β-Cell Regeneration

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β-cell number and/or function is reduced in diabetes. Thus, inducing the formation of new β-cells has been a major goal of diabetes research. However, the pathway(s) by which new β-cells form when preexisting β-cells are decreased in number or cease to function has remained obscure. Many pathways have been proposed, but definitive evidence, particularly in humans, has been lacking. Replication of preexisting β-cells, neogenesis from ducts, redifferentiation from β-cells that dedifferentiated under metabolic stress, and transdifferentiation from other cell types, particularly within the islet, are the major mechanisms that have been proposed for generating increased numbers of functional β-cells.

β-cell islet diabetes

1. Introduction

That β-cell function and β-cells themselves are decreased in both major forms of diabetes has been known for many decades [1]. Strikingly, there continues to be controversy about the mechanism by which β-cell function is compromised. Questions, as fundamental as whether diabetes (either type 1 or 2) leads to β-cell death or merely to loss of markers of β-cell identity, whether β-cell replication can occur in adults with diabetes, and whether β-cell neogenesis from precursors is possible, remain without definitive answers.

2. β-Cell Replication

It is clear that β-cell replication occurs early during development [2]. However, it is also clear that β-cell replication declines to undetectable levels in adults, and particularly in adult humans [3][4][5]. Thus, inducing β-cell replication in adults depends on reactivating a process that does not normally occur and that may be impossible due to β-cell alterations that are irreversible. Regardless, there have been many attempts to induce adult β-cell replication, most prominently by the use of inhibitors of the protein kinase Dyrk [6]. However, evidence of a substantial increase in the number of β-cells by stimulation of β-cell replication in adults remains lacking.

An important issue with studies of β-cell replication is the endpoint used to define that a β-cell has replicated. While the gold standard should be to demonstrate an increase in the number of β-cells, that has proven to be elusive. Most studies have used surrogate markers to indicate DNA replication. The most common are uptake of nucleoside analogs, such as BrdU or EdU, and expression of proteins, such as Ki67, that are expressed only in cells that have entered the cell cycle. However, nucleoside analog uptake, as an indicator of replication, can also be an indication of a DNA damage response, where repair involves incorporation of nucleosides [7]. The pattern of nucleoside

uptake can indicate which is taking place, with an irregular, incomplete uptake in the nucleus being more consistent with a damage response, while a strong, uniform uptake being more consistent with true replication [8]. The presence of doublets of such cells further supports true replication, as the formation of new cells by replication will result in two daughter cells in close proximity. The expression of H2aX, a marker of a DNA damage response, has been found in some studies, including in some where β-cell replication has been claimed [7]. Ki67 expression can also be problematic as a marker of replication, as it has become clear that it plays a broader role in the cell [9]. In summary, studies that claim to show beta cell replication should demonstrate appropriate expression of markers, such as CENPA (Centromere Protein A) that are required for replication and the absence of markers of DNA damage that can produce false positive results in replication assays. Ultimately, strong evidence of an increase in β-cell number needs to be shown.

3. β-Cell Neogenesis from Ducts

Embryonic islet formation occurs by budding from embryonic ducts [10]. This gives rise to the hypothesis that β-cell regeneration in the adult occurs by reactivation of the embryonic program of islet development [11]. There is considerable evidence for the reactivation of embryonic programs in a number of tissues [12] as well as in cancer. Thus, it is a highly attractive notion and has dominated the field of β-cell regeneration for much of its history. Evidence to support β-cell regeneration from ducts through reactivation of the embryonic program of islet development has been tested primarily by the use of models in which various types of pancreatic injury is induced, followed by monitoring to detect islet or β-cell neogenesis. Many investigators have observed cells expressing insulin within ducts [13][14][15]. A transgenic mouse model expressing interferon-gamma in β-cells was claimed to lead to massive formation of insulin-positive cells within ducts [16], but there were no follow up studies from other laboratories to demonstrate reproducibility. While multiple models have been used to study the appearance and fate of intra-ductal insulin-positive cells, including partial pancreatectomy, duct ligation, pregnancy, and secreted factors from the fetal pancreas and other states—discussed below—substantial controversy remains about whether pancreatic ductal cells in adult mammals give rise to mature β-cells.

