Diabetes is a disease of epidemic proportions and is on the rise worldwide. Gene therapy has been actively pursued but limited by technical hurdles and profound inefficiency of direct gene transfer to the pancreas in vivo. Here, we show that, for the first time, appropriate serotypes of adeno-associated virus (AAV)coupled with a double-stranded vector DNA cassette, enable extensive and long-term in vivo gene transfer in the adult mouse pancreas by three different delivery methods. Intraperitoneal and intravenous delivery of AAV8 effectively transduced exocrine acinar cells as well as endocrine β-cells, while local pancreatic intraductal delivery of AAV6 showed the best efficiency in the β-cells among all AAV serotypes tested in this study. Nearly the entire islet population showed gene transfer but with distinct gene transfer efficiency and patterns when different delivery methods and vectors were used. Importantly, localized gene delivery coupled with an insulin promoter allowed extensive yet specific gene expression in the β-cells. These effective new methods should provide useful tools to study diabetes pathogenesis and gene therapy. 

Widespread and Stable Pancreatic Gene Transfer by Adeno-Associated Virus Vectors via Different Routes

Zhong Wang,1 Tong Zhu,1 Khaja K. Rehman,2 Suzanne Bertera,3 Jian Zhang,1 Chunlian Chen,1 Glenn Papworth,4 Simon Watkins,3 Massimo Trucco,3 Paul D. Robbins,2 Juan Li,1 and Xiao Xiao1,2

Original Article

RESEARCH DESIGN AND METHODS

AAV vector construction and viral production. The AAV vector plasmids single-stranded (ss)-AAV–cytomegalovirus enhancer/chicken β-actin promoter (CB)–green fluorescent protein (GFP), dsAAV-CB-GFP, and dsAAV-CB-GFP as well as the production of all serotypes of AAV vectors were described previously (24,26,27). The AAV vector plasmid dsAAV-mouse insulin promoter (mIP)-GFP was made by replacing the CB promoter of dsAAV-CB-GFP with a 1.13-kb constitutive mouse preproinsulin gene II promoter (28), which was obtained by PCR from plasmid Ad.Ins-C-GFP (29,30) using forward primer 5′-TCGACCGGTTGATCCCCCTCCTCCTTG-3′ and reverse primer 5′-AGG-TACCGGGTGTGAAAACAATACCTTG-3′, respectively. It includes full-length promoter, intron 1, noncoding sequence of exon 1, and exon 2 of mouse preproinsulin gene II (28). AAV vectors were purified twice with CsCl gradient, and the titers of viral genome particles were determined by a standard dot-blot assay (27).

Mice and in vivo vector administration. All experimental mice (ICR-CD1, C57BL10, 8–10 weeks old) were purchased from Charles River and the National Cancer Institute. In vivo viral administration was performed via different routes (intraperitoneal, intraductal, and intravenous). To perform intraductal infusion of viruses, a 32-gauge catheter (Braintree Scientific) was inserted into the cystic duct through a small opening on the bottom of the gallbladder. The catheter was then advanced into the common bile duct and secured in place with a slipknot of 0/0 suture around the bile duct and catheter to prevent vector reflux into the liver. With a microclamp being placed on the

From the 1Department of Orthopedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; the 2Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; the 3Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; and the 4Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Address correspondence and reprint requests to Xiao Xiao, PhD, Department of Orthopedic Surgery, University of Pittsburgh School of Medicine, Room E1644 BST, Pittsburgh, PA 15261. E-mail: xiaox@pitt.edu.

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AAV, adeno-associated virus; CB, cytomegalovirus enhancer/chicken β-actin promoter; dsAAV, double-stranded AAV; GFP, green fluorescent protein; H-E, hematoxylin-eosin; mIP, mouse insulin promoter; ssAAV, single-stranded AAV.

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sphincter of Oddi to avoid leakage of the vector into the duodenum, 100 µl AAV vector was slowly injected into the pancreatic duct through the catheter. The intravenous injection plus liver block was performed as described (18).

Islet isolation and in vitro infection. Islets were isolated from the mouse pancreas as described (31,32). The handpicked islets were maintained for in vitro infection or examination of GFP expression. For in vitro infection of the islet isolated from untreated adult mice, the AAV vectors were directly added.

