

Prospects and Challenges for Islet Regeneration as a Treatment for Diabetes: A Review of Islet Neogenesis Associated Protein

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Abstract

Diabetes mellitus results from inadequate insulin action, which can be viewed as a consequence of the limited ability to restore β cells after they are lost as the result of metabolic exhaustion, autoimmune destruction, or surgical insult. Arguably, a uniformly effective therapeutic pathway to address all forms of diabetes would be to reverse the restrictions on β -cell and islet regeneration. The development from progenitor cells of islets with normal endocrine function does occur in adult humans; it is referred to as islet neogenesis. The induction of islet neogenesis is an important, if not essential, therapeutic approach for curing type 1 diabetes mellitus (T1DM) and could be valuable in the treatment of type 2 diabetes mellitus (T2DM) as well.

Islet neogenesis associated protein (INGAP) is the first therapeutic candidate to be identified as the result of a purposeful search for an endogenous molecule with islet neogenic activity. It was found that partial obstruction of the pancreatic duct in hamsters induced islet neogenesis; under this condition, a neogenesis-promoting activity was identified and partially purified from a soluble tissue fraction. A 168-kDa protein product of the cloned gene was found to be responsible for the neogenesis activity. This molecule named INGAP contains an active core sequence of amino acids called INGAP peptide. Results from *in vitro*, animal, and human studies suggest that INGAP and INGAP peptide are neogenic in at least several vertebrate species, including humans. INGAP has since been found to be a member of the family of Reg proteins, which are found across and in multiple versions within species and are closely associated with embryonic and regenerative processes.

Clinical results suggest that INGAP peptide can be a suitable neogenesis therapy, but optimization of the therapy and more data are required to fully access this potential. Understanding of the signaling pathways of INGAP and other related Reg proteins is a promising means of advancing therapeutic development for people with T1DM and T2DM. The quest for the fundamental restorative approach to lost insulin secretion is an enticing target for drug development.

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Abbreviations: (CK+) cytokeratin positive, (DLS) duct-like structures, (FANs) focal areas of neogenesis, (GLP-1) glucagon-like peptide-1, (IGF-1) insulin-like growth factor-1, (INGAP) islet neogenesis associated protein, (ILS) islet-like structures, (NOD) nonobese diabetic, (PDX-1) pancreatic and duodenal homeobox gene-1, (T1DM) type 1 diabetes mellitus, (T2DM) type 2 diabetes mellitus

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Introduction

Diabetes mellitus results from inadequate insulin action, which can be viewed as a consequence of the limited ability to restore β cells after they are lost as a result of metabolic exhaustion, autoimmune destruction, or surgical insult. Arguably, a uniformly effective therapeutic pathway to address all forms of diabetes would be to reverse the restrictions on β -cell and islet regeneration imposed during the developmental process. Definitive therapeutic approaches will also include control of the autoimmune processes of type 1 diabetes (T1DM)¹ and the metabolic defects underlying type 2 diabetes (T2DM),² but the *sine qua non* for curing any form of established diabetes is to restore lost β cells. A cure of established diabetes—particularly T1DM—will therefore almost certainly involve a form of islet regeneration. The induction of islet regeneration from existing progenitor cells is termed neogenesis, the subject of this review. This review focuses on a particular candidate therapy for islet neogenesis, in part, because human trials of islet neogenesis associated protein (INGAP) peptide conducted in both T1DM and T2DM patients provide clinical experience and teach a number of principles and lessons for the development of any neogenesis therapy. This review can only present high-level summaries of the relevant advances that have been made in developmental biology, immunology, and transplantation technology. Each of these important facets of neogenesis has been discussed in depth in recently published excellent reviews.³⁻⁷

Addressing Unmet Clinical Need

After Banting and Best's animal experiments, which culminated in the discovery of insulin therapy, there were probably many who believed that diabetes had largely been conquered. Despite the significant advances in insulin manufacture, modification, and delivery since that time, the treatment of the absolute insulin deficiency resulting from T1DM is very challenging. Insulin administration remains relatively hazardous and not fully effective in preventing complications, even if managed meticulously.⁸ In the meantime, T2DM has become a global health crisis.⁹ Diabetes in general and insulin use in particular have become impediments to maintaining employment and licenses to operate equipment, including motor vehicles.¹⁰ Fortunately, new therapies have been developed, which not only improve the metabolic disorder of T2DM but appear to reduce the loss of β -cell function.¹¹ These therapies may help reduce the proportion of people with T2DM¹² who require insulin therapy, but it is doubtful that this need will be eliminated.

In contrast to these approved therapies and others soon to follow, which appear to improve β -cell health and perhaps even stimulate β -cell replication, a true neogenesis agent gives the prospect of restoring lost islet function (not simply maintaining β -cell mass and function). An effective neogenesis therapy will give people with either T1DM or T2DM the chance to reduce or eliminate their need for insulin therapy. Although animal data have suggested that GLP-1 agonists such as exenatide (Byetta™, Amilyn) have neogenesis activity, direct evidence of such activity in humans has yet to be reported. Some evidence from nonhuman primates implies that these agonists may not have neogenesis activity in humans.¹³

A safe and fully effective neogenesis agent paired with an appropriate immunomodulatory approach would provide definitive therapy for T1DM, resulting in a lifelong remission or an effective "cure" for a population that approaches 1 million people in North America and is growing.¹⁴ Such a therapy could also restore β -cell function in an even larger number of insulin-requiring people with T2DM, thereby returning their endogenous insulin production to levels found in earlier stages of their disease. Even a modest return of islet function and autoregulated insulin secretion would provide a therapeutic advance and substantial clinical benefits for people with either T1DM or T2DM. From a regulatory perspective, the greater unmet need is found among people with T1DM, and neogenesis therapy would provide these patients with an important therapeutic breakthrough in treating their disease. It is emphasized that people with either T1DM or insulin-requiring T2DM would qualitatively benefit clinically from neogenesis in the same ways. However, returning endogenous insulin secretion in T1DM provides the ultimate test and highest hurdle for islet cell regeneration. This review focuses on the application of a neogenesis therapeutic approach to T1DM.

Emerging Technologies to Address This Need

Intermediate technologies for restoring glucose-sensitive insulin secretion include islet transplantation from humans and other animals^{15,16} and "closed-loop" insulin pumps and adult and embryonic stem cell technologies (**Figure 1**). Allotransplantation of human islets has shown some clinical value and has provided important understanding of islet biology and immunology.^{17,18} Although promising, it is unlikely that transplantation and mechanical devices can achieve a high level of safety, flawless and enduring

performance, and convenience, which would fully satisfy patients and clinicians.¹⁹⁻²⁴ Manufacturing of human islets *ex vivo* for transplantation is a technologically hybrid approach. A number of approaches for producing islets have been reported, including involving INGAP.^{4,25-28} These technologies could address the current scarcity of human islets for transplantation, but without encapsulation, would not avoid the need for immune therapy required by allo-islet transplantation. Broadly speaking, islet transplantation and all versions of the artificial pancreas can be viewed as intact systems that sense ambient glucose concentrations and appropriately release insulin. Any closed loop system, whether tissue transplantation or mechanical device, will require careful installation and maintenance.

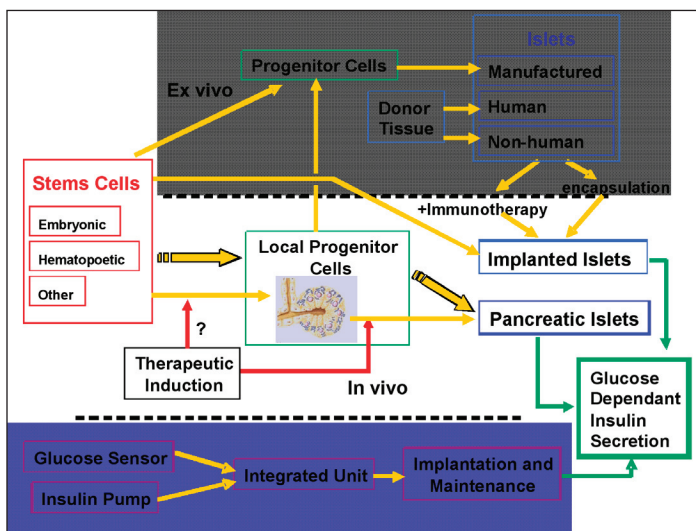


Figure 1: General categories for restoring glucose-dependent insulin secretion. Three major categories are depicted. The biomechanical approach weds two major technologies, glucose sensing and insulin pumping, to achieve a system that maintains normal glucose levels. This system cannot achieve completely normal physiology because only insulin and no other islet hormones are delivered. The site of insulin delivery by this approach is also nonphysiologic. The *ex vivo* category involves transplantation of islets that may be donated or produced from progenitor cells. Approaches in this category require either systemic immunotherapy of the recipient or encapsulation to prevent immune destruction. Transplanted islets reside outside the pancreas. Reintroduced stem cells may possibly home to the pancreas and develop into islets. The third general category simply involves therapeutically inducing endogenous stem or progenitor cells—either within the pancreas or elsewhere—to developing into islets. The normal developmental pathway is indicated by .

