Regulation of compensatory β-cell proliferation by inter-organ networks from the liver to pancreatic β-cells

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Abstract. In insulin-resistant states such as obesity, pancreatic β-cells proliferate to prevent blood glucose elevations. However, the mechanism(s) by which obesity induces compensatory β-cell responses is not fully understood. Recently, several studies have shown that signals from the liver, such as neuronal signals or humoral factors, regulate β-cell proliferation during obesity development. We previously reported a liver-brain-pancreas neuronal relay, consisting of afferent splanchnic nerves, the central nervous system and efferent vagal nerves, to promote this compensatory β-cell proliferation. Furthermore, we recently clarified the molecular mechanisms by which efferent vagal signals induce β-cell proliferation in this inter-organ neuronal network system. Herein, these liver-β-cell inter-organ networks are reviewed, focusing mainly on the neuronal network. The significance of the neuronal network system in the maintenance of glucose homeostasis is also discussed with reference to the relevant literature.

Key words: β-cell proliferation, Inter-organ network, Vagal nerve, FoxM1, Obesity

Introduction

In insulin-resistant states, β-cells proliferate and secrete more insulin to meet the increased systemic demand for insulin [1], thereby maintaining glucose homeostasis at the whole-body level. Therefore, these responses appear to be an endogenous preventive mechanism acting to prevent diabetes development. However, the mechanism(s) by which obesity induces compensatory β-cell responses is not fully understood. It was previously reported that humoral factors such as glucose [2] can serve as a regulator of β-cell proliferation in these processes. However, obese humans [3] and rodents [4, 5] reportedly exhibit compensatory β-cell responses prior to the onset of detectable hyperglycemia, indicating the involvement of unknown triggers, other than glucose, in these processes.

Recently, several studies have shown that signals from the liver regulate β-cell proliferation. These signals are mediated through neuronal pathways or humoral factors.

Humoral Factor-Mediated Inter-Organ Networks from the Liver to Pancreatic β-Cells

Liver specific insulin receptor knockout (LIRKO) mice reportedly exhibited marked islet hyperplasia [6]. Ouaamari et al. reported that parabiosis of LIRKO mice with wild type mice induced enhancement of β-cell proliferation in wild type partners joined with LIRKO mice. In addition, conditioned media from liver explant cultures from LIRKO mice induce β-cell proliferation in wild type mouse islets [7]. These results suggest that liver-derived humoral factors induce β-cell proliferation in LIRKO mice.

Recently, the same group identified Serpin B1, a serine protease inhibitor, as a humoral factor which enhances β-cell proliferation in LIRKO mice. High fat diet (HFD)-induced β-cell proliferation was inhibited in Serpin B1 knockout mice [8], suggesting involvement of liver-derived humoral factors in compensatory β-cell proliferation in LIRKO mice.

Another research group reported the involvement of hepatocyte growth factor (HGF) in compensatory β-cell proliferation. Feeding of a cafeteria diet or HFD loading increased circulating HGF levels in rodents and, furthermore, circulating HGF levels were found to correlate
with β-cell mass in such animal models. Cafeteria diet-induced β-cell proliferation was inhibited by an inhibitor of the HGF receptor \textit{in vivo} [9].

These humoral factors are possibly involved in the compensatory β-cell proliferation that occurs during obesity, although the trigger(s) which upregulates these hepatic humoral factors during obesity development remains unclear.

**Neuronal Signal-Mediated Inter-Organ Network from the Liver to Pancreatic β-Cells**

The extracellular-signal regulated kinase (ERK) pathway is one of the mitogen-activated protein kinase (MAPK) pathways and plays important roles in cell proliferation [10]. Meanwhile, the hepatic ERK pathway is reportedly activated in ob/ob mice, a murine obesity model [11]. We previously showed β-cell proliferation to be markedly enhanced by activation of the hepatic ERK pathway [12]. Mice treated with adenovirus containing constitutively active mutant of MAPK/ERK kinase 1 (MEK1) [13], a kinase which phosphorylates ERK, exhibited activation of the ERK pathway selectively in the liver (L-MEK mice). L-MEK mice showed marked enhancements of both glucose stimulated insulin secretion and β-cell proliferation. Activation of the hepatic ERK pathway can thus induce insulin secretion and β-cell proliferation \textit{via} the inter-organ network from the liver to pancreatic β-cells.

