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ORIGINAL ARTICLE

Comparisons of p53, KI67 and BRCA1 expressions in patients with different molecular subtypes of breast cancer and their relationships with pathology and prognosis

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Summary

Purpose: *To compare the expressions of p53, a tumor suppressor gene, KI67, a proliferating cell nuclear antigen, and breast cancer 1 (BRCA1), a breast cancer susceptibility gene, in patients with different molecular subtypes of breast cancer (BC) and investigate their relationships with pathology and prognosis.*

Methods: *A total of 134 BC postoperative tissue specimens preserved from January 2012 to August 2013 were selected. The expressions of p53, KI67, and BRCA1 in different molecular subtypes of BC were compared, their relationships with pathological features were explored, and the expression correlations among p53, KI67, and BRCA1 were analyzed at the same time.*

Results: *P53 expression was the lowest in Luminal A subtype and similar in human epidermal growth factor receptor 2 (HER-2)-overexpression subtype and triple-negative subtype, with higher expression rates than those in other molecular subtypes. The expression of KI67 was the lowest in Luminal A subtype, showing a significant difference ($p<0.05$) from that in other molecular subtypes and it was the highest in Luminal B subtype ($p<0.05$). BRCA1 exhibited the lowest expression in Luminal B-like subtype but the highest expression in Luminal A subtype. The protein expressions of p53 and KI67 were not related to age but correlated with tumor size, histological grade, lymph node metastasis, estrogen receptor (ER)/progesterone receptor (PR) status, and HER-2 status. The expression of p53 was increased with larger tumor size, higher histological grade, presence of lymph node metastasis (n), lower expression of ER/PR, and higher expression of HER-2. BRCA1 expression had no relation with age, tumor size, histological grade, lymph node metastasis (n), ER/PR status, and HER-2 status. A positive correlation was found between p53 and KI67 ($r=0.893$, $p=0.021$). There were negative correlations between p53 and BRCA1 ($r=-0.921$, $p=0.011$), and between KI67 and BRCA1 ($r=-0.821$, $p=0.032$). The median survival time of patients with positive expressions of p53, KI67 and BRCA1 was significantly shorter than those of patients with negative expressions.*

Conclusion: *The expressions of p53, KI67 and BRCA1 in different molecular subtypes of BC are evidently different and related to pathological features. The above protein expressions are helpful in predicting the prognosis, diagnosis, and treatment of BC.*

Key words: *p53, KI67, BRCA1, molecular subtype, breast cancer*

Introduction

Breast cancer (BC) is related to various factors including hormone receptor status, oncogenes activation, and tumor suppressor genes inactivation [1,2]. It is found that the molecular classification

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of BC is closely correlated with its clinicopathological indexes and prognosis [3,4]. P53 is a tumor suppressor gene discovered earlier in clinical practice, the mutation of which is associated with cancer progression. Clinical studies have discovered that p53 loses its anti-tumor effect if a gene mutation occurs and the overexpression of p53 leads to infinite proliferation of cells and accelerates the growth and development of cancer cells, increasing infiltration and metastasis and aggravating malignant tumors. Clinical researches have shown that most patients with BC have p53 protein mutation and the expression of p53 is increased with severity of malignancy [5]. KI67, a proliferating cell nuclear antigen, is clinically considered as a marker evaluating the proliferation activity of tumor cells. A study has found that KI67, whose expression is evidently increased in patients with malignant tumors, is closely associated with the growth of tumor cells and the prognosis of tumors [6]. The presence of breast cancer gene 1 (BRCA1) mutation, which is a BC susceptibility gene as well as BC suppressor gene, may lead to disorder of cell proliferation and metabolism [7]. Therefore, detecting the expressions of p53, KI67 and BRCA1 in the body can predict the development and prognosis of BC in clinical practice [8]. However, the clinical research on the relationship of the protein expressions of p53, KI67 and BRCA1 with BC via immunohistochemistry (IHC) is rare so far, without any comprehensive study of the correlations among the expressions of p53, KI67 and BRCA1. Thus, in this study, the expressions of p53, KI67 and BRCA1 in patients with BC were determined separately in order to compare their expressions in patients with BC with different molecular subtypes, as well as exploring their correlations with pathology and prognosis.