The other broad category for restoring glucose-sensitive insulin secretion entails inducing progenitor cells to develop into islets that appropriately secrete insulin as well as other endogenous hormones present in normal islet cells. Glucagon, for example, is an important glucose counter-regulatory hormone also secreted by islets.²⁹⁻³⁰ It should be stated in passing that pure β -cell neogenesis

is theoretically possible, but for various reasons, appears unlikely and not as desirable as neogenesis of fully functioning islets. Neogenesis used in the context of diabetes is therefore generally understood to refer to islets and not just insulin-secreting β cells.

Islet neogenesis can be achieved by at least two kinds of intervention. *Gene therapy* involves altering genomic DNA so that the development of islets is turned on in some way. This approach has the inherent limitations of any gene therapy targeted at somatic cells—the proportion of genetically altered cells will steadily decline as the progeny of non-altered cells overwhelm the latter. This therapy therefore would generally require repetitive administration. Gene therapy can target transcription factors involved in the earliest stages of islet developmental control, or gene therapy may function as a delivery approach for a trophic hormone that triggers islet formation at a downstream locus.

The more practical approach for inducing neogenesis is to expose embryonic or adult *stem cells/progenitor cells* to conditions that will cause these cells to differentiate into islets. This could involve removing tissue, such as bone marrow³¹ or spleen,³² treating it in some manner, and re-injecting the activated stem cells contained therein either locally or systemically. More plausibly, trophic agents would be administered to activate stem cells that are further committed toward islet differentiation and are located within pancreatic tissue.

Embryogenesis and Development of Islets

Stem cells have the ability to divide in a way that leads to both differentiation of its progeny and maintenance of the stem cell population. It appears that truly undifferentiated or totipotent stem cells appear at the blastocyst stage of embryogenesis. During development, these early stem cells lose their totipotency to varying degrees. It is now well established that stem cells must exist in some form in every tissue that depends on cellular division to maintain its integrity.³³ In this context, the modifiers, stem and progenitor, have similar meanings but somewhat different connotations. A “progenitor cell” means a cell that is relatively more immediately proximate to the fully differentiated cell than the “stem cell” of the same tissue.

The embryonic development of the pancreas in mice illustrates the general concept of functional tissue developing from stem cells as well as the very relevant biology. The expression of various transcription factors is tied to the progressive differentiation of cells during development. These markers have allowed the delineation

of progenitor cells into mature pancreatic cells.³⁴⁻³⁵ During fetal pancreatic organogenesis, the proliferation of endocrine cells ensues from progenitor cells and not by the division of fully differentiated endocrine cells³⁶ (Figure 2). There is some debate about the nature and role of progenitor cells in the adult pancreas. Some evidence supports that β -cell replication is the predominate means of maintaining adult β -cell mass.³⁷⁻³⁸ In addition to insulin-secreting β cells, islets contain α cells, which produce glucagon; γ cells, which secrete pancreatic polypeptide; and δ cells, which secrete somatostatin.³⁹⁻⁴⁰

Islet Progenitor Cells

Despite continuing debate about the role of β -cell replication, abundant evidence supports the existence of islet progenitor cells in adult animals. Neogenesis has been observed in reaction to various stresses, including induced hyperglycemia,⁴¹ partial pancreatectomy,⁴² and partial obstruction of the pancreas by cellophane wrapping, which led to the discovery of INGAP.⁴³ The neogenesis observed under these and other conditions appears to originate from the pancreatic ducts, which serve the exocrine function of this organ.⁴⁰

Induction of Differentiation to Islets

As evidence for the persistence of islet progenitor cells in the mature pancreas mounts, the search has continued for agents that could induce these progenitor cells to develop into islets. Indeed, evidence of neogenesis activity has been reported for a number of hormones in animal models, including insulin-like growth factor-1 (IGF-1),^{44,45} prolactin,⁴⁶ gastrin,⁴⁷⁻⁴⁹ transforming growth factor- α ,⁵⁰⁻⁵³ epidermal growth factor,^{45,47,54} glucagon-like peptide-1 (GLP-1),⁵⁵⁻⁵⁷ exendin-4,⁵⁸⁻⁶² and INGAP peptide.^{26,63,64} It is important to note that some of these agents, such as GLP-1 and related agonists, directly influence metabolism. Their metabolic effect—lowering high levels of blood glucose—is mainly due to its stimulatory effect on insulin synthesis and release. They may thereby indirectly improve β -cell function through these metabolic effects in addition to direct trophic effects on the β cell. Demonstration of neogenesis in animal models does not necessarily mean that an agent is neogenic in humans.⁶⁵⁻⁶⁷

Islet Neogenesis Associated Protein

History

Islet neogenesis associated protein is the first therapeutic candidate to be identified as the result of a purposeful search for an endogenous molecule with islet neogenic activity.⁶⁸ It was found that partial obstruction of the

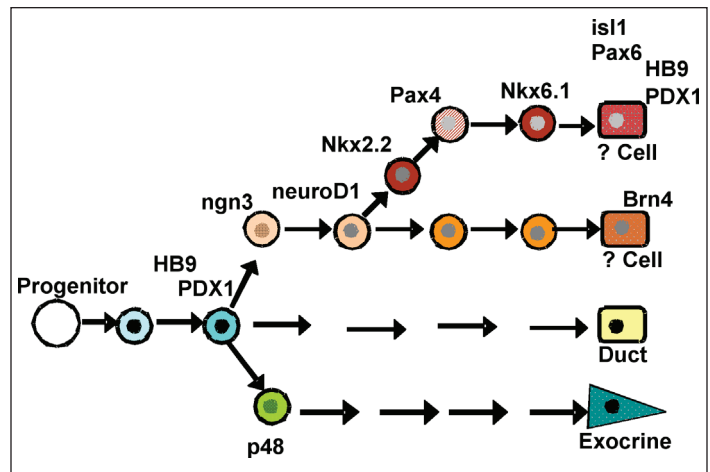


Figure 2: The association of islet transcription factors with stepwise differentiation of progenitor cells to islet endocrine cells and other pancreatic tissue. The proposed position for each transcription factor is based on its timing of expression, timing of predominant functional role, or both. Clearly some factors function at several steps, but a single step is shown for simplicity. From Wilson ME, Scheel D, German MS: Gene expression cascades in pancreatic development. *Mech Dev.* 120:65–80. Copyright 2003, with permission from Elsevier.

pancreatic duct of hamsters during surgery induced islet neogenesis. A parabiotic study, in which cross-circulation was surgically established between two animals—one with partial obstruction of the pancreatic duct—suggested that induction of cellular proliferation and differentiation involved paracrine signaling.⁶⁹ A neogenesis-promoting activity was identified under the condition of incomplete obstruction and partially purified from a soluble tissue fraction. The activity was termed ilotropin.⁷⁰ Using an mRNA differential display, a novel gene was identified, sequenced, and cloned.⁷¹ The 168-kDa protein product of this gene was found to be a major constituent of the ilotropin extract and to be responsible for the neogenesis activity.⁶⁶ This molecule was then named islet neogenesis associated protein.^{66,72,73} An active core sequence of 15 residues within INGAP was identified; antibodies raised against this peptide abolished the neogenesis activity in the ilotropin extract. Subsequent animal and human studies have been performed with this 15 amino acid peptide termed INGAP peptide.⁶⁶

INGAP—A Reg Protein

In parallel with INGAP research, investigations in different areas of interest converged with the discovery of the kinship of various regulatory proteins within and across species. A consequence of this interdisciplinary effort is that different names evolved for the same protein. Investigation of regeneration in the endocrine pancreas originated the Reg terminology.⁷⁴ A focus on exocrine proteins overexpressed during pancreatitis and other clinical conditions led to

the names pancreatitis-associated proteins,⁷⁵ pancreatic thread protein,⁷⁶ pancreatic stone protein,⁷⁷ lithostathine,⁷⁵ and hepatocarcinoma-intestine-pancreas protein.⁷⁸⁻⁷⁹ The exocrine focus led to the observation that the production of certain nonenzymatic proteins in the acinar tissue rises during chronic or acute pancreatitis.^{73,80} A number of activities have been ascribed to these proteins.^{73,81-85} Increased circulating Reg levels have been associated with pancreatitis and cystic fibrosis,⁷⁸ but no correlation has been found between serum levels of Reg and clinical markers of pancreatitis such as trypsin and lipase.⁸⁶

Reg proteins fall into three families, each named for the patriarchal protein, Reg I, Reg II, Reg III, and Reg IV⁸⁷ (see Figure 3). INGAP from hamster and a similar protein in mice, INGAP-related protein,⁸⁸ form a Reg subfamily. Reg genes have remarkably similar structural motifs. Except for the Reg IV family members, the components of the gene are clustered. Each gene characteristically spans 3 kilobases, contains 6 exons, and encodes a single 165–180 amino acid protein. Six cysteine residues are consistently conserved in most Reg proteins. Disulfide bonds form among these cysteine residues in a 1-2, 3-6, 4-5 pattern.⁸⁹ Finally, all Reg proteins possess a C-type lectin moiety.⁹⁰

