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Original Article

Increased expression of FAS is a prognostic marker for patients with breast cancer

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Abstract: Purpose: Understanding the difference of expression of FAS between breast cancer and benign lesion tissues of breast and discuss the expression of FAS in tissues of breast cancer and its clinical value. Methods: FAS mRNA and protein expression were determined by quantitative real-time reverse transcriptase-polymerase chain reaction and Western blotting in 20 pairs of fresh frozen breast cancer tissues, corresponding noncancerous tissues and normal tissues. Additionally, FAS expression was analyzed by immunohistochemistry in 108 cases of clinicopathologically characterized breast cancer patients. The correlation of FAS expression with patients' survival rate was assessed by Kaplan-Meier. Results: The result shows that expression of FAS in mRNA and protein in the breast benign lesion is low level while in the noncancerous tissues is high, but in the tissues of breast cancer is revealed as excessive expression. FAS expression positive rate and staining intensity within the cancer tissues is higher than that of breast lesions (P<0.01). The high expression of FAS is significantly correlated with the diameter of tumor of the breast cancer (P=0.014) and transferring quantity of the surrounding lymph node (P=0.047). There is no statistical correlation found between FAS and ages of patients, menopause or not, tumor tissue ER, PR, c-erbB-2 (P>0.05). Kaplan-Meier survival analysis showed that a high expression level of FAS resulted in a significantly poor prognosis of breast cancer patients. Conclusion: In conclusion, overexpression of FAS is closely related to progression of breast cancer and might be regarded as an independent predictor of poor prognosis for breast cancer.

Keywords: Fatty acid synthetase, clinical significance, breast cancer

Introduction

Some proteins were found in the 1980s that can react with anti-haptoglobin antibody and they were proved to be Fatty Acid Synthase (FAS) later. The molecular weight of FAS is 500 kD. It has 7 kinds of enzymatic activity including condensation, transaldolase, restoration and dehydration. It is a kind of multifunctional enzyme. Apoenzyme is located within cell cytoplasm. It can adjust the level that cells use metabolic product of carbodydrate to synthesize fatty acid and directly affect the speed of synthesis of endogenous fatty acid [1, 2]. Enzymatic activity is NADPH dependent and it synthesizes palmitic acid, stearic acid and myristic acid by adding Acetyl CoA sucessively. Plamitic acid accounts for 80% and the latter two ones account for 10% respectively [3]. FAS is also regulated by the various kinds of factors and closely related to the internal energy material, especially state of fat metabolism. When the lipid diet is ingested, the exogenous fatty acid can restrain this enzymatic activity. Insulin, glucagon, glucocorticoid, thyroid hormones and sex hormone participate into composition and decomposition of the energy material of the body and can also adjust FAS activity [4].

FAS exist in normal cells and tumor cells. Exogenous fatty acid can completely satisfy the requirement of most normal tissues to energy supply of lipid and structured lipid. Therefore, the synthesis of endogenous fatty acid is weak and FAS lies in depressed state and the expression level is low. However, the expression of FAS in some malignant tumor is excessive, including breast cancer and the expression is obviously higher than normal tissue [5]. It means that the synthesis of endogenous fatty acid is active. Many clinical researches show that FAS is one of important prognosis factors for breast can-

Table 1. The correlation of FAS and Clinicopathological variable

Clinicopathological variable	*n*	FAS		*P* value
		+	-	
Age (yr)				
<35	23	15	8	
35-60	59	47	12	0.813
>60	26	14	12	
Tumor diameter (cm)				
≤5	89	53	36	0.021
>5	19	12	7	
TNM stage				
I+II	79	56	23	0.604
III+IV	29	17	12	
Lymph node involvement				
0	41	27	14	0.018
≥1	67	49	18	

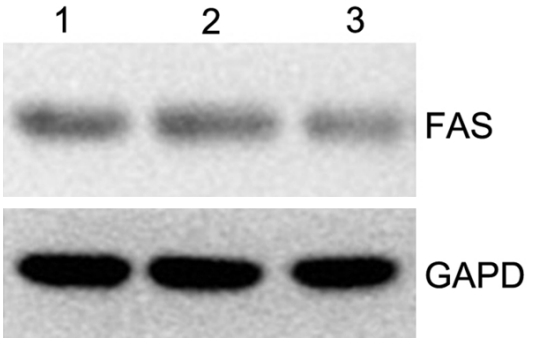

Figure 1. Western blotting results. 1: cancer tissues; 2: para-carcinoma tissues; 3: normal tissues.

