Establishment of a pancreatic β cell proliferation model in vitro and a platform for diabetes drug screening

Jing Jia · Xiaoli Liu · Yongxia Chen ·
Xiaoliang Zheng · Linglan Tu ·
Xiaoming Huang · Xiaojun Wang

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Abstract Diabetes, a disease resulting from loss of functional β cells, is globally an increasingly important condition. Based on the islet-differentiation ability of ductal epithelial cells and stimulating β cell proliferation ability of the Reg I α gene, we aimed to establish an in vitro pancreatic β cell proliferation model for screening therapeutic drugs of diabetes in the future. Pancreatic ductal epithelial cells were isolated from male Wistar rats, and induced to differentiate into pancreatic β cells. Immunofluorescence staining assay, western blot, RT-PCR analysis, and dithizone staining were used to characterize the cells. Rat Reg I α protein was transiently

expressed in vitro by transfection of HEK 293 cells with the PCMV6-entry-REG Ia plasmid, and expression was verified by RT-PCR analysis, proliferation assay, and apoptosis assay. The pancreatic β cell proliferation model was further validated by a proliferation assay using differentiated pancreatic β cells treated with transfection supernatant. Finally, we have successfully established an in vitro pancreatic β cells proliferation model using transiently expressed rat Reg I α protein and differentiated pancreatic β cells from pancreatic ductal epithelial cells. This model could be used as a platform to screen new drugs for islet neogenesis to cure diabetes, especially Chinese herbal drugs in the future.

Jing Jia and Xiaoli Liu contributed equally to this manuscript.

J. Jia · X. Zheng · L. Tu · X. Wang (✉)
Center for Molecular Medicine, Zhejiang Academy of
Medical Sciences, Hangzhou 310013, Zhejiang Province,
People's Republic of China
e-mail: wangxj@zjams.com.cn

X. Liu
Department of Neurology, Zhejiang Hospital,
Hangzhou 310013, Zhejiang Province,
People's Republic of China

Y. Chen
Department of Biomedical Research Center, Sir Run Run
Shaw Hospital, Hangzhou 310000, Zhejiang Province,
People's Republic of China

X. Huang
Institute of Hygiene, Zhejiang Academy of Medical
Sciences, Hangzhou 310013, Zhejiang Province,
People's Republic of China

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Introduction

Diabetes is a severe global health problem, affecting 366 million people over the world in 2011 (Whiting et al. 2011), which increased from 200 million in 2010 (Shaw et al. 2010), and the number is expected to be 552 million by 2030 (Whiting et al. 2011). Type 1 diabetes (T1DM) and Type 2 diabetes (T2DM) both result from inadequate insulin action, a consequence of loss of functional β cells. The loss of β cell mass combined with β cell dysfunction is caused by autoimmune destruction in T1DM, while by metabolic exhaustion in T2DM (Mohan et al. 2013).

Hormonal substitutive therapy, such as insulin administration, has been the main treatment for diabetes. This therapy, however, has to be taken life-long, and could cause long-term complications, such as vascular degeneration, blindness, and kidney failure. Islet transplantation is an alternative therapeutic approach with paramount clinical interest, which has been proven to successfully revert the symptoms for years (Bretzel et al. 2001, 1995). However, the widespread use of this therapy has been hindered by the shortage of donor organs and graft failure risk (Federlin et al. 2001; Jamiolkowski et al. 2012). Therefore, there is an urgent need for alternative islet supply for transplantation.

Previous observations found that islet progenitor cells may reside in the pancreatic ducts (Bonner-Weir et al. 2004; Lin et al. 2006). After culturing in vitro, pancreatic ductal cells often exhibit a combinational phenotype of both mesenchymal- and pancreatic progenitor-like cell (Seeberger et al. 2006). Further, in vitro the glucose responsive islet tissues could also be induced to differentiate from ductal tissue or ductal epithelial cells (Bonner-Weir et al. 2000; Liu et al. 2007), and these tissues were proven to be stable enough for transplantation (Katdare et al. 2004).

