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Transplantation of Human dgHPSCs Overexpressing Insulin and ERRγ can Efficiently Decrease the Glucose and HbA1c levels, Increase the Secretion of C-peptide and Repair the Complications of Coronary Heart Disease in T2D Patient (Case #1-A)

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ABSTRACT

Daily injection of insulin is a widely used treatment for human diabetes. Yet, exogenous insulin injection cannot efficiently prevent the development of diabetes complications. Recently, we reported that the transplantation of directly-generated human pluripotent stem cells (dgHPSCs) overexpressing human insulin or estrogen-related receptor γ (ERRγ) gene can not only reduce the glucose levels of human diabetes patients, but also restore the function of human pancreatic β cells, and therefore with the potential to prevent the patients from developing diabetic complications. Here, we further reported that transplantation of dgHPSCs overexpressing insulin (INS) and ERRγ genes can significantly decrease the glucose and HbA1c levels and increase the secretion of C-peptide and repair the complication of coronary heart disease in type 2 diabetes (T2D) patient. Our data proved that this strategy can secrete high level insulin in vitro, increase the secretion of C-peptide (C-P) in vivo, decrease the patient's glucose (GLU) and HbA1c levels, and repair the patient's diabetic complications efficiently. Thus, our report is the first to prevent and reverse human diabetic complications and with the potential to completely cure human T2D.

Keywords

dgHPSCs, ERRγ, INS, C-peptide, Human T2D, HbA1c, Coronary heart disease.

Abbreviations

INS: insulin; ERRγ: Estrogen-related Receptor γ; hADSCs: Human Adipose-derived Stem Cells; dgHPSCs: Directly Generated Human Pluripotent Stem Cells; iPSCs: induced pluoripotent stem cells; F-GLU: Fasting Glucose; F-C-P: Fasting C-peptide; F-INS: Fasting Insulin; HbA1c: Glycosylated Haemoglobin; FFT-CBG: Fasting Fingertip Capillary Blood Glucose; T1D: Type 1 Diabetes; T2D: Type 2 Diabetes; CDU: Color Doppler Ultrasonography; ECG: Electrocardiogram.

Introduction

Although almost 100 years ago, Banting et al. (1922), demonstrated that daily INS injection could control the blood glucose levels of type I diabetes (T1D), the administration of INS is only a treat,

not a cure, for human diabetes [1,2]. Therefore, even to date, the present available therapies for diabetes still have limited effects in preventing the development of diabetes complications and repairing existing tissue damages [3,4]. Thus, it is urgent to innovate more efficient methods for treating and curing human diabetes.

It was a long-time controversy for the safety and efficacies of stem cell therapy due to the concerns of tumor formation and immune rejection [5-8]. Hence, despite great progressions achieved in human stem cells biology, including, but not limited, the establishment of human induced pluripotent stem cells [9], the successful differentiation of human functional pancreatic β-like cells [10,11], etc, yet, to our knowledge, few clinical stem cell therapies for diabetes patients were reported thus far.

Previously, we designed a "Mouse clone model" to rigorously assess the tumorigenicity and immunogenicity for pluripotent stem cell transplantation therapy theoretically [8]. More importantly, during the past decade, our hospital treated various human diseases with stem cell transplantation, and never found tumour formation in any patient cases, therefore, our data demonstrated that stem cell transplantations are safe and efficient, at least to date [12,13].

Recently, we investigated the efficacies of transplantation of dgHPSCs overexpressing human INS or ERRγ genes for the treatment of human T2D, and our results revealed that this strategy not only can efficiently decrease the glucose and HbA1c levels, but also can increase the secretion of C-P and repair diabetes-derived prophase cataract [4,14]. Here, we further reported that, besides the aforementioned functions of our method, the overexpression of human INS and ERRγ genes simultaneously can also efficiently repair the diabetes-derived coronary heart disease complications of the patient. Taken together, to our knowledge, we are the first to report that transplantation of stem cells overexpression INS and/or ERRγ genes can potentially completely cure human diabetes eventually.

Materials and Methods

Statement of Ethical Approval

The treatments for the patients and the use of human stem cells were approved by the Ethics Committee of Interventional Hospital of Shandong Red Cross Society (Shengjieyi 2003, No. 26) in compliance with Helsinki Declaration. The Ethics Committee of Interventional Hospital of Shandong Red Cross Society approved this clinical study and treatments. The participants provided their written confirmed consent to participate the clinical study and treatments. The Ethics Committee of Interventional Hospital of Shandong Red Cross Society approved this consent procedure. All the treatments for the patients and use of human stem cells were performed in accordance with the guidelines established in Interventional Hospital of Shandong Red Cross Society approved by the Ethics Committee. After traditional daily INS injection for about four years, the patient agreed to try the stem cell therapy with overexpression of INS and ERRγ genes in our hospital to treat and cure his diabetes and coronary heart disease complications. The stem cells used in these clinical treatments are dgHPSCs Line #2 and Line #3 stored at our Stem Cell Bank. All these stem cells were isolated and proliferated with the written confirmed consent of the participants and their parents [4,14].

