Review Article
PI3K/AKT pathway in modulating glucose homeostasis and its alteration in Diabetes

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Introduction

Type 2 diabetes mellitus (T2DM), a complex disease which is characterized by a mixture of impaired insulin action, insulin secretory deficiency and increased endogenous glucose production. With theIn exception of β-cell failure, the resistance of target tissues to insulin is the main contributing incident to the development of T2DM [1,2]. Despite feeding and fasting phase in non-diabetic individuals, blood sugar remains in a narrow fluctuation and tightly controlled through the balance between glucose uptake by the intestine, glucose synthesis by the liver and ultimately glucose absorption by the peripheral tissues. Insulin is the main regulator and key hormone of glucose balance. It increases glucose absorption in muscles and adipose, and decreases glucose synthesis in the liver. In addition, cell growth and differentiation is stimulated by this hormone, which promotes the lipogenesis, protein and glycogen synthesis, and inhibiting lipolysis, proteolysis and glycogenolysis. It has shown that insulin promotes glucose absorption in cells by stimulating the glucose transporter GLUT4 transcription and translocation from the intracellular part to the cell membrane.

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ABSTRACT

The prevalence of type II diabetes is rapidly increasing worldwide which the primary causes of it is the insulin resistance in peripheral tissues. Tightly coordinated control of both insulin function and secretion is required to maintain glucose homeostasis. Phosphatidyl Inositol 3-Kinase pathway (PI3K) is crucial in mediating insulin's metabolic effects. A key downstream effector is AKT/protein kinase B (PKB), which in activated/ phosphorylated form, regulates the activity of numerous targets, including kinases, transcription factors and other regulatory molecules. On the other hand, studies have been performed to realize the role of negative modulators (specially the role of PTEN and SHIP2) of insulin signal transduction, in order to find therapeutic targets for reducing insulin resistance. In this article, the current understanding of involved molecular mechanisms in the impaired insulin signaling pathway that causes Type II diabetes mellitus is reviewed.

Insulin resistance causes deregulation of these pathways, whereas skeletal muscle and fat tissue do not respond to insulin properly, causing elevations in blood glucose and lipid concentration and increase in insulin secretion by pancreas [3]. Clinically insulin resistance refers to the higher than normal levels of insulin to maintain blood sugar at normal levels, while at the cellular level, insulin resistance refers to the defects of insulin signal transduction pathway [2]. The elevated insulin levels may cause poor insulin response and finally causes disruption of the balance between the function of β -cell and the response of peripheral tissue to insulin which ultimately leads to the

manifestation of T2DM [4]. Obesity may cause insulin resistance indicating that adipose tissue has a crucial role in modulation of metabolism through its function in glucose uptake [5]. Although the preliminary measurable defect in predisposed individuals to develop T2DM is insulin resistance and recent studies have demonstrated a profound understanding of the underlying molecular mechanisms, the precise pathophysiological states are still mainly unknown. The present article tries to look at the possible mechanisms involved [6].

Insulin signaling pathway

Insulin function involves various signaling cascades, which are initiated by insulin binding to its receptor (IR) of target cells, stimulating autophosphorylation of the receptor, eliciting the activation of receptor tyrosine kinases and subsequently stimulating the tyrosine phosphorylation of insulin receptor substrates (IRSs). It should be noted that, phosphorylation of IRS promotes the activation of phosphatidylinositol 3-kinase (PI3K), consequently, AKT/protein kinase B (PKB) and protein kinase C (PKC), which are all of them are serine/threonine kinases [7, 8]. Phosphorylated/activated AKT initiates the translocation of GLUT4 from the intracellular site to the surface of the cell to transport glucose into the cell [9]. Conversely, protein tyrosine phosphatases (PTPs) dephosphorylated/inactivated the IR. PTPs involve a family of proteins with negative effects on insulin function [10]. Furthermore, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) a dual function, lipid and protein phosphatase is considered a negative regulator of the insulin signal transduction. PTEN initially identified as a deleted or mutated tumor suppressor gene in different human cancers. PTEN antagonizes PI3-kinase/AKT pathway by reconverting phosphatidylinositol 3,4,5-triphosphate (PIP3) back to phosphatidylinositol 3,4-bisphosphate (PIP2) [11]. Therefore, regulation of insulin function is performed by the balance between phosphorylation and dephosphorylation (Fig. 1; briefly describes the insulin signal transduction pathway in target cells). The PI3K is supposed to be a crucial constituent of the insulin signal transduction and indispensable for the effects of insulin on GLUT4 translocation and glucose uptake [12]. The reduction of PI3K activity in skeletal muscle of diabetic patients (T2DM), provides evidence for impairment in the insulin

