

# Human epidermal growth factor receptor 2 protein expression between primary breast cancer and paired asynchronous local-regional recurrences

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**Abstract.** Knowledge concerning concordance of epidermal growth factor receptor 2 (HER2) expression between primary breast cancers and asynchronous local-regional recurrences is sparse. Receptor characteristics could be altered with time and may be affected by anticancer treatment. It remains uncertain whether recurrences have the identical or similar HER2 receptor expression pattern as the primary breast cancer. The aim of the present study was to evaluate whether HER2 is stable during the process of recurrence. Expression of HER2 was investigated immunohistochemically in paired samples of primary breast cancers and corresponding asynchronous local-regional recurrences (n=35). HER2 expression was scored as 0, 1+, 2+ or 3+. HER2 overexpression (2+ or 3+) was found in 48.57% (17/35) of the primary breast cancers and 45.71% (16/35) of the corresponding local-regional recurrences. A concordance of HER2 overexpression between the primary lesions and matching regional recurrences was observed in 85.71% of the breast cancer cases. Five out of the 35 paired samples (14.28%) were discordant. Only 3 patients who had 2+ HER2 expression in the primary tumors showed HER2 down-regulation (0 or 1+) in the recurrences, while the HER2 score in 2 patients changed oppositely. Moreover, all of the cases with 3+ HER2 staining in the primary lesions retained HER2 overexpression in the recurrences. The HER2 is commonly expressed in breast cancer, and its expression in the primary tumors and the corresponding recurrences was concordant in the majority of the cases. As the receptor

expression may lose or gain in recurrences at a probability of approximately 10%, assessment of the receptor status in recurrences is still encouraged.

## Introduction

Breast cancer represents the most common malignancy affecting women in developed countries, with more than 200,000 new cases diagnosed yearly in the US (1). Moreover, the incidence rates have increased rapidly in previously low-incidence areas, such as China (2), partly due to changes in life style and professional habits, as well as the progression of urbanization.

The last decade has witnessed significant achievements in the management of advanced breast cancer, including the introduction of novel chemotherapeutic agents (3,4), the use of aromatase inhibitors in post-menopausal women (5,6), and the benefits derived from molecular-targeted agents, e.g., trastuzumab (7,8) and lapatinib (9), in patients with epidermal growth factor receptor 2 (HER2)-overexpressing tumors. Despite aggressive multidisciplinary treatment approaches, the prognosis of metastatic breast cancer remains poor, with a median survival of 20 months (10).

HER2, a member of the epidermal growth factor receptor family, is a major target for molecular-targeted therapy in breast cancer. HER2 locates at human chromosome 17q11.2-12, encoding a transmembrane tyrosine kinase that is composed of three distinct regions: an extracellular region containing a ligand-binding domain, a transmembrane domain and an intracellular region harboring a tyrosine kinase domain. Ligand binding leads to receptor dimerization, autophosphorylation and subsequent activation of intrinsic tyrosine kinase activity. Activation of HER2 receptors initiates a series of downstream signaling pathways that regulate various cellular functions, including cell proliferation, apoptosis, angiogenesis and motility.

Although HER2 is not expressed on the cell surface of many normal tissues (11), HER2 gene amplification and protein overexpression are present in 20-30% of breast cancers (12-14). HER2 receptor has become an important target for targeted

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cancer therapy with trastuzumab (Herceptin®). Trastuzumab, a humanized monoclonal antibody (15), has revolutionized therapy for patients with metastatic breast cancers. Studies have indicated that trastuzumab is particularly effective in the treatment of HER2-positive metastatic breast cancer.

Overexpression of HER2 has been identified in human breast cancers (12-14). Although similar HER2 receptor expression between primary breast cancers and metastatic lymph nodes has also been reported (16-18), there are only a few reports regarding the comparison of the HER2 status between the primary breast cancer and the distant metastatic lesions (19,20). To date, the literature regarding the concordance of HER2 receptor expression between primary and local-regional recurrences is sparse. It remains uncertain whether recurrences have the identical or similar HER2 receptor expression pattern as the primary breast cancer.