Patient Case

The diagnosis and previous treatment of this patient (designed as patient #1, initials T. S. D., male, born at 1958) was reported earlier [4]. After the first round treatment, the patient wanted to continue for further transplantations of our stem cells, and the treatment period is from March 19 to June 30 of 2018 (Table 1). Because the transplanted stem cells can also improve the appetite of the recipients, and the patient did not restrain his diet, the patient's daily GLU levels decreased slowly and slightly from March 20 to July 27 of 2018. After our instructions to the patient for proper dieting, he did restrain his daily diet, and his body weight decrease from 70kg to 67.5kg, approximately, and afterwards, and his daily GLU levels decreased significantly (Tables 2 and 3, Figure 5). At October 22 of 2018, the patient performed diagnostic tests in the hospital, including his venous fasting GLU, fasting INS, fasting C-P, HbA1c, Color Doppler Ultrasonography (CDU) and Electrocardiogram (ECG) examinations (Tables 2 and 4; Figures 1, 2, 3, and 4).

Cell preparation

The isolation of lipoaspirate cells and the induction of dgHPSCs were exactly the same as described [14-16]. The cell lines were designated as Line #2 and Line #3 and stored at our stem cell bank. These dgHPSCs Line #2 and Line #3 show TRA-1-60 positive, which is a pluripotent marker of human stem cells (Data not shown) [16].

Date (dd-mm-yy)	Cell types	LV volumes (ml)	Cell number
19-03-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	7.5×10^7
26-03-18	dgHPSCs (Line#3) + INS + ERRγ	50 + 50	3.49×10^7
31-03-18	dgHPSCs (Line#3) + INS + ERRγ	50 + 50	6.8×10^7
31-05-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	6.3×10^7
06-06-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	6.03×10^7
12-06-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	6.75×10^7
18-06-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	9.99×10^7
24-06-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	7.2×10^7
30-06-18	dgHPSCs (Line#2) + INS + ERRγ	50 + 50	4.5×10^7

Table 1: Time table of human stem cell transplantations.

Test	20/01/18 TEST#1	08/03/18 TEST#2	07/07/18 TEST#3	18/08/18 TEST#4	22/10/18 TEST#5	Reference ranges
F-C-P	0.584 [4]	0.890 [4]	1.060	N/A	N/A	0.37-1.47 (nmol/L)
F-INS	59.88 [4]	53.06 [4]	69.27	N/A	N/A	21.53-121.98 (pmol/L)
F-GLU	9.46 [4]	11.23 [4]	10.03	9.51	7.03	3.9-6.1 (mmol/L)
HbA1c	6.0 [4]	8.3 [4]	8.2	8.16	6.58	4.2-6.3 (%)

Table 2: The tested venous blood F-C-P, F-INS, F-GLU and HbA1c levels.

Date (dd-mm-yy)	FFT-CBG (mmol/L)	Date (dd-mm-yy)	FFT-CBG (mmol/L)	Date (dd-mm-yy)	FFT-CBG (mmol/L)
20-03-18	10.7	21-03-18	10.5	22-03-18	12.5
23-03-18	11.6	24-03-18	12.1	25-03-18	12.7
26-03-18	12.2	27-03-18	12.0	28-03-18	10.6
29-03-18	10.0	30-03-18	10.6	31-03-18	9.4
01-04-18	9.8	02-04-18	10.1	03-04-18	9.3
04-04-18	10.0	05-04-18	9.9	06-04-18	10.3
07-04-18	9.5	08-04-18	11.2	09-04-18	9.9
10-04-18	9.4	11-04-18	10.1	12-04-18	8.3
13-04-18	10.2	15-04-18	11.1	16-04-18	9.7
17-04-18	9.6	18-04-18	9.8	19-04-18	9.3
20-04-18	9.4	21-04-18	8.7	22-04-18	8.7
23-04-18	9.8	24-04-18	9.2	25-04-18	8.8
26-04-18	9.3	27-04-18	9.1	29-04-18	9.7
30-04-18	9.4	01-05-18	8.8	02-05-18	9.2
03-05-18	9.1	04-05-18	9.4	05-05-18	10.4
06-05-18	10.0	07-05-18	9.4	08-05-18	9.5
09-05-18	10.3	10-05-18	10.5	13-05-18	10.4
14-05-18	10.3	15-05-18	10.0	16-05-18	10.4
17-05-18	12.5	18-05-18	10.7	19-05-18	9.5
20-05-18	9.0	21-05-18	9.9	22-05-18	8.7
23-05-18	10.7	24-05-18	10.2	25-05-18	10.1
26-05-18	9.9	27-05-18	9.8	28-05-18	9.9
29-05-18	9.1	30-05-18	9.1	31-05-18	9.7
01-06-18	9.7	02-06-18	9.9	03-06-18	9.6
04-06-18	10.0	05-06-18	8.4	07-06-18	10.9
08-06-18	10.9	09-06-18	10.2	10-06-18	10.2
11-06-18	10.5	12-06-18	9.4	13-06-18	8.7
14-06-18	9.3	15-06-18	8.7	16-06-18	8.3
17-06-18	8.0	18-06-18	8.2	19-06-18	8.1
20-06-18	8.9	21-06-18	9.3	22-06-18	9.2
23-06-18	9.3	24-06-18	9.4	25-06-18	10.8
26-06-18	9.5	27-06-18	8.1	28-06-18	8.3
29-06-18	9.2	30-06-18	7.7	01-07-18	8.3
02-07-18	7.9	03-07-18	7.0	04-07-18	8.9
05-07-18	8.2	06-07-18	8.5	08-07-18	7.8
09-07-18	8.9	10-07-18	9.5	11-07-18	10.2
12-07-18	8.2	13-07-18	9.5	14-07-18	8.1
15-07-18	8.7	16-07-18	8.9	17-07-18	9.0
18-07-18	10.2	19-07-18	8.5	20-07-18	7.6
21-07-18	9.0	22-07-18	7.2	23-07-18	6.8
24-07-18	7.3	25-07-18	8.3	26-07-18	8.5

