

Research Progress on the Intervention of Recurrent Miscarriage by Regulating the PI3K/AKT Signaling Pathway with Traditional Chinese Medicine

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Abstract: Recurrent spontaneous abortion (RSA) is a common gynecological complication during pregnancy, characterized by recurrent spontaneous miscarriages. The incidence rate of the disease is increasing year by year, and the risk of recurrent miscarriage increases with the increase in the number of miscarriages, which seriously affects women's physical and mental health. At present, targeted intervention after screening the causes is adopted as the treatment plan, but there are limited treatment options and high recurrence rates. The phosphatidylinositol 3-kinase (PI3K)/protein kinase B(AKT) signaling pathway plays a central regulatory role in cell growth, proliferation, metabolism, and apoptosis, and is closely related to the occurrence and development of RSA. Traditional Chinese medicine has significant therapeutic effects in the prevention and treatment of RSA. It can regulate the PI3K/AKT signaling pathway, affecting the proliferation, invasion and migration of trophoblasts, placental angiogenesis, immune regulation, embryo implantation, etc., thereby maintaining pregnancy. This article extensively collects relevant literature published domestically and internationally, focusing on the regulation of the PI3K/AKT signaling pathway by traditional Chinese medicine to intervene in RSA. It systematically summarizes and organizes the active ingredients of traditional Chinese medicine, including flavonoids, alkaloids etc., as well as traditional Chinese medicine formulas such as Shoutai Wan, Taining Fang, and Zishen Yutai Wan, which can intervene in RSA by regulating the PI3K/AKT signaling pathway and interaction pathway, inhibiting cell apoptosis, promoting decidual angiogenesis, and improving placental interface angiogenesis. This article reviews the relevant research on the regulation of PI3K/AKT signaling pathway by traditional Chinese medicine in the intervention of RSA, in order to provide research ideas for the treatment of RSA with traditional Chinese medicine and lay the foundation for the development of new clinical drugs.

Keywords: Traditional Chinese Medicine, PI3K/AKT signaling pathway, Recurrent spontaneous abortion, Research progress.

1. Introduction

Recurrent spontaneous abortion (RSA) refers to the loss of pregnancy before 28 weeks, with two or more consecutive natural miscarriages, including consecutive biochemical pregnancies [1]. The incidence of RSA is usually between 1% and 2% in couples who are trying to conceive [2]. Its etiology is complex, mainly involving multiple factors such as genetics, immunity, endocrine, anatomy, etc. and the etiology of most patients is unknown [3]. This disease has the characteristics of high recurrence rate, limited treatment methods, and significant differences in efficacy, which seriously affects women's mental health. Therefore, the prevention and treatment of RSA has become an urgent problem in the field of reproduction. The PI3K/AKT signaling pathway, as an intracellular signaling pathway, participates in various processes such as cell division, differentiation, growth, metabolism, and apoptosis, affecting placental formation and early embryonic development [4]. Traditional Chinese medicine has the characteristics of multi-target, multi pathway, and multi pathway effects in the treatment of RSA, and is widely used with excellent therapeutic effects. This article summarizes the regulatory mechanism of traditional Chinese medicine on the PI3K/AKT signaling pathway, laying the foundation for the development of new drugs in clinical practice.

