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Improved transplantation outcome through delivery of DNA encoding secretion signal peptide-linked glucagon-like peptide-1 into mouse islets

Hee Young Chae,[1†] Minhyung Lee,[2†] Hyo Jeong Hwang,[1] Hyun Ah Kim,[2] Jun Goo Kang,[1] Chul Sik Kim,[1] Seong Jin Lee[1] and Sung-Hee Ihm[1]

1 Department of Internal Medicine, Hallym University College of Medicine, Chuncheon, Korea
2 Department of Bioengineering, College of Engineering, Hanyang University, Seoul, Korea

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Correspondence
Sung-Hee Ihm MD, Department of Internal Medicine, Hallym University Sacred Heart Hospital, 896 Pyungchon-dong, Dongan-gu, Anyang, Kyonggi-do 431-070, Korea.
Tel.: +82 31 380 3714;
fax: +82 31 386 2269;
e-mail: ihmsh@hallym.ac.kr

Conflicts of Interest
The authors have declared no conflicts of interest.

[†]Contributed equally.

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Summary

Glucagon-like peptide-1 (GLP-1) stimulates cell proliferation and has anti-apoptotic effects on pancreatic islet β cells. In our previous study, the transduction of mouse islets with a recombinant adenovirus containing GLP-1 cDNA enhanced islet graft survival. In this study, we sought to deliver the GLP-1 gene using a nonviral vector, which raises fewer safety issues in clinical application. We constructed a plasmid, pβ-SP-GLP-1, in which a secretion signal peptide (SP) was inserted to increase GLP-1 secretion, and transfected mouse islets using the nonviral carrier Effectene. Transfection of pβ-SP-GLP-1 induced a significant increase in bioactive GLP-1 levels in islet cultures. Islets transfected with pβ-SP-GLP-1 were protected from H_2O_2-induced cell damage *in vitro*. In addition, glucose-stimulated insulin secretion was significantly increased in pβ-SP-GLP-1-transfected islets. Diabetic syngeneic mice transplanted under the kidney capsule with a marginal mass of pβ-SP-GLP-1-transfected islets rapidly became normoglycemic, with 88% of recipients being normoglycemic at 30 days post-transplantation compared with 52% of mice that received pβ-transfected islet grafts ($P < 0.05$). Islet grafts retrieved 7 days after transplantation revealed that the pβ-SP-GLP-1-transfected group had significantly more Ki67-positive cells as compared with the pβ-transfected group. In conclusion, delivery of a plasmid containing a secretion SP and GLP-1 cDNA using a nonviral carrier leads to efficient secretion of GLP-1 in mouse islet cells, enhances islet cell survival during the early post-transplant period, and improves islet transplantation outcome.

Introduction

Pancreatic islet transplantation is a promising strategy for treating insulin-deficient diabetes. Currently, allogeneic single-donor islet transplantation usually fails to achieve long-term insulin independence in diabetic recipients because of the early loss of transplanted islets [1,2]. Isolated islets are avascular and are therefore ischemic during 7–14 days required for revascularization [3]. Immediately after transplantation, the majority of islets undergo cell death as a result of hypoxic and inflammatory insults [4,5]. One strategy to improve transplanted islet survival rates is genetic modification in islet cells to make them resistant to apoptotic cell death caused by these insults during the early post-transplantation period. Anti-apoptotic genes, such as XIAP and bcl-2, and angiogenic genes, such as Vascular endothelial growth factor (VEGF), have been investigated as therapeutic genes [6–10]. Glucagon-like peptide-1 (GLP-1) gene represents another possibility [11].

GLP-1 is a 30-amino-acid peptide hormone secreted by the L cells in the intestinal epithelium in response to food intake [12,13]. In addition to its glucose-dependent insulinotropic effect on glucose metabolism, which results from its stimulation of β cells, GLP-1 acts as a growth factor for rodent islet β cells, expanding the β-cell mass via the proliferation of islet cells, stimulating the neogenesis of

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pancreatic ductal cells and their subsequent differentiation into insulin-producing cells. GLP-1 also reduces β-cell apoptosis in purified rodent and human islets after exposure to many cytotoxic agents [14–20]. GLP-1 and its long-acting analogs could therefore potentially enhance islet transplantation by maintaining or enhancing the β-cell mass over time, and they have generated particular interest in the field of islet transplantation [21–24]. The expected β-cell 'trophic' effect of GLP-1/analogs could be enhanced by higher drug levels at islet graft sites. However, their tolerated doses in islet allograft recipients with type 1 diabetes mellitus were lower than in patients with type 2 diabetes, posing a significant risk of hypoglycemia to the islet recipients [21,22]. Thus, efforts have been made to directly induce GLP-1 production within islets by gene delivery. Wideman *et al.* [11] showed that the adenovirus-mediated expression of prohormone convertase 1/3 in α cells increased islet GLP-1 secretion, resulting in an improved transplantation outcome in a mouse model. In our previous study, the transduction of mouse islets with a recombinant adenovirus containing GLP-1 cDNA under the CMV promoter/enhancer and an albumin leader sequence enhanced islet survival and improved transplantation outcome [25].

