Islet neogenesis associated protein transgenic mice are resistant to hyperglycemia induced by streptozotocin

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Abstract

Islet neogenesis associated protein (INGAP) is a protein factor that can stimulate new islet mass from adult pancreatic progenitor cells. In models of islet neogenesis, INGAP expression is elevated in pancreatic acinar cells. Using a transgenic model to drive a sustained expression of INGAP in pancreatic acinar cells, we have identified a protection to chemical-induced hyperglycemia. A sustained expression of INGAP during development did not perturb islet development or basal blood glucose homeostasis, although β-cell mass and pancreatic insulin content were significantly increased in the INGAP transgenic mice. When challenged with a diabeticogenic dose of streptozotocin (STZ), mice carrying the INGAP transgene did not become hyperglycemic. In contrast, wild-type mice became and remained hyperglycemic, blood glucose > 550 mg/dl. The serum insulin levels and islet morphology were preserved in the transgenic mice after STZ treatment. These data suggest that the sustained expression of INGAP in the acinar pancreas confers resistance to a diabetogenic insult. The INGAP transgenic mouse provides a new model to uncover factors that are protective to diabetes onset and biomarkers to track β-cell pathology.

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Introduction

A common pathology associated with diabetes onset is a loss of the functional pancreatic β-cell mass (Bell & Polonsky 2001). The development of type 1 diabetes originates from an autoimmune-mediated destruction of β-cell mass (Mathis et al. 2001) and the onset of type 2 diabetes follows a progressive loss of β-cell mass (Butler et al. 2003), in which there is an approximately 50% reduction in β-cell mass upon diagnosis of type 2 diabetes (UKPDS Group 1995) and a steady annual decline thereafter. A central approach to treat diabetes, therefore, involves the replacement/replenishment of functional β-cell mass (Vinik et al. 2004a, 2004b). Strategies to restore β-cell mass include islet transplantation, proliferation of existing β cells or re-deriving β cells from embryonic or adult stem cells. The later method incorporates islet neogenesis, a process thought to involve inductive factors to promote new pancreatic islet mass from endogenous precursor cells (Vinik et al. 1997, Baggio & Drucker 2002, Bonner-Weir et al. 2004, Paris et al. 2004, Vinik et al. 2004a, 2004b). Unlike strategies based upon transplantation that are limited by donor supply and immunosuppressant toxicity (Hirshberg et al. 2003), islet neogenesis relies on autologous precursor cells and therefore circumvents the complications associated with long-term immunosuppression.

Islet neogenesis associated protein (INGAP) was first identified in hamsters undergoing neogenesis (Rafaeloff et al. 1996, 1997, Vinik et al. 1997). Endogenous INGAP expression and islet neogenesis occur concurrently (Del Zotto et al. 2000). INGAP has a molecular weight of 16–8 kDa and is related to the type 2 C-lectins (Taylor-Fishwick et al. 2003, Vinik et al. 2004a, 2004b). The organization of INGAP’s 175 amino acids classifies it as a member of the group 2 superfamily of reg-related proteins (Okamoto 1999). In addition to the biological efficacy of INGAP, a pentadecapeptide derived from the INGAP holoprotein retains biological activity (Rosenberg et al. 2004). INGAP or INGAP-peptide administered to rodents (Rosenberg et al. 1996, 2004) or dogs (G Pittenger & D Taylor-Fishwick, unpublished observations) stimulates new islet growth as evidenced by elevated β-cell mass identified in quantitative histological and molecular analyses of insulin. Further, INGAP therapy reversed established hyperglycemia in rodent models of diabetes (Gold et al. 1998, Rosenberg et al. 2004). Additionally, in human clinical trials, INGAP peptide increased c-peptide secretion in subjects with type 1 diabetes and reduced HbA1c levels in subjects with type 2 diabetes (Ratner & Feeley 2005a, b).
Moreover, INGAP peptide enhances β-cell-secretory responses (Borelli et al. 2005) and promotes duct to islet transdifferentiation \textit{in vitro} (Jamal et al. 2005). During the process of islet neogenesis, inductive factors, such as INGAP, stimulate protodifferentiated cells residing in the pancreatic duct to differentiate, expand, and bud to initially form islet-like clusters (Rosenberg 1995, Baggio & Drucker 2002, Bonner-Weir et al. 2004, Vinik et al. 2004a,b, Suarez-Pinzon et al. 2005). Last, INGAP secretion is upregulated in rodent models of injury-induced neogenesis, such as the partial duct occlusion model in hamster (Rafaeloff et al. 1997), mouse duct ligation model, and the rat partial pancreatectomy model (Song et al. 2005). Upregulated INGAP expression during islet neogenesis predominates in the pancreatic acinar tissue (Rafaeloff et al. 1997, Vinik et al. 1997).

