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Expression of FOXA1 in breast cancer and the significance

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Summary

Objective: The aim of this study to examine the expression of FOXA1, ER, and PR in breast cancer and investigate the association between FOXA1 expression and clinical parameters. *Materials and Methods:* One hundred one cases with primary breast cancer were collected and immunohistochemistry was employed to examine the expression of FOXA1, ER, and PR. The correlation of FOXA1 and ER, PR expression with menopausal status, tumor size, clinical stage, histological grade, recurrence, and clinical index relationship was further analyzed. *Results:* The positive expression of FOXA1 was 67.3%. The positive expression rate of ER in positive FOXA1 group was 66.2% (45/68). The positive expression rate of PR in positive FOXA group was 57.4% (39/68). There was no correlation between FOXA1 expression and menopausal status. Analysis of the relationship between FOXA1 and tumor diameter showed that the percentage of tumor diameter ≤ 2 cm, 2-5 cm, and > 5cm was 52.9%, 32.4%, and 14.7% in positive FOXA1 group. The correlation with clinical staging showed that the percentage of Stages I, II, and III was 33.8%, 41.2% and 25%, respectively, in positive FOXA1 group. Analysis of histological grade showed that the percentage of grades 1, 2, and 3 was 48.5%, 20.6%, and 23.5%, respectively, in positive FOXA1 group. The positive expression rate of P53 was 30.9%, the recurrence rate was 14.7%, and the positive expression of FOXA1 had better DFS.

Key words: Breast cancer; FOXA1; ER; PR; Immunohistochemistry.

Introduction

Breast cancer is one of the most common cancers in female. It accounts for 29% new cancer cases and 14% cancer deaths in women [1, 2]. Although the breast surgery remains the original and most effective therapy [3], the clinical trials showed that the adjuvant therapy is also necessary for these patients. Endocrine targeted therapy is widely used in clinical practice.

For the treatment and prognosis of breast cancer, several maker molecules, such as ER, PR, and HER2, along with lymph node metastases and histological grade, were used to manage the breast cancers [4-6]. Among them, ER positive is the basis of endocrine therapy. Studies showed that 70% cases are ER positive, suggesting that they are candidates for endocrine therapy. However, some ER+ cancers have primary resistance or develop resistance to endocrine therapy during treatment, which greatly limits the effectiveness of endocrine therapy [7].

FOXA1 was first discovered in liver development [8]. The subsequent studies showed that it was involved in a variety of tumors, such as mammary gland and prostate [9]. As a transcriptional activator, FOXA1 controls nearly 50% of ER target genes through facilitating binding of ERs to gene promoters. It was believed as a significant maker for good prognosis [10]. A meta-analysis also showed that FOXA1 expression was related to the ER α status, PR status, lymph node metastasis, and to the histological grade in breast cancer [6].

In this study, the authors will examine the expression of FOXA1, ER, and PR in breast cancer and investigate the association between their expression and clinical parameters, and estimate the value of FOXA1 in treatment and prognosis of breast cancers.

Materials and Methods

One hundred one specimens of breast lesions after breast cancer radical surgery were obtained from the First Affiliated Hospital of Jinan university from October 2009 to October 2011.The samples were all invasive breast cancer, including 62 invasive breast cancers, 15 special type breast cancers, and 24 mixed types of breast cancer. Average age of the patients was 48.9 ± 10.53 years-old. Forty-five cases were postmenopausal and 21 cases suffered from tumor recurrence.

The tissues were fixed in 10% paraformaldehyde and embedded in paraffin. Four-micrometer sections were cut. Two-step envision immunohistochemistry kit was used to examine the expression of FOXA1, ER, and PR. FOXA1 antibody was ordered and used at 1/200 dilution. ER and PR antibody was purchased and used at 1/50 dilution.

According to the percentage of positive FOXA1, the specimens were scored 1 (0-10%), 2 (10-20%), 3 (20-30%), 4 (30-40%), 5 (40-50%), 6 (50-60%), 7(60-70%), 8 (70-80%), 9 (80-90%), and 10 (90-100%). The positive expression intensity was divided into grade I (yellow), grade II (claybank), and grade III (brown). The final score was equal to the product of the above two items. If the score was 0-3, the sample was considered negative and score (4-30) as positive [11]. For ER and PR, more than 10% positive cells was believed as positive.

