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Original Articles

Discordant Human Epidermal Growth Factor Receptor 2 and Hormone Receptor Status in Primary and Metastatic Breast Cancer and Response to Trastuzumab

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Background: Recent studies have shown that the human epidermal growth factor receptor 2 status of a metastatic site may differ from that of the primary site. This difference may influence patient prognosis and response to therapy.
Methods: We conducted a retrospective study using immunohistochemistry and/or fluorescent *in situ* hybridization to compare human epidermal growth factor receptor 2 and hormone receptor status in primary and metastatic breast cancers.
Results: Fifty-six patients were included in this study. Conversion from hormone receptor positive in the primary tumor to hormone receptor negative in the metastasis occurred in 12 patients (21.4%), and hormone receptor negative to hormone receptor positive conversion occurred in two patients (3.6%). Human epidermal growth factor receptor 2 status was discordant between primary and metastatic lesions in seven patients (12.5%). All of the five patients who converted from human epidermal growth factor receptor 2 negative status to human epidermal growth factor receptor positive received trastuzumab-based chemotherapy. Overall response rate and median progression-free survival for concordant human epidermal growth factor receptor 2 positive patients were 69.2% and 16.9 months, whereas that of patients with positive conversion of human epidermal growth factor receptor 2 were 40.0% and 7.6 months, respectively (overall response rate; $P = 0.169$ and progression-free survival; $P = 0.004$).
Conclusion: Discordance in human epidermal growth factor receptor 2 and hormone receptor status between primary and metastatic tumors was observed, which led to altered treatment decisions. Evaluation of human epidermal growth factor receptor 2 and hormone receptor in metastatic tumors should be considered in patients with breast cancer.

Key words: HER2 – hormone receptor – trastuzumab – breast cancer

INTRODUCTION

Breast cancer is the most common malignancy in women. Breast cancer is newly diagnosed in approximately 200 000 patients annually and is estimated to have caused more than 70 000 deaths in the USA in the year 2009 (1). In Korea, the incidence of breast cancer has continuously increased and has become the most common malignancy (2). Treatment of breast cancer is based on tumor stage, histopathologic features, hormone receptor (HR) status and human epidermal growth factor receptor 2 (HER2) status (3).

Approximately 70% of breast cancers are HR positive. The estrogen receptor (ER) is an important regulator of both physiologic and pathologic mammary growth and differentiation (4). HR expression is also the most important predictor of response to endocrine therapies such as tamoxifen or aromatase inhibitors (5). HER2 oncogene, which is located on chromosome 17q21, encodes a 185-kDa transmembrane tyrosine kinase receptor with extensive homology to the epidermal growth factor receptor (EGFR) (6). Approximately 25–30% of patients with breast cancer demonstrate tumor amplification of the HER2 gene or over-expression of the HER2 protein, which are associated with poor prognosis and decreased overall survival (OS) (7).

Therapies targeting the HER2 receptor and its pathway have been developed for patients with HER2-positive breast cancer (8). Trastuzumab, a monoclonal antibody that binds to the HER2 receptor, has markedly increased survival time for HER2-positive patients. Trastuzumab has significantly improved treatment outcomes for HER2-positive metastatic breast cancer and is currently the standard treatment in such patients (9). In early breast cancer, the addition of trastuzumab to the adjuvant chemotherapy significantly decreased recurrence in HER2-positive breast cancer patients (10,11). Lapatinib is a reversible small-molecule dual-tyrosine kinase inhibitor that targets both HER2 and EGFR. Lapatinib shows activity in HER2-positive breast cancer as a single agent or in combination with chemotherapy. Lapatinib also produces response in patients who had progression with previous trastuzumab treatment and lapatinib plus capecitabine is an effective option in these patients (12–14).

