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Expression and Contribution of Insulin Signaling Pathway to the Development of Polycystic Ovary Syndrome

Qingqiang Lin, Hong Zhang, Jiuhua Zhao and Zhengchao Wang

Abstract

Our previous studies have demonstrated that insulin signaling pathway has an important role in the pathophysiology of polycystic ovary syndrome (PCOS), including phosphatidylinositol 3-kinase and protein kinase B signaling, which is critically implicated in insulin resistance, androgen secretion, obesity, and follicular development. PCOS manifests as defective ovarian steroid biosynthesis and hyperandrogenemia, and 50–70% of women with PCOS exhibit insulin resistance and are hyperinsulinemic, indicating that insulin resistance and hyperinsulinism may have an important role in the pathophysiology of PCOS. Therefore, the present article will review the contribution of insulin signaling pathway to the abnormal regulation of follicular growth and ovulation, which can cause corresponding reproductive endocrine diseases and affect women's reproductive health. Exploring the mechanism of insulin signaling pathway in PCOS will help not only to understand the physiology and pathology of follicular development but also to provide theoretical basis for the treatment of PCOS.

Keywords: insulin receptor substrates, protein kinase B, hypoxia-inducible factor-1, granulosa cells, polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder syndrome with reproductive dysfunction and abnormal glucose metabolism, which is characterized by excessive androgen. It is usually accompanied by insulin resistance (IR) and is also a most common endocrine disorder in women of reproductive age [1]. Although the exact cause of PCOS is not clear, it belongs to endocrine and metabolic diseases. It has been concluded that there are not only endocrine disorders but also metabolic abnormalities with many subtypes. Burghen firstly proposed that insulin resistance (IR) was involved in the pathogenesis of PCOS in 1980 [2], and a large number of studies have subsequently confirmed the close relationship between IR and PCOS. IR is a metabolic state in which the normal insulin-promoted glucose uptake and utilization decreased and the compensatory insulin secretion from tissues and organs of the body maintained the stability of blood glucose.

Clinically, PCOS presents after puberty menarche, and patients have the abnormal glucose metabolism and the pathophysiology related to the physiological changes of puberty. IR is not only a normal physiological change of adolescent girls but also an important pathophysiological change of PCOS, that is, IR and compensatory hyperinsulinemia. IR is one of the important pathophysiological mechanisms in the occurrence and development of many PCOS and also an important cause of hyper-androgenism and ovarian dysfunction.

2. Historical overview

The defect of insulin signaling pathway is one of the important mechanisms of PCOS after two centuries of research, and the historical development process is summarized as the following [2–11]. Achard and Thiers firstly described the relationship between abnormal glucose metabolism and hyperandrogenism in 1921, and Kierland et al. believed that hyperandrogenism was related to higher incidence of acanthosis nigricans in female patients with diabetes mellitus in 1947. Kahn et al. mainly focused on the relationship between the abnormal metabolism in the adolescent and the hyperandrogenism, insulin resistance, and acanthosis nigricans in the postmenopausal women in 1976 and believed the postmenopausal pathogens are caused by the result of endogenous IR and the onset of adolescent women is caused by the mutation of insulin receptor. Until 1980, Burghen et al. reported that hyperinsulinemia is closely related to PCOS for the first time and found that hyperinsulinemia existed in PCOS patients with hyperandrogen-ism under the basal state and after glucose stimulation compared with normal people of the same age and weight, suggesting IR existed and insulin was highly correlated with androgen levels. During the mid-1980s, Dunaif et al. found the follicular membrane cells of typical PCOS women proliferated significantly and the morphological changes of their hyperplasia were more common in PCOS patients with IR, suggesting that hyperinsulinemia affected ovarian morphology and functions.