Reg (regenerating gene) I α , a C-type lectin super-family gene, was first identified in 1987 by screening a rat regenerating islet-derived cDNA library. It is conserved through species, and is composed of six exons and five introns. The rat Reg I α gene encodes a 165 amino acid protein with a 21 amino acid signal peptide (Okamoto 1999; Terazono et al. 1988), and human Reg I α cDNA encodes a 166 amino acid protein with a 22 amino acid signal peptide, which is 68 % homologous with rat Reg I α gene (Watanabe et al. 1990).

Reg proteins are mitogenic in both islet and ductal cells in cell lines, isolated primary islets and duct cultures. The well-known function of Reg I α gene is its stimulator role in β cell growth and regeneration (Okamoto 1999), which has been confirmed by Reg I α gene knockout mice. The administration of Reg I α protein could also ameliorate diabetes in non obese diabetic mice or other animal diabetes models (Akiyama et al. 2001; Gross et al. 1998; Unno et al. 2002; Watanabe et al. 1994). The expression of Reg I α was upregulated in regenerating islets, while the Reg receptor was not, suggesting that Reg I α protein could induce β -cell proliferation via the Reg I α receptor (Akiyama et al. 2001). In T1DM, upregulated Reg I α stimulates β cell

mass regeneration, however, the ongoing autoimmunity would destroy the newly regenerated cells, and diabetes is setup finally (Singh et al. 2011). So, Reg I α was not so applicable in diabetes therapy, anyhow, it could help to find new therapeutic drugs for diabetes.

Based on the islet-differentiation ability of ductal epithelial cells and stimulating β cell proliferation ability of Reg I α gene, in this study we created an in vitro pancreatic β cell proliferation model. This model could be used as a platform for screening therapeutic drugs for the islet neogenesis of diabetes.

Materials and methods

Cell isolation and culture

The whole pancreas was isolated from a male Wistar rat (6–8 weeks of age, 220–260 g) after perfusion of pancreas using 1.5 mg/ml collagenase (Type IV, Sigma, St. Louis, MO, USA) through biliary and pancreatic duct. The tissue was then cut into 2–3 mm small pieces and digested with 1.5 mg/ml collagenase (Type V, Sigma) at 37 °C. Digestion was terminated by cold D-Hanks supplemented with 10 % fetal bovine serum (FBS, Zhejiang Tianhang Biological Technology Co., Ltd, Hangzhou, China), and the tissue was filtered through 180 μ m stainless steel mesh. The filtrate was centrifuged at 1,500 rpm, 4 °C, and the pellet was collected and washed by D-Hanks (Boster, Wuhan, China). Pancreas ductal epithelial cells were separated and purified on a Ficoll gradient by centrifugation at 1,000 rpm for 5 min at 4 °C, and the top and middle layers were collected. The pancreatic ductal epithelial cellular pellets were collected and cultured in CMRL1066 medium (5.6 mM glucose, Gibco, Grand Island, NY, USA) supplemented with 10 % FBS, 100 IU/ml penicillin and 100 μ g/ml streptomycin, in non-treated Falcon T-25 flasks at 37 °C, 5 % CO₂. Cells were expanded for up to 1 week with additional medium changes every 2–3 days until most, if not all, adherent cells were in monolayer (Bonner-Weir et al. 2000; Liu et al. 2007). All animal studies were approved by the Animal Care and Use Committee at the Zhejiang Academy of Medical Science.

In vitro differentiation of β cells

When the pancreatic ductal epithelial cells were grown to 90 % confluence in CMRL1066 medium with 10 %

FBS, the medium was changed to differentiation medium: serum-free DMEM/F12 (8 mM glucose) medium supplemented with 1 mg/ml ITS (Sigma), 100 units/ml penicillin (Beyotime, Haimen City, China), 100 µg/ml streptomycin (Beyotime), 2 mg/ml BSA (Beyotime), 10 mM nicotinamide (Sigma), and 10 ng/ml keratinocyte growth factor (KGF) (Pepro-tech, Rocky Hill, NJ, USA) (Bonner-Weir et al. 2000; Zulewski et al. 2001).

