Chapter

Role of PI3K/AKT Pathway in Insulin-Mediated Glucose Uptake

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Abstract

Glucose uptake is regulated by several mechanisms, where insulin plays the most prominent role. This powerful anabolic hormone regulates the transport of glucose into the cell through translocation of glucose transporter from an intracellular pool to the plasma membrane mainly in metabolically active tissues like skeletal muscles, adipose tissue, or liver (GLUT4). This translocation occurs through multiple steps of PI3K/AKT signaling pathway. In this chapter, we will focus on molecular events leading to GLUT4 translocation, starting with activation of insulin receptors through signaling cascade involving phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) and finally, the action of their effectors. We will present regulatory mechanisms and modulators of insulin-mediated glucose uptake.

Keywords: insulin, PI3K, AKT, glucose uptake, GLUT4, insulin resistance

1. Introduction

Nowadays, when society is leading an increasingly sedentary lifestyle with constant access to food without the need for effort, we observe the raising occurrence of diseases with metabolic dysregulation. This financial and social burden has caused the great need for understanding mechanistic details of metabolic response pathways, causes of their impairment, and following consequences. Carbohydrate metabolism is mainly related to glucose. Its level should remain in a narrow range (4–7 mM) by balancing glucose release into the circulation, its absorption from the intestine, the breakdown of stored glycogen in liver, and the uptake of blood glucose by peripheral tissues. These processes are regulated by a few metabolic hormones with insulin being the most important one.

2. Mechanism of insulin action

2.1 Insulin

Insulin is an anabolic peptide hormone secreted by pancreatic β cells, whose mature form arises in two stages [1]. First, preproinsulin is processed via cutting of the signal fragment and forming proinsulin [2]. This is followed by the excision of the middle fragment (C chain—35 aa), which gives dipeptide made up of two chains (A—21 aa, B—30 aa) connected by two disulfide bonds [3]. Insulin is a multitask

	Upregulation	Downregulation
Carbohydrate metabolism	Glucose uptake via GLUT4 Glycogen synthesis Glycolysis Conversion of pyruvate to acetyl CoA	Glycogenolysis Gluconeogenesis
Lipid metabolism	Fatty acids synthesis Triglycerides synthesis Cholesterol synthesis	Lipids oxidation Triglycerides breakdown
Protein metabolism	Transcription of proteins involved in energy stores generation	Transcription of proteins involved in energy stores release

Table 1.
Metabolic functions of insulin.

protein involved, among others, in the regulation of carbohydrate and lipid metabolism (**Table 1**). The most important stimulus for insulin production is a postprandial increase of blood glucose level. By increasing insulin production and its impact on effector cells (myocytes, adipocytes, and hepatocytes), glucose transport to the inside of the cells gets increased while reducing blood glucose level. This is achieved by an increased translocation of the insulin-dependent glucose carriers (GLUT), with GLUT-4 being found in skeletal muscle, hepatocytes, and adipocytes [4].

When glucose concentration exceeds 30 mM in the small intestine, glucose transport to the inside of the pancreatic β cells is initiated in an insulin-independent way *via* GLUT2 (**Figure 1**). GLUT2 facilitates transport with a concentration gradient. Inside the cell, glucose is converted into glucose-6-phosphate, which prevents the equalization of glucose levels and sustained transport to the cell. Glucose-6-phosphate enters the glycolysis, which results in the production of ATP molecules. As a result of a continuous glucose supply, the level of ATP is constantly increasing. This causes an inhibition of the potassium channel with the outflow of K^+ ions from the cell being blocked. K^+ ions concentration increases inside the cell, which becomes electropositive until the charges on the membrane are aligned and membrane becomes depolarized. Depolarization activates the voltage-dependent calcium channel, promoting the influx of Ca^{2+} ions to the cell. Ca^{2+} ions activate the ryanodine channel located in the membrane of insulin-accumulating vesicles, inducing their migration into the cell membrane and releasing their content [5].

2.2 Insulin signaling pathway

Released insulin participates in many metabolic actions, such as glycogen deposition in liver and skeletal muscles, a stimulation of lipogenesis and inhibition of lipolysis, and repression of gluconeogenesis in liver, but mainly in increased glucose uptake through insulin receptor signaling pathway [6]. Signal transmission from the blood to the inside of the cell is a complicated and strongly integrated process. It begins with binding of the hormone to the insulin receptor (IR), eliciting the large protein signal complex formation just below the surface of the cell membrane around IR's cytoplasmic domains (**Figure 2**) [7]. IRs are heterotetrameric glycoproteins containing two extracellular (α) and two intracellular (β) subunits. They occur mainly on the cell surface of metabolically active tissues like muscles, liver, and fat. The binding of insulin by extracellular subunits leads to IR dimerization, which allows ATP binding to β-subunits [8]. This causes the activation of the catalytic domains of tyrosine kinases in the cytoplasm [9]. In the first stage, there is an autophosphorylation of the receptor followed by phosphorylation of several substrate proteins, where IRS (insulin receptor substrate) proteins seem

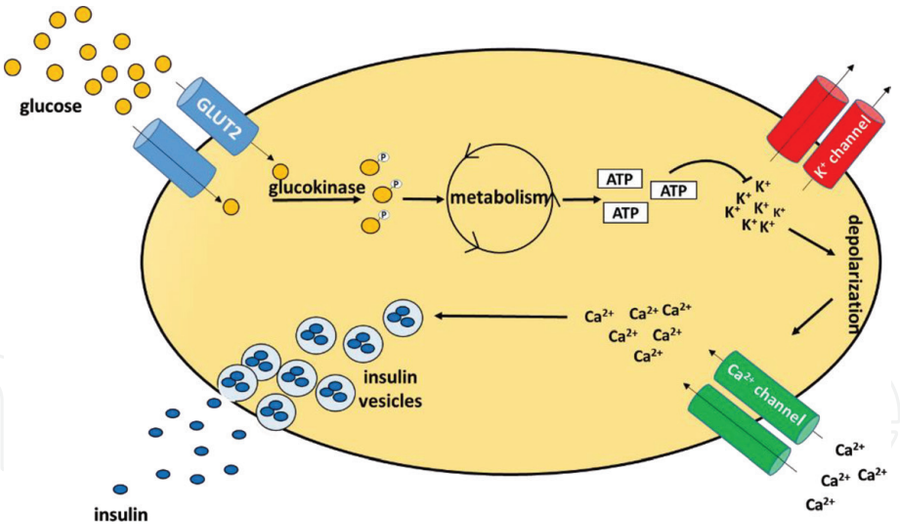

Figure 1.
Insulin release. Glucose is transported into β-cells via GLUT2 in an insulin-independent way with concentration gradient. Then, glucose is phosphorylated by glucokinase to glucose-6-phosphate, which allows for its inclusion to metabolic processes and ATP production. Raised ATP level triggers accumulation of K^+ ions along with membrane depolarization. The latter activates Ca^{2+} channels, leading to increased concentration of Ca^{2+} ions inside the cell and consequent release of insulin from vesicles. For details see text.