On the basis of this positive result, we sought to deliver the GLP-1 gene using a nonviral vector, which raises fewer safety issues and is easier to produce for possible clinical application in islet transplantation trials than are viral vectors. We constructed a GLP-1 expression plasmid, pβ-SP-GLP-1, in which a secretion signal peptide (SP) was inserted to increase the secretion of GLP-1. In this study, we investigated the effect of local production of GLP-1 within the mouse islet graft, achieved through transfection of pβ-SP-GLP-1 with the nonviral carrier Effectene, on the cytoprotection of islets *in vitro* and *in vivo*.

Materials and methods

Plasmid construction and preparation

GLP-1 cDNA was synthesized chemically and inserted into the pβ vector at the *Kpn*I and *Xba*I sites. The DNA fragment encoding the secretion SP was synthesized chemically and inserted into pβ-GLP-1 at the *Kpn*I sites (Fig. 1) to create pβ-SP-GLP-1. Restriction enzyme studies and direct sequencing confirmed correct construction of the plasmids. pβ and pβ-SP-GLP-1 were amplified in *Escherichia coli* strain DH5α, and purified using Plasmid Maxi Kits (Qiagen, Valencia, CA, USA). Plasmid DNA (pDNA) purity was determined by measuring the OD_{260}/OD_{280} ratio. pDNA concentration was determined based on the assumption that an OD_{260} of 1 corresponds to 50 mg of DNA. pDNA was stored at −20 °C until use.

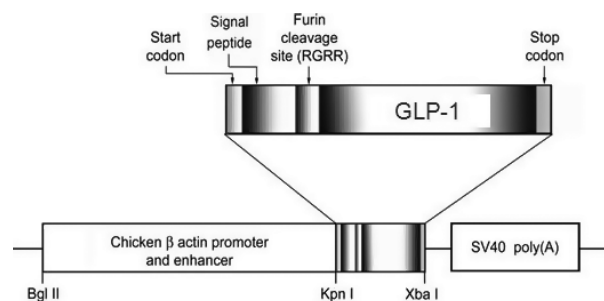

Figure 1 Structures of the pβ-SP-GLP-1 construct.

Islet isolation

Inbred male Balb/c mice aged 9–10 weeks were purchased from Koatech (Pyeongtaek, Korea). Pancreatic islets were isolated from 12-week-old mice by digesting pancreatic tissues with 1 mg/ml collagenase P (Roche, Mannheim, Germany) followed by gradient purification with Ficoll (Biochrom AG, Berlin, Germany). The isolated islets were maintained in a 5% CO_2 incubator in Medium 199 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS). Individual islets were selected using an inverted microscope and quantified by dithizone staining, with a standard islet diameter of 150 μm for one islet equivalent (IEQ).

Transfection

Mouse islets were washed with serum-free Medium 199 and fresh serum-free medium was then applied. An Effectene (Qiagen)/pβ or Effectene/pβ-SP-GLP-1 complex was added to each well, and the cells were then incubated for 4 h at 37 °C in a 5% CO_2 incubator. After 4 h, the transfection mixtures were removed, and fresh medium containing 10% FBS was added.

In vitro test of GLP-1 expression and secretion following pβ-SP-GLP-1 transfection

To check the expression of GLP-1 mRNA, islets transfected with pβ or pβ-SP-GLP-1 were harvested 24 h after infection, and RT-PCR was performed using the following primers: sense, 5′-ATGCGTCAACGTCGTCATGC-3′; antisense, 5′-GCCTTTCACCAGCCAAGCAA-3′. Expression of mouse β-actin mRNA was analyzed as an internal control using the following primers: sense, 5′-CCTAGACTTCGAGCAAGA-GA-3′; antisense, 5′-AATGTAGTTTCATGGATGCC -3′. To measure GLP-1 secretion, supernatants were collected every 24 h for 6 days after transfection, and the amount of GLP-1 was determined using an active GLP-1 enzyme-linked immunosorbent assay (ELISA) kit (Millipore, Tullagreen, Ireland).

Measuring cell viability and apoptosis

To evaluate the effect of GLP-1 expression on islet cell viability and apoptosis, mouse islets transfected with pβ or

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pβ-SP-GLP-1 for 24 h were treated with 200 μmol/l H_2O_2 for 30 min. As controls, nontransfected islets and islets pretreated with GLP-1 (10 nmol/l for 24 h) were also exposed to H_2O_2. Islet cell viability was assessed using fluorescence staining with AO/PI (Sigma-Aldrich, St Louis, MO, USA), as described previously [26]. Approximately 100 individual islets were photographed and the percentage of live (green) versus dead (red) cells among total cells was estimated. Mitochondrial membrane potential ($\Delta\psi m$) was determined by staining with the fluorescent cationic indicator JC-1 (Molecular Probes, Eugene, OR, USA) [26,27]. Islet cell apoptosis was assessed using a caspase-3/CPP32 assay kit (BioVision, Mountain View, CA, USA).