In vivo infection. AAV vector was slowly injected into the pancreatic duct through the catheter. The intravenous injection plus liver block was performed as described (18). Islet isolation and in vitro infection. The handpicked islets were maintained for in vitro infection or examination of GFP expression. For in vitro infection of the islet isolated from untreated adult mice, the AAV vectors were directly added into the culture medium at a dose of 10,000 viral genome particles/cell. The number of cells was determined based on the estimation of about 2,000 cells per islet on the average (33,34).

Examination of GFP expression. The gross fluorescence photography and microphotography were performed as described (35). All the photographs of cryosections in the current study were taken under the same exposure time (10 s) to keep data comparable. The confocal images of islets were performed from the top to the bottom of islets with 5 µm per layer.

For quantification of fluorescence intensities of GFP expression in islets, the midway confocal images of pooled islets were used. The average integrated intensities of GFP fluorescence were obtained with MetaMorph software (Meta Imaging Series, Version 6.2) (35,37,38). Total intensity of 100 islets from multiple areas were accumulated and considered as the value of GFP expression of islets from one mouse; the average value from 3 to 4 mice was considered the GFP fluorescence intensity of specific AAV serotype. Due to differing dosages of AAV vectors being used in various routes (intraperitoneal, intravenous, and intraductal) as well as the differences between in vivo transduction and in vitro infection, we present the final GFP intensity level as a relative value, that is, the percentage of the highest fluorescence level of the different serotypes of AAV vectors in vitro for their infectivity on freshly isolated mouse islets. All of the serotype vectors carried the same gene expression cassette containing the GFP gene driven by a ubiquitous CB promoter (24,39).

RESULTS

Intraperitoneal delivery leads to efficient islet gene transfer in adult mice. To achieve efficient gene transfer to the pancreatic islet, we initially examined different serotype AAV vectors in vitro for their infectivity on freshly isolated mouse islets. All of the serotype vectors carried the same gene expression cassette containing the GFP gene driven by a ubiquitous CB promoter (24,39). Since our previous study showed dsAAV2 yielded 5–15 times greater transduction to in vitro primary mouse islets and human islets (34), we then used the dsAAV vector in all of our current studies. After infection in vitro with different serotypes of dsAAV-CB-GFP, fluorescent confocal microscopy of the islets revealed that AAV6 was the most efficient vector in vitro, followed by AAV1 and further trailed by AAV2 and AAV8, while AAV5 had the lowest infectivity to the mouse islets (Fig. 1, top panels; Table 1). In addition, cells on the peripheral zone of the islets were more effectively infected than those in the central zone, suggesting limited diffusion of the viral particles within the islets.

We next investigated the efficiency of the previously mentioned AAV vectors for the pancreas islets in vivo. The

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<td>AAV1</td>
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<td>Intraperitoneal</td>
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Data are means ± SE. Each data point contained three to four mice. Quantitative analysis of GFP fluorescence intensities was obtained using the Metamorph Software. For each delivery route, values were expressed as relative GFP fluorescence intensity achieved by different serotype AAV vectors, thus, as percentage of the highest fluorescence level (labeled in bold) achieved by a specific serotype. The experimental protocols and corresponding photographs can be found elsewhere (in vitro infection and intraperitoneal administration seen in Fig. 1, intraductal seen in Fig. 3B, and intravenous + liver blockade seen in Fig. 5). ND, not determined.

Blood glucose reading. Ten microliters of blood were extracted from the tail vein of nonfasted mice. The blood was then analyzed with a handheld glucometer (Precision Q.I.D; MediSense).

Southern blot analysis. Total DNA from various tissues was prepared as described (24). Ten micrograms total DNA per sample was digested with BamHI I and XhoI, which dropped an internal fragment from vector genomes. The digested DNA was then separated on a 0.8% agarose gel. Southern hybridization was performed with 32P-labeled GFP DNA fragment as a probe (24).
AAV vectors were first examined by the intraperitoneal route in adult mice because our initial experiments showed that intraperitoneal delivery of AAV vectors yielded more efficient pancreatic gene transfer than tail vein delivery (Fig. 2A; data not shown). Considering the fact that islets only account for ~1% of the pancreatic mass and are scattered throughout, we opted to isolate and concentrate the islets from the pancreas after in vivo transduction to analyze gene transfer efficiency with more accuracy. Two months after intraperitoneal injection of $5 \times 10^{11}$ viral genome of various serotype vectors in adult mice, fluorescent confocal microscopy of the islets revealed widespread gene transfer but with highly diverse efficiencies dependent on the serotypes used (Fig. 1, middle panels). In addition, the pattern of gene transfer efficiency in vivo was different from that in vitro. Thus, AAV8, instead of AAV6 and AAV1, was now the most efficient vector in transducing the islets via the intraperitoneal route. Both AAV2 and AAV5 had similarly low efficiency in vivo in the islets, although AAV2 was equivalent to AAV8 in vitro (Fig. 1, middle panels; Table 1). In addition, AAV8 was also the most robust vector in gene delivery to the exocrine acinar cells. It was distantly followed by AAV6, while AAV1, -2, and -5 were all very poor in transducing the acinar cells (Fig. 1, lower panels; Table 1).

