

Stem Cell & Regenerative Medicine

Transplantation of Human dgHPSCs Overexpressing Human Insulin/ERR γ Genes Can Not Only Decrease the Glucose Levels but Also Decrease the Hypertension in T2D Patient (Case #1-B)

Taihua Wang^{1,2,3*#}, Xiaohui Cui^{1,2,3#}, Zhenzhen Yang^{1,2,3#}, Rongrong Li^{1,2#}, Xinyi Shi^{3#}, Linyu Cui^{1,2,3}, Mengqian Wang^{1,2,3}, Ning Zuo^{1,2,3}, Xiaojuan Diao^{1,2}, Kun Li^{1,2}, Limin Zhang^{1,2}, Guoke Yang^{1,2}, Ying Meng^{1,2}, Shuangshuang Cai^{1,2}, Ying Sun^{1,2} and Gang Zhang^{1,2,3,4*}

¹Interventional Hospital of Shandong Red Cross Society, Room 509, 5th Floor, 2766 Yingxiu Road, Jinan, Shandong Province, China.

²Shandong New Medicine Research Institute of Integrated Traditional and Western Medicine, Room 509, 5th Floor, 2766 Yingxiu Road, Jinan, Shandong Province, China.

³Cell Biotechnology Co., Ltd, Room 401, Building 10, Libin Road, Songshan Lake, Dongguan, Guangdong Province, China.

⁴School of Life Sciences, Shandong Normal University, 88 Wenhua East Road, Jinan, Shandong Province, China.

[#]These authors contributed equally to this work.

Citation: Taihua Wang, Xiaohui Cui, Zhenzhen Yang, et al. Transplantation of Human dgHPSCs Overexpressing Human Insulin/ERR γ 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): 1-5.

*Correspondence:

Taihua Wang, Interventional Hospital of Shandong Red Cross Society, Room 509, 5th Floor, 2766 Yingxiu Road, Jinan, Shandong Province, China, E-mail: drwangtaihua@hotmail.com.
Gang Zhang, School of Life Sciences, Shandong Normal University, 88 Wenhua East Road, Jinan, Shandong Province, China, E-mail: sdzbzhanggang@163.com.

Received: 01 November 2019; **Accepted:** 16 December 2019

ABSTRACT

Transplantation of directly-generated human pluripotent stem cells (dgHPSCs) overexpressing human insulin (INS) and/or estrogen-related receptor γ (ERR γ) genes can efficiently improve the symptoms of type 2 diabetes (T2D), such as the glucose (GLU) and glycosylated haemoglobin (HbA1c) levels, diabetes-derived complications, etc. Therefore, human stem cell transplantation might be a potential strategy to replace exogenous INS administration, and further to prevent the patients from developing diabetic complications. However, little is known concerning the efficacy duration of the transplanted stem cells. Here, we reported the following-up visits of one patient for a year. We found that the patient not only maintained almost normal blood glucose levels, but also his blood pressure decreased to normal range without taking anti-hypertensive drugs. Thus, our data indicated that the efficacy duration of transplanted stem cells can last about two years. At the same time, the transplanted stem cells can not only decrease the blood glucose levels, but also reduce the hypertensions in T2D patient.

Keywords

dgHPSCs, ERR γ , INS, Human T2D, Hypertension.

Abbreviations

INS: insulin; ERR γ : Estrogen-related Receptor γ ; hADSCs: Human Adipose-derived Stem Cells; dgHPSCs: Directly Generated Human Pluripotent Stem Cells; ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; F-GLU: Fasting Glucose; HbA1c: Glycosylated Haemoglobin; FFT-CBG: Fasting Fingertip Capillary Blood Glucose; T1D: Type 1 Diabetes; T2D: Type 2 Diabetes.

Introduction

With the widespread of diabetes, particularly type 2 diabetes (T2D), it becomes a great threat for human health and lifespan. Even worse, the current therapies for diabetes including insulin (INS) administration only have limited effects in preventing the processing of diabetes-derived complications [1,2]. So far, many fundamental investigations in human stem cell biology already substantially performed, such as the induction of human pluripotent stem cells (iPSCs) [3], the successful production of human pancreatic β -like cells from human embryonic stem cells (ESCs) and iPSCs, etc. [4,5], the major concerns regarding tumour

formation and immune rejection of transplanted human stem cells greatly impede the translation from human stem cell biology into clinical medicine [6,7].

Previously, we reported that transplantation of directly generated human pluripotent stem cells (dgHPSCs) overexpressing INS and/or $ERR\gamma$ genes can efficiently decrease the blood glucose (GLU) and glycosylated haemoglobin (HbA1c) levels, and significantly improve the physical and mental conditions of T2D patients [8-10]. But how long is the efficacy duration of these transplanted stem cells? Although little is known, it is a critical question we must answer.

