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GENERATING INSULIN SECRETING CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR TREATING HYPERGLYCEMIA IN DIABETIC MICE MODEL

Chan HL[1], Sudtha A/P Murthy[2], Okechukwu PN[2], Amini F[1*]

[1]School of Healthy Aging, Medical Aesthetics and Regenerative Medicine, Faculty of Medicine and Health Science, UCSI University, Kuala Lumpur, Malaysia
[2]Faculty of Applied Science, UCSI University, Kuala Lumpur, Malaysia

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*Corresponding Author: Assoc. Prof Farahnaz Amini, PhD
Email: farahnaz@ucsiuniversity.edu.my

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ABSTRACT

Type 1 diabetes and late stages of Type 2 diabetes are associated with little or no insulin production from the pancreatic β cells. In this view, insulin production in a patient is one of the potential approach for treating diabetes. One of the innovations in stem cell research is creating induced pluripotent stem cells (IPSC) from adult cells. This study was aimed at reviewing the i. success of induced pluripotent stem cell (iPSC) in producing functional insulin secreting cells (ISC), ii. potential of ISC to treat hyperglycemia in diabetic mice. A literature search was done in PubMed, Google Scholar and Cochrane for all English publications from year 2001 to 2016. A total of 5 studies met inclusion and exclusion criteria to be included in this review. Except one study which used cells from diabetic patients to create iPSCs the remaining four studies used healthy human cells. Study duration was ranged from 3 to 8 months. Three out of 5 studies have reported the successful generation of ISC and correction of hyperglycemia in the diabetic mice until the end of their studies. The two studies which the hyperglycemia mice were not treated, have not measured the expression of β cell markers including PDX1 and NCX6.1 in their generated ISCs before transplantation. In conclusion, the prospect of human iPSC generated ISC to treat DM is still unreliable as not all the studies showed capability of the ISC to function and/or maintain function *in vivo*. The functional capability of an individual ISC is less than normal β cells (less insulin production) and there is variable results in the efficacy of insulin production in the generated ISC. Further studies is needed to examine the functional capability of ISCs generated especially when autologous iPSCs are used from diabetic patients to generate ISC.

1.0 Introduction

The prevalence of diabetes mellitus (DM) is increasing around the world. In 2014, the World Health Organization (WHO) reported that 8.5 % of adults above 18 years old has DM (1). According to International Diabetes Federation, there are 3.3 million cases of diabetes in Malaysia and the prevalence of diabetes in adults of age 20-79 is 16.6 % in 2015. There are two distinct types of diabetes, i.e. Type 1 and Type 2 (T1DM & T2DM) in which T1DM results from destructive of β cell by

autoimmunity and T2DM results from inadequate insulin secretion. Both T1DM and T2DM will eventually cause body systems to collapse which leads to mortality (2).

The pancreas is an organ with both endocrine and exocrine function. The exocrine glands plays a role in the digestive system and acinar cells of the pancreas secrete digestive enzymes (3). The endocrine glands consist of many islets of Langerhans which produces pancreatic hormones. The main cells in the islets of Langerhans are the β-cells and the α-cells which secrets insulin and glucagon respectively. During development of the pancreas, certain genes expression is observed. For instance, insulin promoter factor 1 (PDX)1 is expressed by the foregut endoderm during pancreatic specification whilst other genes like NKX6.1, NKX2.2, NGN3 and HLXB9 are expressed during development of the pancreatic islets. Some of these genes are continuously expressed in adults in order for β-cells count and function to continue (4).

Currently, the available treatments for DM are through oral medications, where patients are monitored for a period of 3 months with modified lifestyle, if the glucose level remains the same then oral hypoglycemic agents (OHA) is started. The more advanced treatment is the insulin pump and artificial pancreas. Pancreatic islets transplant is another promising therapy. However, it is limited by the fact that there is more patients than organ donors (5). Furthermore, there is a need for lifelong immunosuppressive therapy in the patients.

Advances in regenerative therapy now looks towards employing cells that are able to secrete insulin on its own adaptive to the body's glycemic status. Multiple types of stem cells are being employed in *in vivo* studies to produce insulin secreting cells (ISC) such as mesenchymal stem cells (6) and embryonic stem cells (7). In particular interest is induced pluripotent stem cells (iPSC) which are generated from adult cells and have been shown to be capable of differentiating into ISC (8). This area of research offers the opportunity of regenerating ISC from the same patient and bypassing the need for immunosuppressive therapy.