cer. If FAS expresses excessively, the risk of recurrence of tumor will rise. The higher the extent of expression is, the bigger the risk of recurrence [6]. Thus, the rate of death for patients will be increased. FAS can be treated as one of prognosis indexes to predict breast cancer. To explore the vital role of FAS in the tumorigenesis and progression of breast cancer, we examined expression patterns of FAS in breast cancer tissues and analyzed the relationship between FAS expression and clinicopathological factors of breast cancer.

Materials and methods

Specimens

The patients and tissues specimens used to do PCR and WB is from Nanxishan Hospital of Guangxi Zhuang Autonomous Region. Fresh frozen tissues include breast cancer, para-carcinoma tissue and normal breast tissues from breast cancer patients. QPCR is used to analyze the expression difference of breast cancer, para-carcinoma tissue and normal breast cancer tissues.

Quantitative real-time reverse transcription polymerase chain reaction assay

Total RNA was extracted and purified from 20 pairs of fresh frozen breast cancer tissues, corresponding noncancerous tissues and normal tissues.

Total RNA extraction, quality control, and one-step qRT-PCR were performed as previously reported. FAS-specific oligonucleotide primers (forward, 5'-CCTTCCCATCCTCCTGACCA-3'; reverse, 5'-TCGTAAACCGCTTCCCTCACT-3') were designed to yield a 92-bp PCR product. The data were normalized using β-Actin (ACTB) as a reference gene (forward primer, 5'-ACTGGGACGACATGGAGAAAATC-3'; reverse primer, 5'-CTCGCGGTTGGCCTTGG-3').

Western blotting analysis

The breast cancer samples, including para-carcinoma tissues and normal tissues, were lysed in lysis buffer, and the lysates were harvested by centrifugation (12,000 rpm) at 4°C for 5 min. Protein samples of approximately 12 μg were then separated by electrophoresis in a 10% sodium dodecyl sulfate polyacrylamide gel and were transferred onto a PVDF membrane. After blocking the nonspecific binding sites for 60 min with 5% nonfat milk, the membranes were incubated overnight at 4°C with a mouse monoclonal antibody against FAS (CST, USA; at a 1:1,000 dilution). The membranes were then washed three times with Tris-buffered saline with Tween-20 (TBST) for 10 min and were probed with the horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibody (ZSGB-BIO, China, at a 1:5,000 dilution) at room temperature for 1 h. After three washes with TBST, the membranes were developed using an enhanced chemiluminescence system (Applygen Technologies Inc, China). The FAS protein level was normalized to the level of GAPDH detected using goat anti-mouse GAPDH monoclonal antibody (ZSGB-BIO, China, at a 1:5,000 dilution).

Immunohistochemical staining

Paraffin-embedded sections (4-μm thick) were deparaffinized, rehydrated, and heated for 10

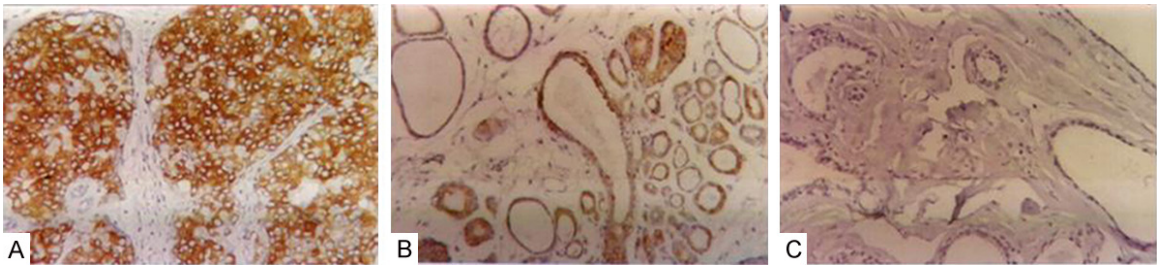

Figure 2. Immunohistochemical results. A: FAS expression in breast cancer tissues 200 ×; B: FAS Expression in the para-carcinoma tissues 200 ×; C: FAS Expression in normal tissues 200 ×.