3. Pathological changes and clinical characteristics of PCOS

Typical polycystic ovaries have stromal hypertrophy, and their volume is 2 times larger than that of normal ovaries. The ovaries showed bilateral sclerosing polycystic degeneration and gray-white or oyster-colored [12]. There is "strand of pearls" appearance on USN, 2-7 mm diameter cystic follicles or large retention follicular cysts under the capsule. Microscopic examination showed atresia follicles increased, no matured follicles formed, cortical surface fibrosis, fewer cells, obvious blood vessels, a large number of outer follicles luteinized, and no signs of ovulation.

Menstrual disorders are the main manifestations of adolescent PCOS patients, with common symptoms such as hirsutism, acne, and body mass increase [12]. But these symptoms can also be the normal physiological manifestations after puberty, so most patients continue to see a doctor for infertility after several years. In addition to the physical changes caused by infertility and excessive androgens, the metabolic syndrome is more prone to cause, mainly including androgenism and metabolic abnormalities such as insulin, glucose, and lipid. Hypertension is more common in the late stage, and cardiovascular diseases such as type 2 diabetes mellitus and coronary heart disease are induced. Many PCOS patients often have IR and hyperinsulinemia as the first manifestation, followed by excessive androgen and reproductive dysfunction.

4. Insulin signaling pathway

Insulin is a multifunctional protein polypeptide, which binds to its specific insulin receptor and causes a series of signal amplification cascade reactions with two main signaling pathways; one is the phosphatidylinositol 3 kinase/protein kinase B (PI3K/PKB) pathway, and the other is the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway. These two signaling pathways are related to the self-phosphorylation of tyrosine residues in receptors, and insulin mainly mediates its metabolic regulation through PI3K pathway [3, 13–16].

PI3K is a kind of kinase that catalyzes phosphatidylinositol, which can be activated by receptor tyrosine kinase or G protein-coupled receptor. It consists of a catalytic subunit P110 and an inhibitory regulatory subunit p85, and it has the activity of lipid kinase and serine/threonine protein kinase. Many growth factors, such as insulin, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF), bind to the corresponding receptors; then tyrosine phosphorylation of the receptor itself and binding of p85 to the phosphorylated receptors relieve the inhibitory effect on p110, and PI3K is then activated. PKB is a member of the serine/threonine protein kinase family and a direct target protein downstream of PI3K. The phosphorylation of 308-site threonine (Thr308) and 473-site serine (Ser473) required for complete activation of AKT depends on PI3K catalysis. After the activation of PI3K/PKB pathway, PI3K/PKB pathway acts on a variety of substrates, mainly regulating the material metabolism of cells, besides participating in cell survival and anti-apoptosis. After insulin binds to insulin receptor alpha subunit located in the cellular membrane, it further triggers tyrosine phosphorylation of beta subunit itself and further phosphorylates tyrosine subunit of insulin receptor substrate (IRS). p-IRS can further activate PI3K and then produce PIP3, a second messenger. PIP3 binds to signal proteins PKB and phosphoinositide dependent kinase-1 (PDK1), which contain the PH domain in cells, and assists PDK1 to phosphorylate 308-site threonine of PKB to make it an active form. Activated-PKB can promote the transfer of glucose carrier-4 (GLUT-4) from the cytoplasm to the envelope and promote the absorption of glucose. PKB then phosphorylates tuberous sclerosis complex protein 1/2 (TSC1/2) to the inactive state, releases its inhibition on the downstream Rheb-GDP, converts it into Rheb-GTP, and further activates target of rapamycin complex 1 (TORC1). In addition, PKB also phosphorylates PRAS40, an inhibitor of mTORC1, to separate it from mTORC1, thereby activating mTORC1 and further exerting downstream cascade reactions, such as hypoxia-inducible factor-alpha (HIF-1alpha)/endothelin-2 (ET-2) signaling pathway-dependent ovulation [3, 13–16].