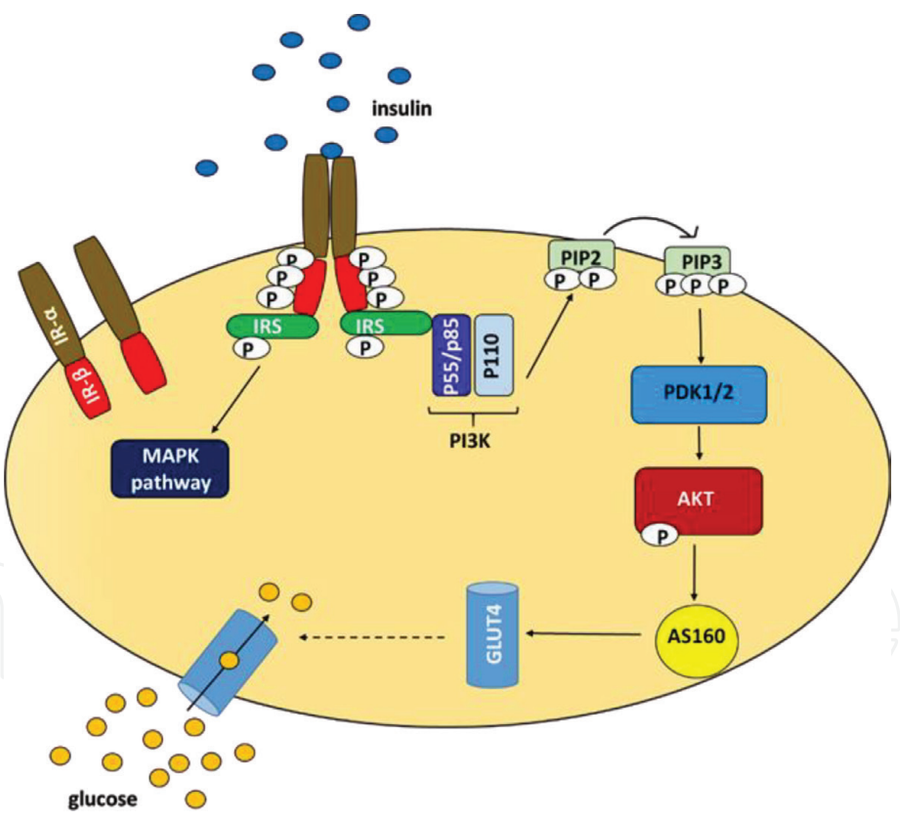

Figure 2.
Insulin signaling pathway. Insulin attaches to insulin receptors triggering its dimerization and intracellular autophosphorylation of their tyrosine residues, which constitute an attachment for IRS proteins. These molecules also undergo phosphorylation and form a complex with PI3K utilizing SH2 domains. PI3K phosphorylates PIP2, which results in PIP3 formation and activation of PDK1/2. AKT gets phosphorylated and activated by PDK1/2, subsequently eliciting phosphorylation of AS160. The latter is responsible for GLUT4 translocation to cellular membrane and glucose inflow.

to be most significant ones. The phosphorylation occurs on tyrosine residues, and then, phosphorylated IRS proteins can trigger two major signaling pathways. First pathway leads from Ras to mitogen-activated kinases (MAPK), being involved in the expression regulation of genes playing a role in cell growth and differentiation.

The second one, phosphatidylinositol 3 kinase (PI3K) pathway, elicits AKT/PKB kinase phosphorylation, and it is responsible for the metabolic action of insulin.

3. PI3K/AKT pathway

As shown in **Figure 2**, activation of PI3K/AKT pathway starts with binding of IRS proteins via SH2 domains to PI3 kinase regulatory subunits. This results in the activation of PI3K that phosphorylates phosphatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol(3,4,5)-triphosphate (PIP3). This, in turn, leads to the activation of PIP3-dependent kinases: PDK-1 and PDK-2 and eventually to the activation of AKT/PKB kinase and atypical PKCs [10]. Subsequently, AKT catalyzes the phosphorylation of AS160 substrate protein that stimulates the translocation of GLUT glucose transporters from the cytoplasmic vesicles onto the cell membrane surface and thereby increases the insulin-dependent transport of glucose into the cell. GLUT4 occurs mainly in the interior of the nonstimulated cell, due to the proper proportion of two actions: slow exocytosis and rapid endocytosis. AS160 increases GLUT4 exocytosis and inhibition of its endocytosis via its downstream target, Rab10, in adipocytes. This results in GLUT4 accumulation in the plasma membrane [11]. Besides the activation of insulin-dependent glucose uptake via GLUT4, AKT has many intracellular targets and mediates numerous metabolic effects. For instance, AKT triggers phosphorylation of glycogen synthase kinase 3 (GSK3), which leads to stimulation of glycogen synthesis in liver and skeletal muscle [12].

4. PI3K/AKT regulation

The PI3K/AKT pathway is under strict control, and its disturbances are the cause of many diseases, including primarily insulin resistance. Further knowledge of the mechanisms regulating this signaling is one of the most important challenges of modern science. Currently, three specific signaling nodes have been distinguished: (a) IRS proteins, (b) regulatory-PI3K kinase subunits, and (c) kinase isoform Akt/PKB [13]. Disturbances of any of these nodes are mainly responsible for the reduction of the signal transmission efficiency and related diseases.

4.1 IRS protein node

IRS family consists of six proteins (IRS1–6), where two representatives, IRS1 and IRS2, are crucial in insulin signaling transduction. IRS proteins show tissue-specific expression and functionality [14]. They have three characteristic domains: (a) pleckstrin homology domain at N-terminus, (b) a phosphotyrosine-binding domain enabling binding to IR in the center, and (c) several sites of phosphorylation on tyrosine and serine residues at C-terminus. After tyrosine residues become phosphorylated, IRS binds by C-terminus domain to molecules containing an Src-homology-2 domain (SH2) [15]. IRS-1 and IRS-2 are widely expressed in all tissues, playing major roles in the maintenance of energy balance: muscle, liver, fat, and pancreatic islets. However, it seems that IRS1 plays the main role in myocytes and adipose tissue, while IRS2 is a key player in hepatocytes and islet cells [16, 17].

Generally, there are three ways allowing the regulation of IRS (**Figure 3**). Crucial control occurs mainly by multiple serine and threonine residues, which may be phosphorylated by different kinases. The phosphorylation of serine residues may inhibit insulin signaling by blocking tyrosine phosphorylation, which is necessary for