2. Overview of PI3K/AKT Signaling Pathway

The PI3K/AKT signaling pathway plays a central regulatory role in cellular physiological processes, participating in key processes such as cell growth, proliferation, metabolism, and apoptosis [5]. The PI3K protein family is an important class of signaling lipases, whose main function is to catalyze phosphatidylinositol (PI) on the plasma membrane and phosphorylate the 3-hydroxy site of PI [6]. The PI3K family can be divided into three categories: I, II, and III. Class I PI3Ks belong to heterodimeric molecules, which are further divided into IA and IB categories. IA class PI3Ks are composed of p85 (α , β , γ) regulatory subunit and p110 (α , β , δ) catalytic subunit, while IB class PI3Ks are composed of p101 regulatory subunit and p110 γ catalytic subunit [7]. Class I PI3K recruits Akt kinase to regulate biological processes such as cell growth, proliferation, survival, and metabolism [8]. Class II PI3Ks have monomeric catalytic isomers, mainly including three catalytic subtypes: C2 α , C2 β , and C2 γ , which play a catalytic role. Catalytic conversion of PI into phosphatidylinositol-3-phosphate and phosphatidylinositol-3, 4-diphosphate [9]. Class III PI3Ks are composed of Vps34 and catalyze the production of phosphatidylinositol - 3 - phosphate [10]. On the one hand, it can be phosphorylated, and on the other hand, it can promote the aggregation of catalytic subunits towards the cell membrane, thus playing a key role in cellular physiological processes [11].

AKT, also known as protein kinase B (PKB), is a type of serine/threonine protein kinase that plays a precise role in regulating the cell cycle. There are three subtypes of AKT1,

AKT2, and AKT3, which have high homology with each other. AKT1, as a proto oncogene, exists in all tissues, inducing cell apoptosis and affecting cell survival. AKT2 plays a role in glucose homeostasis and is located in muscle tissue and adipocytes [8]. AKT3 is expressed in the brain and participates in the development of neurons [12]. AKT itself can regulate multiple cellular processes in the cell cycle. In terms of cell survival, it can inactivate pro apoptotic factors such as BAD and Procaspase-9. In addition, AKT targets can also participate in processes such as protein synthesis and glycogen metabolism [13].

3. The Mechanism of PI3K/AKT Signaling Pathway in Recurrent Miscarriage

The PI3K/AKT signaling pathway plays an important role in the occurrence and development of recurrent miscarriage, affecting the migration, apoptosis, invasion of trophoblast cells, placental angiogenesis, embryo implantation, immune regulation, and other aspects, and influencing the occurrence of RSA through multiple pathways [14]. This article collects relevant research literature to clearly demonstrate the role of the PI3K/AKT signaling pathway in RSA.

3.1 The Impact on Nourishing Cells

Nourishing cells play an important role in the process of embryo implantation and placental formation. Trophoblast cells are the early form and source basis of nourishing cells. In early pregnancy, trophoblast cells shoulder a crucial mission. Not only is it the foundation for successful embryo implantation, but it is also a necessary condition for maintaining the smooth progress of the entire pregnancy [15]. FKBP5, also known as FK506 binding protein 5, when overexpressed, can interfere with the signaling pathways within the trophoblast cells and significantly inhibit their migration and invasion abilities. A study has found that FKBP5 expression is increased in the placenta of mice with miscarriage and RSA females. The experimental results fully confirm that upregulating the expression of FKBP5 can cause obstruction of the PI3K/AKT signaling pathway, while reducing FKBP5 can promote the expression of the PI3K/AKT signaling pathway, thereby promoting the invasion and migration of trophoblast cells, maintaining normal pregnancy, and preventing miscarriage [16, 17]. HTR-8/SVneo is derived from human placental trophoblast cells, and activation of the PI3K/AKT pathway can significantly promote the proliferation of HTR-8/SVneo cells and normal migration of trophoblast cells, while inhibiting cell apoptosis. The activation of pathways significantly increases the expression level of anti apoptotic protein Bcl-2, while the levels of pro apoptotic proteins Bax and Bim are significantly reduced. This further confirms the significant role of the PI3K/AKT pathway in inhibiting apoptosis of HTR-8/SVneo cells, which is crucial for maintaining the normal survival of trophoblast cells and stable development of the placenta [18].

