RNLS Knockout iPSC Differentiated β–cells Transplantation: A Treatment Option for Type 1 Diabetes.

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ABSTRACT

Type 1 Diabetes Mellitus is a genetic disease that allows the autoimmune system to destroy β–cells that are responsible for glucose transportation. Diabetes is a gateway disease to many other extreme complications such as diabetic ketoacidosis (DKA), blindness, hypoxia, and many more. We create an alternate treatment option by compiling pre-existing studies and procedures in the attempts of tackling type 1 diabetes. We assess multiple options to create lab-grown β–cells that have the highest success rate of being functional glucose-responsive insulin-secreting cells. We settle on human induced pluripotent stem cells (iPSC) technology to differentiate into β–cells, which becomes the base of our project as we build on to other aspects. We further search for a way to protect the lab-grown cells from being targeted by the autoimmune response once again, looking at multiple options we stray away from immunosuppression and microencapsulation technology for various rationales. Rather we choose a genetic approach by implementing studies that suggest genes like RNLS, which´s knockout using technologies such as CRISPR, are proven to have an active role in preventing β–cells from being targeted. Furthermore, we locate an appropriate transplantation site, while addressing and tackling additional dissonance such as 50 % of transplanted cells dying. Although many presented studies are still not clearly understood and have other economic and social issues, further continuation of studies regarding iPSC cells, RNLS genome, CRISPR, location site, and proliferation drugs in conjunction have the potential of being the future cure of Type 1 Diabetes Mellitus.

INTRODUCTION TO DIABETES MELLITUS TYPE 1

Diabetes mellitus is a disease where the body is unable to transport glucose efficiently, from the blood into the cells. Leaving a high concentration in the blood and not enough in cells that require glucose as a source of energy. Diabetes mellitus essentially starves the cells for energy despite

having the resources. The amount of glucose in the cells relative to the amount in the bloodstream is credited to two hormones produced by clumps of cells in the pancreas called islets of Langerhans. Firstly, Insulin is secreted by beta-cells to reduce blood glucose levels by binding to insulin receptors planted in the cell membrane of insulin-responsive tissues (skeletal muscles, adipose tissue and liver). When initiated, the insulin receptors prompt glucose transporters to fuse with the phospholipid bilayer membrane, enabling glucose to be moved into the cell. Contrary to insulin, glucagon secreted by alpha-cells works to increase blood glucose levels, by making the liver produce new molecules of glucose and turning glycogen into glucose for it to be deposited into the blood (Osmosis, 2019). In this case, we focus on Type 1 diabetes mellitus where the body doesn't make enough insulin because of a type IV hypersensitivity response which is an immune action by the patient's T-cells, that proceeds to combat the pancreas. A genetic anomaly is responsible for the loss of self-tolerance amongst T cells that explicitly target the beta-cell autoantigen. In conclusion, the immune attack causes the loss of β–cells which means the lack of insulin, which directly translates into high blood glucose levels.

SYMPTOMS

Type 1 can begin and initiate beta-cell destruction very early in life, usually, 90% of the β–cells are exterminated before any sort of symptoms are shown. Major symptoms include polyphagia, where the inability of the cells to attain glucose as an energy source causes a reaction called lipolysis where adipose tissue begins to break down fat and muscle tissue breaks down proteins, which results in drastic weight loss and weakness. Glycosuria, where the high levels of glucose when filtered through the kidneys begins to deposit in the urine because glucose of being osmotically active will cause an increase in urination otherwise known as polyuria. Excessive urination causes polydipsia where the patient will be dehydrated (Osmosis, 2019).

DKA

DKA is the most dangerous complication that arises from type 1 diabetes. During polyphagia in the process of lipolysis, the fat is further broken down into fatty acids and with the assistance of the liver the fatty acids into ketone bodies. These ketone acids are the cell's substitute for glucose to be used as energy, but this comes with a huge setback the blood becomes acidic. This causes patients to have Kussmaul respiration which is how the body compensates for the acidic blood by

breaking the ketone bodies into acetone gas and moving it with carbon dioxide out of the blood at a rapid rate. Furthermore, the rise in acidity correlates directly to a rise in protons or hydrogen ions (H+) in the blood that is meant to be sent into the cells in exchange for potassium (K+). Now that the K+ is outside surrounding the cell fluid it begins to pile up because insulin is also responsible for the sodium-potassium ATPases just as it is for the glucose. The excessive K+ outside the cell makes its way down to the bloodstream and leads to hyperkalemia. As a whole DKA can cause vomiting, nausea, cerebral edema, pulmonary edema, and death (Osmosis, 2019).

