

DISCOVERY OF A HUMAN PEPTIDE SEQUENCE SIGNALING ISLET NEOGENESIS

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ABSTRACT

Objective: To identify triggers for islet neogenesis in humans that may lead to new treatments that address the underlying mechanism of disease for patients with type 1 or type 2 diabetes.

Methods: In an effort to identify bioactive human peptide sequences that might trigger islet neogenesis, we evaluated amino acid sequences within a variety of mammalian pancreas-specific *REG* genes. We evaluated GenBank, the Basic Local Alignment Search Tool algorithm, and all available proteomic databases and developed large-scale protein-to-protein interaction maps. Studies of peptides of interest were conducted in human pancreatic ductal tissue, followed by investigations in mice with streptozocin-induced diabetes.

Results: Our team has defined a 14-amino acid bioactive peptide encoded by a portion of the human *REG3a* gene we termed Human proIslet Peptide (HIP), which is well conserved among many mammals. Treatment of human pancreatic ductal tissue with HIP stimulated the production of insulin. In diabetic mice, administration of HIP improved glycemic control and significantly increased islet number. Bioinformatics analysis, coupled with biochemical interaction studies in a human pancreatic cell line, identified the human exostoses-like protein 3 (EXTL3) as a HIP-binding protein. HIP enhanced EXTL3 translocation from the membrane to the nucleus, in support of a model whereby EXTL3 mediates HIP signaling for islet neogenesis.

Conclusion: Our data suggest that HIP may be a potential stimulus for islet neogenesis and that the differentiation of new islets is a process distinct from beta cell

proliferation within existing islets. Human clinical trials are soon to commence to determine the effect of HIP on generating new islets from one's own pancreatic progenitor cells. (*Endocr Pract.* 2008;14:1075-1083)

Abbreviations:

DAPI = 4',6-diamidino-2-phenylindole dihydrochloride; **EXTL3** = exostoses-like protein 3; **HIP** = Human proIslet Peptide; **INGAP** = islet neogenesis-associated protein; **NGN3** = neurogenin-3; **SFM** = serum free medium; **STZ** = streptozocin

INTRODUCTION

Development of diabetes is associated with a substantial loss in pancreatic islet mass. At the time of diagnosis, patients with type 1 diabetes have lost 90% of their islet mass. This loss is approximately 50% in patients with newly diagnosed type 2 diabetes (1,2). Several groups have shown that pancreatic islets can be generated through differentiation of the nonendocrine fraction of the human pancreas (3,4). Recently, investigators have shown that human pancreatic progenitor cells, located throughout the adult pancreas, are capable of directly differentiating into fully functional islets (5-7).

The terms "islet neogenesis" and "beta cell regeneration" are often used synonymously, even in the basic science literature. The process of new islet formation occurs almost exclusively during fetal development, when the pancreas is being populated with islets for the first time (8,9). Formation of new islets in adults is rare, occurring primarily in response to pancreatic injury and stress. In contrast to islets, Dor et al (10) demonstrated that beta cells within existing islets are generated through self-renewal, a process occurring through adulthood. Autopsy studies conducted among patients with both type 1 and type 2 diabetes demonstrate that not only are there reductions in beta cell numbers but also there are significant reductions in both the islet numbers and the islet mass (11,12).

The *REG* genes are associated with islet neogenesis in mice, rats, and hamsters and are expressed primarily dur-

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ing fetal development (13-15). After fetal development, the *REG* genes are expressed only rarely, as part of the slow natural turnover process and, more noticeably, in response to acute pancreatic injury (for example, pancreatitis, pancreatic stones, or partial pancreatectomy) or conditions of increased insulin demand, such as pregnancy (16-18). The hamster *Reg* gene product, islet neogenesis-associated protein (INGAP) that is expressed in a hamster pancreatitis model, has restored normoglycemia in diabetic hamsters and mice through new islet formation (19,20).

During pregnancy, islet neogenesis precedes beta cell expansion; thus, a new pool of beta cells is provided (21). Islet neogenesis has also been considered to increase among pregnant women with type 1 diabetes (22,23). Jovanovic et al (22) demonstrated that as many as 30% of women with undetectable C-peptide levels before pregnancy have normalization of their C-peptide levels by 10 weeks of gestation. Contributing factors include immune downregulation and upregulation of the *REG* genes (22-24).

The beta cell transcription factor, neurogenin-3 (NGN3), signaling beta cell proliferation, has been shown by some groups to be upregulated after pancreatic ductal ligation, whereas other studies have found that NGN3 and other beta cell transcription factors, including PAX1 and PDX1, are downregulated during islet neogenesis after partial pancreatectomy and autoimmune pancreatic destruction attack (7,25-27). This differential expression of beta transcription factors may be reconciled by immunologic and morphologic evidence of colocalization of alpha, beta, and gamma cells within islets of the midgestational fetus along with data demonstrating that islet neogenesis temporally precedes beta cell proliferation (8,9,21).