Glucose-stimulated insulin secretion

To determine the effect of GLP-1 expression on insulin secretion in response to glucose, a static glucose stimulation test was performed using mouse islets 24 h after their transfection with pβ or pβ-SP-GLP-1. Twenty islets were incubated sequentially in 2 ml of RPMI-1640 medium containing 1.67 mmol/l glucose (basal), 16.7 mmol/l glucose (stimulatory), and 1.67 mmol/l glucose (recovery) (1 h per incubation). Media were collected and assayed for insulin levels using an insulin ELISA kit (Shibayagi, Gunma, Japan). The glucose stimulation index (GSI) was calculated as follows: GSI = (2 × stimulatory insulin level)/(basal insulin level + recovery insulin level).

Islet transplantation

Mice were fed standard rodent chow in a barrier animal facility under a 12-h light/dark cycle. All animal experiments were in compliance with the guidelines of the Institutional Animal Care Committee of Hallym University. At 12 weeks of age, diabetes was induced through a single intraperitoneal injection of 200 mg/kg streptozotocin (STZ, Sigma) 3–5 days before transplantation. Mice were considered diabetic when their blood glucose levels exceeded 300 mg/dl on two consecutive days. At 24 h after transfection with pβ or pβ-SP-GLP-1, 180 IEQs were transplanted under the capsule of the left kidneys of diabetic syngeneic mice. After transplantation, nonfasting blood glucose levels were measured 3 days per week for 30 days. Reversal of diabetes was defined as a consistent reduction in the blood glucose level to <200 mg/dl. At 30 days post-transplantation, left nephrectomy was performed in the cured mice to confirm the recurrence of hyperglycemia. To assess the effect of GLP-1 expression on cell proliferation in early islet grafts, 180 pβ or pβ-SP-GLP-transfected IEQ were transplanted into diabetic recipients and a left nephrectomy was performed 3 or 7 days after transplantation. To evaluate the systemic delivery of GLP-1 expressed in islet grafts, serum GLP-1 levels were measured in tail vein samples obtained at 1, 3, 5, and 7 days after transplantation using active GLP-1 ELISA kit.

Immunohistochemistry of islet grafts

The graft-bearing kidneys retrieved 3 or 7 days after transplantation were fixed in 10% formaldehyde, processed, and embedded in paraffin. The 4-um-thick sections were stained with Ki67 antibody (1:100 dilution; Bethyl, Montgomery, TX, USA) and streptavidin–biotin complexes to detect cell proliferation, and were counterstained with hematoxylin. The fraction of Ki67-positive cells in the islet grafts was quantified under a light microscope using a direct point-counting method, as described previously [25]. Double immunofluorescence staining for insulin and Ki-67 was carried out on formalin-fixed and paraffin-embedded sections after deparaffinization. For insulin labeling, sections were incubated with mouse anti-insulin (Abfrontier, Seoul, Korea), and then incubated in the dark with FITC-conjugated, donkey antimouse immunoglobulin (Bethyl). For Ki-67 labeling, sections were incubated with rabbit anti-mouse Ki-67 (Bethyl), and then incubated in the dark with DyLightR594-conjugated, goat anti-rabbit immunoglobulin (Bethyl). Sections were observed under a confocal microscope (LSM 700; Carl Zeiss, Oberkochen, Germany).

Statistical analysis

Values for all measurements were expressed the mean ± SEM. Statistical significance was calculated using a Student's *t*-test or one-way analysis of variance (ANOVA). The Kaplan–Meier log-rank test was used to determine significance in the marginal islet mass transplantation experiments. A P-value < 0.05 was deemed statistically significant. All statistical analyses were performed using the program MedCalc (Mariakerke, Belgium).

Results

Expression of GLP-1 in pβ-SP-GLP-1-transfected mouse islets

To assess the expression of GLP-1 from pβ-SP-GLP-1, we transfected mouse islets with pβ or pβ-SP-GLP-1 using Effectene as a transfection agent and examined the expression of GLP-1 mRNA using reverse transcriptase-polymerase chain reaction (RT-PCR). GLP-1 mRNA was clearly expressed 24 h after pβ-SP-GLP-1 transfection, whereas GLP-1 mRNA was not detected in pβ-transfected islet cells (Fig. 2a). We examined GLP-1 secretion in pβ- and pβ-SP-GLP-1-transfected islet cells in culture for 6 days after transfection using ELISA. Transfection with pβ-SP-GLP-1 induced a significant increase in bioactive GLP-1 levels in