In this study, we have explored the anti-diabetogenic effect of a sustained pancreatic acinar tissue expression of INGAP by developing a transgene construct, in which INGAP is driven by the \( -500/+8 \) rat elastase-1 enhancer. Analysis of mice carrying this transgene has identified a unique phenotype that could facilitate the discovery of biomarkers for diabetes pathogenesis and resistance to β-cell damage.

Materials and Methods

\textit{FVB/N mice}

All studies were performed with the approval of the Animal Care and Use Committee. Mice kept in a temperature and humidity-controlled room with a 12 h light:12 h darkness cycle, were provided standard Harlan rodent diet (Harlan Teklad, Indianapolis, IN, USA) and water \textit{ad libitum}. Mice were weaned at an age of 28 days. Genotyping of mice was performed on DNA extracted (Promega Wizard Genomic Purification kit; Promega) from a 1 cm tail clip. Mice studied were 3–4-months old. Blood glucose was measured at specified time points, using samples obtained in tail pricks, with a pre-calibrated Advantage Accu-Chek glucometer (Boehringer–Mannheim) and mice were provided 10% sucrose water \textit{ad libitum}. Mice were fasted for 16 h, weighed, and given a single bolus i.p. injection of 140 mg/kg Zanosar (clinical grade STZ; Sicor Pharmaceuticals, Irvine, CA, USA) into the lower left abdominal quadrant. Two hours post-injection with STZ, food was replaced and 3 days post-injection, the 10% sucrose water was replaced with water. Non-fasting blood glucose levels and body weight were measured in the morning at days 3, 5, 8, 10, and 13 post-injection of Zanosar. Mice were defined hyperglycemic once the non-fasting blood glucose exceeded 275 mg/dl. The study was terminated at day 14, with blood and pancreata being harvested for analysis.

\textit{Islet isolation}

Pancreata were minced and placed in Hanks balanced salt solution (HBSS)/Hepes buffer containing 0.23 mg/ml liberase R1 (Roche Applied Science, Indianapolis, IN, USA) and 0.1 mg/ml DNase1 (Roche Applied Science), being digested for 30 min at 37°C in an agitating water bath. Upon termination with ice-cold HBSS containing 10% fetal bovine serum (FBS), the digested tissue was filtered through a 380 μm mesh, washed and separated on an Optiprep gradient (Axis-Shield, Norton, MA, USA). Recovered islets were washed in PBS prior to RNA extraction in Trizol (Invitrogen). Primers used for INGAP PCR were 5'-TATTATTGAAGCTCATATGGACAAAGG-3' and reverse 5'-CAGCTGATCCACGATGCCCGCGC-3' or primers to generate a 536 bp product, which crossed the elastase enhancer/INGAP junction (forward 5'-TGTTGTTTCTCCTGCTTCTAC-3' and reverse 5'-ACCTCCATCCACTTCCGTTGG-3'). Detection of both PCR products confirmed incorporation of the complete transgene.

