Am J Stem Cells 2018;7(2):48-55
www.AJSC.us /ISSN:2160-4150/AJSC0076363

Original Article

Analysis of the regulation pathways via microarray and miRNA studies: human embryonic stem cells to treat diabetes mellitus type-II

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Received February 7, 2018; Accepted April 15, 2018; Epub June 1, 2018; Published June 10, 2018

Abstract: Diabetes mellitus occurs either due to an autoimmune destruction of β cells (Type 1) or resistance to insulin effects (Type 2). Diverse conventional medications are used for treatment of diabetes, which is associated with long term complications such as kidney failure, blindness, and stroke. We recently showed the potential of human embryonic stem cells (hESCs) in 95 patients with type 2 diabetes. In the present study, we use the microarray and miRNA studies to prove why hESCs are effective in diabetes. Three samples of hESCs were cultured and microarray technology was used for the analysis of diabetic pathways. The gene targets for miRNA were analyzed using gene ontology (GO) and DAVID database. Genes involved in the diabetic pathways were classified in accordance with GO analysis. Pathways for these genes were determined using Reactome and Panther databases. The up and down-regulation of all the genes involved were confirmed with the significant *p*-values. Pathways for insulin secretion, binding and its positive regulation were up-regulated while the pathways for negative regulation of insulin were significantly down-regulated. hESCs cultured at our facility have the capability to regenerate the pancreatic β cells after transplantation; as the insulin secretion pathways were significantly up-regulated.

Keywords: Diabetes, human embryonic stem cells, microarray technology

Introduction

Diabetes mellitus (DM) is one of the most common syndrome of disordered metabolism that may develop either due to diminished insulin production (Type 1 DM) or reduced insulin sensitivity (Type 2 DM) [1]. The global prevalence of diabetes among adults over 18 years of age has elevated from 4.7% in 1980 to 8.5% in 2014 [2]. In 2015, The International Diabetes Federation reported that 415 million adults were suffering globally from diabetes, with a prevalence of 8.8% [3] and an estimated mortality of 1.6 million [4]. Almost half of all the deaths attributable to high blood glucose levels occur before 70 years of age. The WHO reported that diabetes will be the seventh leading cause of death in 2030 [2].

DM leads to hyperglycemia which is associated with various complications such as blindness, kidney failure, heart attacks, stroke and lower limb amputation over a significant period of time [5]. Patients with Type 2 DM are more vulnerable to these complications sometimes resulting in premature death. Several medications are available for the treatment of diabetes but long term complications of this disease are still a major issue. Advanced research on stem cells has proposed that the stem cells have the potential to replace destructive β-cells in the patients with diabetes and this could be an important milestone in the management of diabetes [6].

Previous studies reported that human embryonic stem cells (hESCs) differentiate into pancreatic progenitor cells followed by the *in vivo* formation of mature pancreatic endocrine cells and these cells could be a good candidate for the treatment of diabetes [7, 8]. hESCs derived pancreatic progenitors cells showed promising results to cure diabetes in mice [7, 9]. In our previous published study, the patients with dia-

Table 1. RNA concentration analyzed by nanodrop spectrophotometer

S. No	Sample Name	Absorbance value 260/280	Absorbance value 260/230	RNA concentration ng/µl	QC concentration/ yield
1	SO_5327_Repl_A	1.75	1.29	10.2	Optimal
2	SO_5327_Repl_B	1.76	0.64	16.7	Optimal
3	SO_5327_Repl_C	2.15	1.58	18.3	Optimal

ng/µl: Nanogram per microlitre.

betes reported a remarkable improvement after hESCs therapy [10]. A reduction in long term complications associated with heart, kidneys, vision and polyneuropathy, was observed without any adverse event (AE) or teratoma formation [11]. Moreover, no immunosuppressive therapy was given to these patients and the hESCs were well tolerated. The present study describes the differentially expressed gene profile and their related pathways in diabetes with the hESCs cultured at our facility.