Receptor overexpression, together with a similar expression in both primary tumors and disseminated lesions, is considered necessary for the success of targeted therapy, particularly targeted nuclide radiotherapy. In receptor-mediated tumor targeting nuclide radiotherapy, tumor cells are killed with delivered radiation, and therapeutic efficiency is mainly dependent on the receptor expression (21). However, in most studies, samples for analysis are usually obtained from the primary lesion, and the status of the targeted molecules is determined based only on the primary tumor. In the present study, the expression of HER2 was investigated immunohistochemically in a series of primary breast cancer samples and corresponding local-regional recurrent lesions.

## Materials and methods

**Patients and samples.** Thirty-five breast cancer patients with formalin-fixed, paraffin-embedded tumor samples available from untreated primary tumors and later clinically manifested local or regional recurrent tumor deposits were identified in the specimen database of the Department of Pathology, Second Affiliated Hospital Zhejiang University and Shaoxing Hospital. The time period between surgical removal of the primary tumor and surgery or biopsy of the recurrent tumor lesion ranged from 5 to 61 months (median 20). Two separate metastatic lesions (chest wall and supraclavicular) were available for this study from 2 patients. The site of recurrence was chest wall in 32 cases, supraclavicular recurrence in 1 case and 2 were both chest wall and supraclavicular recurrences. The patients were diagnosed between the years 1998 and 2010, and the patient age at diagnosis ranged from 31 to 74 years (median 51). Twenty-five (71.4%) cases were invasive ductal carcinomas, 1 (2.8%) was invasive lobular carcinoma, 2 (5.7%) were mucinous adenocarcinomas, 6 (17.1%) were carcinoma simplex and 1 (2.8%) was medullary cancer. Six cases had no lymph node metastasis, 9 cases had 1-3 metastatic lymph nodes, 15 cases had  $\geq 4$  metastatic lymph nodes, and the lymph node status was not available in the other 5 cases. All patients had no distant metastasis at the time of diagnosis.

**HER2 staining.** The study was carried out under approval of the Institutional Review Board in accordance with the Declaration of Helsinki. The tissues were fixed in 4% buffered formalin, processed and embedded in paraffin. Sections

Table I. HER2 scores for the analyzed primary breast cancers and the paired asynchronous local-regional recurrences (n=35).

	Local-regional recurrences HER2-scores			
	0	1+	2+	3+
Primary tumor				
HER2 scores				
0	7	0	0	0
1+	2	7	1	1
2+	0	3	3	2
3+	0	0	5	4

0, completely negative staining; 1+, faint perceptible staining of the tumor cell membranes; 2+, moderate staining of the entire tumor cell membranes; 3+, strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern.

(4- $\mu$ m) were then cut and deparaffinized in xylene and hydrated through graded concentrations of ethanol to distilled water. The HER2 immunohistochemical staining was carried out as previously described (22). After deparaffinization, the sections were incubated in methanol and hydrogen peroxide for 30 min to quench the endogenous peroxidase. Antigen retrieval was performed in a waterbath at 98°C, pH 6.0 for 40 min. Thereafter, the slides were cooled at room temperature and then washed in distilled water. Immunohistochemical staining was performed using the Elite ABC kit (Vectastain; Vector Laboratories, Burlingame, CA, USA). Blocking serum was applied for 15 min followed by incubation with rabbit anti-human c-erbB-2 oncoprotein (code no. A 0485; Dako), diluted 1:350. Sections were then incubated with the biotinylated secondary antibody and were visualized using the peroxidase substrate 3-amino-9-ethyl-carbazole (AEC) (A-5754; Sigma) as chromogen. Finally, the sections were counterstained with Mayer's hematoxylin and mounted.

**HER2 scores.** HER2 expression was scored using the HercepTest scoring criterion. The HER2 score was based on a scale where 0 corresponded to tumor cells that were completely negative, 1+ corresponded to faint perceptible staining of the tumor cell membranes, 2+ corresponded to moderate staining of the entire tumor cell membranes and 3+ was strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern. The Canadian and the Dako HercepTest guidelines were applied, which require >10% of the tumor cells to be stained (23). Cytoplasmic staining was considered non-specific and was not included in the scoring. As positive controls, in-house positive control tissue sections were used, as well as positive control sections supplied by Dako. As negative controls, normal tissues were used, which are expected not to express HER2, such as connective tissue observed in the same sections as the tumor cells. In sections of lymph node metastases, lymphocytes and the surrounding capsule of the lymph nodes were used as negative internal controls.