It is important to determine the HR and HER2 status of each breast cancer patient to select the appropriate therapy. Metastatic breast cancer is usually diagnosed by a combination of clinical signs and symptoms as well as radiological evaluation. Confirmatory biopsy of metastatic lesions is not a routine clinical practice in most centers, and previous studies have suggested that HER2 expression in metastatic breast cancer is stable throughout the course of the disease (15,16). However, recent studies have revealed discordance between primary and distant metastatic tumors in up to one-third of cases (17,18). These studies suggest that determination of HER2 status at metastatic sites may have value for therapeutic decisions and may influence prognosis.

The purpose of the present study was to compare tumor HR and HER2 status between primary and distant metastatic sites. Furthermore, we sought to evaluate the impact of HER2 conversion in metastatic lesions on prognosis and response to trastuzumab treatment.

PATIENTS AND METHODS

Patients

In this retrospective study, we analyzed cases of metastatic breast carcinoma and evaluated HR and HER2 expression from primary and metastatic lesions treated at the Seoul National University Hospital between January 2003 and June 2009. Patients with HR and HER2 results available from primary and metastatic tumors were included in the present analysis. Clinicopathologic data and follow-up information, including results from treatment with adjuvant hormone therapy, trastuzumab and lapatinib, were retrieved from medical records. Patients were classified by change (or lack of change) in HER2 status from the primary to metastatic sites as follows: Group 1 (negative to negative), Group 2 (positive to positive), Group 3 (negative to positive) and Group 4 (positive to negative). The tumor response was evaluated using the Response Evaluation Criteria in Solid Tumors criteria.

This study protocol was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB protocol number 0906-091-284). Because this study was a retrospective analysis that involved no more than minimal risk for patients, the IRB approved our request for a waiver of informed consent.

Tumor Tissue Samples

Pathological evaluation included tumor type, stage according to the criteria established by the 6th edition of the American Joint Committee on Cancer (19) and histologic and nuclear grade according to the Elston and Ellis modification of the Scarff-Bloom-Richardson grading system (20). The assessments of ER, HER2 and progesterone receptor (PR) expressions for primary and metastatic tissues were performed using immunohistochemistry (IHC). Sections (4 μm thick) of the tissue array block were cut, dried, de-paraffinized in xylene and then rehydrated through a graded alcohol series to distilled water. For antigen retrieval, slides were placed in citric acid and heated in a microwave oven. Tissue sections were incubated with mouse primary antibodies against ER (Dako Corporation, Carpinteria, CA), PR (Dako Corporation) and HER2 (Novocastra Laboratories Ltd., New Castle-Upon-Tyne, UK). Membrane staining was evaluated for HER2 protein expression using the DAKO HercepTest scoring system (Dako Corporation, Carpinteria, CA). Fluorescence *in situ* hybridization (FISH) was performed using the PathVysion kit (Vysis, Downers Grove, IL) with 4-μm tissue sections according to the manufacturer's instructions. The genetic variables reported were HER2 gene copy number, chromosome 17 copy number and mean HER2 gene to chromosome 17 centromere (HER2/CEP17) ratio. The HER2 gene was considered to be amplified in tumors with mean HER2/CEP17 ratios $\geq$2.0 (21,22). HR positivity was defined as positivity for ER or PR, or both. ER- and PR positive results were defined as nuclear staining in $\geq$10% of the tumor cells. HER2-positive was defined as an IHC score of 3+, HER2 amplification as assessed by FISH in case of IHC 2+, whereas HER2 negative was defined as an IHC score of 0 or 1+, and no HER2 amplification by FISH in case of IHC 2+ (23). All of the testing was performed in the same clinical pathology facility using

standard clinical protocols and was reviewed by two pathologists.

Statistical Analysis

Statistical analyses were performed with SPSS for Windows, version 17.0 (SPSS, Inc., Chicago, IL, USA). Comparisons between groups were analyzed using Pearson's χ^2 test or Fisher's exact test, as appropriate. Two-sided *P*-values of <0.05 were considered statistically significant. Progression-free survival (PFS) was calculated from the first day of palliative trastuzumab- or lapatinib-based chemotherapy to the day of progression or last follow-up visit. OS was calculated from the first day of initial diagnosis or metastasis diagnosis to the day of death or last follow-up visit. PFS and OS were calculated with the Kaplan–Meier technique. Survival was compared between groups using log-rank test.

