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Correlation between p53 and epidermal growth factor receptor expression in breast cancer classification

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ABSTRACT. This study aimed to explore new opportunities for developing targeted therapy for triple-negative breast cancer (TNBC) by analyzing the significance and association between p53 and epidermal growth factor receptor (EGFR) expression in different molecular subtypes of breast cancer. The clinical and pathological data of 264 patients with breast cancer receiving surgery in our hospital from January 2012 to August 2013 were retrospectively analyzed. According to the expression of estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), Ki-67, CK5/6, p53, and EGFR detected by immunohistochemical methods, breast cancer was divided into four molecular subtypes. Then, the expression of p53 and EGFR as well as their correlation in the different subtypes were determined. Among the four subtypes, luminal B breast cancer was the most common type. TNBC and HER2-enriched breast cancer had larger tumor sizes with higher expression of Ki-67 as compared with the luminal types. TNBC had a lower lymph node metastasis rate but

higher CK5/6 and EGFR expression than the other three types. The expression of p53 was higher in luminal B, HER2-enriched, and triple-negative breast cancers, and this was positively correlated with the expression of EGFR in TNBC but not in the other subtypes. p53 and EGFR expression was positively correlated in TNBC, which enables us to explore the molecular biological characteristics of TNBC, so as to provide new ideas for the treatment of TNBC.

Key words: Breast neoplasm; Molecular subtypes; p53; EGFR; Immunohistochemistry

INTRODUCTION

Breast cancer is the most common malignancy in women, as well as the main reason for female cancer-related deaths. With the advances in molecular biological research, a more in-depth understanding of breast cancer has been developed. Nowadays, breast cancer can be generally classified into luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and triple negative breast cancer (TNBC) based on the presence or absence of the estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 (Ihemelandu et al., 2007; Muñoz et al., 2009; Wiechmann et al., 2009; Park et al., 2012). Breast cancer is a heterogeneous disease consisting of different molecular subtypes with different molecular biological characteristics and clinical behaviors. Sometimes, different molecular biological characteristics and different outcomes can even be observed in one molecular subtype of breast cancer (Raica et al., 2009). Luminal A breast cancer tends to have a better outcome, while TNBC has a strong aggressiveness, fast disease progression, easier metastasis and recurrence, poor prognosis, and other negative clinical features (Carey et al., 2006; Lin et al., 2008; Dawood et al., 2009). To date, there is no effective targeted therapy for TNBC. Because these tumors are not sensitive to endocrine therapy, chemotherapy is the main treatment for TNBC. Therefore, it is essential to determine additional reliable molecular markers to further characterize the molecular biological features of breast cancer, so as to better determine patient prognosis and guide their individual treatment.

p53 is a tumor suppressor gene that can induce apoptosis after DNA damage (Sjögren et al., 1996). p53 mutations play an important role in tumorigenesis. It was reported that p53 is not highly expressed in breast cancer (Sjögren et al., 1996), but it has been found to be highly expressed in TNBC (Grob et al., 2012). Epidermal growth factor receptor (EGFR) can promote tumor cell migration and increase the invasiveness of tumor cells. The EGFR positivity rate is about 57% in TNBC but only 8% in non-TNBC (Sparano et al., 2009). Studies have shown that mutations in the *p53* gene can lead to loss of function of the *p63* gene and thereby cause *EGFR* gene amplification. This ultimately promotes the formation of TNBC (Shapira et al., 2013). Both p53 and EGFR may be involved in the formation of TNBC, resulting in decreased effectiveness for breast cancer treatments that target EGFR alone (Masuda et al., 2012). Recent studies have shown that EGFR inhibitor has a lethal effect on lung cancer cells expressing wild-type p53 and mutated EGFR, but it is tolerated by the lung cancer cells expressing both p53 and EGFR mutations (Huang et al., 2011), indicating that there may be some association between p53 and EGFR expression in TNBC, which may assist in the deter-

mination of therapeutic targets for TNBC. In this study, we retrospectively analyzed the clinical and pathological data of 264 patients with operable breast cancer and their immunohistochemical results, so as to explore the correlation between p53 and EGFR expression in the different molecular subtypes of breast cancer. Our results would provide further understanding of the molecular biological behavior of breast cancer and broaden the treatment strategies for breast cancer, particularly for TNBC.