signal transduction that probably contribute to the impaired translocation of GLUT4 and insulin resistance. Overexpression of PTEN, results in inhibition of the PI3K signaling pathway, hence glucose uptake. In contrast, decreasing of PTEN expression, enhance insulin stimulated AKT and GSK3 phosphorylation [13- 15]. This suggests that higher level of PTEN may make individuals more susceptible to the development of Type II diabetes.

IR and insulin action

Although IRs exist in all the cell surface of body, their gene expression in typical insulin target tissues is extremely high and there is limited information about the mechanism that regulates their gene expression. Besides tyrosine phosphorylation, both the IR and IRS undergo serine phosphorylation, which may reduce insulin signal transduction by decreasing the stimulation of tyrosine phosphorylation [16-17]. These kinds of inhibitory phosphorylations results in negative feedback in insulin signaling cascade also considered as a mechanism for crosstalk from other pathways, which initiates insulin resistance. In this process, several kinases including PI3K, AKT/PKB, and GSK3 are implicated. Recent studies indicate that obesity-induced reduction of insulin signal transduction probably derives from the sequential activation of PKC, but the details have not yet revealed [18]. Insulin function is also reduced by PTPs that catalyze dephosphorylation of the IR and IRS rapidly. In vitro studies have shown that insulin resistance states upregulates PTPs. Also, there is some evidence that shows alterations in functionality of IR in insulin resistance conditions [19,20] which in comparison of to the defects in IR activation between adipocytes and muscle demonstrates that IR of adipocytes is mainly subjected to deregulation [21–28]. The IR activation was assessed by determination of its tyrosine phosphorylation. These kinds of investigations of primary cultured muscle cells from biopsies and muscle strips has demonstrated in most of the cases, but not all of them, a mild to non-existing impaired IR activation. Limitations in the biopsy-derived samples, investigation of insulin signaling explains the current discrepancies in the literature of insulin resistance [29–33].

Role of PI3K in insulin signaling and glucose uptake

PI3K is one of the IRS proteins (IRS-1 and IRS-2) target that convert PIP2 to PIP3 by phosphorylation of specific phosphoinositides; in turn, activates serine/threonine kinases, including phosphoinositide-dependent kinase-1 (PDK1) and subsequently activated PDK1 phosphorylates or activates another serine/threonine kinase, AKT/PKB. The PH domain of AKT interacts with PIP3 directly and plays a key role in insulin signal transduction by activating GLUT4 translocation to the cell surface [34-36]. Although PI3K and PIP3 are promising targets for therapeutic purpose to improve insulin sensitivity, current data of alterations in PI3K pathway in insulin resistance conditions are related to the immunoprecipitates to IRS1/2 or phosphotyrosines performed in in vitro assays. Even though this technique reflects the extent of PI3K interaction to its partners, it is not enough to understand other involved mechanisms (such as the role of the PTEN/SHIP2 for PI3K function) in regulating of intracellular PIP3 levels, thus leaving us in an uncertainty nevertheless monitoring the PI3K lipid products (PIP, PIP2 and PIP3) would be a useful technique.