3.2 The Impact on Placental Angiogenesis

Vascular endothelial growth factor (VEGF) plays a crucial role in the physiological process of pregnancy. It can promote the invasion of trophoblast cells from decidua and placental

villi into spiral arterioles, induce the formation of capillary networks, and thus complete the process of vascular remodeling [19]. Endothelial nitric oxide synthase (eNOS) is a key regulatory factor in angiogenesis, playing a central role in maintaining the homeostasis of the vascular environment at the implantation site, ensuring successful embryo implantation, and ensuring the smooth progress of the entire pregnancy cycle. The activated PI3K/AKT signaling pathway, as a downstream signaling pathway of VEGF, regulates angiogenesis. Research shows [20], When VEGF significantly increases in the body, it specifically binds to the surface receptors of vascular endothelial cells, sequentially activating the PI3K/AKT signaling pathway and downstream eNOS, which is of great significance for the process of angiogenesis. It has been reported [21] that in female patients with a history of miscarriage, the expression of VEGF in their bodies is correspondingly reduced. Research by Zhang Jie et al. [22] has shown that the kidney tonifying and blood activating formula has a positive effect on angiogenesis and remodeling at the maternal fetal interface, mainly by activating vascular endothelial growth factor A (VEGFA), activating the PI3K/AKT signaling pathway, enhancing the expression and activity of proteins related to this pathway, thereby promoting the generation of decidual blood vessels, promoting the remodeling of blood vessels at the maternal fetal interface, and creating a favorable blood supply environment for embryonic development. According to reports, the PI3K/AKT/eNOS signaling pathway can directly participate in the construction of neovascularization by mobilizing endothelial progenitor cells during angiogenesis [23].

3.3 The Impact of Immune Regulation

Maintaining the immune balance at the maternal fetal interface and the normal function of decidual stromal cells (DSCs) during pregnancy is crucial for preventing miscarriage. Research has found that Bushen Huoxue Tang [24] mainly regulates IL-17A/IL-17RA by acting on the PI3K/AKT signaling pathway. It can effectively inhibit the excessive secretion of IL-17A, alleviate inflammatory effects, and regulate the immune microenvironment at the maternal fetal interface, so as to maintain an immune balance conducive to embryo implantation and development. To provide a favorable supportive environment for embryonic development, ultimately achieving the goal of preventing miscarriage. In the physiological environment of the maternal fetal interface, decidual macrophages (dM φ s) play an indispensable and critical role in maintaining immune balance. The polarization state of macrophages is the core link in regulating the immune microenvironment. Once polarization abnormalities occur, it can easily lead to a series of adverse pregnancy outcomes, such as premature birth, recurrent miscarriage, and infertility [25]. In the study of spontaneous abortion cases, it was found that dM φ s exhibited a unique state dominated by M1, and the expression level of nuclear receptor (Rev-erb α) was significantly reduced. After activating Rev-erb α using SR9009, dM φ s showed a tendency to polarize towards the M2 direction. It was found that SR9009 can induce M2 polarization of macrophages through the PI3K/AKT signaling pathway, promote immune tolerance at the maternal fetal interface, and prevent miscarriage [26].

3.4 The Impact on Embryo Implantation

The stable maintenance of normal physiological functions of the endometrium during embryo implantation is a necessary prerequisite for the successful implantation of the embryo [27]. Embryo implantation can be considered one of the most complex and delicate stages in the reproductive process. Its success or failure is jointly regulated by many factors. Among numerous influencing factors, endometrial receptivity plays a crucial role [28]. According to reports, PI3K/AKT plays an indispensable regulatory role in enhancing endometrial receptivity (ER) and promoting successful embryo implantation into the maternal endometrium [29]. Research has found that the transcript LINC00339 can encode a specific peptide segment. This peptide segment can enhance the adhesion between JAR trophoblast cell spheroids and Ishikawa endometrial cells. This adhesion enhancing effect depends on the PI3K/AKT pathway [30].

