ORIGINAL

In vivo direct reprogramming of liver cells to insulin producing cells by virus-free overexpression of defined factors

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Abstract. Direct reprogramming of autologous cells from diabetes patients to insulin producing cells is a new method for pancreatic cell replacement therapy. At present, transdifferentiation among mature cells is achieved mainly by introducing foreign genes into the starting tissue with viral vector, but there are potential safety problems. In the present study, we delivered plasmids carrying *Pdx1*, *Neurog3* and *MafA* genes (PNM) into mouse hepatocytes by hydrodynamics tail vein injection, investigated islet β cells markers in transfected cells from protein and mRNA level, and then observed the long-term control of blood glucose in diabetic mice. We found that hepatocytes could be directly reprogrammed into insulin-producing cells after PNM gene transfection by non-viral hydrodynamics injection, and fasting blood glucose was reduced to normal, and lasted until 100 days after transfection. Intraperitoneal glucose tolerance test (IPGTT) showed that glucose regulation ability was improved gradually and the serum insulin level approached to the level of normal mice with time. Insulin-positive cells were found in the liver tissue, and the expression of various islet β -cell-specific genes were detected at the mRNA level, including islet mature marker gene *Ucn3*. In conclusion, we provide a new approach for the treatment of diabetes by *in vivo* direct reprogramming of liver cells to insulin producing cells through non-viral methods.

Key words: Hydrodynamic gene delivery, Direct reprogramming, Liver cells, Insulin producing cells, Diabetes

PANCREATIC ISLET TRANSPLANTATION has been considered as an effective cell replacement treatment for diabetes, but facing the problem of organ donors' shortage and immunosuppression. Recently, embryonic stem cells and induced pluripotent stem cells can be induced to pancreatic β like cells, but the risks of neoplasia still remain to be serious issue [1, 2]. As an alternative treatment, direct reprogramming of patient's own somatic cells in to pancreatic β cells eliminates the risk of neoplasia. Direct reprogramming is a process of transdifferentiating one somatic cells to other somatic cells bypassing the phase of pluripotency [3]. Several groups have demonstrated that

the feasibility of reprogramming liver cells and exocrine cells towards a pancreatic β cell phenotype *in vivo* by delivering multiple transcription factors with adenovirus. The combination of *Pdx1*, *Neurog3* and *MafA* has been proved to be the most efficient reprogramming factors, which can trigger irreversible reprogramming process [4, 5].

Given the risks posed by viral vectors, such as integration to the host genome and immunogenicity issue [6], virus-free gene delivery approaches have advantages. Hydrodynamics-based gene delivery (HGD) utilizes hydrodynamic pressure produced by the volume and flow of injection as the driving force to achieve gene transfer without virus vector [7]. HGD has been applied as gene therapy for various diseases [8-10]. The standard procedure in small animals involves tail vein bolus injection of plasmid DNA solution in a volume equal to 8-10% of the body weight within 5-7 seconds [11]. The liver shows the highest level of transgene expression among the various organs, and about 40% hepatocytes are transfected by a single injection of

Submitted Oct. 9, 2016; Accepted Nov. 8, 2016 as EJ16-0463
Released online in J-STAGE as advance publication Jan. 18, 2017
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no less than 50 µg of plasmid DNA [12]. The injected plasmid solution directly goes to the heart, inducing cardiac congestion and backflow to the inferior vena cava and hepatic veins. The solution then reaches the sinusoids; the hydrodynamic pressure enlarges the fenestrae of the endothelium, and forces invagination of the cellular membrane of the hepatocytes to allow the DNA to move into the cytoplasm [13]. In small animals, the opened cellular membrane is smoothly resealed usually within a few minutes, thereby trapping the DNA within the cells [14].

In present study, we delivered plasmids encoding *Pdx1*, *Neurog3*, *MafA* by hydrodynamic injection to the liver, to investigate whether this virus-free gene delivery methods can achieve direct reprogramming of liver cells to insulin producing cells *in vivo*.

Material and Methods

Plasmid construction

Transcription factors *Pdx1*, *Neurog3*, *MafA* cDNA were PCR amplified from Total RNA of C57BL/6J Mouse. The fragments were ligated to PcDNA3.1 (+) vector (Invitrogen, USA) separately. DNA sequencing was performed to demonstrate success construction. Luciferase reporter plasmid with same vector backbone was used as control. Plasmids were further purified with E.Z.N.A Endo-Free Plasmid Maxi Kit (Omega biotek, USA).

