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REVIEW

The Role of Cytoskeleton in Glucose Regulation

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Abstract—Cytoskeleton plays an important role in glucose regulation, mainly in the following three aspects. First, cytoskeleton regulates insulin secretion by guiding intracellular transport of insulin-containing vesicles and regulating release of insulin. Second, cytoskeleton is involved in insulin action by regulating distribution of insulin receptor substrate, GLUT4 translocation, and internalization of insulin receptor. In addition, cytoskeleton directs the intracellular distribution of glucose metabolism related enzymes including glycogen synthase and many glycolysis enzymes.

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The regulation of glucose metabolism is a complex physiology process in which a wide variety of hormones including insulin are involved and a series of molecular events happen. According to research work of recent years, cytoskeleton plays an important physiology role mainly in the following three aspects: insulin secretion, insulin action, and the intracellular distribution of glucose metabolism related enzymes. In this essay, the relationship between cytoskeleton and glucose metabolism is reviewed.

CYTOSKELETON AND INSULIN SECRETION

Insulin is one of the most important hormones in balancing glucose metabolism, and β-cells of the pancreas are the only source producing insulin *in vivo*. Under the stimulation of glucose, insulin is secreted in two phases. The first phase secretion happens rapidly (<200 msec) when the intracellular calcium concentration reaches 2-3 nM, or 3 to 5 min within food intake, mainly characterized by the exocytosis of insulin granules from the readily releasable pool near cell membrane. Insulin secretion is slow and constant in the second stage, which happens 120 min after glucose intake and depends on the synthesis and maturity of insulin and transport of insulin-containing vesicles.

The cytoskeleton of β-cells of pancreas islets is mainly constituted of microtubule and microfilament and plays an important role in the process of insulin secretion. Microtubules provide a pathway for the directional transportation of insulin from rough endoplasmic reticulum to Golgi apparatus and to cell membrane. Microfilament mainly has the following three functions: first, it is a barrier to prevent the approach of insulin vesicles to cell membrane; second, it influences the fusion of insulin vesicles and cell membrane; third, it is involved in the intracellular transport of insulin vesicles. Scheme 1 shows the function of cytoskeleton in insulin secretion.

In rough endoplasmic reticulum, preproinsulin is processed into proinsulin which is later transported to Golgi complex along microtubule framework and further processed into insulin. Then, insulin is packed in large dense core vesicle (LDCVs). When treated with colchicine or vincristine, depolymerization of microtubules occurs, and the conversion from proinsulin to insulin will be delayed. While the recruitment of insulin secretion vesicles from Golgi apparatus to cell membrane during which LDCVs move from the deep part of cytoplasm along the microtubule network to cell membrane, microtubule system and ATP are also needed and the energy for this movement is provided by motor protein-mediated ATP hydrolysis [1]. There are two types of

Abbreviations: CaMK II) calmodulin-dependent protein kinase II; CAP) Cbl-associated protein; GLUT4) glucose transporter 4; INSR) insulin receptor; IRS) insulin receptor substrate; LDCV) large dense core vesicle; MAP-2) microtubule associated protein 2; PI3K) phosphatidylinositol 3-kinase; PKA) AMP-dependent protein kinase; SNARE) soluble N-ethylmaleimide-sensitive attachment protein receptor.

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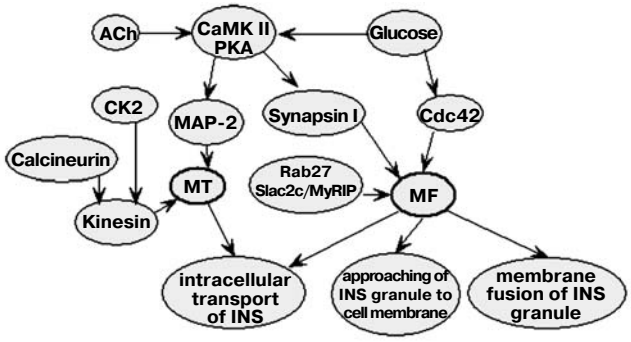


Cytoskeleton and insulin secretion. INS, insulin; ACh, acetylcholine; CK2, casein kinase 2; MT, microtubule; MF, microfilament; CaMK II, calmodulin-dependent protein kinase II; PKA, AMP-dependent protein kinases; MAP-2, microtubule associated protein 2; Cdc42, cell division cycle protein 42

Scheme 1

motor protein in the microtubule system—kinesin (a plus-end-directed motor) and dynein (a minus-end-directed motor), which separately drive the vesicles to move along a microtubule towards the plus end or minus end. Live cell imaging reveals that LDCVs frequently change directions, suggesting that opposite bidirectional motors, notably kinesin and dynein, may be involved. Another study showed that the exocytosis of LDCVs was mediated mainly by kinesin, while the internalization of vesicles after endocytosis mainly by dynein [1]. The homeostasis of the depolymerization and polymerization of a microtubule is the key point to maintain the regular movement of insulin-secreting vesicles. The second phase of insulin secretion will be significantly inhibited when colchicine is used to depolymerize microtubule or nocodazole is used to steady its polymerization state [2].