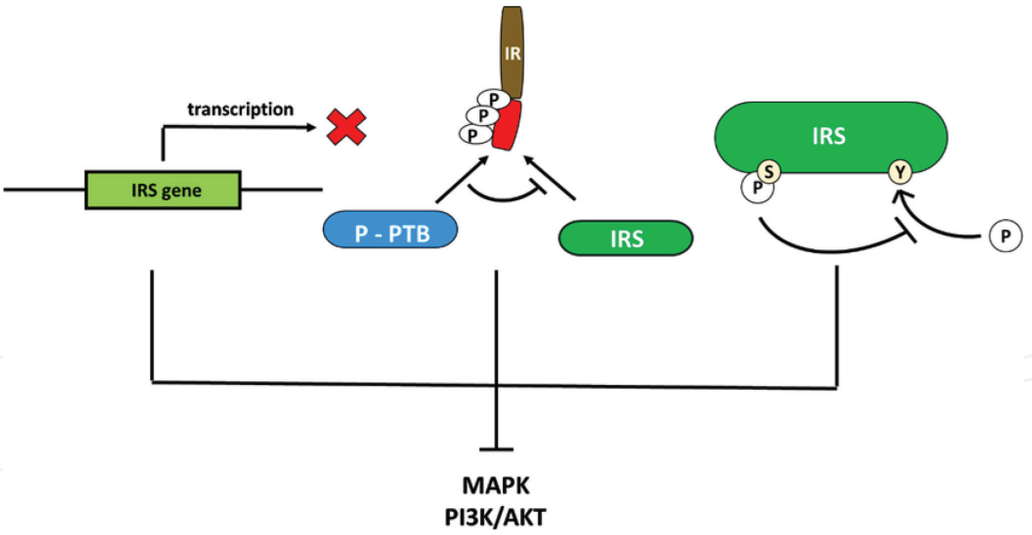

Figure 3.
Overview of three major mechanisms affecting IRS-dependent signal transduction. Signaling via IR may be modulated simply by the decreased rate of IRS gene transcription. Second, proteins with PTB domains may compete with IRS for binding to phosphotyrosines of IR. Finally, IRS phosphorylation of serine residue is known to suppress phosphorylation of its tyrosine, which is indispensable for signal transduction.

signal transduction. However, the details of this inhibitory mechanism are still not well understood. Indeed, there is a strong correlation among serine phosphorylation, decreased tyrosine phosphorylation, and insulin resistance, which is closely related to abnormalities within PI3K pathway. Most critical enzymes being able to phosphorylate IRS in serine residue are stress-induced kinases like ERK, JNK, and AMPK along with inflammatory kinase IKK and other downstream kinases, such as AKT, atypical PKC isoforms, mTOR, or S6K [18, 19]. Blockage of IRS causes the reduced cell response for stimulation with insulin and formation of insulin resistance, the first step toward diabetes. This inhibitory phosphorylation mostly occurs because of low-grade inflammation state, which is caused by lipid accumulation [20]. Studies on palmitate showed that it significantly decreased the insulin-stimulated Ser phosphorylation of Akt and Tyr phosphorylation of IRS-1 [21]. Some drugs exert similar effect. The prominent example is simvastatin, which is commonly used in the prevention and treatment of cardiovascular diseases. Simvastatin reduces the phosphorylation of insulin-induced IR at Tyr, IRS-1 at Tyr, and AKT at Thr [22, 23]. Therefore, therapy with simvastatin or other statins might be a risk factor for the development of insulin resistance or diabetes. This effect can be decreased by many natural substances like silibinin (principal flavonoid contained in silymarin, a mixture of flavonolignans extracted from *Silybum marianum* seeds). Silibinin prevents PI3K/AKT pathway inhibition by decreasing IRS1 phosphorylation on Tyr [24]. Similar mechanism is typical of PTP1B (protein-tyrosine phosphatase 1B), whose overexpression can inactivate the whole PI3K pathway [25]. Since this protein was found to be overexpressed in insulin-sensitive peripheral tissues (fat, muscle) and in hepatic cells during insulin-resistant state, searching for PTP1B inhibitors has become an important area of research in the treatment of impairment of insulin transmission pathway. FYGL (Fudan-Yueyang *G. lucidum* extract) appears to be a promising substance showing PTP1B inhibitory activity with weak cell permeability and bioavailability [26, 27].

IRS function can be also regulated by competitively inhibiting the binding of IR to IRS, primarily by proteins containing phosphotyrosine-binding (PTB) domain. One of them, NYGGF4, is highly expressed in obese individuals. Studies on skeletal myotubes showed the reduced insulin-induced phosphorylation of IRS1 at Tyr and Akt phosphorylation at Ser residue without changes in the insulin-stimulated tyrosine phosphorylation of IR [28, 29].

Among other IRS modulatory mechanisms, it is worth mentioning about expression regulation of IRS mediated by hyperinsulinemia and other hormones [30]. Anjali et al. showed that FSH (follicle stimulating hormone) induces expression of IRS2 in granulosa cells [31]. Also, some natural medicines like Tangzhiqing formula, a mix of five herbs, modulate IRS expression level in HEPG2 cells (IR1 and IRS2) and L-6 myotubes (IRS1) [32].

4.2 PI3K kinase subunits

PI3 kinases constitute protein family, which exhibits activity of phosphorylation of lipids and proteins. They are divided into three groups according to their structural features and substrate preferences (**Table 2**). Members of I class are the most crucial in insulin signaling pathway. PI3K-1 are heterodimers made up of regulatory and catalytic subunits. The regulatory subunit is generally referred to as p85. They all have a similar domain structure: SH3 domain, breakpoint cluster region homology (BH), and two SH2 domains with iSH2 (interSH2) domain in between [33]. Signaling is initiated by p85 interacting through the SH2 domain with IRS phosphotyrosine motif. Subsequently, p85 is joined through its iSH2 domain to the adapter binding domain (ABD) of catalytic subunit called p110. Besides ABD, p110 also contains Ras-binding domain (RBD), which is involved in interaction with Ras protein superfamily, C2 and the helical scaffolding domains, along with kinase domain participating in PIP3 formation [34].

p85 protects p110 from degradation by forming a heterodimer. Furthermore, this binding allows p110 translocation to the cell membrane, where catalytic subunit is able to send a signal via phosphorylation of PIP2 to PIP3, a lipid second messenger. Interestingly, p110α is the most prominent one from all PI3K catalytic subunits in insulin-dependent pathway [35]. Cells with its deletion exhibit hyperglycemia and glucose intolerance [36]. While p110β seems to play a secondary role, its presence is necessary for p110α activity and thus maintenance of basal threshold of PIP3 [37, 38]. PIP3 is bound by proteins with PH domain such as AKT and PDK1. This critical event allows further signal transduction to downstream proteins.

In this control node, a few aspects are taken into account. Firstly, signaling via PI3K is critically dependent upon PI3K regulatory subunit with p85 mediating either its restriction or promotion. In cells deprived of upstream stimuli, p85 reduces p110 activity. It is executed through C2 and helical scaffolding domains, which form inhibitory contacts with p85. Furthermore, monomeric p85 binds to phosphorylated sites of IRS, thus blocking p85-p110 heterodimer attached to IRS [39]. p110, another

Class	Members	Catalytic subunit	Regulatory subunit	Main reaction	Reference
Ia	PI3Kα PI3Kβ PI3Kδ	p110 (α/β/δ)	p85α, p55α, p50α, p85β, p55γ	PtdIns(4,5)P2 → PtdIns (3,4,5)P3	[33]
Ib	PI3Kγ	p110γ	p101 p84/87	PtdIns(4,5)P2 → PtdIns (3,4,5)P3	[33]
II	PI3K-C2α PI3K-C2β PI3K-C2γ	Monomeric		PtdIns(4)P → PtdIns (3,4)P2	[76]
III	PI3K-C3	Vps34	Vps15	PtdIns → PtdIns (3)P3	[77]

Table 2.
Classification of PI3K family members.

essential regulatory molecule, undergoes spatial regulation in some types of human cancer. Studies on HepG2 cells demonstrated that PAQR3 (progestin and adipoQ receptor family member 3) associates with p110α by attracting it to Golgi apparatus, a place of PAQR3 exclusive localization. This event inhibits the interaction between p85a and p110α [40, 41].