INGAP is closely related to Reg III proteins and is almost certainly a member of that family.⁹¹ Reg III family members share a common five amino acid segment. Significantly, INGAP peptide shares this segment at the center of its 15 amino acid sequence.⁶⁶ Based on structural models, this segment would likely be found on an outside turn in the three-dimensional structure of the protein.⁹² It has been suggested that the presence of this segment

might substantially affect ligand binding by altering the distribution of these proteins.⁹⁰

A given species may have two or more Reg proteins in the same Reg family. For example, mice have two Reg I members (Reg I and Reg II) and four Reg III members (Reg3 α , Reg3 β , Reg3 δ , and Reg3 γ). In humans, Reg1 α and Reg 1 β are Reg I members, and Reg3 α and Reg3 γ are Reg III members. Reg proteins are widely distributed throughout the body, although expression patterns differ for individual family members.⁸⁹ In addition to the observed trophic effects of the pancreas, Regs have been implicated as neurotrophic agents.⁹³⁻⁹⁵ This activity is conceivably linked to observations suggesting that pancreatic sensory nerves have a fundamental etiologic role in islet autoimmunity.⁹⁶⁻⁹⁷

Reg Proteins and Islet Biology

Growing evidence links INGAP and other Reg proteins to the developmental control of both exocrine and endocrine tissue of the pancreas. Reg proteins are mitogenic in both islet and ductal cell lines, as well as in isolated islets and primary duct cultures.^{66,98,99} These proteins also have anti-apoptotic properties, specifically against oxidative stress.^{82,100} Most relevant is the association of Reg proteins with regeneration of pancreatic tissue. Reg I was first identified with screening of a rat islet cDNA library under conditions of islet regeneration.⁷⁴ Some Reg protein expression has been found in regenerating islets,¹⁰¹ but most expression occurs in acinar tissue.¹⁰² Reg I treatment improved glycemia in a surgically induced model of diabetes¹⁰³ and reduced the progression to diabetes in the nonobese diabetic (NOD) mouse model.¹⁰⁴ Increased endogenous Reg expression correlated with a delayed onset or prevention of overt diabetes in NOD mice.¹⁰⁵⁻¹⁰⁷ Reg genes are upregulated under multiple conditions of β -cell mass expansion and regeneration, including pancreas-specific IGF-1 knockout mice,¹⁰⁸ exendin-4 administration, and partial pancreatectomy.¹⁰⁹

It is interesting that a Reg I knockout mouse appeared to be normal except for a reduction in the proliferative index of isolated islets. The response to stimuli of islet proliferation was also reduced in these animals.¹¹⁰ However, islet proliferation was noted in transgenic mice expressing Reg I behind the insulin promoter. This Reg I transgenic mouse was crossed with NOD mice to produce NOD mice carrying the Ins-Reg transgene. It was found that development of diabetes was significantly retarded and β -cell mass increased in the resulting Ins-Reg transgenic progeny.⁶⁴ In yet another transgenic construct, INGAP was placed behind

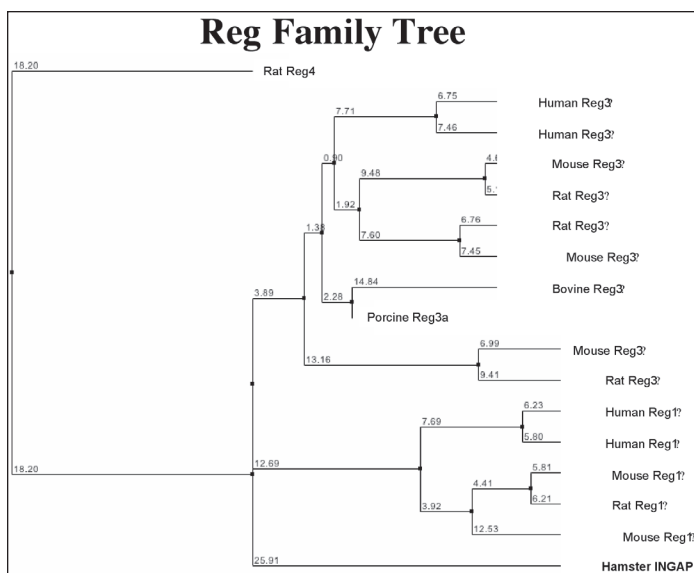


Figure 3: The structural relatedness of Reg proteins across and within species. Reg I, Reg II, and Reg III form the major categories. INGAP is a member of the Reg III family and is closest in amino acid structure to Reg3 α .

the elastase promoter. The result was increased β -cell mass and resistance to streptozotocin-induced diabetes.^{111,112}

INGAP Experimental Data

The identification of INGAP as an important element in the developmental biology of islets has led to three major lines of investigation: (1) study of the role of endogenous INGAP and related proteins in islet neogenesis, (2) use of exogenous INGAP and INGAP peptide as a tool for study of islet biology, and (3) evaluation of INGAP and INGAP peptide as a new therapy. The prospect of INGAP peptide as a pharmaceutical agent is supported by biologic activity in the INGAP pentapeptide and its close homology to those of related human proteins. This work addressed two underlying fundamental questions: (1) Why are islets not regenerated sufficiently in humans following immune destruction? (2) Can islet neogenesis be pharmacologically induced with a novel peptide? A progression of evidence for the role of INGAP in neogenesis and as a therapeutic approach is summarized next.

Studies of Endogenous INGAP

Hamster

INGAP immunoactivity has been noted in the pancreatic tissue of normal hamsters. Most of this activity is seen in acinar tissue while some activity is located within the exocrine ducts and on the periphery of islets, but not within them. Some INGAP expression was found within islet cells but coexpression of islet hormones within these cells was not seen.¹¹³ In hamsters, sucrose added to the drinking water causes increased insulin secretion and β -cell mass while normoglycemic blood levels are maintained. The presence of cytokeratin-positive (CK+) cells within the islets and other evidence suggest that islet neogenesis contributes to the increased β -cell mass. During the conditions of this model, INGAP expression increased in the three pancreas subsectors, namely acinar, ductal, and islet cells. INGAP expression was also associated with glucagon-positive cells in the periphery of islets, with cells of the acinar compartment, and frequently with CK+ cells.¹¹⁴ Sucrose administration to pregnant hamsters led to postnatal hyperinsulemia and lower serum glucose levels (but not hypoglycemia) in their pups from the increased β -cell mass that was induced by the *in utero* sucrose exposure. β -cell proliferation and islet neogenesis increased significantly in these animals.¹¹⁵

These hamster pups also showed a striking increase in the frequency of pancreatic and duodenal cells positive for homeobox gene-1 (PDX1). Within the pancreas, this marker of islet progenitor cells was observed in both

ductal and extra-insular cells. A subset of these PDX1+ cells was also positive for INGAP expression. These PDX1+/INGAP+ cells were found to be negative for all islets hormones and have a very high replication rate, thus suggesting that they probably were early precursor cells. This phenotype accounted for most of the increase in duct- and acinar-associated PDX1+ cells. This cellular phenotype provides evidence for a subpopulation of islet progenitors that express INGAP.¹¹¹

Relevant Data from Other Rodents

Lipsett and Finegood have developed a model of β -cell neogenesis in a rat model based on chronic glucose infusion.¹¹⁶ Immunohistology of tissue from their original study using a polyclonal anti-Reg3 α antibody revealed staining localized in the ducts, but also in acini. Peak activity occurred about 48 hours prior to the observed increase in β -cell mass.⁶ Wang and colleagues suggested the BioBreeding (BBdp) rat as another neogenesis model. They noted an increased abundance of tubular complexes in these animals during the progression of metabolic disease.¹¹⁷ These tubular complexes are postulated to be the result of acinar-to-duct dedifferentiation.¹¹⁸⁻¹²² After observing both amylase+/CK+ and CK+/insulin+ cells and increased expression of both stem and endocrine progenitor cell markers, the authors concluded that the tubular complexes reflected regeneration via islet neogenesis. Subsequent immunohistochemistry of tissues from these pups revealed Reg3 α staining of ducts and acinar tissue. Staining within tubular complexes was particularly prominent.¹²³ These results from two different rat models support the role of a Reg protein closely related to INGAP in islet neogenesis. The temporal sequence of Reg3 expression and neogenesis is consistent with an inductive role of Reg proteins in the neogenesis process.

INGAP Peptide Treatment in Rodents

Daily intraperitoneal injection of normoglycemic hamsters with INGAP peptide was associated with evidence of islet neogenesis within 10 days after start of treatment.⁶⁴ Evidence of neogenesis included increases in islet frequency, total β -cell mass, and both duct- and acinar-associated β -cell mass. This expansion of β -cell mass continued for at least 30 days and then appeared to reach a plateau.

As a requirement for performing clinical trials, acute and subchronic toxicology studies were performed in mice, dogs, and monkeys. These studies provided the opportunity to evaluate INGAP activity in healthy animals. Examination of evidence for islet neogenesis included quantification of β -cell mass and tissue insulin content. In the 90-day study conducted in healthy mice, a dose-related increase in both

duct- and acinar-associated single or small β -cell clusters was observed. A similar pattern was seen for focal areas of neogenesis (FANs), a term originally described in diabetic animals by Lipsett and Finegood.¹¹⁶ Dose-related increases in FANs were associated with both ducts and acinar tissue. The peak effect was generally reached in the 100-mg/kg treated animals with no further increase in the 200-mg/kg animals.¹²⁴ These structures are thought to reflect neogenic activity.^{42,125} Peak effects were generally reached in animals dosed at 100 mg/kg daily, with no further increase noted in the 200-mg/kg group. Additional results are included later under the discussion of the monkey results.