SUBJECTS AND METHODS

Clinical data of the subjects

We retrospectively analyzed the clinical and pathological data of 264 patients with invasive breast cancer collected from the Pathology Department of Shandong Cancer Hospital. All patients underwent surgery at the Breast Disease Center of Shandong Cancer Hospital between January 2012 and August 2013. Patients receiving neoadjuvant therapy or having unknown T stages incised at a different hospital prior to surgery were excluded from the study. All of the pathological and immunohistochemical slices were reviewed by two experienced pathologists. The immunohistochemical indicators included ER, PR, HER2, CK5/6, Ki-67, p53, and EGFR. Clinical data such as age of onset, age of menarche, menopausal status, tumor size, lymph node metastasis, pathological staging, and other indicators were also reviewed. The breast cancer was then classified based on the cancer staging of American Joint Committee on Cancer (AJCC, 7th edition, 2010). This study was approved by the Ethics Committee of Shandong Cancer Hospital. Clinical data involved in this study were used with the consent of the patients themselves.

Interpretation of immunohistochemical results and the defined criteria for molecular typing

The streptavidin-peroxidase immunohistochemical method was used in this study. The DAB kit was from Fuzhou Maxim Biotechnology Co., Ltd. (Fujian, China). The primary antibodies used in this study included ER and PR (Beijing ZSGB Biotechnology Co., Ltd., Beijing, China), HER2, p53, and EGFR (Dako, Carpinteria, CA, USA), as well as CK5/6 and Ki-67 (Maxim, Fujian, China). Tumor cells with nuclei positively stained by ER, PR, p53, and Ki-67 were interpreted as immunopositive, while the positive expression for HER2, EGFR, and CK5/6 was observed in the cellular membrane or cytoplasm (Figure 1). The immunopositive staining of the cells was determined according to their proportion among the total cells. ER- or PR-positive tumors were determined by at least 1% of nuclei positively stained (Hammond et al., 2010), while 20% of nuclei positively stained by Ki-67 could be regarded as high expression (Goldhirsch et al., 2013). According to the recommendations from the American Society of Clinical Oncology and College of American Pathologists, HER2 expression can be classified as HER2-positive (score 3+), suspected HER2-positive (score 2+) and HER2-negative (score 0 or 1+). For those with suspected HER2-positive tumors, fluorescence *in situ* hybridization should be used to confirm its expression. For p53, EGFR, and CK5/6, at least 10% of cells positively stained were considered as positive. According to the Expert Consensus of the 2013 St. Gallen International Breast Cancer Conference (Goldhirsch et al., 2013), breast cancer can be divided into four subtypes on the basis of their molecular markers (Table 1).

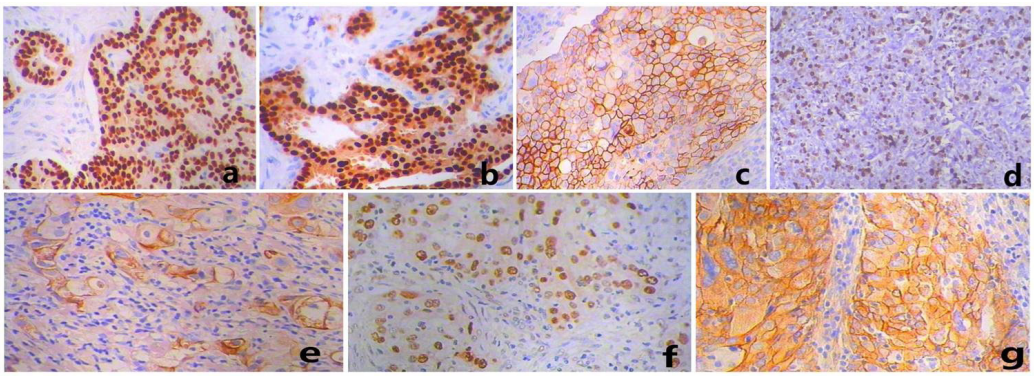

Figure 1. Expression of ER, PR, HER2, p53, EGFR, CK5/6, and Ki-67 in invasive breast cancer determined by immunohistochemistry (SP, 200X). **a.** ER-positive (70%); **b.** PR-positive (60%); **c.** HER2-positive (3+); **d.** Ki-67-positive (50%); **e.** CK5/6-positive; **f.** p53-positive; and **g.** EGFR-positive.