RESULTS

Patient Characteristics

Patient and primary tumor characteristics are summarized in Table 1. Both primary and metastatic lesions were analyzed for HR and HER2 status in a total of 56 patients. All primary lesions were obtained from surgical specimens. Primary lesions were HR positive in 30 patients and HER2 positive in 15 patients. All but three metastases were asynchronous. Median interval between biopsies of primary and metastatic tumors was 23.7 months (range, 0–68.8 months). The most common metastatic site for biopsy was the liver ($n = 23$, 41.0%).

Discordance of HR and HER2 Status

Conversion from an ER-negative primary tumor to an ER-positive metastatic tumor occurred in 9 patients (16.1%), whereas conversion from ER-positive primary tumor to ER-negative metastatic tumor occurred in 8 patients (14.2%). PR status in four patients (7.1%) changed from negative in the primary tumor to positive at the metastatic site, whereas PR status switched from positive to negative in 10 patients (17.9%). As shown in Table 2, combining ER and PR status, 30 patients (53.5%) had HR-positive primary tumors; positive to negative conversion occurred in 8 patients (14.3%) and negative to positive conversion occurred in 6 (10.7%).

Of the total 56 patients, 49 (87.5%) showed a concordant HER2 status between the primary tumor and the metastatic lesion. Among the seven patients (12.5%) with discordant HER2 status, HER2-negative primary tumor and HER-positive metastatic site were observed in five (8.9%), whereas HER2-positive primary tumor and HER2-negative metastatic site were observed in two (3.6%). Table 3 details discordant HER2 statuses between primary and metastatic sites as determined by IHC and FISH.

Table 1. Clinicopathologic characteristics of patients and primary breast cancer tumors

Characteristics	Patients, $n = 56$ (%)
Age (years)	
Median	48
Range	32–73
Tumor size	
T1	10 (17.9)
T2	41 (73.2)
T3	4 (7.1)
T4	1 (1.8)
Histologic grade ($n = 52$)	
I	1 (1.9)
II	24 (46.2)
III	27 (51.9)
Nuclear grade ($n = 52$)	
1	1 (1.9)
2	18 (34.6)
3	33 (63.5)
Hormone status in primary site	
ER+/PR+	17 (30.4)
ER+/PR−	9 (16.1)
ER−/PR−	26 (46.4)
ER−/PR+	4 (7.1)
HER2 status in primary site (immunohistochemistry)	
Negative	25 (44.6)
+	12 (21.4)
++	12 (21.4)
+++	7 (12.5)
Metastatic sites of biopsy	
Liver	23 (41.0)
Lung	12 (21.4)
Lymph node	10 (17.6)
Bone	5 (8.9)
Others	6 (10.7)[a]
Number of metastatic sites	
Median	2
Range	1–4

[a]Others: ovary 2, pleura 2, muscle of hip 1, back muscle 1.

Of these 30 patients who had HR positive in primary breast cancer, positive to negative conversion occurred in 12 (40%). Of the 26 patient s whose primary tumor was HR negative, negative to positive conversion occurred in 2 (7.7%). These two patients received tamoxifen and chemotherapy for adjuvant setting, and metastases were diagnosed during adjuvant tamoxifen therapy. HR-positive/HER2-positive primary tumors were observed in four

Table 2. Hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status in primary and metastatic sites ($n = 56$)

