

KAIYU ZHENGYUAN POWDER AFFECTS EPITHELIAL-MESENCHYMAL TRANSITION AND APOPTOSIS OF PAPILLARY THYROID CARCINOMA THROUGH ER/PI3K/AKT SIGNALING PATHWAY

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ABSTRACT

Introduction: To explore the intervention mechanism of Kaiyu Zhengyuan powder on epithelial mesenchymal transformation and apoptosis of papillary thyroid carcinoma.

Materials and methods: Kaiyu Zhengyuan powder was used to intervene TCP1 cells and divided into blank group, inhibitor group, and traditional Chinese medicine group; The indexes were detected by Western blotting and flow cytometry.

Results: Kaiyu Zhengyuan Powder can effectively reduce ER α , GPER protein expression level in estrogen receptor pathway, increase ER β protein expression level. It can effectively reduce the expression level of angiogenic factors VEGF and BFGF, and also down regulate GSK-3 α , GSK-3 β protein expression level. The expression level of Twist1, HIF-1 α , PFK, PKM2 protein that closely related to epithelial mesenchymal transformation were decreased. Reduce the expression level of PI3K, AKT1, P70S6K, PDK1, cyclin D1, Bcl2 and p21 proteins, promote apoptosis, increase the expression level of Bax protein in apoptotic proteins. Flow cytometry showed that Kaiyu Zhengyuan Powder promoted TCP1 cell apoptosis.

Conclusion: Kaiyu Zhengyuan Powder can inhibit the epithelial mesenchymal transformation of papillary thyroid carcinoma to a certain extent, so as to prevent the metastasis of papillary thyroid carcinoma, and it has a good therapeutic effect on papillary thyroid carcinoma by interfering with PI3K/AKT signal pathway.

Keywords: Papillary thyroid carcinoma, Kaiyu Zhengyuan Powder, ER/PI3K/AKT signaling pathway, epithelial stromal transformation.

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Introduction

Papillary thyroid carcinoma (PTC) is the most common endocrine malignant tumor in the human body and one of the fastest growing malignant tumors in the world. Among thyroid cancer, PTC is well differentiated, but 20% of patients with PTC still have lymph node metastasis, which seriously affects the prognosis and outcome of PTC. Studies have shown that in lymphatic metastasis of papillary thyroid carcinoma mortality rate is much

higher than other endocrine tumor⁽¹⁾, the mortality rate is no lymph node metastasis in patients with 30 times^(2, 3), therefore, to find influence papillary thyroid carcinoma development and infiltration of molecular level change, diagnosis and treatment of papillary thyroid carcinoma has important application significance. Studies have shown that thyroid cancer cell adhesion is reduced, movement and attack ability increases with the transformation of epithelial mesenchymal phenomenon, there is a clear relationship between⁽⁴⁾, epithelial mesenchymal

transformation is the polarity of epithelial cells into activity ability, able to move freely between the cell matrix of mesenchymal cells, the process of the loss of epithelial characteristics and the characteristic of mesenchymal cells⁽⁵⁾ is the main characteristics, Genes that play a key role in EMT are usually expressed at the invasive front of primary tumors and are necessary for primary tumors to cross the basement membrane and enter adjacent host tissues⁽⁶⁾. Therefore, we have reason to believe that lymph node metastasis of papillary thyroid carcinoma is closely related to EMT and that EMT of papillary thyroid carcinoma can be reversed by certain in vitro and in vivo conditions. Modern studies suggest that traditional Chinese medicine intervention in papillary thyroid carcinoma can exert comprehensive effects from multiple targets, including regulating hormone secretion or hormone receptor expression, inducing cell apoptosis, promoting cell differentiation, and inhibiting cell proliferation, etc.

In clinical practice, traditional Chinese medicine, such as promoting blood circulation and removing blood stasis, soothing the liver and relieving depression, and dissipating phlegm and disperses knot are commonly used. To promote blood circulation, remove blood stasis and qi, remove phlegm and toxin, regulate the chongren for the treatment. TCM believes that lymph node metastasis of thyroid cancer is mainly caused by the mutual obstruction of qi deficiency, cancer toxin, phlegm and blood stasis.