We further characterized the in vivo profile of the AAV8 vector after intraperitoneal delivery. One month postintraperitoneal injection of $2 \times 10^{12}$ viral genome particles of dsAAV8-CB-GFP, the entire pancreas expressed so much GFP that it emitted extraordinarily strong green fluorescence under long-wavelength UV excitation and turned greenish under visible light (Fig. 2A, a). However, the vector DNA copy number in the pancreas was not particularly high, much lower than that in the liver and abdominal muscle (Fig. 2B), suggesting superb activity of the CB promoter in the pancreas. Other tissues including the liver, muscle, heart, and testis were also effectively transduced by AAV8 after intraperitoneal delivery in adult mice (Fig. 2B). A quite interesting phenomenon was the GFP expression in the liver. In fact, strong GFP expression was observed in the liver at 2 weeks postinjection; over time, the gene expression decreased significantly. The marked drop of gene expressions in the liver probably resulted from the promoter shut off; a more obvious phenomenon of silence in the liver has been observed with the cytomegalovirus promoter (40).

Vector dose escalation of the AAV8 vector by the intraperitoneal route showed a threshold between $1 \times 10^{11}$ and $3 \times 10^{11}$ viral genome particles/mouse, to achieve nearly complete gene transfer of the acinar cells in the adult pancreas (Fig. 2C). For the islets, however, a dose as high as $1 \times 10^{12}$ viral genome particles could not reach the plateau (Fig. 2D, a-d). At this dose, every islet isolated from the pancreas showed strong GFP expression (Fig. 2D, e and f). But further examination with confocal microscopy revealed gradient gene transfer within each individual islet, with higher efficiency in the peripheral zone than in the central zone (Fig. 2D, g and h).

Time course analysis of the AAV8 vector showed that high levels of GFP expressions were detectable in gross pancreas with UV light at 3 days after intraperitoneal injection of $2 \times 10^{12}$ viral genome particles of dsAAV8-CB-GFP. The gene expressions in gross pancreas gradually increased and reached the plateau at ~2 weeks postvector delivery (Fig. 2A, d; data not shown). Transgene expression also persisted for a long time with minimal decrease. Four months after AAV8 intraperitoneal delivery, extensive GFP expression in the acinar cells as well as the islets was still readily detectable. In situ double staining with anti-GFP and anti-insulin antibodies on thin cross sections of the AAV8-treated pancreas confirmed GFP expressions in both acinar cells and insulin-expressing $\beta$-cells (Fig. 2E) as well as $\alpha$-cells (data not shown). Hematoxylin-eosin (H-E) staining of the pancreas showed normal histology and morphology (Fig. 2E). No cellular immune infiltration was detectable after immunostaining for CD4+ and CD8+ T-cells in the pancreas (data not shown). The serum glucose levels remained within normal range from 5 days to 4 months postvector administration (data not shown), suggesting a lack of functional impairment after pancreatic gene delivery by AAV vectors.

**Pancreatic ductal delivery localizes and enhances gene transfer.** To minimize the unwanted gene transfer to nonpancreatic tissues seen after intraperitoneal or intravenous delivery, we next explored a topical route via retrograde pancreatic intraductal delivery, similar to a commonly used clinical technique endoscopic retrograde cholangiopancreatography. In addition, topical delivery was expected to lower the vector dose requirement as well. Here we compared AAV2, AAV6, and AAV8 by injecting $1.5 \times 10^{11}$ viral genome particles of dsAAV-CB-GFP per mouse via the pancreatic duct. Again, the AAV8 vector achieved the strongest GFP expression in the pancreas as a whole (Fig. 3A). In AAV8-treated mice, the GFP expression could be readily detected by gross pancreas fluorescent photography as early as 5 days. At 2 weeks postduetal injection, GFP expression further increased and surpassed the levels achieved by intraperitoneal injection at a vector dose 10-fold higher (Figs. 2A and 3A), suggesting that the intraductal route is much more efficient than both the intraperitoneal and intravenous routes for pancreatic gene delivery.