INGAP peptide was tested in hyperglycemic mice rendered diabetic by streptozotocin. Hyperglycemia was reversed by 6 weeks in all four animals receiving INGAP peptide treatment. Significant increases were observed in ductal- and acinar-associated neogenesis and β -cell mass.¹¹⁶

Nonobese diabetic mice were treated with INGAP peptide to evaluate activity in an established model of T1DM. Because these animals have an intense islet autoimmunity as the cause of diabetes, it was questioned whether INGAP peptide treatment alone could induce a neogenic response. The animals therefore received INGAP peptide (500 μ g every day intraperitoneally) alone or with immunosuppression (0.1 mg/kg tacrolimus + 0.1 mg/kg sirolimus every day by gavage). In contrast to the acutely diabetic NOD mice used in most immune intervention studies,¹²⁶ the animals in this study were chronically diabetic with blood glucose levels >28 mM for 6 weeks before treatment was begun. The animals used in the study therefore approximated chronic diabetes, which presumably would be more resistant to therapy than new-onset diabetes. INGAP peptide alone was found to improve glycemia and there was some additional effect shown with INGAP peptide in combination with immunosuppression as compared to immunosuppression alone. Moreover, compared to immunosuppression only, treatment with INGAP peptide prolonged survival of these animals.¹²³

INGAP Peptide in Healthy Dogs

In addition to the 90-day toxicology study in mice mentioned earlier, a 34-day toxicology study of INGAP peptide was conducted in healthy dogs (but unlike in mice and monkeys, no 90-day dog study was conducted). In this shorter dog study and consistent with the findings in mice, FANs were observed at the top 10-mg/kg dose group. A dose-ranging study of INGAP peptide was performed in 40 dogs (20 males, 20 females). They were randomized to four daily injected treatment groups (vehicle alone, 0.5, 1.5, or 10 mg/kg INGAP peptide). After 30 days of treatment, pancreatic tissues

were analyzed with immunohistochemistry for insulin content, PDX-1 (the essential islet transdifferentiation transcription factor) and PGP9.5 (a pan-neuroendocrine marker).

In animals treated with INGAP peptide, the percentage of insulin positive cells increased significantly. The 1.5-mg/kg dose produced the highest response—more than doubling the frequency of the controls ($p < 0.05$). Females were also found to be significantly ($p < 0.01$) more responsive than males to the peptide. PGP9.5 staining was increased in large ducts of treated animals, suggesting development of neuroendocrine cells from ductal elements in response to INGAP peptide. PDX-1 was found in isolated cells in ducts and was increased in treated pancreas as well. The increased concurrent expression of PDX-1 in duct cells suggests a role for INGAP at an early stage in islet development.

INGAP Peptide in Healthy Monkeys

As mentioned earlier, a 90-day toxicology study was conducted in healthy cynomolgus monkeys. As in the mouse and dog toxicology studies, pancreatic tissue was examined for evidence of islet neogenesis and included quantification of β -cell mass and tissue insulin content. As was the case in mice and dogs, no difference in total β -cell mass or tissue insulin content was seen in the monkey toxicology study. The presence of FANs in monkeys was more sporadic in dogs, but even more significant in its therapeutic implications for humans. An area of neogenesis, as characterized by a large region of tubular complex formation, was observed in 4 out of 10 monkeys administered the high dose of INGAP peptide (400 mg/kg body weight per day). This area appeared to involve a full pancreatic lobule and the majority of this area was positive for insulin.

Although the study populations are small, these data taken together suggest that INGAP peptide has neogenesis activity in normal animals, which could be expected to be more resistant to induced neogenesis activity than diabetic animals. Similar to other tissues, β -cell mass in the absence of disease appears to be maintained at relatively constant levels but changes in response to physiologic or environmental conditions as needed.¹²⁷⁻¹²⁹ This is likely achieved in normal animals by multiple levels of control applied to islet neogenesis and β -cell growth, atrophy, mitosis, and apoptosis.¹³⁰ The lack of observed net increases in β -cell mass in the face of induced neogenesis in these animals could plausibly be explained by the homeostatic control of these processes.^{127,131,132}

Perhaps more important to supporting the potential value of INGAP peptide as a therapy for people with diabetes is the

direct observation of neogenesis attributable to this peptide in another primate. Comparison of responses to INGAP peptide in monkey and mouse suggests some difference in neogenesis potency.

Effects of INGAP in Human Tissue Culture

Evidence for the neogenic potential of INGAP peptide in humans comes from observations made using cultured human tissue. After isolation of adult human islets, specific culture conditions are used to induce a phenotypic change to highly proliferative duct-like structures (DLS), characterized by the loss of expression of islet-specific hormones and transcription factors, as well as appearance of markers of both duct epithelial and progenitor cells. Based on electron microscopy and immunofluorescent staining, it would appear that transdifferentiating α and δ cells and, to a lesser degree, pancreatic polypeptide cells give rise to the cells of the DLS. Short-term treatment of undifferentiated DLS with INGAP peptide induces their redifferentiation back to islet-like structures (ILS). These ILS resemble freshly isolated human islets with respect to morphology, islet gene expression, hormone production, insulin content, and glucose-stimulated insulin secretion.^{26,133}

This approach extends a fascinating line of investigation, which has broad significance to developmental biology in general and for practical therapeutic applications, which are discussed later. This work and others establish the plasticity of the developmental system that generates and maintains the endocrine and exocrine tissues of the pancreas.¹³⁴⁻¹³⁸ Presumably the demonstration that INGAP peptide can induce human-derived tissue to differentiate into functionally normal islets predicts that humans have the signaling elements necessary for responsiveness to the neogenic effects of INGAP. These data also indirectly support that adults continue to have potentially responsive progenitor cells within the pancreas (Figure 4).

This human-derived tissue also provides a basis for an *in vitro* assay for neogenesis activity. Use of this assay has produced some intriguing if not surprising results: while a synthetic peptide derived from a hamster protein, INGAP peptide, is sufficient to induce neogenesis, the corresponding peptide sequences from the human homologues, Reg3 α and Reg3 γ , do not exhibit this activity. Moreover, islet regeneration in this model is unresponsive to GLP-1, but does respond to both gastrin and hepatocyte growth factor.¹³⁹ Preliminary data also show that Reg3 α does not have activity in this assay.¹²³ Some recent clinical observations are relevant. Reg III protein has been implicated in the case report of hyperinsulinemia

following pancreatic transplant. Florid nesidioblastosis, a rare condition of islet hyperplasia almost always confined to very young children, was found in this patient's pancreas. Neogenic tissue demonstrated heavy staining with a Reg III antibody raised against INGAP¹⁴⁰ but this likely reflects the presence of a closely related Reg III protein. Among the reports of gastric bypass-associated nesidioblastosis cited earlier, these tissues also stained very heavily with this same antibody.

Other Effects of INGAP

Before discussing clinical data from INGAP peptide, it is noteworthy that INGAP peptide may have direct effects on β -cell function. It has been reported that INGAP peptide increases both basal and stimulated insulin secretion from isolated rat islets.¹⁴¹ These effects were observed in islets from both neonatal and adult rats after as little as a 90-minute incubation period. The maximum effect was reached over 4 days of incubation at concentrations in the mid to high nanomolar range. In contrast to GLP-1 agonists, these secretagogue effects were not associated with any effect on cell survival, although administration of INGAP peptide did induce hypertrophy but not hyperplasia of both β and non- β cells within the islet.

INGAP in Human Clinical Trials

Islet neogenesis associated protein peptide was first tested in a rising single-dose safety study. Thirty patients with T1DM or T2DM received single subcutaneous injections; dose levels were 0, 7.5, 15, 30, 60, and 120 mg. A second study

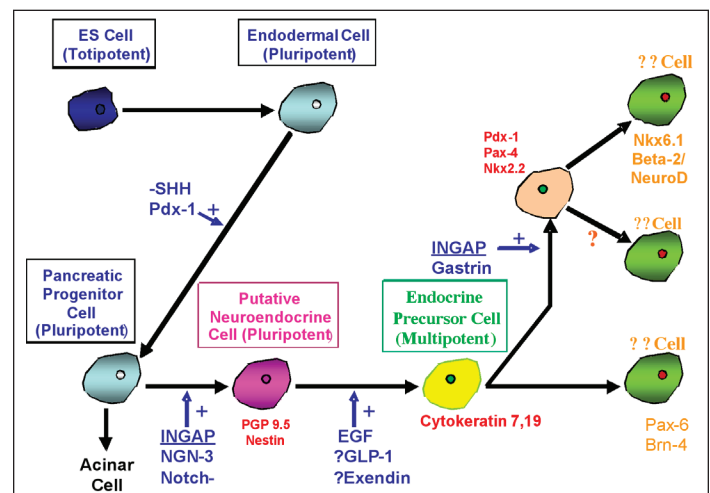


Figure 4: A speculative model for progenitor cell development similar to Figure 1 but with apparent sites indicated for induction by trophic agents. Evidence for these sites and relationships comes primarily from rodent studies. The existence of a pluripotent neuroendocrine cell is suggested by some data but has not been established. Available evidence suggests that humans differ in responsiveness to trophic agents. INGAP appears to work relatively early in this cascade. Used with permission from Gary Pittenger.

administered 34 daily doses to 33 patients randomized to placebo, 75, 30, or 120 mg. These doses were well tolerated by all subjects. The half-life of INGAP peptide was determined to be about 90 minutes. There was no clear effect on basal or stimulated C-peptide levels at these phase 1 dose levels.