Hence, regenerating ISC from iPSC is a revolutionary treatment for DM. In this paper, the success of using iPSC for producing functional ISC and subsequently its potential to treat hyperglycemia will be evaluated.

2.0 Materials and methods

A literature search was done in electronic databases including Cochrane library, PubMed and Google Scholar. The literature search was done using a set of keywords included human induced pluripotent stem cells, iPS cells, iPS cell, hiPSC, fibroblast-derived induced pluripotent stem cells, fibroblast-derived IPS cells, Diabetes Mellitus treatment, Diabetes Mellitus, hyperglycaemia treatment, pancreatic beta cells, beta cells, insulin secreting cells, B cell and pancreatic B cell. Only *in vivo* studies which used human iPSC and published in English language from 2010 to 2016 were included.

3. Results

The numbers of articles from early literature searches in selected databases were narrowed down by combination of different keywords and filters. The title and abstract of the papers were evaluated based on this review inclusion and exclusion criteria. The summary of literature search is presented in Table 1. Finally, a total of 5 articles met the criteria and was selected for this review. The data from these studies are extrapolated out and details of each study is presented in Table 2. The mode of intervention, duration of intervention and comparison groups among the different studies is compared in Table 3. The outcome of each study together with the number of death event during post-transplanted with the iPSC is shown in Table 4.

4. Discussion

The β-cells destruction causes type 1 diabetes, therefore, treatment options for patients are either replacement of pancreatic or β-cells transplantation. Due to shortage of donor, the whole pancreatic organ transplantation is restricted to limited numbers of patients. Recent advancement in stem cell research, especially induced pluripotent stem cell (iPSC), creates huge interest toward treating diseases including diabetes using patients' cells to replace damaged or non-functional cells. Despite of many successful *in vitro* and *in vivo* experiments the clinical applications are still debatable. In this review, the success of iPSC in producing functional insulin secreting cells (ISC) and its potential to treat hyperglycemia in diabetic mice were assessed. To address the objectives of this review, different aspects of experiments in selected articles were appraised.

Different type of adult cells can be used to produce iPSCs. Except study by El Khatib *et al.* (13) who used cells from diabetic patients to create iPSCs the remaining four studies used healthy human cells. In study by El Khatib *et al.* (13), it is noted that the resultant iPSCs produced variable results though implanted in the same type of mice, suggesting that the *in vivo* differentiation of the iPSCs and functionality of the produced ISCs may be partially controlled by the original donor cell. The idea of using patient's own cells to create functional β cells is fascinating since the autologous nature of transplantation increase the rate of success and eliminate the need of immune suppression and its consequences in patients. However, there is a limitation of autologous iPSCs for those

Table 1 Summary of Literature Search

Database	*Keywords*	*No. of Articles Retrieved*	*No. of Articles Selected*
PUBMED	1)Human induced pluripotent stem cells OR IPS cells OR hiPSC OR fibroblast-derived induced pluripotent stem cells OR fibroblast-derived IPS cells	8391	
	2)Insulin producing cells OR pancreatic B cells OR pancreatic beta cells OR beta cells OR insulin secreting cells OR B cells	4867	
	3)Diabetes Mellitus treatment OR hyperglycemia treatment OR in vivo insulin production	213916	
	1 + 2 + 3	21	3
Google Scholar	1)Human induced pluripotent stem cells OR IPS cells OR hiPSC OR fibroblast-derived induced pluripotent stem cells OR fibroblast-derived IPS cells	169000	
	2) Insulin producing cells OR pancreatic B cells OR pancreatic beta cells OR beta cells OR insulin secreting cells OR B cells	34000	
	3)Diabetes Mellitus treatment OR hyperglycemia treatment OR in vivo insulin production	11500	
	1 + 2 + 3	2940	2
COCHRANE	1)Human induced pluripotent stem cells OR IPS cells OR hiPSC OR fibroblast-derived induced pluripotent stem cells OR fibroblast-derived IPS cells	17	0
	2) Insulin producing cells OR pancreatic B cells OR pancreatic beta cells OR beta cells OR insulin secreting cells OR B cells	8	
	3)Diabetes Mellitus treatment OR hyperglycemia treatment OR in vivo insulin production	169	
	1 + 2 + 3	0	

