REVIEW

The endocrine role between β cells and intra-islet endothelial cells

Zilong Cao and Xiaoyu Wang

School of Medicine, Shandong University, Shandong 250012, P.R.China

Abstract. The islets of Langerhans is the endocrine function region of pancreas, which exist in five cell types. The majority of endocrine cells are insulin-secreting β cells, mixed up with glucagon-secreting α -cells. The islets of Langerhans are highly vascularized, and the capillary network around the islet is about five times denser than that in the exocrine tissues. It guarantees endocrine cells adequately contact with the capillary networks. Above mentioned is the basis of deep study the interaction between β cells and capillary. Increasing number of studies contribute to the consensus that endothelial cells have positive effects in the islet microenvironment. Endothelial cells can act as endocrine cells which release many active substances, such as hepatocyte growth factors (HGF), thrombospondin-1(TSP-1), laminins, and collagens by means of different molecule pathways, inducing β cells differentiation, proliferation, survivor, and insulin release next to the vessels. Apart from the effect of endothelial cells on β cells by paracrine fashion, the islets can utilize VEGF-A, angiopoietin-1 and insulin signaling to increase the interaction with endothelial cells. As the endocrine role of endothelial cells to β cells, it may be a novel target to stimulate β cells regeneration, promote vascularization post islet transplantation strategy in the treatment of diabetes mellitus.

Key words: β cells, Endothelial cells, Endocrine, Insulin

THE HUMAN pancreas plays a key role in the digestive and endocrine system. It is a glandular organ functioning as an endocrine gland, which produces many hormones involved in maintaining metabolic homeostasis. These include insulin, glucagon, pancreatic polypeptide, somatostatin (SS), ghrelin [1], and an organ in the digestive system that secretes digestive enzymes containing pancreatic juice, which is responsible for the absorption and digestion of nutrients within the small intestine.

The pancreas can function as an endocrine and exocrine gland. The endocrine function region of the pancreas consists of approximately a million clusters of cells, known as the islets of Langerhans [2]. The islets of Langerhans essentially exist in five cell types, and are classified by their secretion [1]. Specifically, the majority of endocrine cells are insulin-secreting β cells, mixed up with glucagon-secreting α -cells

and somatostatin-secreting δ -cells [1]. The islets of Langerhans are highly vascularized, and the capillary network around the islet is about five times denser than that in the exocrine tissues. It guarantees endocrine cells adequately contact with the capillary networks [3] which is the basis of deep study the interaction between β cells and capillary. Additionally, the capillary wall is composed of one-layer vascular endothelial cells, which show a high permeability, and consist of ten times more fenestrae than those endothelial cells present in the exocrine pancreas [4]. It guarantees a higher capacity in the exchange of oxygen, carbon dioxide, nutrients, and chemical waste substances between the blood and the surrounding tissues [4]. Overall, the hormone secreted by the islets is easily distributed to the whole body, playing a main function in the homeostasis of blood glucose concentration and glucose metabolism.

As we know, type 1 diabetes is due to autoimmune destruction of β cell, which results in lacking of insulin, leading to hyperglycemia [5]. Insulin therapy is the current common method, while the living quality of patients is reduced. Therefore it is crucial to increase the regeneration of self islet β cell and

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Correspondence to: Zilong Cao, Room 1004, Building 7, No.115 Wenhua Xi Road, Jinan, Shandong, 250012 P.R. China

E-mail: xiaocaoshangfei@126.com

production of insulin.

Lots of evidences prove the islet endothelial cells having significant relationship with the function of islets. When suffers hyperglycemia and glucose toxicity, islet endothelial cells are vulnerable to destruction [6]. In the early phase of type 1 diabetes, endothelial cells can attract immune cells by leukocyte-homing receptors to induce β cell destruction [7]. Islet transplantation is an experimental treatment for type 1 diabetes; it uses isolated donor islets into another person. Studies found isolated rat islets can prolong the islet graft survival in diabetic rats by co-transplantation with endothelial cells [8]. In early processes of islet transplantation, when the donor islet is transplanted, donor islet endothelial cells will participate in neovascularization, increasing the survival of β cells [9]. After β cells injury, endothelial cells promote pancreatic stem cell proliferation and islet regeneration [10]. Endothelial cells can enhance embryonic stem cell differentiate into pancreatic progenitors and β cells *in vitro* through bone morphogenetic protein (BMP) pathway, which controls pancreatic cell proliferation and differentiation [11].

Overall, it's definitely that endothelial cells can affect the function of islets. Neovascularization is a very important effect, while recent many studies focus on its endocrine roles to islets.