Methods

Materials

Postoperative tissue specimens from 134 patients with BC (including 132 females and 2 males) preserved from January 2012 to August 2013 were selected and confirmed through pathological examination. The median age was 43.43±6.54 years (range 27-72) and none of the patients had received previous anti-cancer treatment. According to the Union for International Cancer Control (UICC) TNM staging criteria-1997, the number of patients with stage I, II, and III disease were 21, 84 and 29, respectively . Based on the WHO histological classification criteria for BC, there were 120 cases of invasive ductal carcinoma, 10 cases of invasive lobular carcinoma, 2 cases of mucinous adenocarcinoma, and 2 cases of intraductal carcinoma. In terms of disease location, BC occurred in the left breast in 88 cases and in the right in 46 cases.

Immunohistochemical staining assay

Paraffin-embedded specimens (4 μm) were sectioned and stained via immunohistochemistry (two-step method) [9]. Then, the sections were deparaffinized, immersed in citrate buffer (0.01 mol/L, pH 6.0), and placed in a microwave oven (98°C) for 20 min. Next, the sections immersed in the buffer were taken out, cooled for 20 min, treated with 3% H_2O_2 for 5 min, rinsed with phosphate-buffered saline (PBS) for 3 times (3 min/time), added with antibodies, and stored at 4°C overnight. Afterwards, the sections were rinsed for 3 times (3 min/time) and the PBS solution was shaken off. Then, the sections were added with goat antibodies, mouse antibodies, and horseradish peroxidase complex for incubation for 30 min, developed with DAB, counterstained with hematoxylin, permeabilized with xylene, and finally mounted with neutral gum. At the same time, control group was set with PBS instead of antibodies. Monoclonal antibodies included estrogen receptor (ER) (1D5), progesterone receptor (PR) (SP2), human epidermal growth factor receptor 2 (HER-2) (CB11), KI67 (MIB-1), p53 (DO-7) and BRCA1 (D-9). The antibodies and kits were purchased from Sigma (St.Louis, MO, USA).

Interpretation of the IHC results

Interpretation of KI67 staining: Cells with brown-yellow granules in the nucleus were positive cells and after counting positive cells in 10 high-power fields (×400) of tumor cells the expression rate <14% represented low-index expression (negative), while the expression rate ≥14% indicated high-index expression (positive).

Interpretation of ER and PR staining: Cells with brown-yellow granules in the nucleus were positive cells and after counting positive cells in 10 high-power fields (×400) of tumor cells the expression rate <5% repre-

Table 1. Expression status of antibodies in various molecular subtypes of breast cancer patients through immunohistochemistry

Subtype	*Expression status of antibody*
Luminal A subtype	ER and/or PR positive, HER-2 negative, KI67 <14%
Luminal B-like subtype	ER and/or PR positive, HER-2 negative, KI67 in any status
Luminal B subtype	ER and/or PR positive, HER-2 negative, KI67 >14%
HER-2 overexpressing subtype	ER and PR negative, HER-2 positive,
Triple-negative subtype	ER, PR and HER-2 negative

sented negative, while the expression rate ≥5% indicated positive.

Interpretation of p53 staining: Cells with brown-yellow granules in the nucleus were positive cells and after counting positive cells in 10 high-power fields (×400) of tumor cells the expression rate <5% represented negative, while the expression rate ≥5% indicated positive.

Interpretation of HER-2 staining: In accordance with the ASCO/CAP guideline, the staining intensity of cell membrane was rated as 0 (negative), 1+, 2+, and 3+, where 0 and 1+ represented negative, 3+ indicated positive, and 2+ manifested that the results needed to be further determined via fluorescence *in situ* hybridization (FISH). The FISH kit was bought from Sigma and 2+ meant positive in case of expression of gene amplification and negative if there was no expression of gene amplification.