GLUCOSE LEVEL RISE

High levels of blood glucose come with their own set of dangers. Starting with damage to the microvasculature through the process of hyaline arteriolosclerosis where protein begins to deposit making capillaries narrower causing hypoxia. While arteriolosclerosis to the large arterial wall could directly result in a heart attack (Osmosis, 2019). Diabetes is also the leading cause of blindness due to retinopathy. Furthermore, if the arterioles in the kidney are damaged it can result in nephrotic syndrome, slowly reducing the kidney's ability to filter blood, possibly requiring dialysis. Lastly, the nervous system as a whole, sits at a high risk during diabetes with the loss of sensation to disruption of many involuntary systems. Alongside poor blood flow and concentrated nerve damage can lead to ulcers and possible amputation.

CURRENT TREATMENTS

Current treatment includes insulin injection through insulin pumps and insulin pens. Where insulin is injected under the subcutaneous fat where it's absorbed into the bloodstream. Although reliable insulin injections come with many inconveniences. With its risks of dosage levels to hypoglycemia which can cause weakness, loss of consciousness, and in some cases seizures. In addition to scars, rashes, and lumps at the site of injection admissions and an overall daily painful inconvenience to patients. Current physiological solution consists of pancreas or islet transplantation to replace β–cells. Although existing procedures such as the Edmonton protocol have shown to be successful (Nanji & Shapiro, 2006), the lack of donors make the protocol impractical on a larger scale.

SOCIAL ECONOMIC IMPACT

Over the last two decades, people living with diabetes have tripled, making it one of the fastest-growing health crises of the 21st century. This is largely due to a variety of socioeconomic, demographic, environmental and genetic determinants. Globally in the 2000's, the estimate of adult diabetics was 151 million, shortly by 2009 that had increased by 88% to 285 million diabetics worldwide. Today it is estimated that 9.3% of the adult population or 463 million people ranging from 20-47 years old are living with some form of diabetes roughly 10 % of which are type 1. This does not include the additional 1.1 million children and adolescents under the age of 20. Interestingly in 2010, the projected number of diabetics was 438 million by the end of 2025. Currently, 4 more years to go, and the projected number has already been passed by 25 million in 2020. The International Diabetes Federation has estimated 578 million by 2030 and 700 million by 2050, people living with diabetes. Dialing down on Canadians, more than 300'000 live with Type 1 diabetes while DKA being part of the 15%-67% of type 1 diabetics making Canada listed 10th in the world. Such consequential numbers come with even greater costs. Canadians without insurance can expect an annual cost of $5000 for type 1 diabetes. Although collectively North America altogether spends on the upside of $327 Billion (*Worldwide toll of diabetes*, 2019).

COURSE OF ACTION

To tackle type 1 diabetes mellitus, we compile a permanent treatment option derived from many pre-existing studies, methods, and techniques, merging them into a singular procedure. Which entails; source of creating functional glucose-responsive insulin-secreting β–cells to replace the killed original β–cells. Protect and maintain these lab-grown β–cells within the body to prevent the reoccurrence of type 1. We also assign an appropriate transplantation site and cover any dissonance by suggesting an alternative. All through the analysis based on the efficiency and effectiveness of each study.

HUMAN GLUCOSE-RESPONSIVE iPSC β–CELLS

iPSCs can be derived from somatic cells from a human. These cells share the same capability to duplicate and differentiate as the embryonic stem cells (ESC). Likewise, both ESCs and iPSCs possess the excellent potential to be used in many cell therapy techniques. The use of iPSCs has little to no ethical issues and is less frowned upon relative to the ESCs which are obtained from

living embryos. By being able to create tailored fit iPSC induced β–cells for transplantation in patients with β–cells deficiency, we take the first step to a type 1 diabetic cure. Furthermore, using iPSCs ensures the lack of additional immune rejection because iPSC being derived from a patient's somatic cells share identical genetic makeup of the patient. Various protocols to produce glucose-responsive pancreatic β–cells have previously been reported, however, those that most precisely simulated the natural embryonic growth of the endocrine pancreas, were the most successful methods. The procedure begins by deriving many somatic skin cells from a patient, which are later exposed to a combination of five major transcription factors such as Oct3 and Oct4, Sox2, Klf4, and c-Myc (Takahashi et al., 2007). Within 3-4 weeks healthy iPSCs are matured into pluripotent cells sharing the genetic makeup of the patient while having the potential to differentiate into specialized cells **(Figure 1)**. These iPSC-derived cells are further introduced to specific transcription factors to develop glucose-responsive insulin-secreting β–cells. It has been reported that differentiation of the iPSCs into β–cells via specific transcription factors alone isn't enough to develop them into highly functioning insulin-secreting cells (Mayhew & Wells, 2010). Thus, transcription factors must be paired with developmental principles such as signaling pathways that ensure the recapitulation of iPSCs through all the major developmental stages of mature glucose-responsive β–cells. Once a patient's somatic skin cells have differentiated into iPSC's, the developmental process of iPSCs to β–cells can be carried out. The developmental process consists of four major stages: endoderm formation (in vitro), pancreas specification (in vitro), endocrine specification (in vitro), and beta-cell maturation (in vivo).