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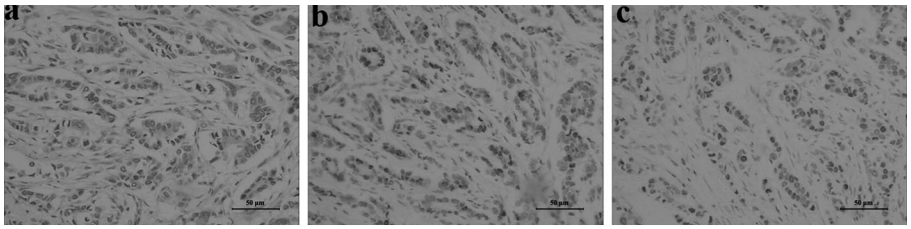

Figure 1. — The expression of FOXA1, ER, and PR; a) shows the expression of FOXA1, b) shows the expression of ER, and c) shows the expression of PR.

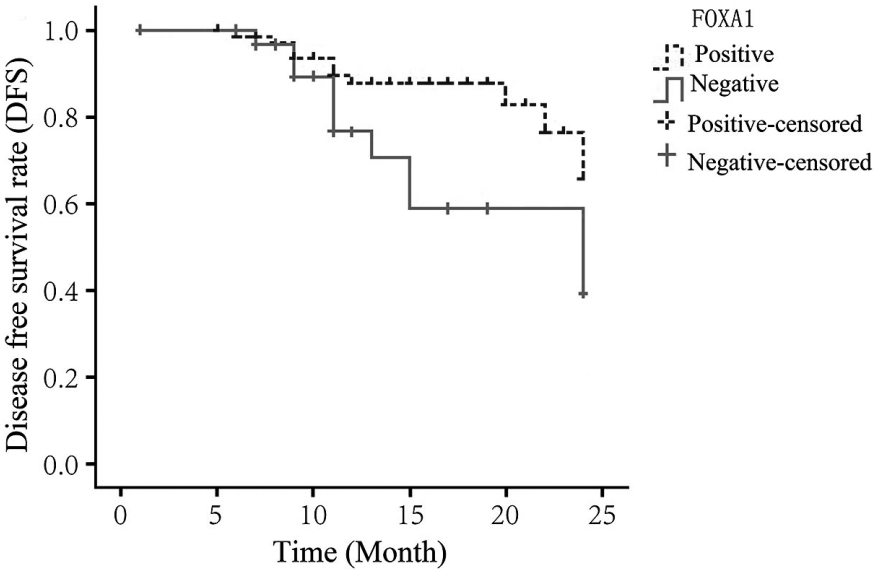

Figure 2. — DFS analysis.

SPSS 13.0 was used to assess the statistical differences. Chi-square test was used to analyze the difference between groups. Kaplan-Meier was used to analyze the DFS.

Results

FOXA1 was mainly expressed in the nucleus of breast cancer cells and also identified in cytoplasm (Figure 1-a). ER and PR was stained in the nucleus of breast cancers cells (Figures 1-a and 1-c). The positive expression of FOXA1 was 67.3% (68/101). The positive expression rate of ER in the positive FOXA1 group was 66.2% (45/68). The positive expression rate of PR in positive FOXA group was 57.4% (39/68).

There was no correlation between FOXA1 expression and menopausal status. Analysis of the relationship between FOXA1 and tumor diameter showed that the percentage of tumor diameter ≤ 2 cm, 2-5 cm, and > 5 cm was 52.9% (36/68), 32.4% (22/68), and 14.7% (10/68) in positive FOXA1 group. The correlation with clinical staging showed that the percentage of Stages I, II, and III was 33.8% (23/68), 41.2% (28/68), and 25% (17/68), respectively, in positive FOXA1 group. Analysis of histological grade showed that the percentage of grades 1, 2, and 3 was 48.5% (33/68), 20.6% (19/68), and 23.5% (16/68), respectively, in positive FOXA1 group. The positive expression rate of p53 in positive FOXA1 group was 30.9% (21/68) and the recurrence rate was 14.7% (10/68) (Table 1).

Two years after surgery, the disease-free survival (DFS) in positive FOXA1 group was 66.7%, which was higher than negative FOXA1 group ($p = 0.041$) (Figure 2).

Discussion

In clinical practice, ER, PR, HER2, and other markers were widely used to manage the patients with breast cancer [4-6]. However, the accuracy of these markers to decide treatment regimen and assess prognosis is still limited. Studies showed that FOXA1 was involved in a variety of tumors and was identified as a significant maker for good prognosis [10]. In this study, the authors found that the positive expression of FOXA1 was related to the positive expression of ER and PR. Patients with positive expression of FOXA1 had better DFS.