Interpretation of BRCA1 staining: Cells with brown-yellow granules in the cytoplasm or nucleus were positive cells and after counting positive cells in 10 high-power fields (×400) of tumor cells the expression rate <10% represented negative, while expression rate ≥10% indicated positive. All pathological sections were reviewed and re-diagnosed by a pathologist.

Determination of molecular subtypes

According to the 2011 St. Gallen Consensus, the molecular subtypes of BC patients were determined based on the expression status of ER, PR, HER-2, and KI67 [10] (Table 1).

Follow-up

Patients were followed-up until May 2018, during which there was no loss to follow-up and the follow-up rate was 100%.

Statistics

Data were processed and analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Enumeration data were expressed as numbers. Chi-square test was employed for difference between groups. The relationships of protein expressions with pathological characteristics were analyzed via the modified Pearson chi-square test. Spearman correlation analysis was applied for correlation analysis. Kaplan-Meier method was utilized to plot survival curves, and Log-rank test was carried out to assess significant differences. P<0.05 suggested that the difference was statistically significant.

Results

Classification of molecular subtypes in breast cancer patients

Among the 134 patients with BC, 18 patients had Luminal A subtype, accounting for 13.4% (18/134), 42 had Luminal B-like subtype, accounting for 31.3% (42/134), 28 had Luminal B subtype, accounting for 20.9% (28/134), 25 had HER-2 overexpression subtype, accounting for 18.7% (25/134), and 21 had triple-negative subtype, accounting for 15.7% (21/134).

Comparisons of the expressions of p53, KI67, and BRCA1 among different molecular subtypes of breast cancer

The expression of p53 was the lowest in Luminal subtype A and similar in HER-2 type and triple-negative group, with higher expression rates than those in other molecular subtypes. KI67 showed the lowest expression in Luminal A subtype, displaying a statistically significant difference from those in other molecular subtypes (p<0.05), while its expression was the highest in Luminal B subtype (p<0.05). BRCA1 expression was the lowest in Luminal B-like subtype and the highest in Luminal A subtype (Table 2).

Correlation between p53 expression and clinical pathology

P53 expression was not related to age but correlated with tumor size, histological grade, lymph node metastasis, ER/PR status, and HER-2 status, indicating that the larger tumor, the higher histological grade, increased lymph node metastasis (n), negative expression of the ER/PR, and higher expression of HER-2 were associated with higher expression of p53 (Table 3).

Table 2. Comparisons of the expressions of p53, KI67, and BRCA1 among different molecular subtypes of breast cancer

Subtype	*n*	*p53 protein expression*		*KI67 protein expression*		*BRCA1 protein expression*	
		+	-	+	-	+	-
Luminal A subtype	18	1 (5.5)*	17 (94.5)	0 (0)*	18 (100)	16 (88.9)*	2 (11.1)
Luminal B-like subtype	42	18 (42.9)	24 (57.1)	34 (80.9)	8 (19.1)	22 (52.4)*	20 (47.6)
Luminal B subtype	28	11 (39.3)	16 (60.7)	28 (100)*	0 (0)	20 (71.4)	8 (28.6)
HER-2 overexpressing subtype	25	21 (84.0)**	4 (16.0)	17 (68.0)	8 (32.0)	17 (68.0)	8 (32.0)
Triple-negative subtype	21	18 (85.7)**	4 (16.0)	17 (80.9)	4 (19.1)	15 (71.4)	6 (28.6)

*p<0.05 vs. other molecular subtypes, **p<0.05 vs. Luminal A, Luminal B-like and Luminal B subtypes

