Original Article Partial hepatectomy promotes pancreatic regeneration through HGF/Gab1 pathway in db/db mice

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Abstract: Both the liver and the pancreas of terrestrial vertebrates are developed from endodermal epithelial cells in the embryonic foregut. Some studies showed that there was an interaction between liver and pancreas regeneration. In the present study, we first examined the effects of partial hepatectomy on β -cell mass, insulin and pancreas weight. Partial hepatectomy significantly improved β -cell proliferation, insulin secretion and pancreas growth. Next, we measured the changes of fasting plasma glucose (FPG) and blood glucose inoral glucose tolerance test (OGTT) in the presence of partial hepatectomy. The results suggested that partial hepatectomy significantly improve carbohydrate metabolism, indicated by the decrease of FPG level and blood glucose in OGTT. The serum hepatocyte growth factor (HGF) in mice gradually increased after partial hepatectomy. The pancreatic HGF expression and Grb2 associated binder 1 (Gab1) activation were also dramatically increased by partial hepatectomy. To further identify the role of HGF and Gab1, we treated mice with PHA-665752, a specific inhibitor of c-Met, the HGF receptor, and found that the ratio of β -cells to non- β endocrine cells, insulin secretion and pancreas weight and Gab1 activation were all decreased by PHA-665752. Furthermore, PHA-665752 significantly increased the FPG levels and blood glucose levels in OGTT compared to Hepatectomy group. These results revealed a positive effect of partial hepatectomy on pancreatic regeneration and a novel mechanism involving HGF/Gab1 signaling.

Keywords: Diabetes mellitus, partial hepatectomy, pancreatic regeneration, hepatocyte growth factor, Grb2 associated binder 1

Introduction

Diabetes mellitus (DM) is defined as a state of abnormality in carbohydrate and lipid metabolism due to defects in secretion (beta cell dysfunction) or action of insulin (insulin resistance), which is characterized by debilitating persistent excess of glucose in circulating blood (hyperglycemia) [1]. As a result of the modern lifestyle, there are currently 382 million DM patients worldwide and the number is still rapidly mounting. By the year 2035, it may reach 592 million [2]. In China, there are over 30 million DM patients. Diabetes has become the third largest non-communicable disease after cardiovascular disease and tumor and a worldwide public health problem that seriously threatens human health.

In DM patients, the loss of pancreatic beta (β) cells and their dysfunction leads to the impaired

proliferation and regeneration capacity of β cells. As a result, a cure for insulin-dependent diabetes requires the reconstitution of a functional β -cell mass by in situ regeneration [3]. The regeneration and stimulatory mechanisms of new ß cell formation are important in the treatment of β cell deficiency in diabetes [4]. Pancreas regeneration has been observed in mammals. This limited ability of regeneration of pancreas makeit has the potential to maintain or increase β-cells, mainly under physiological stimuli such as pregnancy [5] or injury (partial pancreatectomy) [6]. However, under normal circumstance, the limited regeneration capacity of pancreas could not meet compensate the loss of β cells in DM patients [7].

Liver and pancreas both play a pivotal role in the metabolism of the human body. They are connected by the portal vein system and are both derived from the intestinal endoderm. It

has been found that the removal of any part of the organ can affect the size and function of the other organ [8]. But the effects of partial hepatectomy on pancreatic regeneration have not been well studied. It is known that some regulatory factors play a regulatory role in both the liver and pancreas regeneration [9]. Hepatocyte growth factor (HGF) is a polypeptide growth factor that belongs to the plasminogen family. It is originally identified and cloned as a potent mitogen for mature hepatocytes [10]. HGF is mainly expressed in the liver matrix, but can also be found in lung, spleen, placenta, brain and other tissues. Some studies have revealed the possible effects of HGF on pancreatic regeneration. Izumida et al found that the induction of the c-Met/HGF signaling pathway following bone marrow transplantation promotes pancreatic regeneration in diabetic rats [11]. A study of Li et al revealed the involvement of HGF-mediated signaling in differentiation of pancreatic ductal epithelial cells into insulinproducing cells [12]. The Grb2 associated binder 1 (Gab1) is a member of the Gab scaffolding protein family which plays a central role in the intracellular signaling cascades of many extracellular stimuli like growth hormones [13]. A study has shown that Gab1 activation plays a central role in HGF's ability to stimulate intracellular transduction cascades in pancreatic acinar cells and this action likely plays a key role in HGF's ability to alter pancreatic growth/ regeneration [14]. However, the interaction of HGF and Gab1 in the effects of partial hepatectomyon pancreatic regeneration has not been explored yet. Hence, in the present study, we used db/db mice to study the effects of partial hepatectomyon pancreatic regeneration, and then examined the involvement of HGF/Gab1 pathway.