Table II. Discordant data between the primary lesions and paired recurrences.

Case no.	Primary tumor (HER2)	Date	Metastasis (HER2)	Metastatic site	Date
1	Negative	August, 2005	Positive	Chest wall and supraclavicular	April, 2008
2	Positive	November, 1998	Negative	Supraclavicular	May, 2001
3	Positive	May, 1999	Negative	Chest wall	July, 2000
4	Negative	February, 2003	Positive	Chest wall	November, 2004
5	Positive	January, 2003	Negative	Chest wall	January, 2004

Table III. Major results from the HER2-score analyses of breast cancer (n=35).

HER2-score characteristics	Cases (%)
Primary tumors with 2+ or 3+	17 (48.57)
Local-regional recurrences with 2+ or 3+	16 (45.71)
Unchanged HER2 scores in local-regional recurrences vs. the primary tumors	30 (85.71)
Changed EGFR scores in local-regional recurrences vs. the primary tumors	5 (14.29)
Patients who had a score of 0 or 1+ in primary tumors which changed to 2+ or 3+ in local-regional recurrences	2 (5.71)
Patients who had a score of 2+ or 3+ in primary tumors which changed to 0 or 1+ in local-regional recurrences	3 (8.57)

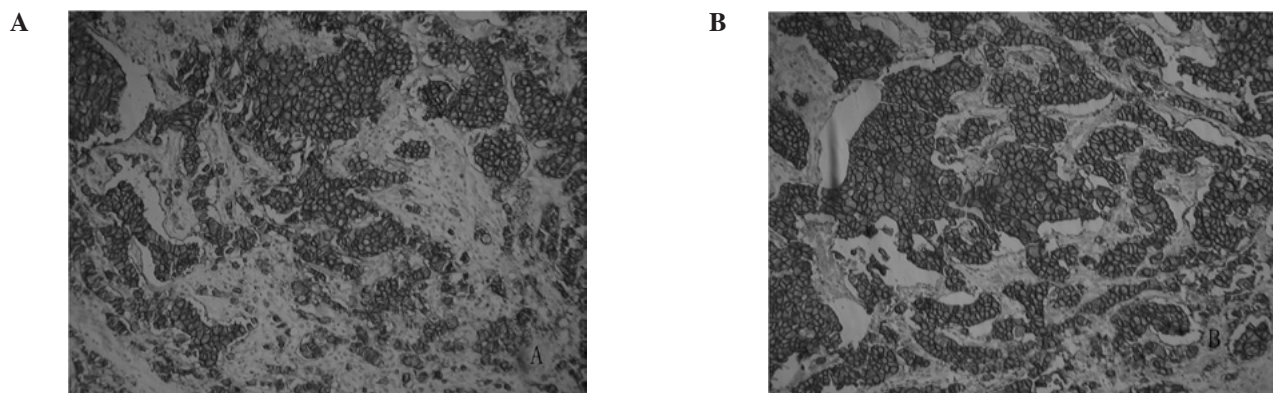


Figure 1. Comparison of the immunohistochemical HER2 staining of (A) primary breast cancer and (B) corresponding chest wall recurrence. Both (from the same patient) scored 3+ (magnification, x40).

**Excluded cases.** In 10 cases, no primary tumor blocks were found in the specimen database, as these cases were previously treated at other hospitals. In another case, there were no tumor cells in the sections supposed to be recurrent breast cancer. Thus, 46 patient cases with local/regional recurrences were initially identified, but finally 35 cases with high-quality material of both primary tumors and the corresponding recurrences were investigated in the study.

## Results

Table I shows the HER2 scores for the analyzed 35 primary breast cancers and the corresponding local-regional recurrences. HER2 overexpression (2+ or 3+) was found in 48.57% (17/35) of the primary breast cancers and 45.71% (16/35) of the corresponding local-regional recurrences. There was a good agreement between the primary tumors and the paired asynchronous local-regional recurrences in the majority of cases.