27-07-18	7.2	28-07-18	6.9	29-07-18	7.2
30-07-18	7.6	31-07-18	7.2	01-08-18	7.7
02-08-18	7.5	03-08-18	7.8	04-08-18	7.2
05-08-18	8.4	06-08-18	7.9	07-08-18	7.9
08-08-18	9.0	09-08-18	8.2	10-08-18	7.9
11-08-18	7.6	12-08-18	7.0	13-08-18	6.6
14-08-18	7.4	15-08-18	7.7	16-08-18	8.1
17-08-18	8.8	18-08-18	8.2	19-08-18	7.5
20-08-18	8.4	21-08-18	7.7	22-08-18	7.1
23-08-18	8.9	24-08-18	8.4	25-08-18	7.5
26-08-18	7.5	27-08-18	7.9	28-08-18	7.1
29-08-18	8.1	30-08-18	6.6	31-08-18	6.7
01-09-18	7.1	02-09-18	7.0	03-09-18	6.8
04-09-18	7.3	05-09-18	7.4	06-09-18	8.6
07-09-18	7.7	08-09-18	7.4	09-09-18	8.3
10-09-18	7.2	11-09-18	8.5	12-09-18	7.4
13-09-18	7.3	14-09-18	7.0	15-09-18	7.1
16-09-18	6.6	17-09-18	6.5	18-09-18	6.1
19-09-18	6.1	20-09-18	5.7	21-09-18	5.8
22-09-18	5.8	23-09-18	5.9	24-09-18	6.4
25-09-18	6.8	26-09-18	6.7	27-09-18	7.1
28-09-18	7.4	29-09-18	7.0	30-09-18	6.7
01-10-18	7.0	02-10-18	6.6	03-10-18	8.2
04-10-18	6.9	05-10-18	7.0	06-10-18	7.6
07-10-18	7.1	08-10-18	7.6	09-10-18	6.4
10-10-18	7.2	11-10-18	6.2	12-10-18	6.3
13-10-18	7.0	14-10-18	5.9	15-10-18	6.5
16-10-18	6.4	17-10-18	6.1	18-10-18	5.9
19-10-18	6.3	20-10-18	6.3	21-10-18	6.4
22-10-18	5.8				

Table 3: Daily monitoring of FFT-CBG levels (from 20-03-2018 to 22-10-2018).

Tests	Date (17-12-2016)	Date (05-07-2018)	Date (22-10-2018)
Two dimensional guided M-mode			
Aortic sinus inner diameter	27 mm	26 mm	24 mm
Left ventricular septal thickness	4-10 mm	10 mm	12 mm
Left ventricular posterior wall thickness	9 mm	10 mm	9 mm
Left ventricular end diastolic diameter	49 mm	45 mm	43 mm
Right ventricular anterior-posterior diameter	19 mm	20 mm	25 mm
Inner diameter of left atrium	32 mm	33 mm	33 mm
Diameter of main pulmonary artery	19 mm	20 mm	17 mm
Maximum flow velocity of aorta	1.26 m/s	1.01 m/s	1.36 m/s
Maximum flow velocity of pulmonary artery	0.75 m/s	0.97 m/s	0.95 m/s
Measurement of heart function			
EF (Ejection fraction)	71%	71%	63%
FS (Short axis shortening fraction)	40%	40%	34%
E peak	0.88 m/s	0.61 m/s	0.86 m/s

