecules were detected in T1D patients' β cells (Foulis et al., 1987) of T1D patients.

Secondly, the environmental factors such as virus infection, toxins, dietary products such as the bovine proteins (e.g. bovine serum albumin, bovine insulin, and casein) are known to cause the development of T1D (Lefebvre et al., 2006). These factors are capable of inducing immune response against the pancreatic β cells, especially for people who have genetic predisposition. For example, it was shown that infection by B4 strain of the coxsackie B virus, german measles, mumps, and rotavirus triggered the development of T1D (Jun and Yoon, 2004). Subsequent studies revealed that these viruses contain very similar antigens to those of β cells. Immune response developed against viral infection attacks β cells, even after the elimination of virus,

resulting in the destruction of β cells, decrease in insulin secretion, and finally T1D (Filippi and von Herrath, 2008).

Antibodies have been found in T1D patients against islet cells' proteins, such as insulin, glutamic acid decarboxylase 65, and islet-specific glucose-6-phosphatase catalytic subunit-related protein (Lieberman and DiLorenzo, 2003), and among which the antibody against glutamic acid decarboxylase is the most common type. Presence of anti-insulin antibodies in some T1D patients represents a major challenge for treatment of T1D because the most commonly used insulin supplement therapy can no longer be considered as a treatment option in this situation. Patients with autoimmune disease tend to have higher tendency in developing T1D due to a high level of anti-glutamic acid decarboxylase antibody, especially in patients with autoimmune thyroiditis (Pilia et al., 2011).

T cells play a very important role in mediating β-cell destruction. In particular, cluster of differentiation 4 ($CD4^+$) and cluster of differentiation 8 ($CD8^+$) T cells appear responsible for β cell elimination. Studies have shown that injection of both $CD4^+$ and $CD8^+$ T cells isolated from T1D mice to non-diabetic mice induces T1D, but not $CD4^+$ or $CD8^+$ alone (Christianson et al., 1993). Immune suppressing drugs such as cyclosporin ameliorate the T1D (Chatenoud et al., 2012). Evidence accumulated so far suggests that activation of $CD4^+$ and $CD8^+$ T cells is the prerequisite for T1D development (Gill and Haskins, 1993).

Naïve CD4+ T lymphocyte cells exist as T helper (Th) 0 cells. It differentiates into Th1 cells (Almawi et al., 1999) and the Th2 cells (Katz et al., 1995) after encountering with antigenic molecules presented by antigen present cells. Both Th1 and

Th2 cells can secrete cytokines such as interferon-γ that sends signals to other immune cells such as $CD8^+$ killer cells and macrophage to proceed for antigen elimination. More recent studies have shown that naive $CD4^+$ T lymphocyte cells can also differentiate into Th17 cells that secret interleukin-17 which is associated with many autoimmune diseases including T1D and upregulates the expression of interferon-γ (Guberski et al., 1989; MacMurray et al., 2002).

$CD8^+$ T cells are believed to be pivotal for the initiation and development of insulitis. Mice without MHC class I molecules and $CD8^+$ T cells do not develop insulitis or T1D (Cito et al., 2018). The initial trigger for activation of T cells, although an ongoing debate, is insulin because a large mass of insulin specific CD4+ and CD8+ T cells were found in T1D mice. The decrease in T1D occurrence in perforin-deficient and Fas-deficient mice suggests the involvement of perforin-dependent and Fas/FasL pathway in T1D development (Kagi et al., 1996; Kagi et al., 1997).

2.3. Pathophysiology of T1D

Once the T1D develops, insufficient insulin in blood circulation blocks intracellular uptake of glucose and production of ATP, the energy form required for cellular functions. Consequently, the body starts to use fat as the energy source for maintaining the activities and at the same time exhibits the symptoms of polyphagia, glycosuria, polyuria, and polydipsia that are the major characteristics of T1D. Fatty acid metabolism to generate energy is a slower process and less efficient compared to glucose, which is the reason that most T1D patients feel hungry and sometimes become polyphagia. The glycosuria occurs when the kidney cannot reabsorb all of the glucose

from the blood, resulting in a high level of glucose in the urine. In addition, since glucose is osmotically active, it draws more water from the blood into the urine, leading to polyuria, and sequentially dehydration and polydipsia. Diabetic ketoacidosis, a life-threatening disease, occurs in about 30% of T1D patients. The development of diabetic ketoacidosis initiates when the body uses fatty acids as the energy source (Kitabchi and Wall, 1995). Fatty acids were metabolized in the liver to generate ketone bodies, resulting in an increase in blood acidity. As the blood gets more acidic, the patients develop Kussmaul respiration, a deep and labored breathing to move carbon dioxide out of the blood in an effort to reduce its acidity. Furthermore, H+/Na+ transporters move more hydrogen ions into the cells and more potassium out. As insulin is essential to activate the sodium-potassium ATPase and facilitate cellular entry of potassium, T1D patients develop hyperkalemia due to extracellular high concentration of sodium. With time, the diabetic ketoacidosis develops accompanied by naoses, vomiting, and if more severe, changes in mental status and acute cerebral edema.

Complications develop in the hyperglycemia environment, among which the sensory and autonomic neuropathy, angiopathy, and nephropathy are most common (Diabetes Control and Complications Trial Research Group, 1993). The neuropathy and nephropathy are caused by the angiopathy of the nerves' blood supplement vessels. The angiopathy is a microvascular disease where T1D patients develop the thickened wall of small arterioles and capillaries around the brain, kidney, retina, and peripheral nerves, leading to the ischemic changes in those vital organs (Wei et al., 1998). The mechanisms of angiopathy in T1D patients (Giacco and Brownlea, 2010) are believed to involve: 1) increased flux through the polyol pathway; 2) intracellular production of advanced

glycation end products precursors; 3) protein kinase C activation, and 4) increased hexosamine pathway activity. Hyperglycemia induces overproduction of superoxide – reactive oxygen species which inhibit the key glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase that is critical for 4 biochemical pathways (Du et al., 2003). Inhibition of glyceraldehyde-3 phosphate dehydrogenase or activation of these pathways exasperates the T1D complications. Damage at the cellular level due to insulin deficiency accumulates, leading to tissue damage and eventual organ failure and amputation. People with T1D, if not properly cared, die prematurely.

Chaper. 3 Current treatment for T1D

3.1. Insulin therapy

Because T1D is caused by insulin insufficiency, insulin supplement is the most common treatment for T1D patients. Nowadays, insulin used for insulin therapy is produced via recombinant DNA technique using *E. coli* expression system (Johnson, 1983). The insulin therapy involves administration of insulin by needle injection, insulin pen, and insulin pump.

3.1.1. Insulin injection

Insulin is a hydrophilic polypeptide that has a large molecular weight and high sensitivity to proteases. Subcutaneous injection is the most common method for insulin delivery in the clinic. On average, patients receive 3-4 insulin injections a day, one after each meal, to control the blood glucose level. It is a very effective method to control blood glucose level. The major drawback, however, is needle insertion associated pain that could become intolerable for some patients who need daily injections for a lifetime. In addition, the infection caused by a not well-sterilized needle also takes place. More importantly, it is difficult to control the insulin dose because blood glucose level often fluctuates depending on the type and the amount of food that patients take, often resulting in a temporary hyper- or hypo-glycemia (Selam, 2010).

3.1.2. Insulin pen

To decrease the pain of injection and risk of infection, the insulin pen was developed. The insulin pen looks like the traditional syringes, but it is used with the disposable needles to deliver the dose. Patients insert the needle into the skin and then press the button at the end of the pen to inject the insulin. They are more accurate and have lower risk of infection because of the safety features such as audible clicks with each dose to improve accuracy and reduce the chances of human errors (Selam et al., 2010; Penfornis et al., 2011). The Insulin pen is a much-improved product, but the price is significantly higher.

3.1.3. Insulin pump

In order to mimic how the pancreas works, insulin pumps are developed. Insulin pumps are small, computerized devices with a tube embedded under the skin to deliver insulin and to monitor the blood glucose changes through an attached biosensor (Selam et al., 2010). This device is capable of releasing insulin based on the blood glucose level. It improves the safety and accuracy of insulin therapy dramatically.

3.2. Transplantation-based therapy

To avoid the disadvantages of insulin therapy, the transplantation-based therapy gains more attention. Researchers tried to transplant the whole pancreas organ, the

pancreatic islet or β cells to patients to overcome hyperglycemia. Each approach shows advantages and also some limitations.

3.2.1. Whole pancreas organ transplantation

With the advances of surgical technologies and development of immunosuppression drugs, the success of transplantation of the whole pancreas has been improved. The transplanted pancreas is able to replace the dysfunctional pancreas to release insulin. About 90% of surgical cases showed that the successful allograft could last for a year, and the long-term studies showed the 5- and 10-year pancreas survival rate at 73% and 56%, respectively (Gruessner and Gruessner, 2016).

Transplantation of the pancreas needs a strong cardiovascular system to prevent the new organ from transplantation-associated complications. Usually, the surgeon connects the iliac vessels, portal (superior mesenteric vein) or systemic (iliac vein) venous drainage of the recipient, and enteric exocrine drainage of the donor's pancreas through an anastomosis between the donor duodenal segment and the recipient ileum (Sneddon et al., 2018) to supply the blood to the donor pancreas. The success rate of this surgical procedure is 90%-95%. The thrombosis of the pancreas allograft or leaks of pancreatic enzymes is the major cause of the failure of pancreatic transplantation. With successful transplantation, T1D patients become insulin-independent immediately. Studies have shown that transplanted pancreas functions stably for a long time and protect T1D patients against hyperglycemia-induced diseases such as retinopathy and neuropathy. A major

challenge facing pancreas transplantation, however, is the lack of sufficient donors that would match the need of a patient.

3.2.2. Pancreatic islet transplantation

Transplantation of isolated islets provides an alternative method for pancreas transplantation. The islet transplantation is performed via the portal vein of the liver. In a few cases, it is also performed intramuscularly (Dardenne et al. 2012). The procedure was first performed on a T1D patient (Shapiro et al., 2000). In the initial trial, all patients received 2 or 3 times of islet infusion and gained insulin independence and 3-year insulin independence is around 50%. The transplanted islets eventually lose their function due to the blood-mediated inflammatory reaction and ischemia among other mechanisms of immune rejections. Improvement has been reported that using immunosuppressive regiments that block immune co-stimulation (CTLA-4Ig, Belatacept) or leukocyte adhesion (anti-LFA1, Raptiva) increased the 3-year insulin independence rate close to 70%.

The transplantation of islet is an easier surgical procedure with a lower cost comparing to pancreas transplantation. However, insufficient islet donors still limit the applications of this promising treatment.