Table 1. Breast cancer typing criteria.

Subtypes	ER	HER2	PR	Ki-67
Luminal A	Positive	Negative	≥20%[a]	Low expression
Luminal B				
HER2-	Positive	Negative	<20%[a] or high Ki-67 expression	
HER2+	Positive	Positive		
HER2 overexpression	Negative	Positive	Negative	
TNBC	Negative	Negative	Negative	

[a]Prat et al. (2013).

Statistical analysis

Data were analyzed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Owing to the non-normal distribution of our enumeration data, the chi-square test was used to compare the differences in clinicopathological features between the different molecular subtypes of breast cancer. Spearman correlation analysis was used to compare the correlation of p53 expression with EGFR expression in the different molecular subtypes of breast cancer. The results were considered to be statistically significant at $P < 0.05$.

RESULTS

Clinical and pathological features

The ages of the 264 patients ranged from 25 to 78 years (mean, 48.3 ± 10.8 years; median, 49 years). Their clinical and pathological characteristics are shown in Table 2. The luminal B subtype of breast cancer accounted for the highest proportion (37.5%, 99/264) of the 264 cases of breast cancer, among which the HER2-negative type accounted for 31.4% (83/264) and the HER2-positive type accounted for 6.1% (16/264). The second most common subtype was TNBC, accounting for 26.1% (69/264), followed by the luminal A subtype (23.9%, 63/264) and the HER2-enriched subtype (12.5%, 33/264) (Table 3).

Table 2. Overview of the clinical and pathological features of breast cancer.

Variables	No. of cases	Proportion (%)
Age of onset		
<49 years	149	56.4
≥49 years	115	43.6
Age of menarche		
<15 years	106	40.2
≥15 years	158	59.8
Menopausal status		
Yes	92	34.8
No	172	65.2
Tumor size		
$\leq T_1$ (≤2 cm)	131	49.6
$>T_1$ (>2 cm)	133	50.4
Lymph node metastasis		
Positive	129	48.9
Negative	135	51.1
Pathological staging		
I	78	29.5
II	136	51.5
III	50	19.0
ER+	163	61.6
PR+	116	43.9
HER2+	50	18.9
Ki-67+ (<20%)	98	37.1
Ki-67+ (≥20%)	166	62.9
CK5/6+	50	18.9
p53+	88	33.4
EGFR+	39	14.8

Table 3. Relationship between the molecular subtypes of breast cancer and the clinicopathological features.

Variables	Luminal A	Luminal B		HER2-enriched	TNBC	χ^2	P value
		HER2 (-)	HER2 (+)				
Total cases	63 (23.9%)	83 (31.4%)	16 (6.1%)	33 (12.5%)	69 (26.1%)		
Age of onset							
<49 years	38	49	11	14	37	4.458	0.348
≥49 years	25	34	5	19	32		
Age of menarche							
<15 years	28	32	9	12	25	2.935	0.569
≥15 years	35	51	7	21	44		
Menopausal status							
Yes	20	31	0	11	30	11.351	0.023
No	43	52	16	22	39		
Tumor sizes							
≤2 cm	39	43	10	9	30	12.658	0.013
>2 cm	24	40	6	24	39		
Lymph node metastasis							
Positive	33	49	8	16	23	10.420	0.034
Negative	30	34	8	17	46		
Pathological staging							
I	24	20	7	4	23	15.559	0.049
II	34	42	7	20	33		
III	5	21	2	9	13		
Ki-67							
<20%	63	18	4	4	11	137.111	0.001
≥20%	0	65	12	29	58		
CK5/6							
Positive	1	8	0	5	36	70.722	0.001
Negative	62	75	16	28	33		
p53							
Positive	6	29	8	15	30	23.545	0.001
Negative	57	54	8	18	39		
EGFR							
Positive	1	3	1	5	29	58.548	0.001
Negative	62	80	15	28	40		

