Journal of Molecular Biomarkers & Diagnosis

He, J Mol Biomark Diagn 2016, 7:3
http://dx.doi.org/10.4172/2155-9929.1000282

Review Article Open Access

The Role of Reg Proteins in Pancreatic Regeneration

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Rec date: Feb 26, 2016; **Acc date:** Mar 29, 2016; **Pub date:** Mar 31, 2016

Abstract

Diabetes mellitus is a widespread disease which an estimated 285 million people in the world suffer from, and these numbers are on the rise making it one of the largest threats to human health in the coming decades. Early research has shown that restoration of the β cell mass by either stimulating β cell replication or β cell neogenesis may be a viable strategy in diabetes therapy. Some proteins such as the INGAP (hamster Reg3delta) have been shown to be involved in β cell regeneration and therefore may be potential sources of new drugs for diabetes treatment. The INGAP protein and peptide as well as other members of the Reg3 family of proteins such as HIP (human Reg3alpha/β) have been shown to have an effect on β cell regeneration, however this effect as well as their mechanism of action in the pancreas is unclear. This review described current knowledge about the mechanism of action and activity of INGAP and other members of the Reg3 family of proteins in β cell regeneration.

Keywords: Diabetes mellitus; Reg proteins; Pancreas; Islet regeneration

The Family of Reg Proteins

The family of Reg proteins have over the past several years received much attention because the Reg proteins are highly upregulated during pancreatic regeneration and in diabetic patients [1-3]. Their roles are very intriguing but poorly understood. The Reg family constitutes a conserved protein family in human and rodents. The Reg proteins are divided into four classes based on their primary protein structure: Reg1, Reg2, Reg3 and Reg4. In the class of Reg1 and Reg3 have different subtypes have been identified. Reg2 is only found in hamster and mouse. All classes except Reg4 have shown to be involved in pancreatic regeneration, for this reason Reg4 will not be described further in this thesis. The different Reg proteins found in mouse, human, rat and hamster, are listed in Table 1 [2].

Models	Class 1	Class 2	Class 3	Class 4
Mouse	Reg1	Reg2	Reg3α, Reg3β, Reg3g, Reg3δ	Reg4
Human	REG 1α, REG1β		REG3α/β, REG3γ	REG4
Rat	Reg1α		Reg3α, Reg3β, Reg3g	Reg4
Hamster		Reg2	INGAP, Reg3g,	

Table 1: Current members of the family of Reg proteins in mouse, human, rat and hamster.

A list of Reg proteins found in four different species. The proteins are grouped into one of four classes, based on their primary structure. For Reg1 and Reg3 different subtypes have been found. The nomenclature of the Reg proteins can be a bit confusing because the proteins have been discovered separately in different fields and therefore given different names. For example, in the literature the Reg3α protein is also referred to as PAP (pancreatitis-associated protein) and HIP (hepatocarcinamo-intestine-pancreas), Reg1 as PTP (pancreatic stone protein), human REG3β as human REG3α and mouse REG3δ as INGAP, which is actually a hamster protein, believed to belong to the Reg3 proteins [2].

The Reg proteins are secreted proteins consisting of 155-180 amino acids, supported by presence of human REG3a/β in serum and Reg1 and Reg2 in the lumen of mouse pancreatic ducts [4,5]. They all contain a calcium-dependent carbohydrate binding recognition domain (c-type lectin), but are not known to bind to carbohydrates. Most Reg proteins have six cysteine residues forming disulphide bonds, suggesting relative similar 3D configurations. The genes encoding the different Reg proteins all span 3 kilobases and contain 6 exons and 5 introns. In mice, human and rat the common chromosomal location of the different Reg genes (except for Reg4), the tandem order on the chromosomes and the similar intron-exon organization suggest that Reg genes have evolved from common evolutionary ancestors [2,6]. All Reg genes are highly expressed in the pancreas; however it is not completely clear what pancreatic cells express them. A recent paper investigating the expression patterns of mouse found that Reg3α, Reg3β and Reg3δ are also expressed in lower levels in the duodenum, stomach and liver, suggesting pleiotropic roles of these proteins. Within the mouse pancreas, Reg3α and Reg3β were found to be expressed in the acinar cells and Reg3δ in the α-cells [7]. The expression of Reg3δ in α-cells is similar to what was discovered by another group investigating the expression of the hamster INGAP

