Original Article

Prognostic Significance of Epidermal Growth Factor Receptor Gene Mutations and Human Epidermal Growth Factor 2 Expression in Breast Carcinoma Metastatic to the Liver

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Abstract

Background: Breast cancer is the most prevalent cancer among females, and metastatic disease is not curable and is treated palliatively. Members of the ErbB family have an important role in the development and progression of breast cancer. The aim of this study was to determine the relationship between epidermal growth factor receptor (EGFR) gene mutation, human epidermal growth factor 2 (HER2) expression, hormone receptor statuses, and clinicopathological parameters in liver metastases from breast cancer. **Materials and Methods:** This study included 41 patients diagnosed with liver metastasis from breast carcinoma, based on morphological and immunohistochemical findings, in our pathology laboratory between 2011 and 2018. EGFR gene mutations were analyzed by polymerase chain reaction (PCR) in these cases. **Results:** EGFR gene mutation analysis was performed by PCR, and no mutations were detected. HER2 and ER statuses of the primary breast tumor were available in 23 cases. HER2 status conversions were present in 9 cases (39.1%); however, this was not statistically significant (P = 0.197). Estrogen receptor (ER) conversions were present in 4 cases (17.4%); however, this was not statistically significant (P = 1.000). Progesterone receptor (PR) conversions were detected in 10 cases (45.5%). There were 10 (45.5%) cases with PR-positive primary tumors and PR-negative liver metastases. No cases with a PR-negative primary tumor developed a PR-positive liver metastasis (P = 0.02). **Conclusions:** No EGFR gene mutations were detected in any of our cases by PCR. There was no statistically significant relationship between clinicopathological parameters and EGFR mutation. The comparison of ER, PR, and HER2 expression between the primary tumor and metastases revealed status conversions in some cases. However, only PR conversion was statistically significant. Studies on EGFR gene mutations that include larger series are warranted to identify the candidates who can benefit from targeted therapies.

Keywords: *Breast carcinoma, epidermal growth factor receptor, hormone receptors, human epidermal growth factor 2, liver metastasis*

Introduction

Breast cancer is the most prevalent cancer among females and 30%–40% of women with breast cancer develop metastatic disease. Metastatic disease is not curable and is treated palliatively.[1,2]

Estrogen receptor (ER) is a nuclear transcription factor that is activated by estrogen. It controls the development and differentiation of normal, hyperplastic, and neoplastic breast tissues. ER-positive and progesterone receptor (PR)-positive tumors have a more favorable prognosis. Approximately 70% of primary breast cancers and 45% of breast carcinoma metastases are ER positive. Hormone receptor-positive tumors respond better to hormonal treatment and manifest a more favorable prognosis.[3,4]

Receptors of the human epidermal growth factor (HER) (ErbB) family have various effects on growth, proliferation, and survival. Activation of the HER pathway results in the growth and spread of cancer cells. Cellular receptors of the HER family, which interact with each other in multiple ways, are categorized into 4 groups: HER1 (epidermal growth factor receptor [EGFR]/ErbB1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4). Members of the ErbB family have critical importance in the development and progression of breast cancer.[5,6]

HER2 is a 185-kDA transmembrane protein coded by the HER2/neu gene localized

How to cite this article: Yilmaz TB, Akatli AN, Samdanci E, Soylu NK, Bag HG, Akpolat N. Prognostic significance of epidermal growth factor receptor gene mutations and human epidermal growth factor 2 expression in breast carcinoma metastatic to the liver. Clin Cancer Investig J 2019;8:247-53.

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Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_70_19

Quick Response Code:

at 17q12.[7] Encountered in 15%–35% of invasive breast cancers, its overexpression decreases survival and augments the predisposition for metastasis.[8,9] Various studies have investigated the relationship between HER2 overexpression and clinical and pathological characteristics of breast cancers. HER2-positive breast tumors manifest clinical differences from negative tumors. These tumors are more prevalent among younger patients and tend to be hormone receptor negative, while hormone receptor-positive tumors are lower grade and have better prognosis. Axillary lymph node metastases are more common in HER2-positive breast tumors. Differently from hormone receptor-positive cancers, they metastasize to solid organs in the early period. In the recent years, therapies targeting HER2 have brought about important changes in the course and prognosis of the disease.[10,11]