5. Insulin resistance and polycystic ovary syndrome

Insulin resistance is the defect of insulin signaling transduction, and PI3K/PKB signaling pathway is the main signaling pathway of insulin. In the state of IR, the role of PI3K signaling pathway induced by insulin stimulation decreased [3, 13]. The factors leading to abnormal insulin signaling transduction can be divided into congenital factors and acquired factors. Inborn abnormal factors, such as genetic defect and gene mutation, and other acquired factors, like lifestyle, obesity, and environment, can all cause IR by affecting PI3K/PKB signaling pathway. It was found that with the increase of free fatty acids in blood, they were converted into acy-CoA, DAG was increased, PKC was activated, serine residues in IRS-1 were phosphorylated, tyrosine phosphorylation in IRS-1 was decreased, PI3K activation was disturbed, and GLUT4 translocation to cell surface was affected, thus reducing

insulin-mediated glucose utilization [3, 13, 17]. The increase of free fatty acids leads to the increase of lipids in muscle, which in turn interferes with the use and storage of insulin to glucose and causes IR.

PCOS patients had no significant difference in insulin receptor quantity and binding ability compared with non-PCOS patients, but the phosphorylation of insulin receptor decreased in PCOS patients, and the maximum glucose transport rate stimulated by insulin decreased [11, 18]. PCOS patients often have IR and hyperandrogenism, and IR plays a greater role in the formation of hyperandrogenism, while hyperandrogenism has no significant effect on the formation of IR. IR is the biological effect of insulin produced at physiological level in the body, which is lower than the actual level. The effect of insulin in promoting the absorption and utilization of glucose by organs, tissues, and cells is reduced, that is, the insulin sensitivity of tissues is reduced [3, 19, 20]. Hyperinsulinemia is a marker that insulin regulates glycometabolism in the compensatory stage under the state of IR [3, 19, 20].

At the cellular level, IR means that insulin signaling transduction is blocked or weakened. This signaling from insulin receptor down to the end-mediated substrates of insulin action involves many aspects of cell metabolism [3, 17–20]. Any link of insulin signaling impairment can lead to IR, which is closely related to the impairment of key molecules in the signaling of insulin, insulin receptor, insulin receptor substrate, PI3K, and GLUT4, specifically divided into the following three aspects [3, 17–20]: First, pre-receptor IR. The mutation of insulin gene leads to the changes of insulin primary structure and the decrease of biological activity, resulting in body IR. Secondly, receptor-level IR. The mutation of insulin receptor gene, which cannot be cleaved into matured alpha and beta receptor subunits, reduced the biosynthesis of insulin receptor or the affinity between insulin receptor and insulin, resulting in the loss of intracellular effect of insulin and leading to IR. Lastly, post-receptor IR. Insulin deficiency refers to a series of abnormal metabolic processes that occur when insulin binds to receptors and transmits signals to cells, which is the main cause of IR in PCOS patients. The physiological effect of insulin on glucose is the result of key enzyme activation such as insulin-dependent GLUT4 and G-kinase and glycogen synthase, and the defects in the structure or function of these enzymes can lead to IR [3, 13, 17–21]. Increased serine phosphorylation of insulin receptor may weaken the tyrosine kinase activity of insulin receptor, which may be the mechanism of post-receptor deficiency in PCOS patients.

6. PCOS ovarian IR

It is still controversial whether PCOS is a reflection of ovarian hypersensitivity to insulin or whether systemic IR is localized in ovaries. The study found that PCOS patients secrete more androgens than normal women after insulin stimulation. In addition, PCOS patients with normal insulin levels were treated with insulin sensitizers, and it was found that insulin levels decreased slowly and ovarian androgen levels also decreased [22].