Two phase 2 trials, one in patients with T1DM and one in T2DM, were then conducted to evaluate the efficacy and safety of INGAP. These trials have been reported in abstracts and full publication is planned by the investigators. In both trials, subjects were randomized to injections of 300 or 600 mg of INGAP peptide versus placebo, and all received single subcutaneous injections for 90 days. The T1DM study enrolled 63 patients, and the T2DM study enrolled 126 patients. The primary efficacy end point was the treatment effect on endogenous insulin secretion as measured by arginine-stimulated C-peptide at baseline and day 120, which was 30 days after end of treatment. HbA1c (measured at day 90 and day 120) and the average daily insulin dose were secondary end points.

In T1DM patients receiving 600 mg daily, there was an increase in stimulated arginine C-peptide response by day 56. The mean change from baseline approached statistical significance ($p = 0.07$) and the pair-wise comparison difference versus placebo was highly significant ($p = 0.0058$). For T2DM patients, there was no statistically significant difference in comparison to placebo for stimulated arginine C-peptide response, and essentially no changes were noted from baseline for the active treatments groups. By day 120, however, the placebo group declined significantly from baseline, resulting in a trend toward a significant difference in the pair-wise comparison of the 600-mg and placebo groups.

In the T1DM trial, overall glycemic control as measured by HbA1c decreased by 0.5 units from baseline to day 90 in the 600-mg treatment group ($p < 0.01$). HbA1c decreased slightly in the placebo and 300-mg groups. By day 120 (30 days after the end of therapy) about half of each of these treatment effects was lost. Average daily glucose levels, as determined by a self-monitored blood glucose meter, fell by 2.15% from baseline in the 600-mg group and rose 0.103% in the placebo at day 90. However, these changes did not reach statistical significance. In the T2DM trial, the effect on HbA1c of the 600-mg treatment group showed a pattern similar to that seen in the T1DM trial, but the effect was greater in magnitude (change from baseline = 0.91 HbA1c units) and greater in statistical significance ($p < 0.01$). Although the placebo group also improved, the pair-wise comparison was still statistically significant ($p = 0.04$) and the difference (treatment effect) was 0.55 HbA1c units (0.8 HbA1c units for the difference between

300- and 600-mg groups). In support of this finding, the 600-mg group mean daily glucose levels as determined by a self-monitored blood glucose meter fell by 9.8% from baseline and 12.6% from placebo at day 90 ($p < 0.01$).

In both trials, no consistent treatment effects on fasting glucose, insulin, and C-peptide were seen. In the T1DM trial, the total insulin dose did appear to fall in a dose-dependent manner from baseline to day 90 with changes of +0.10, -0.28, and -2.15% for the placebo, 300-mg, and 600-mg groups, respectively. The treatment effect for the 600-mg group was about 1.0 unit. However, none of these baseline or pair-wise comparisons was statistically significant. No effect on insulin dose was observed in the T2DM trial.

Anti-GAD antibody titers were monitored in both trials to assure that islet cell destruction was not being stimulated. In the T1DM trial, five, seven, and four patients in each treatment group had positive tests at baseline. Two patients in the 300-mg dose group showed an increase in titer by the end of the study, and one patient in the 600-mg group who had a negative result at baseline converted to seropositive.

Injection site reactions were common and contributed to a significant drop-out rate. T2DM patients had less injection site reactions than T1DM patients. Except for these injection site reactions, the treatment was well tolerated and no evidence of systemic toxicity was observed.

The patterns of response for the two major measures of efficacy—stimulated C-peptide and HbA1c—were similar across both trials, but differed in levels of statistical significance. The latter can be explained by differences between the trials in statistical power for these outcomes. The low variability of the baseline C-peptide levels in the T1DM trial resulted in greater statistical power for this outcome than for the T2DM trial, in which variability was very high. Variability of HbA1c was similar across both studies, but the T2DM trial had twice the number of randomized subjects and fewer drop outs than the T1DM trial. It is therefore possible that the underlying biologic effects of treatment were similar in both studies. The best evidence for islet neogenesis per se comes from the highly statistically significant treatment effect on stimulated C-peptide secretion and the trend toward a reduction in insulin dose seen in the T1DM study. The absolute effect on this measure of endogenous insulin secretion was small but approaches clinical meaningfulness as discussed by Palmer *et al.*¹⁴² Given the once-daily injection used in this trial and the relatively short half-life of the peptide, it is likely that better results and/or improved bioefficacy could be achieved with multiple daily injections, constant infusion, or a depot formulation.

Unanswered Questions and Looking Forward

Much progress has been made in understanding the mysteries of islet biology among vertebrates. Within the context of human health and disease, however, unanswered questions surrounding the birth, development, and death of islets abound. The encouraging conclusion is that humans incontrovertibly have, under some circumstances, the capacity for islet neogenesis. Neogenesis therapy is therefore not a matter of "if" but of "when" and "how." Short of a safe, fully effective, and practical prevention of T1DM, islet neogenesis or a related form of stem cell therapy is a necessary, although probably not entirely sufficient, means of curing T1DM. Therapeutic control or reversal of islet autoimmunity will likely be an important adjunct to any neogenesis treatment of established T1DM. For people with incipient or established T1DM, immunomodulatory therapy can only preserve what secretory function remains, which is typically very little in most T1DM and many T2DM patients today. Many people with insulin-dependent T2DM also have minimal remaining β -cell function. Functioning islet tissue can be surgically returned in the form of either human or encapsulated animal islets, but these have significant limitations and technical challenges. Autologous *ex vivo* regeneration and transplantation of islets is a hybrid and intermediate approach that also requires a means of inducing neogenesis.

While the need of a neogenesis therapy for people with T2DM is not as pressing as for those with T1DM, it is still important and is anticipated to become increasingly important for younger patients with T2DM. Although therapies are now available that slow the progressive loss of β -cell function, preliminary evidence suggests that these therapies will not restore lost function. To the extent that β -cell function continues to decline, despite the use of these therapies, a safe and effective neogenesis therapy will be important in restoring health in people with T2DM.

Is INGAP peptide a practical therapeutic approach? The available evidence is encouraging. Optimization of the therapy and more evidence are needed. An associated puzzle persists, and it goes to the heart of understanding why humans lose the capacity to regenerate lost islets. Although abundant evidence in other animals suggests the importance of Reg proteins to normal islet development, the endogenous expression of human Reg proteins, at least during islet autoimmunity, does not lead to clinically meaningful islet regeneration. Furthermore, some preliminary evidence indicates that the homologue

of INGAP, Reg3 α , and its corresponding sequence to INGAP peptide have no neogenesis activity in a human islet-derived *in vitro* assay. However, a closely related but nonendogenous protein, INGAP, and its core pentapeptide do show evidence of neogenesis activity in several species, including humans. Resolving this apparent paradox with dissection of structure/function relationships among the cross-species signaling pathways could lead to more effective and convenient therapeutic approaches. The urgent needs are to optimize therapy of a molecule in hand that has shown evidence of safety and efficacy and, if safety and effectiveness are confirmed, to deliver this new therapy to people who need it. Optimized INGAP peptide therapy is unlikely to be the ultimate neogenesis agent, but it may well lead to it. The quest for a fundamental restorative approach to lost insulin secretion is an achievable goal, but one that will be reached by taking steps, not leaps.

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References:

1. Atkinson MA. ADA Outstanding Scientific Achievement Lecture 2004. Thirty years of investigating the autoimmune basis for type 1 diabetes: why can't we prevent or reverse this disease? *Diabetes*. 2005;54:1253-63.
2. Porte D, Kahn SE. β -cell dysfunction and failure in type 2 diabetes: potential mechanisms. *Diabetes*. 2001;50:S160-3.
3. Trucco M. Regeneration of the pancreatic beta cell. *J Clin Invest*. 2005;115:5-12.
4. Bonner-Weir S, Weir GC. New sources of pancreatic beta-cells. *Nature Biotechnol*. 2005;23:857-61.
5. Meier JJ, Bhushan A, Butler PC. The potential for stem cell therapy in diabetes. *Pediatr Res*. 2006;59:65-73.
6. Bonner-Weir S, Sharma A. Are there pancreatic progenitor cells from which new islets form after birth? *Nature Clin Prac Endo Metab*. 2006;2:240-1.
7. Lechner A, Habener JF. Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus. *Am J Physiol Endocrinol Metab*. 2003;284:259-66.
8. Gough SC. A review of human and analogue insulin trials. *Diabetes Res Clin Pract*. 2006 Nov 15.
9. Bjork S, Kapur A, King H, Nair J, Ramachandran A. Global policy: aspects of diabetes in India. *Health Policy*. 2003; 66:61-72.
10. Kleinfeld, NR. Costs of a crisis: diabetics confront a tangle of workplace laws. *New York Times*. 2006 December 26.
11. Arulmozhi DK, Portha B. GCP-1 based therapy for type 2 diabetes. *Eur J Pharm Sci*. 2006;28:96-108.
12. Leichter SB. The business of insulin: a relationship between innovation and economics. *Clin Diabetes*. 2003; 21:1.
13. Carpenter T, Trautmann ME, Baron AD. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med*. 2005;353:249-54.