3.2.3. β cells replacement

It is the β cells that secrete insulin to maintain glucose homeostasis. Therefore, transplantation of β cells is considered as a more direct approach for

T1D treatment. This approach is based on our ability to produce a large number of cells in vitro. Major efforts in the past have been made in finding ways to prevent allograft β cells from immune response, including the alloimmune response and autoimmune response.

The immune system has the ability to distinguish self-antigens from non-self-antigens. Therefore, the transplanted β cells are recognized by the immune system via the indirect pathway and direct pathway (Liu et al., 1993). For the indirect pathway, after transplantation, the MHC on transplanted β cells can be taken up by the recipient antigen-presenting cells and then broken down into antigenic fragments that can activate T cells. For the direct pathway, the T cell receptors on the surface of T cells bind to MHC on the transplanted β cells to activate T cells (Liu et al., 1993). After activation, T cells recognize and attack the transplanted β cells leading to graft rejection. Immune suppressors are used to suppressing the activation and proliferation of T cells to protect transplanted β cells. However, immune-suppressing drugs not only prolong the survival of transplantation but also suppress patients' immune systems, which could make patients more vulnerable to infection and tumor development.

For T cell-mediated attack of β cells in patients with transplantation, the MHC I expressed on the surface of β cells are recognized by CD4+ T cells and CD8+ T cells. The activated CD4+ T cells release inflammatory factors or recruit immune cells to kill β cells. The activated CD8+ T cells, on the other hand, differentiate into effector cells to attack β cells (Wang et al., 1991). The regular immune suppression drugs show little effect on controlling the immune response

to β cells. The strategy that helps patients restore immune tolerance appears a promising strategy. Researchers also try to target the β cell-specific effector cells using a tolerogenic vaccine (Herold et al., 2002) or nanoparticle to halt the T cell-mediated autoimmune reaction.

Except for the immune rejection, the problem of the donor's insufficiency of β cells has always been a major bottleneck for cell-based treatment of T1D. Research has been conducted in the past decade to produce β like cells as the ultimate treatment of T1D. The strategy employed is commonly called cell reprogramming aiming at a change of gene expression profile of one type of cells to fit that of β cells for insulin release. The following sections summarize the progress in the area of cell reprogramming to provide sufficient β like cells for the treatment of T1D.

Chaper. 4 Generating β like and insulin producing cells by cell reprogramming

Cell reprogramming is a process to revert mature and specialized cells into a new type of cells with desirable function. It involves the erasure of the natural pattern of gene expression and the re-establishment of the epigenetic marker of designated cell types. The most common approach is to use stem cells or induced pluripotent cells (iPSCs) to differentiate into insulin-producing β like cells. Alternatively, differentiated somatic cells can develop β cell like properties once the gene expression profile of the cells is changed to mimic that of insulin-producing β cells. Different strategies and experimental conditions have been developed and tested to reprogram cells of different origins into β like cells for the treatment of T1D. Nucleus transfer to an embryonic cell with the original nucleus removed could also generate stem cells for targeted differentiation.

The concept of cell reprogramming was first developed in 1962 by John Gurgeon who focused on nucleus transfer to clone frogs (Lensch and Mummery, 2013). In 1988, Austin Smith developed the conditions needed for embryonic cell development and identified factors needed for maintaining pluripotency (Heath and Smith, 1988). In this early study, the authors reported suppression of the spontaneous differentiation of embryonic stem cells (ESCs) in vitro, demonstrating the feasibility to maintain ESCs in vitro. In 2006, Shinya Yamanaka successfully converted mature cells into immature cells and implantation of the converted cells into the body resulted in differentiated cells of many types (Takahashi and Yamanaka, 2006). The principles and methodologies developed in the early work served as guidance in the past 20 years in the field of cell reprogramming for generating β like and

insulin-producing cells. Depending on the starting cells, the efforts can be defined as stem cell-based and somatic cell-based cell reprogramming.

4.1. Stem cell-based cell reprogramming for β cells

4.1.1. Generating β like cells from embryonic stem cells (ESCs)

ESCs are undifferentiated, self-renewable, and highly proliferative cells. These properties make ESCs a great resource for generating not only β cells, but also other types of cells for treatment of any diseases caused by cellular dysfunction. In general, the human ESCs (hESCs) can be obtained from the inner cells of the blastula 4-5 days after fertilization (Godfrey et al., 2012). According to developmental biology, hESCs differentiate into a gastrula with 3 layers: ectoderm, endoderm, and mesoderm. The cells in the endoderm finally differentiate into the pancreas including β cells. By tracing the gene expression profile during the development of β cells, researchers identified a few important transcription factors that are called pancreatic markers including pancreatic and duodenal homeobox 1 (Pdx1), insulin gene enhancer protein, and forkhead box protein A2 (Jiang et al., 2007; Shim et al., 2007). By mimicking the expression pattern of these transcription factor genes during the natural process of development from hESCs to pancreatic into β cells, it is possible to specifically induce the hESCs to differentiate into β cells in vitro.

Studies have shown that hESCs can differentiate into insulin-positive cells spontaneously in vitro (Assady et al., 2001). Assady S. et al. separated and

cultured hESCs on the feeder layer of the culture of embryonic fibroblasts cells, making them spontaneously differentiated into the embryonic body with a similar profile of gene expression of β cells. Insulin secretion was detected, and the cells exhibited a similar profile of gene expression to that of β cells. The process involved hESCs differentiation into endoderm cells and then to insulin-producing cells (D'Amour et al., 2005). Unfortunately, the insulin release by the derived insulin-producing cells is not responsive to and dependent on glucose concentration. In addition, the derived cells tend to be polyhormonal because they secrete both insulin and glucagon. The function of the induced hESCs in vivo was studied by Kroon and colleagues (Kroon et al., 2008). By transforming hESCs into the pancreatic endodermal cells in vitro, the researchers implanted the induced hESCs into the immunocompromising mice with T1D (Kroon et al., 2008). Three months post-implantation, the human C peptide, a marker for insulin release, was detected. The blood C peptide and glucose levels achieved were similar to the levels of T1D mice with transplantation of pancreatic islet cells. These results suggest that the implanted hESCs had successfully differentiated into the insulin-producing cells in mice. When streptozotocin (STZ) was used to destroy the pancreatic β cells of the implanted mice, the C peptide and blood glucose level didn’t change significantly, suggesting that the implanted hESCs functions independent of the pancreas. Despite this exciting result showing potential for hESCs based therapy, progress in hESCs research has been slow due to government regulation prohibiting the use of human embryos in research.

4.1.2. Generating β like cells from induced pluripotent stem cells (iPSCs)

Because of the ethical issue regarding hESCs, induced pluripotent stem cells (iPSCs) were developed and they have provided an alternative approach to generate insulin-producing cells. iPSC technology was developed in 2006 by Shinya Yamanaka who converted mouse fibroblast cells into iPSCs by using retroviral vector-mediated transduction of fibroblast cells with 4 transcription factor genes including Oct3/4, Sox2, Klf4, and c-Myc (Takahashi and Yamanaka, 2006). The converted iPSCs showed similar properties to those of hESCs. Later studies demonstrated successful production of iPSCs without c-Myc although the efficiency was lower (Nakagawa et al., 2008).

Many animal experiments have been conducted demonstrating the feasibility of differentiating the iPSCs to β like cells. Almost all studies have observed successful conversion of iPSCs to insulin-positive cells, but without achieving glucose-induced insulin release. A standard protocol for differentiation includes the culture of iPSCs in media containing serum, activin A or retinoic acid (RA) for a fixed time period. The generated iPSCs-β cells appear capable of secreting insulin with intracellular calcium concentration similar to that of natural β cells and expression of Pdx1 and Nkx6.1, two markers for mature β cells. Implantation of iPSCs-β cells into immunosuppressed mice resulted in insulin secretion and reduction of blood glucose level from 600 mg/dL to 200 mg/dL (Pagliuca et al., 2014). Insulin secretion was long-lasting in these mice as insulin level remained high 18 months after the implantation. Small molecules were also used to induce targeted differentiation of iPSCs to β like cells with high

efficiency. Scientists treated the iPSCs with activin A and glycogen synthase kinase 3β inhibitor to induce differentiation from iPSCs to endodermal cells. The treated cells were incubated with RA and transforming growth factor-β (TGF-β) inhibitor to drive differentiation toward pancreatic progenitor cells. The final step involved the culture of the differentiated cells in a media containing forskolin, dexamethasone, and a TGF-β inhibitor (Kunisada et al., 2012). This procedure generated more than 10% conversion of iPSCs into insulin-producing cells.

A major advantage for iPSCs-based cell reprogramming into β cells is avoidance of allograft rejection because the iPSCs can be derived from patient own cells. However, the autoimmune response originally developed against β cells in T1D patients appears still functional in attacking iPSC-β cells. Different strategies have been used to suppress the immune response and prolong the survival of iPSCs-β cells. It has been shown in animal studies that immune suppressors are highly effective in protecting iPSC-β cells. Devices that are designed to cover the iPSCs-β cells and to protect them from the T-cell mediated cytotoxicity were also evaluated and proven effective (Basta et al., 2011). One major limitation of the device-based approach is a low exchange rate of glucose and insulin between the interior and exterior of the device although the shell is permeable to small molecules such as insulin and glucose. Chen. T and colleagues have shown that inclusion of stromal cell-derived factor 1, a specific immune suppressor against activation of T cells, to the surface of the alginic capsules inhibited immune rejection of iPSCs-β cells and preserved insulin secretion

function with prolonged survival time of the transplanted iPSCs-β cells in mice (Chen et al., 2015).

4.1.3. Generating β like cells from germline stem cells

Oogonia and spermatogonia serve as good resources for generating β cells. There are two methods to develop oogonia derived β cells. The first one is to convert oogonia into pluripotent stem cells via nucleus transfer as demonstrated by Yamada and colleagues (Yamada et al., 2014). The study confirmed that ESCs with nucleus transfer were capable of expressing the markers of pluripotent cells and differentiating into three layers of the gastrula. The ESCs with nucleus transfer could also be stimulated and differentiated into the Pdx1 positive pancreatic precursor cells and insulin-producing cells by treatment of activin A and RA in vitro. The second method is to obtain the female germline stem cells from the ovaries and then culture them to generate stem-like cells. Wang H. et al. cultured the female germline stem cells in a special medium for embryonic stem cells and successfully produced the female embryonic stem-like cells which are confirmed by the expression of stem cell-specific marker genes and the formation of the teratoma (Wang et al., 2014). Conversion of the female embryonic stem-like cells into β cells is achieved by a similar approach employed for iPSCs reprogramming into β cells.