We next analyzed gene transfer efficiencies in the islets that were isolated from the vector-treated pancreas, since overwhelming GFP-positive acinar cells made it difficult for in situ analysis of the islets. As expected, significant enhancement in islet gene transfer as a result of topical delivery was observed in all three serotype AAV vectors (Fig. 3B). Different from the intraperitoneal route, AAV6 was now more efficient than AAV8 in transducing the islets after intraductal route delivery. As expected, AAV2 remained much weaker than both other serotype vectors. Confocal microscopy on the isolated islets after intraductal delivery of AAV6 and AAV8 vectors further confirmed GFP expression in every islet (Fig. 3B). However, the distribution of the GFP-positive cells was almost exclusively in the peripheral zone of the islets. As expected, topical delivery also resulted in dramatically reduced gene transfer to the nonpancreatic tissues. No appreciable GFP expressions in nonpancreatic tissues including the liver, heart, testis, and muscles were observed, except in the liver of the AAV8–treated mice (data not shown). Finally, the serum glucose levels remained in the normal range throughout the time course of 4 months, indicating the lack of discernable impairment of islet function.

**Use of insulin promoter attains $\beta$-cell–specific gene expression.** Since the pancreatic $\beta$-cell is a major target in diabetes gene transfer and therapy, we explored the use of a mIP (29,30,36) to minimize nonspecific transgene expression in the unintended cells and to ensure transcrip-
tional control in the β-cells. The specific transgene expressions in islet β-cells also enabled us to conveniently examine the islet β-cell gene transfer in situ without the interference of transgene expressions from acinar cells. We examined all three different delivery routes (intraperitoneal, intraductal, and intravenous) with the dsAAV-miP-GFP vector.

First we examined the intraperitoneal route. As expected, at 2 weeks after delivery of $1 \times 10^{12}$ dsAAV8-miP-GFP vector in adult mice, strong GFP expression was readily detected exclusively in situ in the β-cell of the islets, but not in the exocrine acinar cells (Fig. 4A) and glucagon-producing islet α-cells (data not shown), despite the fact that both the acinar cells and the α-cells could be

FIG. 2. In vivo transduction of adult pancreas by AAV8-CB-GFP vector via intraperitoneal (i.p.) route. A: GFP expression in the pancreas after i.p. administration of $2 \times 10^{12}$ viral genome particles AAV8. a: Gross fluorescence or daylight photographs of the mice (arrows highlight the pancreas) at 1 month after i.p. injection of dsAAV8. b: Comparison of GFP expression mediated by dsAAV8 and ssAAV8 at 2 months postadministration. c: Comparison of i.p. and intravenous (i.v.) injection of dsAAV8 vector at 2 months postadministration. d: Time course analysis of GFP expression in pancreas after i.p. injection dsAAV8 at 2 weeks, 2 months, and 4 months. B: GFP expression in other organs and tissues and Southern blot analysis of AAV vector DNA distribution at 1 month after i.p. delivery of $2 \times 10^{12}$ viral genome particles of dsAAV8. Note that immunofluorescent (IF) staining with anti-GFP (red) showed that GFP expression in the testis was in interstitial tissues. ABD, abdominal. C: Fluorescent microscopy of cryo-thin sections of adult pancreas at 2 weeks after i.p. injection of dsAAV8 at increasing doses. Scale bar: 100 µm. D: Fluorescent and phase-contrast microscopy of pooled islets of mice at 2 weeks after i.p. injection of dsAAV8 at increasing doses (a–f). Confocal images (g and h) were representative middle planes of series scan of isolated islets. Scale bar: 100 µm. E: Long-term GFP expression in mouse pancreas, shown by fluorescent microscopy of pancreas cryo-thin section (a), confocal microscopy of the isolated islets (b), double immunofluorescence staining of GFP (c) or insulin (d), merged image of both (e), and H-E staining of consecutive sections at 4 months after i.p. injection of $1 \times 10^{12}$ viral genome particles dsAAV8 (f). Scale bar: 100 µm.
effectively transduced by AAV8-CB-GFP vector after intra-peritoneal injection (Fig. 2E and data not shown). Importantly, the vast majority of the islets examined were positive for GFP expression as shown by the precise match between GFP fluorescence and H-E staining on consecutive sections of the pancreas (Fig. 4A). A preference by AAV transduction on cells located on the peripheral zone of the islets was again observed (Fig. 4A). Finally, double staining of the pancreatic thin sections with anti-GFP and anti-insulin antibodies provided additional evidence of β-cell–specific GFP expression by the insulin promoter (Fig. 4A). As expected, GFP expression in nonpancreatic tissues including the liver, heart, muscle, and testis again turned out negative (data not shown).