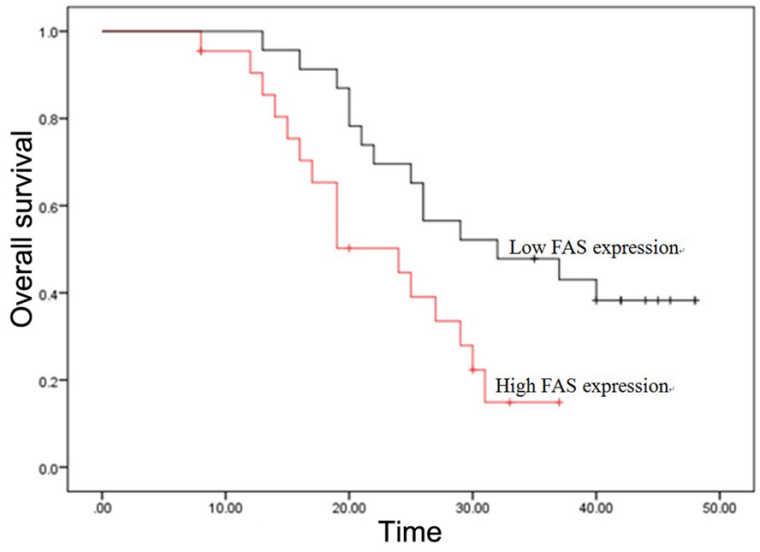

Figure 3. Kaplan-Meier survival analysis.

min in 10 mM of sodium citrate (pH 6.0) to retrieve antigen. Endogenous peroxidase was quenched with 3% hydrogen peroxide. Sections were incubated with anti-FAS (CST; 200) for 1 h, followed by HRP-conjugated secondary antibodies. The sections were developed in diaminobenzidine and counterstained with hematoxylin. Negative controls were included by omitting the primary antibody. The stained sections were independently assessed by two pathologists without prior knowledge of the clinical data. Five random fields of view at × 200 were examined for each section. A modified immunoreactivity score method to evaluate the immunostaining results was performed by multiplying stain intensity by stain area (staining index (SI)). Stain intensity is as follows: no staining (score 0), weak staining (score 1), moderate staining (score 2), or strong staining (score 3). Staining area is as follows: <25% (score 1), 25-50% (score 2), 50-75% (score 3), or more than 75% (score 4) of tumor cells. The expression levels of FAS were determined by the SI, which scores as 0, 1, 2, 3, 4, 6, 8, 9, and 12. An optimal cutoff value was identified as follows: the SI score of 4 or greater was used to define tumors as high FAS expression and the SI score of 3 or less as low FAS expression.

Statistical analysis

Statistical analysis was performed using SPSS 19.0 software (Guilin Medical University, China). The χ^2 test was used to assess the relationship between FAS expression levels and clinicopathological characteristics of GC. Survival curves were drawn according to the Kaplan-Meier method, and the log-rank test was applied to compare the survival curves. ROC curve were also drawn. Differences were considered significant at the $P<0.05$ level.

Results

As shown in **Table 1**, the high expression of FAS is significantly correlated with the diameter of tumor of the breast cancer (P=0.014) and transferring quantity of the surrounding lymph node (P=0.047). There is no statistical correlation found between FAS and ages of patients, menopause or not, tumor tissue ER, PR, c-erbB-2 (P>0.05).

The RT-PCR results showed that expression levels of FAS in the breast benign lesion was lower than that of noncancerous tissues, while it was