Materials and methods

Origin of cell line

Cell lines were cultured from a fertilized egg obtained during natural *in vitro* fertilization (IVF) process with due consent from the donor. The hESCs were cultured and maintained as per our patented technology (United States Granted Patent No US 8592, 208, 52) in a good manufacturing practice (GMP), good laboratory practice (GLP) and good tissue practice (GTP) compliant laboratory. The cell lines were stable and free from any contamination. The detailed cell culture and differentiation techniques are explained in our previous paper [12].

Cell culture and derivation

The fertilized egg was suspended in Roswell Park Memorial Institute (RPMI) medium and broken by mechanical means. After the addition of βhCG and progestin, the cells were placed in a CO_2 water jacketed incubator for a period of 24 hrs. The cell suspension was divided into two and one of them was re-incubated in the same incubator after addition of Dulbecco's Modified Eagle's Medium (DMEM, Hi media Labs, Mumbai, India) under anaerobic conditions. To maintain the cells in their undifferentiated and pluripotent stage, a 0.1 µAmp to 1 mAmp current, was given to the cell culture for a shorter duration. Storage of cell lines is further explained in our previously published paper [12]. The detailed in house cell culture and differentiation techniques for cell lines have also been elaborated in our patent document (detailed compositions comprising human embryonic stem cells and their derivatives, methods of use, and methods of preparation are available athttp://patentscope.wipo.int/search/en/WO2007141657.

RNA extraction and purification

Three samples were collected from media for RNA extraction. The method for RNA extraction used was Qiagen RNeasy micro kit with Dnase Treatment. The RNA concentration was estimated by Nanodrop Spectrophotometer (**Table 1**). The purity and integrity of RNA were checked by employing an Agilent Bioanalyzer. RNA was converted into cDNA using oligo (dT) primers with the help of SuperScript III Cells Direct cDNA Synthesis System. The β-actin gene was considered as house-keeping control gene. The amplified PCR products were analyzed by electrophoresis on 1% agarose gels.

miRNA microarray analysis

The samples were hybridized for microarray experiment. miRNA molecules in total RNA were labeled with Agilent miRNA labeling reagent and Hybridization kit Cat # 5190-0456. Labeling comprised ligation of one cyanine3-pCp molecule to the 3' end of the RNA molecule. The ligation efficiency was more than 90% which was detected by the florescence of ligated miRNA molecules. After hybridization, Agilent scanner was used for scanning the samples and the analysis of the obtained images was carried out by Agilent's Feature extraction software. Raw data was normalized using GeneSpring GX 12.6 software. Complete miRNA in the array was deduced on the basis of intensities. For filtering the high expression miRNA from complete, lobase 2 value ≥ 0.6 was used. The target genes for differentially regulated miRNA's for up- and down-regulated

Table 2. Gene Targets for miRNA's Analyzed by GeneSpring GX

S. No	Mirbase Accession no.	GO Term	Full name	*P*-value
1	MIMAT0002814	GO:0008286	Insulin receptor signaling pathway	0.096
2	MIMAT0005828	GO:0005158	Insulin receptor binding	0.0155
3	MIMAT0004687	GO:0031994	Insulin-like growth factor I binding	0.073
4	MIMAT0005922	GO:0050796	Regulation of insulin secretion	0.047
5	MIMAT0004687	GO:0016942	Insulin-like growth factor binding protein complex	0.0115
6	MIMAT0005828	GO:0032868	Response to insulin stimulus	0.1156
7	MIMAT0005878	GO:0032869	Cellular response to insulin stimulus	0.1156
8	MIMAT0002814	GO:0043567	Regulation of insulin-like growth factor receptor signaling pathway	0.1336
9	MIMAT0004687	GO:0032024	Positive regulation of insulin secretion	0.1466