Therefore, TCM treatment often uses the method of strengthening qi, promoting blood circulation and removing blood stasis, clearing heat and detoxifying and dissipating phlegm, etc., which can effectively prevent lymph node metastasis⁽⁷⁻¹⁰⁾. Therefore, this study aims to investigate the intervention effect of Kaiyu Zhengyuan powder on papillary thyroid carcinoma.

Methods and materials

Materials

TPC-1 Cells were provided by the Shanghai Cell Bank, DMEM medium, and trypsin was kindly provided by Gibco, FBS serum was provided by conning, dimethyl sulfoxide by sigma, Twist1, HIF-1 α , PFK, PKM2, PI3K, AKT1, P70S6K, PDK1, ER α , ER β , GPER, CylinD1, P21, Bcl2, VEGF, BFGF, GSK-3 α , GSK-3 β antibodies were kindly provided by the CST Corporation. Traditional Chinese medicine extraction, white atractylodes

rhizome, dried tangerine peel, Pericarpium Citri Reticulatae Viride, cyperus rotundus, ried coke hawthorn, Sea clam, Platycodon grandiflorum, Poria cocos, fructus amomi, corydalis tuber, medicated leaven, fried malt, prepared licorice, each aliquot, total of 800 grams.

Use 8000ml 75% ethanol for the first reflux extraction for 2 hours; For the second time, reflux continuously for 1 hour, use 5600ml of 75% ethanol, mix the extract, filter it, concentrate it into 1ml/20g concentrated solution by vacuum rotary evaporator, autoclave it for standby, and dilute it at 1:100.

Cell culture

TPC-1 cells were cultured in DMEM medium containing 5% fetal bovine serum in a cell incubator containing 5% carbon dioxide at 37 °C. The cells were divided into blank groups: normal culture, no treatment; Inhibitor group: PI3K inhibitor was added to interfere with cells, while Chinese medicine group: concentrated solution of Chinese medicine was added to interfere with cells.

Western blotting

Total protein was extracted from cells of each group, and the total amount of protein in each group was quantified. Sample loading, electrophoresis, membrane transfer, antibody incubation, and color development were performed.

Apoptosis was measured by flow cytometry

Cells were collected, fixed with cold ethanol, washed, filtered in 400 cells, added staining solution for staining, and cell apoptosis was detected by upper flow cytometry.

Results

Kaiyu Zhengyuan Powder can down regulate the protein expression level of epithelial mesenchymal transformation factor Twist1, HIF-1 α , PFK, PKM2 in TPC-1 cells, thereby inhibiting epithelial mesenchymal transformation of papillary thyroid carcinoma cells and preventing metastasis and invasion of papillary thyroid carcinoma (show as Figure 1). Kaiyu Zhengyuan Powder can regulate the PI3K/AKT signal pathway of TPC-1 cells, and can reduce the protein expression levels of PI3K, AKT1, P70S6K, PDK1 (show as Figure 2).

Kaiyu Zhengyuan Powder can regulate the PI3K/AKT signal pathway of TPC-1 cells, reduce the protein expression level of cyclinD1 and P21,

reduce the protein expression level of apoptosis factor Bcl2, and increase the protein expression level of Bax (show as Figure 3).