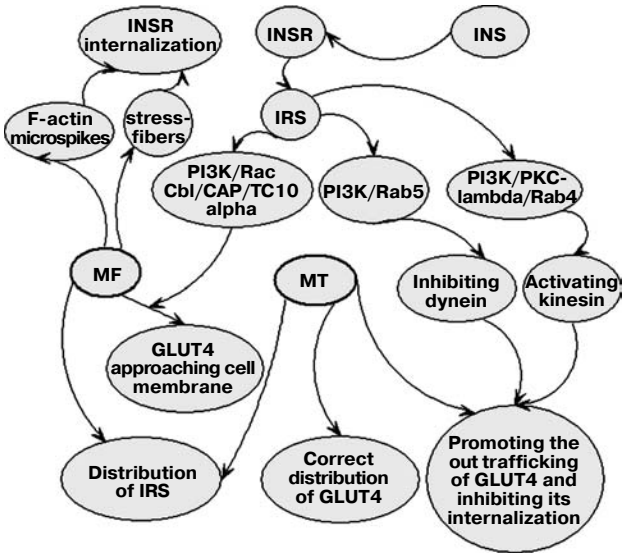
The microfilament network is mainly distributed in the peripheral portion of β-cells and prevents insulin secretion granules from approaching cell membranes; therefore, the disruption and reassembly of microfilaments is crucial for the vesicles to anchor in the membrane. Using cytochalasin or *Clostridium botulinum* C2 toxin to collapse the cell microfilament structure will promote insulin secretion, especially the first phase [3]. Soluble N-ethylmaleimide-sensitive attachment protein receptor (SNARE) is a kind of protein that closely correlates with membrane fusion of insulin granules. Under the sequestering state, the t-SNARE complex located in plasma membrane integrates with polymerized actin, and the transient disruption of this polymerized actin and membrane t-SNARE complex results in the exocytosis of insulin granules, which suggests that microfilaments play a role of preventing the membrane fusion procedure [4].

Besides, microfilaments are also involved in the transport of insulin granules. It has been shown that insulin secretion, especially the second phase, can be inhibited when treating HIT-T15 cells with *C. botulinum* C2 toxin, and it is also slightly inhibited when treating native islet β-cells with the C2 toxin [3]. The modulation mechanism of insulin granule transport mediated by cytoskeleton is still not fully understood. It is probably closely related to the phosphorylation level of motor proteins, microtubule/microfilament binding proteins, and some small G-proteins. Calmodulin-dependent protein kinase II (CaMK II) and cyclic AMP-dependent protein kinase (PKA) are critical elements for the glucose- or acetylcholine-stimulated insulin secretion of β-cells. It has been shown that microtubule-associated protein (MAP-2) and microfilament-associated protein (synapsin I) are substrates of CaMK II and PKA in β-cells [5-9]. MAP-2 has an important influence on the stability of microtubule by mediating the homeostasis of the microtubule network during insulin secretion [10-12]. The significance of phosphorylation of synapsin I by CaMK II is still not well defined.

The general opinion is that it is possibly involved in keeping the integrity of the microfilament network. Besides, phosphorylation of motor protein also plays an important role in the insulin secretion procedure. The phosphorylation of myosin heavy or light chain changes the interaction between myosin and actin, which modulates the movement of insulin granules [13-17]. Kinesin is located in the microtubule network and β-granules. In pancreatic β-cells, kinesin heavy chain is phosphorylated by casein kinase 2 at low levels of Ca^{2+}, but it is rapidly dephosphorylated by protein phosphatase 2B as Ca^{2+} increases. Phosphorylated kinesin heavy chain binds with microtubule, and finally drives the insulin granule moving towards the plus pole of the microtubule [18]. Rab27 and its binding protein Slac2c/MyRIP can regulate the interaction of insulin granules with cortical actin network. Both the overexpression of the actin-binding domain of Slac2c/MyRIP and the selective inhibition of the expression of Rab27 by RNA interference lead to a potent inhibition of exocytosis [19]. Cdc42 is a kind of Rho family GTPase whose activated state can promote actin polymerization. Glucose leads to the rapid and reversible glucosylation of Cdc42, suggesting that cortical actin remodeling maybe induced by this selective glucosylation [20].