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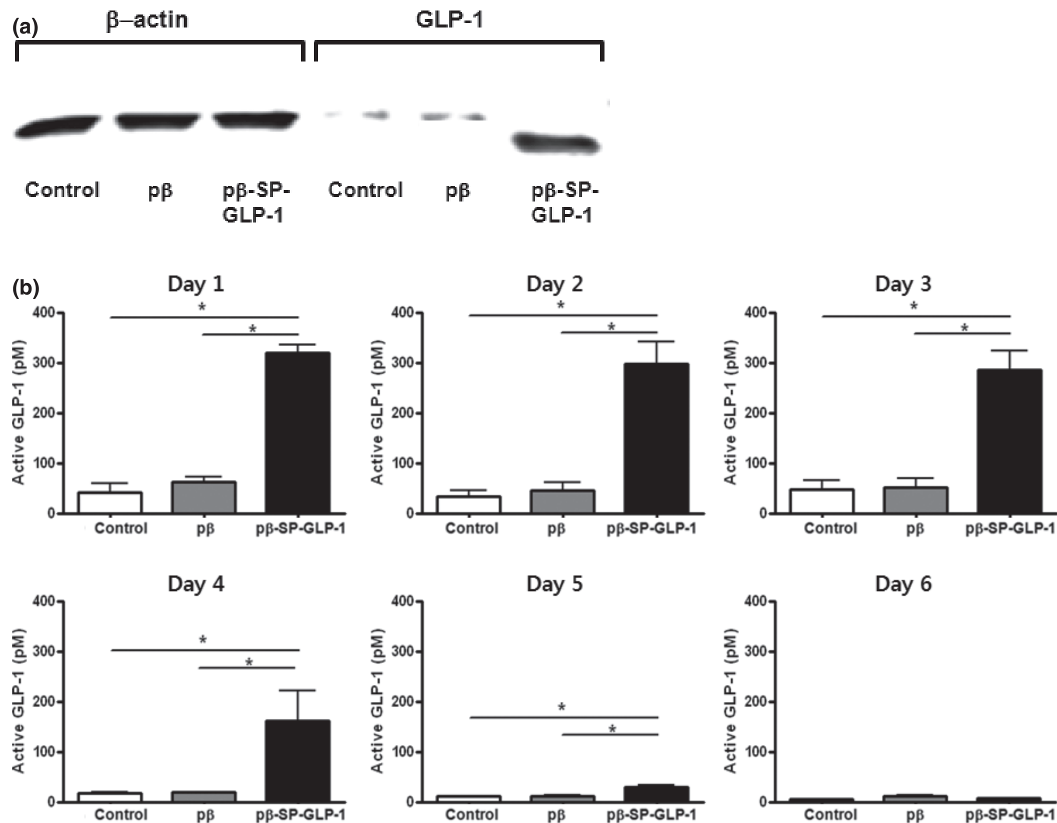

Figure 2 GLP-1 expression in mouse islets after transfection with pβ or pβ-SP-GLP-1. (a) GLP-1 mRNA expression was measured 24 h after transfection using RT-PCR. β-actin mRNA was analyzed as an internal control. (b) GLP-1 levels of culture media were measured every 24 h for 6 days after transfection using ELISA. *$P < 0.05$ versus control or pβ-transfected islets.

the mouse islet culture medium within 24 h, whereas transfection with pβ had no effect on GLP-1 secretion (Fig. 2b). These results indicate that the pβ-SP-GLP-1 construct induces the efficient expression and secretion of GLP-1 in mouse islet cells when delivered using Effectene. GLP-1 secretion in pβ-SP-GLP-1-transfected islets in culture declined over less than a week (Fig. 2b). There was significantly increased secretion of GLP-1 for 5 days after transfection.

Effect of pβ-SP-GLP-1 transfection on the survival and function of mouse islets

To determine whether GLP-1 expression in islets has a cytoprotective effect against cell stress, pβ-SP-GLP-1- and pβ-transfected islets were exposed to 200 μmol/l H_2O_2 for 30 min. Acridine orange/propidium iodide (AO/PI) staining showed that mouse islet cell viability decreased after the H_2O_2 treatment, as indicated by the red PI-positive cells. Pretreatment with 10 nmol/l GLP-1 for 24 h prevented this cytotoxic effect (Fig. 3a). pβ-SP-GLP-1-transfected islets showed significantly decreased H_2O_2-induced cell death compared with pβ-transfected and nontransfected islets. Following JC-1 staining, red aggregates were consistently found in control islet cells, whereas green fluorescence predominated in H_2O_2-treated or pβ-transfected and nontransfected islets, indicating mitochondrial membrane depolarization and a reduced $\Delta\psi m$ (Fig. 3b). Both pretreatment with GLP-1 for 24 h and transfection with pβ-SP-GLP-1 attenuated this decrease in $\Delta\psi m$, as shown by the presence of some red aggregates in islet cells after H_2O_2 treatment. Caspase-3 activity was also significantly lower in pβ-SP-GLP-1-transfected islets after H_2O_2 treatment compared with pβ-transfected and nontransfected islets (Fig. 4). pβ-SP-GLP-1-transfected islets secreted more insulin in response to glucose stimulation than did pβ-transfected and nontransfected islets and had a higher GSI (Fig. 5; 3.07 vs. 1.97 and 1.99, respectively; $P < 0.05$).