Regulation of AKT/PKB in the insulin signaling pathway

AKT/PKB (a serine/threonine kinase with α, β and γ isoforms) is the PI3K downstream target that is activated through phosphorylation mediated by PDK1 and it has been reported as a key enzyme in regulating of insulin function [37,38]. AKT α is considered to perform the regulation of lipid metabolism and the stimulation of glycogen synthesis in muscle as well as the insulin function in adipocytes [39-41]. AKT β which considered as a main isoform in insulin metabolic functions mostly expresses in skeletal muscles [40,42]. Interestingly, reduction of AKT γ activity which is preferentially expressed in non-insulin dependent tissues, has been reported in skeletal muscle of obese and insulin resistant persons [43,44].

AKTs also contributes to the suppression of liver gluconeogenesis by insulin through inhibiting the expression of key enzymes (phosphoenolpyruvate carboxykinase and glucose 6-phosphatase) [45]. Deregulation of AKT has been suggested in insulin resistance conditions and diabetes, for instance an AKT β knockout mice due to decreased glucose absorption by diabetic muscle and adipose cells [46]. Also, dominant negative AKT β mutation may cause hyperinsulinaemia and diabetes in human beings. Even though this event is rare, it confirms the regulatory role of AKT in metabolism [47].

Some controversies have found on studies. For instance, several studies showed significant decreases of insulin-stimulated Serine/Threonine phosphorylation in skeletal muscle of type II diabetic individuals [30,48,49], while other studies reported no alterations between non-diabetic and type II diabetic individuals [13,30,50]. In one study no defects in AKT α/β phosphorylation were seen despite the decreased PI3K activation in type II diabetic patients [13]. This finding was also confirmed by reports of unaltered AKT phosphorylation in skeletal muscle biopsies as well as cultured cells from diabetic patients compared to the control group by using phospho-specific antibodies [30]. In contrast, other researchers revealed that AKT α activation in isolated muscle strips from moderately obese diabetic individuals was altered in comparison to the BMI-matched control group [51]. A more comprehensive study about the action of all AKT isoforms which performed on the skeletal muscle biopsies from morbid obese insulin resistant patients, demonstrated that the only activities of AKT β and γ were decreased [44]. Furthermore, Isoform-specific variations of the phosphorylation form analyzed in cultured primary myotubes from type II diabetic patients in reaction to insulin and defective Serine phosphorylation on AKT β and defective Threonine phosphorylation on AKT β, while the PH-domain leucine-rich replication protein phosphatase (PHLPP1) which can dephosphorylate the Serine residue of AKT β was up regulated in the muscle of type II diabetic patients rather than the control group [49].

The reason of unresolved issues on current understanding of AKT in insulin sensitivity are; i) the investigation of AKT phosphorylation is usually performed, disregarding isoform specificities; ii) the origin of the samples is used for examinations are different, i.e. Biopsy derived muscle strips or cell culture; iii) the characteristics of cohort studies are seldom fully comparable. In addition, there are very few investigations of AKT alteration in adipose tissue from type II diabetic patients. By considering this issue, more consensuses are there about the impaired AKT

function in adipose tissue of type II diabetic patients [52,53].

AKT not only has a key role in in insulin target tissues to uptake glucose, but also it stimulates proliferation of β-cells in the pancreas [54].

