Molecular and Cellular Endocrinology 436 (2016) 33–40

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Sleeve gastrectomy activates the GLP-1 pathway in pancreatic β cells and promotes GLP-1-expressing cells differentiation in the intestinal tract

Feng Li [a, *, 1], Ying Peng [b, 1], Manna Zhang [a], Peng Yang [a], Shen Qu [a, **]

[a] Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tong-Ji University, 301 Middle Yan-Chang Road, Shanghai, 200072, China

[b] Department of Endocrinology and Metabolism, Shanghai Clinical Center for Endocrine and Metabolic Diseases and Shanghai Institute of Endocrinology and Metabolism, Rui-Jin Hospital, Shanghai Jiao-Tong University School of Medicine, 197 Rui-Jin 2nd Road, Shanghai, 200025, China

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form
25 June 2016
Accepted 16 July 2016
Available online 18 July 2016

Keywords:
GLP-1
GLP-1 receptor
Sleeve gastrectomy
L cell differentiation
PAX6
PKCζ

ABSTRACT

Db/db mouse was used to study the underlying mechanisms by which Sleeve gastrectomy (SG) improves β-cell function. We investigated β-cell function, plasma active GLP-1 levels, the GLP-1R pathway in β cells and L cell differentiation. After SG, β-cell function was significantly increased, and the GLP-1R-PKCζ-PDX-1 pathway was active in β cells. Plasma active GLP-1 levels, as well as the number of L cells in the jejunum, were significantly increased after SG. The expression of early transcription factors (TF), including Ngn3, FoxA1 and Nkx2.2, was not compromised by chronic hyperglycemia. In contrast, the expression of the downstream TF PAX6 was affected, and this down-regulation could be reversed by SG. So, SG can maintain L cell differentiation, increase plasma active GLP-1 level, sustain the activation of the GLP-1R pathway and improve β cell function in Db/db mice. Our results show that SG can overall improve the function of the entero-insular axis.

1. Introduction

Bariatric surgery has been shown an effective treatment for the improvement and, in several instances, remission of Type 2 diabetes mellitus (T2DM) (Schauer et al., 2014; Lee et al., 2014; Jiménez et al., 2012; Kim and Richards, 2010; Sjöström et al., 2004; Hsu et al., 2015). β cell function is significantly improved after bariatric surgery (Kashyap et al., 2010; Nannipieri et al., 2011). In recent years, sleeve gastrectomy (SG) has become one of the most performed procedures worldwide as a stand-alone treatment for T2DM. SG was shown to preserve total β-cell mass in Goto-Kakizaki rats (Grong et al., 2016) and improve β-cell function in a non-obese T2DM population (Lee et al., 2015). However, preoperative β-cell failure precludes a complete resolution of T2DM and worsens the outcome of bariatric surgery (Lee et al., 2015; Aarts et al., 2013), suggesting that preventable or "early" SG should be recommended for patients whose β cells are easy to be impaired which is linked to genetic predisposition and environmental factors (Wagner et al., 2014; Stanger et al., 2007; Simmons et al., 2001). Therefore, stratification of T2DM patients and pinpointing those whose β cells are easy to be impaired in the early stages of the disease will contribute to the development of personalized therapeutic interventions.

Glucagon-like peptide-1 (GLP-1) is a gut hormone and functional responses to GLP-1 were improved after SG (Nannipieri et al., 2013; Papamargaritis et al., 2013). However, whether the number of GLP-1-secreting cells increased after SG in different animal models remains controversial (Cavin et al., 2016; Johannessen et al., 2013; Mumphrey et al., 2015). In addition, it remains unclear whether enhanced GLP-1 secretion after SG is sufficient or necessary for diabetes remission (Jiménez et al., 2014; Wilson-Pérez et al., 2013; Vidal and Jiménez, 2013). Further studies are required to answer these open questions.

Abbreviations: SG, sleeve gastrectomy; GLP-1, Glucagon-like peptide-1; GLP-1R, Glucagon-like peptide-1 receptor; Ngn3, Neurogenin 3; FoxA1, Forkhead box A1; Nkx2.2, NK2 homeobox 2; PAX6, Paired box 6; PDX-1, Pancreatic and duodenal homeobox 1; Glut2, Glucose transporter 2; PAX2, Paired box 2.

* Corresponding author.
** Corresponding author.
E-mail addresses: med193@126.com (F. Li), qushencn@hotmail.com (S. Qu).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.mce.2016.07.019
0303-7207/

GLP-1 exerts its actions on the target cells by binding to a specific high affinity receptor (GLP-1R). GLP-1R is abundantly expressed on β cells, but its expression on α cells is controversial (Heller et al., 1997; Tornehave et al., 2008). GLP-1 can potentiate insulin secretion and stimulate β cell proliferation by activating the GLP-1R pathway (Buteau et al., 2001a) and it is an important component of the "incretin effect". GLP-1-based enhancement of glucose dependent insulin release in β-cells is established as being driven mainly via G-protein coupled receptor (GPCR) with downstream PKA and Epac2 activation (Drucker, 2006). Recently, more evidence surport that PKC activation can mediate GLP-1-induced pancreatic β-cell proliferation (Shigeto et al., 2015; Buteau et al., 2001b). The incretin effect accounts for up to 60% of insulin secretion after oral administration of glucose (Nauck et al., 1986). Loss of incretin effect is a very early and specific feature in the pathophysiology of T2DM (Holst et al., 2011). Thus, therapeutic use of GLP-1 to enhance insulin secretion has become an effective treatment for T2DM.