Effect of pβ-SP-GLP-1 transfection on islet graft survival and function

To examine whether GLP-1 expression not only enhances islet cell survival and function *in vitro*, but also improves islet graft survival and function *in vivo*, 180 pβ-SP-GLP-1- and pβ-transfected IEQs were transplanted under the

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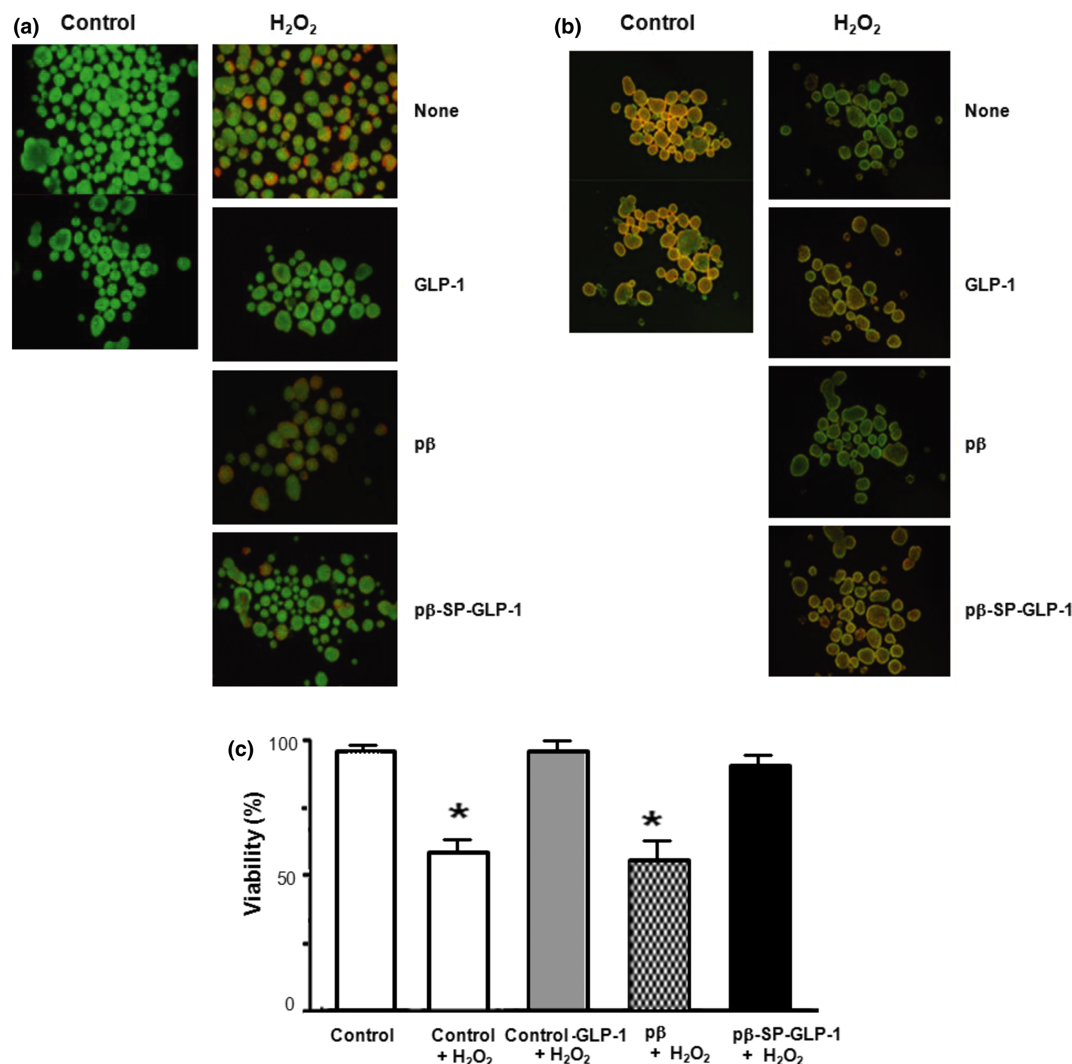

Figure 3 Effect of GLP-1 expression on H_2O_2-induced toxicity in mouse islet cells. (a) AO/PI staining, H_2O_2 treatment (200 μmol/l for 30 min) reduced islet cell viability, as shown by the presence of red PI-positive (dead) cells. The number of red cells was reduced in islets that had been pretreated with GLP-1 (10 nmol/l for 24 h) or transfected with pβ-SP-GLP-1. (b) JC-1 staining. The Δψm was reduced in H_2O_2-treated islet cells, as shown by the predominance of homogenous green fluorescence. Pretreatment with GLP-1 or transfection with pβ-SP-GLP-1 attenuated this decrease in Δψm, as shown by the presence of some red aggregates in the cells. The images shown are representative of three experiments. (c) Islet cell viability (%) quantified in AO/PI staining. *$P < 0.05$ versus Control + H_2O_2 or pβ + H_2O_2.

kidney capsules of diabetic syngeneic mice. Transplantation outcome was evaluated for 30 days post-transplantation (Fig. 6). Diabetic mice transplanted with pβ-SP-GLP-1-transfected islets became normoglycemic more rapidly than did mice transplanted with pβ-transfected islets. After 30 days, grafts containing pβ-transfected islets reversed hyperglycemia in only 52% of the diabetic mice, whereas grafts containing pβ-SP-GLP-1-transfected islets reversed hyperglycemia in 88% of recipients. Based on Kaplan–Meier analysis, the trend for the reversal of hyperglycemia by islet grafts differed between the two groups ($P < 0.05$).