In this report, we describe a human *Reg*-encoded peptide, which we refer to as Human proIslet Peptide (HIP) that signals islet neogenesis. The data presented suggest that islet neogenesis and beta cell proliferation are distinct but not mutually exclusive events.

RESEARCH DESIGN AND METHODS

Human Protein-to-Protein Interaction Maps

To identify candidate peptides that contribute to islet neogenesis, we developed and analyzed large-scale protein-to-protein interaction maps using PubMed, GenBank, Pathway Studio (Ariadne Genomics), the nucleotide sequence database, Map Viewer, Reference Sequence database, and the Basic Local Alignment Search Tool algorithm for sequence comparison. All potential protein sequences were analyzed individually; the result was a manually curated map of potential sequences and protein interactors involved in islet neogenesis. For exclusion of false-positive interactions that sometimes occur by methods such as yeast 2-hybrid screening, which is one method by which proteins were screened, the map was

further refined by using information relating to temporal expression and spatial organization with respect to the plasma membrane and other intracellular structures before confirmation studies were conducted in human pancreatic tissue.

Human Pancreatic Ductal Fractions

Ductal cell cultures were prepared from human donor cadaveric pancreata under a protocol approved by the University of Pennsylvania Institutional Review Board. Islet and ductal tissue fractions were separated with use of the Ricordi method. When a pancreas arrives in the human islet laboratory at the University of Pennsylvania, the pancreas is distended with collagenase and digested in a Ricordi chamber, with the final step being the purification of isolated islets from exocrine tissues. The remaining pancreatic ductal tissues are then cultivated by using a modified method described by Bonner-Weir et al (28).

The ductal tissue is then split to allow the cells to expand into a ductal monolayer of cells. Although islet contamination cannot be excluded, with use of this method, there is typically no staining of insulin in the remaining monolayers of ductal tissue.

Investigators were blinded to the intervention arms. Three HIP sequences that varied in amino acid length along the *Reg3a* protein were evaluated, along with a scrambled peptide serving as a negative control and an INGAP peptide serving as a positive control.

All interventions were plated at the same time from the ductal monolayers prepared from the same cadaveric donors. These studies were repeated to confirm the initial results.

Insulin content was normalized to total protein levels by using the Bradford assay. Ductal cultures were incubated in duplicate with the 3 HIP sequences, which differed in amino acid sequence along the *Reg3a* protein, and with INGAP at a final concentration of 500 nM for 7 days and a scrambled peptide control. At the end of the incubation period, insulin production was determined by means of radioimmunoassay of whole-cell lysates.

Human PANC-1 Culture

Human PANC-1 cells were purchased from the American Type Culture Collection (Manassas, Virginia) and cultured in Dulbecco's Modified Eagle's Medium containing 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 4 mM L-glutamine, and 10% fetal bovine serum. For Western blot and immunostaining analyses, PANC-1 cells were seeded at 50% confluence in standard growth medium. Twenty-four hours after seeding, PANC-1 cells were differentiated in serum free medium (SFM) (F12-K medium supplemented with 17.5 mM glucose, 1% bovine serum albumin, and 1×insulin-transferrin-selenium; Gibco/Invitrogen Corporation, Carlsbad, California) in the presence or absence of HIP (167 nM) for the indicated times at 37°C.

Human Exostoses-Like Protein 3 Immunofluorescence

Human PANC-1 cells grown on coverslips were fixed in 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. After being washed and blocked, cells were incubated with rabbit anti-human exostoses-like protein 3 (EXTL3) antibody (Proteintech Group, Inc., Chicago, Illinois) and appropriate CY3-conjugated secondary antibody. All antibodies were used at a 1:200 dilution. Coverslips were mounted in 4',6-diamidino-2-phenylindole dihydrochloride (DAPI)-containing mounting medium, and staining was analyzed on a Zeiss Axiovert 220M microscope powered by Axiovision 4.0 software with Z-stack acquisition, 3-dimensional deconvolution, and 4-dimensional rendering modules.