14. Boye J, Geiss L, Honeycutt A. Projection of diabetes burden through 2050. *Diabetes Care*. 2001;24:1936-40.
15. Omer A, Duvivier-Kali VF, Trivedi N, Wilmot K, Bonner-Weir S, Weir GC. Survival and maturation of microencapsulated porcine neonatal pancreatic cell clusters transplanted into immunocompetent diabetic mice. *Diabetes*. 2003;52:69-75.
16. Schneider S, Feilen PJ, Brunnenmeier F, Minnemann T, Zimmermann H, Zimmermann U, Weber MM. Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. *Diabetes*. 2005;54:687-93.
17. Hirshberg B, Rother KI, Digion III BJ, Venstrom J, Harlan DM. State of the art: islet transplantation for the cure of type 1 diabetes mellitus. *Rev Endocr Metab Disord*. 2003;4:381-9.
18. Shapiro AM, Lakey JP, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*. 2000;343:230-8.
19. Rother KI, Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. *J Clin Invest*. 2004;114:877-83.
20. Shapiro AMJ, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbitt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*. 2006;355:1318-30.
21. De Vos P, Van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? *Diabetes*. 1999;48:1381-8.
22. King A, Lau J, Nordin A, Sandler S, Andersson A. The effect of capsule composition in the reversal of hyperglycemia in diabetic mice transplanted with microencapsulated allogeneic islets. *Diabetes Technol Ther*. 2003;50:653-63.
23. Hanaire H. Continuous glucose monitoring and external insulin pump: towards a subcutaneous closed loop. *Diabetes Metab*. 2006;32:534-8.
24. Hirshberg B, Rother KI, Harlan DM. Islet transplantation: where do we stand now? *Diabetes Metab Res Rev*. 2003;19:175-8.
25. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes*. 2001;50:1691-7.
26. Hoffman LM, Carpenter MK. Characterization and culture of human embryonic stem cells. *Nat Biotechnol*. 2005;23:669-708.
27. D'Amour KA, Bang KG, Eliazar S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol*. 2006;24:1392-401.
28. Jamal AM, Lipsett M, Sladek R, Laganieri S, Hanley S, Rosenberg L. Morphogenetic plasticity of adult human pancreatic islets of Langerhans. *Cell Death Differ*. 2005;12:702-12.
29. Cryer PE. Glucose counterregulation: the prevention and correction of hypoglycemia in humans. *Am J Physiol*. 1993;264:E144-55.
30. Bolli GB, Fanelli CG. Physiology of glucose counter-regulation to hypoglycemia. *Endocrinol Metab Clin North Am*. 1999;28:467-93.
31. Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thyssen S, Gray DA, Bhatia M. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol*. 2003;21:763-70.
32. Kodama S, Kührtreiber W, Fujimura S, Dale ES, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science*. 2003;302:1223-7.
33. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? *Cell*. 2001;105:829-41.
34. Edlund H. Pancreatic organogenesis developmental mechanisms and implications for therapy. *Nat Rev Genet*. 2002;3:524-32.
35. Thomas MK, Habener JF. Pancreas development. *Immunol Mediat Endocr Dis*. 2002;141-66.
36. Polak M, Bouchareb-Banaei L, Scharfmann R, Czernichow P. Early pattern of differentiation in the human pancreas. *Diabetes*. 2000;49:225-32.
37. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. 2005;433:286-9.
38. Yuval D. β -cell proliferation is the major source of new pancreatic cells. *Nature Clin Prac Endo Metab*. 2006;2:242-3.
39. Wells JM. Genes expressed in the developing endocrine pancreas and their importance for stem cell and diabetes research. *Diabetes Metab Res Rev*. 2003;19:191-201.
40. Jensen J. Gene regulatory factors in pancreatic development. *Dev Dyn*. 2004;229:176-200.
41. Bernard C, Berthault MF, Saulnier C, Ktorza A. Neogenesis vs. apoptosis as main components of pancreatic beta cell mass changes in glucose-infused normal and mildly diabetic adult rats. *FASEB J*. 1999;13:1195-1205.
42. Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE. A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. *Diabetes*. 1993;42:1715-20.
43. Rosenberg L, Brown RA, Duguid WP. A new approach to the induction of duct epithelial hyperplasia and nesidioblastosis by cellophane wrapping of the hamster pancreas. *J Surg Res*. 1983;35:63-72.
44. Smith FE, Rosen KM, Villa-Komaroff L, Weir GC, Bonner-Weir S. Enhanced insulin-like growth factor I gene expression in regenerating rat pancreas. *Proc Natl Acad Sci U S A*. 1991;88(14):6152-6.
45. George M, Ayuso E, Casellas A, Costa C, Devedjian JC, Bosch F. Beta cell expression of IGF-I leads to recovery from type 1 diabetes. *J Clin Invest*. 2002;109(9):1153-63.
46. Nielsen JH, Svensson C, Galsgaard ED, Moldrup A, Billestrup N. Beta cell proliferation and growth factors. *J Mol Med*. 1999;77(1):62-6.
47. Brand SJ, Tagerud SLambert P, Magil SG, Tatarkiewicz K, Doiron K, Yan Y. Pharmacological treatment of chronic diabetes by stimulating pancreatic beta-cell regeneration with systemic co-administration of EGF and gastrin. *Pharmacol Toxicol*. 2002;91(6):414-20.
48. Rومان I, Lardon J, Bouwens L. Gastrin stimulates beta-cell neogenesis and increases islet mass from transdifferentiated but not from normal exocrine pancreas tissue. *Diabetes*. 2002;51(3):686-90.
49. Suarez-Pinzon WL, Lakey JR, Brand SJ, Rabinovitch A. Combination therapy with epidermal growth factor and gastrin induces neogenesis of human islet beta-cells from pancreatic duct cells and an increase in functional beta-cell mass. *J Clin Endocrinol Metab*. 2005;90(6):3401-9.
50. Sandgren EP, Luetke NC, Palmiter RD, Brinster RL, Lee DC. Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell*. 1990;61(6):1121-35.
51. Wang TC, Bonner-Weir S, Oates PS, Chulak M, Simon B, Merlino GT, Schmidt EV, Brand SJ. Pancreatic gastrin stimulates islet differentiation of transforming growth factor alpha-induced ductular precursor cells. *J Clin Invest*. 1993;92(3):1349-56.
52. Wang RN, Rehfeld JF, Nielsen FC, Kloppel G. Expression of gastrin and transforming growth factor-alpha during duct to islet cell differentiation in the pancreas of duct-ligated adult rats. *Diabetologia*. 1997;40(8): 887-93.