CYTOSKELETON AND INSULIN ACTION

Cytoskeleton is vital for keeping the balance of glucose metabolism in which insulin acts in a natural physiological way. Cytoskeleton is involved in the distribution of insulin receptor substrate (IRS), the translocation of glucose transporter 4 (GLUT4), and the internalization of insulin receptor (INSR). Therefore, dysfunction of the cytoskeleton may possibly reduce or block the insulin signal and lead to insulin resistance. (The relationship of cytoskeleton and insulin action is shown in Scheme 2.)



Cytoskeleton and insulin action. INS, insulin; INSR, insulin receptor; IRS, insulin receptor substrate; MT, microtubule; MF, microfilament; GLUT4, glucose transporter 4; PI3K, phosphatidylinositol 3-kinase

Scheme 2

Cytoskeleton and the distribution of IRS. In cells, IRS mainly exists in the cytoskeletal component and the cytosol, and it presents a dynamic flow between the two pools under normal conditions. Only the IRS located on cytoskeleton is functional and can be tyrosyl-phosphorylated under the stimulation of insulin, which is followed by the translocation of phosphatidylinositol 3-kinase (PI3K) from cytosol to cytoskeleton. IRS in cytosol basically has no response to insulin stimulation, which is probably due to the inaccessibility to INSR. Hence, the location of IRS on cytoskeleton provides a more convenient site to the INSR and for the interaction with PI3K, which leads to the highly efficient transport of insulin signal [21].

Naturally, insulin treatment causes the release of IRS from cytoskeleton into the cytosol in an appropriate level. But in the situation of insulin resistance, it has been observed that too much IRS is released into the cytosol, which results in the dramatic reduction in the level of tyrosyl-phosphorylated IRS on the cytoskeletal fraction, while the translocation of PI3K to this fraction is reduced too. This suggests that the excessive collection of IRS proteins in cytosol leads to the state of insulin resistance [21]. It has not been clearly illustrated what causes the excessive release of IRS proteins into cytosol; it is possibly associated with the Ser/Thr phosphorylation level [21].

Cytoskeleton and GLUT4 translocation. GLUT4, mainly existing in the cytosol of fat and muscle cells, is the most important glucose transporter for uptake of glucose in those tissues. The translocation of GLUT4 happens rapidly under stimulation of insulin, and the glucose is taken into cells. It has been previously reported that the structural and functional changes of cytoskeleton triggered by insulin plays a critical role in GLUT4 translocation through the following aspects. First, microfilament is a barrier to prevent the access of GLUT4 to cell membranes. Second, the reassembly of microfilament recruits some certain signal molecules or membrane-fusion-related proteins to collocate with GLUT4 vesicles. Third, microfilament and intermediate filament are essential for the correct intracellular localization of GLUT4-containing vesicles. Fourth, microtubule is involved in the intracellular transport of GLUT4 vesicles.

The structural integrity of the microfilament system is necessary for the insulin-stimulated translocation of GLUT4 vesicles, and the disassembly of microfilament will inhibit the accessibility of GLUT4 to plasma membrane [22-27]. In fat and muscle cells, insulin causes a remarkable reorganization of the cortical actin, which is changed from fibrillar to granular form, and membrane ruffling emerges on the surface of the cell [22-24]. The rearrangement of the microfilament system plays an important physiological role in exocytosis of GLUT4. On one hand, the use of actin filament structure stabilizer can inhibit the transport of GLUT4 to cell membrane, which proved that the reorganization of actin filament is required for GLUT4 to access to the cell membrane. On the other hand, the rearranged actin filament network recruits vesicles containing GLUT4 and other important insulin signal molecules such as PI3K and akt-1 to collocate with GLUT4, which facilitates the insulin signal transfer to GLUT4 [28, 29]. Also, many membrane fusion-related proteins including t-SNARE, syntaxin-4, and SNAP-23 exist in membrane ruffle region supported by rearranged actin filament, which provides spatial conditions for membrane fusion triggered by the interaction of GLUT4 and the membrane fusion-related proteins mentioned above [28].

The mechanism by which insulin stimulates the reorganization of microfilament is still unknown. In muscle cells, it is probably mediated by PI3K and small G-protein Rac, while in lipocytes the dynamic character of actin filaments is probably determined by Cbl/Cbl-associated protein (CAP)/TC10-α-system [30].