Indirect regulation of pathway by PKCs

PKC family are regulated by lipid-derived by-products have been shown to phosphorylate Ser on the insulin receptor and/or its immediate targets IRS1/2, thereby impairing insulin-receptor-mediated Tyr phosphorylation of IRS1 and, consequently, interfering with insulin signaling. The association of aPKCs in GLUT4 translocation and subsequently glucose uptake by muscle and adipose tissue has mainly been examined in animal studies [55]. The classification of PKC isoforms is; i) classical (cPKCα, βI, βII, γ) activated by Ca+2 and diacylglycerol (DAG); ii) novel (nPKC δ, ε, θ, η) activated by DAG and; iii) atypical (aPKC ζ, λ) respond to neither Ca+2 nor DAG [56]. In adipocytes isolated from human, glucose absorption was increased by the expression of PKC-ζ/PKC-λ/ι while it was decreased by expression of kinase-inactive forms [57]. Data from numerous animal studies which have been performed on muscle and adipose tissue, show abnormalities in aPKC activation in insulin resistance states and type II diabetes [55]. Interestingly, these kinds of abnormalities are found in human studies as well. A double increase in aPKC activity in adipose tissue and muscle has been shown in lean non-diabetic participants by insulin stimulation. Furthermore, a positive relationship between insulin-stimulated glucose disposal rate and insulin-induced PKC-ζ/ λ activity was found [32,58-62]. The insulin-induced activation in adipocytes derived from obese glucose intolerant participants and in myotubes derived from obese and/or obese type II diabetic patients was decreased [32,46,58–62]. Another investigation on weight/age-matched diabetic and non-diabetic cases, indicated that insulin-induced activation of aPKC was decreased in diabetic group, allowed to exclude obesity as the responsible factor [59]. Also, in muscle and adipose tissue from non-diabetic individuals, PIP3 directly activated aPKCs in vitro, to a similar extent as acts insulin while in muscle of type II diabetic patients and glucose intolerant cases and in myotubes and adipocytes from obese glucose intolerant women this activation was reduced [57-59].

Several studies show that although there is no evidence of direct interaction between aPKCs with PIP3 in vivo, the direct addition of PIP3 to aPKC immunoprecipitates, causes increase in aPKC activities like insulin effect. the direct addition of PIP3 to aPKC immunoprecipitates cause insulin-like increases in aPKC activitie. All human studies indicate impaired aPKC activity in type II diabetes, obesity and insulin resistance condition, without considering the kind of tissue under study and the methodology used [58,60,63,64].

GSK3 and glucose homeostasis

Binding of insulin to the IR, promotes it autophosphorylation and induces a cascade of phosphorylation events. Subsequently, by activation of PI3K, PIP2 is converted to PIP3 and induces the activation of AKT/PKB.
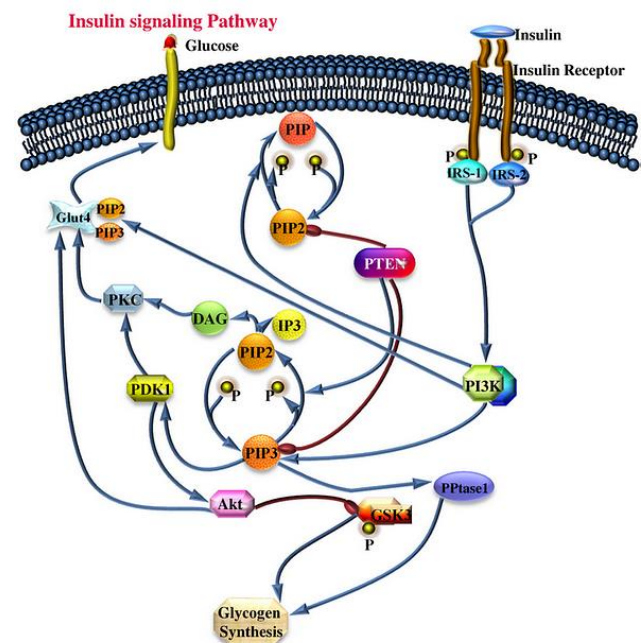

Figure 1. PTEN's role in insulin signaling and glucose metabolism
Insulin interacts with the IR on the surface of the cell membrane, causing autophosphorylation. Consequently, IR promotes the phosphorylation of IRS-1, and binding it to the PI3K. PI3K stimulates the conversion of PIP2 into PIP3, hence activation of AKT by two phosphorylation events (serine and threonine). PTEN antagonizes counteracts PI3K/AKT signaling by dephosphorylating PIP3 back to PIP2. GSK3 is one of AKT's downstream targets. GSK3 activity is reduced by phosphorylation, which induces the activation of GS, leading to increased synthesis of glycogen. On the other hand, stimulation of glucose uptake via Glut4 increases the conversion of blood glucose into muscle glycogen. However, by inhibition of PTEN, PIP3 concentration as well as GLUT4 translocation and AKT downstream events including glycogen synthesis will be increased.