RNA extraction and RT-PCR analysis

Total RNA was extracted following the protocol suggested by the manufacturer using Trizol reagent (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed as described by the manufacturer of PrimeScript RT-PCR kit (Takara, Otsu, Japan). PCR was carried out in 25 µl reaction system using 1 µl of the cDNA reaction product as template mixed with 24 µl of PCR mixture: 10X Taq buffer (Promega, Beijing, China), 10 pm of each primers (forward and reverse) (Invitrogen), 80 mM cold dNTPs (Promega), 5 units DNA polymerase (Promega), and nuclease-free water.

Primers were as follows: rat insulin forward-ccgtcgtgaagtggagga; rat insulin reverse- cagttgga gaggagcagat (153 bp PCR product); rat PDX-1 forward- gaggaccgtacagcctaca, rat PDX-1 reverse- cgttgctcccgtactacgtt, (201 bp PCR product); rat Reg Iα forward-atgactcgcaacaatatattcattctgc, rat Reg Iα reverse- tcaggctttgaactgcagacaaatg (501 bp PCR product); rat beta actin forward-gagagggaaatcgtgcgt gacatt, rat beta actin reverse-caggaaggaaggctggaagaga (189 bp PCR product).

The thermal cycling protocol began with a denaturing step of 95 °C for 3 min, followed by 28 cycles of 94 °C 30 s, 50 °C 30 s, 72 °C 30 s, and finished with 72 °C for 10 min.

RT-PCR products were loaded onto 1.0 % agarose gels, stained with GelRed, and photographed under ultraviolet light in a BioRad Universal Hood II and Quantity One 4.6.1 software.

Immunofluorescence staining

For immunofluorescence staining, cells were washed twice with PBS and fixed with 4 % paraformaldehyde for 10 min at room temperature. After washing, cells were incubated with blocking solutions (5 % BSA in PBS) for 30 min. Cells were then washed two more

times with PBS and incubated with 2 % Triton-X100 for 20 min to make cell membranes permeable. The cells were washed again and incubated with primary antibody at a 1:100 dilution in blocking solution at 4 °C overnight. Subsequently, cells were treated with secondary antibody at a dilution of 1:1,000 for 1 h at room temperature. Images were collected with a confocal microscope (Zeiss LSM710, Oberkochen, Germany). The antibodies used were as following: mouse-anti-rat CK19 (Santa Cruz, Santa Cruz, CA, USA), rabbit-anti-rat insulin (Santa Cruz), rabbit-anti-mouse IgG-FITC (Santa Cruz), goat-anti-rabbit-IgG-PE (Invitrogen).

Western blot assay

The cells were lysed by RIPA lysis buffer, centrifuged for 10 min at 10,000g, and the supernatant was collected for protein analysis. The protein concentration was determined by the Bradford method using the Quick Start Bradford Protein Assay Kit (Bio-Rad, Hercules, CA, USA). Protein lysates were loaded onto 12 % SDS-PAGE, and transferred to PVDF membrane (GE HealthCare, Wuxi, China).

The membranes were blotted with mouse anti-rat antibodies against CK19, pan-cytokeratin, or rabbit anti-rat insulin (Santa Cruz), followed by incubation with horseradish peroxidase-conjugated secondary antibody: sheep anti-mouse IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA), goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories). The protein signal was detected by ECL reagent (GE HealthCare), and photographed using Alpha Innotech Fluor Chem FC2 chemiluminescence image analysis system (Alpha Innotech, San Leandro, CA, USA).

DTZ staining

A DTZ (Sigma) stock solution was prepared with 50 mg of DTZ in 5 ml of dimethyl sulfoxide (DMSO), and the solution was filtered through a 0.2 µm nylon filter and then stored at −20 °C. In vitro DTZ staining was performed by adding 10 µl of the stock solution to 1 ml of culture medium with a final concentration of 100 µg/ml. The culture dishes were incubated at 37 °C for 15 min in the DTZ solution. After the dishes were rinsed three times with D-Hanks, cells were examined under a stereomicroscope.