We next further examined the specificity and efficiency of the dsAAV8-mIP-GFP vector delivered by two additional routes, i.e., the intraductal route ($1.5 \times 10^{11}$ viral genome/mouse) and the intravenous route ($1 \times 10^{12}$ viral genome/mouse). The latter was also coupled with a transient blockade on liver circulation (see in later sections). Again, strong GFP expression was observed exclusively in the islets at 2 weeks after vector delivery with either route (Fig. 4B and C). The green islets were easily visible with the naked eye, when the whole pancreas was illuminated with the long-wavelength UV light for GFP excitation. Microscopic examination on thin sections of the pancreas also showed exclusive and strong GFP expression in nearly every individual islet, which corroborates well with H-E staining on the consecutive sections (Fig. 4B and C). Similar to the intraperitoneal route, the GFP expression in islets with dsAAV8-mIP-GFP persisted for 4 months in the intraductal route (Fig. 4B) and 2 months in the intravenous route (data not shown) at the end of this study. In addition, no appreciable GFP expression was observed in other organ or tissues (data not shown). These results strongly demonstrate that the use of insulin promoter was capable of highly specific transgene expression in the insulin-producing β-cells in vivo.

**FIG. 2—Continued.**
Transient liver blockade enhances pancreatic gene transfer by intravenous route. Finally, we reexamined intravenous delivery of the AAV vectors by applying transient blockade to the liver circulation. Previously, we observed that intravenous route delivery of AAV vectors was dramatically less efficient (5- to 10-fold) than the intraperitoneal route in gene transfer to the pancreas of adult mice (Fig. 2 and data not shown). The liver was primarily responsible for the rapid absorbance of the circulating AAV vectors. Here we chose to investigate AAV6 and AAV8 in the intravascular delivery experiments because these two viruses performed well in intraperitoneal and intraductal routes. Two weeks after injection of dsAAV-mIP-GFP vector \( (5 \times 10^{11}) \) viral genome/mouse aimed at β-cell–specific gene expression, we observed dramatic enhancement in gene transfer to the islets with transient liver blockade for both AAV6 and AAV8 serotypes (Fig. 5).

Without liver blockade, gene transfer to the islets by the intravenous route was virtually undetectable for AAV6 and inefficient for AAV8. With liver blockade, however, gene transfer to the islets increased by \( >15 \)-fold for AAV6 and \( >6 \)-fold for AAV8. All the islets in AAV6- or AAV8-treated mice showed positive GFP expression by fluorescent and phase-contrast microscopy. However, AAV8 was more efficient than AAV6 (Fig. 5). Confocal microscopy of the pooled islets revealed uniform intraislet GFP expression in most of the islets, especially after AAV8 gene transfer (Fig. 5). These results suggest that intravascular delivery coupled with transient liver blockade was an efficient way to deliver the AAV vectors to the pancreas with more uniform vector distribution within individual islets.

**DISCUSSION**

In search for methods of effective and stable in vivo islet gene delivery, we have investigated different AAV serotype vectors using three different delivery routes (intraperitoneal, intraductal, and intravenous). We showed that all three routes were capable of efficient and widespread gene transfer to nearly all islets in the pancreas, which was clearly demonstrated by fluorescent microscopy of the pooled islets as well as by in situ examination of pancreas thin sections of AAV–treated mice. In each individual islet,
FIG. 4. Insulin promoter confers β-cell–specific GFP expression in pancreas. The dsAAV8-mIP-GFP vector was delivered by three different routes and analyzed in situ in the pancreas for β-cell–specific GFP expression. A: Two weeks after intraperitoneal delivery at a vector dose of $1 \times 10^{12}$ viral genome particles/mouse. B: Two weeks and 4 months after intraductal delivery at a vector dose of $1.5 \times 10^{11}$ viral genome particles/mouse. C: Two weeks after intravenous delivery with liver blockade at a vector dose of $1 \times 10^{12}$ viral genome particles/mouse. In gross photos of the whole pancreas, notice the widespread and specific GFP expression in the islets after vector delivery by all three routes. Also notice strong GFP expression in β-cells of individual islet but not in the surrounding acinar cells. It is further confirmed by double immunofluorescent (IF) staining with anti-GFP (red) and anti-insulin (green) antibodies and counterstaining of all cell nuclei with DAPI (blue). Arrows highlight the islets. Scale bar: 100 μm.
which consisted of a few hundred to a few thousand endocrine cells, most of the cells (though not all) also showed GFP marker gene expression following a single injection of the appropriate dsAAV-GFP vector. To our knowledge, such extensive and long-term islet gene transfer observed in this study is unprecedented. We attribute the widespread, strong, and stable gene expression in the islets of the pancreas to the following major factors: (1) the appropriate AAV vectors that provide better infectivity to the pancreatic cells and better vector dissemination in vivo, (2) the improved dsAAV gene expression cassette that enables rapid and strong gene expression (24,25), and (3) the delivery methods that allow widespread vector distribution to the pancreas.