Western Blot Analysis of Cytosolic and Nuclear Fractions

Cytoplasmic extracts were obtained in 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) (pH 8.0), 1 mM ethylenediaminetetraacetic acid (EDTA), 1.5 mM MgCl₂, 10 mM KCl, 0.5 mM dithiothreitol, 200 mM sucrose, and 0.5% Nonidet P-40. Nuclear extracts were obtained in 20 mM HEPES (pH 7.9), 0.75 mM MgCl₂, 210 mM NaCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, and 0.5 mM dithiothreitol. Both extraction buffers contained 0.5 mM phenylmethanesulfonyl fluoride, 1 µg/mL leupeptin, 1 µg/mL aprotinin, 2.5 mM Na₄P₂O₇, 1 mM β-glycerophosphate, and 1 mM Na₃VO₄. Protein extracts were size fractionated on sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose. After blocking in 3% milk in tromethamine-buffered saline (pH 7.4), blots were sequentially incubated with rabbit anti-human EXTL3 antibody overnight at 4°C and appropriate horseradish peroxidase-conjugated secondary antibody. Secondary signals were developed with chemiluminescence substrate (Western Lightning Chemiluminescence Reagent Plus, PerkinElmer Life and Analytical Sciences, Inc., Waltham, Massachusetts) and analyzed by autoradiography.

Streptozocin-Induced Diabetes

Two studies were conducted among 6- to 8-week-old C57BL/6J mice rendered diabetic with streptozocin (STZ). The initial 28-day study evaluating the effect of HIP and placebo was followed by a 39-day intervention study. The initial study included 6 mice per each of the 3 HIP treatment groups and the 1 control group. The second study included 10 mice in the HIP group and in the control group. Mice were injected intraperitoneally with STZ at 50 mg/kg in citrate buffer, pH 4.5, for 5 consecutive days to render them diabetic. Mice were randomized into the study when the blood glucose level was ≥200 mg/dL for 2 consecutive days. Blood glucose was monitored daily, and when glucose levels increased to ≥400 mg/dL, the animal was treated with insulin (1 U/d, glargine insulin, intramuscularly). All treatments were performed in a blinded

manner. On the evening of day 38, mice were fasted overnight, and fasting glucose levels were measured on the morning of sacrifice (day 39).

Immunofluorescence Staining of Pancreatic Tissue From STZ-Treated Mice

Tissue was fixed in 4% paraformaldehyde and sectioned at 50-µm intervals. After antigen retrieval and blocking, sections were sequentially incubated with guinea pig anti-insulin antibody (Dako Cytomation, Carpinteria, California) and CY3-conjugated anti-rabbit antibody. Nuclei were counterstained with DAPI-containing mounting medium, and insulin staining was assessed on a Zeiss Axiovert 220M microscope with Axiovision 4.0 acquisition and quantitation software. The islet area was determined by outlining the insulin-stained regions. Area values were calculated with Axiovision scaling. For every pancreas, 3 sections were analyzed at 50-µm intervals in a blinded fashion.

Statistical Analysis

Differences in mean glucose levels were analyzed by using 2-way repeated-measures analysis of variance and a general linear model with unbalanced nested design. Differences in the daily glucose levels, the number of mice requiring insulin, and the insulin requirement were investigated with use of linear regression and *t* tests to confirm significant differences in regression slopes between groups. Differences in the number of islets per mouse, mean islet size, and mean islet area were assessed with analysis of variance and independent sample *t* tests. The Levene test was used to investigate differences in the distribution of islet size between groups. Correlations among islet mass, glucose levels, and insulin requirements within groups were investigated by using the Pearson product moment correlation. Values of *P* less than .05 were accepted as significant.

RESULTS**HIP Structure and Species Homology**

We define HIP as the bioactive 14-amino acid peptide IGLHDPTQGTEPNG (molecular weight 1,435.5) that is encoded by a region of the human *REG3a* gene. Comparative searches by our team with use of the Basic Local Alignment Search Tool algorithm discovered that the amino acid sequence for HIP is well conserved among many species (Fig. 1), with coding alterations between species usually producing conservative amino acid changes. Three-dimensional modeling of the human *REG3a* protein sequence by SwissProt folding algorithms revealed that the HIP sequence is presented and exposed on the external surface of the protein and not folded within the confines of the protein; thus, it is available for extensive protein-binding interactions.

Human	W	I	G	L	H	D	P	T	Q	G	T	E	P	N	G	E
Chimp	W	I	G	L	H	D	P	T	Q	G	S	E	P	D	G	G
Hamster	W	I	G	L	H	D	P	S	H	G	T	L	P	N	G	S
Mouse	W	I	G	L	H	D	P	T	M	G	Q	Q	P	N	G	G
Norway Rat	W	I	W	L	H	D	P	T	M	G	Q	Q	P	N	G	G
Cow	W	I	G	L	H	D	P	T	E	G	S	E	P	D	A	G
Dog	W	M	G	L	H	D	P	T	E	G	Y	E	P	N	A	D
Sheep	W	I	G	L	H	D	P	T	E	G	S	E	P	N	A	G

Fig. 1. Human proIslet Peptide (HIP) species homology. Comparison of human HIP sequences with homologous sequences among other mammalian species is shown.

