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A Simply way to Generate Pancreatic Beta Cell Formation *In Vivo* through “Cellular Networking, Integration and Processing”

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Introduction

Impaired insulin secretion is a characteristic feature of both type 1 and type 2 diabetes mellitus, and a variety of approaches have been employed to generate beta cells *in vivo* and *in vitro* [1-3]. Herein, we describe a completely novel approach to form beta cells *in vivo* in an adult wild type rodent model of diabetes [4]. Our method integrates multiple process of cellular physiology to regulate organ function and results in the long-term normalization of insulin secretion and glycemia and is not associated with pancreatic injury. We labeled our approach “Cellular Networking, Integration and Processing (CNIP)” to distinguish it from the term “system biology” which is used broadly to refer collectively to different levels of cellular, organ and animal physiology. The fundamental principle underlying CNIP is to simultaneously target and integrate three key levels of cellular physiology/metabolism/molecular biology that have been implicate in beta cell formation. This is in contrast to the non-integrated model, which targets only nuclear reprogramming.

Two important concepts characterize the CNIP approach: (i) it is essential to combine three levels of cellular physiology: carbohydrate metabolism, membrane receptor function, and gene transcription to produce the desired effect, i.e., beta cell formation, and (ii) integration of multiple processes of cellular physiology is essential to produce a synergistic effect on generation of insulin-producing pancreatic beta cells. Synergy is a core factor in which several molecules work together to produce an effect that is greater than the sum of their individual effects. Glycolysis is the first important element in our CNIP approach to induce beta cell formation in the adult pancreas. Glucose is the major energy source consumed by mammalian cells and provides the energy for cellular function and proliferation [5]. We have shown that, if glucose is not phosphorylated to glucose-6-P by glucokinase, it cannot go through further metabolism and cannot produce a signal to the transcriptional machinery to induce gene expression [6,7]. Therefore, the first molecule included in our CNIP cocktail to generate pancreatic beta cell formation is glucokinase.

A second cellular function involved in beta cell formation is ligand binding to tyrosine kinase receptors [8,9]. The expansion in pancreatic beta cell mass in response to physiological stress, i.e., pregnancy, is mediated by growth hormone, prolactin and placental lactogen working through the prolactin receptor [9,10]. The protein-tyrosine phosphatase 1B (PTP1B) has been shown to inhibit the ability of insulin, insulin-like growth factor, prolactin and hepatocyte growth factor to activate tyrosine kinases [11-13]. Consequently, the second molecule included in our CNIP cocktail to generate insulin-producing pancreatic beta cells formation by increasing membrane receptor tyrosine kinase activity is a shRNA which targets PTP1B. The third important step in our CNIP approach is focused at the gene expression level and consist of a transcriptional factor, which is utilized as an attractor to converge and center the glucose metabolic/molecular effects generated by the tyrosine kinase receptor(s) to form new beta cells in the adult pancreas.

A key transcription factor associated in beta cell formation is Pdx-1; it has been demonstrated to be involved in beta cell formation in adult animals [14] and has been used in our CNIP approach for its post-embryonic action to enhance beta cell formation. Overexpression of Pdx-1 plays a crucial role in converging altogether; the signaling mechanisms in the CNIP cocktail to stimulate beta cell formation. To investigate beta cell formation in the adult animal, we employed a novel *in vivo* method to target the adult pancreas using a lentiviral vector [15]. The Lentiviral construct is injected into the mouse pancreas through the pancreatic duct as follows: a 32-gauge catheter is implanted into the cystic duct through a small opening in the gallbladder. With a clamp placed around the sphincter of Oddi to circumvent leakage of the vector into the duodenum, 100 µl of total Lentiviral vector cocktail expressing cDNA glucokinase, Pdx-1, and shRNA PTP1B at 108 to 109 TU/ml is gradually injected into the pancreatic duct via the catheter. The control placebo cocktail was comprised of Lentivirus shRNA scramble with Lentivirus expressed green fluorescent protein (GFP) at the equal concentration and volume as the experimental cocktail. Four weeks post-Lentiviral infection, the entire pancreas is removed for histologic examination.

To test the CNIP therapeutic effect, we utilized a wild-type, partially pancreatectomized rodent model to eliminate the confounding variables that are present in other commonly used mouse models. The partially pancreatectomized mouse represents an insulinopenic model of diabetes and avoids the problem of pancreatic injury associated with streptozotocin and alloxan, as well as the complicating factors associated with genetic mouse models of diabetes, that introduce confounding effects to induce pancreatic beta cell proliferation by themselves. The novelty of CNIP includes: (1) combination of three major levels of beta cell physiology to induce beta cell formation *in vivo* in adult mice; (2) post-embryonic generation of beta cells without formation of other endocrine cell types, such as glucagon secreting alpha cells, that could impair glucose homeostasis; (3) generation of beta cell formation without injury to the pancreas; (4) absence of pancreatic ductal cell proliferation which is associated with pancreatitis; (5) normalization of fasting and postprandial insulin secretion; (6) long-term (in excess of one year) normalization of fasting and postprandial plasma glucose concentrations without hypoglycemia; and (7) lack of injury to the pancreas, as manifested by histologic examination and plasma amylase and lipase levels. The present results provide a completely novel method to generate beta cells using natural physiologic mechanisms that regulate beta cell formation *in vivo* in the adult pancreas.

In contrast to previous approaches, CNIP is designed to target cellular mechanisms implicated in pancreatic function in the organ's adult state and uses a synergistic mechanism that incorporates multiple levels of cellular regulation. The method utilizes post-developmental mechanisms to induce beta cell formation without stem cells and without activating the embryonic pathway; thus, undesired cells are not induced as glucagon or somatostatin. The use of stem cells as a source of beta cell has received recent interest. However, this approach requires an *in vitro* culture system with subsequent islet cell transplantation with all of the problems that have limited this approach. The CNIP approach bypasses the problems associated with *in vitro* petri dish culture, i.e., good manufacturing practice, and the problems associated with islet transplantation that have limited this approach for more than two decades.

Stem cells are highly proliferative and can readily form teratocarcinomas. Further, many of the transcriptional factors employed in stem cell transformation include oncogenes like MafA. Further, the *in vitro* stem cell differentiation protocols do not produce a consistent level of beta cell-like differentiation for each petri dish culture, and the beta-cell-like differentiated stem cells can continue to differentiate into other cell types after injection *in vivo*, resulting in cells with a very different profile than was present in the petri dish. Thus, the *in vitro* cell differentiation method to form beta cells is far from clinical application. Moreover, after the stem cells are injected *in vivo*, one still has to deal with all of the hurdles encountered in islet transplantation, including the immune rejection process. The *in vivo* CNIP approach obviates all of these hurdles.

In summary, the CNIP method provides a novel approach to the regeneration of beta cells using the natural physiological cellular capacity of adult cells in the pancreas to form beta cells *in vivo*. The CNIP approach has the prospective to be used for prevention treatment or cure for both type 1 and type 2 diabetes with the understanding that in type 2 diabetic individuals treatment of the insulin resistance will require a separate approach. For type 1 treatment of diabetic individuals, who are not intrinsically insulin resistant, the CNIP approach provides a potential cure.

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