Mutations of the EGFR are uncommon in breast cancer. However, triple-negative cancers manifest higher amplification. These cancers are usually high grade and demonstrate liver and brain metastases more often.[12-14] Guo *et al*. found that EGFR amplification was related to ER expression, recurrence, and distant metastasis, and demonstrated amplification in high grade tumors. On the other hand, HER2 amplification was found to be associated with a large tumor size, an advanced clinical stage, local recurrence, and distant metastasis. The same study determined EGFR and HER2 co-amplification to be correlated with a markedly short disease-free survival.[14] HER2 overexpression is known to be linked to increased proliferation, invasiveness, recurrence, and an unfavorable prognosis. Besides their prognostic value, EGFR and HER2 are also important in the selection of an appropriate targeted therapy.[15] Studies have revealed the existence of a process named "receptor status conversion." A primary tumor and its metastasis may manifest discordant hormone receptor and HER2 statuses.[16,17] This is clinically significant as it would lead to differences in the treatment regimens of the patients. Studies have reported rates of conversion to HER2 positivity that vary between 0% and 58.3%.[18,19] There are studies that have reported EGFR and HER2 coamplification in breast carcinomas to be linked to a significantly shorter disease-free survival time and metastasis.[14,20] The majority of these studies have focused on brain metastases and lymph node metastases and our literature review identified no studies that have investigated liver metastases with regard to the statuses of EGFR mutation and HER2 protein synthesis. The aim of this study was to determine whether EGFR gene mutations and HER2 expressions in liver metastases from breast cancer are related to the receptor statuses of the tumors and clinicopathological parameters.

Materials and Methods

This study reviewed cases diagnosed with liver metastases from invasive breast carcinoma by the Pathology Laboratory at Inonu University, Faculty of Medicine between 2011 and 2018. The study was approved by the University's Ethics Committee Board. A total of 41 paraffin blocks of patients diagnosed histologically as liver metastases from breast carcinoma, with satisfactory tissue preservation were included in the study. Reports of the primary breast carcinoma were available for review in 23 of these cases. These cases were reevaluated by examining ER, PR, HER2 immunohistochemical staining results in archived reports and slides. EGFR gene mutations were detected using real-time polymerase chain reaction (PCR). ER, PR, and HER2 immunohistochemical staining results of liver metastases cases were compared to the results in the available reports of the primary breast tumor.

Immunohistochemical evaluation of estrogen receptor, progesteron receptor, and human epidermal growth factor 2

In the evaluation of ER and PR, 1% nuclear positive staining was considered the threshold value. In the grading of HER2, scores of 0–1 were considered "negative," a score of 2 was considered "equivocal," and a score of 3 was considered "positive."[21]

Polymerase chain reaction method for detection of epidermal growth factor receptor mutation

Sections were obtained from paraffin-embedded blocks of liver metastases and areas of tumor were identified. Two kits were used in the study and these were the Cobas® DNA Sample Preparation and Cobas® EGFR Mutation Test v2 kits detecting mutations in exons 18, 19, 20, and 21 of EGFR by a multiplex allele-specific PCR based. PCR was performed by Cobas z480 analyzer (Roche Molecular Systems Inc.) Results were analyzed and reported automatically.

Statistical evaluation

Categorical variables were expressed as numbers and percentages. Comparisons involved the Fisher's exact Chi-square, continuity corrected Chi-square, and Pearson exact Chi-square tests. For dependent groups, ratios were compared using the McNemar test, and quantitative data were compared using the Wilcoxon test. Correlations between quantitative variables were determined using the Spearman correlation coefficient. Quantitative variables in independent groups were compared using the Mann–Whitney U-test. The level of significance was accepted as 0.05 for all tests. IBM SPSS Statistics version 22.0 for Windows (New York, USA) was used.

Results

Patients' ages ranged between 27 and 99 years, with a mean age of 53.8 years. 40 patients (97.5%) were female and 1 patient (2.4%) was male.

Forty one liver metastases from breast carcinoma cases were tested for EGFR gene mutations using PCR and no mutations were detected in any of these cases.

Immunohistochemical evaluations determined 34 liver metastases (82.9%) as ER positive and 7 (17%) as negative. Immunohistochemical evaluations identified 22 cases (53.6%) as PR positive and 19 (46.4%) as PR negative. Immunohistochemical evaluation of HER2 graded 9 cases (21.9%) as score 3 (positive), 5 cases (12.1%) as score 2 (equivocal), 4 cases (9.7%) as score 1 (negative), and 21 cases (51.2%) as score 0 (negative) [Figures 1 and 2].

