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The Transplantation of Human Pluripotent Stem Cells is Safe: A Personal Experience during the Past 5 Years (I)

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ABSTRACT

The greatest dilemma of human pluripotent stem cell transplantation therapy clinically is the potential risk to form teratomas in the recipient's body. On the one hand, to date, no data can confirm this risk. On the other hand, no data can confirm the safety of human pluripotent stem cell transplantations, either. To break this dilemma, the correspondence author, G Z, decided to accept human pluripotent stem cell transplantations voluntarily. During the past five years, G Z accepted totally 77 times human stem cell transplantations with/without overexpressing different human genes, and the whole number of human stem cells was up to approximately 6.36 X 10^9. After medical examinations, the results demonstrated that G Z's health conditions were basically normal. Thus, our investigations preliminarily proved that intravenous transplantations of human pluripotent stem cells were safe so far, at least for this case.

Key words

dgHPSCs, Overexpression of Transgenes, Lentiviral Transduction, Safety.

Abbreviations

hADSCs: Human Adipose-derived Stem Cells; PSCs: Placental Stem Cells; dgHPSCs: Directly Generated Human Pluripotent Stem Cells; iPSCs: Induced Pluoripotent Stem Cells.

Introduction

So far, no evidence can confirm the safety of human induced pluripotent stem cell (iPSC) transplantations. On the one hand, iPSCs have the similar pluripotnencies to form all the tissues and cell types as embryonic stem cells (ESCs), which are demonstrated by the formations of "all-iPSC mice" [1-3] and human teratomas in vivo [4,5], which are the "gold standards" for the pluripotencies of mouse and human ESCs and iPSCs, respectively. Therefore, human iPSCs could provide great promise for human stem cell transplantation therapy. On the other hand, the teratoma formation of human iPSCs also evokes great concerns for its clinical application as a renewable source for regenerative medicine.

By scrutinizing the protocols of teratoma formation, we found that the prerequisite to form teratomas, regardless the injection sites, such as subcutaneous injection, intratesticular injection, and injection into the hind limb muscle of nude or SCID mice, etc., is to inject approximately 1 million iPSCs in very small volumes, such as 100µl or 60µl, respectively [1,4-6]. Based on these protocols, we can deduce that the crucial mechanisms for teratoma formation contain three key points. First of all, the injected iPSCs must be of large numbers in very small volumes, so that the iPSCs can be aggregated together and almost touch each other to form iPSC clusters. Secondly, the injected iPSCs are confined in the injection sites, and few of them can disperse into the neighbouring regions. Finally, the recipient mice are immunocompromised. These three conditions facilitate the formation of teratomas.

The major risk of human iPSC transplantations is the formation of teratomas. The aforementioned analyses suggest that if we can avoid the above three conditions, we might avoid the formation of teratomas in clinical human iPSC transplantation therapies. Therefore, we reasoned that if we resuspend appropriate number of human iPSCs in a large volume of saline solution, for example, 100 million iPSCs in 100ml volume, and then transplant them into the patient intravenously, we might avoid the formation of teratomas. Let us do a little math calculation to clarify this point of view. Compared with 1 million iPSCs in 100μl volume, 100 million iPSCs in 100ml volume is up to 10 fold dilution. Under this circumstance, the iPSCs are diluted sufficiently, and very few of them can aggregate together to form clusters. In addition, after intravenous transplantation, iPSCs will circulate along with the blood going to everywhere of the human body, and are further diluted by the blood greatly. Therefore, almost all of the transplanted iPSCs will exist in the patient as single cell. More importantly, the human recipients are with normal immune systems, and not immunocompromised. Therefore, we hypothesized that the above three reasons will ensure the transplanted human iPSCs to absolutely avoid the formation of teratomas.