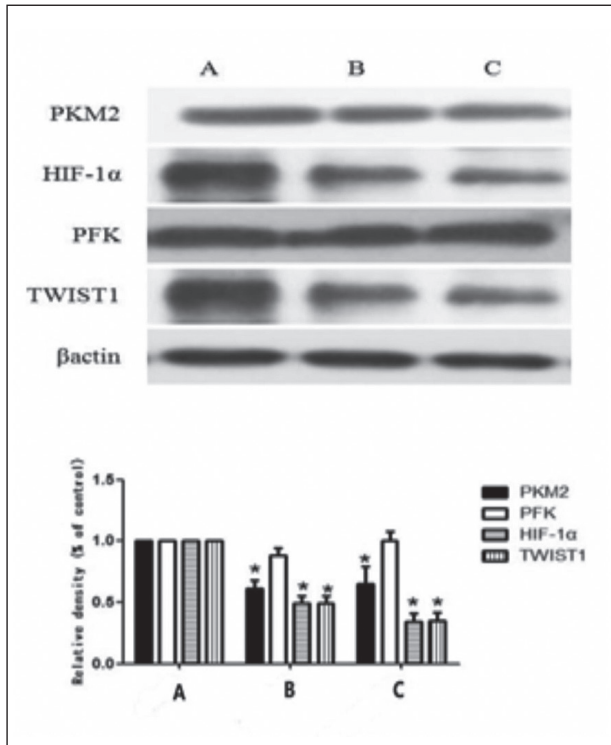


Figure 1: Protein expression levels of Twist1, HIF-1 α , PFK, and PKM2 in each group.
 Note: A: blank group; B: inhibitor group; C: Kaiyuan Zhengyuan bulk group (the same below).

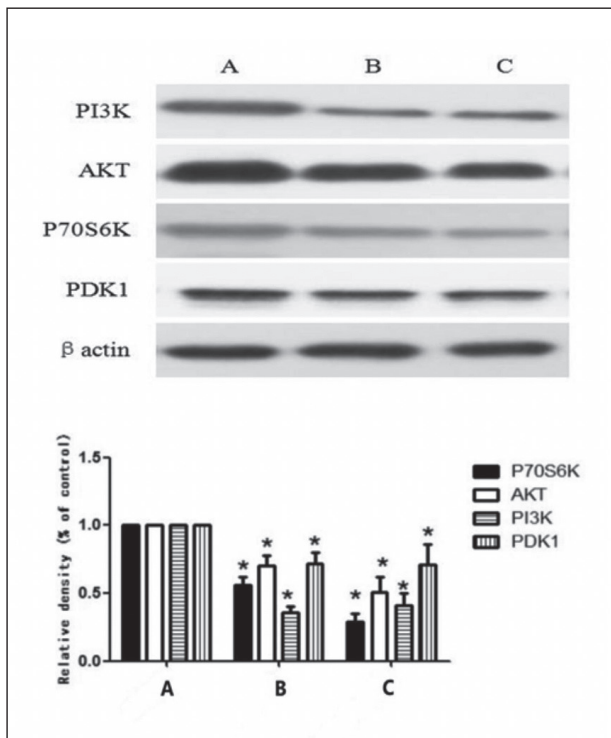


Figure 2: Protein expression levels of P70S6K, AKT, PI3K, and PDK1 in each group.

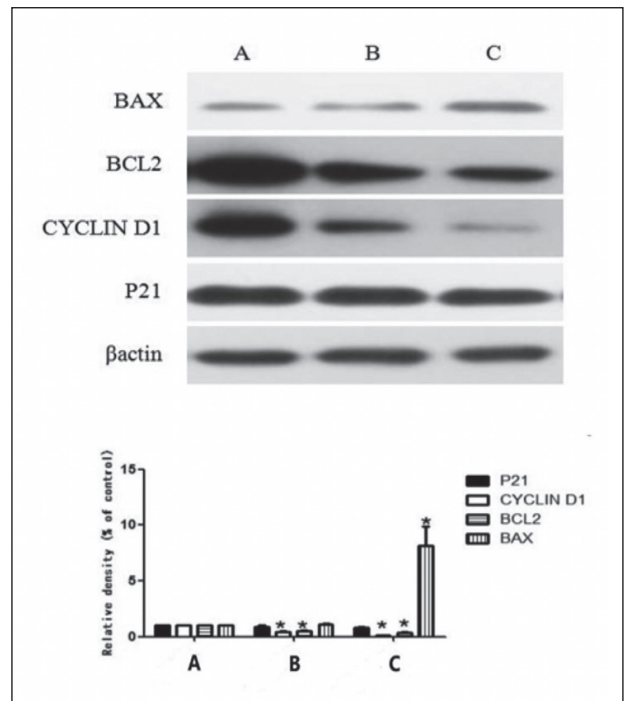


Figure 3: Protein expression levels of CylinD1, P21, BCL2, and BAX in each group.

Kaiyu Zhengyuan Powder can increase the expression level of ER β protein in TPC-1 cells and reduce ER α , GPER protein expression level (show as Figure 4).