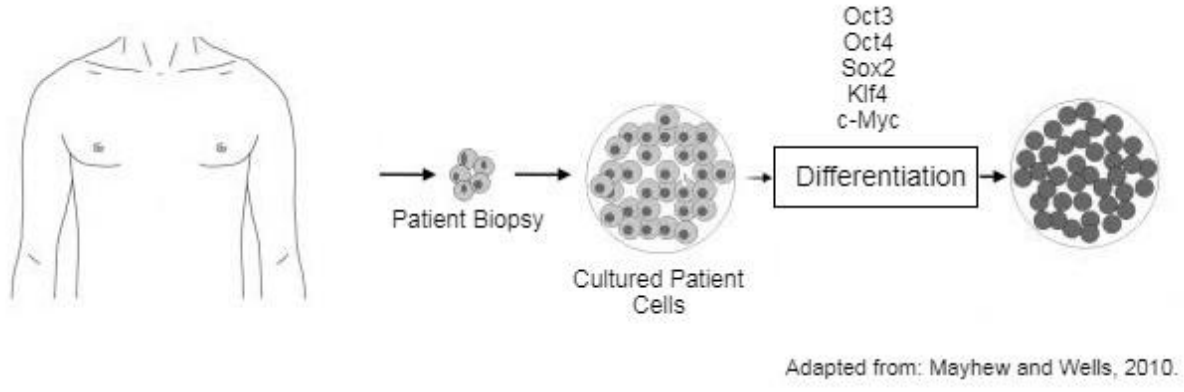

Figure 1: Differentiating Somatic Cells into iPSCs

Firstly, the iPSC must differentiate into prominent definitive endoderm (DE). To produce the DE the patients iPSCs require the transcription factors Oct 4, Nanog, in addition to the signaling pathways Wnt, Nodal and the Tra1-81 gene (Mayhew & Wells, 2010) which will lead the iPSCs through the developmental stages required for the formation of the DE **(Figure 2)**. Formation of the DE is a key step towards producing glucose-responsive insulin-secreting β–cells as it gives rise to a variety of cells and tissues necessary for the development of functional β–cells.

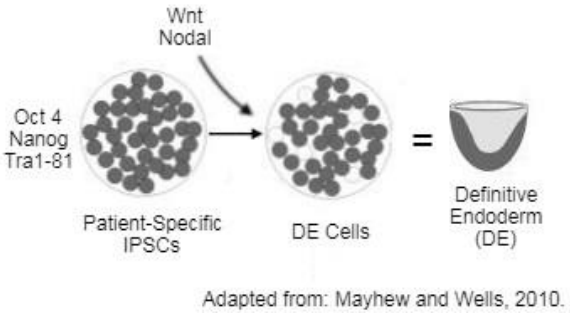

Figure 2: Formation of the Definitive Endoderm

Next the DE must differentiate into the foregut endoderm and then into pancreatic progenitors to successfully carry out pancreas specification. Differentiation of the DE into the forget endoderm is done using the transcription factors Sox17, FoxA2, along with the signaling pathways FGF, BMP and the CXCR4 gene **(Figure 3)**. The foregut endoderm allows for the differentiation of the pancreatic progenitors which is done using the transcription factors HNF1b and HNF4a, along with the signaling pathways FGF, HH and the signaling molecule retinoic acid (RA) (Mayhew & Wells, 2010). The pancreatic progenitors (multipotent stem cells) are further responsible for the development of the pancreatic beta cells **(Figure 4)**.