Transfection

Plasmid PCMV6-Entry-REG Ia (Origene, Rockville, MD, USA) and PCMV6-Entry vector (Origene) without insert as a control were transfected into HEK-293 cells using lipofectamine 2,000 transfection reagent (Invitrogen). A total of 2.5 µl lipofectamine 2,000 reagent and 1 µg plasmid was used to form DNA-reagent complex for one well. At 72 h post-transfection, the supernatant was collected, and stored at -80°C . The transfected cells were collected for RNA extraction and analysis.

Cell proliferation assay by CFSE

To detect proliferation rate, ductal epithelial cells were labeled with Carboxyfluorescein Diacetate (CFSE) dye (CellTrace™ CFSE Cell Proliferation Kit, Invitrogen) for 10 min at 37°C and the staining was ended by adding 5 volumes of ice-cold culture medium to the cells. Labeled ductal epithelial cells were incubated with diluted transfection supernatant (1:10 dilution) for 96 h. Cell proliferation was then assessed per 24 h by flow cytometry (CellQuest; BD, Franklin Lakes, NJ, USA) and analyzed by ModFit™ software version 3.0. In the histograms generated, the x-axis represents CFSE intensity and the y-axis represents the number of cells. Proliferation Index (PI) was defined as the sum of all divisions divided by the number of all cells that proliferated, and it was calculated and given by ModFit™ software. The experiments were repeated three times.

Assay of apoptosis

Pancreatic ductal epithelial cells were incubated with diluted transfection supernatant (1:10 dilution) for 96 h. Then, apoptotic cell fractions were identified by double staining with annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) using an apoptosis detection kit (K101-25, BioVision, Milpitas, CA, USA) according to the manufacturer's instructions. Apoptotic cells were analyzed by flow cytometry (CellQuest; BD). The experiments were performed three times.

Cell proliferation assay by WST-1

Differentiated pancreatic β cells were seeded into 96 well plates with 1×10^4 cells/well. Cell proliferation

assay was carried out in mixtures of 200 µL solution, including cell-suspension (100 µL), transfection supernatants in different dilution, cRPMI-1640 medium. The mixtures containing transfection supernatants of the control plasmid were used as control. The plates were incubated at 37°C in a humidified atmosphere containing 5 % CO_2 . 48 h later, the proliferation responses were detected by the WST-1 (Roche, Basel, Switzerland) method. The OD_{450} value was determined using a Multidetector microplate reader (Bio-Tek, Winooski, VT, USA). The measured absorbance directly correlates to the number of viable cells. The experiments were performed in triplicate and performed three times.

Statistical analyses

Results of proliferation assay are presented as mean $\pm$ SE. The statistical significance of differences was determined with the Student *t* test. The difference between 2 groups was considered significant when $P < 0.05$.

Results

Isolation and culture of pancreatic ductal epithelial cells

The isolated pancreatic ductal epithelial cells exhibited an epithelium-like phenotype (Fig. 1A) at 80–90 % confluence. The pancreatic ductal cells specific markers were validated. CK19 was stained green and detected by immunofluorescence staining assay (Fig. 1B); the protein levels of CK-19 and pancytokeratin were evaluated by Western blot assay (Fig. 1C); mRNA level of PDX-1 was detected by RT-PCR analysis with an expected band of 201 bp, while mRNAs of Reg Ia and insulin were not detected, which is consistent with the characteristics of pancreatic ductal epithelial cells (Fig. 1D).