Table 3. Trancriptomic profile of down-regulated genes

S. No	GO Term	Description	*P* value	Genes
1	GO:0048009	Insulin-like growth factor receptor signaling pathway	0.1585	IGF1R, TSC2
2	GO:0046626	Regulation of insulin receptor signaling pathway	0.3054	SOCS3, TSC2
3	GO:0032868	Response to insulin stimulus	0.8539	IGF1R, SOCS3
4	GO:0046627	Negative regulation of insulin receptor signaling	1.0000	SOCS3
5	GO:0032869	Cellular response to insulin stimulus	1.0000	IGF1R
6	GO:0008286	Insulin receptor signaling pathway	1.0000	IGF1R
7	GO:0043560	Insulin receptor substrate binding	1.0000	IGF1R
8	GO:0005158	Insulin receptor binding	1.0000	IGF1R
9	GO:0043559	Insulin binding	1.0000	IGF1R
10	GO:0005520	Insulin-like growth factor binding	1.0000	IGF1R
11	GO:0031994	Insulin-like growth factor I binding	1.0000	IGF1R

were checked using Genespring GX 12.6 software that had integrated Targetscan database.

Functional annotation analysis

To examine gene pool of detected miRNA, Databasefor Annotation, Visualization and Integrated Discovery (DAVID) (http://www.david.niaid.nih.gov) was used. It covers more than 40 annotation categories, including GO (www.geneontology.org/) terms, protein-protein interactions, protein functional domains, disease associations, biological pathways. GO terms organize genes into hierarchical categories consisting of three main layers and the first layer included three branches: biological process, cellular components, and molecular function.

The potential target genes-associated pathways were analyzed according to Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.ad.jp/kegg) Reactome (http://www.reactome.org) and panther pathway database [14-16]. A p-value of < 0.05 was used as the cut-off criterion.

Results

Study rationale

The present study was done to analyze the mechanistic regulation pathways of hESCs after transplantation into the patients with diabetes. Significant improvements in patients with diabetes after hESCs therapy gave an idea to explore the phenomenon by which hESCs act and results in improvement.

miRNA potential target gene analysis

Hybridized samples predicted the differentially expressed microRNA (miRNA) in the individual test sample. Each miRNA has a unique mirbase accession number and an ability to regulate the expression of several hundred target genes. GeneSpring GX provided the gene target and their location on chromosome for each miRNA

Table 4. Trancriptomic profile of up-regulated genes

S. No	GO Term	Description	*P* value	Genes	Regulation
1	GO:0050796	Regulation of insulin secretion	0.9332	LEP, CAPN10, NNAT	Up
2	GO:0043568	Positive regulation of insulin-like growth factor	1.0000	IGF1	Up
3	GO:0030073	Insulin secretion	1.0000	LEP	Up
4	GO:0043569	Negative regulation of insulin-like growth factor	1.0000	IGFBP5	Up
5	GO:0042567	Insulin-like growth factor ternary complex	1.0000	IGFALS	Up
6	GO:0043559	Insulin binding	1.0000	IGF1R	Up
7	GO:0016942	Insulin-like growth factor binding protein complex	0.0548	IGFALS, IGF1, IGFBP5	Down
8	GO:0005158	Insulin receptor binding	0.1645	IGF1R, PDPK1, IGF1	Up
9	GO:0032868	Response to insulin stimulus	0.3591	SOCS7, FOXO1	Up
10	GO:0005159	Insulin-like growth factor receptor binding	0.3640	YWHAG, REN, IGF1	Up
11	GO:0031994	Insulin-like growth factor I binding	0.4861	IGF1R, IGFBP5	Up
12	GO:0005520	Insulin-like growth factor binding	0.4966	IGF1R, KAZALD1	Up
13	GO:0032869	Cellular response to insulin stimulus	0.5820	IGF1R, PDPK1, FOXO1	Up
14	GO:0048009	Insulin-like growth factor receptor signaling pathway	0.6321	IGF1R, GHR	Up
15	GO:0043567	Regulation of insulin-like growth factor receptor signaling pathway	0.6708	IGF1, IGFBP5	Up
16	GO:0043560	Insulin receptor substrate binding	0.7050	IGF1R, PRKCZ	Up
17	GO:0008286	Insulin receptor signaling pathway	0.7618	FOXO1, FOXO4	Up
18	GO:0046627	Negative regulation of insulin receptor signaling pathway	0.8112	PRKCZ, PTPRF	Up
19	GO:0032024	Positive regulation of insulin secretion	0.8648	CAPN10, NNAT	Up
20	GO:0046626	Regulation of insulin receptor signaling pathway	0.8790	PRKCZ, PTPRF	Up