Effect of pβ-SP-GLP-1 transfection on cell proliferation in early islet grafts

To determine whether the local production of GLP-1 in islets is beneficial for islet cell survival in early islet grafts, the islet grafts retrieved 3 or 7 days after transplantation were stained with Ki67 antibody, which stains proliferating cells (Fig. 7a). Double immunofluorescent staining for insulin (green) and Ki67 (red) in islet grafts showed that Ki67-positive proliferating cells (white arrow) are insulin-positive β cells (Fig. 7a). In the day 7 grafts, the pβ-SP-

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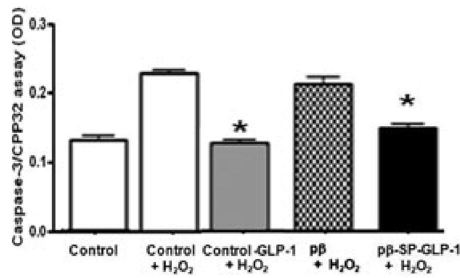

Figure 4 Effect of GLP-1 expression on H_2O_2-induced apoptosis in mouse islet cells. In the caspase-3/CPP32 assay, caspase-3 activity was lower in pβ-SP-GLP-1-transfected islets after H_2O_2 treatment as compared with pβ-transfected or nontransfected islets. *$P < 0.05$ versus Control + H_2O_2 or pβ + H_2O_2.

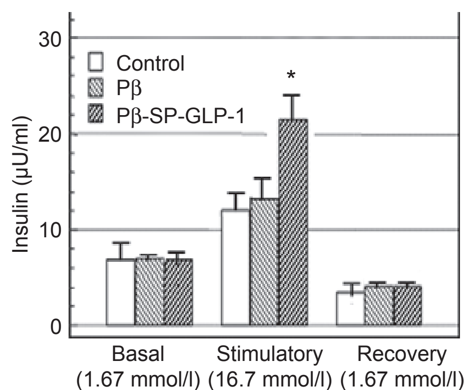

Figure 5 Effect of GLP-1 expression on glucose-stimulated insulin secretion in mouse islets. pβ-SP-GLP-1-transfected islets showed greater insulin secretion in response to glucose stimulation than did pβ-transfected and nontransfected islets. *$P < 0.05$.

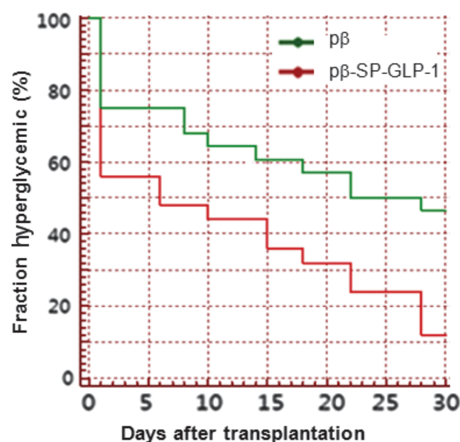

Figure 6 Blood glucose control in mice that had received 180 IEQs [β-SP-GLP-1-transfected ($n = 25$) or pβ-transfected ($n = 23$)]. The proportion of mice that were hyperglycemic at different time points post-transplantation is shown. The groups differed in a Kaplan–Meier analysis ($P < 0.05$).

GLP-1-transfected group had significantly more Ki67-positive cells as compared with the pβ-transfected group (Fig. 7b).

Serum GLP-1 levels after islet transplantation

To assess whether the GLP-1 expressed in islet grafts was delivered to systemic circulation, serum GLP-1 levels were measured in tail vein samples obtained at 1, 3, 5, and 7 days after transplantation. In GLP-1 ELISA assay, serum active GLP-1 levels of the pβ-SP-GLP-1-transfected group were not significantly different from those of the pβ-transfected group (Fig. 8).

Discussion

In this study, we evaluated the effect of the production of GLP-1 within mouse islets on islet survival and function *in vitro* and *in vivo* by means of gene delivery using a nonviral carrier. Nonviral vectors are relatively safe but far less efficient than viral vectors for gene transfection into pancreatic islets [28]. The nonviral system is known to transfect a localized, mostly peripheral population of islet cells. This may be acceptable, however, depending on the target gene chosen. Overexpression of a therapeutic secretory peptide, such as VEGF or GLP-1, by a small portion of islet cells might be sufficient for some therapeutic aims [10,29,30]. We used the synthetic cationic nonliposomal lipid formulation Effectene as a carrier because our previous study demonstrated that it could transfer genes to pancreatic islets with relatively high efficiency and low cytotoxicity [31]. When rat islets were transfected with pEGF-C1 using Effectene, the percentage of islets with GFP-positive cells were 61%. Although up to 50% of INS-1 β cells were transfected in the same transfection condition, we have not assessed what type of islet cells were transfected.