A peak	0.75 m/s	0.72 m/s	0.53 m/s
Ultrasonic findings CDFI (Color Doppler Flow Imaging)	The size and shape of the atrioventricular cavities of the heart are OK; the structure and echo of the vales of each group are OK; the open and close are OK; the continuity of the atrioventricular septum are COMPLETE; parts of the anterior wall of the left ventricle became thinner; the amplitude of motion reduced; the echo and amplitude of motion of the rest of the cardiac muscle were OK. CDFI: Reflux signal was detected in mitral valve area; reflux velocity was Vmax=3.14m/s; the pressure difference was 39mmHg; reflux signal was detected in tricuspid area; reflux velocity was Vmax = 2.71 m/s; the pressure difference was 29 mmHg.	The size and shape of the atrioventricular cavities of the heart are OK; the structure and echo of the vales of each group are OK; the open and close are OK; the continuity of the atrioventricular septum are COMPLETE; parts of the anterior wall of the left ventricle became thinner; the echo enhanced; the thinnest place is about 0.6cm; the amplitude of motion reduced. CDFI: Reflux signal was detected in mitral valve area; reflux velocity was Vmax = 3.31m/s; the pressure difference was 44mmHg; reflux signal was detected in aortic area; reflux velocity was Vmax = 2.29m/s; the pressure difference was 21mmHg; reflux signal was detected in tricuspid area; reflux velocity was Vmax = 2.65m/s; the pressure difference was 28mmHg.	The size and shape of the atrioventricular cavities of the heart are ok; the structure and echo of the valves of each group are OK; the open and close are OK; the continuity of the atrioventricular septum are COMPLETE; the echo of cardiac muscle and motion amplitude are ok; defective areas of abnormal segments of cardiac muscle were not seen. CDFI: Reflux signal was detected in mitral valve area; reflux velocity was Vmax = 3.83m/s; the pressure difference was 58mmHg; reflux signal was detected in tricuspid area; reflux velocity was Vmax = 2.55m/s; the pressure difference was 26mmHg.
Ultrasonic hints	The motion of the left ventricular wall was defective periodically; Reflux of the mitral valve (mild); Reflux of the tricuspid valve (mild)	The motion of the left ventricular wall was defective periodically; Reflux of the mitral valve (mild); Reflux of the tricuspid valve (mild); Reflux of the aortic valve (mild); The filling of the left ventricle was abnormal	Reflux of the mitral valve and tricuspid valve (mild); The adaptability of the left ventricle was OK
Electrocardiogram diagnosis hints	Sinus rhythm; Old myocardial infarct of anterior septum	Sinus bradycardia; The ST segments of some leads were uplifted; The Q wave was abnormal	Sinus bradycardia; The Q wave was abnormal

Table 4: Color Doppler Ultrasonography and Electrocardiogram examination reports.
Note: The different diagnostic symptoms were underlined.

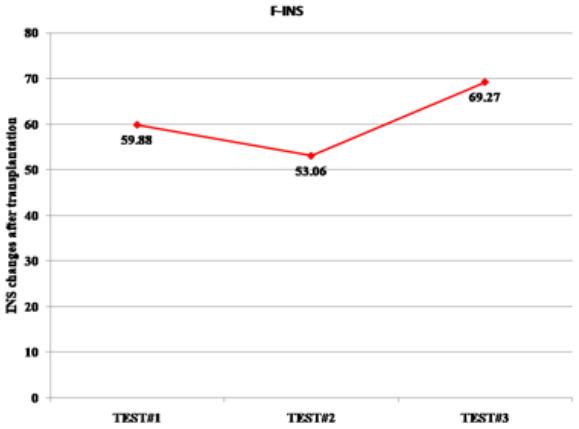

Figure 1: The venous F-INS changes: TEST#1 (20-01-2018); TEST#2 (08-03-2018); TEST#3 (07-07-2018).

Lentivirus vector (LV) construction, production and infection

Clinical level third generation of LVs pWPI/INS and pWPI/ERRγ were constructed as previously described [17-20] from original vector pWPI/hPLKWT/Neo (Addgene plasmid #35385) [21]. The pWPI/INS and pWPI/ERRγ LVs were produced, and infected into dgHPSCs Line #2 and Line #3 cells according to a previous report [21]. Briefly, two 15-cm dishes of dgHPSCs were infected with 50ml pWPI/INS and 50ml pWPI/ERRγ LVs, individually and sequentially (Table 1).

dgHPSCs transplantation

After clinical treatment of dgHPSCs/INS+ERRγ cells, the cells were transplanted into the patient intravenously. Each time, approximately 3.5 x 107 to 1.0 x 108 cells were transplanted into the patient, and the intervals between transplantations were around five to seven days, respectively. Totally, nine times of transplantation were performed (Table 1).