Materials and methods

Animals and treatments

Male C57BL/KsJ db/db mice, whose weight ranged between 40-50 g, were purchased from the Shanghai Laboratory Animal Center and used in this study. Mice were housed in a standard animal room under controlled temperature (24°C) and humidity (55%) as well as a 12-h light/12-h dark cycle and were provided with adlibitum rodent chow. The experimental procedures were approved by the Institute Research Ethics Committee of Fudan University. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. Mice were randomly divided into three groups: Control group; Sham group (undergoing sham operation); Hepatectomy group (undergoing partial hepatectomyoperation). Detailed surgical procedures were described earlier [15]. After the wounds were sutured, animals were kept on a warming mat to avoid hypothermia. For the mice in Hepatectomy + PHA group, after the surgery, mice were given intravenous tail vein injections of the c-met inhibitor PHA-665752 (Pfizer) at a dosage of 20 mg/kg/day in a volume of 150 µl every subsequent 24 h until sacrifice.

Determination of β -cell/non- β endocrine cell ratio, plasma insulin and pancreas weight change

10 sections (at least 200 µm apart) were immunostained with insulin and counterstained with DAPI. Sections were examined using a confocal microscope (A1; Nikon) connected to a computer with NIS elements software (Nikon). Images (4×, spanning the entire tissue for each slide) were acquired using an X-Y motorized microscope with resolution sufficient to identify single β -cells, and "stitched together" using the MetaMorph software program (V.7; Molecular Devices). Insulin staining in each section was calculated by MetaMorph, and then checked manually to remove irrelevant spots or to add β-cells that stained weakly. MetaMorph quantified total tissue area (based on measurement of DAPI-stained area) and insulin-positive area (based on measurement of glucagon/somatostatin/c-peptide cocktail-positive area) to generate β -cell/non- β endocrine cell ratio. For the measurement of insulin, plasma was evaluated using a radioimmunoassay kit (Diagnostics Products Corporation, Los Angeles, CA). The mice were euthanized at 4 weeks post-operation, then pancreases were carefully excised and weighed and used to calculate the Panc/ body weight.

Measurement of plasma glucose and serum HGF

For fasting plasma glucose (FPG), blood was collected from the tail in conscious animals



Figure 1. Effects of partial hepatectomy on β -cell mass, insulin and pancreas weight. A: Shows the ratio of β -cells to non- β endocrine cells; B: Shows the changes in insulin secretion; C: Shows the changes in pancreas weight. All the samples were taken 4 weeks post-surgery. Values were expressed as Mean ± SEM. #P<0.05 compared to Sham.

after fasting overnight. Samples were centrifuged and plasma glucose analyzed using the glucose oxidase method (Roche/Hitachi 917, Roche Diagnostic, Mannheim, Germany). For oral glucose tolerance test (OGTT), after 12-14 hours of fasting, glucose load (2 g/kg body weight) was administered orally. Glucose levels were measured from tail bleed with a glucometer (Roche; Milpitas, CA, USA) at 0, 10, 20, 30, 40, 60, 80 and 100 min after glucose administration. Serum HGF was measured by ELISA similarly to the method described by Xie et al [16].

Western blotting

First, protein was extracted from pancreatic tissues and measured with the Bio-Rad Protein Assay Kit (Bio-Rad, USA). It was separated by 10% SDS-PAGE gel and transferred to PVDF membranes (Millipore, Germany). The membrane was blocked and incubated with specific primary antibodies, then incubated with specific primary antibodies labeled with horseradish peroxidase (HRP) and detected by chemiluminescence (Thermo, USA). β -actin was used as protein loading control. Primary antibodies against HGF, pGab1, Gab1, β -actin and the corresponding secondary antibodies were purchased from Santa (Santa Cruz, USA).

Statistical analyses

Data were expressed as mean \pm SE, which were analyzed statistically with the software SPSS 17.0 (SPSS, USA). Statistical analysis was performed using ANOVA when appropriate, followed by unpaired Student's t-test. P<0.05 was considered statistically significant.