Spermatogonium stem cells (SSC) have also been explored. They locate at the basement membrane of the seminiferous tubules in the testes and are easily accessible. Animal studies have shown successful conversion of SSC into iPS like

cells in a medium containing glial cell-derived neurotrophic factor, leukemia inhibitory factor, epidermal growth factor, and basic fibroblast growth factor (Kanatsu-Shinohara et al., 2004). The converted cells have the ability to differentiate into any layer of the gastrula in mice. Golestaneh N, et al. tested this approach in humans. Their results showed a successful generation of human testis-derived embryonic stem-like cells (htESLCs) with high similarity to iPSCs (Golestaneh et al., 2009). The condition that induced the differentiation from htESLCs to IP cells contained 10μM all-trans-retinoic acid, followed by 2nd culture in a plate for 2 days with RA and 2 weeks of maturation period without RA. Insulin release was detected in the differentiated cells, but not in glucose concentration-dependent manner.

The pluripotency of htESLCs has not been fully established so far. Teratoma developed from htESLCs appears smaller than the ones developed from iPSCs. Expression of pluripotent marker genes in htESLCs is significantly fewer in numbers than in iPSCs, suggesting that the htESLCs are not truly pluripotent (Chikhovskaya et al. 2012). Results from gene chip assay suggest that htESLCs are more like mesenchymal stem cells. Furthermore, it has been shown that the formation of htESLCs is age dependent. Ko K et al. have shown successful conversion of the SSC from young mice into htESLCs but not from adult mice (Ko et al., 2009).

4.1.4. Generating β cells from mesenchymal stem cells

Mesenchymal stem cells (MSCs) exist in many tissues and are self-renewed. MSCs have been derived from adipose tissue and bone marrow with low immunogenicity. Efforts have been made to generate β like cells using MSCs for potential treatment of T1D.

Timper, K. et al. were the first to derive MSCs from the adipose tissue and demonstrated that the adipose-derived MSCs (AD-MSCs) were able to differentiate into the insulin producing cells with expression of the pancreatic marker genes such as Pdx1 (Timper et al., 2006). Xie, Q. et al. reported induction of human bone marrow derived MSCs into β cells capable of reversing the hyperglycemia in T1D (Xie et al., 2009). In the study, bone marrow derived MSCs were isolated from 12 human donors, characterized, and implanted into immune compromised T1D mice. The results showed that the implanted bone marrow derived MSCs differentiated into insulin producing cells to normalize glucose concentration in T1D mice. There was no difference in gene expression profile between BM-MSC derived insulin producing cells and pancreatic β cells.

AD-MSCs when implanted into mice elevated insulin level and decreased blood glucose concentration (Bassi et al., 2012). Unfortunately, the therapeutic effect was short lived. Nine weeks post the implantation, the blood glucose began to increase and reached the highest level at the twelfth week. An important beneficial effect of AD-MSCs implantation observed was protection of pancreatic β cells. Compared to control animals, AD-MSC implantation decreased inflammatory infiltration into pancreas and inhibited $CD4^+$ Th1 cells that are part

of autoimmune attack against β cells. The level of protective regulatory T cells and TGF-β1 were also elevated, confirming the anti-inflammatory activity of AD-MSCs. Mechanistic study by Kono et al. using human AD-MSCs revealed an increased expression of metallopeptidase inhibitor 1 gene when AD-MSCs were co-cultured with pancreatic islets (Kono et al., 2014).

The comparison of characteristics of different cells investigated is shown in Table 1.

Table 1. β cells derived from different types of stem cells

Cell Type	**Protocol**	**Pros**	**Cons**	**Ref**
hESCs	Sequentially expose the hESCs to serum, activin A, and RA, *in vitro*	Relatively simple process; stable function of generated β-like cells	Autoimmune response; safety issue; immature β cells; allograft rejection; ethical issue	Shim et al., 2007
iPSCs	Sequentially expose the iPSCs to activin A and RA, *in vitro*	No allograft rejection; less ethical issue	Autoimmune response; safety issue; immature β cells; complex process; ethical issue	Pilia et al., 2011
Oogonia derived-fESLCs	Exposed to activin A and RA, *in vitro*	No allograft rejection; no ethical issue; ease of obtaining; abundant resources	Autoimmune response; safety issue; immature β cells	Wang et al., 2014
SSC derived-htESLCs	Expose the htESLCs to RA, and culture 2 weeks without RA, *in vitro*	No allograft rejection; no ethical issue; ease of obtaining; abundant resources	The successful rate is related with age; autoimmune response; safety issue; immature β cells	Golestaneh et al., 2009
AD-MSC	Spontaneous differentiation, *in vivo*; or function as immunoregulator	No allograft rejection; abundant resources; ease of obtaining; omit immunosuppression	Immature β cells	Xie et al., 2009; Zhao et al., 2012

4.2. Somatic cell-based cell reprogramming for generating β cells

The use of somatic cells to generate β like cells has been considered for treatment of T1D. Compared to stem cells, somatic cells are easier to be obtained and have less risk of becoming tumor cells, and simpler process for large scale production. A process of converting one type of cell to another is called cell reprogramming or transdifferentiation. It is normally achieved by delivery of pancreatic pivotal genes into non β cells using viral or non-viral vectors. In principle, any type of cells has the potential to be converted into insulin-producing β like cells if appropriate set of genes is up- or down-regulated. Transdifferentiation is reversible when β cell specific gene expression is lost.

4.2.1. Selection of the proper cell types for cell reprogramming

Cell lineage in tissue development is a significant factor to be considered for the selection of cell types for cell reprogramming. The cells being close to pancreatic β cells in the pancreas or those sharing the same lineage during the development of pancreatic β cells are the most appropriate resources for generating β like cells. Usually, the pancreas consists of endocrine and exocrine components, and in mammals, it contains three types of cells: the ductal cells, the acinar cells, and the endocrine cells. In humans, the endocrine cells contain 4 types of cells: the α cells expressing glucagon, the β cells expressing insulin, the δ cells expressing somatostatin, and the pancreatic polypeptide cells expressing pancreatic peptide. For all adult human islet cells, the β cells account for around 50%, the α cells account for around 40%, the δ cells account for around 10%, and

the pancreatic polypeptide cells account for a few (Steiner et al., 2010). The acinar cells and α cells are the most studied cell types for reprogramming into pancreatic β cells.

The pancreas develops from the endoderm layer of the gastrula, which is the same as the gut. It is observed that the pancreas is a part of the duodenum in the early stage of embryonic development. The exocrine cells and endocrine cells develop from ductal cells that connect the pancreas and intestine. Then the endocrine cells aggregate to form the islet of Langerhans, and the exocrine cells aggregate to form the exocrine part of the pancreas. These two parts connect with gut and gut rotation brings them close to each other, and finally, they form the fully functional pancreas. Therefore, intestinal cells and other cells developed from the foregut progenitor could also be good resources for generating β cells.

4.2.2. Finding the proper factors for cell reprogramming

Lineage tracing was used for understanding the development of the pancreas, especially the gene expression profiles of each specific type of pancreatic cells. Branda et al. used site-specific recombinase systems to knock out the target gene to moderate the gene expression and the results showed that only progenitors that expressed Pdx1 and/or pancreas transcription factor 1 subunit alpha (Ptf1A) differentiated into the pancreas. Among these progenitors, only those expressed neurogenin3 (ngn3) differentiated into islet cells (Branda and Dymecki, 2004).

Pdx1 and Ptf1A gene expression facilitates the development of the pancreas. In gastrula, cells expressing Pdx1 facilitate the formation of all 3 types of pancreatic cells: exocrine, endocrine, and duct cells (Gu et al., 2002). Overexpression of Pdx1 in the embryo increases the development of pancreatic cells. However, inhibiting Pdx1 causes pancreas damage (Li et al., 1999). For the function of Ptf1A, researchers showed that Ptf1A mutation resulted in abnormal pancreatic development and diabetes (Sellick et al., 2004). Fukuda A et al. concluded from their study that the dosage of Ptf1A is critical for the pancreas growth, β cell number and mass, and endocrine function. Decreased Ptf1A expression inhibits the development of the pancreas and secretion of insulin (Fukuda et al., 2008).

Ngn3 is a transcription factor of a basic helix–loop–helix family, and it is important for the differentiation of neural precursor cells in the neuroectoderm (Gradwohl et al., 2000). In both pancreas and gastrointestinal endocrine cells, ngn3 is necessary for differentiation from the multipotent endoderm stem cells (Jenny et al., 2002). Gradwohl, G. et al. studied the function of ngn3 in the development of pancreatic endocrine cells. Their results demonstrated the increasing in ngn3 expression when the first glucagon-producing cell was detected, and a lower ngn3 level with time till none ngn3 labeled cells were detected in the birth of adult pancreas. Transgenic mice with ngn3-deletion showed missing islets in Langerhans structure with a high glucose level in blood and urine. These results suggest that ngn3 is required for the differentiation of endocrine precursors.

In addition to Pdx1 and/or Prt1a and ngn3, differentiation of pancreatic β-cells appears to be regulated by many other factors. It was reported that there is a network of transcription factors involved in further differentiation of each islet-specific cell. The transcription factors include nueroD (Naya et al., 1997), the paired and homeodomain genes paired box 4 (Pax4) (Sosa-Pineda et al., 1997) and 6 (Pax6) (St-Onge et al., 1997), Pdx1 (Offield et al., 1996), Nkx6.1 (Øster et al., 1998), Nkx2.2 (Sussel et al., 1998), and members of Maf family. Maf protein functions in later developmental and maturation stages of pancreatic endocrine cells (Artner et al., 2010; Nishimura et al., 2006). In the early stage of β cells, there is a lot of MAF BZIP transcription factor B (MafB) expressed, however in matured β cells, MAF BZIP transcription factor A (MafA) increases expression and replaces MafB, suggesting that the β cells would be affected by MafB during development and by MafA in the adult stage. Research indeed shows that MafB can regulate the expression of genes important for the function of β cells, including glucose sensing and hormone processing during the development, and the MafA maintains the expression of those genes in adults.

The overall development process of pancreatic β cells and the transcription factors involved in regulating the differentiation are shown in Fig. 3.