\textit{STZ treatment in vivo}

Twenty-four hours before the initiation of treatment with streptozotocin (STZ), non-fasting blood glucose was measured using a pre-calibrated Advantage Accu-Chek glucometer (Boehringer–Mannheim) and mice were provided 10% sucrose water \textit{ad libitum}. Mice were fasted for 16 h, weighed, and given a single bolus i.p. injection of 140 mg/kg Zanosar (clinical grade STZ; Sicor Pharmaceuticals, Irvine, CA, USA) into the lower left abdominal quadrant. Two hours post-injection with STZ, food was replaced and 3 days post-injection, the 10% sucrose water was replaced with water. Non-fasting blood glucose levels and body weight were measured in the morning at days 3, 5, 8, 10, and 13 post-injection of Zanosar. Mice were defined hyperglycemic once the non-fasting blood glucose exceeded 275 mg/dl. The study was terminated at day 14, with blood and pancreata being harvested for analysis.

\textit{Transgene construct and genotyping}

INGAP mRNA (region 19–766 of GenBank U41738) was cloned at a BamHI restriction site downstream of the rat elastase 1 enhancer that was kindly provided by Dr Galvin Swift (sequence 240–748 of GenBank L00112; Southwestern Medical Centre, University of Texas, Dallas, TX, USA) and a XhoI restriction site upstream of the rat insulin 1 poly-A tail (sequence 39262–40162 of GenBank AC098563). The resulting 2446 bp transgene was excised by restriction enzymes HindIII and Apal. The transgenic mouse line termed elastage promoter-INGAP (EP-INGAP) was generated through nuclear injection of the transgene as previously described (Harlan et al. 1994). To genotype the mice, genomic PCR was performed using primers to generate a 981 bp product, which crossed the INGAP/poly-A junction (forward: 5'-TATTATTGAAGCTCATATGGACAAAGG-3' and reverse: 5'-CAGCTGATCCACGATGCCCGCGC-3') or primers to generate a 536 bp product, which crossed the elastase enhancer/INGAP junction (forward 5'-TGTTGTTTCTCCTGCTTCTAC-3' and reverse 5'-ACCTCCATCCACTTCCGTTGG-3'). Detection of both PCR products confirmed incorporation of the complete transgene.
INGAP riboprobes were precipitated in LiCl/ethanol and resuspended in TE buffer (pH 7.5). Whole pancreas was cryopreserved in OCT (Ted Pella, Redding, CA, USA) and prepared for hybridization as previously described (Prado et al. 2004). Transcripts were detected using 1:5000 α-digoxigenin IgG–Fab–alkaline phosphatase (Roche Applied Science) by incubating overnight at 4 °C. Following washes in maleic acid buffer containing 0.1% Tween-20, visualization was achieved with BM Purple precipitating solution (Roche Applied Science) by incubating overnight at 4 °C. Following washes in maleic acid buffer containing 0.1% Tween-20, visualization was achieved with BM Purple precipitating solution (Roche Applied Science). Slides were sealed in VectaMount (Vector Labs, Burlingame, CA, USA) and images captured with DP-70 camera on an Olympus BX-51 microscope.