Table 3. Correlation between p53 expression and clinical pathology

Clinicopathologic type	*n*	*p53 expression*		x^2	*p*
		+ *n (%)*	- *n (%)*		
Age, years					
<40	42	209 (47.6)	21 (52.4)	1.642	0.153
40-59	67	27 (40.3)	40 (59.7)		
≥60	25	16 (64.0)	9 (36)		
Tumor size, cm					
≤2	37	11 (29.7)	26 (70.3)	3.274	0.032
2-5	68	39 (57.4)	29 (42.6)		
>5	29	20 (69.0)	9 (31.0)		
Histological grade					
Grade 1	21	10 (47.6)	11 (52.4)	4.974	0.021
Grade 2	84	65 (77.4)	19 (22.6)		
Grade 3	29	23 (79.3)	6 (20.7)		
Lymph node metastasis					
0	45	18 (40.0)	27 (60.0)	3.074	0.028
1-3	28	16 (57.1)	12 (42.9)		
4-9	32	23 (71.9)	9 (28.1)		
≥10	29	22 (75.9)	7 (24.1)		
ER/PR status					
Negative	46	28 (60.9)	18 (39.1)	5.397	0.016
Positive	88	35 (39.8)	53 (60.2)		
HER-2 status					
Negative	25	16 (64.0)	9 (36.0)	3.097	0.021
Positive	109	95 (87.2)	41 (12.8)		

Table 4. Relationship between the expression of KI67 and clinical pathology

Clinicopathologic type	*n*	*KI67 expression*		x^2	*p*
		+ *n (%)*	- *n (%)*		
Age, years					
<40	42	22 (52.4)	20 (47.6)	0.865	1.965
40-59	67	28 (41.8)	39 (58.2)		
≥60	25	17 (68.0)	8 (32.0)		
Tumor size, cm					
≤2	37	19 (51.4)	18 (48.6)	2.664	0.016
2-5	68	39 (57.4)	29 (42.6)		
>5	29	16 (55.2)	13 (44.8)		
Histological grade					
Grade 1	21	11 (52.4)	10 (47.6)	3.783	0.275
Grade 2	84	45 (53.6)	39 (46.4)		
Grade 3	29	16 (55.2)	13 (44.8)		
Lymph node metastasis					
0	45	16 (35.6)	61 (64.4)	1.092	0.043
1-3	28	18 (64.3)	10 (35.7)		
4-9	32	28 (87.5)	4 (12.5)		
≥10	29	27 (93.1)	2 (6.9)		
ER/PR status					
Negative	46	27 (58.7)	19 (41.3)	3.231	0.020
Positive	88	37 (42.0)	51 (58.0)		
HER-2 status					
Negative	25	15 (60.0)	10 (40.0)	2.978	0.019
Positive	109	97 (89.0)	12 (11.0)		

Relationship between the expression of KI67 and clinical pathology

The expression of KI67 had no relation with age but was associated with tumor size, histological grade, lymph node metastasis, ER/PR status, and HER-2 status, showing that the expression of KI67 was elevated with increased tumor size, increased histological grade, lymph node metastasis (n), negative expression of ER/PR, and positive expression of HER-2 (Table 4).

Association between BRCA1 expression and clinical pathology

BRCA1 expression exhibited no correlations with age, tumor size, histological grade, lymph node metastasis, ER/PR status and HER-2 status (Table 5).

Correlation study of p53, KI67, and BRCA1

The results of Spearman correlation analysis showed that there was a positive correlation between p53 and KI67 (r=0.893, p=0.021) and negative correlations between p53 and BRCA1 (r=-0.921, p=0.011), and between KI67 and BRCA1 (r=-0.821), p=0.032) (Table 6).