Although the above analyses seem to be reasonable, it is only a hypothesis necessitated to be confirmed clinically. Previously, we directly generated human pluripotent stem cells (dgHPSCs) from human adipose-derived stem cells (hADSCs) without any genetic modifications [7]. These dgHPSCs showed similar pluripotencies with human iPSCs, such as positively expressing TRA-1-60 marker [7] and formation of embryoid body in vitro (Data not shown). To confirm the safety of human pluripotent stem cell transplantations clinically, one of the corresponding authors of this paper (G. Z) decided to accept human dgHPSC transplantations voluntarily. From October 15 of 2016 until the writing of this paper, G. Z received totally 77 times of human stem cell transplantations, including dgHPSCs, hADSCs, and placental stem cells (PSCs). All of them are either autologous (Auto) or allogenic (Allo) stem cells, respectively. The total stem cell numbers were amounted to approximately 6.36 X 10^9, and the majority of them were overexpressing some human genes (Table 1). Approximately five years later, G. Z is still completely normal with his health, physically and mentally. To our knowledge, this is the first report for large amount transplantations of human pluripotent stem cells. Therefore, our preliminary clinical investigation, for the first time, provided vivid example for the safety of human stem cell-gene transplantation therapy.

Materials and Methods

Statement of Ethical Approval

The treatments for the patient 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. To confirm the safety of human pluripotent stem cell transplantations clinically, one of the correspondence authors of this paper (G. Z) agreed voluntarily to accept human stem cell transplantations overexpressing various human genes (Table 1). The stem cells used in these clinical treatments were stored at our Stem Cell Bank. All these stem cells were isolated and proliferated with the written confirm consent of the participants.

Cell preparation

The isolation of lipoaspirate cells and the induction of dgHPSCs were exactly the same as described [7,8]. The cell lines used in this investigation were Line #1 (derived from Z. G., the correspondence author of this paper), Line #3, Line #4, Line #5, and Line #8 (Table 1), respectively, which were stored at our stem cell bank. 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 second generation of LVs carrying different human genes, including pWPI/p53WT, pWPI/SRY, pWPI/INSULIN (INS), pWPI/ERRγ, pWPI/p53WT-STAT, and pWPI/SIRT1, were constructed and stored in our lab as previously described [9-12]. All the LVs were produced, and infected into different human stem cell lines according to a previous report [13]. Each infection format was shown in details in Table 1.

Human stem cell transplantation

The intravenous transplantations of different human stem cells with/without overexpressing of different human genes were exactly the same as previously described [14-18]. The transplantation dates, cell types, and cell numbers were listed in Table 1.

Assessment of the effects of human stem cell transplantations

The medical examinations were performed by Jinan kingmed Center for Clinical Laboratory (Jinan, Shandong Province, China). The examinations included liver function, blood lipids, renal function, tumor markers, thyroid function, sex hormones, hepatitis B virus, hepatitis A virus, hepatitis C virus, syphilis, AIDS and diabetes. The detailed medical examination results were listed in Table 2.

Results

To investigate the safety of human pluripotent stem cell transplantations, during the past five years (from October 15 of

Table 1: dgHPSCs transplantations of G Z.