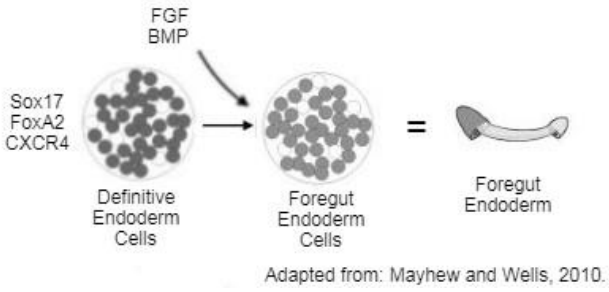

Figure 3: Foregut Endoderm Formation

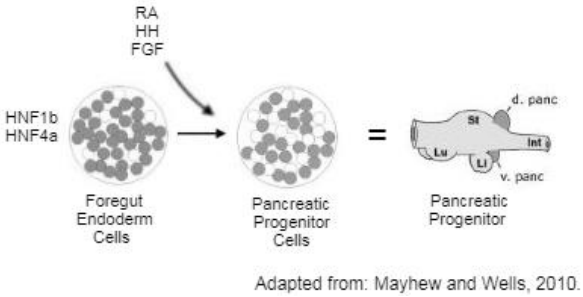

Figure 4: Foregut Endoderm Formation

Now that the development of pancreatic progenitors is complete, endocrine specification is possible where pancreatic progenitors are multiplied and differentiated into pancreatic endocrine progenitor cells using the transcription factors Pdx1, Nkx6.1, Hnf6, and Sox9 along with the

signaling pathways EGF, and Notch (Mayhew & Wells, 2010) **(Figure 5)**. These cells are capable of directly developing into β–cells.

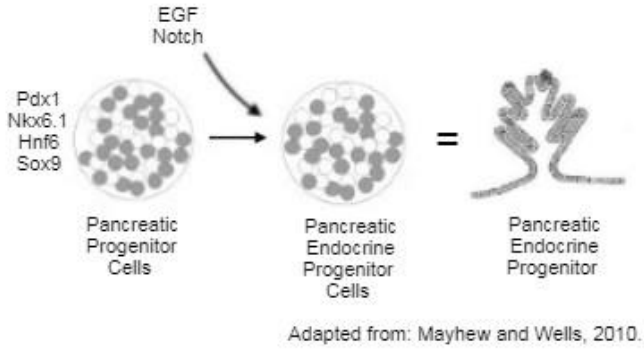

Figure 5: Pancreatic Endocrine Progenitor Formation

The final step of induced iPSC-derived beta cells is the maturation of pancreatic endocrine progenitor cells to glucose-responsive insulin-secreting β–cells. The maturation of the highly functional β–cells capable of secreting insulin can only be achieved in vivo by engrafting the endocrine progenitor cells into mice. The maturation of β–cells can be done in both vivo and vitro (Salinno et al., 2019; Hohmeier et al., 2019) but after the comparative analysis of various studies we conclude that in vivo maturation is the most effective method due to our lack of understanding about the signaling pathways that are responsible for β–cells maturation in vivo which makes us incapable of providing the same environment for the cell in vitro.

PROTECTING iPSC β–CELLS AUTOIMMUNE DESTRUCTION

By creating functional glucose-responsive insulin-secreting β–cells we are able to replace the ones that were targeted by the patient's autoimmune response. However, the same self-tolerance reaction is also subjected towards the lab-grown iPSC β–cells. We approach this issue by not considering immunosuppression. Immunosuppressive drugs come with a variety of complications such as a high risk of infection, diseases, and malignancy. Another way for scientists to protect iPSC β–cells is to incase them in immunoprotective microencapsulation devices (Ludwig, 2020). Although phenomenal advancements have been made regarding this technology it still has its own setbacks such as biocompatibility, graft oxygenation, immunoprotection, and inflammatory responses. Instead, we choose a study that suggests a more efficient method. Harvard Stem Cell Institute researchers have found that targeting the protein renalase can protect β–cells from autoimmune attacks (*Protecting beta cells against type 1 diabetes*, 2020). The study provides evidence that a β–cell's own genes can render it dysfunctional, further triggering the autoimmune attack. To find that genome the researchers used a screening method consisting of CRISP-editing technology, to suppress one gene at a time. They found a genome RNLS responsible for the protein renalase. The researchers then deleted the RNLS genome from mouse induced β–cells while transplanting

them into the diabetic mouse, discovering the control β–cells were destroyed while the ones without the RNLS genome managed to survive (Cai et al., 2020). This is because the renalase protein is correlated with the endoplasmic reticulum stress (ER), the cells with ER are more likely to be attacked by the T-Cells. With CRISPR-Cas9 **(Figure 6)** technology scientists can edit these human iPSC β–cells to knock out the RNLS gene which will prevent ER, lowering the chance of dysfunctionality in the β–cells, and limiting the autoimmune attack towards human iPSC β–cells post-transplantation. Thus, we propose that by eliminating the RNLS genome via CRISPR we are not only able to protect iPSC β–cells from being attacked, but we can also ensure the daughter cells reproduced from iPSC β–cells will also be protected from autoimmune attacks in the future.

