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ORIGINAL PAPER

Expressions of ER, PR, HER-2, COX-2, and VEGF in Primary and Relapsed/Metastatic Breast Cancers

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Abstract In the present study, we evaluated expressions of estrogen receptor (ER), progestin receptor (PR), human epidermal growth factor receptor-2 (HER-2), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF) in primary and relapsed/metastatic breast cancers to elucidate the clinical significance of these markers. The markers were evaluated by immunohistochemistry in specimens of 50 patients with primary or metastatic breast cancer. Positive rates of ER were significantly ($p = 0.002$) higher in primary versus relapsed/metastatic breast cancer (70 vs. 38 %, respectively). The VEGF positive expression rates were also significantly higher in primary versus metastatic cancer (82 vs. 38 %, respectively; $p < 0.001$). By contrast, positive rates of HER-2 and COX-2 were not significantly different between different types of cancer. COX-2 correlated with HER-2 expression in both primary and relapsed/metastatic focuses of breast cancer. COX-2 also correlated with VEGF expression in primary breast cancer. Expressions of ER, PR, HER2, and COX-2 did not correlate between primary and relapsed/metastatic breast cancers, indicating that the treatment decision should be made according to the status of these markers in relapsed/metastatic focuses. The total change rates of ER, PR, HER-2, COX-2, and VEGF were 26, 18, 10, 30, and 58 %, respectively. In conclusion, HER-2 and COX-2, along with VEGF, appear to play a role in the development and progression of breast cancer. In addition, all of the studied markers may serve as indicators of prognosis.

Keywords Breast cancer · Estrogen receptor · Progestin receptor · Immunohistochemistry · HER-2

Introduction

Worldwide, the mortality rates associated with breast cancer are decreasing, although the incidence of breast cancer is still increasing [1]. Similar trends are seen in China [2, 3]. A variety of factors led to decreasing mortality; among those factors, both endocrine and targeted therapies of breast cancer play an important role [4]. The use of endocrine and targeted therapies requires expression of appropriate receptors in primary cancer. Further, in recent years, more attention is drawn to expression of different receptors in metastatic cancer. To further expand the knowledge in this field, we collected 50 specimens of primary and metastatic focuses of breast cancer and evaluated expressions of estrogen receptor (ER), progestin receptor (PR), human epidermal growth factor receptor-2 (HER-2), cyclooxygenase-2 (COX-2), and vascular endothelial growth factor (VEGF) in these specimens by

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immunohistochemistry. The immunohistochemistry data were then used to evaluate the clinical usefulness of these markers.

Materials and Methods

Specimens

The specimens of primary and metastatic breast cancers were obtained from 50 patients with different stages of the disease proved by pathology at the Xuzhou Central Hospital, Xuzhou, Jiangsu Province between January 2001 and December 2011. Forty-nine specimens were obtained from female patients and one specimen from a male patient. The patients' age ranged from 31 to 64 years, with the median age of 52 years. Study individuals were the stages I–III postoperative patients before recurrence and metastasis, and the stage IV patients after recurrence and metastasis.

The time of recurrence and metastasis ranged from 2 to 9 years, and the median time was 5.5 years. All the cases were invasive ductal carcinomas proved by pathology. Three cases were local recurrences after breast-conserving surgery, 17 were the chest wall skin recurrences, 16 were supraclavicular lymph node metastasis, 5 were contralateral axillary lymph node metastasis, 2 were lung metastasis, 1 was brain metastasis, 2 wwere bone metastasis, 3 were liver metastasis, and 1 case was peritoneal metastasis.

Reagents

Primary monoclonal antibodies against ER, PR, HER-2, COX-2, and VEGF receptors, EDTA buffer, DAB, Maxvision reagent, secondary antibody Sunpoly-H, xylene, ethanol, hematoxylin, and other reagents were purchased from Maixin Biotechnology Development Co., Ltd. (Fuzhou, Fujian, China).

Detection Methods

All the waxed specimens were subjected to a 4-μm thick serial sectioning for immunohistochemical staining. After xylene dewaxing, the slides were de-benzened using conventional gradient ethanol and immersed in water. After incubation for 10 min with 3 % H_2O_2, the slides were washed thoroughly using PBS and placed in the phosphate–EDTA buffer solution (50 %, pH 9.0). Antigens were retrieved in the 121° C high-pressure cooker for 5 min, followed by cooling down of the slides to room temperature. Approximately, 50 μl of the ER, PR, HER-2, COX-2, and VEGF monoclonal antibodies were added to each slide. The slides were incubated with antibodies for 2 h at room temperature, and washed with PBS. The Maxvision reagent was added to the slides and incubated for 20 min. The slides were then washed 3 × 3 min with PBS and dry wiped afterwards. 50 μl of secondary antibody Sunpoly-H was added to the slides, the slides were incubated for 30 min at room temperature, washed 3 × 3 min with PBS, and incubated with DAB. The slides were then washed with distilled water, re-stained using hematoxylin, dehydrated, and sealed with neutral resin. The known positive slides were used as positive controls. In negative control slides, PBS was used instead of primary antibody.