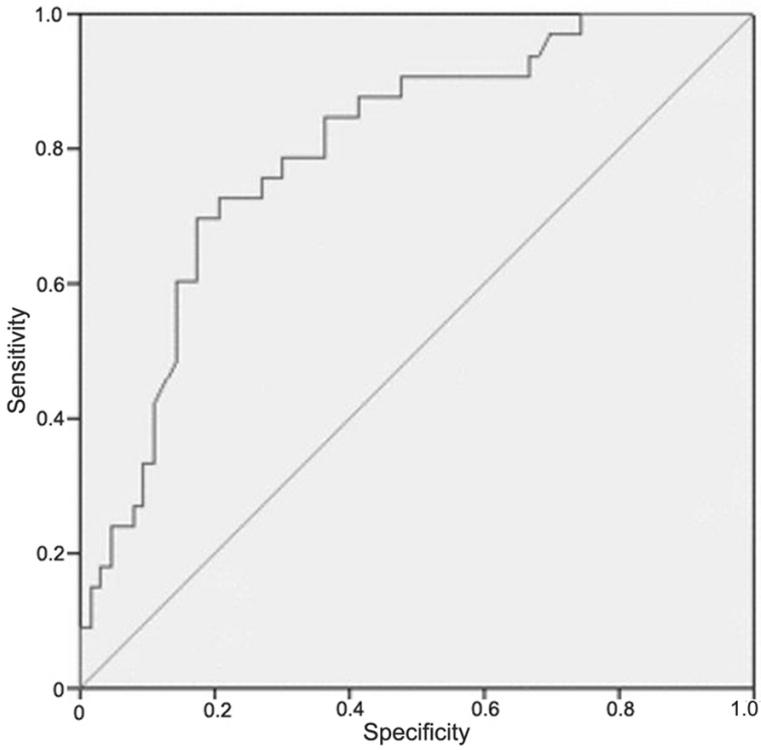

Figure 4. ROC analysis.

the highest in the tissues of breast cancer. The western blotting results were consistant with that of RT-PCR results, it was shown in **Figure 1**. Immunohistochemical results were shown in **Figure 2**. FAS expression positive rate and staining intensity within the cancer tissues is higher than breast lesions (P<0.01). Kaplan-Meier survival analysis was shown in **Figure 3**, we can find that a high expression level of FAS resulted in a significantly poor prognosis of breast cancer patients. ROC analysis was shown in **Figure 4**.

Discussion

FAS is the key enzyme of fatty acid synthesis within cells. Due to down regulation function of the diet lipid, the expression of FAS within most of tissues of human body lies in low level. Excessive FAS expressions in some cancers have been found, such as breast cancer, prostate cancer, endometrial cancer and colorectal cancer [7-9]. Recent research shows that the rising of FAS expression level within the tissues of breast cancer is related to the bad prognosis of tumor [10]. It indicates that the expression level of FAS is possibly relevant to the degree of malignancy of tumor.

Fibroadenoma and cyclomastopathy selected in this research is the most common benign lesion of breast. FAS expression in most tumor tissues is negative. Even if it is the positive dyeing, the positive cells account for a small proportion in all the tumor cells (<25%). According to the views of Rashid and Hennigar, FAS immunity expression is in line with the level of synthesis of endogenous fatty acid synthesis and it can reflect the activity of enzyme [11]. The synthesis of endogenous fatty acid in these benign lesion tissues of breast is not active and thus the activity of FAS is not high as well. Though this research does not get the normal breast tissues specimen as comparison group, FAS dyeing of the normal breast tissues beside the all the tumors is negative. Thus, it indicates that FAS in most of normal breast tissues is possibly negative expression. In conclusion, the FAS expression in the benign lesion of breast is low level and the negative expression is the overall characteristic.

Additionally, in the specimen of positive expression, it is found that cells of glandular epithelium have different degree of hyperplasia phenomenon. Though not all the hyperplasia parts FAS dyeing is positive, it still indicates that the hyperplasia of gland cells have certain relation with the FAS positive. Previous researches argue that in contrast with the normal body tissue, the FAS expression in breast cancer, colorectal cancer, ovarian cancer and endometrial cancer is extremely high [12]. This research selects pathological specimen at random, observes FAS dyeing of tissues with blindness method. The statistical analysis got the similar results. In the tissues of breast cancer, there is nearly 3/4 mass with FAS in it shows to be positive. And dyeing cases of (++)-(++++) emerge. The positive rate of FAS and degree of positive dyeing is obviously higher than the group of breast cancer tissues.

The expression level of FAS is possibly relevant to the degree of malignancy of tumor. There are

only a few clinical research statistics in this regard in China. Therefore, to understand FAS deeply has clinical significance to occurrence and development of the malignant tumor and launching of new therapeutic approach.

Disclosure of conflict of interest

None.

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