Animals

Male C57BL/6J mice were purchased from Guangdong Medical Laboratory Animal Center and housed under specific pathogen free conditions. All animal procedures were approved by Institutional Animal Care and Use Committee of the Second clinical Medical College, Ji'nan University. 6-week-old male mice with an initial body weight of 18-20 g were fasted overnight for 14 h and injected intraperitoneally with 2% STZ (Sigma, USA) at a dosage of 60 mg/kg for 3 consecutive days. None-fasting blood glucose was measured using a glucometer (LifeScan Canada Ltd). Mice with blood glucose of ≥ 300 mg/dL for 3 consecutive days, which was stable for at least 1 week, were considered as diabetic.

Hydrodynamic procedures

The TransIT-EE (Enhanced Expression) Hydrodynamic Delivery Solution (Mirus, USA) was used

to deliver plasmid DNA. The hydrodynamic tail vein injection procedure has been reported previously [11]. Total injection volume was equal to 10% body weight, which includes plasmid and TransIT-EE hydrodynamic delivery solution. Briefly, 1.8-2.0 mL delivery solution containing 20 µg, 50 µg, 80 µg plasmid DNA was respectively injected into the tail vein within 5-7 seconds.

In vivo bioluminescence imaging

The efficiency of luciferase plasmid by hydrodynamic delivery was assessed by *in vivo* bioluminescence imaging of luciferase activity using an IVIS Spectrum system (Perkin-Elmer, Waltham, MA) at 3, 6, 12, 24, 48, and 72 hours post-injection. Mice were anesthetized with isoflurane, followed by intraperitoneally injection of 150 mg/kg D-luciferin (Goldbio, USA). Eight consecutive scans (1 min acquisition time/scan) were taken after 10 min. Images were processed by using Living Image 4.2 software. An ellipsoidal region of interest was drawn over the liver. Data were shown in radiance units (photons/sec/cm²/steradian) for statistical analysis. To determine the distribution of luciferase activity, mice were euthanized, and liver, spleen, kidney, pancreas, heart, stomach, gastrointestinal tract, and brain were dissected 10 minutes after D-luciferin injection.

In vivo hydrodynamic delivery of *Pdx1*, *Neurog3* and *MafA*

In the experiment, three kinds of plasmids respectively encoding *Pdx1*, *Neurog3* and *MafA* were premixed with transIT-EE hydrodynamic delivery solution. The total amount of the plasmids was 50 µg, including each plasmid of 16.7 µg. The mixture was injected into diabetic mouse tail vein once (single injection), or repeatedly for fourth time at a daily interval (multiple injection). Plasmid DNA containing luciferase reporter gene was injected as negative vector control. Normal mice and diabetic mice were injected with the same amount of hydrodynamic delivery solution containing 50 µg luciferase plasmid.

Physiological measurements

Fasting blood glucose levels were measured by glucometer (Life Scan Canada Ltd) every 3 days post injection at 1600 h until day 100. The body weight of all subjects was measured at the same time. The serum was collected on days 7, 14, 28, 56, 100 post

injection. Insulin levels were measured using mouse Insulin ELISA kits (Mercodia, Sweden) according to manufacturer's protocol. IPGTT was carried out on days 7, 14, 28, 56, 100 post injection. Subjects in all groups were fasted for 6 h, and then were intraperitoneally injected a glucose solution at a dosage of 2 g/kg. And blood glucose of tail vein blood samples were measured at 0, 15, 30, 60, 120, 180 min after glucose injection.

Real time RT-PCR

Total RNA was isolated from the liver using TRIzol reagent (TaKaRa, Tokyo Japan). 1 µg RNA was reverse-transcribed into cDNA using a PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's protocol. Quantitative real time PCR using SYBR Green Premix EX Taq (TaKaRa) to determine relative gene expression was performed on Biorad CFX 96 touch real time PCR detection system. Ct values were normalized to β -actin, and expression level was calculated by the $2^{-\Delta\Delta C_t}$ method. All primer sequences are listed in Table 1.