**Immunohistochemistry**

Pancreata were fixed in 10% formalin (Fisher Chemicals, Fairlawn, NJ, USA) and embedded in paraffin. Tissue sections (5–7 μm) were deparaffinized and rehydrated using xylene and ethanol dilutions. Sections were stained with H&E, aldehyde fucin, trichrome and by antigen immunolocalization. Prior to immunodetection sections were blocked with Superblock buffer (Pierce, Rockford, IL, USA) for 30 min. They were incubated for 4 h at room temperature with either 1:1000 mouse anti-glucagon (Sigma), 10 μg/ml mouse anti-Glut-2 (Alpha Diagnostic Labs, San Antonio, TX, USA), 1:1000 mouse anti-insulin (Sigma), 1:2000 Rabbit anti-INGAP or 1:2000 Rabbit Anti-Glucagon (Zymed Labs, San Francisco, CA, USA). Antibodies were diluted in blocking buffer. Following three PBS wash cycles, immunospecific signal was visualized by a 30-min incubation with either 1:1000 anti-mouse tetramethyl rhodamine iso-thiocyanate (TRITC) (Sigma), 1:1000 anti-rabbit FITC (Sigma) or species-specific peroxidase/alkaline phosphatase Vector ABC kits (Vector Labs) following the manufacturer’s instructions. Fluorescent detection was counterstained with 1:100 000 DAPI (Sigma) and mounted using Fluoromount-G (Southern Biotechnology, Birmingham, AL, USA). Non-specific staining was excluded by substituting an irrelevant species-matched primary antibody. Images were recorded using a DP-70 camera with a BX-51 Microscope. Quantitation was measured using established techniques, Birmingham, AL, USA). Non-specific staining was excluded by substituting an irrelevant species-matched primary antibody. Images were recorded using a DP-70 camera with a BX-51 Microscope. Quantitation was measured using established techniques. For BrdU detection, sections were incubated for 5 min in 2 N HCl and washed in PBS prior to 2-h incubation with 1:20 mouse anti-BrdU (Becton-Dickinson, Dickinson Mountain View, CA, USA) in binding buffer (PBS containing 0.2% Triton, 5% goat serum) followed by 30-min incubation with 1:1000 biotinylated goat anti-mouse-IgG (Sigma) in binding buffer. Antigen localization was visualized using streptavidin-HRP Vectastain Elite kit (Vector Labs) according to the manufacturer’s instructions. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end-labeling analysis was performed using the in situ Cell Death Detection Kit (Roche Applied Science) according to the manufacturer’s recommendations. Prior to staining, sections were microwaved at 60% power for 5 min in 100 mM sodium citrate (pH 6.0).

**Quantitation of β-cell mass**

Transverse sections of whole pancreata were serial sectioned at 5 μm intervals and mounted onto the slides. Randomly selected starting sections and then every tenth section (~50 μm apart) were selected for analysis. Sections were stained for insulin. Quantitation used established techniques (Bonner-Weir 2001, Rosenberg et al. 2004); briefly, the insulin–positive area falling into ten randomly chosen areas of a defined size, was determined and normalized to the unified area and weight of the pancreas. For each analysis greater than four sections from three mice of each line were analyzed.

**Pancreatic and serum insulin content**

Freshly extracted pancreas was weighed, homogenized, and extracted in a 20-fold volume of acid ethanol overnight at 4 °C. Insulin content was determined by RIA (Linco Research, St Charles, MI, USA) and normalized to protein concentration determined by the Bradford assay. Serum insulin was measured by ELISA (ALPCO, Windham, NH, USA) per the manufacturers directions. Results are shown as mean ± S.E.M. Significance of the data was evaluated by non-paired Student’s t-test. P < 0.05 was judged significant. Analyses were performed using Graphpad prism V4.0 software (Graphpad software, San Diego, CA, USA).

**Results**

**Creation of EP-INGAP transgene and expression of INGAP in transgenic mice**

For generation of the INGAP transgene, INGAP cDNA was inserted by directional restriction cloning between the rat elastase enhancer (Kruse et al. 1993) and the poly-A tail of the rat insulin gene, as shown in Fig. 1A. In order to direct the expression of INGAP more specifically into the acinar tissue, the region of the elastase enhancer used to drive INGAP was that defined as −500/+8. Expression of the INGAP transgene in the pancreas was confirmed by RT-PCR, in situ hybridization, and immunohistochemistry (Fig. 1). INGAP mRNA was detected in the pancreas of the EP-INGAP transgenic line, but not wild-type mice (Fig. 1B). The expression of INGAP message was localized to the acinar tissue of the pancreas using in situ hybridization of an INGAP–riboprobe and no hybridization signal was detected in control pancreas sections (data not shown). Further, expression of INGAP was detected in the acinar region of the pancreas using an anti-peptide antibody to INGAP (peptide INGAP20–39). INGAP immunoreactivity in pancreatic acinar tissues was patchy in the EP-INGAP mouse (arrows, Fig. 1C), whereas no signal was observed in the wild-type mouse (Fig. 1D). Islets were isolated from EP-INGAP mice...
and analyzed for expression of the INGAP transgene. No endocrine expression of transgenic INGAP was observed by PCR (Fig. 1E, lane 1) compared with whole pancreas (Fig. 1E, lane 3). The housekeeping gene product actin was detected in both isolated islets and whole pancreas (Fig. 1E, lanes 1 and 3). Tissues from EP-INGAP mice and wild-type mice were screened for the expression of INGAP message by Northern blot analysis. No INGAP signal was detected in wild-type mice, and INGAP message in the EP-INGAP mice was restricted to the pancreas and not detected in stomach, spleen, duodenum, liver, kidney, lung, skeletal muscle, brain, heart, or bone marrow (data not shown). Since endogenous elastase is also detected in the stomach (Kruse et al. 1993), this expression pattern supports a restricted expression of INGAP driven by the elastase enhancer.