HIP Stimulates Insulin in Human Pancreatic Ductal Cells

For initial assessment of the effect of HIP on insulin production in nonendocrine pancreatic tissue, human pancreatic ductal cell cultures were prepared from donor cadaveric pancreatic tissue and treated with HIP, INGAP, or a scrambled HIP peptide used as a control at a final concentration of 500 nM for 7 days. Radioimmunoassay of these cell lysates revealed that all HIP-treated cultures exhibited a 2-fold higher insulin content than cultures treated with INGAP ($P = .020$) or scrambled peptide ($P = .017$) (Fig. 2). Duplicate experiments revealed that HIP 2 stimulated the greatest insulin production.

HIP Improves Glycemic Control in Mice Rendered Diabetic With STZ

Next, we analyzed the effect of intraperitoneal injections of HIP on blood glucose levels and insulin dependence in mice with STZ-induced diabetes. Administration of a 28-day, 250- μ g twice daily intraperitoneal regimen of HIP reduced nonfasting glucose levels from baseline by 26.9% in diabetic animals, whereas the placebo treatment reduced glucose levels by 9.5% ($P = .002$). Improvement in the placebo-treated group may be accounted for by the spontaneous partial recovery that is typically seen over

time in the STZ-induced diabetic mouse model. Diabetic animals were treated with insulin if the blood glucose level was ≥ 400 mg/dL. Overall, HIP-treated animals required 32% less insulin than their placebo-treated counterparts ($P = .002$). The difference in glucose levels in the HIP-treated group in comparison with the placebo group is shown in Figure 3. The rate of decrease in insulin requirements was approximately twice as fast in the HIP-treated mice ($y = -0.1235x + 3.5556$; $R^2 = 0.9926$) in comparison with the placebo-treated mice ($y = -0.0648x + 3.1111$; $R^2 = 0.9932$) ($P = .004$) (Fig. 3).

In a similar experiment that followed the initial 28-day study, the HIP intervention was extended to 39 days. There were no significant differences between treatment groups with respect to baseline glucose levels ($P = .301$). The variation in the daily glucose levels between the HIP-treated mice and the control mice during the 39-day study period is depicted in Figure 4. The slopes of the lines in Figure 4 represent the rate of change in glucose levels, which we refer to as “regeneration speed.” HIP treatment resulted in a rate of decline (regeneration speed) in glucose levels of -0.602 as compared with a rate of increase in glucose levels of $+0.381$ within the placebo-treated group during the course of the study from day 1 to day 38.

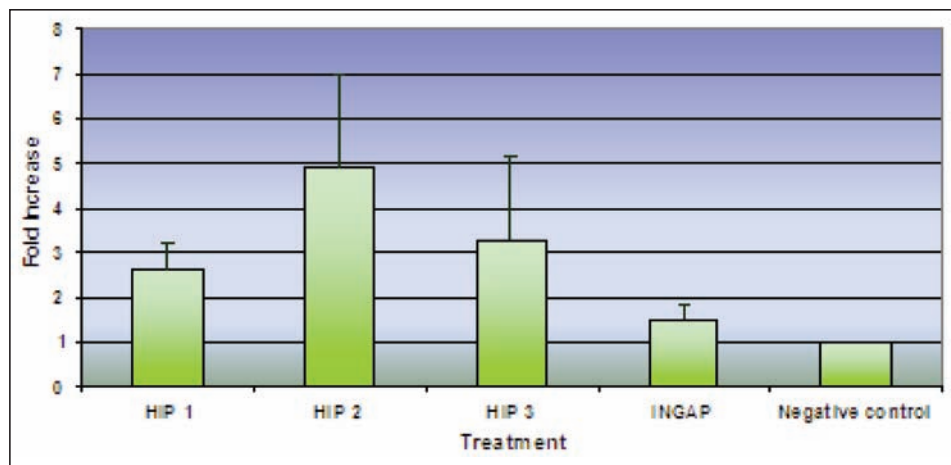


Fig. 2. Insulin concentration measured in human pancreatic cells after incubation with the Human proIslet Peptide (HIP) sequences that varied in amino acid length, islet neogenesis-associated protein (INGAP), and a scrambled peptide control. Fold differences in insulin concentration are expressed on the y-axis.