Specimen Evaluation Criteria

The evaluation criteria for HER-2 were as follows. According to the 2009 Edition of the Breast Cancer HER-2 Detection Guide [5], HER-2 negative staining was ranked "0" or "1+". Specifically, "0" were the specimens with invasive cancer cells that did not stain for HER-2, while the rank "1+" was assigned for any percentage of invasive cancer cells that were weakly stained with incomplete membrane staining, or if there were <10 % cancer cells that were weakly stained with complete membrane staining. An uncertain staining, which was given the rank of "2+", had ≥10 % of tumor cells showing weak or inconsistent complete membrane staining, or ≤30 % of cancer cells showing strong and complete membrane staining. Finally, positive staining, ranked "3+", was the staining with >30 % of invasive cancer cells showing strong and complete membrane staining. Figure 1 demonstrates Her-2 positive staining in metastatic breast cancer.

The ER and PR evaluation criteria were as follows. First, ten high-power fields were randomly selected and more than 500 cells were counted. A nuclear staining was

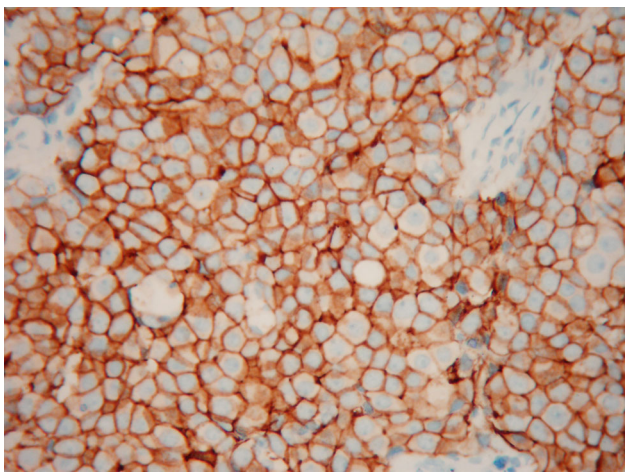

Fig. 1 Positive expression of HER-2 in breast cancer metastasis (magnification ×400)

ranked as positive. The specimens were assigned different ranks, depending on the number of positive cells and staining intensity: specimens with no positive cells were ranked "negative", the presence of 1–25 % positive cells was ranked as "+", 25–50 % positive cells were ranked as "++", 50–75 % positive cells were ranked as "+++", while 75–100 % positive cells were ranked "++++". The staining intensity was further divided into "strongly positive", "positive", "weakly positive", and "negative". Figures 2 and 3 represent positive stainings for ER and PR in metastatic breast cancer.

The COX-2 and VEGF stainings were evaluated as follows. As before, ten high-power fields were selected and more than 500 cells counted. The presence of brown particles in the cytoplasm was considered as positive staining. Depending on the number of positive cells and staining intensity, the specimens were ranked as "negative" (no positive cells), "+" (1–25 % positive cells), "++" (25–50 % positive cells), "+++" (50–75 % positive cells), and "++++" (75–100 % positive cells). The intensity of the staining was further divided into "strongly positive", "positive", "weakly positive", and "negative". Figure 4 represents the COX-2 positive staining in primary breast cancer, while Fig. 5 demonstrates the VEGF positive staining in metastatic breast cancer.

Statistical Analysis

The experimental data were analyzed using the SPSS17.0 statistical software. The differences in expressions of ER, PR, HER-2, COX-2, and VEGF between the primary and metastatic cancers were analyzed using the Chi square test, while correlation analysis was carried out using the Chi square test for paired data.