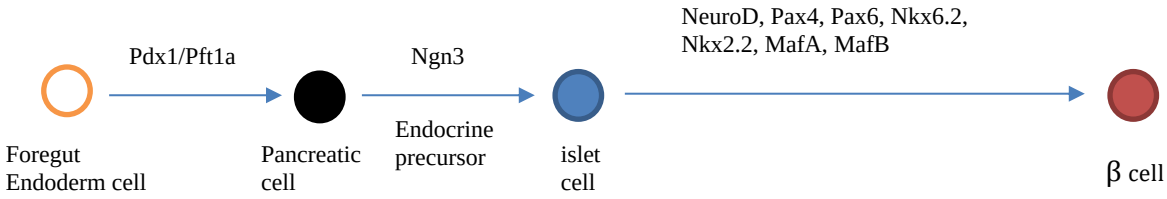

Fig. 3. Critical genes involved in development of pancreatic β cells

4.2.3. Acinar cell-derived β cells

The acinar cell is a good resource for cell reprogramming to generate β cells because acinar cells and β cells develop from similar tracts and locate nearby in the pancreas. Focusing on acinar cells, researchers did many studies to prove the capacity of acinar cells to be converted into β cells. For example, Rooman et al. treated diabetic mice with epidermal growth factor and gastrin and restored the function of β cells (Rooman and Bouwens, 2004). In a separate study, Zhou and Melton converted mouse acinar cells into β cells by overexpression of Ngn3, Pdx1, and MafA via viral transduction (Zhou et al., 2007). Successful conversion of acinar cells into β like cells was confirmed by expression of β cell marker genes for Nkx6.1, Glut2 and glucokinase, and loss of acinar specific marker gene of Ptf1a. With the previous studies, Clayton and colleagues used the transgenic mice that could express MafA, Pdx1 and Neurog3 in pancreatic acinar cells. The results showed that the overexpression of Neurog3 could lead to the generation of acinar-to-ductal cells, and with either reduced expression of Neurog3 or limited reprogramming induced inflammation could lead to the generation of acinar-to-β cells (Clayton et al., 2016). However, after stopping the expression of those transcription factors, the generated cells cannot maintain their functions in T1D mice models. Except for using transcription factors, the cytokines were also used to stimulate the conversion from acinar cells to β cells. Baeyens et al. using a combination of epidermal growth factor and ciliary neurotrophic factor demonstrates that cytokines were also able to induce the acinar cell to differentiate into β cells (Baeyens et al., 2014). The epidermal growth factor acts

via the same receptor as TGF-α, and functions as a growth factor as well as gastrin. The function of the ciliary neurotrophic factor is to reduce inflammation-induced tissue damage. The more detailed mechanistic study revealed that the ciliary neurotrophic factor protects pancreatic β cells against inflammation-induced apoptosis (Rezende et al., 2009). Direct infusion into T1D mice of epidermal growth factor and ciliary neurotrophic factor using an osmotic pump for a period of 8 months resulted in restored β cell mass and function, and the amelioration of hyperglycemia.

4.2.4. α cell-derived β cells

The α cell is very similar to the β cell because both of them are endocrine cells and locate in proximity. These make α cells a good resource for generating β cells. In 2009, Collombat, P. et al. studied the role of Pax4 in the development of β cells. They found ectopic expression of Pax4 can force endocrine precursor cells or mature α cells into β cells and the new β cells can reverse the hyperglycemia in STZ-induced T1D mice (Collombat et al., 2009). In contrast, in the absence of Pax4, β cells cannot be matured, and the mass of β cells is decreased (Sosa-Pineda et al., 1997). The α cells are usually sufficient and they can self-proliferate, therefore using part of α cells to generate β cells will not affect patients' normal function of α cells. An earlier study showed that, when the β cells were ablated, α cells would spontaneously reprogram themselves into β cells and function as β cells in vivo (Thorel et al., 2010). Using transgenic mice with almost all β cells ablated, they confirmed the generation of new β cells with expression of β cell

marker genes of Pdx1, and Nkx6.1. In an animal study, Xiao et al. injected adeno-associated viral vectors carrying Pdx1 and MafA genes into the pancreas of STZ treated T1D mice. Normoglycemia was restored with a significant increase in β cell mass (Xiao et al., 2018). The lineage tracing experiment confirmed the origin of newly generated β cells was α cells. The survival time of new β like cells was around 4 months, much longer than the time achieved by β like cells derived from other cell types. In another research, it was reported that exposure to the GLP-1 could also induce the conversion from pancreatic α cells to the β cells. (Zhang et al., 2019) The STZ-induced T1D mice presented ameliorated hyperglycemia and restored β cells mass after the treatment of GLP-1, moreover, the gene expression profile of those new generated cells was similar with the original pancreatic β cells. In later study, the researchers used the GLP-1 receptor antagonist exendin to block the function of GLP-1, and the results showed the hyperglycemia and loss of β cells appeared again, which confirmed that the GLP-1 could induce the neogenesis of pancreatic β cells in T1D mice.

4.2.5. Intestine cell-derived β cells

In embryogenesis, intestine and pancreas are derived from the same part of gastrulation: endoderm. Therefore, intestinal and pancreatic cells share many similarities in gene expression patterns. Converting intestinal cells to β cells is considered easier than other remote lineages of cells. Earlier studies identified the Notch signaling pathway most critical for development of intestinal or pancreatic cells. Overexpression of neurogenin 3 (ngn3), the downstream factor of the Notch

signaling pathway, leads to the pancreas phenotype (Apelqvist et al., 1999), and inhibiting the expression of helix-loop-helix 1 (Hes1) genes induces pancreatic hypoplasia. Long-term infusion of glucagon like peptide 1 (GLP-1), a gut hormone that activates the Notch signal pathway through the GLP1 receptor, increased the β cell mass in STZ induced T1D mice (Perfetti and Merkel, 2000). In addition, Suzuki, A. et al. showed GLP-1-induced conversion of intestinal progenitors to release insulin in glucose concentration-dependent manner (Suzuki et al., 2003). Similar results were reported by Duan F. et al. who used Lactobacillus gasseri bacteria to express GLP1 in the intestine and successfully detected insulin secretion by intestinal cells (Duan et al., 2015).

4.2.6. Hepatocyte-derived β cells

Hepatocyte development is similar to pancreatic β cells. Both are derived from the same endodermal progenitor cells and have similar glucose-sensing systems. Some studies showed that hepatocyte growth factors can protect β cells from death in T1D mice (Dai et al., 2003). Gene expression profiles between them are also similar. Yang, L. J. demonstrated that overexpression of Pdx1 in hepatocytes can convert the hepatocytes to the precursor of β like cells (Yang, 2006). With the activation of Pax4, the precursors differentiated into functional β cells. Maturation of β cells was achieved by stimulation from high glucose or implantation of hepatocytes into T1D mice. Non-viral vector-mediated delivery of Pdx1 and Ngn3 to the liver of STZ-induced T1D mice resulted in the alleviation of hyperglycemia (Wang et al., 2007). The glucose tolerance test and RT-PCR

results showed that the transduced liver cells secreted insulin in response to glucose and had a similar gene expression profile to that of β cells. A different study using adenoviral vectors showed that the strong immune reaction induced by adenoviral vectors is necessary for the generation of insulin-producing liver cells (Wang et al., 2007). By inspiration of those studies, in 2017 Cerdá-Esteban and colleagues started to unravel the molecular mechanism of transdifferentiation from hepatocytes to pancreatic cells and found that the Three-Amino-acid-Loop-Extension (TALE) homeobox TG-interacting factor 2 (TGIF2) played an important role in the developmental direction of endoderm progenitor cells. They successfully converted the liver cells into β cells by regulating the expression of Tgif2 gene both in vivo, and in vitro. The results showed that the expression of Tgif2 in hepatocytes made a large part of hepatocytes convert into pancreatic β cells. Moreover, the lineage tracing presented that the hepatocytes original features disappeared and the phenotype of pancreatic β cells could be detected. (Cerdá-Esteban et al., 2017) Even though there were many successful hepatocytes-to-β cell, the conversion rate was still low, which limited the efficiency of transdiffrentiation. A scientific group investigated the efficiency of each distinct hepatocytes subpopulation and demonstrated that the Wnt signaling could predispose the hepatocytes cells to increase the conversion rate. This research made a great progress for the clinical application of transdifferentiation in the future. (Cohen et al., 2018)

4.2.7. Biliary epithelium cell-derived β cells

In gastrulation, the biliary system and pancreas are both developed from the foregut of endoderm. For differentiation of the biliary system and pancreas, the Hes1 gene plays an important role. Hes1 gene encodes the basic Hes1 protein that can repress the expression of neurog3, a transcription factor that stimulates differentiation of pancreatic endocrine cells. Hes1 functions as a negative regulator of the differentiation of endodermal endocrine. In Hes1 deficient mice, the differentiation of endodermal endocrine is accelerated. Therefore, biliary epithelial cells are considered for reprogramming into β cells by manipulating Hes1 gene expression. Using Hes1-/- mice, Sumazaki R et al. showed the presence of 4 types of endocrine cells in the biliary epithelium including α cells expressing glucagon, β cells expressing insulin, δ cells expressing somatostatin and pancreatic polypeptide cells expressing pancreatic peptide. Moreover, the results of the electronic microscope showed the secret vesicle structure in Hes1-/- mice biliary epithelium, which is a typical structure of pancreatic β cells (Sumazaki et al., 2004). These cells function like the β cells in the pancreas. In 2009, other researchers successfully converted the biliary epithelial cells in normal mice to the pancreatic β cells by inhibiting activity of Hes1. The results showed after the inhabitation, the biliary epithelial-to-β cells could produce insulin and the pancreatic marker Pdx1, meanwhile the markers of biliary epithelial cells disappeared with time. (Coad et al., 2009)

Although the results of many related studies meet researchers' expectations, more studies are needed to achieve long term function of the reprogrammed β cells and to determine whether the derived cells are immune tolerant. Evidence does exist in support of a long-term insulin production, but more detailed mechanistic studies need to be conducted. Among all protocols developed, the α-to-β cells are most promising as they showed the longest survival up to 4 months (Xiao et al., 2018). The acinar-to-β cells protocol that involved the osmotic pump to continuously release the EGF and CNTF (Baeyens et al., 2014) was also promising. The hepatocytes-to-β cells have also attracted attention in the field.

A comparison of characteristics of different somatic cells investigated is shown in Table 2.