Results

Effects of partial hepatectomy on β -cell mass, insulin and pancreas weight

Animals were sacrificed 4 weeks post-surgery. Pancreases were carefully excised, weighed, and subjected to insulin immunohistochemical staining to observe the morphological changes. As shown in **Figure 1A**, the ratio of β -cells to non- β endocrine cells also did not differ significantly between Control and Sham group, but was notably higher in Hepatectomy group (p< 0.05 between Sham and Hepatectomy). Meanwhile, insulin secretion in Hepatectomy group was significantly higher than Control and Sham group (**Figure 1B**). Pancreas weight, expressed as g per 100 g body weight, was much higher in Hepatectomy group (**Figure 1C**).

Effects of partial hepatectomy on FPG and blood glucose in OGTT in db/db mice

FPG in db/db mice was evaluated weekly for one week before surgery, the day at surgery and for four weeks after surgery. **Figure 2A** shows the time course of FPG levels. The AUC of FPG level of each group is shown in **Figure 2B**. The AUC in Hepatectomy group was significantly lower than Sham group (P<0.05 compared to Sham). **Figure 2C** and **2D** show the time course of blood glucose in OGTT and their AUCs. When the tests began, there was no significant difference between the blood glucose



Figure 2. Effects of partial hepatectomy on FPG and blood glucose in OGTT. A: Shows the time course of FPG changes from two weeks pre-surgery to four weeks post-surgery. Mice were fasted overnight and then blood glucose levels were measured. B: Shows the AUC of FPG. C: Shows the blood glucose in OGTT. Mice were fasted overnight and then orally administered glucose (2 g/kg body weight). D: Shows the AUC of blood glucose. Values were expressed as Mean ± SEM. #P<0.05 compared to Sham.

levels in Control, Sham, and Hepatectomy group. However, the blood glucose levels in Hepatectomy group were significantly lower than Control and Sham group after the tests began. The AUC of glucose in Hepatectomy group was significantly lower than Sham group (P<0.05 compared to Sham).

Effects of partial hepatectomy on serum HGF and pancreatic HGF and Gab1 activation

To examine the possible role of HGF and Gab1, we measured the serum HGF and pancreatic HGF and Gab1 expression after partial hepatectomy. **Figure 3A** shows the time course of serum HGF changes. Day 0 was the day when partial hepatectomy was done. It shows that the serum HGF in mice gradually increased after partial hepatectomy and reached the peak on Day 11. **Figure 3B-D** shows the pancreatic HGF and Gab1 expression on Day 11. The expression of HGF in Hepatectomy group was about 4 times higher than the Control or Sham group (P<0.05 compared to Sham). The Gab1 phosphorylation was about 6 times higher than the Control or Sham group (P<0.05 compared to Sham).

Effects of PHA-665752 on β -cell mass, insulin, pancreas weight and Gab1 activation

PHA-665752 is a specific inhibitor of c-Met, the HGF receptor. We treated mice with PHA-665752, and then examine the changes of



Figure 3. Effects of partial hepatectomy on serum HGF and pancreatic HGF and Gab1 activation. A: Shows the time course of serum HGF post-surgery (Day 0). B-D: Show pancreatic HGF and Gab1 expression. The samples were taken on Day 11. Values were expressed as Mean ± SEM. #P<0.05 compared to Sham.

β-cell mass, insulin, pancreas weight and Gab1 activation after the HGF signaling was blocked. As shown in **Figure 4A-C**, The ratio of β-cells to non-β endocrine cells, insulin secretion and pancreas weight were all decreased by PHA-665752 compared to Hepatectomy group (P<0.05 compared to Sham). As shown in **Figure 4D**, the ratio of pGab1/Gab1 was decreased in Hepatectomy + PHA group, indicating that the treatment of PHA-665752 significantly inhibited the Gab1 activation.

Effects of PHA-665752 on FPG and blood glucose in OGTT in db/db mice

To evaluate the effect of PHA-665752 on pancreas function, we measured the FPG blood glucose in OGTT in db/db mice after partial hepatectomy. **Figure 5A** and **5B** show that the FPG levels was significantly increased by PHA-665752 compared to Hepatectomy group (P<0.05). **Figure 5C** and **5D** show that the blood glucose levels in Hepatectomy + PHA group were significantly higher than Hepatectomy group (P<0.05).