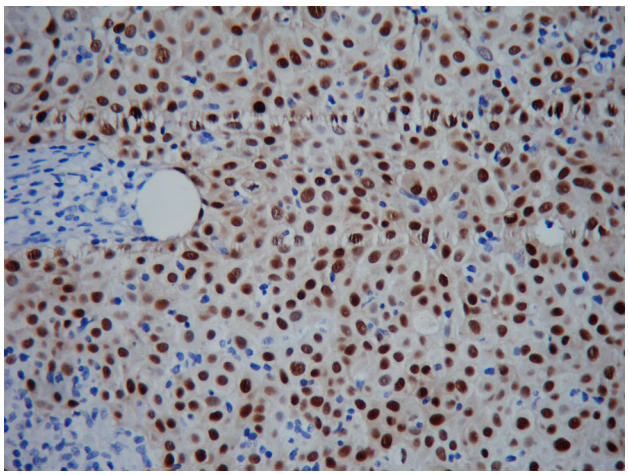

Fig. 3 Positive expression of PR in breast cancer metastasis (magnification ×400)

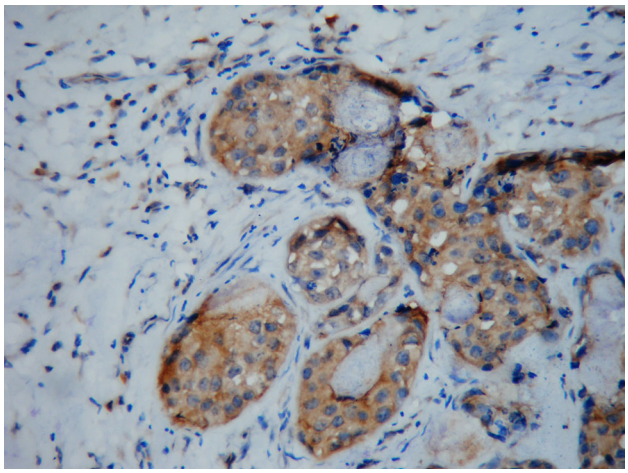

Fig. 4 Positive expression of COX-2 in primary breast cancer (magnification ×400)

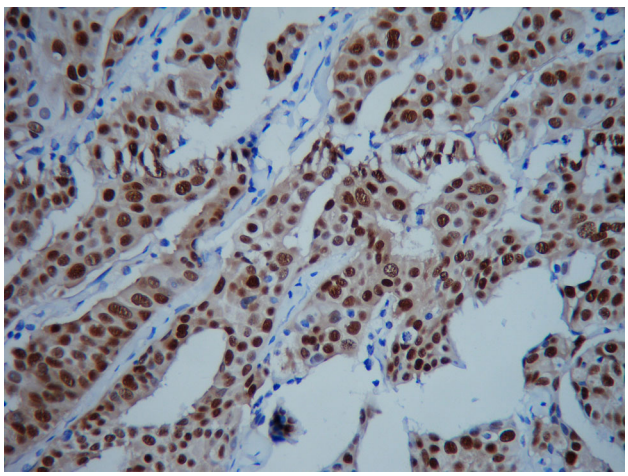

Fig. 2 Positive expression of ER in breast cancer metastases (magnification ×400)

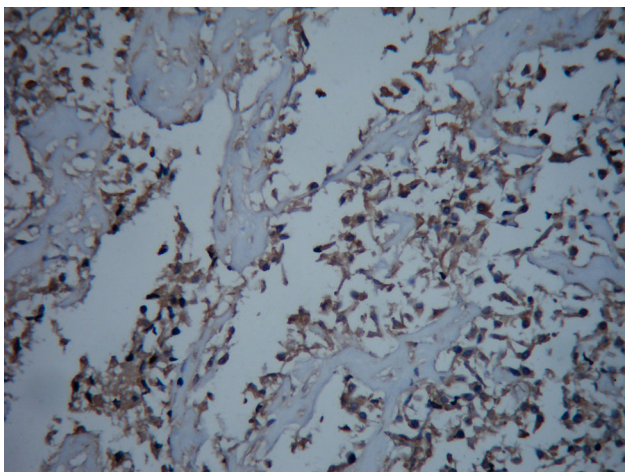

Fig. 5 Positive expression of VEGF in breast cancer metastasis (magnification ×400)

Results

Expressions of ER, PR, HER-2, COX-2, and VEGF in Primary and Metastatic Cancers

There were, respectively, 35, 16, 16, 38, and 34 cases of ER, PR, HER-2, COX-2, and VEGF positive specimens among primary cancers. Thus, the positive rates were 70, 32, 32, 76, and 68 %, respectively.

The ER, PR, HER-2, COX-2, and VEGF positive stainings were found, respectively, in 19, 11, 12, 44, and 18 specimens of metastatic cancers, making the positive rates of, respectively, 38, 22, 24, 88, and 36 %. Expressions of ER and VEGF differed significantly between primary and metastatic cancers ($p = 0.002$ and 0.001, respectively; Table 1).