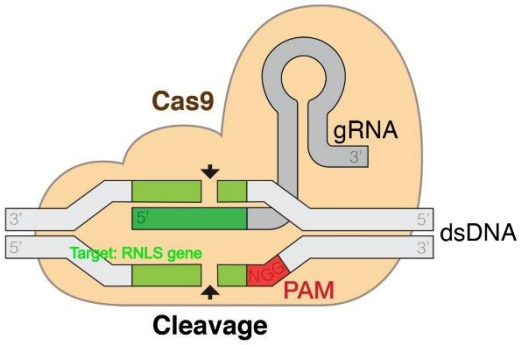

Figure 6: CRISPR-Cas9 mechanism. Guide RNA (gRNA) is used to guide the system to the location of the target gene RNLS. The loop like structure of the tracer RNA allows the gRNA to bind with Cas 9, allowing it to do its job by binding to the RNLS gene and cutting it off, thus shutting down the gene and protecting iPSC β–cells from being attacked.

TRANSPLANTATION

With the functional glucose-responsive insulin-secreting human iPSCs β–cells protected from autoimmune attacks; they are ready for transplantation. Which requires a minimally invasive procedure. The patient will firstly receive local anesthesia paired with a sedative. The procedure includes a catheter inserted into a thin incision on the upper abdomen (National Institute of Diabetes and Digestive and Kidney Diseases, 2021). With the assistance of a radiologist using x-rays and ultrasound, the catheter tube is led to the portal vein of the liver. Next the human iPSCs β–cells are slowly infused into the appropriate site by the catheter using gravity. The portal vein of the liver is chosen as the transplantation site for maximum efficiency. It is easily accessible through a minor procedure while also being the hotspot of insulin utilization (Mayhew & Wells, 2010). Although after transplantation around 50% of the β–cells die, mostly from the lack of oxygenation. Alternate sites have also been tested such as the kidney, subcutaneous layer, and

omentum, while this is safer for the β–cells, it serves to be pointless from a functionality perspective.

iPSC β–CELLS ENHANCEMENT POST TRANSPLANTATION

Additional study is required for a more optimal site, but we are able to compensate for a 50% individual iPSC β–cell loss through the means of drugs. Researchers at the Ichan School of Medicine discovered a combination of drugs when used together are able to induce the highest levels of proliferation in β–cell (*Diabetes: New drug cocktail increases human beta cell proliferation at rapid rates*, 2018). The drug Harmine is responsible for inhibiting an enzyme dual-specificity tyrosine-regulated kinase (DYRK1A) with a β–cells-targeting drug agonist GLP1R which inhibits (TGFβSF) responsible for inhibiting the transforming growth factor of the beta superfamily members. In conjunction, these drugs are able to boost proliferation by 5%-8% (Ackeifi et al., 2020). Post transplantation half the lab-grown iPSC β–cells are bound to die but with further advancement to this procedure, we will be able to initiate a bigger culture of β–cells from the surviving transplanted β–cells.

CONCLUSION

Advancement in health sciences have progressed at a rapid rate over the last 20 years. We most definitely have the capability to create, protect, and sustain lab grown human iPSCs β–cells. That being said, there are multiple aspects of this procedure still not clearly understood, most specifically signaling pathways that are able to instruct progenitor cells to turn into mature and functioning β–cells. Furthermore, the protection of β–cells by knocking out the RNLS gene also requires further study, and we suspect that the method is only able to minimize autoimmune attacks but not completely render them. The appropriate transplantation site is also debated on, to find a balance of preserving β–cell health, but also optimizing its functionality. Lastly the procedure we recommended to proliferate β–cells post transplantation requires additional work, to be more specific to β–cells, and prevent the drug from spreading to surrounding organs, which can potentially uphold many complications such as tumors. Other consequential obstacles must still be tackled. One of which is the poor cost-effectiveness of this method, to move to a larger scale we will need to find a more economically sustainable differentiation method. More testing is required

to solidify each method for it to be a complete procedure. With the rapid rates of advancements of sciences each year, we get one step closer to a complete cure of type 1 diabetes mellitus.

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