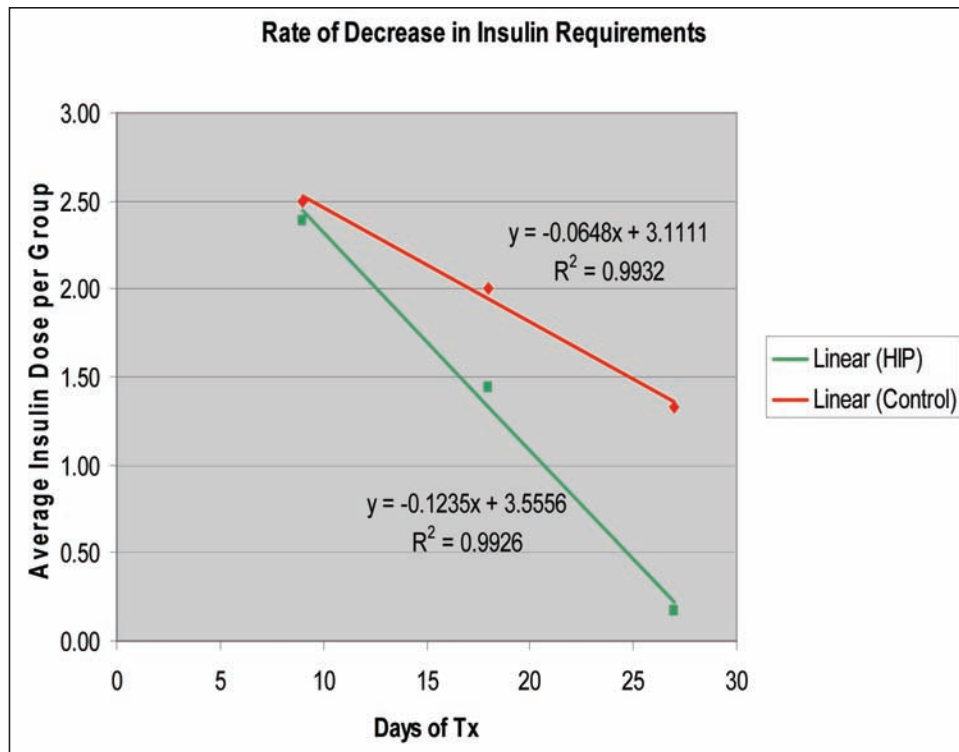


Fig. 3. Comparative rate of decline in insulin requirements between the placebo group (*Control*) and the Human proIslet Peptide (HIP)-treated animals. The rate of decrease in insulin requirements was approximately twice as fast in HIP-treated mice ($y = -0.1235x + 3.5556$; $R^2 = 0.9926$) in comparison with placebo-treated mice ($P = .004$). The *red line* illustrates the rate of decrease in insulin requirements among the placebo-treated animals in comparison with that in the HIP-treated animals (*green line*). *Tx* = treatment.

At the end of the intervention, the mice underwent an overnight fast, and a fasting glucose level was measured on the morning of day 39. The mean fasting glucose level was 132% higher in the placebo-treated group (258.00 ± 84.5 mg/dL) than in the HIP-treated mice, with a mean fasting glucose level of 111.00 ± 11.4 mg/dL ($P = .020$).

HIP Increases Islet Number in the STZ Diabetes Model

Quantitative analysis of insulin immunofluorescent staining of pancreatic tissue revealed that the total islet area in animals receiving the 28-day regimen of HIP ($2,161,782 \mu\text{m}^2$) was 1.5-fold higher (153%) than that in the placebo-treated group ($854,362 \mu\text{m}^2$) ($P = .05$). The number of islets in the HIP-treated mice (454 islets) was 62% greater than that in the placebo-treated mice (280 islets) ($P = .022$) (Fig. 5). Islet size was similar in both groups ($P = .067$). These studies were repeated with a stabilized formulation of HIP, which demonstrated 168% greater number of islets than did placebo ($P = .032$) and an islet mass of 398% higher than did placebo ($P = .040$), with no significant differences in islet size between groups ($P = .518$). This stabilized form is our candidate for clinical trials. Double-labeling with insulin-somatostatin and insulin-glucagon of islets in HIP-treated animals showed positive staining results for glucagon and somatostatin,

with central staining of cells for insulin and peripheral staining of cells for glucagon and somatostatin.

Human EXTL3 Is a Candidate for HIP Signaling

To narrow the field for the identification of the potential HIP receptor and protein interaction partners, we

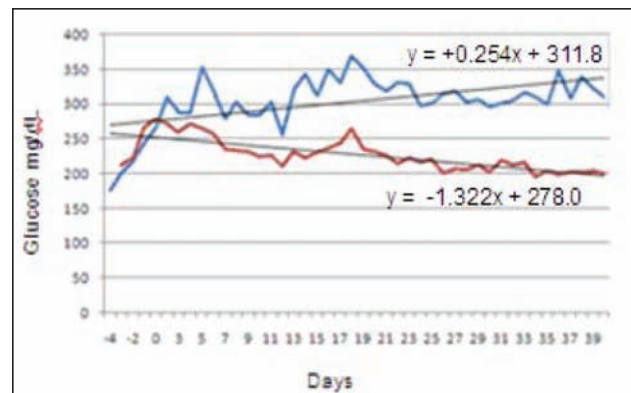


Fig. 4. Daily variations in glucose levels between the Human proIslet Peptide (HIP)-treated mice (*red line*) and the control animals (*blue line*). The slopes of the lines represent the rate of change in glucose levels, which we refer to as “regeneration speed.” HIP treatment resulted in a rate of decline (regeneration speed) in glucose levels of -0.602 as compared with a rate of increase in glucose levels of $+0.381$ within the placebo-treated group during the course of the study from day 1 to day 38.