protein (Taylor-Fishwick et al. Another group demonstrated that mouse Reg3α and Reg3β are expressed in the α-cells [8-10] contradicting the findings of Wang's study [7]. Pancreatic specific expression of the mouse Reg3γ has not been investigated; however one paper states that human REG3γ is expressed predominantly in the pancreas in the acinar cells, based on a lack of PCR amplification of the gene transcript from islets or duct cell lines [11]. Reg1 and Reg2 genes have been found to be expressed primarily in the acinar cells, and occasionally in the ductal cells, but exclusively in the exocrine pancreas [12]. Other groups, however, state that Reg1 and Reg2 are expressed in the β-cells [10].

The contradicting findings of the location of Reg expression might reflect that the genes can be expressed in different cell types depending on different physiological conditions and those antisera to the different Regs' cross react with multiple members making the results difficult to interpret. Suspecting that the Reg proteins serve an important role during pancreatic regeneration, it would be desirable to clarify the expression patterns during such events.

Pancreatic Regeneration

Postnatal regeneration of the pancreas, in which a functional β-cell mass is restored following injury or stress to the pancreas, was for the first time described more than a century ago. The Italian researcher diMattei was the first to observe pancreas regeneration after performing partial pancreatectomy (partial removal of the pancreas) in dogs in 1885 [12]. Since then several researchers have reported regeneration of pancreatic endocrine and exocrine tissue following various models of injury [12]. Today the term "β-cell regeneration" is used to describe endogenous formation of β-cells in the adult pancreas regardless of a previous loss of β-cells, such as expansion of the β-cell mass during obesity and pregnancy, which is not stimulated by injury. The issue of islet regeneration in the human adult pancreas is a controversial topic [13,14]. Most of the knowledge within the field is obtained by studying rodent models, and it is uncertain whether the knowledge gained from animal models translates to human β-cell biology. Today, it is recognized that the functional β-cell mass in human is somewhat dynamic and that maintenance does occur during postnatal life in response to ever-changing physiological demands, such as during disease, aging or pregnancy. However, the degree of islet cell expansion in human is low compared to that in rodents [15,16]. Furthermore, as lineage tracing cannot be done in humans, it has been impossible to determine the origin of new arising β-cells in humans. To unravel the complexity of the mechanisms accounting for pancreas regeneration the field has been studied for many years using a variety of rodent diabetic and regenerating models.

Three major mechanisms have been proposed to account for islet regeneration in rodents:

i) Replication of existing β-cells,

ii) Emergence of new β-cells from precursor cells residing in the pancreatic duct epithelium (referred to as neogenesis) and

iii) β-cell formation from transdifferentiation of α-cells [12,17]. Below is a brief summarization of some of the more important studies that convincingly support each of the three mechanisms [i, ii, iii] proposed to account for pancreas regeneration.

Replication of existing β-cells

Strong data supports that new β-cells form from replication of pre-existing β-cells. Yuval Dor et al. showed by lineage tracing in adult mice that pre-existing β-cells are the major source of new β-cells both in normal conditions and after partial pancreatectomy. The study did not rule out that a smaller pool of the new β-cells were formed from differentiation of precursor cells [18]. Another paper also from Yuval Dor very nicely demonstrated the ability of pre-existing β-cells to proliferate during β-cell regeneration. In a transgenic mouse model expressing diphtheria toxin, specifically in the β-cells in a doxycycline-inducible manner, 70%-80% of all β-cells were ablated rendering the mice diabetic. Withdrawal of doxycycline resulted in spontaneous regeneration and recovery after 5-7 months. Lineage tracing indicated that pre-existing β-cells were the major source of new β-cells [19]. Xu et al. reported a very important study that demonstrated a clear exception to the above studies, and showed that β-cells in adult mice can be formed from non-β-cells. They discovered that in the days that follow partial duct ligation PDL1, Ngn3 expression, which is required for the formation of all endocrine cells types [20], was induced in the pancreas. It was shown that new β-cells originated from these Ngn3 expressing cells, and if you removed Ngn3, the process of regeneration and the formation of new β-cells were severely decreased. Thus, in addition to proliferation of pre-existing β cells, the appearance and subsequent differentiation of Ngn3-positive cells also contributes to new β-cells after this type of injury [21].

