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ORIGINAL RESEARCH ARTICLE

THE EXPRESSION OF PI3K/ AKT SIGNAL PATHWAY IN BREAST TISSUE AND IT S CLINICAL SIGNIFICANCE

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ABSTRACT

This work aimed to investigate the expression of p-AKT in breast tissue and its clinical significance. Methods: Western blot and RT-PCR were used to detect the expression of p-AKT in 100 cases of breast cancer and 30 cases of normal breast tissue. The expression levels of p-AKT in breast cancer were significant higher than that in normal breast tissue. The expressions of p-AKT had correlation with Clinical grade($P<0.05$), but it was irrelevant to the age, tumor size, lymph node metastasis and histological differentiating degree. PI3K/AKT Signal Pathway may take a part in the development and progression of breast cancer.

Key words: *Breast cancer, P-AKT, RT-PCR, Western blot.*

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INTRODUCTION

PI3K/Akt(Phosphatidylinositol3-kinase/Protein Kinase B) signal pathway plays an important role in the development process of tumor. The pathway can affect the activation status of the multiple molecular downstream, so the pathway become a research hotspot in recent years. This pathway was found excessive expressed and activated in a variety of malignant tumors, were associated with metastasis of the tumor and prognosis of patients. The function of the PI3K/Akt pathway also include regulation of the cell cycle and tumor angiogenesis, also involves tumor invasion and metastasis. Akt is an important signaling molecule of the pathway. It was a Ser/Thr protein, many ways can stimulate Akt phosphorylation and activation. We used Western blot and RT-PCR to detect the expression of p-AKT in breast cancer and normal breast tissue, and examined its significant in the early clinical diagnosis and treatment of breast cancer.

MATERIALS AND METHODS

General information One hundred cases of breast cancer at the Affiliated hospital of Hebei University between January 2010 and May 2013 were collected. The age 35 to 78 years old, and the average age was (48.35±5.14). Select 30 cases of corresponding tissue adjacent to carcinoma edge measuring≥5 cm as nomal breast tissue, The age 36 to 72 years old, the average age was (40.52±5.13). In one hundred cases of breast cancer, ≤50 years old, 42 cases, > 50 years old, 58 cases. Tumor diameter≤2cm 37 cases, >2cm 63 cases.; Histologic grade I-II,55 cases, grade III 45 cases. 60 cases of patients with positive lymph node, 40 cases of patients with negative lymph nodes. Clinical staging I-II 76 cases; grade III 24 cases. A manual immunohistochemical procedure was performed using 4μm thick tissue sections. The corresponding fresh tissue of the 100 breast cancer were carried out for Western blot and RT-PCR.

Reagent and instrument Rabbit anti p-AKT monoclonal antibody used in this study were bought from Chinese fir jinqiao biological technology co., LTD., AMV cDNA systhesis kit were bought from Takara co., primers were synthesized by Shanghai biological engineering technology service co.. Mouse anti β-actin monoclonal antibody were purchased from the United States Invitrogen company. Second antibody labeled by horseradish peroxidase were goat anti mouse polyclonal antibody, purchased from the United States K P L company. Ultraviolet spectrophotometer instrument (DU800, beckman companies in the United States), ELASA MK3(Thermo labsystems company),DYY - 6 b type voltage steady flow electrophoresis apparatus(shanghai xinran co., LTD), PTC-220-PCR amplification(Research company), 2020D digital uv fluorescence imaging (GoldSpring company)

METHOD

Western blot Breast cancer tissue and normal breast tissue were keeped in- 80^{0}C, scissors to cut up, add 400 μl single liquid detergent cracking (including PMSF) in homogenizer, 4^{0}C 12000rpm centrifugal, discard the supernatant , subpackage into Eppendorf tube, -20^{0}C. Total protein concentration was determined by BCA working liquid.It was carried out with 12% Separation gel and 5% stacking gel. Loading quantity were according to the calculated result. Load 1×loading Buffer, 80V, control the voltage to 120 v when the marker reach the Separation gel. After the electrophoresis, cut according to the purpose, 130mA, voltage steady, transferred, 2h. After TBST liquid containing 5% skimmed milk closed. each membrane respectively incubated with anti-1. PVDF membrane were washed three times, 15,10,10min, dilute second antibody at 1:5000, add second antibody and keep 1h, developing and fixing. Readed the stripes width and grey value by Image J Image .The data was analyed by statistical Microsoft.