The molecular subtypes of breast cancer were not associated with age of onset or age of menarche (P > 0.05, both). However, the luminal B (HER2-positive) subtype was more common in premenopausal women, showing a significant difference from the other subtypes (P = 0.023). The tumor sizes in the TNBC and HER2-enriched subtypes were larger than those in the other two subtypes (P = 0.013) with a higher expression of Ki-67 (P = 0.001). TNBC had the lowest positive rate of lymph node metastasis (P = 0.034) but higher positive rates of CK5/6 and EGFR (P = 0.0001, both). In the HER2-enriched subtype, the proportion of pathological stage III cases was remarkably higher than among the other three subtypes (P = 0.049). p53 expression was high in the luminal B, HER2-enriched, and TNBC subtypes but low in the luminal A subtype (Table 3).

Correlation between p53 and EGFR expression in different molecular subtypes of breast cancer

The expression of p53 and EGFR was 43.5% (30/69) and 42.0% (29/69) in TNBC, 9.5% (6/63) and 1.6% (1/63) in the luminal A subtype, 34.9% (29/83) and 3.6% (3/83) in the luminal B subtype with HER2-negative, 50% (8/16) and 6.25% (1/16) in luminal B with HER2-positive, 45.5% (15/33) and 15.2% (5/33) in the HER2-enriched subtype and 43.5% (30/69) and 42.0% (29/69) in TNBC, respectively. Spearman correlation analysis showed that p53 expression was positively correlated with EGFR expression in invasive breast cancer (r = 0.226, P = 0.001; Table 4). Further analysis showed that the correlation of p53 expression with EGFR expression only existed in TNBC (r = 0.319, P = 0.007; Table 5) but not in the other molecular subtypes of breast cancer (P > 0.05; Table 5).

Table 4. Correlation between p53 and EGFR expression in invasive breast cancer.

p53 expression	EGFR expression		r	P value
	Positive	Negative		
Positive	23	65	0.226	0.001
Negative	16	160		

Table 5. Correlation between p53 and EGFR expression in different subtypes of invasive breast cancer.

p53/EGFR	Luminal A	Luminal B		HER2-enriched	TNBC
		HER2 (-)	HER2 (+)		
+/+	0	2	1	2	18
+/-	6	27	7	13	12
-/+	1	1	0	3	11
-/-	56	53	8	15	28
r	-0.041	0.129	0.258	-0.046	0.319
P value	0.748	0.246	0.334	0.798	0.007

DISCUSSION

The *p53* gene plays an important role in the regulation of cell cycles. The wild-type *p53* gene is involved in cell cycle regulation, preventing the transition from the G1 phase to the S phase. Thus, it is a negative regulator for cell division and proliferation. However, when