Table 2. Somatic cells studied for conversion into β cells

Cell Type	**Protocol**	**Ref**
Acinar cells	Ngn3, Pdx1, and MafA directed reprogramming, *in vitro*; Infusion of EGF and CNTF by osmatic pump to convert the acinar cells, *in vivo*; MafA, Pdx1 and Neurog3 directed reprogramming, *in vivo*	Zhou et al., 2007; Baeyens et al., 2014; Clayton et al., 2016
α cells	Pdx1 and MafA directed reprogramming, *in vivo*; GLP-1 directed reprogramming, *in vivo*	Xiao et al., 2018; Zhang et al., 2019
Intestine epithelial cells	GLP-1 directed reprogramming, *in vitro*; Oral administration of Lactobacillus gasseri bacteria that can express GLP1 to T1D mice, *in vivo*	Suzuki et al., 2003; Duan et al., 2015
Hepatocyte	Pdx1 and ngn3 directed reprogramming, *in vivo*; Tgif2 directed reprogramming, *in vivo and vitro*; Wnt signaling directed reprogramming, *in vivo*	Wang et al., 2007; Cerdá-Esteban et al., 2017; Cohen et al., 2018
Biliary epithelium cells	Ablate almost all the β cells in Hes1-deficient mice to convert the biliary epithelium cells, *in vivo*; Inhibiting the activity of Hes-1 gene converts the biliary epithelial cells, *in vivo*	Sumazaki et al., 2004; Coad et al., 2009

Chaper. 5 Clinical applications of cell reprograming for treatment of T1D

Clinical evaluation of the established strategies in preclinical studies has been conducted. The primary focus has been on stem cell-derived β like cells such as hESCs/iPSCs derived β cells, MSCs- and hematopoietic stem cells-derived insulin-producing cells. The first clinical trial involved transplantation of autologous nonmyeloablative hematopoietic stem cells into T1D patients and was conducted in 2007 (Voltarelli et al., 2007). Fifteen T1D patients received transplantation of differentiated cells in combination with immunosuppression therapy to protect the transplanted cells. In the 36-month follow up studies, 14 out of 15 patients became insulin independence and the C-peptide level showed a significant increase compared to the initial level before the treatment began. Even though the results showed a good efficiency of transplantation of autologous nonmyeloablative hematopoietic stem cells as a treatment for T1D, the adverse effects caused by immunosuppression therapy, the limitations for lack of control group, the small sample size, and short follow-up period were identified as the shortfalls of the first trial. Therefore, two years later, another clinical study (Couri et al., 2009) was performed, which included the previously tested 15 patients and 8 new patients. The period of follow-up was extended to 4 years. In this study, the insulin and C-peptide level significantly increased in all patients and 20 out of 23 patients became insulin independence. The time for insulin independence increased compared to that of the previous study. These results support the notion that stem cell-derived β like cells have excellent potential for treatment of T1D. Zhang and colleagues conducted a clinical trial involving 9 T1D patients to investigate the potential mechanism of

autologous nonmyeloablative hematopoietic stem cells improving β cells' function (Zhang et al., 2012). They showed that the transplanted autologous nonmyeloablative hematopoietic stem cells helped T1D patients eliminate the islet specific autoreactive T cells, suggesting that the hematopoietic stem cells provided immune suppression against β cells.

After these early encouraging clinic results, additional clinical trials were conducted using different stem cells derived cells. In a study by Liu, et al., $2x10^7$ stem cells (with high expression of CD33 and CD34) from the neonatal amniotic membrane were injected into the pancreatic dorsal artery of the newborn baby's T1D father through the left femoral artery (Liu et al., 2013). Three months post-transplantation, the patient become independent on insulin therapy, and the stable blood glucose level lasted for 6.2 months. During the 36-month follow-up, the blood glucose level was in normal range. This data indicated that implanted amniotic stem cells could improve the islet-cell function in response to blood glucose in T1D patients. However, the survival time of the β like cells was relatively short when immune suppression drugs were not used. In a different study, Dave et al. co-injected AD-MSCs derived insulin-producing cells with hematopoietic stem cell to one T1D patient and observed normalized blood glucose level (Dave et al., 2013). In a single patient study, a 30-year-old man with a history of T1D for 15 years and at the end-stage of renal disease received renal transplantation coupled with injection of insulin-producing stem cells. During the 13 months follow-up, the graft showed stable function and the blood glucose level was at the normal range. In a separate study involving 20 T1D patients (Thakkar et al., 2015), AD-MSCs derived insulin-producing cells and bone marrow derived hematopoietic stem cells was injected (Thakkar et al., 2015). Patients were evenly divided into 2 groups. Patients in group 1 received autologous stem cells, and patients in group 2 received allogenic stem cells.

Patients in both groups showed improvement in blood glucose concentration but patients infused with autologous AD-MSCs derived insulin-producing cells exhibited a longer control of the hyperglycemia. The timeline of the clinical studies involving transporting the stem cells or stem cell-derived insulin-producing cells is shown in Fig. 4.

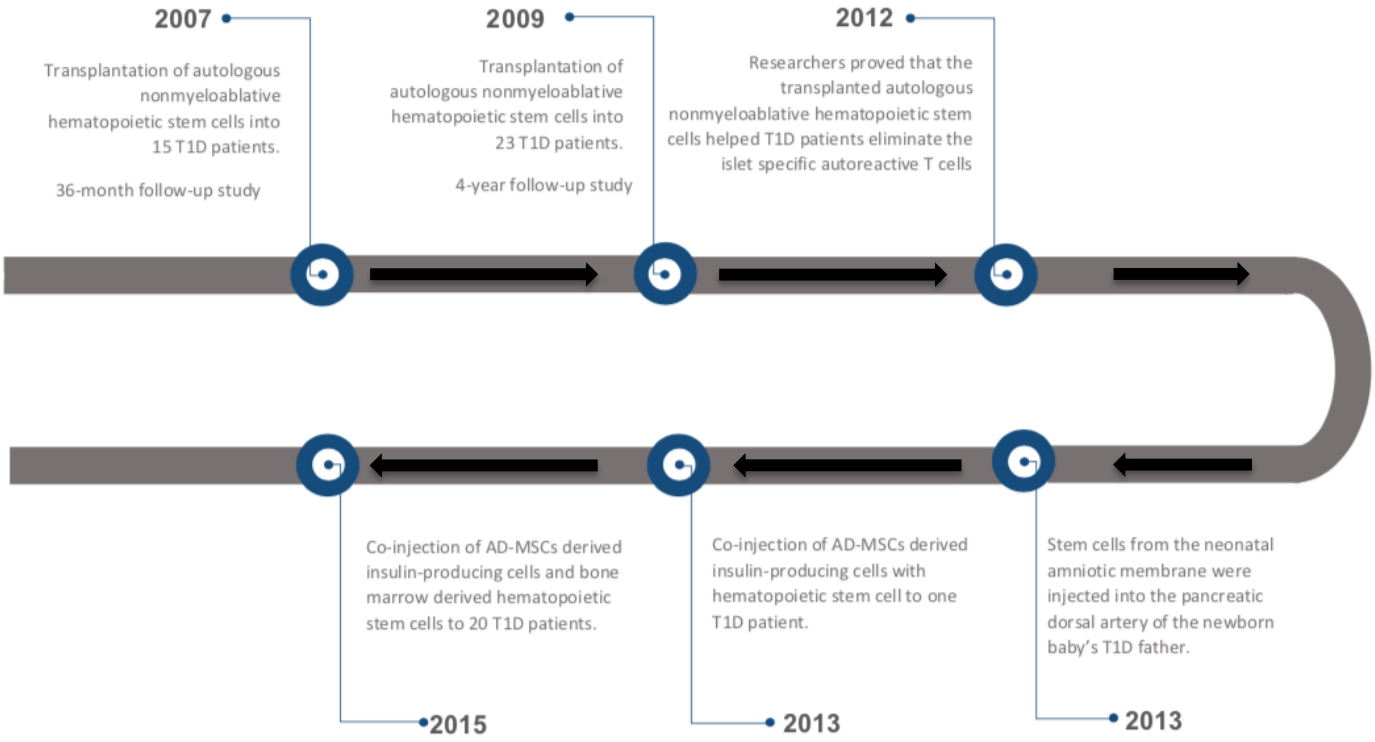

Fig. 4 The Timeline of the Stem Cell-based Clinical Studies

Results from clinical trials of stem cells-based treatment of T1D clearly show a need to protect the implanted cells against immune rejection. Except for the use of immune suppressors or co-injection of MCS cells with β cells, a strategy of encapsulation of β like cells into a semipermeable membrane to protect cells from immune attack has been explored. The approaches have also been approved for clinical trials and showed delight. The most advanced microencapsulation system is a device called VC-01TM that encapsulates hESCs-

derived β cells to isolate the cells from the immune environment. A phase ½ clinical study was conducted to test the safety, tolerability and efficacy of this strategy in the U.S. and Canada in 2014 (Viacyte, 2014). Alginate is used to encapsulate allogeneic islets with a diameter in the range of 300 to 400μm. In this study, 69 T1D patients received the microencapsulated hESCs-derived pancreatic progenitors via subcutaneous implantation. This trial is still in progress and the final data is estimated to be reported around January 2021. This company started another clinical trial for testing the similar product called VC-02TM in 2017 and the phase 1 was completed in 2018 without any reported results. Ludwig et al. (Ludwig et al., 2013) have introduced an improved encapsulation procedure to improve the exchange of oxygen and nutrients of the earlier encapsulates. The new system called the βAir device containing generated islet cells was implanted into T1D patients and monitored for 8-months. Insulin secretion was detected in the treated patients and need for the exogenous insulin was reduced. Moreover, the immune response toward the transplant was detected. A new and microvasculature strategy was also developed. In the design there are two devices (the Theracyte device, and Sernova cell pouch). These two device systems have been approved for phase I/II trial and the results have not been reported (Pepper et al., 2015). Besides, the Stem Cell Educator Therapy is a new developed strategy to overcome the autoimmune response in T1D. This therapy uses an in vitro closed-loop system to separate the lymphocytes from the patients' blood and co-cultures the lymphocytes with certain stem cells and then returns the blood back to the patients. In 2012, Zhao and colleagues utilized the cord blood-derived multipotent stem cells to co-culture the lymphocytes from 15 T1D patients separately, and the results showed in the treated patients, the hyperglycemia was reversed and the regeneration of islet β cells was promoted (Zhao et al., 2012). Then the

same team investigated the safety and efficiency of this therapy in 20 children with T1D, and this clinical trial is still ongoing, and no more data have been reported yet.

The summary of active /completed clinic trials around the world for treatment of T1D by using different types of stem cells is shown in Table 3.

Table 3. Summary of active/completed clinical trials around the world for the treatment of T1D using stem cells.