There are two other possible PI3K activation pathways, both being dependent on ligand-membrane receptor binding. The first mechanism is based on binding the adaptor protein GRB2 to RTK (receptor tyrosine kinase). When GRB2 is already attached to GAB protein, it is allowed to bind p85. By contrast, the second way of PI3K activation is not dependent on p85 subunit. In this scenario, GRB2 binds to SOS, which activates RAS, leading to activation of p110α subunit. In addition, the p110β catalytic subunit may be stimulated in a similar, p85-independent way via G protein-coupled receptors [42].

Another critical regulatory mechanism is associated with the control of PIP3 level. There are several well-known inhibitors which dephosphorylate PIP3 with phosphatase and tensin homolog (PTEN) being the most well-known one. Undoubtedly, PTEN is an intriguing protein for research in the context of diseases with PI3K signaling impairment. For instance, in adipose tissue, it can be blocked by H2S or its precursor, L-cysteine. Diet supplementation of L-cysteine increases PIP3 level and mediates the activation of PI3K, resulting in improvement of glucose metabolism [43, 44]. Expression level of PTEN is also regulated epigenetically in adipocytes via several miRNAs such as miR-21, miR-23a-3p, miR-26a, miR-26b, and miR-181a-5p [45–49]. Another widely known PIP3 inhibitor is SHIP (SH2-containing inositol 5′-phosphatase). SHIP dephosphorylates PIP3 at 5′-inositol position (in contrast to PTEN targeting 3′-inositol position) and inhibits AKT primarily through regulation of its cellular localization [50].

Last but not least, PI3K dysregulation can be also underlain by gene mutations of p110α and p85 subunits or PI3K negative regulators. For instance, loss of function or deletion of PTEN is known to occur in numerous types of cancer. Therefore, enormous attempts are put into research focused on searching compounds targeting PI3K. The most common PI3K regulators are Wortmannin (steroid fungal metabolite) and LY294002 (morpholine-containing chemical compound) [51]. Moreover, there are multiple members of a new generation of more stable molecules such as SF-1126, CAL101, GSK615, XL147, and PF-4989216, which evoke the suppression of overactive PI3K signaling particularly in cancer [52].

4.3 Kinase isoform AKT/PKB

AKT (also named PKB) occurs in mammals in three isoforms (AKT1, AKT2, and AKT3). Although they share a similar domain structure (N-terminal PH domain, a central kinase domain, and C-terminal domain), AKT isoforms exhibit target specificity and play divergent roles. AKT2 is the most essential in glucose uptake [53].

The PH domain enables AKT to be attracted by PIP3 just as PDK1. After binding to PIP3, AKT undergoes conformational changes that allow revealing the phosphorylation site. While they are in nearby, PDK is able to phosphorylate AKT on Thr308. Nevertheless, for full activation of AKT (besides AKT3), second phosphorylation on Ser residue is necessary (AKT1-Ser473 and Ser-474 AKT2). Ser473 is modified by PDK-2/mTORC2 (mammalian target of rapamycin complex 2) [54]. AKT activation is terminated through the action of PP2 (protein phosphatase 2) and PHLPP (PH domain leucine rich repeat phosphatase), which perform dephosphorylation of Thr308 and Ser473, respectively [55].

While phosphorylation status of both of these sites is fundamental for AKT activity, there is plethora of other posttranslational modifications affecting its

performance [56]. For instance, oxidation of Cys124 triggered by PDGF-induced (platelet-derived growth factor) ROS leads to the blockage of AKT2 activity [57]. Besides PI3K-dependent activation, AKT may be switched on by alternative modulators. Namely, two groups of uncommon AKT activators are distinguished: tyrosine kinases (e.g., ACK1, SRC, PTK6) and serine/threonine kinases (e.g., TBK1, IKBKE). ACK1, a non-receptor tyrosine kinase, is capable of regulating AKT recruitment to the plasma membrane due to AKT phosphorylation on Tyr176, making it preferentially binding to phosphatidic acid—a membrane phospholipid. This elicits AKT attachment to plasma membrane even in the presence of some specific PI3K inhibitors. The increase of AKT2 activity occurs in many cancers, which may be underlain by auto-activating mutations of ACK1. Another nonreceptor kinase involved in AKT regulation is Src. Its action takes place on Tyr315 and 326. By contrast, PTK6 responds to epidermal growth factor (EGF), whose overexpression is typical of many cancers, via phosphorylating Tyr215 and 326. Modifications triggered by Src and PTK6 are resistant to some popular PI3K inhibitors. The second group of AKT activators, Ser/Thr kinases, modifies Thr195, Ser378, and Ser473 (TBK1), as well as Ser137, Thr308, and Ser473 (IKBKE). These alternative activation modes may suggest that under some particular conditions, cells can turn on AKT signaling in quick response [58].

Due to the fact that AKT, just like PI3K, is one of the most commonly deregulated molecules in human cancers, AKT inhibitors development constitutes an important field of research. Currently tested molecules utilize two major mechanisms. First group acts as competitors for ATP-binding site of AKT (e.g., GSK690693, GDC-0068, GSK2110183, and GSK2141795). They share features of major pharmacophore with minor differences. The second group is composed of allosteric AKT inhibitors (e.g., 2,3-diphenylquinoxaline and analogs, alkylphospholipids). Many of these molecules are in clinical trial phase and have a potential in the treatment of AKT dysregulations [59].

5. Environmental insulin signaling modulating factors

The relationship between environmental factors like diet, drugs, lifestyle in general, and PI3K pathway remains undeniable. Herein, we will discuss major agents responsible for PI3K modulation. In terms of mediated effect, they can be divided into two types: insulin sensitizing factors and insulin-resistance inducing factors. They do not usually affect a specific protein, but through their action, they dysregulate the entire pathway and the overall metabolism.

5.1 Factors inducing insulin resistance

Due to the fact that insulin is one of the key regulators of metabolism, it is not surprising that the most important factor modulating its action is diet. Impairment of PI3K signaling is well known to be connected with obesity. Depending on the tissue, the mechanism of obesity-induced insulin resistance seems to differ, but it is in general connected with lipid overload. In liver and muscles, the most crucial is elevation of FFA level, which is characteristic for the obese. In consequence, toxic lipids, mainly ceramides and diacylglycerol (DAG), do accumulate. The increased amount of ceramides causes PP2A stimulation, which terminates insulin pathway via AKT dephosphorylation. On the other hand, DAG activates PKC isoforms (ε and θ) [60]. The latter ones are able to obstruct signaling either by IRS (muscles) or IR (liver). PKC isoforms activation leads to increased expression of NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), which takes part

in inflammatory cell response. Subsequently, NFκB activates pro-inflammatory cytokines and stress-induced serine-threonine kinases like JNK, which are able to block insulin signaling pathway via improper IRS phosphorylation. Furthermore, the increasing concentration of lipids in the cells leads to the aggregation of toxic metabolites derived from the incomplete oxidation, and, as a result, the elevated synthesis of free radicals. This is also correlated with increased activation of stress-induced kinases. In overall, these events lead to PI3K pathway impairment and the emergence of insulin resistance [60–62].