In vitro induction of pancreatic β cells

The pancreatic ductal epithelial cells were induced to differentiate in vitro and the cells gradually became round and detached from the bottom. After 4 weeks, some of the differentiated cells clustered together to form an islet-like cell mass (Fig. 2A). After 6 weeks,

Fig. 1 Pancreatic ductal epithelial cells were cultured until 80–90 % confluence (A, bar = 50 μ m, magnification 100 $\times$). For validation, CK19 was stained green and detected by immunofluorescence staining assay (B, bar = 50 μ m, magnification 200 $\times$), and the protein levels of CK19 and pan-cytokeratin were evaluated by Western blot assay (C). mRNA levels of PDX-1 and beta actin were detected in these cells by RT-PCR analysis, while mRNAs of Reg Ia and insulin were not detected (D)

the suspension cells were collected to characterize the differentiated pancreatic β cells. Immunofluorescence staining assay and Western blot found that insulin protein was expressed in the cells (Fig. 2C, D). Dithizone (DTZ) staining demonstrated that these cells were pancreatic β cells with the mature cells dyed red (Fig. 2B).

In vitro expression of Reg Ia protein

The expression of Reg Ia mRNA after PCMV6-Entry-REG Ia transfection was detected by RT-PCR assay (Fig. 3A). There was a DNA band of 501 bp in the lane, which is consistent with the molecular weight of Reg Ia cDNA.

Validation of biological activity of Reg Ia protein

The proliferation stimulating ability of Reg Ia protein was detected by cell proliferation assay using CFSE and apoptosis assay. The transiently expressed Reg Ia protein in culture supernatant of cells transfected with

PCMV6-Entry-REG Ia was used for validation of the biological activity of Reg Ia protein. The culture supernatant of cells transfected with PCMV6-Entry was used as control. The results showed that the CFSE-fluorescence intensity decreased over time as cells divide, and also the peak number corresponds to cell generation (division) number. In both groups, the parent and early generation peaks gradually disappeared while later generation peaks appeared. However, there were always more peaks detected in transiently expressed Reg Ia treated cells than control. The results demonstrated that the cells proliferated faster in the Reg Ia treated group. 96 h later, the proliferation index was 13.85 ± 0.21 in the transiently expressed Reg Ia treated group and 12.34 ± 0.70 in the control. The difference between the two groups was significant with $P < 0.05$. One representative figure of the CFSE assay is shown in Fig. 3B, the proliferation index of the transiently expressed Reg Ia treated cells was 14.01, greater than that of the control's 11.83. 23.87 % of transiently expressed Reg Ia treated cells approached the 6th generation, and only 12.39% cells

Fig. 2 The in vitro cultured pancreatic ductal epithelial cells were induced to differentiate into pancreatic β cells. The cells gradually became round and detached from the culture surface (A, bar = 20 μ m, magnification 100x). After 6 weeks, the differentiated β cells were stained red by dithizone (DTZ) staining (B, bar = 20 μ m, magnification 400x), and the insulin protein was detected by immunofluorescence staining assay (C, bar = 20 μ m, magnification 100x) and Western blot assay (D) in these differentiated pancreatic β cells

reached the 6th generation in control group. Also, the peaks corresponding to 8th and 9th generation could only be detected in Reg Ia treated group. There were 7 division peaks in Reg Ia treated group versus 5 in the control group (Fig 3B). Apoptotic cell fractions were identified by double staining of annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI), and the apoptosis ratio (bottom right), necrosis ratio (top right), and viable cells ratio (bottom left) was evaluated. In Fig 3C, the apoptotic ratio and necrosis ratio were lower in the PCMV6-Entry-REG Ia transfection supernatant treated cells (10.45, 12.17 %) than in the control cells (13.60, 23.30 %), while the viable cells ratio was higher (76.14 versus 58.13 %) at 96 h. The average apoptosis ratio was 10.28 ± 3.40 % in the transiently expressed Reg Ia treated group and 13.81 ± 2.81 % in the control group (Fig 3C). However, there was no significant difference.

Establishment of pancreatic β cell proliferation model

Differentiated pancreatic β cells were added to 96 well plate with 1×10^4 cells/well, 2×10^4 cells/well, 3×10^4 cells/well for each group. After 48 h incubation, the proliferation responses were detected using the WST-1 method (Fig. 4A). The OD₄₅₀ value increased with the seeding cell density, which was 0.324 ± 0.033 , 0.40 ± 0.029 , 0.43 ± 0.053 , respectively. 1×10^4 cells/well was chosen for the following cell proliferation assay for the further study.