Discussion

Both the liver and the pancreas of terrestrial vertebrates are developed from endodermal epithelial cells in the embryonic foregut. Liver is generated by epithelial hyperplasia from the forelimb end of the ventral wall and abdominal midline [17, 18]. The pancreas is also derived from the ventral wall of the foregut endoderm and the anterior midline cells of the anterior gut in the region adjacent and caudal to the lateral side of the liver [19]. Surgical resection or toxic damage can cause rapid and extensive liver cell proliferation. Resection of more than 50% of the rat liver can induce rapid regeneration of liver stem cells, and restore the original size of



Figure 4. Effects of PHA-665752 on β -cell mass, insulin, pancreas weight and Gab1 activation. A: Shows the ratio of β -cells to non- β endocrine cells; B: Shows the changes in insulin secretion; C: Shows the changes in pancreas weight. All the samples were taken 4 weeks post-surgery. D: Shows the change in Gab1 activation. Values were expressed as Mean ± SEM. *P<0.05 compared to Hepatectomy.

the liver in 10-14 days. The pancreas consists of exocrine and endocrine parts, which are involved in the digestion of nutrients and blood sugar regulation, respectively. The regenerative capacity of pancreatic beta cells is limited compared to that of hepatocytes, especially in adults. Limited regenerative capacity of the pancreatic endocrine may be associated with limited cellular capacity of the pancreas for compensation for β cell loss [20]. However, in the present study, we first examined the effects

of partial hepatectomy on β -cell mass, insulin and pancreas weight. The treatment of partial hepatectomy significantly improved β -cell proliferation, insulin secretion and pancreas growth. Next, we measured the changes of FPG and blood glucose in OGTT in the presence of partial hepatectomy. The results suggested that partial hepatectomy significantly improve carbohydrate metabolism, indicated by the decrease of FPG level and blood glucose in OGTT.



Figure 5. Effects of PHA-665752 on FPG and blood glucose in OGTT in db/db mice. A and B: Show the time course of FPG changes from two weeks pre-surgery to four weeks post-surgery and the AUC of FPG. C and D: Show the blood glucose in OGTT and the AUC of blood glucose. Values were expressed as Mean \pm SEM. [&]P<0.05 compared to Hepatectomy.

Some studies showed that there was an interaction between liver and pancreas regeneration. Oval cells with the characteristics of liver appear in the pancreas after partial removal of acinar cells [9]. After transplantation, the pancreas-derived "oval cells" can differentiate into functional liver cells and bile duct cells [21]. It is shown that liver cells and pancreas cells have the possibility of mutual transformation. Liver cells may have functions of pancreatic endocrine cell through gene transformation [22]. When the liver is regenerated, some inducing factors were greatly increased and may simultaneously induce pancreatic ß cell regeneration. HGF in plasma is increased by 10-20% after partial hepatectomy [23]. Portal vein injection HGF can cause liver cells proliferation and hepatomegaly [24]. HGF receptor is activated in within 30-60 min after partial hepatectomy [25] and the block of it is accompanied by reduced or absent liver regeneration [26]. In the present study, we measured the serum HGF and pancreatic HGF and Gab1 expression after partial hepatectomy. It shows that serum HGF in mice gradually increased after partial hepatectomy and reached the peak on Day 11. The pancreatic HGF expression and Gab1 activation were also dramatically increased by partial hepatectomy.

To further identify the role of HGF and Gab1, we treated mice with PHA-665752, a specific inhibitor of c-Met, the HGF receptor, and found that the ratio of β -cells to non- β endocrine cells, insulin secretion and pancreas weight and Gab1 activation were all decreased by PHA-

665752. Furthermore, PHA-665752 significantly increased the FPG levels and blood glucose levels in OGTT compared to Hepatectomy group. These results revealed a positive effect of partial hepatectomy on pancreatic regeneration and a novel mechanism involving HGF/ Gab1 signaling. The scaffolding/adapter protein, Gab1, is a key signaling molecule for numerous stimuli including growth factors and G protein-coupled-receptors. The interaction between HGF and Gab1 has been demonstrated in many studies. Hoffmann et al showed that HGF induced c-Met and Gab1 tyrosine phosphorylation [27]. A more specific study demonstrated that HGF stimulated a 3-fold increase in membrane Gab1association with c-Met, Grb2, SHP2, PI3K, Shc, Crk isoforms and CrkL. It showed that Gab1 activation plays a central role in HGF's ability to stimulate intracellular transduction cascades in pancreatic acinar cells and this action likely plays a key role in HGF's ability to alter pancreatic cell function (i.e., growth/regeneration) [14], which is consistent with our results in the present study. These findings demonstrated that partial hepatectomy in db/db mice produced pancreatic islet regeneration through HGF/Gab1 signaling pathway. This study provides evidence of a novel mechanism by which diabetes remission induced by pancreatic islet regeneration after partial hepatectomy in db/db mice.

Disclosure of conflict of interest

None.

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