The endocrine role of islet endothelial cells to β cells

Besides acting as a barrier, endothelial cell also has endocrine property, and it is attached importance to continuous growth, differentiation, and function of β cell. It can release many vasoactive substances and growth factors, which affect the function of β cell in a paracrine fashion, leading to the production and secretion of insulin [4, 12].

Hepatocyte growth factor

Hepatocyte growth factor (HGF) is a cytokine which promotes cell proliferation, motility, and morphogenesis [13]. It can bind to the tyrosine kinase membrane receptor c-Met [14, 15], which activates RAS and phosphatidylinositol (PI) 3-kinase (PI3K) pathway, mediates proliferation and survival signals [16, 17]. Studies found that HGF has strong positive effects on β cells mitogenesis, differentiation, glucose sensing, and transplant survival [18]. In early development,

HGF and c-Met are highly expressed in the pancreas, and maintained at a low level in adults, which seems HGF and c-met pancreatic gene expression are age dependent [19]. HGF can be released by endothelial cells [4], and bind to c-Met receptor on β cells [20]. It activates phosphatidylinositol (PI) 3-kinase (PI3K) pathway, generates phosphorylated phosphoinositides binding to pro-survival intracellular signaling molecule protein kinase B (PKB/Akt), enhancing β cells proliferation and survival [21]. PKC zeta is a novel downstream target of PI3K–phosphoinositide-dependent kinase-1 (PDK-1) activation, which is found essential for HGF induced β cell proliferation [22]. In rat insulin II promoter (RIP)-HGF transgenic mice, HGF are overexpressed in β cells, leading to a 2-fold increase in insulin; β cells replication rates and islet mass are two to three times higher than control. Moreover, the islet number per pancreatic area is increased by approximately 50% [23], which shows resultant mitogenesis, pro-differentiation, and anti-apoptotic properties [18]. In streptozotocin (STZ)-induced diabetic mice, when islets are transplanted, HGF is essential for the amelioration of hyperglycemia [24]. It's interesting that during pregnancy in adult rats, the production of HGF is increased, concluded by overexpression of prolactin [25]. Overall, these studies suggest that HGF is a important factor to islets, so HGF gene therapy is a novel way to improve successful islet transplantation and treat diabetes [26].

Thrombospondin-1

Thrombospondin-1 (TSP-1) is a matricellular glycoprotein that interacts with growth factors, cytokines, other matrix components and membrane proteins, regulating cell proliferation, migration and apoptosis [27]. It is important to the activation of transforming growth factor β -1 (TGF β -1) *in vivo*, which induce cell growth, differentiation, and immune modulation [28]. In normal islets, TSP-1 is primarily produced by endothelial cells [29], and it can induce the islets produce TGF- β [21], which may initiate a signaling cascade leading to Rho-like GTPases and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) pathway activation [30], to establish and maintain defining features of mature pancreatic β cells [31]. Studies found that in TSP-1-deficient mice, glucose-stimulated insulin release is decreased [29], which therefore suggests the critical role of TSP-1

in insulin production. In high glucose model, both overexpression of PKC and TGF- β 1 regulate TSP-1 expression, and that glucose-induced increase of TSP-1 can synergistically facilitate TGF- β 1 [32]. On the other hand, TSP-1 plays roles in antiangiogenesis [33]. It can inhibit endothelial tubule formation *in vitro* [34], and express antiangiogenic activities *in vivo* [34]. It interacts with the endothelial cell surface receptors CD36 [35], activates Src family kinases Fyn/Yes, and increases expression of death receptors and Fas ligand, leading to cell apoptosis [34]. So it can induce the destruction of islet capillary network in islets, showing the negative aspects to β cell.

Basement membrane

Basement membranes (BMs) are extracellular matrix that lie along the basal surface of endothelial cells, which look like a sleeve around the endothelium, supporting the architecture of vessel [36]. They are primarily composed of collagen, laminin, fibronectin, and heparan sulfate [36]. In islets, as β cells cannot form a basement membrane [37], BMs are primarily formed by endothelial cells [36]. Vascular basement membrane directly contact with the pancreatic islet cells [38], so they play an important role of the physiological microenvironment to pancreatic islet cells [38, 39]. Many evidences found that disruption relationship between islet cells and basement membrane, or the loss of basement membrane, both leading to the apoptosis of islets cells [40].