**Basal characteristics of EP-INGAP mice**

The EP-INGAP mouse line was compared with the wild-type (non-transgenic littermate) mouse line. Both mouse lines were euglycemic (average blood glucose 128±27 and 128±25 mg/dl for wild type and EP-INGAP respectively) and gained equivalent age-related weight and pancreas weight (pancreatic weight expressed as a percentage of total body weight at 12 weeks was 0.758±0.11% and 0.764±0.09% for wild type and EP-INGAP respectively). Additionally, there was no statistical difference in the response of the mouse lines to an i.p. glucose tolerance test. Both lines (n=6 per line) showed equivalent time-dependent increase and normalization in blood glucose concentrations following an i.p. injection of 2 g/kg dextrose (Fig. 2A).

The architecture of the islet in the EP-INGAP mice was compared with non-transgenic littermate controls by immunohistochemistry. Using 5 µm paraffin sections, pancreas tissues were stained with H&E, aldehyde fucin (data not shown) and hormone-specific immunocytochemistry (Fig. 2B). No changes in pancreatic organization were detected. Moreover, the cellular topology of insulin-positive cells (arrows, Fig. 2B) and glucagon-positive cells (arrows, Fig. 2B) was analogous between the mouse lines. Quantitative
analysis for total pancreatic islet mass and insulin content was performed (Fig. 3). The EP-INGAP mice had a significant ($P<0.05$) increase in both $\beta$-cell mass (insulin positive cells; $2.1\pm0.9$-fold) and in the total pancreatic insulin/protein ratio, $2\pm0.8$-fold ($2\pm0.8$-fold) when compared with the wild-type line. Morphometric analysis of $\beta$ cell and islet size was studied (Fig. 4). No significant difference in individual $\beta$-cell area was observed between EP-INGAP and wild-type islets (data not shown). EP-INGAP mice had more small islets than wild-type mice shown in representative low power micrographs (Fig. 4A and B) and histogram ranking the number of islets of defined size (Fig. 4C).

**Resistance to STZ-induced insulinemia**

To explore the functional differences between the strains, mice were challenged using the diabetogenic agent STZ. In wild-type FVB/N mice given a single i.p. STZ injection of $140\text{ mg/kg}$, non-fasting blood glucose levels rose to $\geq350\text{ mg/dl}$ and progressed to severe hyperglycemia (non-fasting blood glucose $\geq550\text{ mg/dl}$) by day 5 post-injection; Fig. 5). The hyperglycemia was sustained for the rest of the 14-day study. All the mice in the wild-type group ($n=6$) became hyperglycemic. In marked contrast, EP-INGAP mice did not become hyperglycemic at any point measured during the study following STZ administration.