RT-PCR analysis The amplified fragment length of P-Akt was 196bp, the Primer sequence as following, F5 ′-GTGCTGGAGGACAATGACT ACGG-3′ ,R: 5 ′-AGCAGCCCTGAAAGCAAGGA- 3′ $_0$β-actin was 250bp, the Primer sequence as following, F: 5′-CATGTACGTTGCTATCCAGGC-3′,R: 5′-CTCCTTAATGTCACGCACGAT-3′$_0$The total RNA were prepared from 100 mg of fresh tissue stored in liquid nitrogen. The purity of the RNA was detected. The results showed that, if OD260/280 between 1.8-2.0, then without RNA degradation and protein pollution. cDNA were synthesized by reverse transcription of 0.3 μg total RNA. Reaction conditions as follows: 30^{0}C,10min;42^{0}C,30 min;99^{0}C, 5min;5^{0}C,5min$_0$ And the reverse transcription reaction system was 10 μL.. P-Akt amplification reaction conditions as follows: 94^{0}C, Pre degenerated 2 min, 94^{0}C, degenerated, 30 s, 59^{0}C, annealing 30s, 72^{0}C for 1 min, 30 cycles, 10^{0}C for 10 min. 20 uL amplification products together with 6 uL DNA Ladder were used for 4% agarose gel electrophoresis (120 v, 45 min). Take photo with ZF type ultraviolet reflex analyzer. Using Quantity one 4.62 statistical software to count the integral optical density, integral optical density value ratio was used to analyze the differences between the groups.

STATISTICS ANALYSIS

SPSS18.0 statistical package were was used to analyze data. RT-PCR and Western blot results were expressed with $\bar{x}$±S,with independent sample t test (Independent sample t text), P<0.05 as statistically significant.

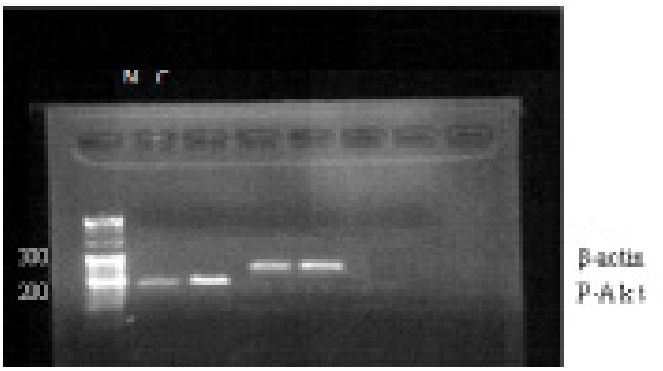
RESULTS

The expression levels of p-AKT in breast cancer tissue and normal breast tissue.

Western blot showed that, the relative expression

of P-Akt in normal breast tissue was 0.37±0.08, significantly lower than that in breast cancer tissue (0.79±0.15). RT-PCR showed that, the relative expression of P-Akt in normal breast tissue was 0.35±0.17, lower than that in breast cancer tissue(0.78±0.16), agreeing with the Western blot results. Figure1, Figure2.

Picture 1:The expression of P-Akt in breast cancer tissue and normal breast tissue. by Western-blot method



Picture 2: The expression of P-Akt in breast cancer tissue and normal breast tissue. by RT-PCR.

The relationship between P-Akt and clinicopathological characteristics.

In breast cancer, the expression of P-Akt protein and mRNA were higher in III period breast cancer, compared with I-II, with significant difference (P<0.05). P-AKT protein and mRNA were higher in lymph node metastasis and high grade group, but irrelevant to age and tumor diameter. Table 1.

Table 1: The relationship between P-Akt and clinicopathological characteristics.