Differentiated pancreatic β cells were added to 96 well plates with 1×10^4 cells/well. Cell proliferation assay was carried out with supernatant containing transiently expressed Reg Ia at different dilutions (1:10, 1:2 and 1:1 equivalent to 10 μ l, 50 μ l, and 100 μ l), the proliferation responses were detected by WST-1 method after 48 h (Fig. 4b). For the transiently expressed Reg Ia treated group, the OD₄₅₀ values varied with the dilution, which was 0.44 ± 0.018 (1:10), 0.54 ± 0.026 (1:2), 0.58 ± 0.056 (1:1), respectively. There was a significant difference between the 1:10 and 1:2 diluted transfection supernatant group. However, there was no significant difference between the 1:2 and 1:1 group. For the control supernatant group, the OD₄₅₀ values varied slightly with the dilution, which was 0.35 ± 0.016 (1:10), 0.38 ± 0.021 (1:2), 0.41 ± 0.017 (1:1), respectively. Significant differences were also observed

Fig. 3 The transient expression of Reg Ia mRNA was detected by RT-PCR assay (**A**). The biological activity of Reg Ia protein was assessed by cell proliferation assay using CFSE (**B**) and apoptosis assay (**C**) by double staining of annexin V and PI, with PCM6-Entry transfection supernatant as control. After 96 h, the cell proliferation indexes (14.01 vs 11.83), division peaks (7

vs 5), and the percentages of cells reached generation 6th (23.87 vs 12.39 %) were measured and compared. The apoptosis ratios (*bottom right*: 10.45 vs 13.60 %), necrosis ratios (*top right* 12.17 vs 23.30 %), and viable cells ratios (*bottom left* 76.14 vs 58.13 %) were also measured and compared

Fig. 4 Establishment of pancreatic β cell proliferation model. **A** Cell proliferation assay by the WST-1 method was used to select seeded cell density from 1×10^4 cells/well, 2×10^4 cells/well, 3×10^4 cells/well. 48 h later, OD₄₅₀ values of the three groups were tested (0.324 ± 0.033 , 0.40 ± 0.029 , 0.43 ± 0.053 , respectively). **B** Cell proliferation assay by the WST-1 method was used to select the dilution of the transfection supernatants (1:10, 1:2, and 1:1). 48 h later, OD₄₅₀ values were measured and compared between the groups (0.44 ± 0.018 (1:10), 0.54 ± 0.026 (1:2), 0.58 ± 0.056 (1:1) versus controls: 0.35 ± 0.016 (1:10), 0.38 ± 0.021 (1:2), 0.41 ± 0.017 (1:1)). * $P < 0.05$ versus control. $^{\Delta}P < 0.05$ versus 1:10 group. **C** Cell proliferation assay by the WST-1 method was used to establish the growth curve of pancreatic β cells under the effect of Reg Ia. Differentiated pancreatic β cells (1×10^4 cells/well) were incubated with transfection supernatants (1:2 diluted) in 96 well plates for 102 h. The number of viable cells was represented by OD₄₅₀ values measured every 24 h (0.14 ± 0.015 (24 h), 0.51 ± 0.013 (48 h), 0.69 ± 0.031 (72 h), 1.33 ± 0.026 (96 h), 1.15 ± 0.042 (102 h))

between each treated group and its control. Finally, 1:2 diluted transfection supernatant was chosen for the proliferation model.

Differentiated pancreatic β cells were added to 96 well plates with 1×10^4 cells/well, and incubated with 1:2 diluted transfection supernatants for 102 h. The numbers of viable cells were represented by OD₄₅₀ values detected every 24 h by the WST-1 method. The growth curve of pancreatic β cells under the effect of Reg Ia is shown in Fig. 4C, for each test point, the OD₄₅₀ values were 0.14 ± 0.015 (24 h), 0.51 ± 0.013 (48 h), 0.69 ± 0.031 (72 h), 1.33 ± 0.026 (96 h), 1.15 ± 0.042 (102 h), respectively. The formula of this growth curve was $y = -0.0524x^3 + 0.4261x^2 - 0.6786x + 0.4754$, with R^2 equal to 0.9432. Finally the proliferation model of pancreatic β cells was setup successfully.