6.1 Molecular mechanism of IR in the follicular membrane cells of PCOS

The most common symptoms of PCOS patients are excessive androgens in the ovary, especially increased testosterone production by follicular membrane cells. Insulin can stimulate the activity of steroid hormones in follicular theca cells through a variety of ways, one of which is through the MAPK pathway, which is still controversial; the other is through LH to induce the accumulation of cyclic adenosine phosphate (cAMP), which stimulates the activity of PI3K [23]. Studies

have shown that LH and insulin have synergistic effects on the gene expression and mRNA accumulation of steroid hormone acute synthesis rapid regulatory protein (StAR) and cytochrome P450c17 (CYP-17). The increased PKB phosphorylation in insulin signaling pathway promotes the production of PCOS clinical symptoms such as follicular cell proliferation, follicular dysplasia, or anovulation, suggesting that insulin induces the synthesis of steroid hormones in follicular cells, which may be regulated by PI3K pathway, and PKB is a downstream regulator of this pathway [24–26]. A special blocker (LY294002) was used to block the PI3K pathway, which inhibited the activation of PKB and weakened the activity of 17a-hydroxylase and also proved the above opinion [26].

6.2 Molecular mechanism of IR in the granulosa cells of PCOS

Insulin can increase the activity of LDL receptor transcription factors through intracellular mechanisms such as protein kinase A (PKA), PI3K, and MAPK signaling pathways, thus promoting steroid hormone synthesis [27]. It has been found that after inhibiting PI3K with wortmannin, IR is produced by interfering with the intracellular glycometabolic signaling pathway [26]. Franks compared anovulatory PCOS patients with ovulatory PCOS patients and found the abnormal glucose metabolism in granulosa cells and the significantly impaired insulin-stimulated lactate production, while insulin-mediated glucose metabolism was resisted besides the steroid hormone secretion remaining normal. Therefore, the complex mechanism of insulin deficiency can also be found in the local of the ovary [28]. Our previous studies further found that PI3K/PKB signaling pathway was impaired in PCOS granulosa cells, which affected the downstream HIF-1a/ET-2-dependent ovulation mechanism, leading to PCOS anovulation [13, 29, 30].

7. Effects of IR on ovarian functions

Insulin can activate or inhibit ovarian hormone synthase and stimulate steroid hormone synthesis in ovarian cells with the species or cell specificity. Human ovarian matrix and follicles have insulin receptor distribution and can produce IGF and binding protein. Therefore, the ovary is one of the important target organs of insulin [7–9].

7.1 Effect of IR on the level of androgen hormone

Insulin enhances the binding ability of LH by increasing the LH receptor of granulosa cells; insulin acts on the pituitary gland to increase the sensitivity of gonadotropin-releasing hormone (GnRH); insulin inhibits the synthesis of sex hormone-binding protein in the liver and increases the level of free insulin [7–9]. PCOS patients taking insulin sensitizer can increase the binding protein in the circulation and decrease the free hormone, thus alleviating hyperandrogenism caused by hyperinsulinemia or IR [7–9].

7.2 Effect of IR on the proliferation of granulosa cells

The proliferation and physiological function of ovarian granulosa cells are very important for the follicular maturation and ovulation, while FSH, IGF2, and insulin are important factors to promote the proliferation of granulosa cells. In PCOS patients, insulin regulates the glucose uptake of ovarian granulosa cells, and the synthesis of lactic acid, a metabolite of glucose utilization, significantly decreases, resulting in the impaired insulin metabolism [7–9]. Insulin can induce IR and affect

the proliferation of granulosa cells in PCOS patients, and most of the PCOS patients are complicated with hyperinsulinemia, high insulin concentration in the follicular fluid. However, the proliferation of granulosa cells was inhibited, and apoptosis was increased. Both of them synergistically enhance the expression of LH and HCG receptors in granulosa cells. The granulosa cells with 50–100 μm follicles have acquired the function of LH receptors only in matured follicles with a diameter of about 20 μm during normal menstrual cycle, which makes the granulosa cells with lumen follicle stage prematurely luteinized and inhibits their proliferation, leading to follicular stagnation [7–9]. Insulin also strengthens the response of granulosa cells to LH, leading to the LH-like peak at the elevated level of LH, which leads to the arrest of granulosa cell proliferation, follicular growth arrest, and anovulation [28].

