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The Cure of Human Type 2 Diabetes via Systematic Transplantations of dgHPSCs Overexpressing Human ERRγ and/or Insulin Genes (I)

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ABSTRACT

Although insulin (INS) injection is widely administrated clinically for the treatment of human type 2 diabetes (T2D), this method cannot effectively prohibit the progressing of diabetes complications. Previously, we demonstrated that daily INS injections could be replaced by transplantations of directly-generated human pluripotent stem cells (dgHPSCs) overexpressing human INS and/or estrogen-related receptor γ (ERRγ) genes. In this investigation, we further revealed that systematic transplantations of dgHPSCs overexpressing INS and/or ERRγ genes could completely replaced the INS injections of T2D patient, and the patient's blood glucose and HbA1c levels were kept around the normal ranges. Hence, this study provided an important strategy for the eventually cure of human T2D disease through human stem cell and gene therapy.

Keywords

dgHPSCs, ERRγ, INS, Human T2D.

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; **HbA1c:** Glycosylated Haemoglobin; **T2D:** Type 2 Diabetes.

Introduction

In 1922, Banting and his colleagues demonstrated that type 1 diabetes (T1D) patients could be treated with insulin (INS) injection [1]. But the daily injection of INS is only a treat, not a cure, for human diabetes [2]. Eventually, human diabetes patients will inevitably develop various complications gradually. Therefore, it is in urgent demand to develop new strategies to effectively prevent and repair human diabetes complications for better treatment of this disease.

Previously, we reported that serial transplantations of directly generated human pluripotent stem cells (dgHPSCs) overexpressing human INS and/or ERRγ genes can efficiently replace the daily injections of INS. More importantly, this strategy can not only control the glucose and HbA1c levels around the normal ranges [3-6], but also alleviate and repair diabetes-derived complications, such as coronary heart disease [4], hypertension [5], prophase cataract [6], etc. To test whether or not our methods are suitable for more T2D patients, we further applied this strategy to treat another T2D patient who needed to inject 36 IU INS daily to maintain his blood glucose levels. After 19 times systematic transplantations of dgHPSCs overexpressing human INS and/or ERRγ genes, the daily injections of INS were completely replaced. Therefore, in general sense, his T2D was cured currently.

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 participant provided his 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 patient 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 a long time, the patient agreed to try the stem cell therapy overexpressing INS and/or ERRγ genes in our hospital to treat his T2D. The stem cells used in these clinical treatments are dgHPSCs Line #1, Line #3, Line #4, Line #5, Line #6, Line #7, respectively, stored at our Stem Cell Bank. All these stem cells were isolated and proliferated with the written confirmed consent of the participants [3-6].

Patient

The patient's name is S. S. R., who was born at 1966. In 2006, he was diagnosed with T2D. After the diagnosis, he was treated with daily injection of INS and oral administration of Metformin to control his blood glucose levels, at first intermittently, and then continuously, and his T2D worsened gradually. Until July of 2018, he was diagnosed with coronary heart disease, and one cardiac stent was implanted. From March of 2019, he decided to accept our stem cell transplantation therapy. At that time, He was 176cm in height and 93.5Kg in weight, and diagnosed with mild obesity. He needed to inject 36 IU INS daily to maintain his blood glucose levels.

Cell preparation

The isolation of lipoaspirate cells and the induction of dgHPSCs were exactly the same as described [3,7,8]. The cell lines used in this investigation were Line #1 (derived from Z. G., the correspondence author of this paper), Line #3 (derived from S. X. Y.), Line #4 (derived from L. H. G.), Line #5 (derived from L. W. F.), Line #6 (derived from L. P.) and Line #7 (derived from H. J.) (Table 1), respectively, which were stored at our stem cell bank. All the dgHPSCs Lines employed for the treatment were allogeneic, among these lines, Line #3 was derived from a woman volunteer, and all the others were from volunteered men.

Lentivirus vector (LV) construction, production and infection

Clinical level third generation of LVs pWPI/INS and pWPI/ERRγ were constructed and stored in our lab as previously described [9-11]. The pWPI/INS and pWPI/ERRγ LVs were produced, and infected into dgHPSCs cell lines according to a previous report [12]. Each infection format was shown in details in Table 1.

dgHPSCs transplantation

The intravenous transplantations of dgHPSCs cell lines were exactly the same as previously described [3-6]. The transplantation dates, cell types, and cell numbers were listed in Table 1.

Table 1: dgHPSC cell transplantations for the patient.