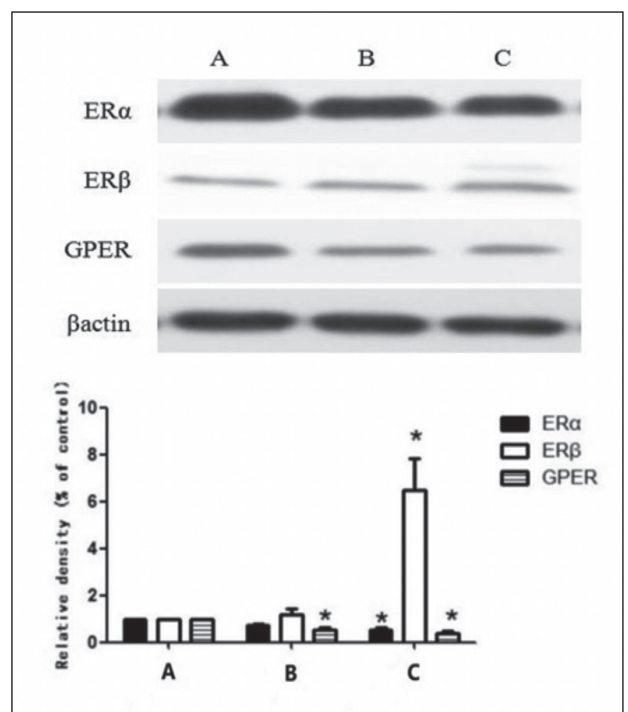


Figure 4: Protein expression levels of ER α , GPER, and ER β in each group.

Kaiyu Zhengyuan Powder can reduce the expression levels of angiogenic factors VEGF

and BFGF, as well as GSK-3 α , GSK-3 β Protein expression level (show as Figure 5).

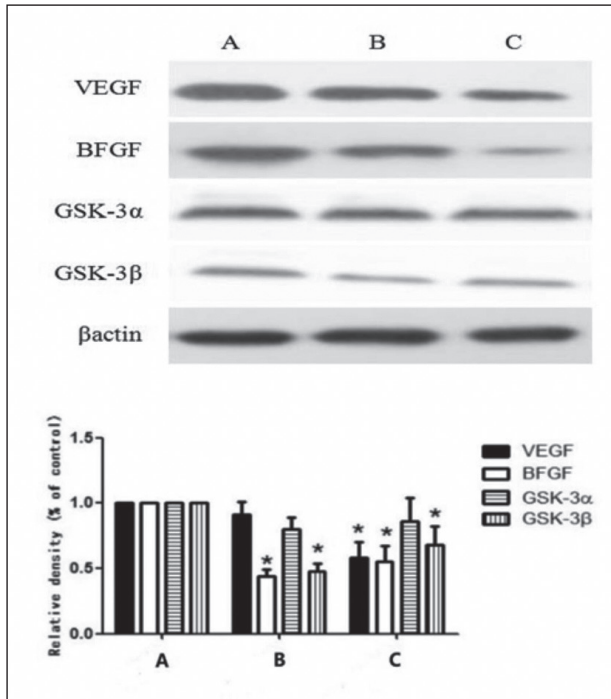


Figure 5: Protein expression levels of VEGF, BFGF, GSK-3 α , and GSK-3 β in each group.

Kaiyu Zhengyuan Powder can promote cell apoptosis (show as Figure 6).

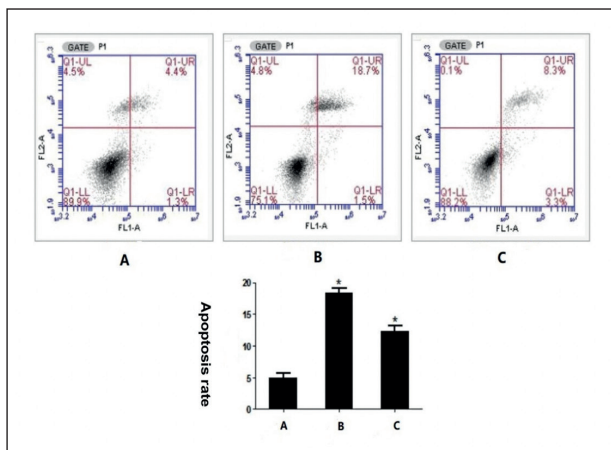


Figure 6: The level of the apoptosis rate of the cells in each group.