No.	Date	Cell types	Cell numbers	Auto/Allo
#1	15/10/2016	hADSCs	1.3×10^7	Auto
#2	21/10/2016	hADSCs	2.1×10^7	Auto
#3	02/11/2016	hADSCs	2.5×10^7	Auto
#4	17/01/2017	hADSCs	3.2×10^6	Auto
#5	26/02/2017	Line #1 dgHPSCs + 25ml p53WT	1.2×10^7	Auto
#6	26/02/2017	Line #1 dgHPSCs	2.0×10^7	Auto
#7	05/03/2017	Line #1 dgHPSCs	5.1×10^7	Auto
#8	12/03/2017	Line #1 dgHPSCs	2.8×10^7	Auto
#9	12/04/2017	Line #1 dgHPSCs	3.24×10^7	Auto
#10	19/05/2017	Line #1 dgHPSCs	1.2×10^8	Auto
#11	13/06/2017	Line #1 dgHPSCs	8.32×10^7	Auto
#12	24/07/2017	Line #1 dgHPSCs	1.8×10^8	Auto
#13	24/07/2017	Line #1 dgHPSCs	5.6×10^7	Auto
#14	04/08/2017	Line #1 dgHPSCs	1.42×10^8	Auto
#15	16/08/2017	Line #1 dgHPSCs + 12.5ml Sry	9.0×10^7	Auto
#16	04/09/2017	Line #1 dgHPSCs + 25ml Sry	5.85×10^7	Auto
#17	10/10/2017	Line #1 dgHPSCs	5.2×10^7	Auto
#18	01/11/2017	Line #1 dgHPSCs + 25ml ERRγ	9.36×10^7	Auto
#19	07/11/2017	Line #1 dgHPSCs + 25ml ERRγ	3.7×10^7	Auto
#20	13/11/2017	Line #1 dgHPSCs + 25ml ERRγ	4.41×10^7	Auto
#21	11/01/2018	Line #1 dgHPSCs + 25ml ERRγ	9.09×10^7	Auto
#22	16/01/2018	Line #1 dgHPSCs + 25ml ERRγ	1.23×10^8	Auto
#23	23/01/2018	Line #1 dgHPSCs + 25ml ERRγ	9.81×10^7	Auto
#24	29/01/2018	Line #1 dgHPSCs + 25ml ERRγ	1.17×10^8	Auto
#25	26/05/2018	Line #1 dgHPSCs	6.75×10^7	Auto
#26	31/05/2018	Line #1 dgHPSCs + 25ml ERRγ	4.5×10^7	Auto
#27	06/06/2018	Line #1 dgHPSCs + 50ml Sry	2.56×10^7	Auto
#28	12/06/2018	Line #1 dgHPSCs + 25ml ERRγ	5.85×10^7	Auto
#29	18/06/2018	Line #1 dgHPSCs + 50ml Sry	7.54×10^7	Auto
#30	24/06/2018	Line #1 dgHPSCs + 50ml Sry	7.38×10^7	Auto
#31	30/06/2018	Line #1 dgHPSCs + 50ml Sry	2.7×10^7	Auto
#32	06/07/2018	Line #1 dgHPSCs + 50ml Sry	6.57×10^7	Auto
#33	13/07/2018	PSCs + 100ml Sry	2.13×10^7	Allo
#34	19/07/2018	Line #1 dgHPSCs + 25ml Sry	1.36×10^7	Auto
#35	24/07/2018	Line #1 dgHPSCs + 75ml Sry	7.65×10^7	Auto
#36	13/10/2018	Line #1 dgHPSCs + 50ml Sry	4.82×10^7	Auto
#37	17/10/2018	PSCs + 50ml Sry	1.3×10^7	Allo
#38	06/11/2018	Line #1 dgHPSCs + 50ml Sry	1.098×10^8	Auto
#39	09/11/2018	Line #1 dgHPSCs + 50ml Sry	1.053×10^8	Auto
#40	26/11/2018	Line #1 dgHPSCs + 75ml Sry	3.85×10^7	Auto
#41	26/11/2018	Line #1 dgHPSCs	1.35×10^8	Auto
#42	05/12/2018	Line #1 dgHPSCs + 50ml Sry	1.17×10^8	Auto
#43	19/12/2018	Line #1 dgHPSCs + 75ml Sry	1.67×10^8	Auto
#44	25/12/2018	Line #1 dgHPSCs + 75ml Sry	9.4×10^7	Auto
#45	29/12/2018	Line #1 dgHPSCs + 75ml Sry	9.72×10^7	Auto
#46	03/01/2019	Line #1 dgHPSCs + 75ml Sry	1.35×10^8	Auto
#47	08/01/2019	Line #1 dgHPSCs + 75ml Sry	3.6×10^7	Auto
#48	25/01/2019	Line #1 dgHPSCs	2.592×10^8	Auto
#49	08/03/2019	Line #1 dgHPSCs + 10ml p53WT-STAT	5.4×10^7	Auto
#50	17/03/2019	Line #1 dgHPSCs + 50ml Sry	1.02×10^8	Auto
#51	22/03/2019	Line #1 dgHPSCs + 25ml SIRT1	8.18×10^7	Auto
#52	29/03/2019	Line #1 dgHPSCs + 50ml Sry	1.18×10^8	Auto
#53	07/04/2019	Line #1 dgHPSCs + 30ml p53WT-STAT	1.22×10^8	Auto
#54	13/04/2019	Line #5 dgHPSCs + 50ml Sry	1.1×10^8	Allo
#55	20/04/2019	Line #5 dgHPSCs + 50ml Sry	9.72×10^7	Allo
#56	05/05/2019	Line #5 dgHPSCs + 50ml Sry	6.75×10^7	Allo
#57	19/05/2019	Line #5 dgHPSCs + 50ml Sry	8.4×10^7	Allo