(**Table 2**). The levels of expression of miRNA analyze the expression of genes which are active in diabetic pathways. miRNA study reveals about the potential target genes which enhance the expression of insulin producing genes. Hence, GO term and their description for each miRNA target gene was determined for diabetic pathways. Genes which are responsible for the up and down-regulation of diabetic pathways were determined with the help of miRNA' target.

Pathway analysis

Diabetic pathways of the predicted miRNAs gene targets were classified by the GO analysis. Genes involved in each pathway are then determined by Reactome, Panther and Kegg databases. Significant *p*-values were used to recognize the up-and down-regulated diabetic pathways. Insulin/IGF pathway-protein kinase B signaling cascade (P=0.02) showed the clear up-regulation of insulin pathways. The insulin binds to its receptor and then to protein kinase B that further controls the levels of blood glucose. The values show that the insulin producing genes are significantly expressed in hESCs further helping to decrease sugar levels in diabetic patients (**Table 3**).

The insulin receptor signaling pathway was not up-regulated (P=1.000). This pathway neither expressed its genes nor showed down-regulation. Reactome and Panther database analysis for Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade (P=0.4607) and signaling by insulin receptor were down-regulated (**Table 4**) which shows that the mitogen doesn't contribute in the activation of insulin receptors.

Functional analysis of genes

Genes involved in the diabetic pathways were classified according to the GO analysis. A significant ($P < 0.05$) up and down-regulation of genes involved in all the diabetic pathways was observed. It was perceived that the binding of insulin to its receptor was enhanced as the genes involved in insulin-like growth factor I binding (IGF1R and IGFBP5) were up-regulated (P=0.4861). Binding of insulin to its receptor activate the various signaling molecules and results in lowering the blood sugar levels (**Figure 1**).

Genes, LEP and IGF1 that are involved in insulin secretion as well as regulation and positive regulation of insulin-like growth factor respective-