Vagal nerve signals to the pancreas reportedly modulate insulin secretion [14, 15] and β-cell mass [16], although the mechanism(s) by which vagal nerves are activated was not addressed in early studies. Therefore, we designed experiments to examine the involvement of vagal nerves in the liver-β-cell inter-organ network from the liver to pancreatic β-cells. Pancreatic vagotomy significantly blunted enhancements of both insulin secretion and β-cell proliferation in L-MEK mice. These results clearly showed vagal signals to be involved in the liver-β-cell inter-organ network. In addition, involvement of the neuronal network system, consisting of efferent vagal nerves, indicates that signals from the liver are first transmitted to the brain. The liver is innervated by splanchnic nerves, both afferent and efferent, and afferent splanchnic nerves reportedly transmit nociceptive information from the liver to the brain [17]. Therefore, we pharmacologically blocked afferent signals of splanchnic nerves using capsaicin. Selective deafferentation of splanchnic nerves significantly blunted enhancement of both insulin secretion and β-cell proliferation in L-MEK mice. These results indicate that signals from the liver are transmitted to the brain \textit{via} afferent splanchnic nerves in the liver-β-cell inter-organ network system. In fact, midbrain transection, which blocked the pathway from the brainstem to upper brain structures such as the hypothalamus [18], also blunted the β-cell phenotypes in L-MEK mice. Thus, the triggers which transmit the signals to vagal nerves are derived from the liver (liver-β-cell inter-organ neuronal network) and, furthermore, the liver-brain-β-cell neuronal network is involved in β-cell proliferation [12, 19] (Fig. 1).

**The Liver-β-Cell Neuronal Network is Involved in Compensatory β-Cell Proliferation during Obesity Development**

To explore the involvement of the liver-β-cell neuronal network in compensatory β-cell proliferation during obesity development, we blocked the neuronal network in ob/ob mice which exhibit marked β-cell proliferation along with obesity development [11]. The liver-β-cell neuronal network was blocked at several points, such as suppression of the hepatic ERK pathway employing adenovirus containing the dominant-negative mutant of MAPK/ERK kinase 1 (MEK1) (DNM) [20], pharmacological deafferentation of splanchnic nerves and pancreatic vagotomy. Each of these blockade strategies resulted in marked inhibition of compensatory β-cell proliferation along with obesity development in ob/ob mice. These results confirmed the liver-β-cell inter-organ neuronal network to indeed be involved in β-cell mass expansion during obesity development (Fig. 1). In addition, these results provided the first evidence that factors stimulating efferent vagal nerves to the pancreas are derived from the liver, at least in the setting of obesity.

**Activation of the Liver-β-Cell Inter-Organ Neuronal Network Reverses Hyperglycemia in Murine Models of Insulin-Deficient Diabetes**

Thus, since activation of the liver-β-cell neuronal network can increase β-cell mass, we attempted to improve insulin-deficient diabetes by activating this network. First, we activated the hepatic ERK pathway in mice with streptozotocin (STZ)-induced diabetes, a murine
model of pharmacological β-cell loss. Activation of the inter-organ network dramatically reduced blood glucose levels of STZ-mice along with an increase in β-cell mass. We then applied this strategy to Akita mice, a murine model of endoplasmic reticulum stress-induced β-cell loss [21, 22], because endoplasmic reticulum stress is involved in the pathogenesis of type 2 diabetes [23]. Findings similar to those in STZ-mice were obtained in Akita mice. Thus, activation of this inter-organ network can increase the β-cell mass of insulin-deficient diabetic mice and manipulating it may lead to the development of novel therapeutic strategies for insulin-deficient diabetes, including both type 1 and type 2 diabetes. In addition, injured tissues or organs may potentially be regenerated by stimulating the endogenous inter-organ neuronal network system.

**Liver-β-Cell Inter-Organ Neuronal Network Activates β-Cell FoxM1 Pathway**

FoxM1 is known to be a critical transcription factor for cell cycle progression. This mitogenic transcription factor affects several aspects of the cell cycle [24], such as stimulation of cell proliferation by promoting G1/S transition via triggering of the transcriptions of several cyclins, including cyclin A (Ccna) [25-27], regulation of G2/M transition by transactivating cyclin dependent kinase 1 (CDK1) [26, 28] and control of proper progression of mitosis by increasing the expressions of several mitotic genes such as polo-like kinase 1 (Plk1) [29]. FoxM1 expression is induced during cellular proliferation in a variety of cell types [30, 31]. The importance of FoxM1 was recently reported in pancreatic β cells as well. FoxM1 deficiency in the whole pancreas induced a gradual decline in β cell mass with age [32]. Furthermore, β cell proliferations after partial pancreatectomy [33] and during pregnancy [34] were markedly blunted in pancreas-specific FoxM1 knockout mice. Additionally, FoxM1 in pancreatic islets is reportedly upregulated in obese mice [35].

Employing microarray analysis or quantitative RT-PCR using isolated islets from L-MEK mice, we found that expressions of FoxM1 and its target genes were significantly increased [36]. Importantly, both FoxM1 pathway activation in islets and β-cell mass increases in L-MEK-mice were completely blocked by the vagotomy procedure. These findings suggest that neuronal signals, transmitted by the liver-β-cell neuronal network, activate the FoxM1 pathway in β-cells. We next explored the significance of β-cell FoxM1 in obesity settings employing ob/ob mice or HFD-fed mice. Expressions of FoxM1 and its target genes were significantly increased in islets from ob/ob mice or HFD-fed mice. When the inter-organ neuronal network was blocked at several points by suppression of the hepatic ERK pathway or by pancreatic vagotomy in ob/ob mice, activation of the β-cell FoxM1

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**Fig. 1  Liver-β-cell inter-organ neuronal network**

The hepatic ERK pathway is activated during obesity development. The neuronal signals triggered by hepatic ERK activation are transmitted to the brain through afferent fibers of splanchnic nerves. After modulation of the signals in the brain, efferent fibers of vagal nerves transmit these signals to pancreatic β-cells and thereby elicit compensatory β-cell responses such as insulin secretion or β-cell proliferation.
pathway and increases in β-cell mass along with obesity development were entirely blocked. Thus, vagal nerve signals transmitted by the inter-organ network apparently activate the β-cell FoxM1 pathway, thereby inducing compensatory β-cell proliferation during obesity.