4. The Role of Traditional Chinese Medicine in Regulating the PI3K/AKT Signaling Pathway and Intervening in Recurrent Miscarriage

RSA belongs to the category of traditional Chinese medicine, including “slippery fetus” and “fetal instability” [31]. The etiology is relatively complex, mainly including congenital deficiencies, emotional injuries, falls and falls, and irregular diet. The main pathogenesis is related to the instability of Chong Ren and fetal loss. Deficiency of kidney qi, weakness of qi and blood, and blockage of blood stasis can all lead to Chong Ren injury and fetal instability. The disease is located in the kidney, spleen, Chong and Ren, and the nature of the disease belongs to the syndrome of deficiency in origin, excess in appearance, and deficiency and excess in combination. The treatment of traditional Chinese medicine is characterized by “conditioning before pregnancy and protecting the fetus after pregnancy”. Before pregnancy, it is important to “pre-train for its damage”, and after pregnancy, it is important to “supplement training to clear vitality”. The treatment of RSA with traditional Chinese medicine has significant therapeutic effects. The organic integration of TCM syndrome differentiation and modern medical pathogenesis, and the multidimensional utilization of the irreplaceable advantages of TCM, have played an important role in promoting the treatment of RSA.

4.1 Active Ingredients of Traditional Chinese Medicine

4.1.1 Flavonoids

Flavonoids are natural organic compounds with polyphenolic structures. It can interact with biological targets and generate a series of biological activities. Such as antioxidant, antibacterial, anti-inflammatory, and free radical scavenging effects [32]. Baicalin belongs to the flavonoid compounds and is the main active ingredient of the traditional Chinese medicine *Scutellaria baicalensis*. Its pharmacological effects mainly include anti-inflammatory, immune regulation, and anti-tumor effects [33, 34]. Experiments have shown that baicalin can promote the expression of CD206 protein, PI3K, and AKT by regulating the PI3K/AKT pathway, while

downregulating tumor necrosis factor- α (TNF- α), interleukin-1 β (interleukin-1 β), CD86 protein, inhibiting macrophage M1 polarization, and inducing M2 polarization. From this, it can be concluded that baicalin activates the PI3K/AKT signaling pathway, promotes the transformation of uterine macrophages towards M2 type, and inhibits the transformation towards M1 type, thereby alleviating miscarriage [35]. Flavonoids belong to the flavonoid class and are one of the active substances in traditional Chinese medicine *Angelica sinensis* and *Paeonia lactiflora*. They exhibit significant pharmacological effects in human immune regulation, hematopoietic function maintenance, anti-inflammatory, and other aspects [36, 37]. Research has shown that *Angelica sinensis* and *Paeonia lactiflora* can reduce the content of endostatin ES, increase the expression levels of CD34, PI3K, and pAKT/AKT, thereby improving the obstruction of angiogenesis at the maternal fetal interface and intervening in RSA. *Angelica sinensis* and *Paeonia lactiflora* exert the above-mentioned effects mainly by activating the PI3K/AKT signaling pathway [38].

4.1.2 Alkaloids

Berberine, also known as berberine, belongs to the light yellow isoquinoline alkaloids [39]. Its main pharmacological effects include anti-tumor, anti-inflammatory, antiviral, etc [40]. Berberine BBR can enhance the proliferation ability of endothelial progenitor cells (EPCs) damaged by TNF- α by activating the PI3K/AKT/eNOS signaling pathway. LY294002, as a PI3K inhibitor, can block the expression of AKT and eNOS mRNA induced by BBR. When the level of TNF - α increases, it not only damages the function of endothelial cells, but also exacerbates the apoptosis of EPCs and causes their loss of function. Berberine (BBR) can significantly upregulate the expression levels of PI3K/AKT/eNOS, thereby reversing the damage caused by TNF- α to EPCs proliferation ability [41]. Influence the construction of neovascularization and intervene in RSA [29]. As a key alkaloid extracted from motherwort, motherwort alkaloids can regulate processes such as oxidative stress, inflammatory response, and cell apoptosis, thereby assisting in pregnancy [42]. Leonurus alkaloids have a significant effect on apoptosis related markers, as they can significantly increase the expression of anti apoptosis marker B cell lymphoma 2 (Bcl-2) and significantly decrease the expression of apoptosis marker Bcl-2 related X protein (Bax). This research result is consistent with other research findings, which have shown that protein kinase B (AKT) plays a practical and effective role in anti apoptotic response as a signaling pathway [43-45]. Research has shown that motherwort alkaloids have the ability to improve the pre thrombotic state of recurrent miscarriage. Their mechanism of action is to affect coagulation factor II and fibrinogen, thereby regulating complement and coagulation cascade reactions, blood coagulation process, and platelet activation process to achieve the goal of preventing and treating RSA [46].