Correlations of p53, KI67, and BRCA1 with prognosis in patients with breast cancer

An obvious difference was detected in median survival time between patients with positive p53,

Table 5. Association between BRCA1 expression and clinical pathology

Clinicopathologic type	*n*	*BRCA1 expression*		x^2	*p*
		+ *n (%)*	- *n (%)*		
Age, years					
<40	42	22 (52.4)	20 (47.6)	2.652	0.275
40-59	67	28 (41.8)	39 (58.2)		
≥60	25	15 (60.0)	10 (40.0)		
Tumor size, cm					
≤2	37	20 (54.1)	26 (45.9)	1.985	0.121
2-5	68	39 (57.4)	29 (42.6)		
>5	29	16 (55.2)	13 (44.8)		
Histological grade					
Grade 1	21	13 (61.9)	8 (38.1)	3.736	0.421
Grade 2	84	45 (53.6)	39 (46.4)		
Grade 3	29	17 (58.6)	12 (41.4)		
Lymph node metastasis					
0	45	22 (48.9)	23 (511)	0.342	0.209
1-3	28	15 (53.6)	13 (46.4)		
4-9	32	16 (50.0)	16 (50.0)		
≥10	29	17 (58.6)	12 (41.4)		
ER/PR status					
Negative	46	27 (58.7)	19 (41.3)	1.091	0.097
Positive	88	46 (52.3)	42 (47.7)		
HER-2 status					
Negative	25	14 (56.0)	11 (44.0)	1.175	0.065
Positive	109	59 (54.1)	50 (45.9)		

Table 6. Correlation study of p53, KI67 and BRCA1

Protein expression		*n*	*p53*		*KI67*		*BRCA1*	
			+	-	+	-	+	-
p53	+	69	-	-	61	8	36	33
	-	65	-	-	35	30	54	11
KI67	+	96	61	35	-	-	59	57
	-	38	8	30	-	-	31	7
BRCA1	+	90	36	54	59	31	-	-
	-	44	33	11	57	7	-	-

Table 7. Correlations of p53, KI67 and BRCA1 proteins with prognosis in patients with breast cancer

Protein		*N*	*Median survival time (months)*	x^2	*p*
p53				11.54	0.001
	+	69	14		
	-	65	30		
KI67				10.09	0.003
	+	96	16		
	-	38	36		
BRCA1				8.78	0.012
	+	90	12		
	-	44	20		

KI67, and BRCA1 and those with negative p53, KI67, and BRCA1. The median survival time of patients with positive expression of each protein was overtly shorter than those patients with negative expression (Table 7).

Discussion

As a common malignant tumor in clinic in females, BC imposes a heavy burden on life safety and physical and mental health of patients. Clinical studies have proved that BC is characterized by high heterogeneity and the disease prognosis may be significantly different even though the clinical stage and treatment method used for patients are the same. Thus, how to predict the prognosis in patients during the treatment is of particular importance [11-13]. Researches have discovered that p53, one of the major tumor suppressor genes, is present in normal cells and mediates the cell cycle through multiple signaling pathways, thus inducing apoptosis and preventing cell carcinogenesis [14]. KI67, a proliferating cell nuclear antigen, is related to cell mitosis, which can be used to evaluate the proliferation of cells. The higher KI67 expression is associated with a more aggressive cell behavior. In clinical practice, KI67 has been used as an important index in evaluating the prognosis of BC [15]. In addition, BRCA1 is considered a BC-associated suppressor gene, which negatively regulates cell growth. A study found that BRCA1 is differently expressed in different molecular subtypes of BC and related to the clinicopathological features of prognosis [16]. With the continuous advances in medicine, it was clarified that accurate clinical treatment cannot be achieved using the anatomical staging and histological classification. Based on 2013 St. Gallen Consensus, the molecular subtypes of BC according the ER, PR, HER-2 and KI67 index are as follows; Luminal A, Luminal B, Luminal B-like, basal-like, and HER-2 overexpression [17].