**FoxM1 is Critical for Compensatory β-Cell Proliferation during Obesity**

To explore the role of FoxM1 in the β-cell proliferation observed in L-MEK-mice and obese mice, our research group generated tamoxifen-inducible β-cell-specific FoxM1 knockout mice (iFoxM1βKO mice) by crossing RIP-CreER mice [37] and FoxM1-floxed mice [38]. β-cell FoxM1 deficiency significantly blocked increases in β-cell mass in both L-MEK-mice and HFD-fed mice, highlighting the key role of β-cell FoxM1 in neuronal signal-dependent β-cell proliferation. In addition, impaired glucose tolerance and insufficient insulin secretion became apparent in iFoxM1βKO mice under the HFD-fed condition. Importantly, hepatic ERK activation and β cell FoxM1 activation were observed as early as one week after HFD loading. Thus, the liver-β-cell inter-organ neuronal network system is likely to maintain glucose homeostasis, allowing insulin resistance to be anticipated, with possible prevention of the resultant diabetes, in the very early phase of obesity development based on promoting compensatory β-cell proliferation via β-cell FoxM1 up-regulation (Fig. 2).

**Combinations of Several Vagal Factors Induce β-Cell Proliferation**

Vagal nerves innervating pancreatic islets have been demonstrated to express several neuropeptides, including pituitary adenylate cyclase activating polypeptide (PACAP) [39], vasoactive intestinal polypeptide (VIP) [40, 41] and gastrin releasing peptide [42, 43] in addition to acetylcholine [44]. Therefore, we treated pancreatic islets, isolated from mice, with a combination of these neural factors. Histological analyses revealed proliferating β-cells to be increased in islets treated with all four neural factors in combination. Further examinations revealed treating acetylcholine plus either PACAP or VIP to maximally increase proliferating β-cells, results similar to those obtained with four neural factors. Importantly, FoxM1 deficiency suppressed neural factor-mediated upregulations of FoxM1 target genes and the
resultant β-cell proliferation. Thus, combined treatment with neural factors promotes β-cell proliferation through a FoxM1-dependent mechanism. Several previously reported findings have suggested the role of FoxM1 in β-cell proliferation. However, the mechanisms by which FoxM1 is activated in β cells were unclear. These results first identified vagal factors as activators of the β-cell FoxM1 pathway. Collectively, vagal nerves which release several neurotransmitters then activate the FoxM1 pathways in β-cells, thereby promoting compensatory β-cell proliferation during obesity development (Fig. 3).

Acetylcholine exerts its insulinotropic effects on β-cells through a Gq-linked G protein-coupled receptor (GPCR) [45]. Meanwhile, PACAP and VIP also induce insulin secretion from β-cells through Gs-linked GPCR [46, 47]. It is likely that, taking advantage of the vagal system in the pancreas, which releases multiple neurotransmitters and achieves high concentrations of these factors locally around β-cells, the Gq- and Gs-signaling pathways in β-cells are both stimulated, in fact simultaneously, leading to efficient β-cell proliferation (Fig. 4). Since low responsiveness of human islets cells to several proliferation-inducing stimulants was previously reported [48], further intensive studies, such as examinations of whether vagal factors promote proliferation of human β-cells, are needed for clinical application of these vagal factors.

Conclusions and Future Perspectives

Recently-obtained evidence has revealed that signals from the liver regulate β-cell proliferation. How neuronal signals and humoral factors cooperate to regulate β-cell proliferation in several physiological settings remains, however, to be elucidated. β-cell loss is critical to the pathogenesis not only of type1 but also of type 2 diabetes mellitus [49]. Although marked advances have been made in transplanting pancreatic islets, immune rejection and donor supply remain major challenges which must be overcome [50]. In this context, targeting of these inter-organ network mechanisms constitutes a promising line of research for regenerating the patients’ own β-cells.

Impairment of limb regeneration [51] and retardation of liver regeneration [52] by surgical denervation have already been reported. Thus, the concept that neuronal signal-regulated tissue regeneration has already been advocated for a few decades. However, the molecular mechanisms underlying these phenomena remain a mystery. It is hoped that the concept of neuronal factors pro-
moting cell proliferation may open a new avenue of research in the field of tissue regeneration, paving the way to novel therapies.

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Conflict of Interest Statement

The author has no competing interests to declare.

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