Primary site HR and HER2 status	Patients, *n*	Metastatic site HR and HER2 status	Patients, *n* (% converted)
HR(+), HER2(−)	26	HR(+), HER2(−)	20 (76.9)
		HR(−), HER2(+)	3 (11.5)
		HR(−), HER2(−)	3 (11.5)
HR(−), HER2(+)	11	HR(−), HER2(+)	7 (63.6)
		HR(+), HER2(−)	1 (9.1)
		HR(−), HER2(−)	1 (9.1)
		HR(+), HER2(+)	2 (18.2)
HR(−), HER(−)	15	HR(−), HER(−)	10 (66.7)
		HR(+), HER(−)	3 (20.0)
		HR(−), HER2 (+)	2 (13.3)
HR(+), HER(+)	4	HR(+), HER(+)	2 (50.0)
		HR(−), HER2 (+)	2 (50.0)

Table 3. HER2 status assessed by immunohistochemistry (IHC) or fluorescence *in situ* hybridization in group had discordance between primary and metastatic breast cancer ($n = 7$)

	HER2 by IHC in primary lesion	HER2 by IHC in metastatic lesion	HER2 status (HER2/CEP17 ratio) in primary lesion	HER2 status (HER2/CEP17 ratio) in metastatic lesion
Case 1	0	3	ND	ND
Case 2	2	3	1.23	ND
Case 3	2	3	1.14	10
Case 4	1	2	ND	4.41
Case 5	1	3	ND	ND
Case 6	2	1	8.17	ND
Case 7	2	1	6.45	ND

ND, not done.

patients. HR-negative/HER2-negative primary tumors were observed in 15 patients; positive conversion of HER2 status, but no HR status conversion occurred in two of these patients (13.3%). Tumors from 2 of 11 patients (18.2%) with HR-negative/HER2-positive status converted to HER2-negative status in the metastatic sites; one of the two patients had concordant HR status in the metastatic site.

HER2 Discordance and Outcome of Trastuzumab Treatment

All patients with HER2 over-expression in the primary and/or metastatic sites received trastuzumab therapy. One patient received trastuzumab as first-line chemotherapy for both the adjuvant setting and the metastatic setting. Four patients received lapatinib; one of the four was treated in the adjuvant setting, and the others were treated in the metastatic setting. Of the five patients who converted from HER2-negative status to HER2 positive, all five received trastuzumab after diagnosis of HER2-positive status and four patients received trastuzumab-based therapy as first-line chemotherapy. Two patients achieved partial response, one experienced stable disease and two had progressive disease with trastuzumab therapy. Therefore, overall response rate (ORR) for first-line treatment—trastuzumab therapy—was 40% (95% CI: 11.8–76.9%) in patients with positive HER2 conversion. Two patients who converted from HER2 positive to HER2 negative were treated with trastuzumab for metastatic disease, but not as adjuvant therapy. One patient had PR and the other had PD with trastuzumab. In patients with concordant HER2-positive status, two patients achieved complete response (15.4%), seven patients showed PR (53.8%) and two patients had SD (15.4%). ORR in Group 2 (positive to positive) was 69.2% (95% CI: 42.4–87.3%). There was no significant difference between ORRs of patients who converted from HER2-positive status to HER2-positive status and patients who converted from HER2-negative status to HER2-positive status ($P = 0.169$).

Survival from the time of primary breast cancer diagnosis was compared among patient groups classified by HER2 status in the primary and metastatic sites. The median OS from diagnosis of primary breast cancer was not reached for Group 1 (negative to negative), but was 60.9 months for Group 2 (positive to positive), and 51.6 months for Group 3 (negative to positive) ($P = 0.434$). The median OS from diagnosis of metastasis was not reached for Group 1, but was 32.3 months for Group 2 (positive to positive), and 16.3 months for Group 3 (positive to negative) ($P = 0.710$). From the initiation of trastuzumab therapy, the median PFS was 5.4 months (95% CI: 4.1–25.5 months) and median OS was 26.6 months (95% CI: 7.1–46.1 months). The median PFS for patients treated with trastuzumab was 16.9 months for Group 2 (positive to positive) and 7.6 months for Group 3 (positive to negative) ($P = 0.004$) (Fig. 1A). The median OS for patients treated with trastuzumab was 31.8 months for Group 2 (positive to positive) and 11.1 months for Group 3 (positive to negative) ($P = 0.023$) (Fig. 1B).