Immunostaining

Mice were euthanized at 7, 14, 28, 56 and 100 days post transfection. Livers were removed and fixed in 4% paraformaldehyde. Then tissue blocks were embedded in O.C.T. compound (SAKURA Tissue-Tek, Pennsylvania, USA). 7 µm sections were prepared for immunostaining. Frozen tissue sections were permeabilized and blocked with 0.2% Triton X-100 plus 1% BSA. The sections were then incubated with Guinea pig anti-insulin (1:100; Abcam, Cambridge, UK) or with sheep anti-glucagon (1:200; Abcam, Cambridge, UK) at 4°C overnight. The sections were incubated with secondary antibodies Alexa Fluor 568 conjugated Goat Anti-Guinea Pig IgG (1:1,000; Abcam) or FITC-conjugated Rabbit Anti-sheep IgG (1:300, Abcam). The nuclear were staining with 4', 6-diamidino-2-phenylindole (DAPI) (1:800, Invitrogen). Images were observed and captured under a LSM 510 confocal microscope (Zeiss, Germany).

Histopathological analysis

The livers were removed from mice in the multiple injection group (n=3) on days 7 and 100 post-transfection, and then fixed in 10% formalin. Tissues were embedded in paraffin and sectioned to 5 µm for routinely staining with hematoxylin and eosin.

Table 1 Primer sequences and sizes

Gene name	Primers (F/R 5'-3')	Size (bp)
<i>Actb</i>	GAGACCTCAACACCCACAGC ATGTCACGCACGATTTCCCT	266
<i>Pdx1</i>	AGTGGGCAGGAGGTGCTTAC ATGTGTCTCTCGGTCAAGTTCA	172
<i>Mafa</i>	TTCAGCAAGGAGGAGGTCAT CCGCCAACTTCTCGTATTTC	217
<i>Neurog3</i>	CTGCGCATAGCGGACCACAGCTTC CTTCACAAGAAGTCTGAGAACACCAG	233
<i>Neurod1</i>	CGTTGCCTTAGCACTTCTTTCT CATTTCGGTTTTTCATCCTCTTG	167
<i>Pax4</i>	CCACAGGAATCGGACTATCTTC TGTTAGAAAACCAACCCCTCAC	152
<i>Nkx6-1</i>	GGAAGAGAAAACACACCAGAC GAACCAGACCTTGACCTGACTC	153
<i>Alb</i>	GAAGTATGCTGAGGCCAAGG TCCACACAAGGCAGTCTCTG	399
<i>Afp</i>	AGCGAGGAGAAATGGTCCGG GGACATCTTACCATGTGG	147
<i>Ins1</i>	GCTGTGATAAACCTGACAAG TGTGGGGATAATAGGAGCAGTT	144
<i>Ins2</i>	CAGAAGCGTGGCATTGTAGAT AGAGGGGTAGGCTGGGTAGT	102
<i>Gcg</i>	CAGCATGCCTCTCAAATTCATC ACATTCACCAGCGACTACAG	130
<i>Isl1</i>	GCCTGTAAACCACCATCATGT GTGCAAGGACAAGAAACGC	138
<i>Abcc8</i>	ACCAGCACATACGACACCAG TGGCTTTGATCCTTCCGAGT	164
<i>Kcnj11</i>	TCCACTCCTTTTCGTCTGCC CTACGCGAAGCATGAAGCAC	267
<i>Ucn3</i>	ATACCAATCCCAGGCACAGC GCTCGCAAATCTTGGCCTT	147

Abbreviations: *Actb*, β -actin; *Pdx1*, pancreatic and duodenal homeobox 1; *Mafa*, v-mafmusculoaponeurotic fibrosarcoma oncogene family, protein A; *Neurog3*, neurogenin 3; *Neurod1*, neurogenic differentiation 1; *Pax4*, paired box 4; *Nkx6-1*, NK6 homeobox 1; *Alb*, albumin; *Afp*, alpha fetoprotein; *Ins1*, insulin I; *Ins2*, insulin II; *Gcg*, glucagon; *Isl1*, ISL1 transcription factor, LIM/homeodomain; *Abcc8*, ATP-binding cassette, sub-family C (CFTR/MRP), member 8; *Kcnj11*, potassium inwardly rectifying channel, subfamily J, member 11; *Ucn3*, urocortin 3.

Pathological changes were observed under the microscope (Olympus IX71, Japan).

Statistical analysis

All data were analyzed using Graphpad Prism software. Continuous variables are expressed as the mean $\pm$ standard deviation. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to compare differences among different groups. *T*-tests were used to compare differences between two groups. Blood glucose levels and body weight were compared using repeated measures ANOVA. All experiments were performed in technical triplicate. Differences with a *p* value less than 0.05 were considered statistically significant.