it is mutated, it can no longer arrest cell proliferation or induce apoptosis, but instead leads to uncontrolled cell growth and tumor formation. Our findings showed that the overall expression of p53 was low in invasive breast cancer (33.4%), with 37.4% positivity in the luminal B subtype, 43.5% in TNBC, and 45.5% in the HER2-enriched subtype. Curtis et al. (2012) analyzed the *p53* gene in 2000 cases of breast cancer specimens and found that the mutation rate of *p53* in the luminal A subtype was 5%, while it was 13% in luminal B, 34% in TNBC, and 22% in the HER2-enriched subtype. It has been demonstrated that overexpression of EGFR in breast tumors is associated with larger tumor size, poor differentiation, and poor prognosis. EGFR overexpression can be seen in each of the molecular subtypes of breast cancer, but it is found at a higher rate in TNBC and inflammatory breast cancer (Burness et al., 2010; Masuda et al., 2012). In this study, the positive rate of EGFR was 42% in TNBC, slightly lower than that reported by Dent et al. (2007). Other subtypes of breast cancer showed lower EGFR expression, which was 1.6% in luminal A, 4.0% in luminal B, and 15.2% in the HER2-enriched subtype. We found that p53 expression was positively correlated with EGFR expression in invasive breast cancer, and a further analysis found that the correlation existed only in TNBC but not in the other molecular subtypes. It was reported that mutation in the *p53* gene leads to *p63* gene mutation and loss of function, which would cause *EGFR* gene amplification and ultimately contribute to the formation of TNBC (Shapira et al., 2013). EGFR inhibitors exert their lethal effect on lung cancer cells expressing wild-type *p53* gene and mutated *EGFR* gene but they have no effect on the lung cancer cells expressing both mutated *p53* and mutated *EGFR* gene (Huang et al., 2011), suggesting that p53 may have a regulatory effect on EGFR inhibitors. The low expression of p53 in breast cancer overall, but its high expression in TNBC suggests that we need to launch a new understanding of the EGFR signaling pathway by targeting p53 expression, so as to explore a new therapeutic approach for TNBC. Reconstruction of the wild-type *p53* gene may overcome the resistance to EGFR inhibitors in patients with TNBC, and *p53* gene therapy combined with targeted inhibition of EGFR may change the treatment model for some patients with TNBC.

In this study, the Expert Consensus of the 2013 St. Gallen International Breast Cancer Conference was used to analyze the subtypes of breast cancer and showed that luminal B was the most common subtype (37.5%) in this group of subjects, followed by TNBC (26.1%), luminal A (23.9%), and the HER2-enriched subtype (12.5%), findings consistent with the results from El Fatemi et al. (2012), Howland et al. (2013), and Goldhirsch et al. (2013). In this study, the positive rate of CK5/6 was 52.2% in TNBC. CK5/6 is a basal keratin expressed in the basal lamina of epithelial tissues, which may be a marker of basal-like breast cancer (BLBC). Thus, CK5/6-positive TNBC is more likely to be BLBC. There is an overlap of about 80% of BLBC with TNBC, thus CK5/6 expression can be used as one marker for BLBC. HER2-enriched breast cancer and TNBC are more malignant, more aggressive and grow more rapidly. In this study, we also found that the tumor sizes were larger in HER2-enriched breast cancer and TNBC, which was also reported by Park et al. (2012). Currently, HER2-targeted therapies have offered more benefits for patients with HER2-enriched breast cancer, but there is still no effective targeted therapy for patients with TNBC. Thus, it is necessary to develop a new treatment modality for TNBC. This study also determined that the lymph node metastasis rate was higher in luminal B with HER2-negative (59.0%) but lower in TNBC (33.3%). Bennis et al. (2012) also found that the luminal B subtype of breast cancer is prone to lymph node metastasis (74%), while TNBC appears to have a lower incidence of lymph node metastasis (55.2%), suggesting that the luminal B subtype of breast cancer is more likely to metastasize via lymph

nodes, but TNBC may be more likely to metastasize via blood circulation.

No molecular subtype of breast cancer was associated with the age of onset in this study, but the luminal B (HER2-positive) subtype was more common in premenopausal women, while the other molecular subtypes displayed no correlation with menopausal status ($P > 0.05$). Li et al. (2013) also reported that molecular subtypes of breast cancer were not correlated with age of onset or menopausal status, while TNBC is more common in patients below 40 years in California, U.S. (Bauer et al., 2007). In addition, researchs from the Carolina Breast Cancer Study showed that TNBC more commonly occurs in premenopausal women in North Carolina (Carey et al., 2006; O'Brien et al., 2010). The disparities between these studies may result from the racial differences in the samples. Furthermore, it is possible that the fact that all samples were from a single center in this study may also contribute to the differences. Thus, a larger sample size from multiple centers will be included in future studies to verify the results of this study.

In conclusion, different molecular subtypes of breast cancer demonstrated different clinical and pathological features. The expressions of p53 and EGFR were higher and positively correlated with each other in TNBC, while in the other subtypes, their expressions were lower and had no correlation with each other. Gene therapy to reconstruct wild-type *p53* combined with therapy targeting the inhibition of EGFR may bring new opportunities for patients with TNBC.

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