Collagen-I and IV are primarily collagen in basement membrane [39; 41], and α 1 β 1-integrin and α 3 β 1-integrin are the primary collagen receptors in both fetal and adult β cells [42; 43]. *In vitro*, cultured β cells can adhere to and migrate on collagen [43]. When collagen bind to α 1 β 1-integrin, it can activate focal adhesion kinase and extracellular signal-regulated kinases phosphorylation (FAK-ERK) pathway, increasing pancreatic and duodenal homeobox 1 (Pdx-1) expression, which is a transcription factor necessary for pancreatic development and β cell maturation, and protect from caspase 3 cleavage, which is activated in the apoptotic cell [42; 44]. It regulates glucose metabolism, contributes to the survival and function of β cell *in vivo* [44]. When binding to α 3 β 1-integrin, it can activate phosphatidylinositol (PI) 3-kinase (PI3K), which increase the lipid phosphatidylinositol (3,4,5) P3, leading to activation of protein kinase B (PKB /Akt) pathway,

enabling the differentiation and survival of human islet cells [42].

Laminin-411 and laminin-511 are two major laminin isoforms in vascular basement membranes [37]. Through activation of the FAK/ERK pathway, they can bind to β 1-integrin on β cells, increasing the expression of Pdx-1 and insulin, contributing to the differentiation and survival of human fetal pancreatic islet cells [45]. Other studies found that laminin-1 can bind α 6-integrin and α -DG on β cells to promote cell survival and β cell differentiation [46, 47]. When islets are exposed to interleukin-1 β , which is an important mediator of the inflammatory response, the expression of laminin-332 is unregulated, protecting β cells from apoptosis through phosphatidylinositol 3-kinase (PI3-K) pathway [48].

In addition to laminin and collagen, many other basement membrane may also exert beneficial effects to β cell. Fibronectin is a glycoprotein that binds to integrins, which mediates a wide variety of cellular interactions. It plays roles in cell adhesion, migration, growth and differentiation [49]. It can maintain integrity of islet structure *in vitro*, and increase insulin release by decreasing expression of SUR1 [50], which is a subunit of ATP-sensitive K-channels, closing the channel can result in insulin release [51]. Heparan sulfate is a linear polysaccharide that is expressed at high levels in mouse islets [42]. It can protect β cells resistant to damage from ROS *in vitro*; and when heparan sulfate is degraded islets will be autoimmune destructed in NOD mice, showing essential for β cell survival [52].

Studies found that collagen, laminin, fibronectin, and other basement membrane can bind to TSP-1, which can activate TGF- β and affect the composition of basement membrane, such as inducing the synthesis of collagens and other matrix molecules [53]. It may shows the extracellular proteins which have synergistic effect to β cells.

Connective tissue growth factor

Connective tissue growth factor (CTGF) is protein that is highly expressed in endothelial cells during development [54, 55], and functions as an inducer of β cell proliferation, differentiation, and islet morphogenesis [56]. The loss of CTGF derived from endothelial cells results in embryonic β cell proliferation and islet vascularity decreased [56]. Studies found that CTGF can induce the production of basement membrane components, such as collagen and fibronectin

by mediating transforming growth factor β (TGF- β), and it has a domain that is thrombospondin-1 (TSP-1) repeat, all of which have shown beneficial effects on β cell [54]. Furthermore, CTGF induces expression of platelet-derived growth factor subunit B (PDGF-B) in endothelial cells, required for pericytes recruitment and retention, which are embedded in basement membrane [54]. Studies found that pericytes can increase eNOS levels and vasodilatation, which have contribute to the development of insulin resistance [57].

Endothelin-1

Endothelin-1 (ET-1) is a potent endothelium-derived vasoconstrictor [58]. Insulin can stimulate the expression and secretion of ET-1 from bovine endothelial cells [59], and hyperinsulinemia stimulates the production of plasma ET-1 in humans [60]. Studies found it can stimulate insulin secretion *via* ETA receptors in β cell [61], which increase calcium ion channels influx [62] and activate PKC pathway [61], acting as an important intracellular amplifier in insulin secretory process in islets [61]. On the other hand, ET-1 can upregulate the expression of Forkhead box O1 (FOXO1) gene on endothelial cells, which plays an important role in endothelial cell survival [63].

The endocrine role of β cells to islet endothelial cells

Apart from the effect of endothelial cells on β cells by paracrine fashion, β cells can also secrete many pro-angiogenic factors which promote endothelial cells proliferation, influence the function of endothelial cells, and increase vascularization [3, 64].

Vascular endothelial growth factor-A (VEGF-A) is a major contributor to the proliferation, migration survival and permeability of endothelial cells [65]. If VEGFR is blocked by antagonists, such as AG-013736, a small-molecule VEGFR tyrosine kinase inhibitor, the density of capillaries is significantly reduced [66]. In islets, β cells secrete large amounts of VEGF-A early in development and throughout adult life [67], and it's controlled by pancreatic and duodenal homeobox 1 (Pdx1) promoter [57].