The next step is the phosphorylation of glycogen synthase kinase (GSK3) by activating AKT (Figure 1), which consequently phosphorylation/inactivation of GSK3 causes reduced phosphorylation/activation of Glycogen synthase (GS) [56].

Data from type II diabetic patients shown an increased expression level of GSK3 and its activity in skeletal muscle moreover studies in diabetic mice (by using the pharmacological inhibitors of GSK3) suggesting the role of GSK3β in impaired insulin signal transduction and diabetes because of improvement in insulin signaling and blood sugar in diabetic animal [65,66].

Insulin regulates the activation of GS by stimulating glycogen associated form of protein phosphatase 1 (PP1) which dephosphorylates the phosphorylated residues by GSK3 [67]. Findings suggest that the activation of hepatic GS by insulin is mediated by inactivation of GSK3 while its activation of glucose is mainly performed through the stimulation of a protein phosphatase [68]. However, GSK3 inhibitors stimulate liver GS and have a glucose lowering effects in animal models of T2DM [69]. The GS activity in liver and muscle is modulated by insulin and GSK3α/β [70], and deposition of glycogen in skeletal muscle is reduced by over-expression of GSK3β that indicates the main kinase in modulation of GS in muscle is GSK3β [71].

In addition to its role in the modulation of GS and hence glucose homeostasis, GSK3 is implicated in the translocation of GLUT4 to the cell membrane (hence mediates glucose uptake) which inhibits GSK3 action [72]. On the other hand, GSK3 inactivation by insulin may increase glucose absorption. Moreover GSK3 can phosphorylate the IRS1 and affect the insulin function [73]. Upon insulin-stimulation, IRS1 undergo tyrosine phosphorylation [74].

Hyper phosphorylation of IRS1 on serine/threonine residues decreases the IR ability to phosphorylate IRS1, hence may develop insulin resistance [75]. It has been reported that GSK3 mediates the phosphorylation of IRS1 on Serine residues, also overexpression of GSK3 can cause an increase in the phosphorylation of serine residue and decrease in the phosphorylation of tyrosine residue of IRS1 and IR [73,76]. In proteasomes, hyperphosphorylation of IRS1 on serine residues causes its degradation; therefore,

inhibition of GSK3 might decrease IRS1 phosphorylation. By increasing the expression level of IRS1 in skeletal muscle, the function of insulin will be increased. There are discrepancies regarding the modulation of gluconeogenic enzymes by GSK3. Some findings suggest that inhibition of GSK3 results in suppression of the gluconeogenic enzymes (phosphoenolpuruvate carboxy kinase and glucose-6-phosphatase) hence reduces hepatic glucose synthesis and influence glucose homeostasis [77], while other investigations show that these enzymes, can be suppressed without inhibiting GSK3 [78]. In the short term, GSK3 can inhibit GS activity while in long term GSK3 can negatively regulate insulin signaling and glucose absorption. Moreover, over-expression of GSK3α in skeletal muscle impaired insulin-induced (but not basal) glycogenesis and glucose uptake, indicating that GSK3α has a role in insulin signaling cascade in skeletal muscle [79]. Contrarily, one study [80] by using GSK3α knockout mice demonstrated that the regulatory role of GSK3α in glycogen metabolism is limited to the liver and not muscle [80]. Besides knockout animals, show higher insulin sensitivity and glucose tolerance compared with wild type with a significant decrease in body fat mass. It can be concluded that besides GSK3 effects on GS and hepatic glucose production, it is also involved in insulin signal transduction.

Inhibition of insulin-receptor signaling by protein tyrosine phosphatases

In type II diabetes the insulin-regulated processes in liver, skeletal muscle and adipocytes is impaired. It can occur due to a mutation or loss of expression of an enzyme involved in the insulin signal transduction. In adipose tissue from type II diabetic patients; defective insulin-stimulated IRS-1 tyrosine phosphorylation, decreased association of PI3K with IRS-1, impaired stimulation and activity of PI3K, and reduced GLUT4 expressio, was observed [81-82].