#58	25/05/2019	Line #5 dgHPSCs +50ml Sry	9.0 X 10^7	Allo
#59	08/06/2019	Line #5 dgHPSCs + 50ml Sry	7.9 X 10^7	Allo
#60	15/06/2019	Line #5 dgHPSCs + 75ml Sry	6.15 X 10^7	Allo
#61	23/06/2019	Line #5 dgHPSCs + 50ml Sry	1.02 X 10^8	Allo
#62	30/06/2019	Line #5 dgHPSCs + 75ml Sry	9.7 X 10^7	Allo
#63	07/07/2019	Line #5 dgHPSCs + 75ml Sry	1.404 X 10^8	Allo
#64	14/07/2019	Line #5 dgHPSCs + 75ml Sry	1.85 X 10^8	Allo
#65	05/10/2019	Line #5 dgHPSCs	9.8 X 10^7	Allo
#66	13/10/2019	Line #5 dgHPSCs	6.3 X 10^7	Allo
#67	05/11/2019	Line #3 dgHPSCs + 25ml p53WT	6.1 X 10^7	Allo
#68	29/12/2019	Line #5 dgHPSCs + 50ml ERRγ	1.7 X 10^8	Allo
#69	05/01/2020	Line #5 dgHPSCs + 50ml ERRγ	1.44 X 10^8	Allo
#70	29/04/2020	Line #4 dgHPSCs	6.5 X 10^7	Allo
#71	08/05/2020	Line #4 dgHPSCs + 30ml p53WT-STAT	1.31 X 10^8	Allo
#72	24/05/2020	Line #4 dgHPSCs + 40ml p53WT-STAT	1.18 X 10^8	Allo
#73	31/05/2020	Line #4 dgHPSCs + 40ml p53WT-STAT	8.4 X 10^7	Allo
#74	07/06/2020	Line #5 dgHPSCs + 75ml ERRγ	7.07 X 10^7	Allo
#75	10/01/2021	Line #8 dgHPSCs + 75ml Sry	1.3 X 10^8	Allo
#76	17/01/2021	Line #8 dgHPSCs + 75ml Sry	1.1 X 10^8	Allo
#77	22/01/2021	Line #4 dgHPSCs + 75ml INS + 75ml ERRγ	2.9 X 10^7	Allo
Total			6.36 X 10^9	

Table 2: Medical examinations of G Z after human stem cells transplantations.

Medical examinations	**Methods**	**Results**	**Reference ranges**
LIVER FUNCTION			
Alanine aminotransferase (ALT/GPT)	Rate assay	19 U/L	9-50
Aspartate aminotransferase (AST)	Rate assay	16 U/L	15-40
AST/ ALT (S/L)	Calculation		
Alkaline phosphatise (ALP)	Rate assay	79 U/L	45-125
Lactate dehydrogenase (LDH)	Rate assay	169 U/L	120-250
Total protein (TP)	Biuret	73.5 g/L	65.0-85.0
Albumin (ALB)	Bromocresol green (BCG)	51.4 g/L	40.0-55.0
Globulin (GLOB)	Calculation	22.1 g/L	20.0-40.0
γ- Glutamyl transpeptidase (GGT)	Rate assay	22 U/L	10-60
ALB/ GLOB (A/G)	Calculation	2.3 Ratio	1.2-2.4
BLOOD LIPIDS			
Triglyceride (TG)	GPO-PAP	**3.73** mmol/L	0.00-1.70
Cholesterol (CHOL)	COD-CE-PAP	5.03 mmol/L	0.00-5.18
High density lipoprotein cholesterol (HDL-CH)	CAT	**0.90** mmol/L	1.04-1.55
Low density lipoprotein cholesterol (LDL-CH)	CAT	3.07 mmol/L	0.00-3.37
RENAL FUNCTION			
Glucose (GLU)	Hexokinase	5.32 mmol/L	3.90-6.10
Urea	UV-GLDH	5.96 mmol/L	3.10-8.00
Creatinine (Cr)	Enzyme	68.6 µmol/L	57.0-97.0
Uric acid (UA)	URO-PAP	404 µmol/L	208-428
TUMOR MARKERS			
Alpha fetoprotein (AFP)	Chemiluminescence	4.75 ng/mL	≤12.00
Carcinoembryonic antigen (CEA)	Chemiluminescence	2.88 ng/mL	≤5.00
Neuron specific enolase (NSE)	Chemiluminescence	10.38 ng/mL	≤15.20
Cytokeratin 19 fragment (CYFRA21-1)	Chemiluminescence	1.25 ng/mL	≤3.30
Sugar chain antigen 50 (CA50)	Chemiluminescence	7.20 U/mL	≤25.00
Sugar chain antigen 242 (CA242)	Chemiluminescence	4.87 U/mL_	≤20.00
β-Human chorionic gonadotropin (β-HCG)	Chemiluminescence	<0.50 mIU/mL	<5.00
Sugar chain antigen 19-9 (CA-19-9)	Chemiluminescence	11.98 U/mL	≤40.00
Carbohydrate antigen 724 (CA724)	Chemiluminescence	1.31 U/mL	≤8.30
Ferritin (FER)	Chemiluminescence	177.90 ng/mL	27.00-375.00
Total prostate specific antigen (TPSA)	Chemiluminescence	0.87 ng/mL	≤4.00
Free prostate specific antigen (FPSA)	Chemiluminescence	0.13 ng/mL	≤0.80
FPSA/ TPSA	Calculation	0.15	