Experiments discovered that VEGF is important to the success of islets transplantation. During the first few days after transplantation, if subjected to hypoxia, it induces VEGF producing, thereby initiating revas-

cularization and maintaining the vascular permeability of the rat grafted islets [68]. Upregulation of VEGF accelerates islet revascularization, which increases the survival of transplanted islets [69]. Experiments have shown that VEGF knocked-out mice contain defective remaining capillaries. Consequently, which results in the decreased release of glucose-stimulating insulin, and the impairment of glucose homeostasis [70]. On the other hand, when suffers hyperglycemia, VEGF-A expression is decreased because of islet inflammation, inducing islet vasculature density reducing [6, 71]. Is the VEGF-A the more, the better? The answer is negative. VEGF-A overexpression can increase intra-islet endothelial cells proliferation and vascularization, but the endothelial cells may not form functional vessels, which limit β cell proliferation, and alter islet morphology [72].

VEGF-A mainly acts on VEGFR-2 (Flk-1) on endothelial cells [73]. Studies found that VEGFR-2 is highly expressed in intra-islet capillaries, while in microvasculature of exocrine tissue, and even in islet arterioles and venules, it is downregulated [74]. When VEGF-A binds to VEGFR-2, the activating mechanism of signal transductions are multiple. 1) It results in autophosphorylation of tyrosine residues, activate protein kinase C (PKC)-Ras pathway, which induces mitogen-activated protein kinase (MAPK) /extracellular regulated kinase (ERK) pathway activating transcription factors, causing cells proliferation [65]. 2) It activates phosphatidylinositol (PI) 3-kinase (PI3K)/protein kinase B (Akt/PKB) pathway, through increasing lipid phosphatidylinositol (3,4,5) P3, activates downstream protein kinase B (PKB /Akt) pathway and endothelial nitric oxide synthase, promotes cells survival and increase of vascular permeability and cellular migration [65]. 3) It activates p38MAPK and focal adhesion kinase, inducing cytoskeletal reorganization ad cell migration [65]. Other opinions demonstrate that it can activate signal transducer and activator of transcription 3 (STAT3) signaling, developing pancreatic microvasculature [75]. Sitagliptin, a Dipeptidyl Peptidase IV Inhibition, can activate cAMP response element-binding (CREB) in β cell, increase VEGF secretion, inducing neovascularization of islets [76].

Angiopoietin-1(Ang-1), which functions as a ligand that binds to endothelium-specific receptor tyrosine kinase Tie-2, and activates the p13k/Akt pathway on endothelial cells, as well as prevents endothelial cells apoptosis [77], is a powerful vasoactive factor.

Fig. 1 The following model shows the intra-islet crosstalk between endothelial cell and β -cell mediated by different endocrine factors. Hepatocyte growth factors (HGF), thrombospondin-1(TSP-1), laminins, collagens are vasoactive substances secreted by endothelial cell, promoting β -cell continuous growth, differentiation, and production of insulin. Vascular endothelial growth factor-A (VEGF-A), angiopoietin-1 and insulin are important factors for endothelial cell proliferation, which is mainly produced by β -cell in islet.

Ang-1 can inhibit the expression of inflammation-associated adhesion molecules, including E-selectin and Inter cellular Adhesion Molecule 1 (ICAM-1), protecting against damage induced by inflammatory factor, and regulates the integrity of blood vessels [78]. In islets, Ang-1 is expressed only by the β cells [74]. Unlike the overexpression of VEGF-A impact the function of β cells, increased expression of Ang-1 just alter islet endothelial cells ultrastructure, not do islet differentiation, development, or morphology [72].

As β cells and intra-islet endothelial cells have close relationship, insulin may influence the function of endothelial cells. Insulin receptor can be detected on endothelial cells [79]. It can activate PI-3 kinase which regulate the expression of eNOS gene in endothelial cells, promoting intra-islet blood flow and modulates vascular tone [67, 80]. When suffering diabetes, the inhibition of PI-3 kinase activity may lead to endothelial dysfunctions [80]. Study found insulin

can be synergistic with VEGF-A *in vivo* and *in vitro*, which can induce endothelial cells secreting HGF and enhance β cells proliferation [25].

Conclusion

Increasing number of studies contribute to the consensus that endothelial cells have positive effects in the islet microenvironment. Endothelial cells can act as endocrine cells. Hepatocyte growth factors (HGF), thrombospondin-1(TSP-1), laminins, collagens and other active substances from endothelial cells induce β cells proliferation, insulin secretion next to the vessels. The islets utilize VEGF-A, angiopoietin-1 and insulin signals to increase the interaction with endothelial cells (Fig. 1). As the endocrine role of endothelial cells to β cells, it may be a novel target to stimulate β cells regeneration, promote vascularization post islet transplantation strategy in the treatment of diabetes mellitus.

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