To investigate the efficacy duration of transplanted stem cells, we request the recipient patient to monitor his fasting fingertip capillary blood glucose (FFT-CBG) daily, and also keep his close eyes on the other possible changes of his health. The follow-up visits revealed that the efficacy duration for controlling the GLU levels can last almost two years. More surprisingly, after about one and a half years of transplantation, the patient's hypertension decreased to normal range without taking anti-hypertensive drugs. The patient will continue to monitor his GLU levels and hypertension changes subsequently. Our data demonstrated that the efficacy duration of transplanted stem cells can last at least about two years. And besides decreasing the blood GLU levels, the stem cells can also reduce the hypertensions in T2D patient.

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 (Shengjiei 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 $ERR\gamma$ genes in our hospital to treat and cure his diabetes and coronary heart disease complications. All these stem cells were isolated and proliferated with the written confirmed consent of the participants [8,10].

Patient Case

The detailed description of this patient, T. S. D., was described in References [8,10]. After he finished the stem cell transplantation therapy at June 30 of 2018, he kept monitoring his FFT-CBG levels daily (Table 1), and tried his best to keep diabetic diet and exercise physically. He felt his body was full of strength. Sometimes, he would walk about 10,000 to 15,000 steps daily. Some other times, he would do other exercises, such as whipping a top for one to

two hours. From June 1st of 2019 on, he stopped taking anti-hypertensive drugs, and his blood pressure was kept around the normal range (Tables 2 and 3).

Date (dd/mm/yyyy)	F-GLU	Date (dd/mm/yyyy)	F-GLU	Date (dd/mm/yyyy)	F-GLU
23/10/2018	6.0	24/10/2018	5.9	25/10/2018	6.1
26/10/2018	5.9	27/10/2018	6.5	28/10/2018	6.8
29/10/2018	6.8	30/10/2018	6.1	31/10/2018	6.1
01/11/2018	5.3	02/11/2018	5.9	03/11/2018	6.0
04/11/2018	5.8	05/11/2018	6.6	06/11/2018	5.5
07/11/2018	6.4	08/11/2018	6.8	09/11/2018	6.2
10/11/2018	6.1	11/11/2018	6.4	12/11/2018	6.3
13/11/2018	6.3	14/11/2018	5.9	15/11/2018	5.8
16/11/2018	5.9	17/11/2018	6.0	18/11/2018	6.0
19/11/2018	5.3	20/11/2018	5.7	21/11/2018	5.7
22/11/2018	6.2	23/11/2018	5.5	24/11/2018	5.4
25/11/2018	5.8	26/11/2018	5.1	27/11/2018	6.1
28/11/2018	5.7	29/11/2018	6.1	30/11/2018	5.6
01/12/2018	5.7	02/12/2018	6.2	03/12/2018	6.5
04/12/2018	5.5	05/12/2018	6.2	06/12/2018	6.1
07/12/2018	5.9	08/12/2018	6.0	09/12/2018	6.5
10/12/2018	7.0	11/12/2018	6.2	12/12/2018	6.3
13/12/2018	6.1	14/12/2018	6.1	15/12/2018	4.8
16/12/2018	5.0	17/12/2018	5.5	18/12/2018	6.3
19/12/2018	5.4	20/12/2018	5.7	21/12/2018	5.9
22/12/2018	6.1	23/12/2018	5.9	24/12/2018	5.6
25/12/2018	5.8	26/12/2018	5.4	27/12/2018	6.1
28/12/2018	5.9	29/12/2018	6.4	30/12/2018	5.1
31/12/2018	5.6	01/01/2019	7.5	02/01/2019	6.2
03/01/2019	6.8	04/01/2019	6.9	05/01/2019	7.7
06/01/2019	7.1	07/01/2019	7.6	08/01/2019	7.5
09/01/2019	7.8	10/01/2019	7.2	11/01/2019	7.3
12/01/2019	8.1	13/01/2019	7.1	14/01/2019	7.0
15/01/2019	5.8	16/01/2019	6.0	17/01/2019	5.4
18/01/2019	5.9	19/01/2019	5.8	20/01/2019	5.9
21/01/2019	6.4	22/01/2019	7.4	23/01/2019	6.4
24/01/2019	5.6	25/01/2019	5.7	26/01/2019	6.2
27/01/2019	6.1	28/01/2019	6.6	29/01/2019	5.9
30/01/2019	6.1	31/01/2019	6.8	01/02/2019	6.5
02/02/2019	6.2	03/02/2019	7.3	04/02/2019	7.1
05/02/2019	7.3	06/02/2019	7.4	07/02/2019	7.2
08/02/2019	8.2	09/02/2019	7.1	10/02/2019	6.5
11/02/2019	7.2	12/02/2019	6.9	13/02/2019	6.0
14/02/2019	6.3	15/02/2019	6.6	16/02/2019	6.2
17/02/2019	5.8	18/02/2019	6.0	19/02/2019	5.8
20/02/2019	6.0	21/02/2019	6.0	22/02/2019	6.6
23/02/2019	6.3	24/02/2019	5.1	25/02/2019	6.5
26/02/2019	5.8	27/02/2019	5.9	28/02/2019	5.8
01/03/2019	5.7	02/03/2019	6.2	03/03/2019	6.8