STZ-treated mice were further analyzed by collecting blood samples at the end of the study for insulin (Fig. 6). Consistent with the reported mechanism of action for STZ-induced hyperglycemia (Wilson et al. 1988), circulating insulin levels in the STZ-dosed wild-type mice were significantly ($P<0.001$) reduced being just $5\%$ of that detected in non-STZ-treated mice. In contrast, EP-INGAP mice, which did not become hyperglycemic following STZ, had circulating insulin levels, which were comparable to the insulin levels detected in non-STZ-treated EP-INGAP and wild-type mice. Taken together, these data suggest that STZ at $140\text{ mg/kg}$ destroys pancreatic $\beta$ cells in wild-type FVB/N mice such that the islets fail to produce insulin. However, administration of STZ at $140\text{ mg/kg}$ to EP-INGAP transgenic mice did not result in marked $\beta$-cell destruction, hyperglycemia, or decreased insulin levels. We also performed immunohistochemical analysis of mouse pancreata following STZ. Pancreas sections from STZ-treated wild-type FVB/N mice compared with non-STZ-treated controls revealed a reduction of $87\%\pm4\%$ ($n=5$) of the intraislet insulin immunoreactive $\beta$ cells (Fig. 7A, C and E top). Whereas a comparison between STZ-treated and non-STZ-treated EP-INGAP transgenic mice revealed an overall preservation of islet structure with $91\%\pm5\%$ ($n=5$) of the immunoreactive islet $\beta$-cell mass being preserved (Fig. 7B, D, and E, bottom).

**Discussion**

Previous studies have provided support for the hypothesis that INGAP is an important factor in an islet neogenesis cascade (Rafaeloff et al. 1997; Del Zotto et al. 2000, Gagliardino et al. 2003, Rosenberg et al. 2004, Vinik et al. 2004a,b). Despite these observations, the mechanisms by which INGAP acts on pancreatic cells to stimulate new islet growth is unclear. As INGAP expression is upregulated in the acinar pancreas during islet neogenesis, we have investigated the consequences of a sustained acinar tissue INGAP expression in terms of pancreas development, physiology, and diabetes susceptibility in transgenic mice with INGAP expressed during islet neogenesis, we have investigated the consequences of a sustained acinar tissue INGAP expression in terms of pancreas development, physiology, and diabetes susceptibility in transgenic mice with INGAP expressed under the control of the elastase-1 enhancer. INGAP expression was acinar pancreas-specific albeit with an irregular pattern of expression (Fig. 1). The elastase-1 promoter used in the EP-INGAP transgene was the $−500/+8$ enhancer, which includes a repression element (located $−500$ to $−206\text{ nt}$) that acts to suppress gene expression in $\beta$ cells driven by the B element in the shorter $−205/+8$ enhancer sequence (Swift et al. 1989, MacDonald & Swift 1998). This enhancer sequence has been described as producing a patchy expression (Zhu et al. 2004), when
compared with the truncated enhancer termed -205/+8, and is consistent with the INGAP expression observed in the EP-INGAP mouse. Furthermore, the EP-INGAP transgene incorporates the INGAP secretion signal, thus INGAP should be secreted from cells as seen in established in vitro models (data not shown). Sustained INGAP expression did not disrupt pancreas development, rather an enhancement in β-cell mass was evident in terms of elevations in both islet mass and total pancreatic insulin content. This increase in β-cell mass was associated with an increase in the number of smaller islets rather than the creation of large super islets. Additionally, no significant change in proliferation/apoptotic

Figure 3 Increased β-cell mass in EP-INGAP mice. (A) β-cell quantitation and (B) determination of insulin/total protein ratio reveal a significant elevation in overall β-cell mass and pancreatic insulin content of EP-INGAP mice. *P<0.05.

Figure 4 Low power (4× objective) bright field micrographs of (A) wild-type and (B) EP-INGAP pancreas stained with trichrome. (C) Quantitation of islet size. Graph shows number of islets defined by area ranges (arbitrary morphometric units) for equal randomly selected fields (n≥7) for wild-type (open) and EP-INGAP (filled) mice.
ratios of β cells or ducts (not shown) were detected. Thus, these results suggest that the increase in islet mass may arise from a different pancreas modeling program (Slack 1995) in the EP-INGAP mice. These changes are being characterized in ongoing studies of the developing pancreas.