53. Song SY, Gannon M, Washington MK, Scoggins CR, Meszoely I, Goldenring JR, Marino CR, Sandgren EP, Coffey RJ, Jr, Wright CV, Leach SD. Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology*. 1999;117(6):1416-26.
54. Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. *Diabetes*. 2003;52(1):124-32.
55. Pospisilik JA, Martin J, Doty T, Ehses JA, Pamir N, Lynn FC, Piteau S, Demuth HU, McIntosh CH, Pederson RA. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes*. 2003;52(3):741-50.
56. Nauck MA. Glucagon-like peptide 1 (GLP-1) in the treatment of diabetes. *Horm Metab Res*. 2004;36(11-12):852-8.
57. Gallwitz B. Glucagon-like peptide-1 as a treatment option for type 2 diabetes and its role in restoring beta-cell mass. *Diabetes Technol Ther*. 2005;7(4):651-7.
58. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*. 1999;48(12):2270-6.
59. Turrel C, Bailbe D, Meile MJ, Kergoat M, Portha B. Glucagon-like peptide-1 and exendin-4 stimulate beta-cell neogenesis in streptozotocin-treated newborn rats resulting in persistently improved glucose homeostasis at adult age. *Diabetes*. 2001;50(7):1562-70.
60. Holz GG, Chepurny OG. Glucagon-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. *Curr Med Chem*. 2003;10(22):2471-83.
61. Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes DG, Young AA. Exenatide (exendin-4) improves insulin sensitivity and [beta]-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. *Endocrinology*. 2005;146(4):2069-76.
62. Xu G, Kaneto H, Lopez-Avalos MD, Weir GC, Bonner-Weir S. GLP-1/exendin-4 facilitates beta-cell neogenesis in rat and human pancreatic ducts. *Diabetes Res Clin Pract*. 2006 Jul;73(1):107-10.
63. Lipsett M, Hanley S, Radzioch D, Compton O, Wang GS, Scott F, Fine-Goed D, Rosenberg L. INGAP: A critical mediator of islet neogenesis? *Diabetes*. 2003;52(Suppl 1):A360.
64. Rosenberg L, Lipsett M, Yoon JW, Prentki M, Wang R, Jun HS, Pittenger GL, Taylor-Fishwick D, Vinik AI. A pentadecapeptide fragment of islet neogenesis-associated protein increases beta-cell mass and reverses diabetes in C57BL/6J mice. *Ann Surg*. 2004;240(5):875-84.
65. Service FJ, Thompson GB, Service FJ, Andrews JC, Collazo-Clavell ML, Lloyd RV. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med*. 2005;353(3):249-54.
66. Kaiser AM. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med*. 2005;353(20):2192-4.
67. Carpenter T, Trautmann ME, Baron AD. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *N Engl J Med*. 2005;353(20):2192-4.
68. Rafaeloff R, Pittenger GL, Barlow SW, Qin XF, Yan B, Rosenberg L, Duguid WP, Vinik AI. Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. *J Clin Invest*. 1997;99(9):2100-109.
69. Rosenberg L, Duguid WP, Brown RA, Vinik AI. Induction of nesidioblastosis will reverse diabetes in the Syrian golden hamster. *Diabetes*. 1988;37:334-41.
70. Pittenger GL, Rosenberg L, Vinik AI. Partial purification and characterization of ilotropin, a pancreatic-islet specific growth factor. *J Cell Biol*. 1991;115:270a.
71. Rafaeloff R, Qin XF, Barlow SW, Rosenberg L, Vinik AI. Identification of differentially expressed genes induced in pancreatic islet neogenesis. *FEBS Lett*. 1996;378:219-23.
72. Rosenberg L, Rafaeloff R, Clas D, Kakugawa Y, Pittenger GL, Vinik AI, Duguid WP. Induction of islet cell differentiation and new islet formation in the hamster. Further support for a ductular origin. *Pancreas*. 1996;13:38-46.
73. Vinik AI, Rafaeloff R, Pittenger GL, Rosenberg L, Duguid W. Induction of pancreatic islet neogenesis. *Horm Metab Res*. 1997;29:278-93.
74. Terazono K, Yamamoto H, Takasawa S, Shiga K, Yonemura Y, Tochino Y, Okamoto H. A novel gene activated in regenerating islets. *J Biol Chem*. 1988;263(5):2111-4.
75. Iovanna J, Orelle B, Keim V, Dagorn JC. Messenger RNA sequence and expression of rat pancreatitis-associated protein, a lectin-related protein overexpressed during acute experimental pancreatitis. *J Biol Chem*. 1991;266(36):24664-9.
76. Gross J, Carlson RI, Brauer AW, Margolies MN, Warshaw AL, Wands JR. Isolation, characterization, and distribution of an unusual pancreatic human secretory protein. *J Clin Invest*. 1985;76(6):2115-26.
77. Sarles H, de Caro A, Multinger L, Martin E. Giant pancreatic stones in teetotal women due to absence of the "stone protein"? *Lancet*. 1982;2(8300):714-5.
78. Lasserre C, Christa L, Simon MT, Vernier P, Brechot C. A novel gene (HIP) activated in human primary liver cancer. *Cancer Res*. 1992;52(18):5089-95.
79. Motoo Y, Itoh T, Su SB, Nakatani MT, Watanabe H, Okai T, Sawabu N. Expression of pancreatitis-associated protein (PAP) mRNA in gastrointestinal cancers. *Int J Pancreatol*. 1998;23(1):11-6.
80. Orelle B, Keim V, Masciotra L, Dagorn JC, Iovanna JL. Human pancreatitis-associated protein. Messenger RNA cloning and expression in pancreatic diseases. *J Clin Invest*. 1992;90(6):2284-91.
81. Multigner L, De Caro A, Lombardo D, Campese D, Sarles H. Pancreatic stone protein, a phosphoprotein which inhibits calcium carbonate precipitation from human pancreatic juice. *Biochem Biophys Res Commun*. 1983;110:69-74.
82. Bimmler D, Graf R, Scheele GA, Frick TW. Pancreatic stone protein (lithostathine), a physiologically relevant pancreatic calcium carbonate crystal inhibitor? *J Biol Chem*. 1997;272(5):3073-3082.
83. Dieckgraefe BK, Crimmins DL, Landt V, Houchen C, Anant S, Porche-Sorbet R, Ladenson JH. Expression of the regenerating gene family in inflammatory bowel disease mucosa: Reg I alpha upregulation, processing, and antiapoptotic activity. *J Invest Med*. 2002;50(6):421-34.
84. Vasseur S, Folch-Puy E, Hlouschek V, Garcia S, Fiedler F, Lerch MM, Dagorn JC, Closa D, Iovanna JL. p8 improves pancreatic response to acute pancreatitis by enhancing the expression of the anti-inflammatory protein pancreatitis-associated protein I. *J Biol Chem*. 2004;279(8):7199-207.
85. Christa L, Carnot F, Simon MT, Levavasseur F, Stinnakre MG, Lasserre C, Thepot D, Clement B, Devinoy E, Brechot C. HIP/PAP is an adhesive protein expressed in hepatocarcinoma, normal Paneth, and pancreatic cells. *Am J Physiol*. 1996;271(6 Pt 1):G993-1002.
86. Carrere J, Guy-Crotte O, Gaia E, Figarella C. Immunoreactive pancreatic Reg protein in sera from cystic fibrosis patients with and without pancreatic insufficiency. *Gut*. 1999;44(4):545-51.
87. Graf R, Schiesser M, Reding T, Appenzeller P, Sun LK, Fortunato F, Perren A, Bimmler D. Exocrine meets endocrine: pancreatic stone protein and regenerating protein—two sides of the same coin. *J Surg Res*. 2005;80(3):1120-2.