04/03/2019	6.1	05/03/2019	6.2	06/03/2019	6.7
07/03/2019	6.5	08/03/2019	6.8	09/03/2019	6.8
10/03/2019	7.1	11/03/2019	7.2	12/03/2019	7.0
13/03/2019	7.6	14/03/2019	6.8	15/03/2019	7.1
16/03/2019	7.6	17/03/2019	6.7	18/03/2019	6.6
19/03/2019	6.6	20/03/2019	6.8	21/03/2019	6.9
22/03/2019	7.1	23/03/2019	7.2	24/03/2019	7.6
25/03/2019	7.0	26/03/2019	6.9	27/03/2019	8.1
28/03/2019	8.0	29/03/2019	7.2	30/03/2019	6.8
31/03/2019	7.2	01/04/2019	7.8	02/04/2019	7.9
03/04/2019	7.0	04/04/2019	7.6	05/04/2019	6.7
06/04/2019	7.5	07/04/2019	7.7	08/04/2019	6.7
09/04/2019	6.4	10/04/2019	6.2	11/04/2019	5.8
12/04/2019	5.8	13/04/2019	5.9	14/04/2019	6.0
15/04/2019	6.1	16/04/2019	5.2	17/04/2019	5.9
18/04/2019	5.6	19/04/2019	6.0	20/04/2019	4.9
21/04/2019	5.5	22/04/2019	5.4	23/04/2019	4.8
24/04/2019	5.6	25/04/2019	4.7	26/04/2019	4.7
27/04/2019	5.1	28/04/2019	5.0	29/04/2019	5.4
30/04/2019	6.0	01/05/2019	6.2	02/05/2019	6.2
03/05/2019	5.8	04/05/2019	6.7	05/05/2019	5.7
06/05/2019	6.0	07/05/2019	5.9	08/05/2019	5.8
09/05/2019	5.4	10/05/2019	5.3	11/05/2019	6.0
12/05/2019	6.2	13/05/2019	6.2	14/05/2019	5.5
15/05/2019	5.4	16/05/2019	5.3	17/05/2019	6.6
18/05/2019	6.7	19/05/2019	5.7	20/05/2019	5.9
21/05/2019	6.3	22/05/2019	5.7	23/05/2019	5.9
24/05/2019	5.7	25/05/2019	6.0	26/05/2019	6.5
27/05/2019	6.9	28/05/2019	6.8	29/05/2019	6.5
30/05/2019	5.8	31/05/2019	6.5	01/06/2019	5.5
02/06/2019	5.9	03/06/2019	5.2	04/06/2019	5.9
05/06/2019	6.7	06/06/2019	6.7	07/06/2019	6.8
08/06/2019	6.1	09/06/2019	5.9	10/06/2019	6.8
11/06/2019	6.8	12/06/2019	5.7	13/06/2019	5.4
14/06/2019	5.7	15/06/2019	5.4	16/06/2019	6.0
17/06/2019	6.3	18/06/2019	5.1	19/06/2019	5.5
20/06/2019	5.4	21/06/2019	5.9	22/06/2019	6.2
23/06/2019	5.5	24/06/2019	5.6	25/06/2019	5.2
26/06/2019	5.8	27/06/2019	5.9	28/06/2019	6.1
29/06/2019	5.9	30/06/2019	5.1	01/07/2019	5.0
02/07/2019	5.3	03/07/2019	5.0	04/07/2019	5.5
05/07/2019	5.7	06/07/2019	5.2	07/07/2019	6.8
08/07/2019	6.8	09/07/2019	5.5	10/07/2019	6.2
11/07/2019	6.4	12/07/2019	5.8	13/07/2019	6.4
14/07/2019	6.2	15/07/2019	7.1	16/07/2019	6.0
17/07/2019	6.3	18/07/2019	6.3	19/07/2019	6.2
20/07/2019	5.7	21/07/2019	6.4	22/07/2019	5.6
23/07/2019	6.3	24/07/2019	7.1	25/07/2019	7.2
26/07/2019	6.7	27/07/2019	6.4	28/07/2019	6.3