DISCUSSION

HER2 status is an important predictive and prognostic factor in breast cancer. HER2 over-expression is associated with a poor prognosis (7), and is an important indicator for responsiveness to trastuzumab treatment. Therefore, conversion of HER2 status between primary and metastatic tumors in patients with metastatic breast cancer is important. Until recently, initiation of trastuzumab therapy was determined according to the HER2 status of the primary lesion, because gene expression was thought to remain constant between the primary tumor and distant metastases. However, recent studies have reported discordance in HER2 status ranging

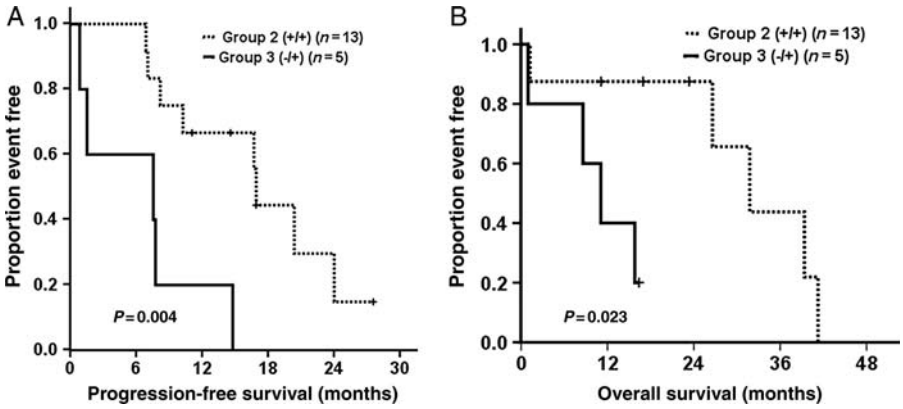

Figure 1. Progression-free survival (PFS) and overall survival (OS) from initiation of trastuzumab based on HER2 status in the primary and metastatic lesions. Median PFS was higher in Group 2 (positive/positive) compared with Group 3 (negative/positive) ($P = 0.004$) (A), and median OS was higher in Group 2 (positive/positive) compared with Group 3 (negative/positive) ($P = 0.023$) (B).

from 0 to 33.3% between the primary tumor and metastatic sites (17,18). And Amir et al. (24) demonstrated discordance of receptor status in largest prospective analysis. In our study, 12.5% of the 56 cases showed discordance between primary and metastatic HER2 expression; this result is similar to those of recent studies.

Another powerful prognostic and predictive factor is HR status. Recent studies have demonstrated the mutual effect of ER and HER2. The ER signaling interacts with other growth factor signaling pathways in breast cancer cells, and members of the HER family may contribute to the development of tamoxifen resistance (25). In this setting, tamoxifen may lose its estrogen antagonist activity and acquire more agonist-like activity, resulting in tumor growth stimulation (26). Massarweh *et al*. (27) demonstrated in an *in vivo* model of MCF-7 breast cancer cells that EGFR/HER2 may mediate tamoxifen resistance in ER-positive breast cancer, despite continued suppression of ER genomic function by tamoxifen. This result suggests that combining HER inhibitors with tamoxifen may be a useful strategy, even in tumors that do not initially over-express HER2. This acquired endocrine resistance can be associated with modest adaptive increases in HER2 and suppressed ER expression and function (28). In this aspect, we analyzed the concordance of HR connection with HER2 status. Our study showed conversion of HR-positive status to HR-negative in three of five patients who had discordant HER2 status with positive conversion; they were treated with tamoxifen as adjuvant hormone therapy and experienced metastasis during tamoxifen.