The mechanism of obesity-induced insulin resistance formation in adipose tissue is also related to lipid overload but has a different course. It is connected to the constant enlargement of adipocytes, which along with dysregulation of adipogenesis leads to the introduction of hypoxia. Reduced oxygen supply introduces cellular stress response, which includes activation of stress-induced kinases, pro-inflammatory cytokines, and tissue infiltration by pro-inflammatory macrophages. These events result in low-grade inflammation state characteristic of PI3K impairment. Adipose tissue is not only an energy reservoir but also an active endocrine organ, which produces hormones called adipocytokines. They are sensors of nutritional and metabolic homeostasis. Accumulation of visceral fat and inflammation development alters the secretary profile of adipocytokines. Adipocytes start to send pro-inflammatory signals like TNF-α and interleukin1 (IL1). Other typical insulin resistance-inducing cytokines are resistin and IL-6, which activate pro-inflammatory pathways of NFκB and JNK kinase, leading to defective response to insulin [63].

5.2 Insulin-sensitizing factors

While prolonged high-calorie diet undeniably leads to insulin resistance, proper dietary style can be a sensitizing factor as well. There are many diet supplements improving insulin signaling. Herein, we will point out only a few members of this enormous group. For instance, glutamine (Gln) supplementation Gln increases the expression of key PI3K signaling molecules (PI3K, PDK1, and GLUT4) and promotes AKT phosphorylation, GLUT4 translocation, and glucose uptake in the presence of insulin during exposure to hyperglycemia [64]. An epidemic of obesity and numerous side effects of drugs that increase insulin sensitivity has caused the great interest among scientists to search for natural sensitizers. They include dieckol (an extract from a brown seaweed), which enhances translocation of GLUT4 in peripheral tissues [65]. Another seaweed improving glucose uptake is *Gelidium amansii*. It exhibits antihyperglycemic, antioxidant, and antiobesity effects potentially *via* PI3K/AKT/GLUT4 signaling [66]. Also, carnosol, a compound found in spices such as sage or rosemary, increases glucose uptake *via* GLUT4 [67]. Interestingly, it has been proven that 1,25-dihydroxyvitamin D3 (active form of vitamin D3), which is mainly provided with food, can improve glucose uptake and has a potential in acting as an anti-inflammatory factor [68]. It seems that an alternative for typical drugs like metformin or pioglitazone, which cause side effects, may be products containing natural substances like Jiangtang Xiaoke granule. The latter is composed of 10 herbs, and it can significantly increase the expression of vast PI3K proteins in mice even upon hyperglycemia [69].

Components of the diet are not the only ones able to improve the signaling via discussed pathway. Studies on rat model demonstrated that long-term caloric restriction may enhance AKT2-dependent mechanism for improving insulin-stimulated glucose uptake. Moreover, a lot of research has been carried out to indicate that physical exertion has a positive effect on insulin [70–73].

6. PI3K/AKT pathway impairment

PI3K pathway impairment is related to many diseases, among which the most common and worth attention are insulin resistance and numerous types of cancers.

Insulin resistance may be defined as a subnormal glucose response to endogenous and/or exogenous insulin. Peripheral tissues are not able to respond to the hormone by increasing glucose uptake from the bloodstream. Initially, pancreatic β-cells are not harmed yet, and in response to high glucose level, they synthesize more and more insulin. However, if this state lasts for a long time, islet cells start to overgrow, and deterioration of their function and/or decline of β-cell mass do occur. As normalization of glucose level does not occur, cells are becoming more and more resistant to insulin simultaneously forming a vicious circle of insulin resistance. The most affected tissues are the most metabolically active ones like liver, muscles, and fat. Although the pathogenesis of insulin resistance is getting better understood, the exact mechanism is still not clear. The causes may be connected to abnormal insulin production, but in most cases, the changes in insulin receptors and their substrates along with defects in post-receptor signaling play the role.

PI3K pathway is one of the most frequently deregulated signaling pathways in human cancers. As it plays an essential role in many biological processes like cell survival, proliferation, migration and differentiation, its dysregulation may result in tumorigenesis. The most common changes are mutations (*PIK3CA*, *AKT1*, and *PTEN*), genes amplification (*PIK3CA*, *AKT1*, and *AKT2*), and loss of expression or deletion of the tumor suppressor PTEN [74]. The highest prevalence of mutations within PI3K pathway is typical of lung cancer, breast cancer, endometrial cancer, and head and neck cancer along with glioblastoma [75].

7. Conclusions

Insulin is the most crucial agent in glucose metabolism. It stimulates glucose uptake from the bloodstream to peripheral tissues. Furthermore, it is responsible for energy storage through accelerating glycogen synthesis and lipogenesis. In general, it promotes cellular events leading to energy storage and represses processes of energy release (**Figure 4**). Insulin action takes place mainly through PI3K pathway and results not only in metabolic effects but also in mitotic response. Insulin is also

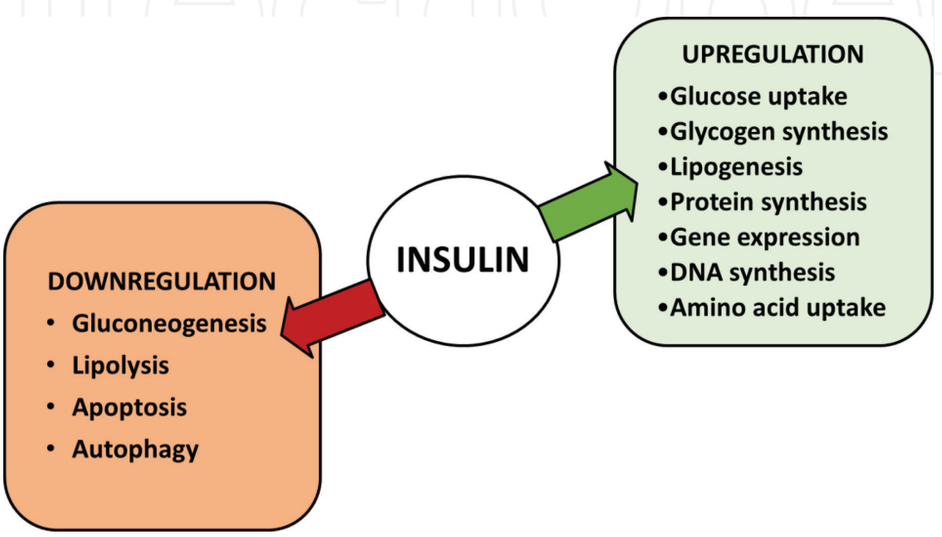

Figure 4.
Critical actions and pathways controlled by insulin.