THYROID FUNCTION			
Hypersensitive thyroid stimulating hormone (H-TSH)	Chemiluminescence	4.523 µIU/mL	0.350-5.100
Free triiodothyronine (FT3)	Chemiluminescence	4.82 pmol/L	2.76-6.45
Total thyroxine (TT4)	Chemiluminescence	85.44 nmol/L	64.36-186.64
Total triiodothyronine (TT3)	Chemiluminescence	1.44 nmol/L	0.89-2.49
Free thyroxine (FT4)	Chemiluminescence	13.53 pmol/L	6.44-18.02
Anti thyroid peroxidase antibody (TPOAb)	Electrochemiluminescence	**74.72** IU/mL	≤34.00
Antithyroid globulin antibody (TGAb)	Electrochemiluminescence	**182.60** IU/mL	0.00-115.00
SEX HORMONES			
Follicle stimulating hormone (FSH)	Chemiluminescence	8.10 mIU/mL	0.95-11.95
Luteinizing hormone (LH)	Chemiluminescence	2.04 mIU/mL	0.57-12.07
Estradiol (E2)	Chemiluminescence	24.00 pg/mL	11.00-44.00
progesterone (P)	Chemiluminescence	0.30 ng/mL	0.00-0.30
testosterone (T)	Chemiluminescence	14.15 nmol/L	4.94-32.01
Pituitary prolactin (PRL)	Chemiluminescence	4.19 ng/mL	3.46-19.40
HEPATITIS B VIRUS			
Hepatitis B virus surface antigen (HBsAg)	ELISA	Negative (-)	Negative (-)
Hepatitis B virus surface antibody (HBsAb)	ELISA	**Positive (+)**	Negative (-)
Hepatitis B virus E antigen (HBeAg)	ELISA	Negative (-)	Negative (-)
Hepatitis B virus E antibody (HBeAb)	ELISA	Negative (-)	Negative (-)
Hepatitis B virus core antibody (HBcAb)	ELISA	Negative (-)	Negative (-)
HEPATITIS A VIRUS			
Hepatitis A virus antibody IgG (HAV-IgG)	ELISA	**Positive (+)**	Negative (-)
Hepatitis A virus antibody IgM (HAV-IgM)	ELISA	Negative (-)	Negative (-)
HEPATITIS C VIRUS			
Hepatitis C virus antibody IgG (HCV-IgG)	ELISA	Negative (-)	Negative (-)
Hepatitis C virus antibody IgM (HCV-IgM)	ELISA	Negative (-)	Negative (-)
SYPHILIS SEROLOGICAL TEST+ SYPHILIS ANTIBODY TEST			
Characterization of **T**re**p**onema **p**allidum specific **a**ntibody (TPPA)	Agglutination reaction	Negative (-)	Negative (-)
Syphilis toluidine red unheated serum anti-stress test (TRUST)	Agglutination reaction	Negative (-)	Negative (-)
AIDS			
Preliminary screening test of human immunodeficiency virus antibody (Anti-HIV)	Enzyme-linked immunosorbent assay (ELISA)	Negative (-)	Negative (-)
DIABETES			
Fasting insulin (F-INS)	Electrochemiluminescence	7.21 µU/mL	2.60-24.90
Fasting C-peptide (F-C-P)	Electrochemiluminescence	1.76 ng/mL	1.10-4.40
Glycosylated hemoglobin (HBA1C)	High performance liquid chromatography (HPLC)	5.2%	4.3-6.1