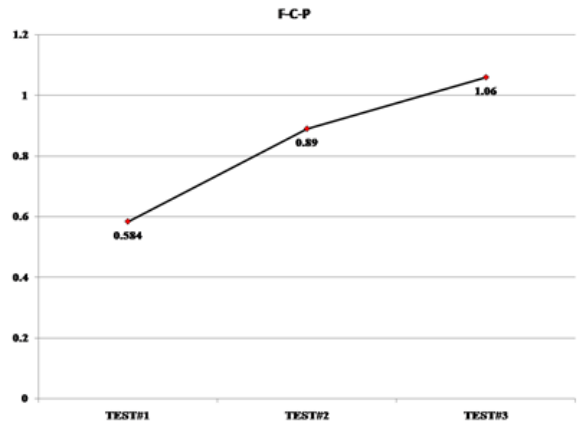

Figure 2: The venous F-C-P changes: TEST#1 (20-01-2018); TEST#2 (08-03-2018); TEST#3 (07-07-2018).

INS secretion test

The INS secreted into cell culture supernatant was tested via electrochemiluminescence method performed by Kingmed Diagnostics (Jinan, Shandong Province, China) [4,14].

Clinical responses and treatment efficacy assessment

The venous blood C-P and INS were tested by Jinan ADICON

Clinical Center (Jinan, Shandong, China) (Table 2, Figures 1 and 2). The fasting venous blood GLU and HbA1c were tested by the Hospital of Traditional Chinese Medicine of Zhangdian District (Zibo, Shandong, China) (Table 2, Figures 3 and 4). The fasting fingertip capillary blood GLU was monitored daily (Table 3, Figure 5). The CDU and ECG examinations were also performed by the Hospital of Traditional Chinese Medicine of Zhangdian District (Table 4). The subjective symptoms were reported by the patient during the following-up visit.

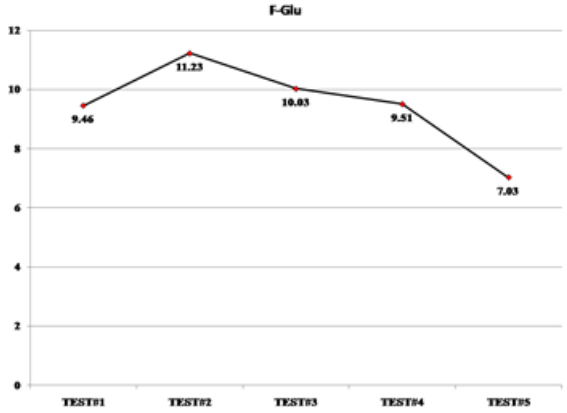

Figure 3: The venous F-GLU changes: TEST#1 (20-01-2018); TEST#2 (08-03-2018); TEST#3 (07-07-2018); TEST#4 (18-08-2018); TEST#5 (22-10-2018).

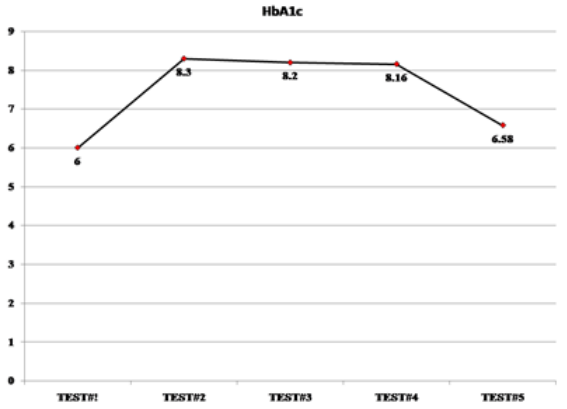

Figure 4: The venous HbA1c changes: TEST#1 (20-01-2018); TEST#2 (08-03-2018); TEST#3 (07-07-2018); TEST#4 (18-08-2018); TEST#5 (22-10-2018).

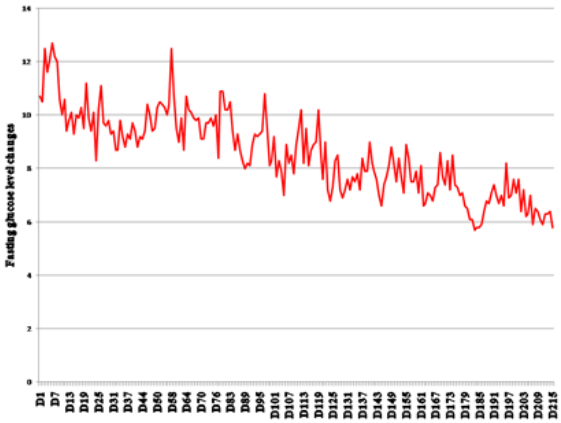

Figure 5: Fasting glucose changes during daily monitoring of FFT-CBG levels (from 20/03/2018 to 22/10/2018).