A concordance of HER2 overexpression between the primary lesions and matching regional recurrences was observed in 85.71% of the breast cancer cases. Five changes were observed. However, there were only 3 patients who had HER2 overexpression (all 3 cases had 2+ HER2 staining) in the primary tumors which changed to 1+ in the chest wall recurrences; in another 2 patients, the score of 1+ in the primary tumors switched to 2+ or 3+ in local-regional recurrences. Moreover, all cases with 3+ HER2 staining in the primary lesions retained HER2 overexpression in the recurrences. The major results from the HER2-score analyses are summarized in Tables II and III. Examples of staining patterns for a primary tumor and the corresponding metastases (which were both scored as 3+) are shown in Fig. 1A and B.

Two cases had both chest wall and supraclavicular recurrent samples. The same HER2 scores were noted between the chest wall and supraclavicular lesions; 1 case was scored as 2+ and the other had negative staining.



## Discussion

Anti-HER2-targeted therapy, e.g., trastuzumab, for recurrent or metastatic breast cancer is generally thought to be reasonable when the primary lesions of the breast overexpress HER2 receptor. In several cases, however, the recurrent lesions show no response to trastuzumab treatment, even though the primary breast cancer exhibits strong HER2 expression (8). Heterogeneity of the receptor status in primary and metastatic breast cancer has been confirmed, and a loss of HER2 receptor in recurrent lesions, which may be affected by the intervening treatment, is known to be associated with a poor response to anti-HER2-targeted therapy. Yet, knowledge of HER2 expression in local-regional recurrences of breast cancer is relatively limited. For a receptor to be of interest for targeting, a similar expression in both the primary tumors and the disseminated lesions is required. Investigation of the concordance of the receptor status between recurrences or metastases and the primary tumors will provide valuable information on whether the receptor is suitable as a target for diagnostic and/or therapeutic procedures. In the present study, the expression of HER2 was investigated immunohistochemically in paired samples from a series of primary breast lesions and corresponding local-regional recurrences.

HER2 overexpression (2+/3+) was found in 48.57% of the primary lesions and 45.71% of the local-regional recurrences. HER2 expression in breast cancer has been commonly reported to range from 20 to 30% (12-14). Our result showed a higher percentage since our analyzed cases represented a more malignant subgroup which developed recurrences. Studies reporting a higher HER2 expression rate were also noted in the literature. Carlsson *et al* (17), using the same scoring criteria, found HER2 expression in 55% of the analyzed primary breast cancers and lymph node metastases. Braun *et al* (24) reported HER2 overexpression in 60% of breast cancers with bone marrow metastases.

Furthermore, a good agreement was noted between the primary tumors and the paired asynchronous recurrences in the majority of our studied cases. A concordance of HER2 overexpression between the primary lesions and matching regional recurrences was observed in 85.71% of the breast cancer cases. Previous studies mainly focused on the concordance of the HER2 status between primary breast cancer and synchronous lymph node metastases, or between primary tumors and distant metastases, while reports concerning local-regional recurrences are relatively limited. The reported prevalence of concordance of the HER2 status between primary tumors and synchronous lymph node metastases ranges from 82 to 94% (16,25), and that between primary tumors and distant metastases ranges from 92.4 to 97% (19,26,27). Our data of local-regional recurrences are consistent with these former findings; only 3 patients with HER2 overexpression (scored as 2+) in the primary tumors had lower HER2 scores in the corresponding recurrences, and in another 2 patients the score of 1+ in the primary tumors switched to 2+ or 3+ in the local-regional recurrences. Moreover, all cases with 3+ HER2 staining in the primary lesions retained HER2 overexpression in the recurrences.

Although trastuzumab-based therapy is commonly used to treat metastatic disease, HER2 status is generally evaluated in the primary lesions since, in most cases, the metastatic lesions

are not removed or biopsied prior to treatment. With regards to more recent clinical trials (8,28), only 50% of the metastatic breast cancer patients with HER2 overexpression respond to trastuzumab treatment. There may be many reasons for the poor response to trastuzumab (29). One of the explanations may be the heterogeneity of expression of HER2 between the primary lesions and metastatic tumors, as receptor characteristics change with time and may be affected by anticancer treatment. However, based on our result and other reports, it appears that heterogeneity is an unlikely explanation.

The HER2 is commonly expressed in breast cancer, and its expression in primary tumors and the corresponding recurrences was concordant in the majority of cases. Our results add to the body of data on the subject. As the receptor expression may lose or gain in recurrences at a probability of approximately 10%, assessment of the receptor status in recurrent lesions is encouraged.

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