B-cell neogenesis from ductal precursors

Several independent studies in various animal models of β-cell injury provide evidences for the existence of facultative stem cells that are able to give rise to functional β-cells. Yet, whether or not adult β-cell progenitor exists is a controversial issue in diabetes research. Evidence of β-cell neogenesis from ductal precursors arise from *in vitro* studies demonstrating a differentiation of cells in the duct tissue into insulin-producing cells [21,22] and from morphological studies where insulin-positive cells are observed in close proximity to ducts or within the duct epithelium after injury inducing pancreatic regeneration [23]. Despite several studies supporting the hypothesis that duct cells can serve as progenitor cells and differentiate into insulin-producing cells, evidence requires lineage tracing which is considered the "gold standard" of proof. The group of Susan Bonner-Weir has for many years worked to prove the existence of β-cell neogenesis from ductal precursor cells in rodent models. In 2008, a lineage tracing experiment using the human carbon anhydrase II (CAII) promoter to label duct cells, showed that CAII-expressing cells within the pancreas act as progenitors that give rise to both new islets and acini normally after birth and after injury (PDL), indicating that duct precursor cells can in fact give rise to postnatal formation of new β-cells (neogenesis) [24]. This finding is still controversial as other lineage tracing experiments using Sox9 and Hnf1b to mark the ducts were not able to show that the endocrine cells formed following PDL, originated from ducts. Partial duct ligation (PDL) is a model of pancreatic injury forcing the generation of new β-cells (islet regeneration). However, these negative findings could be due to very poor labeling of the duct cells [25].

Transdifferentiation of α-cells to β-cells

In the line of neogenesis, two convincing studies have recently shown that α-cells can give rise to the postnatal formation of new functional β-cells by transdifferentiation [26]. In one of the studies,

extreme loss of β-cells was induced in a transgenic mouse model by expressing diphtheria toxin specifically in the β-cells in a doxycycline-inducible manner. The α-cells were YFP labeled using the Cre-lox system before β-cell ablation and lineage tracing convincingly showed that a large fraction of regenerated β-cells were derived from α-cells (they were YFP positive) [27]. In the other study, PDL and administration of alloxan treatment 2 resulted in an elimination of β-cells (99%) followed by a dramatically regenerative response. The study ruled out that the new forming β-cells originated from proliferation of pre-existent β-cells as there were no proliferation markers or 5-bromo-2'-deoxyuridine (BrdU) incorporation detected in β-cells during the regenerative period. Furthermore, no insulin-positive cells were observed in the ductal epithelium, which lead to the conclusion that the new β-cells did not originate from ductal precursor. Intermediate cells expressing both β- and α-cell markers were observed in the regenerative areas supported the evidence for α- to β-cell transdifferentiation [26]. While β-cell replication is the best supported mechanism to account for islet regeneration in rodents, human β-cells only possess a limited capacity to replicate, and in both human and rodents the capacity declines with age [28]. This could suggest that neogenesis might be the most common mechanism to drive endogenous formation of β-cells in the human pancreas, and observations of single cells and small insulin

positive clusters found near the ducts in human pancreas is suggestive for progenitor cells residing in 2 Alloxan treatment selectively destroys insulin-producing cells in the pancreas when administered to rodents and thereby induce pancreatic regeneration [29-34]. However, this is only speculation. The different mechanisms proposed to account for pancreatic regeneration reflect a highly complex system that is dependent on several factors, and that different mechanisms can be set into play which are dependent on for example the type of injury model and the amount of β-cells present in the initial phase of the regenerative process. No matter what the origin of new β-cells is, it is desirable to identify, investigate and translate protein targets that can induce β-cell regeneration/β-cell expansion, and therefore it is highly relevant to keep studying the mechanisms of regeneration. Even though the regenerative mechanism of rodents and human most likely differ, model-organisms are necessary as the human pancreas, for obvious reasons, is difficult to study.