88. Sasahara K, Yamaoka T, Moritani M, Yoshimoto K, Kuroda Y, Itakura M. Molecular cloning and tissue-specific expression of a new member of the regenerating protein family, islet neogenesis-associated protein-related protein. *Biochem Biophys Acta*. 2000;1500:142.
89. Itoh T, Tsuzuki H, Katoh T, Teraoka H, Matsumoto K, Yoshida N, Terazono K, Watanabe T, Yonekura H, Yamamoto H, et al. Isolation and characterization of human reg protein produced in *Saccharomyces cerevisiae*. *FEBS Lett*. 1990;272(1-2):85-8.
90. Drickamer K. Two distinct classes of carbohydrate-recognition domains in animal lectins. *J Biol Chem*. 1988;263(20):9557-60.
91. Okamoto H. The Reg gene family and Reg proteins with special attention to the regeneration of pancreatic beta-cells. *J Hepatobiliary Pancreat Surg*. 1999;6(3):254-62.
92. Laurine E, Manival X, Montgelard C, Bideau C, Berge-LeFranc JL, Erard M, Verdier JM. PAP IB, a new member of the Reg gene family: cloning, expression, structural properties, and evolution by gene duplication. *Biochem Biophys Acta*. 2005;1727(3): 177-87.
93. Namikawa K, Fukushima M, Murakami K, Suzuki A, Takasawa S, Okamoto H, Kiyama, H. Expression of Reg/PAP family members during motor nerve regeneration in rat. *Biochem Biophys Res Commun*. 2005;332:126-34.
94. Nishimune H, Vasseur S, Wiese S, Birling MC, Holtmann B, Sendtner M, Iovanna JL, Henderson CE. Reg-2 is a motoneuron neurotrophic factor and a signalling intermediate in the CNTF survival pathway. *Nat Cell Biol*. 2000;2:906-14.
95. Livesey F J, O'Brien JA, Li M, Smith AG, Murphy LJ, Hunt SP. A Schwann cell mitogen accompanying regeneration of motor neurons. *Nature*. 1997;390:614-18.
96. Bour-Jordan H, Bluestone JA. Sensory neurons link the nervous system and autoimmune. *Diabetes*. 2006;127:1097-99.
97. Razavi R, Chan Y, Afifiyan FN, Liu XJ, Wan X, Yantha J, Tsui H, Tang L, Tsai S, Santamaria P, Driver JP, Serreze D, Salter MW, Dosch HM. TRPV1+ sensory neurons control β cell stress and islet inflammation in autoimmune diabetes. *Cell*. 2006 Dec 15;127(6):1123-35.
98. Zenilman ME, Magnuson TH, Swinson K, Egan J, Perfetti R, Shuldiner AR. Pancreatic thread protein is mitogenic to pancreatic-derived cells in culture. *Gastroenterology*. 1996;110:1208-14.
99. Levine JL, Patel KJ, Zheng Q, Shuldiner AR, Zenilman ME. A recombinant rat regenerating protein is mitogenic to pancreatic derived cells. *J Surg Res*. 2000;89(1):60-5.
100. Ortiz EM, Dusetti NJ, Vasseur S, Malka D, Bodeker H, Dagorn JC, Iovanna JL. The pancreatitis-associated protein is induced by free radicals in AR4-2J cells and confers cell resistance to apoptosis. *Gastroenterology*. 1998;114(4):808-16.
101. Terazono K, Uchiyama Y, Ide M, Watanabe T, Yonekura H, Yamamoto H, Okamoto H. Expression of reg protein in rat regenerating islets and its colocalization with insulin in the beta cell secretory granules. *Diabetologia*. 1990;33(4):250-2.
102. Rouquier S, Verdier JM, Iovanna J, Dagorn JC, Giorgi D. Rat pancreatic stone protein messenger RNA. Abundant expression in mature exocrine cells, regulation by food content, and sequence identity with the endocrine reg transcript. *J Biol Chem*. 1991;266(2):786-91.
103. Watanabe T, Yonemura Y, Yonekura H, Suzuki Y, Miyashita H, Sugiyama K, Moriizumi S, Unno M, Tanaka O, Kondo H, et al. Pancreatic beta-cell replication and amelioration of surgical diabetes by Reg protein. *Proc Natl Acad Sci U S A*. 1994;91(9):3589-92.
104. Gross DJ, Weiss L, Reibstein I, van den Brand J, Okamoto H, Clark A, Slavin S. Amelioration of diabetes in nonobese diabetic mice with advanced disease by linomide-induced immunoregulation combined with Reg protein treatment. *Endocrinology*. 1998;139(5):2369-74.
105. Baeza NJ, Moriscot CI, Renaud WP, Okamoto H, Figarella CG, Vialettes BH. Pancreatic regenerating gene overexpression in the nonobese diabetic mouse during active diabetogenesis. *Diabetes*. 1996;45(1):67-70.
106. Baeza N, Sanchez D, Vialettes B, Figarella C. Specific reg II gene overexpression in the non-obese diabetic mouse pancreas during active diabetogenesis. *FEBS Lett*. 1997;416(3):364-8.
107. Sanchez D, Baeza N, Blouin R, Devaux C, Grondin G, Mabrouk K, Guy-Crotte O, Figarella C. Overexpression of the reg gene in non-obese diabetic mouse pancreas during active diabetogenesis is restricted to exocrine tissue. *J Histochem Cytochem*. 2000;48(10):1401-10.
108. Lu Y, Ponton A, Okamoto H, Takasawa S, Herrera PL, Liu JL. Activation of the Reg family genes by pancreatic-specific IGF-I gene deficiency and after streptozotocin-induced diabetes in mouse pancreas. *Am J Physiol Endocrinol Metab*. 2006;291:E50-8.
109. De Leon DD, Farzad C, Crutchlow MF, Brestelli J, Tobias, J, Kaestner KH, Stoffers DA. Identification of transcriptional targets during pancreatic growth after partial pancreatectomy and exendin-4 treatment. *Physiol Genomics*. 2006;24:133-43.
110. Unno M, Nata K, Noguchi N, Narushima Y, Akiyama T, Ikeda T, Nakagawa K, Takasawa S, Okamoto H. Production and characterization of Reg knockout mice: reduced proliferation of pancreatic beta-cells in Reg knockout mice. *Diabetes*. 2002;51(Suppl 3):S478-83.
111. Hamblet NS, Bowman A, Vinik AI, Taylor-Fishwick DA. Analysis of islet and ductal pancreatic proliferation in transgenic mice expressing INGAP. *Keystone Symposia, Taos, NM*; 2006.
112. Taylor-Fishwick DA, Bowman A, Malik N, Hamblet NS, Vinik AI. INGAP transgenic mice show resistance to streptozotocin-induced diabetes. *Keystone Symposia, Taos, NM*; 2006.
113. Flores LE, Garcia ME, Borelli MI, Del Zotto H, Alzugaray ME, Maiztegui B, Gagliardino JJ. Expression of islet neogenesis-associated protein in islets of normal hamsters. *J Endocrinol*. 2003;177(2):243-8.
114. Del Zotto H, Massa L, Rafaeloff R, Pittenger GL, Vinik AI, Gold G, Reifel-Miller A, Gagliardino JJ. Possible relationship between changes in islet neogenesis and islet neogenesis-associated protein-positive cell mass induced by sucrose administration to normal hamsters. *J Endocrinol*. 2000;165(3):725-33.
115. Gagliardino JJ, Del Zotto H, Massa L, Flores LE, Borelli MI. Pancreatic duodenal homeobox-1 and islet neogenesis-associated protein: a possible combined marker of activateable pancreatic cell precursors. *J Endocrinol*. 2003;177(2):249-59.
116. Lipsett M, Finegood DT. Beta-cell neogenesis during prolonged hyperglycemia in rats. *Diabetes*. 2002;51(6):1834-41.
117. Wang GS, Rosenberg L, Scott FW. Tubular complexes as a source for islet neogenesis in the pancreas of diabetes-prone BB rats. *Lab Invest*. 2005;85(5):675-88.
118. Iovanna JL. Redifferentiation and apoptosis of pancreatic cells during acute pancreatitis. *Int J Pancreatol*. 1996;20(2):77-84.
119. Bouwens L. Transdifferentiation versus stem cell hypothesis for the regeneration of islet beta-cells in the pancreas. *Microsc Res Tech*. 1998;43(4):332-6.
120. Wagner M, Luhrs H, Kloppel G, Adler G, Schmid RM. Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. *Gastroenterology*. 1998;115(5):1254-62.
121. Bockman DE, Guo J, Buchler P, Muller MW, Bergmann F, Friess H. Origin and development of the precursor lesions in experimental pancreatic cancer in rats. *Lab Invest*. 2003;83(6):853-59.

122. Lardon J, Huyens N, Rومان I, Bouwens L. Exocrine cell transdifferentiation in dexamethasone-treated rat pancreas. *Virchows Arch.* 2004;444(1):61-5.
123. Lipsett M, Rabinovitch A, Rosenberg L, et al. The role of islet neogenesis-associated protein (INGAP) in islet neogenesis. *Biochem Biophys.* In press 2007.
124. Lipsett M, Castellarin M, Lemay J, Rosenberg L. Evidence for regulation of beta-cell mass expansion in response to induction of islet neogenesis. *Diabetes.* 2004;53(Suppl 2):A382.
125. Rosenberg L, Duguid WP, Healy, M, Clas, D, Vinik AI. Reversal of diabetes by the induction of islet cell neogenesis. *Transplant Proc.* 1992;24(3):1027-8.
126. Suarez-Pinzon WL, Yan, Y, Power R, Brand SJ, Rabinovitch A. Combination therapy with epidermal growth factor and gastrin increases beta-cell mass and reverses hyperglycemia in diabetic NOD mice. *Diabetes.* 2005;54(9):2596-601.
127. Bernard-Kargar C, Ktorza A. Endocrine pancreas plasticity under physiological and pathological conditions. *Diabetes.* 2001;50(Suppl 1):S30-5.
128. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes.* 2003;52(1):102-10.
129. Butler AE, Janson J, Soeller WC, Butler PC. Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes.* 2003;52(9):2304-14.
130. Finegood DT, Scaglia L, Bonner-Weir S. Dynamics of beta-cell mass in the growing rat pancreas. Estimation with a simple mathematical model. *Diabetes.* 1995;44(3):249-56.
131. Lipsett MA, Austin EB, Castellarin ML, Lemay J, Rosenberg L. Evidence for the homeostatic regulation of induced beta cell mass expansion. *Diabetologia.* 2006;49:2910-9.
132. Taylor-Fishwick DA, Shi W, Pittenger GL, Vinik AI. PDX-1 can repress stimulus-induced activation of the INGAP promoter. *J Endocrinol.* 2006;188:611-21.
133. Jamal AM, Lipsett M, Hazrati A, Paraskevas S, Agapitos D, Maysinger D, Sladek, R., Laganriere, S., Hanley, S, Rosenberg, L. Signals for death and differentiation: a two-step mechanism for in vitro transformation of adult islets of Langerhans to duct epithelial structures. *Cell Death Differ.* 2003;10(9):987-96.
134. Zulewski H. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes.* 2001;50:521-33.
135. Gruner S, Jechlinger M, Beug H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. *Nat Rev Mol Cell Biol.* 2003;4:657-65.
136. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science.* 2004;306:2261-4.
137. Linning KD, Tai MH, Madhukar BV, Chang CC, Reed DN Jr, Ferber S, Trosko JE, Olson LK. Redox-mediated enrichment of self-renewing adult human pancreatic cells that possess endocrine differentiation potential. *Pancreas.* 2004;29:e64-76.
138. Lechner A, Nolan AL, Blacken RA, Habener JF. Redifferentiation of insulin-secreting cells after in vitro expansion of adult human pancreatic islet tissue. *Biochem Biophys Res Commun.* 2005;327:581-8.
139. Hanley S, Jamal AM, Lipsett M, Rosenberg L. Inducers of islet neogenesis from adult human pancreatic progenitors. *Diabetes.* 2004;53(Suppl 2):A379.
140. Semakula, C., Pambuccian, S., Gruessner, R., Kendall, D., Pittenger, G., Vinik, A, Seaquist ER. Clinical case seminar: hypoglycemia after pancreas transplantation: association with allograft nesidiodyplasia and expression of islet neogenesis-associated peptide. *J Clin Endocrinol Metab.* 2002;87(8):3548-54.
141. Borelli MI, Stoppiglia LF, Rezende LF, Flores LE, Del Zotto H, Boschero AC, Gagliardino JJ. INGAP-related pentadecapeptide: its modulatory effect upon insulin secretion. *Regul Pept.* 2005; 131:97-102.
142. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β -cell function. *Diabetes.* 2004;53:250-4.