Times	Dates	Cell types	Cell No.
#1	30/03/2019	dgHPSCs (Line #1) + 50ml ERRγ	6.05×10^7
#2	07/04/2019	dgHPSCs (Line #1) + 50ml ERRγ	1.70×10^8
#3	14/04/2019	dgHPSCs (Line #1) + 50ml ERRγ + 50ml INS	1.08×10^8
#4	20/04/2019	dgHPSCs (Line #4) + 50ml ERRγ + 50ml INS	1.51×10^8
#5	27/04/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	5.77×10^7
#6	05/05/2019	dgHPSCs (Line #6) + 50ml ERRγ + 50ml INS	1.24×10^8
#7	12/05/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	9.14×10^7
#8	12/05/2019	dgHPSCs (Line #7) + 50ml ERRγ	3.87×10^7
#9	18/05/2019	dgHPSCs (Line #4) + 50ml ERRγ + 50ml INS	7.02×10^7
#10	25/05/2019	dgHPSCs (Line #4) + 50ml ERRγ + 50ml INS	9.0×10^7
#11	02/06/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	1.22×10^8
#12	08/06/2019	dgHPSCs (Line #7) + 75ml ERRγ	1.20×10^8
#13	15/06/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	6.10×10^7
#14	23/06/2019	dgHPSCs (Line #4) + 50ml ERRγ + 50ml INS	1.05×10^8
#15	30/06/2019	dgHPSCs (Line #3) + 50ml ERRγ + 50ml INS	9.20×10^7
#16	07/07/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	1.24×10^8
#17	14/07/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	5.99×10^7
#18	21/07/2019	dgHPSCs (Line #5) + 50ml ERRγ + 50ml INS	6.39×10^7
#19	27/07/2019	dgHPSCs (Line #5) + 75ml ERRγ + 50ml INS	8.64×10^7
Total			1.80×10^9

Assessment of the efficacy of transplantation treatment

The fasting fingertip capillary blood glucose was monitored daily during the first half months or so after stem cell transplantations (Table 2). The levels of fasting venous blood glucose and glycosylated Haemoglobin (HbA1c) were tested respectively by different Hospitals as listed in Table 3. The subjective symptoms were described by the patient during the following-up visit.

Table 2: the fasting fingertip capillary blood glucose levels and the dosages of daily insulin injections after dgHPSC cell transplantations.

Dates	Fasting glucose (mmol/L)	Morning INS injection (IU)	Evening INS injection (IU)
08/04/2019	6.4	16	12
09/04/2019	7.9	16	12
11/04/2019	7.2	16	12
13/04/2019	8.2	16	12
14/04/2019	7.5	16	12
15/04/2019	8.1	16	12
16/04/2019	7.6	14	10
17/04/2019	7.4	14	10
18/04/2019	7.6	14	10
19/04/2019	7.5	12	10
20/04/2019	6.7	12	10
21/04/2019	8.4	12	10
22/04/2019	8.2	12	10
23/04/2019	7.8	12	10
24/04/2019	7.8	12	10
25/04/2019	7.9	12	10
26/04/2019	6.7	12	10

Table 3: Tests of fasting venous blood glucose and glycosylated Haemoglobin (HbA1c) before and after dgHPSC cell transplantations.

Dates	Test results (**F-GLU/ HbA1c**)	Hospitals
20/07/2018	7.3 (before)	ShiboHigh-Tech Hospital, Zibo, Shandong
26/07/2018	5.53/6.73 (before)	Shandong Police General Hospital, Jinan, Shandong
2019-4-10	8.22 (after)	Shandong Luzhong Prison Hospital, Zibo, Shandong
2019-4-25	7.91 (after)	Shandong Luzhong Prison Hospital, Zibo, Shandong
25/07/2019	7.23 (after)	Physical Examination Center of Zichuan District People's Hospital, Zibo, Shandong
28/08/2019	6.58 (after)	Shandong Luzhong Prison Hospital, Zibo, Shandong
31/12/2020	8.72/7.40 (after)	Physical Examination Center of Zichuan District People's Hospital, Zibo, Shandong

Note: Reference ranges: F-GLU: 3.9-6.1 mmol/L; HbA1c: 4-6%

Results

The gradual decreases of INS daily injection dosages after dgHPSCs transplantations

The patient was transplanted with dgHPSC stem cells overexpressng ERRγ alone and ERRγ + INS intravenously. The transplantation times were 19, and the stem cells amounted to approximately 1.80 X 10^9 in total (Table 1). Before stem cell transplantation therapy, the patient needed to inject 36 IU INS to keep his blood glucose levels around the normal range. After he accepted the stem cell transplantations, which was about once a week, he gradually decreased the INS daily injection dosages. As shown in Table 2, he roughly decreased 2 IU of INS daily after each time of transplantation. Besides the INS injections in the morning and at evening as shown in Table 2, the patient also needed to inject INS before lunch (not shown in Table 2). After totally 19 times transplantations, the patient completely replaced daily INS injections, and his blood glucose and HbA1c levels were maintained around the normal ranges. Importantly, about one and half years later after the last stem cell transplantation, his blood glucose and HbA1c levels were still kept around the normal ranges (Table 3). The treatment efficacy of this patient was consistent with our previous reports [3-6]. Therefore, this strategy might be an efficient treatment for more T2D patients.