4.2 Tcm

Clever formulation of traditional Chinese medicine formulas, using Chinese medicinal materials with different properties, flavors, and meridians. According to the compatibility criteria of monarchs, ministers, and envoys, multiple medicinal herbs

can be flexibly combined. Play a synergistic role to enhance therapeutic efficacy. Based on the core idea of traditional Chinese medicine, which emphasizes the holistic concept and dialectical treatment, it provides unique and effective ideas for disease treatment. Research shows that traditional Chinese medicine The compound intervenes in RSA by regulating the PI3K/AKT signaling pathway.

4.2.1 Shoutai Pill

When Shoutai Wan intervenes in RSA, it can enhance the activity of key aerobic glycolytic enzymes, such as hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), and pyruvate kinase M2 (PKM2), while lactate levels increase. The acidic microenvironment is maintained in a stable state, and the PI3K/AKT/mTOR signaling axis is also upregulated. The expression of angiogenesis related factors such as vascular endothelial growth factor receptor 2 (VEGFR2) and vascular endothelial growth factor A (VEGFA) at microvascular density (MFI) is increased, resulting in improved angiogenesis. Research has shown that Shou Tai Wan has a significant improvement effect on early pregnancy outcomes, laying a solid foundation for the subsequent remodeling of the uterine spiral artery. Shoutai Pill can resist the phenomenon of decreased glycolysis caused by an increase in oxygen content. Once LDHA is knocked down or PI3K is inhibited, the recovery effect of Shoutai Pill will be adversely affected [47]. Forkhead box transcription factor O3 (FOXO3) is a key downstream effector molecule in the PI3K/AKT signaling pathway. With its spiral loop spiral DNA binding domain, it is widely involved in cellular processes such as oxidative stress, cell proliferation, DNA damage repair, apoptosis, influencing angiogenesis, and immune regulation [48, 49]. Jianwei Shoutai Wan upregulates forkhead transcription factor O3, regulates the PI3K/AKT pathway, inhibits trophoblast apoptosis, and prevents RSA [50].

4.2.2 Tire Ning Formula

Taining Formula is an in-house preparation of the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, which has multiple positive effects on recurrent miscarriage rats. It can increase the uterine organ index of rats with recurrent miscarriage, reduce embryo loss and miscarriage rates, and improve the status of decidua tissue. In terms of serum related substances, it can promote the increase of HCG, t-PA, APC, and AT-III levels in serum, optimize progesterone levels, regulate fibrinolytic activity, and inhibit the coagulation process. From a molecular mechanism perspective, Taining Formula can increase the levels of PI3k, p-AKT protein, and PI3k, p-AKT mRNA in recurrent miscarriage rats. By regulating the PI3k/AKT pathway, it achieves a regulatory effect on angiogenesis between mother and fetus, providing support for maintaining normal pregnancy [51].