Distinct gene transfer patterns within the islets were observed to be dependent on which delivery route was used. The intraductal route gave rise to gene transfer predominantly in the peripheral zone of the islets, whereas the intravenous route coupled with liver blockade led to fairly uniform gene transfer. The intraperitoneal route, on the other hand, resulted in a combined intraislet gene transfer pattern of the two. The differences could be explained by the vector dissemination pathways in each delivery route. The more uniform intraislet gene transfer by the intravascular route could be partly explained by the blood-borne dissemination of viral vector through capillary blood vessels within the islets. With transient liver blockade, more viral particles became available through the blood supply to the pancreas. On the other hand, the concentrated peripheral zone gene transfer by the intraductal retrograde delivery is likely due to direct access by the AAV viral particles to the peripheral cells of the islets.

Different serotypes of AAV vectors perform very differently in islet gene transfer. While the AAV8 vector was apparently more efficient when both intraperitoneal and intravenous routes were used, the AAV6 vector had significant advantages when delivered by the intraductal route. An interesting phenomenon observed in our study is the profoundly better in vivo performance of the AAV8 vector over its in vitro infectivity on the islets. This improved performance of AAV8 vector in vivo is most likely due to the superior capability of AAV8 in crossing the in vivo barriers, such as the peritoneal epithelium and vasculature endothelium, to eventually reach the islets for infection. The remarkable in vivo dissemination by AAV8 may also explain the widespread transgene expression in the liver, heart, muscle, etc., after intraperitoneal delivery while the universal CB promoter was used; it has also been recently observed by us and others (35,41) during gene delivery to the muscle and heart after systemic vector administration. On the other hand, the excellent in vivo performance of AAV6 by the localized delivery is likely due to its better direct infectivity to the islet β-cells. This phenomenon is similarly observed in gene transfer to the muscle by direct intramuscular injection of AAV6 vectors (35,42).

Based on our data, we consider the intraductal administration of AAV6 the best way to deliver genes to β-cells in vivo. The big advantage for the intraductal route is its necessity of much fewer viruses and less spread of viruses to nonpancreas organs and tissues, compared with the intravenous route and intraperitoneal routes. This method can be also applied to large animals and even humans. In fact, an equivalent procedure, called endoscopic retrograde cholangiopancreatography, has been a routine clinical application. The disadvantage of the intraductal route is the lack of gene transfer in the core zone of the islets. However, the majority of the islet cells are still able to be transduced because the peripheral zone has a larger volume, hence more cells, in a sphere-like islet. Intravascular delivery of AAV8 is a good alternative, especially when transduction to nearly every islet cell is needed. On the other hand, administration of AAV8 via the intraperitoneal route provides a convenient and effective method for pancreatic gene delivery, although it may only be practical for small rodents mainly due to their unique
diffused anatomical structure of the pancreas. In addition, we also showed that the exocrine acinar cells are highly susceptible to AAV8's transduction. This highly efficient gene transfer to exocrine acinar cells may also be applicable for gene therapy of pancreatitis, pancreatic malignancy, and exocrine enzyme insufficiency, etc.

Our study showed that dsAAV vectors offer particular advantages over the traditional ssAAV vectors for much more effective pancreatic gene delivery. However, a trade off using the dsAAV vector is its shortened packaging capacity of 2.5 kb, which may limit the use of certain genes. Nonetheless, numerous therapeutic genes can still be suitable candidates for dsAAV vectors, for example, growth factor (IGF-1, glucagon-like peptide 1, exendin-4) and antiapoptotic (Bcl-XL, heme oxygenase-1) and immune modulating factor genes (interleukin-4, interleukin-10, and CTLA4-Ig).

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