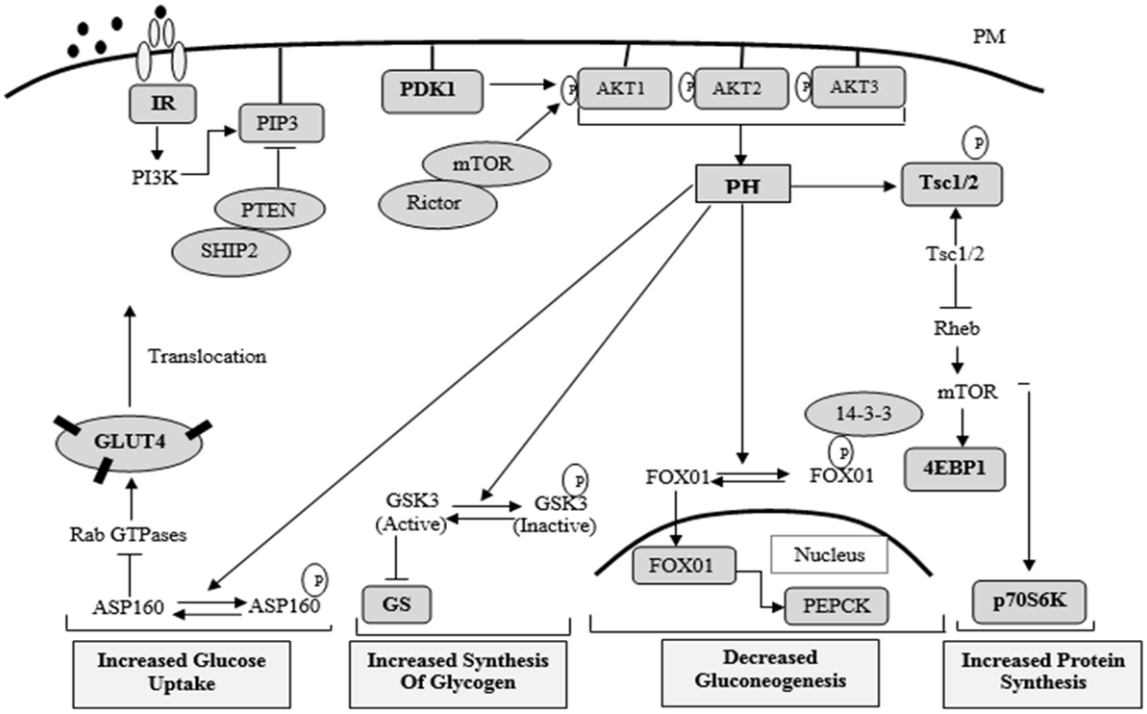

Figure 1. Insulin signaling pathway-AKT/PKB. PKB-Protein kinase b, PI3K-phosphatidylinositol 3-kinase, PDK1-phosphoinositide-dependent kinase-1, Mtor-mammalian target of rapamycin, GSK3-Glycogen synthase kinase-3, forkhead box o1-foxo1, PTEN-phosphatases phosphatase and tensin homologue, ship2-Src-homology-2 domain-containing inositol phosphatase-2.

ly, are also up-regulated (P=1.00). So we assume that after transplantation hESCs results in the increased insulin secretion in diabetic patients which shows the positive results for control of blood sugar levels. FOXO1 is involved in response to insulin stimulus (P=0.3591) and cellular response to insulin stimulus (P=0.500) and both of these pathways were significantly up-regulated which means that the process of gluconeogenesis is decreased as FOX01 is directly involved in the management of process of gluconeogenesis and results in lowering the sugar levels in diabetic patients.

Regulation of insulin receptor signaling pathway, Insulin-like growth factor I binding, insulin-like growth factor binding all were up-regulated as shown in **Table 4** which means that the hESCs cultured at our facility have higher potential to secrete insulin. As one of our previously published study reported that the hESCs were safe and effective in the treatment of patients with diabetes because all the patients after hESCs therapy showed a significant improvement.

We also assume that the hESCs might have the capability to migrate and regenerate the damaged insulin producing cells of pancreas. Only one pathway is down-regulated, *i.e.,* insulin-like growth factor binding protein complex which involve IGFALS, IGF1, IGFBP5 genes. All the other genes involved for the management of diabetes were up-regulated (**Table 5**). Gene SOCS3 inhibits the insulin receptor by negatively regulating the insulin receptor so this gene is down-regulated. Gene IGF1R is involved in various pathways of insulin which includes cellular response to insulin stimulus, insulin receptor signaling pathway and insulin like growth factor binding. The *p*-value for all these pathways was non-significant (P=0.05) which means that the IGF1R was down-regulated in all the pathways (**Table 5**).

Discussion

Diabetes is a chronic condition characterized by hyperglycemia, insulin resistance and insulin deficiency. The American Diabetes Association has recommended various therapeutic

Table 5. Description analysis of pathways

Pathways-up regulation					
S. No	GO Term	Description	*P* value	Genes	Regulation
1	hsa04910	Insulin signaling pathway	0.4649	PPP1CA, SOCS3, TSC2, PPP1CB	Down
2	P00032	Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	1.0000	IGF1R	Down
3	REACT_498	Signaling by Insulin receptor	1.0000	TSC2	Down
4	P00033	Insulin/IGF pathway-protein kinase B signaling cascade	0.0275	FOXH1, IGF1R, FOXQ1, TSC2, FOXB2, FOXB1	Up
5	P00033	Insulin/IGF pathway-protein kinase B signaling cascade	0.4663	FOXQ1, FOXR2	Down
Pathway Down regulation					
1	hsa04910	Insulin signaling pathway	0.1327	CALML6, PIK3R2	
2	P00032	Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	0.4607	IGF1R, FOS, GRAP, IGF1, RTL1	
3	REACT_498	Signaling by Insulin receptor	0.8047	PDPK1, PIK3R2	

options for the management of diabetes that include sulphonylureas, thiazolidines and metformin along with insulin therapy, but their long term use results in cardiovascular diseases, hypoglycemia and various other AEs [17].