29/07/2019	7.1	30/07/2019	8.1	31/07/2019	7.3
01/08/2019	7.3	02/08/2019	7.2	03/08/2019	6.4
04/08/2019	7.4	05/08/2019	7.0	06/08/2019	6.2
07/08/2019	6.5	08/08/2019	6.0	09/08/2019	7.0
10/08/2019	6.6	11/08/2019	6.1	12/08/2019	6.9
13/08/2019	7.5	14/08/2019	6.9	15/08/2019	6.3
16/08/2019	6.6	17/08/2019	6.5	18/08/2019	5.6
20/08/2019	6.8	21/08/2019	6.4	22/08/2019	5.8
23/08/2019	6.5	24/08/2019	6.8	25/08/2019	6.6
26/08/2019	7.0	27/08/2019	6.5	28/08/2019	4.3
29/08/2019	7.4	30/08/2019	7.6	31/08/2019	6.7
01/09/2019	7.3	02/09/2019	6.6	03/09/2019	6.8
04/09/2019	6.8	05/09/2019	5.7	06/09/2019	5.5
07/09/2019	6.5	08/09/2019	6.2	09/09/2019	6.1
10/09/2019	7.4	11/09/2019	6.5	12/09/2019	6.3
13/09/2019	5.5	14/09/2019	8.3	15/09/2019	7.4
16/09/2019	6.6	17/09/2019	6.2	18/09/2019	6.2
19/09/2019	6.2	20/09/2019	7.2	21/09/2019	7.0
22/09/2019	7.0	23/09/2019	7.0	24/09/2019	5.7
25/09/2019	5.8	26/09/2019	5.4	27/09/2019	6.1
28/09/2019	5.7	29/09/2019	5.8	30/09/2019	5.3
01/10/2019	6.9	02/10/2019	7.4	03/10/2019	6.7
04/10/2019	7.3	05/10/2019	6.9	06/10/2019	7.1
07/10/2019	7.5	08/10/2019	7.1	09/10/2019	8.1
10/10/2019	7.7	11/10/2019	6.8	12/10/2019	6.5
13/10/2019	6.4	14/10/2019	5.8	15/10/2019	5.8

Table 1: Daily monitoring of FFT-CBG levels (from 23/10/2018 to 15/10/2019).

Date (dd/mm/yyyy)	Blood pressure (Relax/contract)	Date (dd/mm/yyyy)	Blood pressure (Relax/contract)	Date (dd/mm/yyyy)	Blood pressure (Relax/contract)
15/09/2019	70/110	16/09/2019	70/120	17/09/2019	65/105
18/09/2019	65/110	19/09/2019	75/120	20/09/2019	70/110
21/09/2019	75/115	22/09/2019	70/120	23/09/2019	70/115
24/09/2019	65/105	25/09/2019	65/105	26/09/2019	70/110
27/09/2019	70/110	28/09/2019	65/100	29/09/2019	60/95
30/09/2019	60/100	01/10/2019	60/100	02/10/2019	75/125
03/10/2019	70/120	04/10/2019	65/105	05/10/2019	65/105
06/10/2019	70/110	07/10/2019	70/120	08/10/2019	70/120
09/10/2019	80/140	10/10/2019	75/125	11/10/2019	75/130
12/10/2019	80/135	13/10/2019	75/125	14/10/2019	70/110
15/10/2019	78/135				

Table 2: The decrease of blood pressure after human stem cell transplantations without taking anti-hypertensive drugs.

Cell preparation

Refer to the references [8-10].

Lentivirus vector (LV) construction, production and infection

Refer to the references [8-15].

dgHPSCs transplantation

Refer to references [8,10].

Follow-up visits

In order to investigate the efficacy duration of the transplanted human stem cells overexpressing human INS and/or ERR γ genes, the patient was requested to monitor his FFT-CBG levels daily (Table 1) [8-10]. And from June 1 of 2019 on, he stopped taking anti-hypertensive drugs. From September 15 of 2019 on, he began to measure his blood pressure every day (Tables 2 and 3).