Insulin stimulates glucose transport by promoting translocation of GLUT4 proteins from the perinuclear compartment to the surface of the cell. In unstimulated adipocytes, the GLUT4 exists mostly in the perinuclear compartment, and it is acutely translocated to the cell surface in response to insulin only when microtubule and intermediate filament are functioning normally [31, 32]. In adipocytes, GLUT4 resides in two kinds of pools, endosomal pool and a unique pool termed "GLUT4 storage vesicles"; only the latter pool relates with insulin-stimulated GLUT4 trafficking. Two kinds of motor proteins are involved in the directional movement of

GLUT4, in which kinesin supplies the energy for insulin-stimulated GLUT4 trafficking to the cell membrane [33-36] and dynein for the movement of GLUT4 vesicle towards the perinucleus along a microtubule [31, 37].

In adipocytes, insulin treatment increases the total polymerized α-tubulin, during which microtubule-associated motor protein kinesin is activated by PI3K/PKCλ and Rab4, and dynein is inhibited by PI3K and Rab5, thus promoting the trafficking of GLUT4 to the cell membrane and inhibiting its internalization [35-37]. All the research achievements described above were obtained with adipocytes, and the functional study of microtubules in muscle cells still remains to be done. Depolymerization of actin filaments with latrunculin B only causes a significant inhibition of GLUT4 translocation in response to insulin, not to PDGF (a growth factor which is also involved in actin remodeling), which shows that in muscle cells there were two mechanisms on GLUT4 translocation, and only in the insulin-induced GLUT4 translocation pathway the integrity of the microfilament is necessary [38, 39].

Cytoskeleton and the internalization of insulin receptor (INSR). The internalization of insulin–insulin receptor complex, which is the critical requirement for maintaining the sensitivity of cells to insulin, happens immediately after their amalgamation. The complex is transported to a lysosome where insulin is digested and INSR is then recycled to the cell surface. If CHO-T cell line (a Chinese hamster ovary cell line overexpressing INSR) is treated with latrunculin B inducing the disassembly of the microfilament system, the internalization of INSR is inhibited, but the disassembly of microtubule has no effect on it [40]. The modulation mechanism of the internalization of INSR is still not fully understood, but it is probably related with certain extracellular matrix proteins. When cells adhere onto fibronectin, collagen, or laminin, microfilament stress-fibers appear, which probably result in the promotion of the INSR internalization. On the contrary, when cells adhere onto galectin-8, F-actin generates microspikes, which may be the reason of the decreased rate of internalization [40].

CYTOSKELETON AND THE INTRACELLULAR DISTRIBUTION OF GLUCOSE METABOLISM RELATED ENZYMES

In muscle and liver cells, cytoskeleton participates in glycogen synthesis and glycolysis, and it has a close relationship with the intracellular distribution of glycogen synthase and many glycolysis enzymes.

The disassembly of the microfilament–microtubule system will lead to a reduction in glycogen synthesis [41, 42]. Glycogen synthase is an important rate-limiting enzyme in glycogen synthesis whose intracellular distribution has certain characteristics. Under the stimulation of glucose, glycogen synthase interacts with cytoskeleton, which results in its translocation from cell plasma to periphery of the cell and collocation with actin filaments. Microfilament disassembly will inhibit the translocation of glycogen synthase [43, 44].

The glycolytic enzymes phosphofructokinase, enolase, and pyruvate kinase collocate in the cytoskeletal structure, which gives the facility for the transport of substrates [45]. The research of our laboratory proved that pantothenate kinase-4 is involved in glucose modulation via its interaction with pyruvate kinase [46] and also via down-regulating the expression of some cytoskeleton components such as F-actin. Phosphofructokinase is the rate-limiting enzyme in the glycolysis pathway, and its binding with cytoskeleton is reversible. In contrast to soluble phosphofructokinase, cytoskeleton-bound phosphofructokinase is not sensitive to allosteric regulation mediated by glucose 1,6-bisphosphate [47]. Insulin can rapidly stimulate the binding of phosphofructokinase to cytoskeleton resulting from the increase in the local ATP concentration, which supplies sufficient energy for the rapid rearrangement of cytoskeleton [48, 49]. The cytoskeleton-bound phosphofructokinase distinctively decreased in gastrocnemius muscle cells of STZ rats with diabetes [50].

Diabetes, a major phenotype of metabolic syndrome, is now one of the most common diseases throughout the world. The cytoskeleton has a close relationship with several key steps of glucose regulation, which suggests that its dysfunction probably plays an important role in the onset of diabetes.

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