Recent advancement in the therapeutic potential of stem cells have given a new hope for the management of diabetes. Mazzola *et al* showed that the stem cell therapy can replace the insulin producing cells of the pancreas which are destroyed by the patient's own immune system [6]. Rosa *et al* showed that the stem cells have the capability to migrate and regenerate the damaged cells. The study also proved that the stem cells are also helpful in reducing the autoimmune process and assist in a faster recovery [18]. Chengwei *et al* reported that the stem cells differentiate into islet like cells and have immuno-modulatory abilities. They secrete the growth factors that enhances the differentiation of progenitor cells into endogenous progenitor cells (EPCs), which then promote tissue regeneration and angiogenesis of the defective tissues [19].

Zhou *et al* showed that the stem cells derived from umbilical cord exert trophic effects on the pancreatic β islets by secreting β-cell growth factors such as IGF1. The study revealed multiple mechanisms and pathways involved in the management of diabetes with hESCs [20]. Rezania *et al* suggested that the hESCs derived pancreatic progenitors are capable of treating pre-existing diabetes [21]. Hua *et al* opined that hESCs are a strong therapeutic candidate for the treatment of diabetes as they can produce an unlimited number of pancreatic islet cells and these cells then lead to *in vivo* formation of mature pancreatic cells [7]. Pagluica *et al* proved that the hESCs have a much lower risk of tumorogensis as these are isolated from fertilized egg and are pluripotent in nature [22]. Li *et al* reported that the hESCs have very few chances of immune mediated rejection as they have unique immune privileged properties [23].

Our previously published studies reported that hESCs were effective in the treatment in patients with diabetes with a good tolerability profile. A reduction in the secondary complications associated with high blood sugar such as the heart, kidneys, vision and polyneuropathy was also observed [10, 11]. No immunosuppressants were given to these patients; no tumor formation was observed. In the present study, the mechanistic pathways for the effectiveness of hESCs in the patients with diabetes were explored. The highly expressed miRNA's target genes involved in various diabetic pathways were analyzed. The analysis showed that the pathways like regulation of insulin secretion, positive regulation of insulin like growth factor, insulin secretion, and insulin like growth factor ternary complex were up-regulated. Up-regulation of insulin producing pathways might help the hESCs to regenerate the damaged pancreatic cells.

Various registered clinical trials using stem cells for diabetes proposed the same hypothesis that the hESCs after transplantation in human pancreas result in an increased angiogenesis, secretion of various cytokines and up-regulation of pancreatic transcription factors and insulin pathways for the activation of β

cells and survival [24]. Similar results were also observed in our study such as the Insulin/IGF pathway-protein kinase B signaling cascade is up-regulated and Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade is down-regulated.

Conclusion

The present study indicated that the hESCs cultured at our facility had the capability to regenerate the pancreatic β cells after transplantation; as the diabetic pathways are significantly up-regulated. However, well designed studies are required to prove the long term efficacy of hESCs in the treatment of patients with diabetes.

Acknowledgements

The author acknowledges Knowledge Isotopes Pvt. Ltd. (http:// www.knowledgeisotopes.com) for the medical writing assistance.

Disclosure of conflict of interest

None.

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References

[1] Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman Med J 2012; 27: 269.

[2] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3: e442.

[3] World Medical Association. Available on: http://www.wma.net/en/30publications/10policies/b3/17c.pdf,.

[4] World Health Organisation. Available on: http://www.who.int/mediacentre/factsheets/fs312/en/.

[5] Siddiqui AA, Siddiqui SA, Ahmad S, et al. Diabetes: mechanism, pathophysiology and management-a review. International Journal of Drug Development and Research 2013.