A previous study revealed that patients with HER2-negative primary cancer and HER2-positive metastasis experienced the best survival (18). In contrast, Liedtke *et al*. (29) evaluated ER, PR and HER2 status in patients with recurrent breast cancer and showed that patients with concordant receptor-positive breast cancer had significantly better post-recurrence survival compared with discordant cases. In the present study, patients in Group 3 (negative to positive) had a trend of lower response rate for trastuzumab than Group 2 (positive to positive) ($P = 0.169$). And they experienced shorter PFS and OS from initiation of trastuzumab therapy compared with patients in Group 2 (positive to positive). Group 3 (negative to positive) also had a shorter OS from diagnosis of metastasis compared with Group 2 (positive to positive) (16.3 vs. 32.3 months), although this difference was not significant ($P = 0.244$).

There could be two possible explanations for these results. First, accurate interpretation of HER2 status can be difficult. In a recent study, considerable variability occurred in interpretation of cases with low-level or borderline amplification (30). They defined 'borderline' HER2/CEP17 ratio as slightly lower or higher than the threshold of 2.0. Moreover, in an international ring study (31), the concordance rate for IHC scoring was 45% in categories of negative, equivocal, and positive, and the rate for FISH scoring was 80%. The concordance rate was similar in a Japanese ring study, despite the increased number of participants (32). Both studies showed that equivocal IHC and borderline FISH cases are difficult to interpret. Although we evaluated HER2 status in a single institute, equivocal IHC and borderline FISH data could affect the interpretation of results. However, patients in the present study who showed discordance between the primary and metastatic lesions did not have borderline HER2/CEP17 ratios. A second possible explanation for our results is the genetic heterogeneity of breast cancer. Genetic heterogeneity within individual breast carcinomas is believed to result from the growing genetic instability of subclonal populations of tumor cells subjected to different host selection pressures, such as variations in growth environment, which lead to clonal diversification and differences in genetic composition (33). Intratumoral heterogeneity in breast cancer has been reported by many researchers. Kuukasjarvi *et al*. (34) reported that the genetic composition of 315 metastases differed almost completely from that of the paired primary breast cancer, and Moeder *et al*. (35) suggested that heterogeneity of HER2 gene amplification within a given tumor may affect treatment response to therapy by selecting resistant subclones. These findings suggest that intratumoral HER2 heterogeneity may explain

some of the unexpected failures of trastuzumab therapy, and it also may explain our results showing the effect of HER2 status on PFS and OS after initiation of trastuzumab, that is, heterogeneity of breast cancer suggests that patients with positive conversion may have fewer subclones that responded to trastuzumab chemotherapy compared with HER2-positive concordant patients.

Limitations of the present study include its retrospective design and the small number of subjects. As mentioned above, in a Japanese ring study, inter-institutional and inter-observer discrepancies in HER2 status were attributed to the evaluation process in 33.0% of the samples, staining procedures in 25.0% and a combination of both factors in 41.7%. However, analysis by FISH, resulted in consistency across multiple institutions (32). A major limitation of the current study, however, is that it was performed retrospectively, and we were not able to reanalyze all of the molecular markers.

In particular, the number of patients who were treated with trastuzumab was very small. Moreover, we only included the patients who were available for HR and HER2 results in primary and metastatic tumors. This inclusion could induce a potential selection bias. However, our study demonstrated that HER2-positive conversion of metastatic site critically changed the treatment of metastatic breast cancer, although patients with HER2-positive conversion had an inferior outcome of trastuzumab. Therefore, we cannot conclude whether a similarity or difference of HER2 status between the primary and metastatic sites predicts the outcome of treatment with trastuzumab. Additional studies with larger numbers of patients and prospective studies are needed to verify our results, and moreover further studies are needed to research on explainable underlying mechanism.

In conclusion, this study demonstrates a relatively high discordance rate (12.5%) in HER-2 amplification between primary and metastatic lesions of the same breast cancer, which led to trastuzumab treatment in patients who had a positive conversion of HER2. Therefore, we recommend evaluating HER2 status in metastatic sites to establish optimal treatment strategy in individual patients.

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