Table 3: Comparison of health conditions before (Bef.) and after (Aft.) human stem cell transplantations.

Examinations	13/05/2015 (Bef.)	21/06/2017 (Aft.)	11/10/2021 (Aft.)
Anti-HIV	Negative	Negative	Negative
Syphilis serology	Negative	Negative	Negative
HBsAg	Negative	Negative	Negative
Anti-HCV	Negative	Negative	Negative
ALT (GPT)	24 U/L	20 U/L	19 U/L
Abdomen Ultrasound	Fatty liver	Mild fatty liver	N/A

2016 to January 22 of 2021), one of the correspondence authors (G Z) of this paper, transplanted totally 77 times of human stem cells, and the total number of the stem cells was approximately 6.36 X 10^9 (Table 1). Among those transplanted stem cells, four times are hADSCs from G Z himself (Auto), two times are PSCs (Allo), and the rest 71 times are dgHPSCs, including either autologous (Auto) or allogenic (Allo) stem cells, respectively (Table 1). In addition, seven times of the stem cells were overexpressing human tumor suppressor p53WT [19] and p53WT-STAT (the protein secreting signal and plasma membrane transduction domain TAT were engineered into p53WT gene sequence at the N-terminal) [20] genes, 36 times were overexpressing human SRY (sex-determining region in Y chromosome, also called testis-determining factor, TDF, which can initiate male development in humans) gene [21], 12 times were overexpressing human ERRγ (estrogen-related receptor γ, which is a master regulator of β cell maturation in vivo) gene [22], One time was overexpressing human ERRγ and INS genes, and One time was overexpressing human SIRT1 gene (SIRT1 is a member of sirtuins. Supposedly, up-regulating sirtuins might provide a pathway toward increased longevity in humans. This mechanism was already evaluated by US FDA for their ability to prevent diabetes and heart disease with a possible "side effect" of longer life.) [23], respectively (Table 1).

Five years later, on October 11 of 2021, G Z took detailed health examinations. The medical examinations were performed by Jinan kingmed Center for Clinical Laboratory. The examination results were listed in Table 2, which included liver function, blood lipids, renal function, tumor markers, thyroid function, sex hormones, hepatitis B virus, hepatitis A virus, hepatitis C virus, syphilis, AIDS and diabetes. The medical examination results preliminarily proved the following conclusions. First of all, G Z's health conditions are basically normal, except the triglyceride (TG), high density lipoprotein cholesterol (HDL-CH), anti-thyroid peroxidase antibody (TPOAb) and antithyroid globulin antibody (TGAb) are higher than the reference ranges (Table 2). In addition, G Z's tumor markers are all within the normal ranges, which demonstrated that human pluripotent stem cell transplantations could not induce the formation of tumors and thus are safe so far, at least for the case of G Z (Table 2). Most importantly, G Z's preliminary screening test of human immunodeficiency virus antibody (Anti-HIV) is negative (Table 2), which indicated that the second generation lentiviral vectors are an effective and safe transgene vehicle in human stem cell-gene transplantation therapy clinically.