Disease and complications	Treatment before transplantation	After transplantation
T2D	24 IU INS were injected daily, 12 IU in the morning, 12 IU at evening [8,10].	Totally stopped INS injection.
Diabetes-derived coronary heart disease and myocardial infarct	Three cardiac stents were implanted [8,10].	Myocardial Infarct disappeared, coronary heart disease improved significantly.
Hypertension	Taking Benazepril Hydrochloride Tablets, 10 mg/Tablet, half tablet per day.	Stopped taking Benazepril Hydrochloride Tablets, the blood pressure became normal.

Results

The efficacy duration of transplanted dgHPSCs-INS/ERR γ cells can last almost two years.

In order to investigate the efficacy duration of transplanted dgHPSCs overexpressing INS and/or ERR γ for controlling the blood GLU levels of the recipient, we requested the patient (T. S. D.) to monitor his FFT-CBG levels daily [8,10] (Table 1), who totally accepted 14 times of human stem cell transplantations from January 20 to June 30 of 2018 [8,10]. The results showed that the patient's FFT-CBG remained around normal range from October 23 of 2018 to October 15 of 2019 (Table 1). When the patient began to accept his first human stem cell transplantation, he stopped daily injection of 24 IU insulin at January 20 of 2018 [8]. Therefore, if we counted from January 20 of 2018, the efficacy duration of transplanted dgHPSCs overexpressing INS and/or ERR γ for decreasing the blood GLU levels was almost 2 years. It was worth of particular note that the patient's GLU levels would continue to be around the normal range in the near future, therefore, the exact efficacy duration of the transplanted human stem cells needs to be investigated further.

The transplanted dgHPSCs can decrease blood pressure efficiently. The patient was also diagnosed hypertension about ten years ago. Before he accepted human stem cell transplantations, he took Benazepril Hydrochloride Tablets (10 mg/Tablet, NOVARTIS, Beijing, China), half tablet a day, to control his blood pressure. After 14 times transplantations, from June 1 of 2019 on, he stopped taking Benazepril Hydrochloride Tablet and his blood pressure decreased to normal range (Tables 2 and 3). These data demonstrated that the transplanted human stem cells not only can

maintain the blood GLU levels around the normal range, but also can improve the patient's cardiovascular function and decrease the hypertension.

The follow-up visits of the patient

His body weight keeps around 68kg, and he feels strong. He adheres to diabetic diet fairly well. He persists in exercising every day, sometimes, he walked about 10,000 to 15,000 steps daily, at other times, he did some other exercises, such as whipping a top. If he decreased his exercises due to the weather, etc., the next day fasting blood glucose would increase a little bit. Or when he caught a cold and had less exercises, his fasting glucose also would go up somewhat. When he had a feast with his friends and had more food, his fasting glucose levels would also rise up to some extent. Mostly, he could keep his fasting blood glucose levels within normal range (Table 1). As mentioned above, he stopped taking anti-hypertensive drugs from June 1 of 2019 until present, and his blood pressure kept within normal range (Tables 2 and 3). The comparison between before stem cell transplantation and after transplantation was listed in Table 3.

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. Palmer AK, Tchkonja T, LeBrasseur NK, et al. Cellular Senescence in Type 2 Diabetes: A Therapeutic Opportunity. *Diabetes*. 2015; 64: 2289-2298.
3. 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.
4. Pagliuca FW, Millman JR, Gürtler M, et al. Generation of functional human pancreatic β cells in vitro. *Cell*. 2014; 159: 428-439.
5. Yoshihara E, Wei Z, Lin CS, et al. ERR γ is required for the metabolic maturation of therapeutically functional glucose-responsive β cells. *Cell Metabolism*. 2016; 23: 622-634.
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. Wang T, Wang X, Zhang B, et al. Transplantation of human pluripotent stem cells overexpressing ERR γ can efficiently improve the symptoms of type 2 diabetes patient. *Adv Tissue Eng Regen Med Open Access*. 2018; 4: 457-462.
9. Wang T, Chen X, Cui X, et al. Transplantation of human pluripotent stem cells overexpressing insulin/ERR γ can efficiently decrease the HbA1c levels of type 2 diabetes patient (Case #2-A). *International Journal of Current Research*. 2018;10: 74690-74696.
10. Wang T, Wang X, Zhang B, et al. Transplantation of human dgHPSCs overexpressing insulin and ERR γ 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 Cell & Regenerative Medicine*. 2018; 2: 1-9.
11. Zhang G, Tandon A. Quantitative assessment on the cloning efficiencies of lentiviral transfer vectors with a unique clone site. *Sci Rep*. 2012; 2: 415.
 12. 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.
 13. 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.
 14. Zhang G, Zhang Y. On the “All or Half” law of recombinant DNA. *American Journal of Biomedical Research*. 2016; 4: 1-4.
 15. 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.