4.2.3 Zishen Yutai pill

Zishen Yutai Wan has the effects of tonifying kidney qi, nourishing blood, and stabilizing pregnancy. In clinical applications, it is mainly used for the prevention and treatment of habitual miscarriage and threatened miscarriage, with

significant therapeutic effects [52]. Zishen Yutai Wan can significantly increase the expression levels of VEGF, microvascular density (MVD), PI3K, AKT, p-AKT, and hypoxia inducible factor-1 α (HIF-1 α) proteins in decidual tissue. Through this enhancing effect, Zishen Yutai Wan can promote decidual angiogenesis and improve the embryo loss rate in RSA mice. Research has shown that Zishen Yutai Wan exerts the above-mentioned effects and is closely related to the regulation of VEGF/PI3K/AKT pathway protein expression, providing strong support for improving symptoms related to recurrent miscarriage [53].

5. Summary and Outlook

The PI3K/AKT signaling pathway is closely related to the pathogenesis of recurrent miscarriage and has become a research focus in the treatment of recurrent miscarriage. Traditional Chinese medicine can intervene in recurrent miscarriage by regulating this signaling pathway. In summary, it was found that the PI3K/AKT pathway often interacts with signaling pathways such as VEGF/PI3K/AKT, PI3K/AKT/mTOR, PI3K/AKT/GSK3 β , and PI3K/AKT/eNOS. The mechanisms of action of active ingredients in traditional Chinese medicine and traditional Chinese medicine compound interventions for recurrent miscarriage differ. Huangqin, Danggui, and Baishao, traditional Chinese medicines derived from flavonoids; Alkaloids derived from traditional Chinese medicine Huanglian and motherwort. Pregnancy is maintained through the regulation of PI3K/AKT and its interaction pathways, including immune regulation, maternal fetal interface angiogenesis, cell apoptosis, proliferation, migration and invasion, and embryonic development. Traditional Chinese medicine formulas such as Shou Tai Wan, Tai Ning Fang, and Zi Shen Yu Tai Wan also intervene in RSA by activating the PI3K/AKT pathway and interaction pathway, inhibiting cell apoptosis, promoting decidual angiogenesis, and improving placental interface angiogenesis.

At present, traditional Chinese medicine has made some progress in regulating the PI3K/AKT signaling pathway for the intervention of recurrent miscarriage, but there are still several issues to be resolved. Firstly, existing research mainly focuses on the effects of traditional Chinese medicine formulas and extracts on this signaling pathway, and there is a lack of exploration on specific targets and upstream and downstream regulatory mechanisms. Secondly, the research forms are mostly animal experiments and cell experiments, and the number of clinical studies is small and the sample size is small. There is a lack of multi center, large sample randomized controlled trials, which lacks persuasiveness. Thirdly, the composition of traditional Chinese medicine is complex, and its efficacy may be affected due to differences in origin and processing methods. At the same time, there is a lack of standardized quality control systems.

Looking ahead to the future, it is urgent to focus on strengthening the basic research work of traditional Chinese medicine regulating the PI3K/AKT signaling pathway to intervene in recurrent miscarriage. Thoroughly study the specific molecular mechanisms, clearly define the target of action, and lay a theoretical foundation for precision treatment in traditional Chinese medicine. At the same time, actively

conducting multicenter, large-scale, high-quality randomized controlled trials to objectively and accurately evaluate the efficacy and safety of traditional Chinese medicine in clinical applications. Furthermore, efforts should be made to establish a comprehensive quality control system for traditional Chinese medicine, standardizing various aspects such as the selection of production areas, processing procedures, and formulation processes to ensure the reliability of research results. These measures will effectively promote the development of more efficient traditional Chinese medicine drugs for the prevention and treatment of recurrent miscarriage, and open up new ideas and methods for clinical treatment. I firmly believe that with the continuous advancement of research, traditional Chinese medicine will demonstrate more significant efficacy in the prevention and treatment of recurrent miscarriage.

Fund Project

National Natural Science Foundation of China (No. 81603656); Construction Project of National Famous Old Chinese Medicine Experts Inheritance Studio of State Administration of Traditional Chinese Medicine (No. [2022] 75); Pilot project of clinical collaboration between Chinese and Western medicine for major and difficult diseases (infertility).

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