NCT number[a]	Study title	Status	Treatment method
NCT01121029	Hematopoietic stem cell transplantation in type 1 diabetes mellitus	Completed (12/2012, results not posted)	IV infusion of in vitro cultured autologous hematopoietic stem cells
NCT00315133	Safety and efficacy study of autologous stem cell transplantation for early onset type 1 diabetes mellitus	Completed (12/2014, results not posted)	IV infusion of in vitro cultured bone marrow stem cells
NCT02239354	A safety, tolerability, and efficacy study of vc-01™ combination product in subjects with type 1 diabetes mellitus	Active, not recruiting	Subcutaneous implantation of VC-01TM microencapsulating hESCs-derived pancreatic progenitors
NCT03162926	A safety and tolerability study of vc-02™ combination product in subjects with type 1 diabetes mellitus	Completed (02/2018, results not posted)	Subcutaneous implantation of VC-02TM microencapsulating hESCs-derived pancreatic progenitors
NCT01350219	Stem cell educator therapy in type 1 diabetes	Recruiting	Co-culture the umbilical cord blood-derived multipotent stem cells with T1D patient's lymphocytes and then retune the lymphocytes back to the patient's circulation system
NCT00690066	Prochymal® (human adult stem cells) for the treatment of recently diagnosed type 1 diabetes mellitus (t1dm)	Completed (12/2011, results not posted)	IV infusion of ex vivo cultured human MSCs
NCT01068951	Treatment of patients with newly onset of type 1 diabetes with mesenchymal stem cells	Completed (09/2013)	IV infusion of autologous MSCs

NCT02940418	Use of stem cells in diabetes mellitus type 1	Recruiting	Two IV infusions of allogenic adipose derived-MSCs 6 months apart
NCT03484741	MSCs therapy for type 1 diabetes mellitus patients	Recruiting	IV infusion of autologous BM-MSCs, allogeneic UC-MSCs, and PRP
NCT03484741	MSCs administration for the management of type 1 diabetic patients	Active, not recruiting	IV infusion of allogeneic, ex vivo expanded MSCs

[a]Data taken from: https://clinicaltrials.gov.
NCT, National Clinical Trial; NCT number, an identification code given to each clinical study registered on ClinicalTrials.gov.

The somatic cell-based approach is at its early stage and till now, there is no clinical data available for the somatic cells derived β cells. However, the abundant resources and simple process of transdifferentiation make the somatic cell-based treatment a promising research field. In fact, progress has been made with very interesting and encouraging results. Duan and his colleagues provided patients, by oral administration, with Lactobacillus gasseri bacterial that are engineered to produce GLP1 and observed a successful conversion of intestinal epithelial cells into β-like cells (Duan et al., 2015). Although additional work is needed, these results show a great potential for high level of patient compliance because oral administration of an engineered bacterial for transdiffereniating intestinal cells into insulin generating cells is convenient comparing to other approaches often involving cell implantation.

Chaper. 6 Conclusions and future prospects

T1D is one of the oldest diseases in human history. There are 46 million T1D patients worldwide (Saeedi et al., 2019) and 1.3 million Americans suffer from this disease. T1D is caused by the loss of pancreatic β cells leading to deficiency of insulin, a peptide hormone critical for maintaining glucose homeostasis. T1D is considered an autoimmune disease due to the presence of anti- β cell antibodies and $CD4_+$ and $CD8_+$ cells in T1D patients. Genetic predisposition, although no specific genetic defect has been identified so far, and the environmental factors are believed to be responsible for the initiation of the immune attack on β cells. T1D associated symptoms include hyperglycemia, polyphagia, glycosuria, polyuria, polydipsia, and diabetic ketoacidosis persistent hyperglycemia, often leads to sensory and automatic neuropathy, angiopathy, nephropathy, amputation, organ failure, and premature death.

Significant efforts have been made in the past to reduce patients' need for daily insulin injection. Among the strategies explored so far, cell reprogramming to convert the non-insulin-producing cells to β like cells has been explored for its potential use in the treatment of T1D. Different types of cells have been explored as the potential source to generate β like cells including ESC cells, iPSCs, and somatic cells sharing the origin of differentiation into pancreatic β cells. The major advantage of the stem-cell-based approach is that stem cells are capable of self-renew and proliferation and differentiating into β like cells. Ethical concern on the use of human embryonic cells is the major limitation of clinical applications. The discovery of iPSCs has had a significant impact on cell reprogram research in general and

has advanced the field of β cell generation for T1D. As summarized in section 4.1 of this thesis, iPS cells have a great advantage in avoiding MHC-based immune rejection because cells from T1D patients can be used. Pluripotency induced by the introduction of Yamanaka factor genes, while essential for proliferation and differentiation, has been a concern on iPS due to possible change of iPSCs into tumor cells. It is possible that future research will lead to the elimination of such a possibility to make iPSCs the optimal choice for generating not only β like cells but also any other type of cells for the treatment of disease caused by dysfunctional cells.

The somatic cell-based cell reprogramming avoids the ethical and safety issues associated with stem cells. It can be performed directly in T1D patients by the introduction of proper genes into cells that share the same lineage as the pancreatic β cells. Successful conversion α cells to insulin-producing β cells have been achieved in animals by adeno-associated virus-mediated gene delivery to pancreatic α cells (Xiao et al., 2018). In the T1D mouse model, the β like cells were alive and continued insulin release for about 4 months before the insulin level returned to the background level. Additional work is needed to enhance the efficiency of delivering the right genes to enough number of cells that share the embryonic lineage of β cells including pancreatic α cells, epithelial and endothelial cells, intestinal epithelial cells or hepatocytes in the liver. Further research is also needed to achieve full regulation of insulin production and release in a glucose-dependent manner.

Progresses in preclinic studies have made it possible to evaluate different designs in clinic. Indeed, studies have been conducted to evaluate the feasibility of cell reprogramming approach for the treatment of T1D. As summarized in section 5, promising results have been obtained but additional work seems needed. One important finding is that immune response,

the initial cause of T1D as an autoimmune disease, remains active against β like cells regardless of their origin for differentiation. Such an observation was not anticipated when the cell re-programming approach was taken because it was generally believed that cells of different origins do not share exactly the antigens of β cells and insulin-producing β like cells generated will not be rejected immunologically. This observation has led the use of immune suppressor to prolong the survival of the β like cells either implanted or directly derived through gene transfer. Two approaches are currently pursued solving immune rejection problems, genetic engineering approach, and encapsulation of β like cells.

Genetic engineering is a technique to change the genetic composition of cells for a desirable phenotype. It is commonly achieved by insertion or knockout of the target element in a target cell or organism. Transporters (membrane transport/carrier proteins) are specialized membrane-spanning proteins that assist in the movement of ions, peptides, small molecules, lipids and macromolecules across a biological membrane.has been used to eliminate the MHC molecules from the membrane surface of hESCs (Lin et al., 2014) in order to block antigen presentation in the hESCs-derived β cells. The results show that hESCs-derived β cells are protected against autoimmune rejection. Elimination of both MHC class I and II from the hESCs also represents a very promising strategy solving the T-cell mediated immune rejection of reprogrammed β like cells (Young et al., 2004). More recent studies revealed that knockout the whole HLA genes that encode MHC molecules triggers a nature killer cells-mediated attack on β like cells (Sneddon et al., 2018). It seems necessary to knockout the selected HLA genes but keeps a balance between suppression of T-cell mediated immune rejection and nature killer cell-mediated elimination in order to achieve long-term survival of β like cells.

Cell encapsulation strategy has gained more attention recently because of its safety and efficacy. The technique involves the use of semi-permeable membranes made of biomaterials to encapsulate β like cells inside. The membrane used allows free diffusion of glucose and insulin but not the cytokines to avoid the allograft immune response and cell damage by antibodies or T-cells originally generated at the initial stage of T1D development. One advantage of this approach is protecting patients from the risk of cancer induced by the implanted hESCs derived β cells because abnormal cell growth in the capsule if occurs, can be stopped by physical removal of the cell containing capsules. The most advanced device for cell encapsulation is the βAir device developed by Beta-O2 the company capable of not only offering a selected exchange of glucose and insulin across the membrane but also providing exogenous oxygen to the implanted β like cells (Ludwig et al., 2013). This device contains two compartments, one for cell encapsulation and another as a gas chamber. These two compartments are separated by a gas-permeable membrane and the oxygen can diffuse through freely. The gas chamber is connected with a port that is implanted subcutaneously to fill oxygen to the chamber. In addition, enhancing the microvasculature strategy is also used to prolong the survival time of implanted cells. It is highly possible that the ongoing clinic test of this new technology will bring significant benefits to T1D patients making the reprogramming-based approach for T1D treatment a reality.

In summary, treatment of T1D by cells mimicking pancreatic β cells is in its early stage, but at present a lot of work is ongoing to improve the effectiveness of the techniques for directed differentiation into β like cells with prolonged survival after implantation, full responsiveness to glucose concentration, and avoidance of immune rejection. It is possible in a few years all the technical limitations will be solved and β like cells derived from either

stem or somatic cells become one of the most effective therapies to treat not only T1D but also diseases caused by dysfunctional cells.

REFERENCES

Almawi, W. Y., Tamim, H., and Azar, S. T. (1999). T helper type 1 and 2 cytokines mediate the onset and progression of type I (insulin-dependent) diabetes. *JCEM*, **84**: 1497-1502.

American Diabetes Association Position Statement (2017). 2. Classification and diagnosis of diabetes. *Diabetes Care*, **40**: 11-24.

Aoyagi, K., Ohara-Imaizumi, M., and Nagamatsu, S. (2011). Regulation of resident and newcomer insulin granules by calcium and SNARE proteins. *Front Biosci*, **16**: 1197-1210.

Apelqvist, Å., Li, H., Sommer, L., Beatus, P., Anderson, D. J., et al. (1999). Notch signaling controls pancreatic cell differentiation. *Nature*, **400**: 877-811.

Artner, I., Hang, Y., Mazur, M., Yamamoto, T., Guo, M., et al. (2010). MafA and MafB regulate genes critical to β-cells in a unique temporal manner. *J Diabetes*, **59**: 2530-2539.

Assady, S., Maor, G., Amit, M., Itskovitz-Eldor, J., Skorecki, K. L., et al. (2001). Insulin production by human embryonic stem cells. *J Diabetes*, **50**: 1691-1697.

Baeyens, L., Lemper, M., Leuckx, G., De Groef, S., Bonfanti, P., et al. (2014). Transient cytokine treatment induces acinar cell reprogramming and regenerates functional beta cell mass in diabetic mice. *Nat Biotechnol*, **32**: 76-83.

Bassi, Ê. J., Moraes-Vieira, P. M., Moreira-Sá, C. S., Almeida, D. C., Vieira, L. M., et al. (2012). Immune regulatory properties of allogeneic adipose-derived mesenchymal stem cells in the treatment of experimental autoimmune diabetes. *J Diabetes*, **61**: 2534-2545.