The role of Reg proteins in pancreatic regeneration

Since discovery of Reg1 and INGAP proteins, promising data have emerged to support their function in pancreatic regeneration. Reg1 can stimulate β-cell replication, increase β-cell mass and delay the onset of diabetes in *in vitro*and *in vivo*models. Reg1-induced increase in β-cell proliferation was shown by an increase in [3H]-thymidine uptake in both rat islets grown in culture with Reg1 protein supplied in the culture medium and in islets isolated from transgenic mice overexpressing human REG1α protein specifically in the β- cells [33]. These data were supported by a paper reporting a reduced proliferation of pancreatic β-cells in Reg1 knockout mice [34]. Reg1-induced increase in β-cell mass have been demonstrated by a 2 month long intrapertioneal (IP) injection study of Reg1 in 90% pancreatectomized rats. The Reg1 treated animals had remarkably larger islets compared to non-treated animals [33]. This study was supported by another study suggesting that Reg1 injections protects NOD mice from becoming diabetic, by increasing the β-cell mass [34]. A transgenic mouse overexpressing Reg1 in β-cells did not have larger islets compared to wild type, suggesting that Reg1 only increases the β-cell mass, when the pancreas is challenged by a diabetic state or during regeneration. The majority of the Reg1 studies have been done by the same Japanese group. The second Reg protein to be discovered was the hamster INGAP protein. An extract prepared from cellophane-wrapped pancreas 10 days after surgery, termed ilotropin, was shown to be capable of inducing islet neogenesis and reversing streptozotocin (STZ)-induced diabetes in hamsters [35]. A year after this discovery, a gene highly upregulated in the ilotropin extract was identified by comparing gene expressions from cellophane wrapped hamster pancreata versusm sham-operated pancreata [36]. Cellophane wrapping is a model of pancreatic regeneration thought to induce formation of new islets by neogenesis and Rosenberg who developed the technique, called the identified gene "islet neogenesis associated protein" (INGAP). The phenomenon of neogenesis after cellophane wrapping and induction by INGAP injections was concluded based on findings that many small insulin-positive cells and small clusters were in close proximity to the ductal epithelium suggesting that they arose from ductal precursors. Lineage tracing experiments have never been performed to prove the neogenesis theory. As written previously, INGAP is proposed to belong to the class 3 of the Reg protein family, but there is no human, rat or mouse equivalent of the protein. Since its discovery, a pentadecapeptide (INGAPPP) containing the 104-118 amino acid sequence has been proposed to carry the core regenerative activity of INGAP, as it shows the same effect as the intact molecule with regards to inducing proliferation of ductal cells [36], increasing β-cell mass [37] and reversing STZ induced diabetes [38]. Intramuscular injections of INGAP-PP for 30 days can increase the β-cell mass in mice [4]. In the same study INGAP-PP IP injections of hamster showed same response, and an increase in β-cell mass for INGAP treated dogs, has been shown in another study [37]. These findings are very interesting, as this indicates, in contrast to Reg1, that INGAP/INGAP-PP can induce an increase of the β-cell mass without the pancreas being challenged by a diabetic state or in the phase of regeneration. INGAP has also been tested in clinical trials. Here it showed promising results by increasing C-peptide secretion in human type 1 diabetes and provided improved glycemic control in type 2 diabetics. However, due to the large amount of injected INGAP required inducing an effect (600 mg/day), many test subjects suffered from injection-site reactions, and withdrew from the experiment. Therefore, it was concluded that a better tolerated formulation is needed [38]. The majority of the work on INGAP has been done by an Argentinean and Canadian group. Despite their effort very little is still known about the mechanism of action and the intracellular pathways activated by INGAP as no specific receptor has been clearly identified. Despite the unknown mechanism(s), INGAP has shown positive effects on diabetes improvement of diabetic phenotypes by stimulating islet cell expansion, and therefore it remains an interesting candidate for a therapeutic use.

Conclusion

In conclusion, β cell regeneration refers to the putative ability of β cells to restore their mass is one of the most promising new fields of diabetes research and may one day provide an answer to curing this problem. This however requires a lot more research as there are multiple methods and candidates that have been implicated in β cell regeneration. Reg3 subfamily of proteins with emphasis on the INGAP protein, which has been shown to play a role in β cell regeneration in animal and may one day represent a new form of therapy for diabetes mellitus.

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