The follow-up visits of the patient

The patient reported that, after he accepted the first three times stem cell transplantations, he had a transient fever after each times of transplantation. The fever faded away next day. With the transplantation times going up, he said the fever was very mild and did not need special care anymore. At the same time, he felt stronger physically and happier mentally. Even though we repeatedly advised him to restrain from drinking too much wine, he still often drank high concentration alcohol wine a lot, for example, sometimes up to 400ml 53% volume wine made in China at lunch or dinner. We will continue to try our best to suggest him keeping on a healthier lifestyle. In summary, his overall health conditions improved significantly, physically and mentally, and he was satisfied with our stem cell transplantation therapy for him very much.

Discussion

Because T2D is mounting more and more risk for human health, and to date, traditional methods cannot prevent from the occurrence of diabetes complications eventually, it is urgent to develop new strategies for the treatment and cure of type 2 diabetes. Previously, we demonstrated that transplantations of dgHPSCs overexpressing human INS and/or ERRγ genes can greatly improve the overall physical and mental conditions of T2D patients. And after sufficient times of dgHPSCs transplantations, the treated patients could completely give up insulin injections [3-6].

In this report, we provided a further example to demonstrate the efficacy of our strategy. After totally 19 times of intravenous transplantations of dgHPSCs overexpressing human INS and/or ERRγ genes, the patient did not need administration of exogenous insulin injections completely (Before the stem cell transplantation therapy, the patient needed to inject 36 IU insulin daily to control his blood glucose levels). The total number of stem cells transplanted added up to approximately 1.80 X 10^9 cells. And the patient physical and mental conditions improved greatly.

Another great concern for human stem cell and gene therapy is the risk to form tumours after the transplantations. During the past more than three years, we treated several diabetes patients with this strategy, and no tumour formations were observed ever [3-6]. In addition, during the past five years, the correspondence author of this paper (Z. G.) transplanted intravenously 77 times of stem cells (including adipose stem cells and dgHPSCs), including both autologous and allogeneic stem cells, and the total number of stem cells reached to 6.36 X 10^9 approximately, and I (Z. G.) did not feel any abnormal symptoms (Data not shown). Taken together, our preliminary investigations revealed that using allogeneic dgHPSCs overexpressing human INS and/or ERRγ to treat human type 2 diabetes was effective and safe, at least so far.

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 patient but are available from the corresponding author on reasonable request.

Authors Contributions

G Z instructed and supervised the whole experimental work. T W instructed and supervised the whole clinical work. X C and S D performed the vector construction. L C and M W charged the lentiviral production and transduction. Z Y and N Z did the stem cell culture. R L, X S, X J, G Y and Y M worked on the clinical treatments of the cells. All the authors discussed, 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. 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.
4. Wang T, Wang X, Zhang B, et al. 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). Stem Cells Regen Med. 2018; 2: 1-9.
5. Wang T, Cui X, Yang Z, et al. Transplantation of Human dgHPSCs Over-expressing Human Insulin/ERRγ Genes Can Not Only Decrease the Glucose Levels but Also Decrease the Hypertension in T2D Patient (Case #1-B). Stem Cells Regen Med. 2019; 3: 1-5.
6. Wang T, Chen X, Cui X, et al. Transplantation of human pluripotent stem cells over expressing insulin/ERRγ can efficiently decrease the HbA1c levels of type 2 diabetes patient (Case #2-A). International Journal of Current Research. 2018; 10: 74690-74696.
7. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002; 13: 4279-4295.
8. 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.
9. Zhang G, Tandon A. Quantitative assessment on the cloning efficiencies of lentiviral transfer vectors with a unique clone site. Sci Rep. 2012, 2: 415.
10. 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.
11. Zhang G, Zhang Y. On the "All or Half" law of recombinant DNA. American Journal of Biomedical Research. 2016; 4: 1-4.
12. Zhang G, Wang T. Efficient Lentiviral Transduction of Different Human and Mouse Cells. Int J Bioprocess Biotech: IJBBT-104. 2018; 1: 1-11.