Results

dgHPSCs-INS+ERRγ cells can secrete more INS immediately after transduction

It was reported that the overexpression of ERRγ gene in human iPSC-derived β-like cells can result in secretion of human INS in vitro, and therefore, ERRγ is a master regulator of β cell maturation in vivo [11]. Recently, we also confirmed that dgHPSCs overexpressing ERRγ (dgHPSCs-ERRγ) can secret human INS into the cell culture supernatant immediately after transduction, and the concentration of INS can reach up to 30.84μIU/ml, whereas, the concentration of secreted INS is 11.61μIU/ml when dgHPSCs overexpressing INS (dgHPSCs-INS) using the same method [4, 14]. Based on these findings, we reasoned that the overexpression of ERRγ and INS, simultaneously, may increase the secretion of human INS into the cell culture supernatant. Thus, we infected dgHPSCs with pWPI/INS and pWPI/ERRγ, individually and sequentially, to make dgHPSCs double overexpressing INS and ERRγ genes (dgHPSCs-INS+ERRγ). Surprisingly, we found that the concentration of INS in the supernatant was 84.47μIU/ml, which was much higher than our previous reports, i.e. 11.61 μIU/ml and 30.84μIU/ml for dgHPSCs-INS and dgHPSCs-ERRγ, respectively. Consistent with previous reports, no human C-P was detected in the supernatant of dgHPSCs-INS+ERRγ as well, indicating that these cells can produce and secrete human INS at the "stem" state [4,14]. Therefore, to our knowledge, this is the first report that the double overexpression of human INS and ERRγ genes can synergistically promote the synthesis and secretion of human INS at the "stem" cell state, and do not need to differentiate into matured β-like cells.

Transplantation of dgHPSCs-INS+ERRγ can efficiently decrease GLU and HbA1c levels and increase the secretion of C-P

The detailed results were showed in Tables 2 and 3, Figures 1, 2 3, 4 and 5. From January 10th of 2018 on, when the patient accepted the first stem cell transplantation, he stopped the daily INS injections completely [4]. The results revealed that the patient's F-GLU increased from around 7mmol/L (with daily INS injection) up to 9.46mmol/L in the morning of January 19th of 2018 (without INS daily injection) [4]. With the continued stem cell transplantations, the patient's F-GLU and HbA1c decrease gradually. Because, at the first stage, the patient did not restrain his diet properly, his GLU levels and HbA1c values decreased slowly and slightly during March 20 to July 27 of 2018. According to our instructions, the patient began to diet his dieting afterwards, and gradually, his body weight decreased from 70kg to 67.5kg, approximately. As a result, his GLU levels and HbA1c values decreased significantly (Tables 2 and 3, Figures 3, 4 and 5), and almost reach to the normal ranges, such as 6.58% of HbA1c and 7.03mmol/L of F-GLU, etc. These data demonstrated that the transplantation of our stem cells (dgHPSCs-INS, dgHPSCs-ERRγ, dgHPSCs-INS+ERRγ, respectively) can completely replace the daily injection of exogenous INS.

More importantly, after totally 14 times transplantations [4], the patient's F-INS and F-C-P increase from 53.06pmol/L to 69.27pmol/L, and 0.584nmol/L to 0.890nmol/L up to 1.060nmol/L, respectively (Table 2, Figures 1 and 2). These results vividly manifested the improvement of the pancreatic β cell functions for secreting more INS and C-P, and demonstrated that the transplanted stem cells not only can decrease the GLU and HbA1c levels, but also can repaired pancreas and improve the β cell functions for secreting INS. Whereas, the daily injection of INS is lack of this role to restore the pancreas functions. Obviously, our method stated here is much better than daily administration of INS. Furthermore, our data also shown that our strategy can maintain its GLU level control function for at least 9 months (from January 19th to October 22th of 2018) [4]. To our knowledge, this is the first report using stem cells transplantations overexpressing INS or/and ERRγ to repair the pancreatic β cell functions for human T2D patients [4,14].

Transplanted dgHPSCs-INS+ERRγ cells can repair and improve diabetes-derived coronary heart disease complications

Besides the above-mentioned restoration and improvement of pancreatic β cell functions of diabetes patient, we also investigated the functions of our transplanted dgHPSCs-INS or/and ERRγ cells for the repairing of diabetic complications. As diagnosed, the patient has diabetes-derived coronary heart disease and was implanted 3 cardiac stents before [4]. The patient accepted CDU and ECG examinations, respectively and sequentially, as described in Table 4. The detailed diagnoses were listed in Table 4, and the different diagnostic symptoms were underlined. Both the CDU and ECG diagnoses indicated that the patient's heart structure and functions were improved significantly. To our knowledge, this is the first report to repair diabetes complications of coronary heart disease. Therefore, together with our former report [14], our strategy can repair the diabetes-derived complications, such as prophase cataract and coronary heart disease.