Correlation Between Expressions of HER-2, COX-2, and VEGF in Primary and Metastatic Cancers

Expressions of VEGF and COX-2 correlated significantly with primary but not metastatic cancers (Table 2). Further, HER-2 and COX-2 expressions correlated with both primary and metastatic breast cancers (Table 3). Finally, ER, PR, and HER-2 did not correlate with either primary or metastatic breast cancers (Table 4).

Differences in Expressions of ER, PR, HER-2, COX-2, and VEGF Between Primary and Metastatic Breast Cancers

There were five cases of ER switch positive to negative from primary to metastatic cancers (5/50, 10 %), and eight cases of ER switch negative to positive from primary to metastatic cancers (8/50, 16 %), making the total number of cases for ER switch to 13; and the rate of the switch of 26 % (13/50) (Table 5).

Further, there were six cases of PR switch positive to negative from primary to metastatic cancers (6/50, 12 %), and three cases of PR switch negative to positive from primary to metastatic cancers (3/50, 6 %). The total cases with PR switch were nine, and the rate of change was 18 % (9/50) (Table 5).

Eight cases were HER-2 positive in primary cancers and negative in metastatic specimens (8/50, 16 % each), while two were HER-2 negative in primary cancers and positive in metastatic specimens (2/50, 4 %), making five cases of HER-2 switch and the rate of change of 10 % (5/50) (Table 5).

There were 8 COX-2 positive primary cancers and negative metastatic cancers (8/50, 16 %), and 7 COX-2 negative primary and 7 positive metastatic cancers (7/50, 14 %). Thus, there were 15 cases of COX-2 switch, while the rate of change was 30 % (15/50) (Table 5).

Finally, VEGF switch from primary to metastatic cancers was 40 % (20/50), and the probability of turning positive from primary to metastatic cancers was 18 % (9/

Table 2 Correlation between expressions of HER-2 and COX-2 in primary focuses of breast cancer

	COX-2 positive	COX-2 negative	Total	*p*
HER-2 positive	9	7	16	0.036
HER-2 negative	29	5	34	
Total	38	12	50	

Table 3 Correlation between expressions of VEGF and COX-2 in primary focuses of breast cancer

	COX-2 positive	COX-2 negative	Total	*p*
VEGF positive	34	7	41	0.027
VEGF negative	4	5	9	
Total	38	12	50	

Table 4 Correlation between expressions of HER-2 and COX-2 in metastatic focuses of breast cancer

	COX-2 positive	COX-2 negative	Total	*p*
HER-2 positive	8	4	12	0.024
HER-2 negative	36	2	38	
Total	44	6	50	

Table 1 Expressions of ER, PR, HER-2, COX-2, and VEGF in primary and metastatic focuses of breast cancer

Receptor	Primary focus		Metastatic focus		Chi square value	*p*
	Positive	Negative	Positive	Negative		
ER	35 (70 %)	15 (30 %)	19 (38 %)	31 (62 %)	9.058	0.002
PR	16 (32 %)	34 (68 %)	11 (22 %)	39 (78 %)	0.812	0.368
HER-2	16 (32 %)	34 (68 %)	12 (24 %)	38 (76 %)	0.446	0.504
COX-2	38 (76 %)	12 (24 %)	44 (88 %)	6 (12 %)	1.694	0.192
VEGF	34 (68 %)	16 (32 %)	18 (36 %)	32 (64 %)	10.256	0.001

Table 5 Expression switch of ER, PR, HER-2, COX-2, and VEGF in primary and metastatic focuses of breast cancer

Switch	ER	PR	HER-2	COX-2	VEGF
Positive to negative	5/50 (10 %)	6/50 (12 %)	3/50 (6 %)	8/50 (16 %)	20/50 (40 %)
Negative to positive	8/50 (16 %)	3/50 (6 %)	2/50 (4 %)	7/50 (14 %)	9/50 (18 %)
Total discordance	13/50 (26.0 %)	9/50 (18 %)	5/50 (10.0 %)	15/50 (30.0 %)	29/50 (58 %)

50). The total probability of the VEGF change was 58 % (29/50) (Table 5).