involved in phenomena connected with cell survival. Multitasking nature of this hormone causes that any abnormality in its signal transmission can result in serious consequences, such as diabetes and cancer. These two diseases are the scourge of the modern world. The steadily increasing percentage of people suffering from insulin resistance or full-blown diabetes and the high incidence of cancer have caused scientists to focus on seeking therapeutic goals that may contribute to the prevention or treatment of these disorders. In insulin-resistance, the main target constitutes the improvement of insulin sensitivity. Among common approaches, it is worth to highlight two of them: increasing fatty acids oxidation and elongation of IR activation state by blocking PTP1B activity. Promising therapeutic targets seem to be also pro-inflammatory cytokines and other proteins involved in inflammation response. On the other hand, cancer cells show mainly hyperactivity of PI3K pathway and the increased glucose uptake. Therefore, it seems that blockage of impaired signal transduction may contribute to suppression of the growth of the tumor. For this reason, intensive search for selective inhibitors or silencers of the insulin pathway are underway. Conducting further research may become the basis for the development of new methods of prevention and more effective treatment strategies for these diseases.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

List of abbreviation

ABD	adapter binding domain
ACK-1	activated CDC42 kinase 1
AKT (PKB)	protein kinase B
AMPK	5'AMP-activated protein kinase
AS160	Akt substrate of 160 kDa
BH domain	breakpoint cluster region homology domain
EGF	epidermal growth factor
ERK	extracellular signal-regulated kinase
FSH	follicle stimulating hormone
FYGL	Fudan-Yueyang *G. lucidum* extract
GAB	GRB2-associated binding protein
GLUT1–4	glucose transporter type 1–4
GSK3	glycogen synthase kinase 3
GRB2	growth factor receptor-bound protein 2
HEPG2	human liver cancer cell line
IKBKE	inhibitor of nuclear factor kappa-B kinase subunit epsilon
IKK	IκB kinase
IR	insulin receptor
IRS	insulin receptor substrate
JNK	c-Jun N-terminal kinase

MAPK	mitogen-activated protein kinase
mTOR	mammalian target of rapamycin kinase
mTORC2	mammalian target of rapamycin complex 2
NYGGF4 (PID1)	phosphotyrosine interaction domain-containing protein 1
PAQR3	progestin and adipoQ receptor family member 3
PDGF	platelet-derived growth factor
PDK1	pyruvate dehydrogenase lipoamide kinase isozyme 1
PH domain	pleckstrin homology domain
PHLPP	PH domain leucine rich repeat phosphatase
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP2	phosphatidylinositol 4,5-bisphosphate
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
PKC	protein kinase C
PP2	protein phosphatase 2
PTB domain	phosphotyrosine-binding domain
PTB1	polypyrimidine tract binding protein-1
PTEN	phosphatase and tensin homolog
PTK6	tyrosine-protein kinase 6
PTP1B	protein-tyrosine phosphatase 1B
RBD	Ras-binding domain
ROS	reactive oxygen species
S6K	ribosomal S6 kinase
SH2 domain	Src-homology-2 domain
SHIP	SH2-containing inositol 5′-phosphatase
SOS	son of sevenless, guanine nucleotide exchange factor
SRC	proto-oncogene tyrosine-protein kinase Src
TBK1	TANK binding kinase 1

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References

[1] Banting FG, Best CH. The internal secretion of the pancreas. The Journal of Laboratory and Clinical Medicine. 1922;**7**(5):251-266

[2] Steiner DF, Cunningham D, Spigelman L, Aten B. Insulin biosynthesis: Evidence for a precursor. Science. 1967;**157**(3789):697-700

[3] Dodson G, Steiner D. The role of assembly in insulin's biosynthesis. Current Opinion in Structural Biology. 1998;**8**(2):189-194

[4] Olson AL, Pessin JE. Structure, function, and regulation of the mammalian facilitative glucose transporter gene family. Annual Review of Nutrition. 1996;**16**:235-256

[5] Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. Sugar absorption in the intestine: The role of GLUT2. Annual Review of Nutrition. 2008;**28**:35-54

[6] Newsholme EA, Dimitriadis G. Integration of biochemical and physiologic effects of insulin on glucose metabolism. Experimental and Clinical Endocrinology & Diabetes. 2001;**109**(Suppl 2):S122-S134

[7] Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell. 1990;**61**(2):203-212

[8] Perlman R, Bottaro DP, White MF, Kahn CR. Conformational changes in the alpha- and beta-subunits of the insulin receptor identified by anti-peptide antibodies. The Journal of Biological Chemistry. 1989;**264**(15):8946-8950

[9] Baron V, Kaliman P, Gautier N, Van Obberghen E. The insulin receptor activation process involves localized conformational changes. The Journal of Biological Chemistry. 1992;**267**(32):23290-23294

[10] Manna P, Jain SK. PIP3 but not PIP2 increases GLUT4 surface expression and glucose metabolism mediated by AKT/PKCzeta/lambda phosphorylation in 3T3L1 adipocytes. Molecular and Cellular Biochemistry. 2013;**381**(1-2):291-299

[11] Sano H, Eguez L, Teruel MN, Fukuda M, Chuang TD, Chavez JA, et al. Rab10, a target of the AS160 Rab GAP, is required for insulin-stimulated translocation of GLUT4 to the adipocyte plasma membrane. Cell Metabolism. 2007;**5**(4):293-303

[12] Friedrichsen M, Birk JB, Richter EA, Ribel-Madsen R, Pehmoller C, Hansen BF, et al. Akt2 influences glycogen synthase activity in human skeletal muscle through regulation of NH(2)-terminal (sites 2 + 2a) phosphorylation. American Journal of Physiology. Endocrinology and Metabolism. 2013;**304**(6):E631-E639

[13] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: Insights into insulin action. Nature Reviews. Molecular Cell Biology. 2006;**7**(2):85-96

[14] Cai D, Dhe-Paganon S, Melendez PA, Lee J, Shoelson SE. Two new substrates in insulin signaling, IRS5/DOK4 and IRS6/DOK5. The Journal of Biological Chemistry. 2003;**278**(28):25323-25330

[15] Gustafson TA, He W, Craparo A, Schaub CD, O'Neill TJ. Phosphotyrosine-dependent interaction of SHC and insulin receptor substrate 1 with the NPEY motif of the insulin receptor via a novel non-SH2 domain. Molecular and Cellular Biology. 1995;**15**(5):2500-2508

[16] Thirone ACP, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose

transport. Trends in Endocrinology and Metabolism. 2006;**17**(2):72-78

[17] Rother KI, Imai Y, Caruso M, Beguinot F, Formisano P, Accili D. Evidence that IRS-2 phosphorylation is required for insulin action in hepatocytes. The Journal of Biological Chemistry. 1998;**273**(28):17491-17497

[18] Nandipati KC, Subramanian S, Agrawal DK. Protein kinases: Mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. Molecular and Cellular Biochemistry. 2017;**426**(1-2):27-45

[19] Greene MW, Garofalo RS. Positive and negative regulatory role of insulin receptor substrate 1 and 2 (IRS-1 and IRS-2) serine/threonine phosphorylation. Biochemistry. 2002;**41**(22):7082-7091

[20] Engin A. The pathogenesis of obesity-associated adipose tissue inflammation. Advances in Experimental Medicine and Biology. 2017;**960**:221-245