GLP-1 is secreted by specialized intestinal enteroendocrine cells known as L-cells. L-cells differentiate from intestinal stem cells residing in the crypt and are renewed every 4–5 days (van der Flier and Clevers, 2009). An increasing number of reports indicate that a hierarchical network of transcription factors regulates L-cells differentiation, including Ngn3, NKX2.2, FoxA1/A2 and Pax6 (VanDussen and Samuelson, 2010; Jenny et al., 2002; Ye and Kaestner, 2009; Schonhoff et al., 2004). In addition, although it is known that high-fat diet impairs L-cells differentiation (Sakar et al., 2014), the impact of long-term severe hyperglycemia on the differentiation of L-cells is not known.

In this study, we used the leptin-receptor (LPR)-deficient mouse model (db/db), a spontaneous model of T2DM with early β-cell failure and severe hyperglycemia, to determine the molecular mechanisms mediating the increased function of the entero-insular axis after SG.

2. Materials and methods

2.1. Animals

C57BLKS/J-Leprdb/m (Db/m) heterozygotes for the diabetes spontaneous mutation (Leprdb) with normal in body weight, blood glucose, and plasma insulin, regarded as Control in our study and C57BLKS/J-Leprdb/Leprdb (Db/db) homozygous for Leprdb were purchased from the Shanghai Laboratory Animal Center, Chinese Academy of Sciences (SLAC, CAS). All of the mice were housed in pathogen-free facilities with a 12-h light/dark cycle. Sleeve gastrectomy and sham operation were performed respectively on the Db/db mice (Db-Surgery and Db-Sham) at the age of 6 weeks under a microscope as previously described (Bruinsma et al., 2015). Briefly, under a microscope, laparotomy was followed by removal of ~80% of the stomach by cutting in near parallel to the greater curvature, 5 mm from gastroesophageal junction to 5 mm from the pylorus, leaving a narrow gastric tube in the lesser curvature approximately 5–6 mm wide. For sham operation, only retract the liver cranially and bluntly dissect the stomach from its surrounding ligaments. During the 7 days after the surgery, Db-Surgery and Db-Sham were offered liquid diet and after that an *ad libitum* normal diet was resumed to be offered. All animals were killed at the age of 12 weeks. All of the animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

2.2. Glucose tolerance test and metabolic measurements

The mice were fasted for 12 h before the glucose tolerance test. The mice were injected intraperitoneally 1 g kg^{-1} glucose. The glucose measurements were taken up to 2 h post injection using One-Touch Ultra glucometers (LifeScan). The serum insulin levels were measured by a mouse insulin ELISA kit (Crystal Chem). The mice received oral gavage of 2 g kg^{-1} glucose in sterile PBS and GLP-1 levels were measured by a mouse GLP-1 (active) ELISA kit (Merck Millipore).

2.3. Histologic and immunostaining analyses

Pancreata were harvested and fixed in 4% buffered formaldehyde. The immunohistologic analyses were performed on paraffin serial sections, as described previously (Li et al., 2015). The antibodies used for the immunochemistry and immunofluorescence assays are the following: polyclonal rabbit anti-GLP-1 antibody (Abcam), polyclonal rabbit anti-FoxA1 antibody (Abcam), polyclonal rabbit anti-PAX6 antibody (Abcam), monoclonal rabbit anti-PAX2 antibody (Abcam), polyclonal rabbit anti-Ngn3 antibody (Developmental Studies Hybridoma Bank), polyclonal rabbit anti-PKCαβγ antibody (Abcam), polyclonal rabbit anti-PDX-1 antibody (Abcam), polyclonal rabbit anti-Nkx2.2 antibody (Abcam), polyclonal rabbit anti-GLP-1R antibody (Abcam), polyclonal rabbit anti-Glut2 (Abcam), monoclonal mouse anti-insulin antibody (Sigma), polyclonal rabbit anti-glucagon antibody (Cell Signaling Technology), polyclonal rabbit anti-Ki67 antibody (Cell Signaling Technology), monoclonal rabbit anti-PKCζ (Abcam). The images were acquired using a Zeiss confocal microscope or an Olympus system.

2.4. Isolation of mouse pancreatic islets and glucose-stimulated insulin secretion (GSIS)

Pancreatic islets were isolated as previously described (Li et al., 2015). Briefly, the pancreases were digested with collagenase and dissociated vigorously by mechanical pipetting. The islets were "hand-picked" from dark-field dishes under a dissecting microscope and pooled for further analysis.

Islets were incubated over a period of 60 min in 1 ml Krebs–Ringer bicarbonate Hepes buffer (KRBH, 140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM Hepes (pH 7.4), 0.25% BSA) containing 2.8 mM/l glucose or 16.7 mM/l glucose. Experiments were conducted with three to five tubes for each condition. The insulin levels in the supernatant were measured by a mouse insulin ELISA kit (Crystal Chem).

2.5. Quantitative PCR analysis

The total RNA extraction was performed on hand-picked islets using an RNeasy kit (Qiagen) according to the manufacturer's instructions. Quantitative real-time polymerase chain reactions (PCRs) were performed as previously described (Li et al., 2015). The following primer pairs were used in this study:

Nkx2.2: TCGCTCTCCCCTTTGAACTTT, GTTAACGTTGGGATGGT TTGG.

FoxA1: CAAGGATGCCTCTCCACACTT, TGACCATGATGGCTCTCT GAA.

Ngn3: TGACCCTATCCACTGCTGCTT, CCTCATCCACCCTTTGGAGTT.

2.6. Western blot

Protein preparation and Western blots were performed as described previously (Jiang et al., 2014). The following primary antibodies were used: polyclonal rabbit anti-GLP-1 antibody (Abcam), polyclonal rabbit anti-PAX6 antibody (Abcam), polyclonal rabbit anti-Ngn3 antibody (Abcam), polyclonal rabbit anti-ER81