Basta, G., Montanucci, P., Luca, G., Boselli, C., Noya, G., et al. (2011). Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care*, **34**: 2406-2409.

Bottazzo, G., Florin-Christensen, A., and Doniach, D. (1974). Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *The Lancet*, **304**: 1279-1283.

Branda, C. S. and Dymecki, S. M. (2004). Talking about a revolution: The impact of site-specific recombinases on genetic analyses in mice. *Dev Cell*, **6**: 7-28.

Cerdá-Esteban, N., Naumann, H., Ruzittu, S., Mah, N., Pongrac, I. M., et al. (2017). Stepwise reprogramming of liver cells to a pancreas progenitor state by the transcriptional regulator Tgif2. *Nat Commun*, **8**: 1-13.

Chatenoud, L., Warncke, K., and Ziegler, A. G. (2012). Clinical immunologic interventions for the treatment of type 1 diabetes. *CSH Perspectives in Medicine*, https://doi.org/10.1101/cshperspect.a007716

Chen, T., Yuan, J., Duncanson, S., Hibert, M. L., Kodish, B. C., et al. (2015). Alginate encapsulant incorporating CXCL12 supports long-term allo-and xenoislet transplantation without systemic immune suppression. *Am J Transplant*, **15**: 618-627.

Chikhovskaya, J. V., Jonker, M. J., Meissner, A., Breit, T. M., Repping, S., et al. (2012). Human testis-derived embryonic stem cell-like cells are not pluripotent, but possess potential of mesenchymal progenitors. *Hum Reprod*, **27**: 210-221.

Christianson, S. W., Shultz, L. D. and Leiter, E. H. (1993) Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8 + T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *J Diabetes*, **42**: 44–55

Cito, M., Pellegrini, S., Piemonti, L., and Sordi, V. (2018). The potential and challenges of alternative sources of β cells for the cure of type 1 diabetes. *Endocr Connect*, **7**: 114-125.

Clayton, H. W., Osipovich, A. B., Stancill, J. S., Schneider, J. D., Vianna, P. G., et al. (2016). Pancreatic inflammation redirects acinar to β cell reprogramming. *Cell Rep*, **17**: 2028-2041.

Coad, R. A., Dutton, J. R., Tosh, D., and Slack, J. M. W. (2009). Inhibition of Hes1 activity in gall bladder epithelial cells promotes insulin expression and glucose responsiveness. *Biochem. Cell Biol*, **87**: *975–987.*

Cohen, H., Barash, H., Meivar-Levy, I., Molakandov, K., Ben-Shimon, M., et al. (2018). The Wnt/β-catenin pathway determines the predisposition and efficiency of liver-to-pancreas reprogramming. *J Hepatol*, **68**: 1589-1603.

Collombat, P., Xu, X., Ravassard, P., Sosa-Pineda, B., Dussaud, S., et al. (2009). The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells. *Cell*, **138**: 449-462.

Couri, C. E., Oliveira, M. C., Stracieri, A. B., Moraes, D. A., Pieroni, F., et al. (2009). C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*, **301**: 1573-1579.

D'Amour, K. A., Agulnick, A. D., Eliazer, S., Kelly, O. G., Kroon, E., et al. (2005). Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol*, **23**: 1534–1541.

Dai, C., Li, Y., Yang, J., and Liu, Y. (2003). Hepatocyte growth factor preserves beta cell mass and mitigates hyperglycemia in streptozotocin-induced diabetic mice. *J Biol Chem*, **278**: 27080-27087.

Dardenne, S., Sterkers, A., Leroy, C., Da Mata, L., Zerbib, P., et al. (2012). Laparoscopic spleen-preserving distal pancreatectomy followed by intramuscular autologous islet transplantation for traumatic pancreatic transection in a young adult. *JOP*, **13**: 285–288

Dave, S. D., Vanikar, A. V., and Trivedi, H. L. (2013). Co-infusion of adipose tissue derived mesenchymal stem cell-differentiated insulin-making cells and hematopoietic cells with renal transplantation: a novel therapy for type 1 diabetes mellitus with end-stage renal disease. *BMJ Case Rep.*, **2013**: 009901. https://doi.org/10.1136/bcr-2013-009901

Diabetes Control and Complications Trial Research Group. (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med*, **329**: 977-986.

Du, X., Matsumura, T., Edelstein, D., Rossetti, L., Zsengellér, Z., et al. (2003). Inhibition of GAPDH activity by poly (ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest*, **112**: 1049-1057.

Duan, F. F., Liu, J. H., and March, J. C. (2015). Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. *J Diabetes*, **64**: 1794-1803.

Efrat, S. (1997). Making sense of glucose sensing. *Nat Genet*, **17**: 249-250.

Filippi, C. M., and von Herrath, M. G. (2008). Viral trigger for type 1 diabetes: pros and cons. *J Diabetes*, **57**: 2863-2871.

Foulis, A. K., Farquharson, M. A., and Hardman, R. (1987). Aberrant expression of class II major histocompatibility complex molecules by B cells and hyperexpression of class I major histocompatibility complex molecules by insulin containing islets in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia*, **30**: 333-343.

Fukuda, A., Kawaguchi, Y., Furuyama, K., Kodama, S., Horiguchi, M., et al. (2008). Reduction of Ptf1a gene dosage causes pancreatic hypoplasia and diabetes in mice. *J Diabetes*, **57**: 2421-2431.

Giacco, F., and Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circ Res*, **107**: 1058–1070.

Gill, R. G., and Haskins, K., (1993). Molecular mechanisms underlying diabetes and other autoimmune diseases. *Immunol Today*, **14**: 49-51.

Godfrey, K. J., Mathew, B., Bulman, J. C., Shah, O., Clement, S., et al. (2012). Stem cell-based treatments for Type 1 diabetes mellitus: bone marrow, embryonic, hepatic, pancreatic and induced pluripotent stem cells. *Diabet Med*, **29**: 14-23.

Golestaneh, N., Kokkinaki, M., Pant, D., Jiang, J., DeStefano, D., et al. (2009). Pluripotent stem cells derived from adult human testes. *Stem Cells Dev*, **18**: 1115-1125.

Gradwohl, G., Dierich, A., LeMeur, M., and Guillemot, F. (2000). neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *PNAS*, **97**: 1607–1611.

Gruessner, A.C., and Gruessner, R.W.G. (2016). Long-term outcome after pancreas transplantation: a registry analysis. *Curr Opin Organ Transplant*, **21**: 377–385.

Gu, G., Dubauskaite, J., and Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development*, **129**: 2447-2457.

Guberski, D. L., Butler, L., Kastern, W., and Like, A. A. (1989). Genetic studies in inbred BB/Wor rats: analysis of progeny produced by crossing lymphopenic diabetes-prone rats with nonlymphopenic diabetic rats. *J Diabetes*, **38**: 887-893.

Heath, J. K., and Smith, A. G. (1988). Regulatory factors of embryonic stem cells. *J Cell Sci*, **1988**: 257-266.

Herold, K. C., Hagopian, W., Auger, J. A., Poumian-Ruiz, E., Taylor, L., et al. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*, **346**: 1692-1698.

Jenny, M., Uhl, C., Roche, C., Duluc, I., Guillermin, V., et al. (2002). Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *The EMBO Journal*, **21**: 6338-6347.

Jiang, J., Au, M., Lu, K., Eshpeter, A., Korbutt, G., et al. (2007). Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells*, **25**: 1940-1953.

Johnson, I. S. (1983). Human insulin from recombinant DNA technology. *Science*, **219**: 632-637.

Jun, H. S., and Yoon, J. W. (2004). A new look at viruses in type 1 diabetes. *ILAR Journal*, **45**: 349-374.

Kagi, D., Odermatt, B., Ohashi, P. S., Zinkernagel, R. M., and Hengartner, H. (1996). Development of insulitis without diabetes in transgenic mice lacking perforin-dependent cytotoxicity. *J Exp Med*, **183**: 2143–2152.

Kagi, D., Odermatt, B., Seiler, P., Zinkernagel, R. M., Mak, T. W., et al. (1997). Reduced incidence and delayed onset of diabetes in perforin-deficient nonobese diabetic mice. *J Exp Med*, **186**: 989–997.

Kanatsu-Shinohara, M., Inoue, K., Lee, J., Yoshimoto, M., Ogonuki, N., et al. (2004). Generation of pluripotent stem cells from neonatal mouse testis. *Cell*, **119**: 1001-1012.

Katz, J. D., Benoist, C., and Mathis, D. (1995). T helper cell subsets in insulin-dependent diabetes. *Sci*ence, **268**: 1185-1188.

Kitabchi, A. E., and Wall, B. M. (1995). Diabetic ketoacidosis. *Med Clin N Am*, **79**: 9-37.

Ko, K., Tapia, N., Wu, G., Kim, J. B., Bravo, M. J. A., et al. (2009). Induction of pluripotency in adult unipotent germline stem cells. *Cell Stem Cell*, **5**: 87-96.

Kono, T. M., Sims, E. K., Moss, D. R., Yamamoto, W., Ahn, G., et al. (2014). Human adipose-derived stromal/stem cells protect against STZ-induced hyperglycemia: analysis of hASC-derived paracrine effectors. *Stem Cells*, **32**: 1831-1842.

Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*, **26**: 443-452.

Kunisada, Y., Tsubooka-Yamazoe, N., Shoji, M., and Hosoya, M. (2012). Small molecules induce efficient differentiation into insulin-producing cells from human induced pluripotent stem cells. *Stem Cell Res*, **8**: 274-284.

Lefebvre, D. E., Powell, K. L., Strom, A., and Scott, F. W. (2006). Dietary proteins as environmental modifiers of type 1 diabetes mellitus. *Annul Rev Nutr*, **26**: 175-202.

Lensch, M. W., and Mummery, C. L. (2013). From stealing fire to cellular reprogramming: a scientific history leading to the 2012 Nobel Prize. *Stem Cell Rep*, **1**: 5–17.

Li, H., Arber, S., Jessell, T. M., and Edlund, H. (1999). Selective agenesis of the dorsal pancreas in mice lacking homeobox gene Hlxb9. *Nat Genet*, **23**: 67-70.

Lieberman, S. M., and DiLorenzo, T. P. (2003). A comprehensive guide to antibody and T-cell responses in type 1 diabetes. *Tissue Antigens*, **62**: 359–377.

Lin, S., Staahl, B.T., Alla, R.K., and Doudna, J.A. (2014). Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *eLife*, **3**: e04766. https://doi.org/10.7554/eLife.04766

Liu, Y., Cao, D. L., Guo, L. B., Guo, S. N., Xu, J. K., et al. (2013). Amniotic stem cell transplantation therapy for type 1 diabetes: a case report. *Glob J Med Res*, **41**: 1370-1377.