[21] Ajuwon KM, Spurlock ME. Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3-L1 adipocytes. The Journal of Nutrition. 2005;**135**(8):1841-1846

[22] Li W, Liang X, Zeng Z, Yu K, Zhan S, Su Q, et al. Simvastatin inhibits glucose uptake activity and GLUT4 translocation through suppression of the IR/IRS-1/Akt signaling in C2C12 myotubes. Biochemical and Biophysical Research Communications. 2016;**83**:194-200

[23] Yaluri N, Modi S, Kokkola T. Simvastatin induces insulin resistance in L6 skeletal muscle myotubes by suppressing insulin signaling, GLUT4 expression and GSK-3beta phosphorylation. Biochemical and Biophysical Research Communications. 2016;**480**(2):194-200

[24] Li HB, Yang YR, Mo ZJ, Ding Y, Jiang WJ. Silibinin improves palmitate-induced insulin resistance in C2C12 myotubes by attenuating IRS-1/PI3K/Akt pathway inhibition. Brazilian Journal of Medical and Biological Research. 2015;**48**(5):440-446

[25] Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. The Journal of Biological Chemistry. 2000;**275**(6):4283-4289

[26] Yang Z, Wu F, He Y, Zhang Q, Zhang Y, Zhou G, et al. A novel PTP1B inhibitor extracted from *Ganoderma lucidum* ameliorates insulin resistance by regulating IRS1-GLUT4 cascades in the insulin signaling pathway. Food & Function. 2018;**9**(1):397-406

[27] Wang CD, Teng BS, He YM, Wu JS, Pan D, Pan LF, et al. Effect of a novel proteoglycan PTP1B inhibitor from *Ganoderma lucidum* on the amelioration of hyperglycaemia and dyslipidaemia in db/db mice. The British Journal of Nutrition. 2012;**108**(11):2014-2025

[28] Wu WL, Gan WH, Tong ML, Li XL, Dai JZ, Zhang CM, et al. Over-expression of NYGGF4 (PID1) inhibits glucose transport in skeletal myotubes by blocking the IRS1/PI3K/AKT insulin pathway. Molecular Genetics and Metabolism. 2011;**102**(3):374-377

[29] Zhang CM, Chen XH, Wang B, Liu F, Chi X, Tong ML, et al. Over-expression of NYGGF4 inhibits glucose transport in 3T3-L1 adipocytes via attenuated phosphorylation of IRS-1 and Akt. Acta Pharmacologica Sinica. 2009;**30**(1):120-124

[30] Hirashima Y, Tsuruzoe K, Kodama S, Igata M, Toyonaga T, Ueki K, et al. Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. The Journal of Endocrinology. 2003;**179**(2):253-266

[31] Anjali G, Kaur S, Lakra R, Taneja J, Kalsey GS, Nagendra A, et al. FSH stimulates IRS-2 expression in human granulosa cells through cAMP/SP1, an inoperative FSH action in PCOS patients. Cellular Signalling. 2015;**27**(12):2452-2466

[32] Gao J, Li J, An Y, Liu X, Qian Q, Wu Y, et al. Increasing effect of Tangzhiqing formula on IRS-1-dependent PI3K/AKT signaling in muscle. BMC Complementary and Alternative Medicine. 2014;**14**:198

[33] Fruman DA. Regulatory subunits of class IA PI3K. Current Topics in Microbiology and Immunology. 2010;**346**:225-244

[34] Jimenez C, Hernandez C, Pimentel B, Carrera AC. The p85 regulatory subunit controls sequential activation of phosphoinositide 3-kinase by Tyr kinases and Ras. The Journal of Biological Chemistry. 2002;**277**(44):41556-41562

[35] Bi L, Okabe I, Bernard DJ, Wynshaw-Boris A, Nussbaum RL. Proliferative defect and embryonic lethality in mice homozygous for a deletion in the p110alpha subunit of phosphoinositide 3-kinase. The Journal of Biological Chemistry. 1999;**274**(16):10963-10968

[36] Nelson VL, Jiang YP, Dickman KG, Ballou LM, Lin RZ. Adipose tissue insulin resistance due to loss of PI3K p110alpha leads to decreased energy expenditure and obesity. American Journal of Physiology. Endocrinology and Metabolism. 2014;**306**(10):E1205-E1216

[37] Yu J, Zhang Y, McIlroy J, Rordorf-Nikolic T, Orr GA, Backer JM. Regulation of the p85/p110 phosphatidylinositol 3′-kinase: Stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. Molecular and Cellular Biology. 1998;**18**(3):1379-1387

[38] Berenjeno IM, Guillermet-Guibert J, Pearce W, Gray A, Fleming S, Vanhaesebroeck B. Both p110alpha and p110beta isoforms of PI3K can modulate the impact of loss-of-function of the PTEN tumour suppressor. The Biochemical Journal. 2012;**442**(1):151-159

[39] Mauvais-Jarvis F, Ueki K, Fruman DA, Hirshman MF, Sakamoto K, Goodyear LJ, et al. Reduced expression of the murine p85alpha subunit of phosphoinositide 3-kinase improves insulin signaling and ameliorates diabetes. The Journal of Clinical Investigation. 2002;**109**(1):141-149

[40] Wang X, Wang L, Zhu L, Pan Y, Xiao F, Liu W, et al. PAQR3 modulates insulin signaling by shunting phosphoinositide 3-kinase p110alpha to the Golgi apparatus. Diabetes. 2013;**62**(2):444-456

[41] Li X, Li M, Chen D, Shi G, Zhao H. PAQR3 inhibits proliferation via suppressing PI3K/AKT signaling pathway in non-small cell lung cancer. Archives of Medical Science. 2017. DOI: 10.5114/aoms.2017.72220

[42] Castellano E, Downward J. RAS interaction with PI3K: More than just another effector pathway. Genes & Cancer. 2011;**2**(3):261-274

[43] Manna P, Jain SK. Hydrogen sulfide and L-cysteine increase phosphatidylinositol 3,4,5-trisphosphate (PIP3) and glucose utilization by inhibiting phosphatase and tensin homolog (PTEN) protein and activating phosphoinositide 3-kinase (PI3K)/

serine/threonine protein kinase (AKT)/protein kinase Czeta/lambda (PKCzeta/lambda) in 3T3l1 adipocytes. The Journal of Biological Chemistry. 2011;**286**(46):39848-39859

[44] Manna P, Jain SK. L-cysteine and hydrogen sulfide increase PIP3 and AMPK/PPARgamma expression and decrease ROS and vascular inflammation markers in high glucose treated human U937 monocytes. Journal of Cellular Biochemistry. 2013;**114**(10):2334-2345

[45] Ling HY, Hu B, Hu XB, Zhong J, Feng SD, Qin L, et al. MiRNA-21 reverses high glucose and high insulin induced insulin resistance in 3T3-L1 adipocytes through targeting phosphatase and tensin homologue. Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association. 2012;**120**(9):553-559

[46] Seeger T, Fischer A, Muhly-Reinholz M, Zeiher AM, Dimmeler S. Long-term inhibition of miR-21 leads to reduction of obesity in db/db mice. Obesity (Silver Spring, Md). 2014;**22**(11):2352-2360