7.3 Effect of IR on the development of ovarian follicles

Intraovarian follicular development undergoes a series of physiological processes, such as recruitment, selection, dominance, and ovulation. The basic manifestations of follicular dysplasia in PCOS patients are excessive follicular recruitment, blocked follicular selection and dominance, follicular stagnation, and anovulation [7–9]. Hyperinsulinemia in PCOS patients increases the sensitivity of preantral follicles to FSH, leading to excessive follicular recruitment, androgen synthesis in thecal cells, and conversion to estradiol. Furthermore, estradiol decreased the secretion of pituitary FSH due to negative feedback regulation, and the follicles lacked FSH stimulation and grew slowly, leading to the accumulation of preantral follicles and small sinusoidal follicles. The immaturity of follicles resulted in the accumulation of a large number of sinusoidal follicles and the formation of a unique polycystic ovary [31, 32]. Insulin is also one of the most powerful factors affecting plasma plasminogen activator inhibitor-1 (PAI-1). Hyperinsulinemia causes the excessive PAI-1 production in the liver and inhibits the ovulation by inhibiting the conversion of plasminogen to plasmin. The effects of hyperinsulinemia and IGF-2 on ovarian primordial follicles were continuously activated and finally formed the characteristic ovarian morphological changes of PCOS [33, 34].

7.4 Effect of IR on the signaling of ovarian PI3K

In the IR state, the decrease of PI3K signaling induced by insulin leads to the abnormal insulin signaling transduction. PI3K signaling is mainly related to the regulation of insulin on glucose metabolism. The signal molecule downstream of IRS is the key protein for insulin signaling to regulate the glucose metabolism. Activated PI3K, on the one hand, triggers vesicles rich in GLUT4 to translocate to the cell surface through the form of exocytosis from the endokaryon via Golgi apparatus, increases GLUT4 on the cell surface, and regulates the uptake of glucose by myocytes, adipocytes, and hepatocytes. On the other hand, activated PI3K inhibits gluconeogenesis by inhibiting enol pyruvate carboxykinase and ultimately increases the utilization of glucose and glycogen [7–9, 28]. Our previous studies have clearly demonstrated that the mechanism of IR in PCOS ovaries is related to post-insulin receptor signaling disorder [13, 29, 30]. At the same time, IR is also a key link in the pathogenesis of PCOS, so the causal relationship between PCOS and IR is still unclear.

8. Conclusion

PCOS is a disease involving many factors such as heredity and environment, and IR plays an important role in the development of PCOS. After insulin binds

to receptors, it exerts its biological effects through a series of signaling pathways. Obstacles in any link of the pathways can lead to the signaling disorders and cause IR. Compensatory hyperinsulinemia and IR are considered to be the pathological basis of abnormal glucose metabolism and reproductive dysfunction in PCOS patients. Although the signaling pathway of insulin and its mechanism during the occurrence and development of insulin resistance are still poorly understood, PI3K/PKB pathway, as the main signal pathway of insulin, is involved in the metabolism of glucose and lipid in vivo. Further study about the regulatory mechanism of PI3K/PKB pathway and the interaction between the molecule and targeted molecule is needed and has high clinical value and application prospects to develop PI3K/Akt signaling pathway-specific drugs as a new target of IR therapy. At the same time, using modern molecular biology technology to comprehensively understand insulin signaling pathway and in-depth study of IR molecular mechanism of PCOS can provide a solid theoretical basis for clinical diagnosis and treatment.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Qingqiang Lin[1], Hong Zhang[1], Jiuhua Zhao[2] and Zhengchao Wang[1]*

1 Provincial Key Laboratory for Developmental Biology and Neurosciences, College of Life Sciences, Fujian Normal University, Fuzhou, China

2 West Anhui Health Vocational College, Liuan, China

*Address all correspondence to: zcwang@fjnu.edu.cn

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