Discussion

T1DM and T2DM results from different pathogenesis and there is no lasting therapeutic treatment for them. To restore β cells has been considered to be an effective treatment for curing both T1DM and T2DM. Islet neogenesis, induction of islet regeneration from existing progenitor cells, is a theoretically possible way to enrich β cells (Halban et al. 2010). The most promising way to achieve islet neogenesis, is therapeutically inducing endogenous stem or progenitor cells to develop into islets (Guo and Hebrok 2009).

The islet progenitor cells could be obtained from different sources, including pancreatic islets (Smukler et al. 2011), pancreatic ductal tissue (Lin et al. 2006), brain derived pluripotent cells (Saljooque et al. 2011), bone marrow and umbilical cord blood (Gao et al. 2008; Paz et al. 2011), and so on. However, hES and pluripotent stem cells are easy to induce but have a high tumorigenicity. Adult stem cells are easy of procurement, but would need different induction protocols because they have different intrinsic potentials (Satija et al. 2009). However, the islet neogenesis still faces problems of progenitor cells sources, tumorigenicity, patient-safety, and so on. Clinically applicable approaches to regenerate β cells or enrich them in vivo or in vitro are an urgent need.

Until now, lots of pharmaceutical agents have been identified in labs to promote β -cell proliferation and cure diabetes, including GLP-1, GLP-1 mimetics, DPP-IV inhibitors, exendin-4, and the INGAP peptide

(Lavine and Attie 2010; Nauck et al. 2006; Ritzel et al. 2007; Shimoda et al. 2011). But none of them has proven to be the ultimate neogenesis agent. Chinese medicinal herbs have fewer and less severe side effects than single pure drugs. This advantage as well as their compatibility are making Chinese medicine especially attractive. While Western medicine employs pure, single compounds, either natural or synthetic, Chinese medicine focuses on combined therapy to relieve disease symptoms, including diabetes (Agrawal et al. 2013; Ji et al. 2011; Lee 2000; Liu et al. 2011). But research is still needed to identify and improve the efficacy of the active principals of herbs both used alone and in combination.

Thus, β cell neogenesis technology, and Reg Ia gene, were combined together to establish a model of β cell regeneration in vitro, which could be used as a platform for screening new drugs for diabetes, especially Chinese herbal drugs. The screened drugs could be used for islet neogenesis and β -cell restoration in vivo, together with immune modulation, and will be safer, more cost effective, and clinically more applicable compared to present drugs.

In this study, a proliferation model of pancreatic β cells was established successfully, using pancreatic β cells induced from pancreatic ductal cells, and transiently expressed Reg Ia protein as stimulator. The growth curve of the pancreatic β cells stimulated by Reg Ia protein was obtained, which could be used as a reference for drug screen. The Reg Ia protein was expressed by HEK-293 cells transfected with PCMV6-Entry-REG Ia plasmid, and its bioactivity was validated by cell proliferation and apoptosis assay. The transfection supernatant was used for the proliferation model with proper dilution. In the future, purified and quantified Reg Ia protein should be used for this model and to screen diabetes drugs. Another disadvantage of this model is the differences between human beings and rats. The rat is a usually used model organism for study of human disease, but there are many differences between human and rats. This is a disadvantage of this pancreatic β cell proliferation model. Anyhow, to compensate this disadvantage, we could do verification studies on human cells with drugs screened out on this model in the future, or try to establish a model based on human cells in advance.

In conclusion, a proliferation model of pancreatic β cells was established successfully, and it could be used

as a platform for screening new drugs for diabetes, especially Chinese herbal drugs in the future.

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Conflict of interest None of the authors has any potential financial conflict of interest related to this manuscript.

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