[47] Xu G, Ji C, Song G, Zhao C, Shi C, Song L, et al. MiR-26b modulates insulin sensitivity in adipocytes by interrupting the PTEN/PI3K/AKT pathway. International Journal of Obesity (2005). 2015;**39**(10): 1523-1530

[48] Li G, Ning C, Ma Y, Jin L, Tang Q, Li X, et al. miR-26b promotes 3T3-L1 adipocyte differentiation through targeting PTEN. DNA and Cell Biology. 2017;**36**(8):672-681

[49] Lozano-Bartolome J, Llaurado G, Portero-Otin M, Altuna-Coy A, Rojo-Martinez G, Vendrell J, et al. Altered expression of miR-181a-5p and miR-23a-3p is associated with obesity and TNFalpha-induced insulin resistance. The Journal of Clinical Endocrinology and Metabolism. 2018;**103**(4):1447-1458

[50] Carver DJ, Aman MJ, Ravichandran KS. SHIP inhibits Akt activation in B cells through regulation of Akt membrane localization. Blood. 2000;**96**(4):1449-1456

[51] Ui M, Okada T, Hazeki K, Hazeki O. Wortmannin as a unique probe for an intracellular signalling protein, phosphoinositide 3-kinase. Trends in Biochemical Sciences. 1995;**20**(8):303-307

[52] Maira SM, Stauffer F, Schnell C, Garcia-Echeverria C. PI3K inhibitors for cancer treatment: Where do we stand? Biochemical Society Transactions. 2009;**37**(Pt 1:265-272

[53] Hanada M, Feng J, Hemmings BA. Structure, regulation and function of PKB/AKT—A major therapeutic target. Biochimica et Biophysica Acta. 2004;**1697**(1-2):3-16

[54] Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Developmental Cell. 2006;**11**(6):859-871

[55] Chen R, Kim O, Yang J, Sato K, Eisenmann KM, McCarthy J, et al. Regulation of Akt/PKB activation by tyrosine phosphorylation. The Journal of Biological Chemistry. 2001;**276**(34):31858-31862

[56] Risso G, Blaustein M, Pozzi B, Mammi P, Srebrow A. Akt/PKB: One kinase, many modifications. The Biochemical Journal. 2015;**468**(2):203-214

[57] Wani R, Qian J, Yin L, Bechtold E, King SB, Poole LB, et al. Isoform-specific regulation of Akt by PDGF-induced reactive oxygen species.

Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(26):10550-10555

[58] Mahajan K, Mahajan NP. PI3K-independent AKT activation in cancers: A treasure trove for novel therapeutics. Journal of Cellular Physiology. 2012;**227**(9):3178-3184

[59] Nitulescu GM, Margina D, Juzenas P, Peng Q, Olaru OT, Saloustros E, et al. Akt inhibitors in cancer treatment: The long journey from drug discovery to clinical use (review). International Journal of Oncology. 2016;**48**(3):869-885

[60] Szendroedi J, Yoshimura T, Phielix E, Koliaki C, Marcucci M, Zhang D, et al. Role of diacylglycerol activation of PKCtheta in lipid-induced muscle insulin resistance in humans. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(26): 9597-9602

[61] Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014;**59**(2): 713-723

[62] Goetze S, Blaschke F, Stawowy P, Bruemmer D, Spencer C, Graf K, et al. TNFalpha inhibits insulin's antiapoptotic signaling in vascular smooth muscle cells. Biochemical and Biophysical Research Communications. 2001;**287**(3):662-670

[63] Stafeev IS, Vorotnikov AV, Ratner EI, Menshikov MY, Parfyonova YV. Latent inflammation and insulin resistance in adipose tissue. International Journal of Endocrinology. 2017;**2017**:5076732

[64] Wang C, Deng Y, Yue Y, Chen W, Zhang Y, Shi G, et al. Glutamine enhances the hypoglycemic effect of insulin in L6 cells via phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT)/glucose transporter 4 (GLUT4) signaling pathway. Medical Science Monitor. 2018;**24**:1241-1250

[65] Kang J, Ge C, Yu L, Li L, Ma H. Long-term administration of dehydroepiandrosterone accelerates glucose catabolism via activation of PI3K/Akt-PFK-2 signaling pathway in rats fed a high-fat diet. PLoS ONE. 2016;**11**(7):e0159077

[66] Choi J, Kim KJ, Koh EJ, Lee BY. *Gelidium elegans* extract ameliorates type 2 diabetes via regulation of MAPK and PI3K/Akt signaling. Nutrients. 2018;**10**(1):51

[67] Vlavcheski F, Baron D, Vlachogiannis IA, MacPherson REK, Tsiani E. Carnosol increases skeletal muscle cell glucose uptake via AMPK-dependent GLUT4 glucose transporter translocation. International Journal of Molecular Sciences. 2018;**19**(5):1321

[68] Jin W, Cui B, Li P, Hua F, Lv X, Zhou J, et al. 1,25-Dihydroxyvitamin D3 protects obese rats from metabolic syndrome via promoting regulatory T cell-mediated resolution of inflammation. International Journal of Molecular Sciences. 2018;**8**(2):178-187

[69] Yu N, Fang X, Zhao D, Mu Q, Zuo J, Ma Y, et al. Anti-diabetic effects of Jiang Tang Xiao Ke granule via PI3K/Akt signalling pathway in Type 2 diabetes KKAy mice. PLoS ONE. 2017;**12**(1):e0168980

[70] Cao S, Li B, Yi X, Chang B, Zhu B, Lian Z, et al. Effects of exercise on AMPK signaling and downstream components to PI3K in rat with type 2 diabetes. PLoS ONE. 2012;**7**(12):e51709

[71] Cao SC, Zhao G, Chang B, Zhang H. Effects of exercise on expression and phosphorylation of PI3K and PKB in insulin signaling in the skeletal muscles of type 2 diabetic rats. Nan fang yi ke da xue xue bao = Journal

of Southern Medical University. 2010;**30**(6):1217-1221

[72] Liu Y, Liu C, Lu ML, Tang FT, Hou XW, Yang J, et al. Vibration exercise decreases insulin resistance and modulates the insulin signaling pathway in a type 2 diabetic rat model. International Journal of Clinical and Experimental Medicine. 2015;**8**(8):13136-13144

[73] Maarbjerg SJ, Sylow L, Richter EA. Current understanding of increased insulin sensitivity after exercise—Emerging candidates. Acta Physiologica. 2011;**202**(3):323-335

[74] Li A, Qiu M, Zhou H, Wang T, Guo W. PTEN, insulin resistance and cancer. Current Pharmaceutical Design. 2017;**23**(25):3667-3676

[75] Faes S, Dormond O. PI3K and AKT: Unfaithful partners in cancer. International Journal of Molecular Sciences. 2015;**16**(9):21138-21152

[76] Falasca M, Maffucci T. Regulation and cellular functions of class II phosphoinositide 3-kinases. The Biochemical Journal. 2012;**443**(3):587-601

[77] Backer JM. The regulation and function of Class III PI3Ks: Novel roles for Vps34. The Biochemical Journal. 2008;**410**(1):1-17