EP-INGAP transgenic mice were resistant to STZ-induced hyperglycemia and β-cell damage when treated with up to 140 mg/kg STZ. At a lower dose of STZ (120 mg/kg), the EP-INGAP mice remained euglycemic, whereas approximately half the wild-type mice became hyperglycemic. Further, administration of STZ at 160 mg/kg and above resulted in toxicity and morbidity that was not related to β-cell destruction (data not shown). Possible mechanisms for the protection to STZ-induced hyperglycemia in EP-INGAP mice include: (a) reduction in STZ bio-distribution, (b) active islet neogenesis, or (c) resistance to STZ. STZ is a DNA-alkylating agent (Yamamoto et al. 1981) that preferentially enters pancreatic β cells through the glucose transporter 2 (GLUT2) transporter due to a glucose-like moiety within its chemical structure (Wilson et al. 1988). Using immunohistochemical staining and semi-quantitative RT-PCR analysis, we found no difference in the GLUT2 transporter expression between EP-INGAP and wild-type mice (data not shown). Possible mechanisms for the protection to STZ-induced hyperglycemia in EP-INGAP mice include: (a) reduction in STZ bio-distribution, (b) active islet neogenesis, or (c) resistance to STZ. STZ is a DNA-alkylating agent (Yamamoto et al. 1981) that preferentially enters pancreatic β cells through the glucose transporter 2 (GLUT2) transporter due to a glucose-like moiety within its chemical structure (Wilson et al. 1988). Using immunohistochemical staining and semi-quantitative RT-PCR analysis, we found no difference in the GLUT2 transporter expression between EP-INGAP and wild-type mice (data not shown). Possible mechanisms for the protection to STZ-induced hyperglycemia in EP-INGAP mice include: (a) reduction in STZ bio-distribution, (b) active islet neogenesis, or (c) resistance to STZ. 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protection. Resistance to STZ-induced hyperglycemia has been reported in mice deficient in pancreatic insulin-like growth factor-I (Lu et al. 2004), in mice lacking the phosphatase and tensin homolog deleted on chromosome ten (PTEN) in adipose tissue (Kurlawalla-Martinez et al. 2005) and in mice that are deficient for poly (ADP-ribose) polymerase (Berkart et al. 1999). Whether INGAP plays a direct role in these pathways is unknown. INGAP has been reported to activate protein kinase B (AKT) phosphorylation (Jamal et al. 2005) and constitutive AKT activation results in an increased pancreatic β-cell mass and protection to STZ-induced diabetes (Bernal-Mizrachi et al. 2001, Tuttle et al. 2001). In the adipose tissue, AKT activation is a hallmark of PTEN loss (Kurlawalla-Martinez et al. 2005). While indirect actions of INGAP expression may also be relevant, the generation of biomarkers for pathways conferring protection to β-cell damage would have utility in identifying individuals that are disease susceptible amongst pre-diabetics in addition to monitoring the efficacy of therapeutic regimens in established diabetes. A complete characterization of these candidate protein biomarkers and the cellular mechanisms associated with INGAP efficacy are ongoing researches.

This study is the first report of efficacy for full-length INGAP in the mouse. The sustained expression of INGAP in pancreatic acinar tissue resulted in increased β-cell mass and resistance to STZ-induced diabetes. While these two observations may not be causally linked, this exciting and novel animal model could provide a gateway to identify neogenesis-related pathways and new molecules to target in diabetes prevention and/or treatment.

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References


Ratner RE & Feeley D 2005c Double-blind, placebo-controlled trial of Ine Neogenesis Gene Associated Protein (INGAP) in subjects with Type1 Diabetes Mellitus (T1DM). *American Diabetes Association Scientific Sessions Abstracts* 11-LB.


Song L, Taylor-Fishwick DA & Park I 2005 Chronological expression of INGAP (islet neogenesis associated protein) and clusterin during pancreas regeneration. *Diabetes* 54 A650.


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