Results

Efficient and targeted gene delivery to the liver by hydrodynamic injection

Hydrodynamic tail vein injection was used to deliver luciferase plasmids mixed with Mirus hydrodynamic delivery solution. To examine the efficiency of the method, the bioluminescence emission was measured from 3 hours to 72 hours after injection. Luciferase gene expression was observed in the liver 3 hours after injection. The bioluminescence signals peaked at 24 hours ($p < 0.01$), declined rapidly and maintained in a lower level thereafter (Fig. 1A, B). At 24 hours, injection of 50 μ g plasmids induced the highest bioluminescence signals ($1.17 \pm 0.39 \times 10^7$ /photons/sec/cm²/steradian; $n=4$), comparing to 20 μ g ($7.28 \pm 1.41 \times 10^5$ /photons/sec/cm²/steradian; $n=4$) and 80 μ g ($1.24 \pm 0.12 \times 10^6$ /photons/sec/cm²/steradian; $n=4$). Gene expression was confined to the liver (Fig. 1C). No signal was found in pancreas, kidney, spleen, gut, heart, lung, and brain.

Repeated hydrodynamic injection of PNM plasmids relieved hyperglycemia

Plasmids encoding *Pdx1*, *Neurog3*, *MafA* separately was delivered by hydrodynamic injection. When transferred once, fasting blood glucose rapidly decreased at day 3, but the anti-hyperglycemia effect disappeared at day 7. When plasmids mixture were repeatedly delivered forth time, fasting blood glucose rapidly decreased to 200 mg/dL, and maintained normal until 100 days. As shown in Fig. 2D, Glucose tolerance test on day 7, day 14 and day 28 showed

that there was no difference between the PNM treated and diabetic control groups. On day 56 and day 100, the PNM treated group showed lower glucose levels at each time point after the glucose challenge, indicating more insulin secretion in the PNM treated group. On day 100, Insulin levels in serum of the PNM treated group (4.19 ± 0.87 ng/mL) increased significantly, which was close to that of the normal control group (4.40 ± 0.44 ng/mL) and much higher than that of diabetic control group (0.97 ± 0.45 ng/mL, $p < 0.01$).

Reprogramming of hepatocytes to insulin producing cells by hydrodynamic injection

Repeated hydrodynamic injection of PNM plasmids reversed hyperglycemia significantly. To examine whether the anti-hyperglycemia effects came from the transdifferentiation of hepatocytes to insulin producing cells, pancreatic markers in the liver were investigated at both gene and protein level.

To examine the gene expression profiles related to pancreatic β cells, we performed quantitative RT-PCR analysis using RNAs extracted from whole liver cells (Fig. 3). As shown in Fig. 3F-H, the expression of *Pdx1*, *Neurog3* and *MafA* elevated dramatically after hydrodynamic injection. Overexpression of these transcription factors induced expression of insulin genes, including *Ins1* and *Ins2*. On day 100, insulin gene mRNA levels reached the peak, which is the same as insulin levels in the serum (Fig. 3A, B). Other hormone genes, like glucagon from pancreatic α cells was enhanced in the mRNA level (Fig. 3C). Liver specific markers *Alb* and *AFP* slightly decreased on day 100 (Fig. 3D, E). Pancreatic development related transcription factors began to express in the liver after hydrodynamic injection, such as *Nkx6.1*, *NeuroD1*, *Pax4* and *Isl1* (Fig. 3I-J). Expression of genes related to insulin release (*Abcc8*, *Kcnj11*) was elevated over time (Fig. 3M, N). *Ucn3*, which is required for glucose stimulated insulin release of β cells, was slightly elevated on day 100 (Fig. 3O). At protein level, on day 7, single insulin positive cells could be seen in the liver (Fig. 4A1). 4-6 insulin positive cells were found to be clustered in the liver at day 56. More insulin positive cells, 10-20 cells, was observed in a bigger cell cluster, similar to normal islet (Fig. 4B1, C1, D1). These data suggest that overexpression of *Pdx1*, *Neurog3*, *MafA* delivered by hydrodynamic injection in the liver can induce transdifferentiation process of liver cells to insulin procuring cells.