P53 has tumor suppressing effect, which was used by ASCO as one of the important indicators for evaluating the prognosis of BC. The mutation of p53 gives rise to malignant proliferation of cells and infinite growth and proliferation of BC cells, thereby promoting the deterioration of BC [18]. It was found in this study that the expression of p53 was clearly higher in HER-2 overexpressing subtype and triple-negative subtype than those in other molecular subtypes, implying that the prognosis was poorer in patients with these two subtypes of BC. The overexpression of p53 protein indicates an aggravated p53 gene mutation. Mutant p53 has no anti-cancer effect, inhibits the anti-cancer effect of p53 protein, and interacts with various proteins in cells to further aggravate BC. This study discovered that p53 was correlated with tumor size, histological grade and lymph node metastasis. These results indicate that in patients with BC, the higher expression of p53 is associated with high lymph node metastasis and higher disease deterioration, thus a preliminary judgement may be suggested that the invasiveness and metastasis of tumors have a strong association with higher p53 expression. Bae et al [19] found that the positive rate of p53 protein in HER-2 overexpressing and triple-negative subtypes was evidently higher than those in Luminal A, Luminal B1 and Luminal B2 subtypes, with statistically significant differences.

P53 protein was positively correlated with KI67, indicating that the two genes have a synergistic effect. The overexpressed p53 promotes the growth of tumor cells, resulting in enhanced tumor invasiveness and metastasis. Positive expression of KI67 (14%) has been used as an independent factor for prognosis of BC. KI67 >14% represents accelerated proliferation of tumor cells, increased deterioration of BC, aggravated infiltration, and poorer prognosis. This study found that KI67 showed the highest expression in Luminal B subtype and the lowest expression in Luminal A subtype, which might be related to ER and PR. Under normal cir-

cumstances, BC patients with positive ER and PR have relatively high differentiation, hence the deterioration of tumor and the expression of KI67 are relatively low. This study revealed that KI67 was associated with tumor size, histological grade, and lymph node metastasis in patients with BC, suggesting that the higher expression of KI67 was associated with higher grade of malignancy, faster growth rate of tumor cells, higher degree of invasion and metastasis, and poorer clinical prognosis. Besides, the survival analysis revealed that the median survival time of patients with positive p53 expression was significantly shorter than that of patients with negative p53 expression. Sungu et al [20] found that the expression of KI67 was not correlated with tumor size, histological grade, and lymph node metastasis, which is inconsistent with the results of this study. Such an inconsistency may be caused by the small number of cases and individual patient variation. Therefore, more patients should be enrolled in the later stage to minimize individual variation.

The results showed that KI67 was negatively correlated with the expression of BRCA1. It is preliminarily confirmed that BRCA1 is able to inhibit the growth and proliferation of tumor cells. When the expression of BRCA1 declines, the prognosis is poor. As an important BC suppressor gene recently discovered in the clinic, BRCA1 negatively regulates the growth of tumor cells. When it is dysfunctional, its inhibitory effect on cell growth will be lost, thus leading to the cancerization of cells. This study discovered that BRCA1 had the highest expression in Luminal A subtype, indicating that Luminal A BC has the poorest recurrence and prognosis. This study showed that BRCA1 expression showed no clear association with tumor size, lymph node metastasis and histological grade. The survival analysis showed that the median survival time of patients with positive expressions of KI67 and BRCA1 was notably shorter than those of patients with negative expressions of KI67 and BRCA1. This study found that BRCA1 was negatively associated with p53 and KI67, further proving that BRCA1 plays a negative regulatory role in tumor cells. Decreased expression of BRCA1 promotes the proliferation of tumor cells, indicating that the clinical prognosis is poor in case of declined BRCA1 protein expression. In conclusion, p53, KI67 and BRCA1 are related to pathological features and clinical prognosis in different molecular subtypes of BC. Detecting the protein expressions of p53, KI67 and BRCA1 in patients with BC helps evaluate the treatment effect and prognosis, implying that multiple biological indicators detected via IHC can be utilized as supplementary reference standards for the clinical treatment of patients with BC, thereby providing a more sound scientific basis for the accurate prediction of the prognosis of BC.

Conflict of interests

The authors declare no conflict of interests.

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