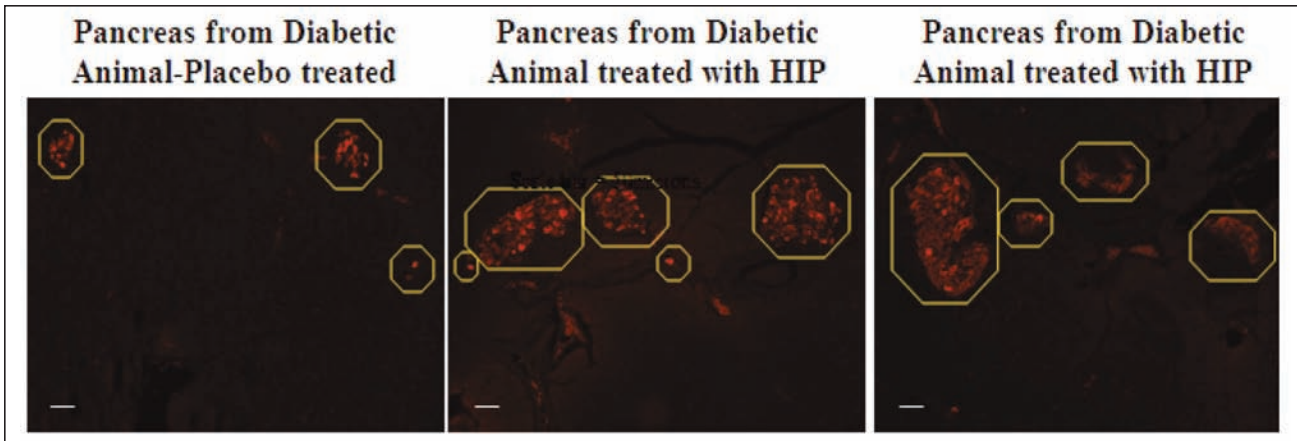


Fig. 5. Insulin staining of pancreatic islets from diabetic mice. Representative images show insulin immunostaining in placebo-treated and Human proIslet Peptide (HIP)-treated mice. The insulin-stained islets are outlined in yellow to distinguish these structures from autofluorescent blood cells. All images are at the same magnification. Scale bar = 50 μ m in all images.

developed a large-scale protein-to-protein interaction map using *in silico* methodology, which evaluated databases that encompassed the human genes and the largest non-redundant human protein interactions. The primary candidate protein for potential interaction with HIP was identified as the human EXTL3. Consistent with this finding, EXTL3 has been implicated as a binding protein for Reg1 in rats (29). To begin our examination, we asked whether HIP modulated the expression and subcellular localization of EXTL3.

Western blot and immunofluorescence analyses of EXTL3 localization were performed in human PANC-1 cells grown under conditions that cause differentiation of these cells into islet-like cells (30,31). EXTL3 is predominantly located on the external surface of the cytoplasmic membrane, and its structure includes a single transmembrane region located inside the cytoplasmic membrane. PANC-1 cells were grown in a standard growth medium or in SFM containing HIP. Western blot and immunofluorescence analyses of EXTL3 localization in the PANC-1 cells revealed that, when HIP was added to PANC-1 cells grown in SFM, EXTL3 appeared to translocate from the plasma membrane to the nucleus (Fig. 6). Western blot analyses of EXTL3 levels in cytosolic and nuclear fractions were isolated. Western blot analyses demonstrated that nuclear levels of EXTL3 were observed at 6 hours after culture in SFM without HIP. Inclusion of HIP in the culture medium enhanced EXTL3 nuclear translocation at 30 minutes (Fig. 6).

Using an antibody directed against human EXTL3, we immunostained PANC-1 cells and compared cells grown in SFM in the presence or absence of HIP. In standard growth medium, the well-defined cell borders indicated surface expression of EXTL3 on the plasma membrane in conjunction with undetectable levels of EXTL3 in the nucleus (Fig. 7). Among cells grown in SFM, EXTL3 was localized in the cytoplasm, as indicated by Cy3 staining in the cytoplasm and lack of EXTL3 staining in the nuclei. In cells grown in SFM and HIP, EXTL3

was present in both the cytoplasm and the nucleus, as indicated by the overlap of Cy3 and DAPI staining; this result suggested that HIP facilitated a translocation of EXTL3 into the nucleus.