Discussion

In 1890, Beatson first described clinical phenomenon that the breast cancer metastases are attenuated in premenopausal breast cancer patients after oophorectomy [6]. This observation led to the endocrine treatment of breast cancer. In 1966, ER was identified [7]. Subsequently, beneficial effects of ovariectomy in the treatment of breast cancer were confirmed [6]. Endocrine therapy has been included in the National Comprehensive Cancer Network guidelines [8] and has become an important method in breast cancer treatment. However, all present treatment regimens are based on hormone receptor expressions in primary tumor, while the expression of these receptors in metastasic lesions received significantly less attention. In our study, we found that the ER expression is statistically different between primary and metastatic breast cancers. This indicates that the expressions of hormone receptors are different between primary and metastatic cancers. Previous studies [9–12] found that the expressions of ER, PR, and HER-2 can change between primary and metastatic breast cancers. While the studies report different receptor changing rates, there is a consensus that receptor expressions can change in recurrence and metastasis. Our results are consistent with the results from the literature and also reflect expressions of these markers in Chinese patients with breast cancer. Both the 2010 European Society for Medical Oncology (ESMO) and 2011 the United States NCCN guidelines [8, 13] recommend redoing the biopsy on metastatic lesion in breast cancer recurrence patients to better direct the clinical treatment, underlining the importance of receptor expressions in metastatic lesions for more optimal treatment options based on receptor expression levels.

In our study, we found that the probability of the switch of HER-2 in primary breast cancer and its metastases was 10 %. However, the difference in HER-2 expression between primary and metastatic cancers was not statistically significant. This may be related to the relative small sample numbers. In a larger study (i.e., 151 cases), Wilking et al. [14] reported that HER-2 re-detection has important clinical significance. In another study, HER-2 overexpression was found in about 20 % of breast cancer patients [15]. Indeed, beneficial effects of the HER-2 receptor monoclonal antibody Herceptin were confirmed in multiple clinical trials. Re-biopsy in metastatic breast tumor lesions enables breast cancer patients with overexpressed HER-2 to benefit from Herceptin. Many studies found that the HER-2 expression is an important prognostic factor for breast cancer. HER-2 expression closely correlates with the initiation, development, and prognosis of cancer [16].

The expression of VEGF in metastatic lesions was statistically less frequent than in primary cancer. This may be due to the fact that there is no angiogenesis during the early stage of relapsed/metastatic focuses of breast cancer. During the growth of metastatic cancer, VEGF expression gradually increases to facilitate the angiogenesis. Angiogenesis is important for the growth of many cancers, as it is involved in cancer survival, growth, and metastasis [17]. VEGF is the main regulatory factor in tumor angiogenesis [16]. Therefore, the inhibition of angiogenesis is one of the pursued anti-cancer therapeutic strategies. Vidaurreta et al. [18] found that VEGF increases permeability of small veins, resulting in extravasation of plasma proteins and fibrinogen, altering extracellular matrix, fibrin deposition, and new matrix formation, thus providing the basis for cancer invasion and metastasis. This suggests that the overexpression of VEGF in primary breast cancers may be one of the mechanisms of breast cancer dissemination and metastasis, closely correlated with the prognosis in breast cancer patients, further highlighting the role of VEGF as a factor to predict the prognosis of breast cancer.

In this study, we also found that the rates of COX-2 expression were 76 % in primary cancers and 88 % in metastatic lesions. High COX-2 expression indicates a poor prognosis [19]. COX-2 plays an important role in the initiation and development of breast cancer, and is closely related to the breast cancer metastasis. Moreover, COX-2 expression correlates well with cancer size, histological grading, and increased number of metastatic lymph nodes. Therefore, COX-2 is considered as an important poor prognostic factor in breast cancer [20]. COX-2 can be carcinogenic through many mechanisms, including promotion of tumor angiogenesis [21, 22].

We found a correlation between expressions of HER-2 and COX-2 in both primary and metastatic breast cancers.

Further, COX-2 expression also correlated with the expression of VEGF in primary breast cancers. Combined, these findings indicate a close association between HER-2, COX-2, and VEGF to increase the degree of cancer malignancy, leading to earlier recurrence and metastasis. Therefore, the detection of these three proteins may facilitate a more precise prognosis. One could also speculate that simultaneous inhibition of several targets may boost the treatment success rates. Supporting this, Pierga et al. [23] found that a combination of COX-2 inhibitor Celecoxib and HER-2 inhibitor Herceptin improved the efficiency of the treatment of patients with advanced breast cancer.

In conclusion, expressions of ER, PR, HER2, and COX-2 are discordant in primary and relapsed/metastatic focuses of breast cancer. Therefore, treatment decisions should be made according to the expression statuses in relapsed/metastatic focuses. HER-2, COX-2, and VEGF are important markers, and their combined detection may facilitate the prognosis of breast cancer.

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