To create a GLP-1 expression plasmid, we constructed pβ-GLP-1 and inserted a secretion SP to facilitate the secretion of GLP-1. The SP linked to GLP-1 in pβ-SP-GLP-1 was intended to target the peptide to the constitutive secretion pathway [32]. We previously showed that enhancement of exendin-4 secretion through incorporation of this secretion SP resulted in the increased cytoprotection of INS-1 cells against hypoxic injury [33]. In this study, the transfection of mouse islets with pβ-SP-GLP-1 using Effectene supported the efficient secretion of GLP-1 *in vitro*. Islets transfected with pβ-SP-GLP-1 were protected from H_2O_2-induced cell damage *in vitro*, confirming the anti-apoptotic action of locally expressed GLP-1. In addition, glucose-stimulated insulin secretion was significantly higher in pβ-SP-GLP-1-transfected islets than in pβ-transfected and nontransfected islets. This insulinotropic effect of local GLP-1 expression in pβ-SP-GLP-1-treated islets

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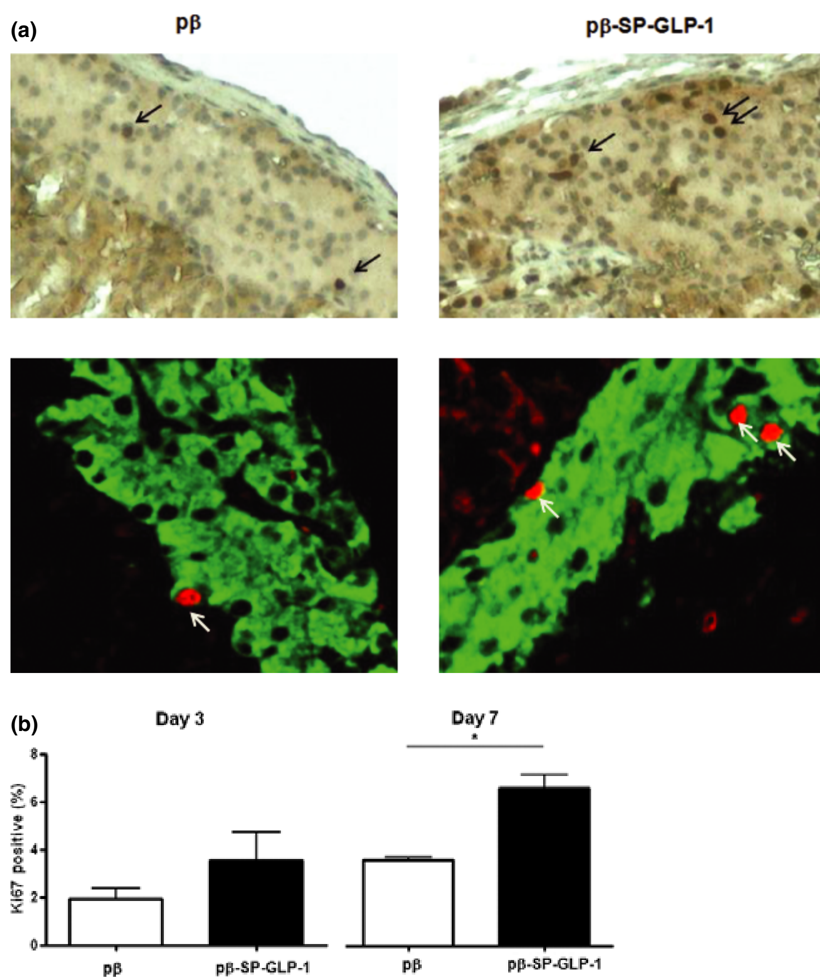

Figure 7 Ki67 staining of day 3 or day 7 islet grafts. (a) In the day 7 grafts, Ki67-positive cells (black arrow) were scarce in the pβ-transfected group, while the number of Ki67-positive cells increased in the pβ-SP-GLP-1-transfected group. Double immunofluorescent staining for insulin (green cytoplasm) and Ki67 (red nuclei) revealed that Ki67-positive cells (white arrow) in islet grafts are insulin-positive β cells. (b) The fraction of Ki67-positive cells in the islet grafts at day 3 or 7, obtained using a direct point-counting method. *$P < 0.05$ versus pβ-transfected islet grafts.

might be owing to the autocrine or paracrine effect of GLP-1 on β cells.

We next examined whether GLP-1 expression in islets also improves islet graft survival and function *in vivo* by transplanting a marginal mass of pβ-SP-GLP-1-transfected islets into diabetic syngeneic mice. Diabetic mice transplanted with pβ-SP-GLP-1-transfected islets became normoglycemic more rapidly than did mice transplanted with pβ-transfected islets. Function was superior for 30 days after transplantation in pβ-SP-GLP-1-transfected islet grafts, with significantly higher hyperglycemia reversal rates compared with pβ-transfected islet grafts (88% vs. 52%). This transplantation outcome was not inferior to that obtained when the same number of mouse islets was transduced with recombinant adenovirus containing GLP-1 cDNA in our previous study [25].