Previously, G Z took two times of Entry-Exit Inspection and Quarantine in the People's Republic of China at May 13 of 2015 (Qianlingshan Road, Guiyang City, Guizhou Province, P. R. C.) and June 21 of 2017 (No. 9 Jianshe Road, Dongguan, Guangdong Province, P. R. C.), respectively (Table 3). The comparison of health conditions before and after human stem cell transplantations showed that, after human stem cell transplantations, G Z's alanine aminotransferase (ALT/GPT) levels were decreased from 24 U/L to 20 U/L and 19 U/L, respectively (Table 1, 2 and 3). Furthermore, via abdomen ultrasound examination, the results showed that G Z's liver was significantly ameliorated, from fatty liver to mild fatty liver (Table 3). Therefore, our data revealed that our strategy and paradigm of intravenous human stem cell transplantations were not only safe but also beneficial to improve human health conditions.

Discussion

Although the teratoma formation of the transplantation of human pluripotent stem cells (including hiPSCs, hESCs and dgHPSCs) is the greatest concern for their clinical applications as very promising renewable sources for regenerative medicine, G Z's own dgHPSCs transplantation experience preliminarily suggests that human pluripotent stem cell transplantation therapy is safe. Previously, we envisaged that the tumorigenicity of human pluripotent stem cell transplantations might be avoided and analysed theoretically in a "Mouse Clone Model" [24]. To put the theory into practice, from 2016 on, we invented an efficient protocol to directly induce hADSCs into human pluripotent-like stem cells without any genetic modifications [7,8], therefore removed the potential risks of tumour formation caused by the transgenes, particularly the oncogene c-Myc [4,6]. These human pluripotent-like stem cells manifested similar pluripotencies with human ESCs and iPSCs, such as positively expression of pluripotency marker TRA-1-60 and formation of embryoid body in vitro (Data not shown), and were coined as directly-generated human pluripotent stem cells (dgHPSCs) [7,25].

After large amount transplantations of human stem cells (77 times separately, and the total numbers are up to about 6.36 billion) and relatively long time duration (about five years), the medical examinations of the recipient (G Z) showed that his health conditions are basically normal (Table 2). Among the transplanted human stem cells, the majority was dgHPSCs (71 times in total, Auto and/or Allo, Table 1), which revealed similar pluripotentcies with human iPSCs [4, 25]. These data vividly demonstrated the safety of intravenous transplantation strategy of human pluripotent stem cells clinically. In addition, 57 times of the transplanted human stem cells were with transgenes via lentiviral vector transduction, which was derived and engineered from human immunodeficiency virus (HIV) [9]. Yet, five years after the transplantations, G Z's Anti-HIV test result was negative. This data strongly suggested that the second generation lentiviral vectors could be a safe tool for clinical human gene therapy (Table 1 and 2). More importantly, the comparison of G Z's ALT (GPT) and abdomen ultrasound examinations before and after human stem cell transplantations showed that G Z's ALT (GPT) values decreased and his live became better from fatty liver to mild fatty liver (Table 3). Altogether, our data demonstrated that human pluripotent stem cell-gene therapies are not only safe but also beneficial to human health.

Previously, we reported that transplantations of dgHPSCs overexpressing human ERRγ and/or INS genes could significantly decrease the daily dosages of insulin in type 2 diabetes patients, and even further completely cure the disease by sufficient serial transplantations. Furthermore, the diabetic complication symptoms were gradually improved and repaired effectively, and the patient's physical and mental conditions were ameliorated greatly [7, 14-18]. (G Z started his own human stem cell-gene transplantation trials much earlier than the treatments of these type 2 diabetics). Taken together, these data preliminarily proved that dgHPSC transplantations overexpressing proper human genes were not only safe but also very promising in curing different human diseases. To our knowledge, this is the first report for large amount transplantations of human pluripotent stem cells intravenously with overexpressing various human genes transduced by lentiviral vectors. These data laid very important foundations for the application of large scale human pluripotent stem cell-gene transplantation therapies in the foreseeable future.

Conclusions

Based on our preliminary data, we could make the following conclusions:

1. Intravenous human pluripotent stem cell (such as dgHPSCs)

transplantations are safe.
2. The second generation lentiviral vectors are safe tools for transducing human genes clinically.
3. The transplantations of dgHPSCs are beneficial to human health, and combined with overexpressing of proper human genes, such as ERRγ and INS, could effective treat and even cure some human diseases, for example, human type 2 diabetes.

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 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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