Fig. 1 *In vivo* DNA transfection efficiency demonstrated by bioluminescence imaging

A) Time dependent curve of transfection efficacy with different luciferase plasmid concentrations; **B)** Mouse liver bioluminescence images at 24 hours after transfection with different luciferase plasmid concentrations ($n = 4$); **C)** High concentration of bioluminescence signals in mouse liver after transfection by hydrodynamic delivery.

Fig. 2 Repeated *in vivo* *Pdx1*, *Neurog3* and *MafA* transfection by hydrodynamic delivery for anti-diabetic treatment

A) Diagram of study design; repeated *in vivo* DNA transfection was conducted from day 4 to day 0 followed by 100 days of glucose monitoring every 3 days; **B)** Fasting blood glucose level in diabetic mice after transfection; **C)** Weight change in diabetic mice after transfection; **D)** Glucose tolerance results in diabetic mice at day 7, 14, 28, 56, and 100 after transfection; **E)** Fasting insulin levels in diabetic mice at day 7, 14, 28, 56, and 100 after transfection. $n = 4$ in each mouse group.

Fig. 3 mRNA expression levels of genes relevant to pancreatic β cells and hepatocytes in liver tissue after *in vivo* transfection. mRNA levels were analyzed on day 7 ($n = 3$), 14 ($n = 4$), 28 ($n = 5$), 56 ($n = 4$), and 100 ($n = 3$) after transfection. Ct values were normalized to β -actin, and expression level was calculated by the $2^{-\Delta\Delta C_t}$ method. Pancreas-related genes: *Ins1*, insulin I; *Ins2*, insulin II; *Gcg*, glucagon. Hepatocyte-related genes: *Alb*, albumin; *Afp*, alpha fetoprotein. Key transcription factors related to pancreatic development: *Pdx1*, pancreatic and duodenal homeobox 1; *Neurog3*, neurogenin 3; *Mafa*, v-maf musculoaponeurotic fibrosarcoma oncogene family, protein A; *Nkx6-1*, NK6 homeobox 1; *Pax4*, paired box 4; *Neurod1*, neurogenic differentiation 1; *Isl1*, ISL1 transcription factor, LIM/homeodomain. Pancreatic β cell function-related genes: *Abcc8*, ATP-binding cassette, sub-family C (CFTR/MRP), member 8; *Kcnj11*, potassium inwardly rectifying channel, subfamily J, member 11; *Ucn3*, urocortin 3. * $p < 0.05$, ** $p < 0.01$ compared to diabetic control.

Fig. 4 Immunofluorescence images of hepatic insulin producing cells

Liver tissue was sectioned and stained on days 7 ($n = 3$), 56 ($n = 4$), and 100 ($n = 3$) after transfection. Red fluorescence: insulin; Green fluorescence: glucagon; Blue fluorescence: cellular nuclei. Scale bar = 25 μm (A1-3, B1-3, D1-3), 10 μm (C1-3).

Effects of hydrodynamic injection on the liver

Hydrodynamic injection procedure may bring potential injury to the mouse liver. To test the possible effects of hydrodynamic injection to the liver, the concentration of liver specific enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined at indicated time point. As shown in Fig. 5, on each time point, serum AST level in the PNM treated mice were in the normal range as

compared with those of normal animals without hydrodynamic injection. On day 7, an increase of the ALT value to 139.0 ± 38.12 ng/mL was seen in PNM treated animals, compared with approximately 48.4 ± 3.80 ng/mL in normal animals. It was noted that the ALT value falls into a normal range on day 100 after the injection. Histological staining showed no obvious injury to the liver tissue after transfection (Fig. 6).

Fig. 5 Liver functions after DNA transfection in diabetic mice

AST, aspartate aminotransferase; ALT, alanine aminotransferase; * $p < 0.05$, ** $p < 0.01$ compared to normal control or blank transfection control as indicated.

Fig. 6 Liver tissue pathology in diabetic mice

A) Liver tissue from normal control mice; B) Liver tissue from mice at 100 days ($n = 3$) after transfection. Scale bar = 50 μ m.

Discussion

We delivered plasmids encoding *Pdx1*, *Neurog3* and *MafA* by virus-free hydrodynamic injection repeatedly to the diabetic mice liver, and found that fasting blood glucose decreased to normal level for three months. At the early stage after transfection, few of insulin positive cells were found, and at the later stage, more insulin positive cells gradually clustered as aggregates. In mRNA level, a plurality of islet β cell-specific transcription factor genes began to increase at early stage. At later stage after transfection, mRNA levels of β cell function-related proteins gradually increased, including islet β cell maturation marker *Ucn3* [15], but expression level of liver specific marker *Alb* decreased. These data indicated that the reprogramming process from hepatic cells into insulin-secreting cells initiated at the early stage after transfection, and the maturity of the reprogrammed insulin producing cells gradually increased with time.