As GLP-1 secretion in pβ-SP-GLP-1-transfected islet grafts declines over less than a week, the superior function of pβ-SP-GLP-1-transfected islet grafts at 30 days after transplantation does not seem to be owing to the insulinotropic action of locally produced GLP-1. It is known that the majority of islets fail to engraft and rapidly undergo cell death as a result of hypoxic and inflammatory insults immediately after transplantation [4,5]. Considering the cytoprotective effect of pβ-SP-GLP-1 delivery on islets in our *in vitro* experiments, the improved function of pβ-SP-GLP-1 transfected islets over time suggests that pβ-SP-GLP-1 delivery may favor islet survival by reducing apoptosis during the immediate, early vulnerable post-transplantation period, which may lead to maintenance of β-cell mass and function. In addition, the islet grafts retrieved 7 days after transplantation revealed that the

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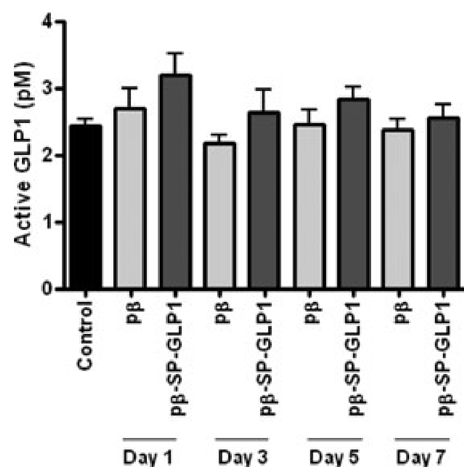

Figure 8 Serum GLP-1 levels in tail vein samples obtained at 1, 3, 5, and 7 days after transplantation. In GLP-1 ELISA assay, serum active GLP-1 levels of the pβ-SP-GLP-1-transfected group were not significantly different from those of the pβ-transfected group (*n* = 4/group).

pβ-SP-GLP-1- transfected islets contained significantly more Ki67-positive cells than the pβ- transfected group. These results suggest that the local GLP-1 secreted by the islet grafts was sufficient for GLP-1 to act as a growth factor for β cells, leading to expansion of the β-cell mass via the proliferation and thus contributing to the improved transplant outcome.

GLP-1/analogs have several potential uses in clinical islet transplantation that may exploit its insulinotropic, anti-apoptotic, and β-cell growth- promoting actions. Although whether GLP-1/analogs can stimulate β-cell replication in human islet grafts had been unknown, a recent study showed that GLP-1 agonists can stimulate β-cell replication in human islets from young donors transplanted to diabetic mice [34]. Thus, GLP-1/analogs could be an excellent alternative to insulin for patients who require additional therapy to improve glycemic control after transplantation [21–24]. As type 1 diabetic patients with functional transplanted islets showed a significant risk of hypoglycemia with systemic GLP-1/analog therapy in clinical trials, this strategy needs to be optimized through further studies. The blood levels of GLP-1/analogs achieved after peripheral injection of tolerable doses in these patients might not be sufficient to induce β-cell-preserving effects.

We are especially interested in inducing GLP-1 production through direct gene delivery to islets to increase the local concentration because the doses/concentrations of GLP-1/analogs that stimulate cell proliferation and/or inhibit apoptosis in rodents and *in vitro* experiments far exceed the clinically applicable range [34–39]. Our data show that the local expression of GLP-1 in islet grafts during the early post-transplantation period maintained or enhanced β-cell mass and function over time in a rodent transplantation model. In an islet transplantation context, the short duration of expression of GLP-1 may prevent over-regeneration of β cells and possible tumorigenesis. In addition, local and transient production of GLP-1 within islets could limit systemic delivery and thereby prevent unwanted side effects, such as vomiting and nausea [21,22]. This potential benefit is supported by our finding that serum active GLP-1 levels of the pβ-SP-GLP-1-transfected group were not significantly different from those of the pβ-transfected group at 1, 3, 5, and 7 days after transplantation.

Although we did not examine the endogenous pancreas and thus could not completely rule out the contribution of regenerative pancreatic β cells to better transplant outcome in the pβ-SP-GLP-1-transfected group, the immediate recurrence of hyperglycemia after removal of graft-bearing kidney also supports that there was no significant, if any, pancreatic β-cell regeneration by the systemic delivery of GLP-1 from the pβ-SP-GLP-1-transfected islet grafts.

In conclusion, delivery of a plasmid containing a secretion SP and GLP-1 cDNA to islets using the nonviral carrier Effectene induced the efficient expression and secretion of GLP-1 in mouse islet cells and preserved islet mass and function in transplants over time. These results suggest that, as *ex vivo* gene therapy in clinical islet transplantation, local GLP-1 expression in islets may be a viable strategy for improving transplant outcome.

Authorship

HYC: performed research and wrote the manuscript. ML: designed and performed research. HJH: performed research. HAK: performed research. JGK: collected and analyzed data. CSK: collected and analyzed data. SJL: performed research. SHI: designed research, performed research and wrote the manuscript.

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