Discussion

PI3K/AKT is a signaling pathway closely related to proliferation and apoptosis. The activation and high expression of AKT can activate the PI3K/AKT signaling pathway, thereby further participating in the pathogenesis of thyroid cancer. Some animal experimental models and human tumor related studies have found that PI3K/AKT signaling

pathway is related to the invasion and metastasis of thyroid cancer. If AKT gene is knocked-down, it will delay and prevent the development of thyroid cancer, angiogenesis and distant metastasis. According to the epidemiology statistics, in patients with thyroid cancer, women are significantly more than men, and men's and women's estrogen level difference is an important factor of thyroid carcinoma, so suggest thyroid cancer may be associated with estrogen levels, many studies have shown that in thyroid carcinoma tissues can detect estrogen receptors, estrogen may promote the growth of cancer cells is an important matter, The enhanced 2-hydroxylation reaction during its metabolism may be related to the occurrence of thyroid cancer⁽¹¹⁾. The migration, invasion and adhesion of thyroid cancer cells are all related to the action of estrogen⁽¹²⁾.

Estrogen and its receptor have a proliferation effect on differentiated thyroid cancer. Estrogen in female body mainly includes estrone, estradiol and estriol, among which estradiol has the strongest biological activity. Animal studies have long demonstrated that estradiol significantly increases the incidence of differentiated thyroid cancer. Estrogen receptor (ER) includes two subtypes, ER α and ER β , which can exert opposite regulatory responses by specifically binding to estrogen⁽¹³⁾. The abnormal estradiol level leads to the abnormal expression of certain genes and proteins and promotes angiogenesis, which leads to the invasion and metastasis of differentiated thyroid cancer. The surrounding tissues of thyroid cancer are rich in blood vessels, which can transport thyroid cancer cells to distant target organs, which is a necessary condition for the distant metastasis of thyroid cancer.

Epithelial-to-mesenchymal transition (EMT) is a process in which polar epithelial cells transform into mesenchymal cells with migratory ability, and acquire the ability of invasion and migration. Epithelial-mesenchymal transition (EMT) is a dynamic, multistep process in which epithelial-cell interactions are lost, the tissue structure is loose, and the cuboidal epithelial cells change to a spindle-shaped fibrocytic morphology and exhibit invasiveness. The central cells of solid tumors such as papillary thyroid carcinoma are epithelial cells, and the surrounding cells are often mesenchymal cells. Their strong movement ability causes tumor cells to infiltrate locally, invade blood and lymphatic vessels, and transfer to target organs. After reaching the target organ, cancer cells can undergo mesenchymal epithelial transition (MET) to

reconstruct intercellular junctions and cytoskeleton to form metastatic foci⁽¹⁴⁾. Epithelial mesenchymal transformation is closely related to tumor metastasis, the change on the form, mainly epithelial cell polarity, gathered together, each other between adhesion molecules mediated cells maintain cell structure integrity and cell differentiation, cell can also make it difficult to from the primary tumor into the surrounding tissue and blood vessels, and in the process of transformation of epithelial mesenchymal, lost cell adhesion, motility, To get stronger ability of migration and invasion, and tumor cells after transferred to distant organs, can change again to epithelial morphology, engraftment in organs, epithelial mesenchymal transformation in the development of papillary thyroid carcinoma, can break through the basement membrane and vascular lymphatic metastasis, making papillary thyroid carcinoma metastasis to other parts, the serious influence the treatment and prognosis of papillary thyroid carcinoma⁽¹⁵⁾.

Kaiyu Zhengyuan Powder can effectively reduce ER α , GPER protein expression level in estrogen receptor pathway, increase ER β Protein expression level. It can effectively reduce the expression level of angiogenic factors VEGF and BFGF, and also down regulate GSK-3 α , GSK-3 β Protein expression level. The expression level of Twist1, HIF-1 α , PFK, PKM2 protein that closely related to epithelial mesenchymal transformation was decreased. Reduce the expression level of PI3K, AKT1, P70S6K, PDK1, cyclin D1, Bcl2, and p21 proteins, promote apoptosis, increase the expression level of Bax protein in apoptotic proteins.

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