The follow-up visit of the patient

When the patient accepted the first round stem cell transplantations, he reported that he had a transient fever after the transplantations of about 1 x 10^8 cells, and the body temperature reached up to 37°C. The fever faded away next day [4].

When the patient accepted his second round transplantations, he did not report fever ever since then. Instead, the patient continued to describe that he felt stronger physically, felt happy mentally, and his knees felt much better, he could exercise much longer than before and did not feel tired. After he restrained his diet properly, his body weight decreased from 70kg to 67.5kg, approximately, and his daily GLU and HbA1c levels also decrease significantly as stated in Tables 2 and 3, and Figures 3, 4 and 5. Each time when he did his CDU and ECG examinations in the hospital, his physician informed him that his heart became better, and he was very happy about this. In a word, his overall physical and mental conditions were improved significantly, and he was very happy about our treatment for him [4].

Discussion

T2D is an increasing threat to human health span, and approximately 25.9% of Americans of 65 years or older have diabetes, whereas, 9.3% of those in the general population [3, 4]. Even worse, up to date, there are no effective methods which can prevent from the development of diabetes complications. At present, the daily administration of exogenous INS is the final option for late stage diabetes. Yet, the injection of INS cannot maintain blood glucose levels within the narrow physiological range and further protect from development of various diabetic complications, due to INS injection cannot exactly mimic pancreatic β cells to adjust INS secretion in response to varying blood GLU levels [22]. Therefore, there are three goals must be achieved in order to completely cure human diabetes. The first goal is to control blood GLU levels effectively, and this can be realized by INS administration. The second goal is to repair the functions of pancreatic β cells to improve the secretion of INS. And the third is to restore and even reversed existed diabetes complications. So far, the last two goals are not satisfactorily achieved clinically, although, in mouse models, it was reported that the transplanted differentiated human pancreatic β cells could incorporate into the recipients and repair their pancreatic β functions [10, 11]. Particularly, to our knowledge, besides our recent reports [4, 14], there are no successful reports for clinically treatment of human diabetes patients using human pluripotent stem cells thus far.

In our previous investigations, we found that dgHPSCs-INS and dgPSCs-ERRγ can secrete INS effectively in vitro after transduction. The concentrations of INS in cell culture supernatant could reach up to 11.61 μIU/ml and 30.84μIU/ml, respectively [4, 14]. Encouraged by this finding, we reasoned that the double expression of INS and ERRγ genes simultaneously may produce and secrete more INS in vitro. Surprisingly, we found that dgHPSCs-INS+ERRγ could secrete much more INS than the overexpression of INS and ERRγ genes, separately, and the concentration of INS in the supernatant was up to 84.47μIU/ml, which was significantly higher than our previous reports [4, 14]. Because our transduction format was performed in 50ml volume, in theory, our dgHPSCs-INS+ERRγ cells could secrete approximately 4.22IU INS into the patient blood after each transplantation. More importantly, the patient's F-C-P and F-INS were improved significantly, from 0.584nmol/L (before transplantation) to 0.890nmol/L and up to 1.060nmol/L (after transplantation), and from 53.06pmol/L to 69.27pmol/L (after transplantation), respectively [4] (Table 2; Figures 1 and 2). These data demonstrated that the functions of the patient's pancreatic β cells were repaired and improved significantly. As a result, the patient's GLU and HbA1c levels decreased gradually but significantly after the transplantations without exogenous injections of INS. Both of them were almost down to the normal ranges (Tables 2 and 3; Figures 3, 4, and 5). Altogether, it is very promising to completely restore the normal functions of pancreatic β cells, and effectively cure T2D via stem cell transplantations.

To investigate whether or not our strategy can reverse the existed diabetes complications of the patient, we suggested the patient

did proper examinations after transplantations sequentially. The heart examinations of the patient by CDU and ECG examinations indicated that the coronary heart disease of the patient was restored significantly. This data manifested that the transplanted dgHPSCs could repair the existed diabetes-derived complications. Combined with our recent report [14], it is evident that transplantation of dgHPSCs can prevent from diabetes complication development and eventually repair and reverse existed complications, including prophase cataract and coronary heart disease.

It is hard to describe by words the overall benefits of dgHPSCs transplantations to the improvement of the patient conditions physically and mentally, including but not limited to the strengthened muscles and the knees, and the vitality of the patient. All these changes vividly manifested the positive effects of our dgHPSCs-INS-and/or-ERRγ transplantation therapy, particularly for the repair and reverse of the existed diabetes-derived complications. Therefore, our investigations laid an important foundation for stem cell therapy for eventually cure human diabetes diseases [4,14].