A combination of transcription factors *Pdx1*, *Neurog3* and *MafA* was first to be screened out and applied directly to *in vivo* cellular reprogramming from pancreatic exocrine cells [4]. Liver is another starting tissue for reprogramming of insulin producing cells [16, 17]. A series of studies have shown that hepatocytes can be directly reprogrammed into insulin producing cells *in vivo* [5, 18-21]. The reprogrammed cells can significantly improve the STZ diabetic mice symptoms of hyperglycemia, and insulin secretion can reach 23% of the normal insulin secretion level. Currently, adenoviral vectors have been widely used in the reprogramming system for exogenous gene transfer because of its high transfection efficiency [22-27], but still face the risks of integration and immune rejection. Studies have shown that up to 2×10^{10} PFU adenovirus of PNM infection was needed to achieve reduction of blood glucose to normal levels in immunocompetent mice, and can only maintain 6-7 weeks [28]. Non-viral gene transfection approach has low toxicity and immunogenicity characteristics [29]. Cim *et al.* reported that insulin gene expression could be induced in the liver after delivery of plasmids encoding *Pdx1*, *Neurog3* and *MafA* to rat liver cells by hydrodynamic injection. Fasting blood glucose reduced to normal level in a week, but elevated again [30]. They believed that a single hydrodynamic delivery of *Pdx1*, *Neurog3* and *MafA* to the liver cells can initiate the trans-differentiation of liver cells to islet β

cells, but the process is reversible, considering the reason may be the short expression time and low expression level. In our study, we delivered mixed plasmids encoding *Pdx1*, *Neurog3* and *MafA* separately with Mirus hydrodynamic delivery solution once, blood glucose only shortly decreased. Next, we injected repeatedly for four consecutive days, and found that fasting blood glucose in diabetic mice got long-term improvement with enhancement of glucose regulation ability. Reprogrammed cells in the liver expressed β cells mature marker gene *Ucn3*.

Hydrodynamic based gene delivery is an efficient non-viral gene transfection method. Luciferase signal in the liver was up to 10^7 RLU/mg protein after hydrodynamic delivery of plasmids encoding luciferase reporter gene [31]. Liu *et al.* found that when 25 μ g pCMV-Luciferase was delivered by hydrodynamic injection the strongest signal of luciferase in the liver was at 8 h, followed by a sharp decline of the signal. When plasmids carrying hAAT or FGF21 were transfected by hydrodynamics tail vein injection, they observed the highest gene expression at 24 h and sustainable expression in the liver for more than two weeks [8, 12]. In our study, we found that luciferase signal in the whole liver reached the highest level at 24 h after hydrodynamic delivery of 50 μ g pCMV-Luciferase plasmids DNA. Elevated mRNA level of ectopic transcription factors in the liver continued until the end of the observation. Similarly, hAAT could still be detected in the serum 8 weeks after hydrodynamic delivery, considering the possible reason may be partial integration of exogenous plasmid DNA to the host genome [12].

Hydrodynamic injection procedure can induce some damage to liver, resulting in rapid weight loss after transfection. We found that ALT level elevated post injection but finally returned to normal level afterwards. Pathological findings showed minor liver tissue damage at day 7, which disappeared later. A novel image-guided hydrodynamic technique might help reduce the amount of liver tissue damage [32].

In summary, the application of hydrodynamics based gene delivery in optimal transfection dose by repeated injections of a combination of direct reprogramming factor PNM plasmids can directly reprogram hepatocytes into insulin producing cells, and improve long-term blood glucose levels, but injection procedure in small animal models can bring some liver injury. Further studies in other animal models

are warranted to elucidate the treatment effect and safety of hepatocyte reprogramming by hydrodynamic injection.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81270857, 81670702), The Natural Science Foundation of Guangdong (No.

2015A030313762), The Science and Technology Project of Shenzhen (JCYJ20140416122811921, JCYJ2016031115823245, CXZZ20140903103747568) and the China Postdoctoral Science Foundation (No. 2016M592603).

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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