Further investigation is under way on the signaling pathway, including other interacting proteins with HIP that were indicated by our protein-interaction maps. Potential HIP interactors include Syndecan2, an isoform Fibronectin1, and Annexin3. We hypothesize that the *REG3a* protein is a rate-limiting factor for islet neogenesis

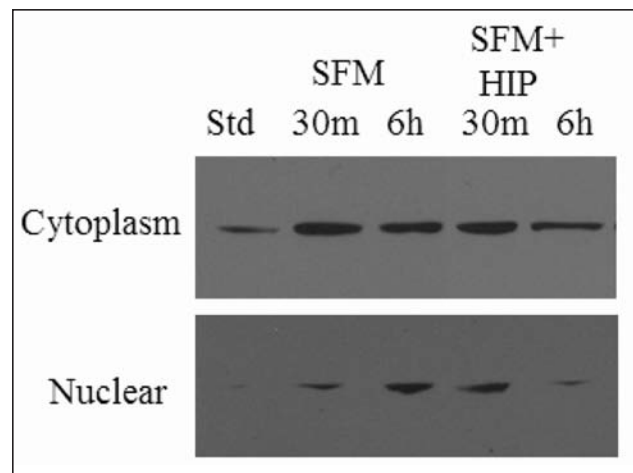


Fig. 6. Human proIslet Peptide (HIP) enhances exostosin-like protein 3 (EXTL3) translocation from the cytoplasmic membrane to the nucleus in PANC-1 cells. Western blot analyses of EXTL3 levels in cytosolic and nuclear fractions were isolated at the indicated times shown at top. Western blot analysis demonstrates that higher nuclear levels of EXTL3 were observed at 6 hours after culture in serum free medium (SFM) without HIP. Addition of HIP to the culture medium enhanced EXTL3 nuclear translocation, demonstrated by higher nuclear levels of this protein at 30 minutes. These comparisons demonstrate that, in the presence of HIP, there is enhanced translocation of EXTL3 from the cytoplasmic compartment to the nucleus and that EXTL3 nuclear translocation can be modulated by the presence of HIP. Western blots are repeated results with use of these cells and time points. *Std* = standard growth medium.