Liu, Z., Sun, Y. K., Xi, Y. P., Maffei, A., Reed, E., et al. (1993). Contribution of direct and indirect recognition pathways to T cell alloreactivity. *J Exp Med*, **177**: 1643-1650.

Ludwig, B., Reichel, A., Steffen, A., Zimerman, B., Schally, A. V., et al. (2013). Transplantation of human islets without immunosuppression. *Proc Natl Aca Sci USA*, **110**: 19054-19058.

Maahs, D. M., West, N. A., Lawrence, J. M., and Mayer-Davis, E. J. (2010). Epidemiology of type 1 diabetes. *Endocrin Metab Clin*, **39**: 481-497.

MacMurray, A. J., Moralejo, D. H., Kwitek, A. E., Rutledge, E. A., Van Yserloo, B., et al. (2002). Lymphopenia in the BB rat model of type 1 diabetes is due to a mutation in a novel immune-associated nucleotide (Ian)-related gene. *Genome Res*, **12**: 1029-1039.

Müller, T. D., Finan, B., Clemmensen, C., DiMarchi, R. D., and Tschöp, M. H. (2017). The new biology and pharmacology of glucagon. *Physiol Rev*, **97**: 721-766.

Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., et al. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol*, **26**: 101-106.

Naya, F. J., Huang, H. P., Qiu, Y., Mutoh, H., DeMayo, F. J., et al. (1997). Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in B2/neuroD-deficient mice. *Genes Dev*, **11**: 2323-2334.

Nishimura, W., Kondo, T., Salameh, T., El Khattabi, I., Dodge, R., et al. (2006). A switch from MafB to MafA expression accompanies differentiation to pancreatic β-cells. *Dev Biol*, **293**: 526-539.

Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., et al. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development*, **122**: 983-995.

Øster, A., Jensen, J., Serup, P., Galante, P., Madsen, O. D., et al. (1998). Rat endocrine pancreatic development in relation to two homeobox gene products (Pdx-1 and Nkx 6.1). *J Histochem Cytochem*, **46**: 707-715.

Pagliuca, F. W., Millman, J. R., Gürtler, M., Segel, M., Van Dervort, A., et al. (2014). Generation of functional human pancreatic β cells in vitro. *Cell*, **159**: 428-439.

Palmer, J. P., Asplin, C. M., Clemons, P., Lyen, K., Tatpati, O., et al. (1983). Insulin antibodies in insulin-dependent diabetics before insulin treatment. *Science*, **222**: 1337-1339.

Penfornis, A., Personeni, E., and Borot, S. (2011). Evolution of devices in diabetes management. *Diabetes Technol Ther*, **13**: 93-102.

Pepper, A. R., Pawlick, R., Gala-Lopez, B., MacGillivary, A., Mazzuca, D. M., et al. (2015). Diabetes is reversed in a murine model by marginal mass syngeneic islet transplantation using a subcutaneous cell pouch device. *Transplantation*, **99**: 2294–2300.

Perfetti, R., and Merkel, P. (2000). Glucagon-like peptide-1: a major regulator of pancreatic beta-cell function. *Eur J Endocrinol*, **143**: 717-725.

Pilia, S., Casini, M. R., Cambuli, V. M., Ibba, A., Civolani, P., et al. (2011). Prevalence of type 1 diabetes autoantibodies (GAD and IA2) in Sardinian children and adolescents with autoimmune thyroiditis. *Diabetic Med*, **28**: 896-899.

Pugliese, A. (2004). Genetics of type 1 diabetes. *Endocrin Metab Clin*, **33**: 1-16.

Rezende, L. F., Vieira, A. S., Negro, A., Langone, F., and Boschero, A. C. (2009). Ciliary neurotrophic factor (CNTF) signals through STAT3–SOCS3 pathway and protects rat pancreatic islets from cytokine-induced apoptosis. *Cytokine*, **46**: 65-71.

Röder, P. V., Wu, B., Liu, Y., and Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Exp Mol Med*, **48,** e219. https://doi.org/10.1038/emm.2016.6

Rooman, I., and Bouwens, L. (2004). Combined gastrin and epidermal growth factor treatment induces islet regeneration and restores normoglycemia in C57Bl6/J mice treated with alloxan. *Diabetologia*, **47**: 259-265.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., et al. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from

the international diabetes federation diabetes atlas, 9th Edition. *Diabetes Res Clin Pract*, **157**: Article107843. https://doi.org/10.1016/j.diabres.2019.107843

Selam, J. L. (2010). Evolution of diabetes insulin delivery devices. *J Diabetes Sci Technol*, **4**: 505–513.

Sellick, GS., Barker, KT., Stolte-Dijkstra, I., Fleischmann, C., Coleman, RJ., et al. (2004) Mutations in PTF1A cause pancreatic and cerebellar agenesis. *Nat Genet*, **36**: 1301–5.

Shapiro, A.M., Lakey, J.R., Ryan, E.A., Korbutt, G.S., Toth, E., et al. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *New Engl J Med*, **343**: 230–238.

Shim, J. H., Kim, S. E., Woo, D. H., Kim, S. K., Oh, C. H., et al. (2007). Directed differentiation of human embryonic stem cells towards a pancreatic cell fate. *Diabetologia*, **50**: 1228-1238.

Sneddon, J. B., Tang, Q., Stock, P., Bluestone, J. A., Roy, S., et al. (2018). Stem cell therapies for treating diabetes: progress and remaining challenges. *Cell Stem Cell*, **22**: 810-823.

Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G., and Gruss, P. (1997). The pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature*, **386**: 399-402.

St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A., and Gruss, P. (1997). Pax6 is required for differentiation of glucagon-producing α-cells in mouse pancreas. *Nature*, **387**: 406-409.

Steiner, D. J., Kim, A., Miller, K., and Hara, M. (2010). Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. *Islets,* **2**: 135-145.

Sumazaki, R., Shiojiri, N., Isoyama, S., Masu, M., Keino-Masu, K., et al. (2004). Conversion of biliary system to pancreatic tissue in Hes1-deficient mice. *Nat Genet*, **36**: 83-87.

Sussel, L., Kalamaras, J., Hartigan-O'Connor, D. J., Meneses, J. J., Pedersen, R. A., et al. (1998). Mice lacking the homeodomain transcription factor Nkx2. 2 have diabetes due to arrested differentiation of pancreatic β cells. *Development*, **125**: 2213-2221.

Suzuki, A., Nakauchi, H., and Taniguchi, H. (2003). Glucagon-like peptide 1 (1–37) converts intestinal epithelial cells into insulin-producing cells. *Proc Natl Aca Sci USA*, **100**: 5034-5039.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126**: 663-676.

Thakkar, U. G., Trivedi, H. L., Vanikar, A. V., and Dave, S. D. (2015). Insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow–derived hematopoietic stem cells from autologous and allogenic sources for type 1 diabetes mellitus. *Cytotherapy*, **17**: 940-947.

Thorel F., Népote V., Avril I., Kohno K., Desgraz R., et al. (2010). Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature*, **464**: 1149-1154.

Timper, K., Seboek, D., Eberhardt, M., Linscheid, P., Christ-Crain, M., et al. (2006). Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun*, **341**: 1135-1140.

Viacyte. (2014). A safety, tolerability, and efficacy study of VC-01™ combination product in subjects with type 1 diabetes mellitus. https://clinicaltrials.gov/ct2/show/NCT02239354

Voltarelli, J. C., Couri, C. E., Stracieri, A. B., Oliveira, M. C., Moraes, D. A., et al. (2007). Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*, **297**: 1568-1576.

Wang, A. Y., Ehrhardt, A., Xu, H., and Kay, M. A. (2007). Adenovirus transduction is required for the correction of diabetes using Pdx-1 or Neurogenin-3 in the liver. *Mol Ther*, **15**: 255-263.

Wang, H., Jiang, M., Bi, H., Chen, X., He, L., et al. (2014). Conversion of female germline stem cells from neonatal and prepubertal mice into pluripotent stem cells. *J Mol Cell Biol*, **6**: 164-171.

Wang, Y., Pontesilli, O., Gill, R. G., La Rosa, F. G., and Lafferty, K. J. (1991). The role of CD4+ and CD8+ T cells in the destruction of islet grafts by spontaneously diabetic mice. *Proc Natl Aca Sci U S A*, **88**: 527-531.

Wei, M., Gaskill, S. P., Haffner, S. M., and Stern, M. P. (1998). Effects of diabetes and level of glycemia on all-cause and cardiovascular mortality: The San Antonio Heart Study. *Diab Care*, **21**: 1167-1172.

Xiao, X., Guo, P., Shiota, C., Zhang, T., Coudriet, G. M., et al. (2018). Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. *Cell Stem Cell*, **22**: 78-90.

Xie, Q. P., Huang, H., Xu, B., Dong, X., Gao, S. L., et al. (2009). Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. *Differentiation*, **77**: 483-491.

Yamada, M., Johannesson, B., Sagi, I., Burnett, L. C., Kort, D. H., et al. (2014). Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells. *Nature*, **510**: 533-536.

Yang, L. J. (2006). Liver stem cell-derived β-cell surrogates for treatment of type 1 diabetes. *Autoimmun Rev*, **5**: 409-413.

Young, H.Y., Zucker, P., Flavell, R.A., Jevnikar, A.M., and Singh, B. (2004). Characterization of the role of major histocompatibility complex in type 1 diabetes recurrence after islet transplantation. *Transplantation*, **78**: 509–515.

Zhang, X., Ye, L., Hu, J., Tang, W., Liu, R., et al. (2012). Acute response of peripheral blood cell to autologous hematopoietic stem cell transplantation in type 1 diabetic patient. *PLoS One*, **7**: e31887. https://doi.org/10.1371/journal.pone.0031887

Zhang, Z., Hu, Y., Xu, N., Zhou, W., Yang, L., et al. (2019). A new way for beta cell neogenesis: transdifferentiation from alpha cells induced by glucagon-like peptide 1. *J. Diabetes Res*, **2019**: 1-11.

Zhao, Y., Jiang, Z., Zhao, T., Ye, M., Hu, C., et al. (2012). Reversal of type 1 diabetes via islet β cell regeneration following immune modulation by cord blood-derived multipotent stem cells. *BMC Med*, **10**: 3. https://doi.org/10.1186/1741-7015-10-3

Zhou, Q., Law, A. C., Rajagopal, J., Anderson, W. J., Gray, P. A., et al. (2007). A multipotent progenitor domain guides pancreatic organogenesis. *Dev Cell*, **13**: 103-114.