[6] Mazzola N. Review of current and emerging therapies in type 2 diabetes mellitus. Am J Manag Care 2012; 18 Suppl: S17-26.

[7] Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, Gauvin R, Narayan K, Karanu F, O'Neil JJ, Ao Z, Warnock GL, Kieffer TJ. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012; 61: 2016-2029.

[8] Hua XF, Wang YW, Tang YX, Yu SQ, Jin SH, Meng XM, Li HF, Liu FJ, Sun Q, Wang HY, Li JY. Pancreatic insulin-producing cells differentiated from human embryonic stem cells correct hyperglycemia in SCID/NOD mice, an animal model of diabetes. PLoS One 2014; 9: e102198.

[9] Liang D, Zhang Y, Han J, Wang W, Liu Y, Li J, Jiang X. Embryonic stem cell-derived pancreatic endoderm transplant with MCT1-suppressing miR-495 attenuates type II diabetes in mice. Endocr J 2015; 62: 907-920.

[10] Shroff G. Therapeutic potential of human embryonic stem cells in type 2 diabetes mellitus. World J Stem Cells 2016; 8: 223.

[11] Shroff G. A novel approach of human embryonic stem cells therapy in treatment of Friedreich's ataxia. Int J Case Rep Imag 2015; 6: 261-266.

[12] Shroff G. Establishment and characterization of a neuronal cell line derived from a 2-cell stage human embryo: clinically tested cell-based therapy for neurological disorders. Int J Recent Sci Res 2015; 6: 3730-3738.

[13] DAVID. Avaiable from: http://www.david.niaid.nih.gov Accessed on: 1 May, 2017.

[14] Reactome. Available on: http://www.reactome.org Accessed on: 22 Aug, 2017.

[15] KEGG. Available on: www.genome.ad.jp/kegg Accessed on: 18 April, 2017.

[16] Panther. Available from: http://pantherdb.org/ Accessed on: 21 August, 2017.

[17] Choe C, Edelman S. New therapeutic options for treating type-2 diabetes: a review of insulin analogs and premixed insulin analogs. J Natl Med Assoc 2007; 99: 357.

[18] Rosa SB, Voltarelli JC, Chies JA, Pranke P. The use of stem cells for the treatment of autoimmune diseases. Braz J Med Biol Res 2007; 40: 1579-1597.

[19] Gu C, Huang S, Gao D, Wu Y, Li J, Ma K, Wu X, Fu X. Angiogenic effect of mesenchymal stem cells as a therapeutic target for enhancing diabetic wound healing. Int J Low Extrem Wounds 2014; 13: 88-93.

[20] Zhou Y, Hu Q, Chen F, Zhang J, Guo J, Wang H, Gu J, Ma L, Ho G. Human umbilical cord matrix-derived stem cells exert trophic effects on beta-cell survival in diabetic rats and isolated islets. Dis Model Mech 2015; 8: 1625-1633.

[21] Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, Gauvin R, Narayan K, Karanu F, O'Neil JJ, Ao Z, Warnock GL, Kieffer TJ. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional is-

lets capable of treating pre-existing diabetes in mice. Diabetes 2012; 61: 2016-2029.

[22] Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, Peterson QP, Greiner D, Melton DA. Generation of functional human pancreatic β cells in vitro. Cell 2014; 159: 428-439.

[23] Li L, Baroja ML, Majumdar A, Chadwick K, Rouleau A, Gallacher L, Ferber I, Lebkowski J, Martin T, Madrenas J, Bhatia M. Human embryonic stem cells possess immune-privileged properties. Stem Cells 2004; 22: 448-456.

[24] Bhansali A, Upreti V, Khandelwal N, Marwaha N, Gupta V, Sachdeva N, Sharma RR, Saluja K, Dutta P, Walia R, Minz R, Bhadada S, Das S, Ramakrishnan S. Efficacy of autologous bone marrow-derived stem cell transplantation in patients with type 2 diabetes mellitus. Stem Cells Dev 2009; 18: 1407-1416.