Forkhead box (Fox) proteins are evolutionarily conserved transcription factors. So far, 17 members of FOX family were cloned, from FOXA to FOXR. They function in diverse aspects, including growth, proliferation, differentiation, apoptosis, invasion, and metastasis. The abnormal expression or mutation of FOX proteins are related to various diseases [12]. The tumor related FOX proteins include FOXO, FOXM, FOXP, FOXC, and FOXA [13]. In breast

Table 1. — *The correlation between FOXA1 expression and clinical parameters.*

	Group	FOXA1 (number)		X^2	*p* value
		Negative	Positive		
Menstruation	Non-menopause	16	40	4.645	0.098
	Menopause	17	28		
Tumor diameter	≤ 2cm	3	36	20.591	0.000
	2-5cm	14	22		
	≥ 5cm	16	10		
Clinical Stage	I	2	23	9.340	0.009
	II	18	28		
	III	13	17		
Histological Grade	1	6	33	8.673	0.013
	2	14	19		
	3	13	16		
ER	-	28	23	23.14	0.000
	+	5	45		
PR	-	30	29	21.30	0.000
	+	3	39		
p53	-	6	47	23.114	0.001
	+	27	21		
Recurrence	No	22	58	4.681	0.030
	Yes	11	10		

cancer, the positive expression rate of FOXA1 is high. Studies showed that FOXA1 function through binding to chromatinized DNA and open chromatin and enhance the combination of ER to the promoter of target genes [14-16]. Half of target genes of estrogen has the binding site of FOXA1 and co-expression of ER-α, and FOXA1 can optimize the function of estrogen. GATA4 plays important role in interaction between ER-α and FOXA1 [17]. PR was also reported to relate to ER-α through increasing the interaction between ER-α and growth factor signaling. Studies showed that ER-positive/FOXA1-negative/PR-negative breast cancers respond less well to selective ER modulator therapy than ER-positive/FOXA1 positive/PR-positive tumors. It is possible that PR increases the function of signalling pathway or independent hormonal growth characteristics [18]. FOXA1 also can activate the growth factor to increase the interaction between hormones and sensitivity [19]. Hence FOXA1 can be used as an independent index or as an associated indicator with ER, PR, growth factor signaling pathway, and GATA pathway. In the present study, there was a positive correlation between FOXA1 and ER and PR.

The 2011 St. Gallen Consensus Conference recommended that the luminal A subtype breast cancer ER+, PR+, Her-2-, and Ki57 < 14% should receive endocrine therapy. If the tumor is large, the new adjuvant endocrine therapy can be carried out [20]. However, clinical studies showed that 30-40% hormone receptor-positive breast cancer were resistant to agents, especially tamoxifen resistance in the treatment of hormone-dependent breast cancers. Studies showed that mutations in the ER at a low frequency do not account for tamoxifen-resistant breast tumors [21]. Caryn *et al.* reported that drug-resistant cancers still recruited ER to the chromatin, but the sensitivity and specificity between hormone and hormone ER was lacking. They believed it was due to the FOXA1-mediated reprogramming of ER binding on a rapid timescale [22]. Basal like breast cancer (BLBC) refer to the subtype that both hormone receptor and Her-2 are negative, which accounts for 15% of breast cancers. BLBC lacks on the basis of endocrine therapy due to negative hormone receptor; single treatment is difficult to obtain a therapeutic effect and the patients have high risk of recurrence. However, some studies showed that 5-45% BLBC expressed ER and 15% BLBC expressed Her-2 [23]. In fact, the examination of FOXA1 in BLBC will be helpful to provide more basis for endocrine therapy. FOXA1 can act synergistically with ER and PR and increase the effectiveness between hormone and receptor. Hence FOXA1 can work as a marker for sensibility to endocrine therapy in breast cancer.

Fisher *et al.* studied 1,157 cases with node negative breast cancer and found that patients with ER and/or PR have great DFS, DDFS, and S [24]. Badve *et al.* reported that FOXA1 expression correlates with luminal subtype A breast cancer and it is a significant predictor of cancer-specific survival in patients with ER-positive tumors, which was consistent with the present study [11].

In summary, in this study the authors found that FOXA1 was expressed in breast cancer and has a positive correlation with ER and PR. Breast cancer with positive FOXA1 has the following characteristics: clinical early stage, small diameter, histologically well-differentiated, and low recurrence. FOXA1 can be used as an indicator for endocrine therapy in breast cancer.

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