Grp.	N	mRNA			Protein		
		$\bar{x}\pm S$	t	p	$\bar{x}\pm S$	t	p
age							
≤50	42	0.781±0.051	1.025	.12	0.754±0.025	0.651	.361
>50	58	0.774±0.063			0.793±0.018		
tumor diameter (cm)							
≤2	37	0.745±0.024	0.610	.16	0.761±0.034	0.083	.584
>2	63	0.793±0.015			0.794±0.020		
histological differentiating degree							
I-II	55	0.771±0.041	1.667	.235	0.778±0.015	1.493	.963
III	45	0.786±0.059			0.783±0.028		
lymph node							
-VE	40	0.727±0.057	3.810	.146	0.761±0.021	3.863	.584
+VE	60	0.799±0.013			0.786±0.014		
Clinical grade							
I-II	76	0.663±0.050	4.252	.001	0.519±0.013	3.146	.041
III	24	0.914±0.012			0.788±0.018		

DISCUSS

PI3K/Aktsignalingpathways,B(Phosphatidylinositol 3-kinase /Protein Kinase B), were researched mostly in recent years, which played an important role in the the occurrence and development of tumor. PI3K (phosphatidylinositol 3-kinase,PI3K) is an important signaling molecular in the signaling pathways, can be activated by a variety of ways. [1,2] The intracellular signaling proteins can be further activated by the activation of PI3K, whit PIP2,PIP3 as second messenger, finally activate Akt by phosphorylation of Thr308 and Ser473. PKB (Protein Kinase B, PKB), also known as Akt, homologous with v-Akt. Akt belongs to a Ser/Thr Protein, is also an important Protein Kinase in the PI3K/Akt signal transduction pathway. Its activation is regulated by the many factors, including: hormones, growth factors and cytokines, etc. [3] Activation of Akt can further active the downstream molecules involved in cell proliferation, cell differentiation and inflammatory process.[4] The expression of any signal molecule will

cause tumor cell apoptosis, proliferation and invasion of abnormal.

The expression of PI3K/Akt signal pathway has changed in most malignant tumor. Akt express high levels in lung cancer and related with differentiation, lymph node metastasis and TNM stages. [5] Zhang, etc.[6] detected 11 cases of normal intestinal tissue and 53 cases of intestinal carcinoma tissue by immunohistochemical and fluorescence quantitative, found that, PI3K/Akt signal pathway were high activated in adenocarcinoma, prompting PI3K/Akt signaling pathway can be used as a clinical potential therapeutic targets. Peng[7] detected the PI3K/Akt signaling pathway in breast cancer , normal tissue , and breast fibroadenoma, coming to an conclusion, PI3K/Akt signaling pathway is probably one of the factors that lead to occurrence and development of breast cancer, and play a role in the invasion and distant metastasis of breast cancer cells. Akt played an important role in the process of malignant tumor. After Akt activation, can activate or suppress the downstream target protein, including Bad, caspase-9 and Forkhead etc.[8,9] Bad belongs to the BCL-2 family, which can promote apoptosis. Bad can promote apoptosis by combining into complex with BCL- XL. Activated Akt can block the formation of the complex by Bad phosphorylation. [10] After the activation of caspase-9 by Akt, it can directly inhibit cytochrome C inducing caspase activation. Ser183 and Ser196 were two Akt phosphorylation sites on caspase- 9, and Ser196 is relatively important.After the phosphorylation of caspase-9 by Akt, the protease activity of AKT reduced, resulting in a decline in its effect on promoting apoptosis. Forkhead family factor can induce the expression of many apoptosis. Akt can promote the phosphorylation of Forkhead family, and play a role of resistance to apoptosis.by transcriptional regulation.

Western blot showed that, the relative expression of P-Akt in normal breast tissue was lower than that in breast cancer tissue. RT-PCR showed that, the relative expression of P-Akt mRNA in normal breast tissue was lower than that in breast cancer tissue,too, according with the result above. In breast cancer, the expression of P-Akt protein and mRNA were higher in III period breast cancer, compared with I-II, with significant difference($P<0.05$), P-AKT protein and mRNA were higher in lymph node metastasis and high grade group, but irrelevant to age, tumor diameter. The expression of P-Akt mRNA and protein had correlation with breast cancer. PI3K/Akt signaling pathways involved in the development of breast cancer, and plays an important role in the process of cancer.

Through the above experiments, we confirmed the up-regulated expression of P-Akt. In the next stage experiment, we will use more experimental methods, increase experimental cases, to explore significance of P- P-Akt in breast cancer, to provide a new direction for the diagnosis and treatment of breast cancer.

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