As discussed, PI3k has a crucial role in the insulin signaling pathway, which its activity is determined by phosphatidylinositol-3-phosphatases such as SH2-domain-containing inositol-5-phosphatase (SHIP2) and PTEN. Overexpression of these lipid phosphatases results in reduced levels of PIP3 in cells, which inhibit insulin signaling. It has been demonstrated that negative regulation of

insulin signal transduction is performed by PTEN. Over-expression of PTEN in cultured cells, inhibits insulin-stimulated synthesis of PIP2 and PIP3, activation of AKT and translocation of GLUT4 to the cell surface hence it affects glucose absorption by the cells [15]. In contrast, basal and insulin-induced GLUT4 translocation is increased by microinjection of anti-PTEN antibody [83]. The overexpression of inactive PTEN mutant can't adversely regulate the insulin signal transduction, demonstrating that lipid phosphatase function is necessary for the action of PTEN on the insulin signaling pathway [15]. It has been observed that PTEN inhibition results in AKT phosphorylation and inhibiting PTEN gene expression caused a 80% reduction in liver and adipocytes mRNA level result in normal blood glucose level in the db/db mice [79-81]. These studies, highly suggesting the negative regulatory role of PTEN in insulin signal transduction and insulin sensitivity. As PTEN hydrolyses PIP3 and convert it back to PIP2, it can be a potential target for drug discovery and the inhibition of PTEN could increase the amounts of PIP3 thus affect insulin signaling pathway (Figure 1). However, there is controversy about the role of PTEN in insulin-induced glucose uptake in the literature. Whereas some findings indicate that knock-out of PTEN by siRNA increases insulin-dependent glucose uptake into adipocytes, other investigators based on their experiments with cells overexpressing a dominant inhibitory PTEN mutant, demonstrate that PTEN isn't implicated in GLUT4 translocation and activity of insulin under normal physiological states [84,85]. Nevertheless, it has been clarified that overexpression of wild-type PTEN antagonizes the metabolic function of insulin dependent on PI3K pathway.

Overexpression of SHIP2 which is a negative regulator of insulin signal transduction, reduces the production of insulin-dependent PIP3, insulin-induced AKT activation, as well as GSK3 inactivation/GS activation [86]. The genetic loss of SHIP2 has been shown to enhance insulin sensitivity *in vivo*, and may as well be a target for diabetes treatment. Tissues derived from SHIP2−/− new born mice showed reduced expression level of gluconeogenesis enzymes with severe hypoglycemia and morbidity that can be improved by infusion of insulin-neutralizing antibody or glucose, while interestingly SHIP2+/− mice showed increased translocation of GLUT4, enhanced glycogen synthesis in skeletal muscles and improved insulin resistance [87,88]. It is postulated that, SHIP2 inhibitors could be a therapeutic target for treatments of type II diabetes. Excessive inhibition of both PTEN and SHIP2 could be deleterious [88]. From a practical standpoint, there is no known specific inhibitor for either PTEN or SHIP2 in the literature. Competitive inhibitors of PTEN or SHIP2 due to the nature of their physiological substrates such as PIP3are probably high charged. High charged inhibitors are not very efficient due to the low cell permeability.

Conclusion

The main risk factors for developing T2DM are either low level of insulin or the inability of insulin-targeted tissues to respond properly to insulin. Insulin resistance might occur as a result of an impaired insulin signaling pathway at multiple levels including reduced insulin receptor concentration and kinase activity as well as defects in concentration/phosphorylation/activity of the intracellular enzymes involved in the insulin signaling pathway and ultimately GLUT4 translocation. Impairments including intracellular and PI3K pathway is the main defect in type II diabetes.

Mediators that improve the function of PI3K pathway by IRS might enhance peripheral insulin sensitivity particularly in obesity-induced insulin resistance. The PI3K-independent mechanisms of AKT signaling pathway, which are poorly understood, should be revealed and the regulators of these mechanisms are required to be recognized. Furthermore, more investigations on SHIP2 and PTEN could elucidate some of the discrepancies reported about the PI3K/AKT pathway.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgement

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