Table 2 Characteristics of selected studies

Author & Year	*Title*	*Journal*	*Study design*	*Characteristic of the animal*	*Sample size (No. of Mice treated)*
Pagliuca et al. 2014 (9)	Generation of functional human pancreatic β cells in vitro	Cell	Animal study	NRG Akita mice	pancreatic islet transplant – 12 functional β cells -37 polyhormonal – 8
Raikwar et al. 2015 (10)	Human iPS cell-derived insulin producing cells form vascularized organoids under the kidney capsules of diabetic mice	PLOS One	Animal study	Streptozotocin treated Rag $2^{-/-}$ $\gamma c^{-/-}$ male mice at 8 weeks old	ISC transplant – 10 Non-transplant - 10
Pellegrini et al. 2015 (11)	Human induced pluripotent stem cells differentiate into insulin-producing cells able to engraft in vivo	Acta Diabetol	Animal study	Normoglycemic NOD/SCID mice at 8 weeks old	ISC transplant – 12 Precursor transplant - *ND
Zhu et al. 2016 (12)	Human pancreatic beta-like cells converted from fibroblasts	Nat Commun	Animal study	Streptozotocin treated NSG male mice at 6-10 weeks old	12
El Khatib et al. 2016 (13)	Tumor-free transplantation of patient derived induced pluripotent stem cell progeny for customized islet regeneration	Stem Cells Transl Med	Animal study	SCID-beige mice	LV-IPSC – 18 TGF-IPSC -29

Table 3 Intervention, duration of intervention and comparison group

Study	*Intervention*	*Duration of Intervention*	*Comparison Groups*
Pagliuca et al. (9)	5 x 10^6 insulin producing cell from human IPSC was transplanted under the kidney capsule of the mice	18 weeks	Diabetic mice transplanted with functional insulin producing cell Diabetic mice transplanted with human pancreatic stem cells(PSC) Diabetic mice transplanted with non-functional polyhormonal cells (control)
Raikwar et al. (10)	5 x 10^6 insulin producing cell from human IPSC was transplanted under the kidney capsule of the mice	150 days	ISC transplanted Streptozotocin induced diabetic Rag $2^{-/-}$ $\gamma c^{-/-}$ Non-transplanted Streptozotocin induced diabetic Rag $2^{-/-}$ $\gamma c^{-/-}$
Pellegrini et al. (11)	4-5 x 10^6 human IPSC at stage of posterior foregut or endocrine cells was transplanted under the kidney capsule of the mice	12 weeks	ISC transplanted NOD/SCID mice Pancreatic endoderm transplanted NOD/SCID mice
Zhu et al. (12)	5 x 10^6 insulin producing cell from human IPSC was transplanted under the kidney capsule of the mice	24 weeks	Diabetic mice transplanted with insulin producing cells (CPB) Diabetic mice transplanted with human fibroblast Diabetic mice transplanted with pancreatic endodermal progenitor cell(CPE)
El Khatib et al. (13)	1 x 10^6 human IPSC was transplanted under the kidney capsule of the mice	8 months	Diabetic mice transplanted with lentiviral vector IPSC (LV-IPSC) Diabetic mice transplanted with transgene free IPSC (TGF-IPSC) from A patient (DM type 1) Diabetic mice transplanted with transgene free IPSC (TGF-IPSC) from B patient (DM type 1) Diabetic mice transplanted with transgene free IPSC (TGF-IPSC) from C patient (DM type 2)

Table 4 Details of finding in post-transplant stage

Study	*No of dead mice post-transplant*	*Insulin secretion after transplant*	*C-peptide in mice*	*PDX1, NCX6.1 expression in iPSC*	*Histopathology finding*	*Test for teratoma formation*
Pagliuca et al. (9)	Post-transplant 8 weeks -2 human PSC -0 human ISC 18 weeks -5 human PSC -1 human ISC	Insulin was secreted within 2 weeks	C-peptide: detected up to 1 week after transplant in mice serum	-PDX1: not tested -NCX6.1: not tested	presence of C-peptide cells, absence of GCG and DAP1 in mice kidney	Not done