Conclusion

Our investigations demonstrated the following conclusions:

- dgHPSCs cells can be produced from human adipose-derived stem cells without any genetic modifications [4,14].
- dgHPSCs-INS+ERRγ can produce and secrete much more human INS into cell culture medium in vitro immediately after transduction and do not need to differentiate into functional pancreatic β-like cells [4,14].
- Transplantation of dgHPSCs-INS+ERRγ can decrease the patient's blood GLU and HbA1c levels to nearly the normal ranges, increase C-P level and repair the functions of pancreatic β cells significantly, and replace daily INS injections completely.
- Transplantation of dgHPSCs-INS+ERRγ can improve the patient health conditions greatly, physically and mentally.
- Our strategy can repair and reverse the existed diabetic complications, such as prophase cataract and coronary heart disease, and can potentially completely cure human diabetes [4,14].

Availability of supporting data

The datasets generated and/or analysed during the current study are not publicly available due to the protection of the confidential information of the participated patients but are available from the corresponding author on reasonable request.

Authors Contributions

G Z and T W instructed and supervised the whole experimental and clinical work. X W and X C performed the vector construction. B Z and L Z charged the lentiviral transduction. M F, X C, Z Y and X Y did the cell culture. R L, Q F, X D, L Z, G Y and Y M worked on the clinical treatments of the cells. All the authors discussed, wrote, read and approved the final manuscripts.

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References

1. Banting FG, Best CH, Collip JB, et al. Pancreatic extracts in the treatment of diabetes mellitus preliminary report. Canadian Medical Association Journal. 1922; 12: 141-146.
2. Banting FG. Nobel Lecture. 1925.
3. Palmer AK, Tchkonia T, LeBrasseur NK, et al. Cellular Senescence in Type 2 Diabetes A Therapeutic Opportunity. Diabetes. 2015; 64: 2289-2298.
4. Wang T, Wang X, Zhang B, et al. Transplantation of human pluripotent stem cells overexpressing ERRγ can efficiently improve the symptoms of type 2 diabetes patient. Adv Tissue Eng Regen Med Open Access. 2018; 4: 457-462.
5. Freed CR, Greene PE, Breeze RE, et al. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl JMed. 2001; 344: 710-719.
6. Hagell P, Piccini P, Björklund A, et al. Dyskinesias following neural transplantation in Parkinson's disease. Nat Neurosci. 2002; 5: 627-628.
7. Olanow CW, Goetz CG, Kordower JH, et al. A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol. 2003; 54: 403-414.
8. Zhang G, Zhang Y. Mouse Clone Model for evaluating the immunogenicity and tumorigenicity of pluripotent stem cells. Stem Cell Res Ther. 2015; 6: 255.
9. Yu J, Hu K, Smuga-Otto K, et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science. 2009; 324: 797-801.
10. Pagliuca FW, Millman JR, Gürtler M, et al. Generation of functional human pancreatic β cells in vitro. Cell. 2014; 159: 428-439.
11. Yoshihara E, Wei Z, Lin CS, et al. ERRγ is required for the metabolic maturation of therapeutically functional glucose-responsive β cells. Cell Metabolism. 2016; 23: 622-634.
12. Wang T, Zhang G. On the safety and efficacy of human patient stem cell transplantation therapy. Adv Tissue Eng Regen Med Open Access. 2017; 2: 00030.
13. Wang T, Li R, Zhang G. Significant physical improvement of spinal cord injured patients after neural stem cell transplantation. Adv Tissue Eng Regen Med Open Access. 2017; 2: 00018.
14. Wang T, Chen X, Cui X, et al. Transplantation of human pluripotent stem cells overexpressing insulin/ERRγ can efficiently decrease the HbA1c levels of type 2 diabetes patient Case #2-A. Bio Rxiv. 2018.
15. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multi potent stem cells. Mol Biol Cell. 2002; 13: 4279-4295.
16. Dowey SN, Huang X, Chou BK, et al. Generation of integration-free human induced pluripotent stem cells from postnatal blood mononuclear cells by plasmid vector expression. Nat Protoc. 2012; 7: 2013-2021.
17. Zhang G, Tandon A. Quantitative assessment on the cloning efficiencies of lentiviral transfer vectors with a unique clone site. Sci Rep. 2012; 2: 415.

18. Zhang G, Tandon A. Combinatorial Strategy A highly efficient method for cloning different vectors with various clone sites. American Journal of Biomedical Research. 2013; 1: 112-119.
19. Zhang G. A new overview on the old topic The theoretical analysis of Combinatorial Strategy for DNA recombination. American Journal of Biomedical Research. 2013; 1: 108-111.
20. Zhang G, Zhang Y. On the All or Half law of recombinant DNA. American Journal of Biomedical Research. 2016; 4: 1-4.
21. Visanji NP, Wislet-Gendebien S, Oschipok LW, et al. Effect of Ser-129 phosphorylation on interaction of alpha-synuclein with synaptic and cellular membranes. J Biol Chem. 2011; 286: 35863-35873.
22. Minami K, Seino S. Current status of regeneration of pancreatic β-cells. Journal of Diabetes Investigation. 2013; 4: 131-141.