3.1. Partial Pancreatectomy

The classical model used to induce pancreatic damage has been a partial pancreatectomy [17]. In fact, the origin of the hypothesis that β-cell regeneration occurred by reactivation of an embryonic program came from a study of partial pancreatectomy [11]. That study demonstrated regenerating ducts following a partial pancreatectomy that had a high degree of BrdU incorporation, indicating that they were derived from replicating ductal precursors. An adjacent islet was claimed to be neogenic, with a ductal origin, but few of the islet cells exhibited BrdU incorporation, indicating that they had not arisen from a replicating precursor. The presence of ducts in which most cells had replication was taken as evidence of pancreatic regeneration and so the presence of an islet in that region was thought to indicate islet neogenesis. However, the islet shown had few cells that had taken up BrdU, indicating that most cells in that islet were preexisting rather than new.

Despite many publications on islet neogenesis following partial pancreatectomy, definitive evidence was lacking. If islet neogenesis occurred following partical pancreatectomy, new islets marked by many BrdU-positive endocrine cells should be found in neogenic areas marked by BrdU-positive duct cells. Not only were neogenic islets not found, but serial pancreatomy led to an almost complete absence of insulin-positive cells in neogenic areas. Rather, the few islets found in areas of regenerating pancreas appeared to be derived from the area of the pancreas that had not been pancreatectomized [18]. In humans, no evidence for β-cell regeneration was found after partial pancreatectomy [19]. β-cell neogenesis during development requires the transcription factor geurogenin-3, but it appeared to not play a role in β-cell regeneration following partial pancreatectomy [20]. Thus, there does not appear to be support for β-cell regeneration by reactivation of an embryonic program following partial pancreatectomy, and the evidence for any significant β-cell regeneration following partial pancreatectomy is equivocal.

3.2. Pancreatic Duct Ligation

Under the hypothesis that β-cell regeneration in adults occurs from ducts, duct ligation has been studied intensively as a model for β-cell regeneration [21]. Most studies [13], have found small numbers of cells within ducts that express insulin following duct ligation. Multiple studies, including with genetic lineage tracing, have been performed with the goal of demonstrating β-cell neogenesis from duct precursors. While often regarded as a gold standard method, lineage tracing is prone to artifacts, most often arising from leakiness of the promoter used to drive the gene used to mark the duct cell population being studied. The results have varied, with positive [22][23][24] and negative [25][26] studies having been reported. Most recently, a careful lineage-tracing study demonstrated β-cell neogenesis from ducts [27].

3.3. Inductive Factors

A number of the potential stimuli for β-cell regeneration involve secreted factors. For instance, a corollary to the fact that islets and β-cells arise from embryonic ducts is that there should be inductive factors in the embryonic pancreas that promote islet and β-cell formation. Epidermal growth factor (EGF) has been studied as a factor that can induce expansion of endocrine progenitors in the embryonic and adult pancreas [28][29], but robust increase in β-cell mass has proven elusive. HGF (hepatocyte growth factor) is expressed in both the fetal and adult pancreas and has been claimed to induce the replication of human β-cells [30], but this is controversial [31].

In a more general approach to testing the hypothesis that factors from the fetal pancreas could induce β-cell formation from adult duct cells, it was purified non-endocrine epithelial cells from the adult human pancreas, consisting of a population of cells with duct-like properties that did not express insulin, and genetically marked them using a lentiviral vector expressing GFP (green fluorescent protein). Those cells were combined with human fetal pancreatic tissue and transplanted into immunodeficient mice. Following harvest, the existence of cells coexpressing GFP and insulin was determined. There was clear evidence for induction of insulin-positive cells from the duct-like cells under the influence of factors from the co-transplanted human fetal pancreatic tissue. However,

the number of insulin-GFP co-positive cells was small, similar to the small number of insulin-positive cells observed in damage models, such as duct ligation [14].

Prolactin, a factor that is at a high level during pregnancy, has been the subject of much investigation as an inducer of β-cell replication during pregnancy [32]. However, while it is clear that there is substantial expansion of β-cell mass in rodents, the evidence for expansion of β-cell mass in humans is weaker than in rodents [33] and if it does occur is likely to be of a much lower magnitude.

Reg is a protein secreted by pancreatic exocrine cells following injury that has been proposed to induce β-cell regeneration [34]. A peptide derived from Reg termed INGAP (islet neogenesis-associated protein) has also been studied as a promoter of β-cell regeneration [35] but has not proven to be robustly active [36].

Overall, no factor from the fetal or adult pancreas has demonstrated robust and reproducible ability to induce β-cell regeneration.

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