Raikwar et al. (10)	1 post transplanted mice 10 non-treated control mice	blood glucose reduced from 400-500mg/dL to <200mg/dL	C-peptide: detected up to 1 week after transplant in mice serum	-PDX1: not tested -NCX6.1: not tested	-lacking of islets in pancreas in post-transplanted mice -transplanted cells were positive for insulin and glucagon	MRI at day 150 shown no teratoma formation
Pellegrini et al. (11)	ND	Mice remained normoglycemic during the follow-up	-C-peptide: detected up to 1 week after transplant in serum	-PDX1: upregulation was observed -NCX6.1: not tested	1 week post-transplant: significant number of insulin positive cells and PDX1 on histology 12 weeks post-transplant: – PDX1 positive but no cell positive for insulin on histology	Not done
Zhu et al (12)	ND	significant insulin secretion 2 months after transplantation	2.1x increase in C-peptide in both treated groups	-PDX1: similar level of expression compared to human islets -NCX6.1: similar level of expression compared to human islets	CPE mice: histology at 15 weeks was positive for insulin and PDX1, NKX6.1 but negative for glucagon CPB mice: histology showed islet like structures containing insulin and immunofluorescence positive for C- peptide, PDX1 and NKX6.1 but negative for glucagon and SST	no tumour in graft up to 24 weeks
El Khatib et al. (13)	ND	LV-IPSC: at 2 months post-transplant, glucose challenge observed	LV-IPSC: negative C-peptide increment in all	-PDX1: In 80% of cells expression was detected -NCX6.1: expressed in all cells	-LV-IPSC: PDX1 and NCX6.1 positive -TGF-IPSC: positive for insulin cells, glucagon cells, PDX1 and C-peptide B TGF-IPSC – no insulin positive cells observed C TGF-IPSC – few islet like structure seen. Glucose challenge negative	-LV-IPSC – 17 out of 18 mice developed solid tumour larger than 2cm within 4-6 weeks -TGF-IPSC – no tumour detected at 3 and 8 months

who are genetically inherited T1DM. Because all the cells in their tissues carried same genetic defects then a genetic manipulation is needed to correct or replace the defective gene before creating iPSCs.

The ability of created ISC to continuously function on its own and maintain normoglycemia in the recipient mice varied in each study. In two studies normoglycemia was observed in all ISCs transplanted mice up to the end of the studies (9,12). Whilst in study by Raikwar *et al*, (10) normoglycemia only attained in 50% of mice at 3 months. Pellegrini *et al*. (11) saw dismaying results at the end of 12 weeks as in 5 out of 6 mice, the transplanted cells was not visible. However, both studies by

Raikwar *et al*. (10) and Pellegrini *et al*. (11) did not measured the NKX6.1 expression in ISCs before transplantation which might explain the failure of secreting insulin by transplanted cells. It is believed that NKX6.1 play an important role in identifying β cells and studies have shown that the outcome of the transplants is directly proportional to the amount of NKX6.1 levels (12). Furthermore, a study done by Taylor *et al*. (13) showed that NKX6.1 is essential for normal β cell function in mice. Besides NKX6.1, expression of PDX1 and C-peptide also measured as β cells characteristics in three studies (14-16). Furthermore, some of the cell cluster developed during the differentiation process also tested positive for glucagon, showing that they are polyhormonal instead of monohormonal like β cells. The term of ISC or insulin producing cell are used instead of β cells since it seems to be more appropriate to recognize the IPSC generated cells from primary β cells.

Another factor which might had contribution to the outcome of iPSCs to generate ISC is the number of cells transplanted into the mice. It was noted that in all the studies the transplanted cells were much higher than required number. To establish normoglycemia in diabetic mice, it is considered that 2 $x10^5$ cells is required (13). Four studies transplanted 5 x 10^6 ISC and 1 of the studies transplanted 1 $x10^6$ iPSC. This suggest the possibility of the engineered cells not producing similar insulin levels compared to normal β cells. Comparatively in humans, the required amount of β cells to maintain normoglycemia is 5 x 10^6 cells per kilogram of body weight. Hence if the ISC generated is only able to produce a fraction of insulin as compared to normal β cells, the amount of ISC if ever transplanted in a human patient would be a massive amount which would lead to the question of safety and carcinogenic development. In the studies examined, the transplanted cells did not infiltrate the surrounding tissue nor form tumor as compared to multiple ESC studies which noted teratoma formation (17-19). However, it is noted that the longest duration of the iPSC study was conducted for 8 months and there is the possibility that the tumor occurrence can be after that period of time.

5.0 Conclusion

The prospect of using human iPSC to generate ISC for treating DM is promising but not all the studies showed capability of the ISC to maintain its function *in vivo* for long period. Furthermore, the functional capability of generated ISCs were less than normal β cells (less insulin production). It might be assumed that the genetic makeup of adult cells which are used to create iPSCs may influence the efficacy of insulin production in the generated ISC. This is especially important in the case of autologous iPSC in a patient with hereditary diabetic which may results in less amount of secreted insulin from transplanted ISC.

Further *in vivo* studies using human iPSC is needed especially when cells from an affected diabetic patient are used to further understand the complex behavior of transformation into ISC and the micro-components that affects the resultant insulin production. Longer duration of study is also advocated to evaluate any potential carcinogenic effect of the resultant cell before transplantation in humans.

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