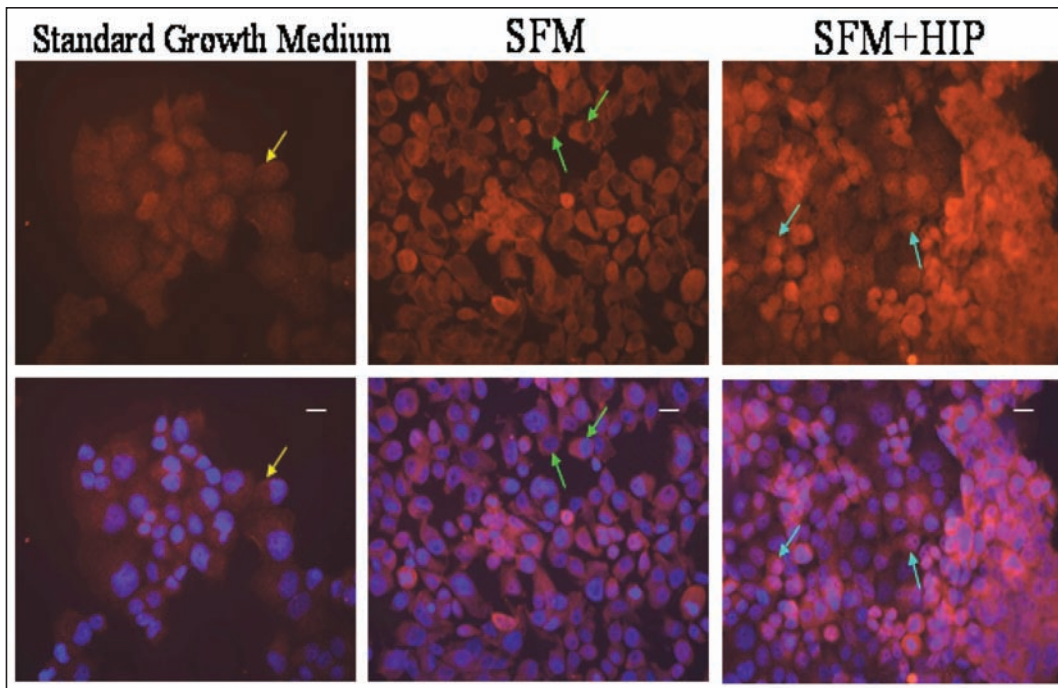


Fig. 7. Immunofluorescent analyses of exostosin-like protein 3 (EXTL3) are shown in the upper panels, indicated by the Cy3 immunofluorescent staining of EXTL3 (red). In the lower panel of images, the Cy3 immunostaining of EXTL3 has been overlaid with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) staining (blue) of the nuclei. Cells were grown in standard growth medium as a control and compared with cells grown in serum free medium (SFM) in the presence or absence of Human proIslet Peptide (HIP). The yellow arrows demonstrate examples of the surface expression of EXTL3 grown in standard growth medium. The well-defined cell borders indicate surface expression of EXTL3 on the plasma membrane. The yellow arrows delineate the cell borders, whereas the nuclei are shown in blue. The middle images are cells grown in SFM. EXTL3 is localized in the cytoplasm, as indicated by cytoplasmic Cy3 staining. The green arrows show the lack of staining in the position of the nuclei. The green arrows in the lower image of cells grown in SFM demonstrate intense blue DAPI staining of the nuclei, indicating a lack of EXTL3 in the nucleus. In the upper image of the cells grown in SFM and HIP, the presence of EXTL3 immunostaining in the nucleus (indicated by the blue arrows) suggests a translocation of EXTL3 into the nucleus. In the lower image of cells grown in SFM and HIP, the blue arrows indicate the position of the nuclei. In the lower image, there is an overlap of EXTL3-Cy3 staining and nuclear DAPI staining that corroborates the nuclear localization of EXTL3 (blue arrows). Scale bar = 20 μ m in all images.

in humans and that the binding of the HIP region of the *REG3a* protein initiates an interaction that may trigger signaling cascades toward differentiation of progenitors into new islets. Syndecan2 and Fibronectin1 may participate in the process by aiding in the signaling of progenitor cell differentiation. Annexin3 may promote angiogenesis and innervation of developing islets.

DISCUSSION

In 1920, Moses Barron (32) made the paradoxical observation that pancreatic stones cause islet neogenesis. Barron's observation led Frederick Banting to design his initial studies of ligating the pancreatic ducts in dogs and collecting the remaining pancreatic secretions, which resulted in the discovery of insulin (33,34). Before the widespread availability of insulin, surgeons performed partial pancreatectomies on children with diabetes in the hopes of regenerating islets (35,36). In 1983, Rosenberg

and Vinik generated a pancreatitis model in hamsters, which resulted in the formation of new islets (37).

Our results demonstrate that HIP, derived from the human *REG3a* gene, stimulates insulin production in human pancreatic ductal cells and improves glycemic control in mice with STZ-induced diabetes. The finding that HIP increased the number of islets in diabetic animals suggests that HIP improves glucose metabolism by stimulating the formation of new islets. On the basis of our findings, we hypothesize that (1) the human *Reg3a* protein may be a rate-limiting component of islet neogenesis, (2) progenitor cells that are capable of differentiating into islets are present in the ductal compartment of the adult human pancreas, and (3) newly formed human islets may be the source of beta cells for subsequent beta cell expansion and proliferation.

In contrast to islet neogenesis, which predominantly occurs in utero when the pancreas is being populated with islets for the first time, beta cells are capable of consider-

able plasticity throughout one's life span, undergoing changes in number depending on the insulin demands of an individual. Despite the low abundance of *REG3a* after fetal development, evidence suggests that new islet formation can occur in the adult by means of the differentiation of progenitor cells that are dispersed throughout the pancreas.

Our data suggest that the pathway for the differentiation of new islets is separate from beta regeneration because HIP was able to stimulate insulin production in human nonendocrine pancreatic tissue and increase islet number, but not islet size, among diabetic mice. Nonetheless, islet neogenesis and beta regeneration may be linked, as evidenced by the capacity in adulthood to generate new islets in times of acute stress. Similar to the process of beta cell proliferation requiring a number of transcription factors, including NGN3, PDX1, and PAX1, recruitment of other proteins, including Syndecan2, an isoform of Fibronectin1, and Annexin3, may be required for the development of new islets from progenitor cells.

Our data suggest that generation of new islets through islet neogenesis from one's own progenitor cells may serve as a novel approach for future therapies for diabetes. Human clinical trials are soon to commence evaluating the potential of HIP as a novel therapy for both type 1 and type 2 diabetes. Additional studies are necessary for further elucidation of the signaling pathways for islet neogenesis, which may lead to novel treatment strategies for diabetes that address the underlying mechanism of this disease.

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DISCLOSURE

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ty of interest based on their interest in CureDM, now incorporated as CureDM, Inc. Dr. Roger Nolan, also an author, has a duality of interest because of his role as a senior scientific director at Calvert Research Institute.

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