Twenty-three cases had accessible pathology reports and archive slides of the primary breast tumor. Hormone receptor and HER2 statuses of the primary and metastatic tumors of the cases have been summarized in Table 1.

The year of the initial diagnosis of the primary breast tumor could be determined for 31 patients. Metastasis-free survival times varied between 0 and 18 years, with the mean determined as 4.4 years.

Age, tumor grade, tumor size, and tumor type did not have a statistically significant relationship with hormone receptor and HER2 expression [Table 2].

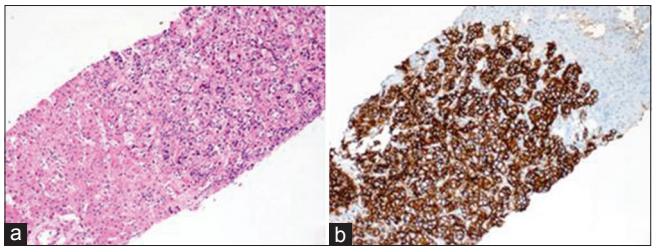

Figure 1: (a) Invasive ductal carcinoma metastatic to the liver (H and E, ×200), (b) positive human epidermal growth factor 2 expression in the tumor cells (×200)

The HER2 status of the primary breast tumor was stated in the reports of 23 cases. Nine cases (39.1%) showed HER2 status conversions, but this was not statistically significant (P = 0.197). Fourteen (60.8%) cases did not show any receptor status conversions [Table 3].

The ER status of the primary breast tumor was stated in the reports of 23 cases. 4 cases (17.4%) showed ER status conversions; however, this was not statistically significant (P = 1.000) [Table 4].

The PR expression status of the primary breast tumor was stated in the reports of 22 cases. 10 cases (45.5%) showed PR status conversions, and this was found to be statistically

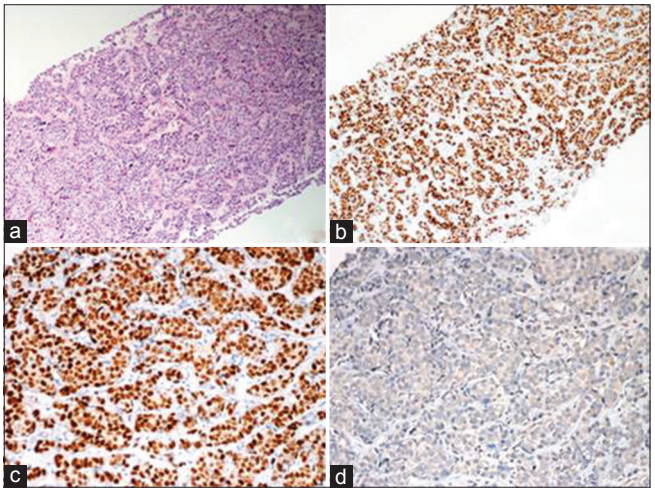

Figure 2: (a) Invasive lobular carcinoma metastatic to the liver (H and E, ×100), (b) nuclear expression of estrogen receptor in the tumor cells (×100), (c) progesterone receptor nuclear positivity in the tumor cells (×200), (d) human epidermal growth factor 2 negativity in the tumor (×200)

Table 1: Comparison of estrogen receptor, progesterone receptor, and human epidermal growth factor 2 percentages between primary and metastatic tumors

Receptors	Primary breast carcinoma			Liver metastasis		
	Positive (%)	Negative (%)	Equivocal (%)	Positive (%)	Negative (%)	Equivocal (%)
ER (*n*=23)	19 (82.6)	4 (17.4)	-	19 (82.6)	4 (17.4)	-
PR (*n*=22)	21 (95.5)	1 (4.5)	-	11 (50)	11 (50)	-
HER2 (*n*=23)	3 (13)	16 (69.6)	4 (17.4)	5 (21.7)	13 (56.5)	5 (21.7)

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2

Table 2: Comparison of estrogen receptor, progesterone receptor, and human epidermal growth factor 2 statuses of liver metastases with clinical parameters

	Liver metastasis									
	ER status			PR status			HER2 status			
	Positive (%)	Negative (%)	*P*	Positive (%)	Negative (%)	*P*	S 0-1	S2	S3	*P*
Age										
<50	13 (86.7)	2 (13.3)	1.00	8 (53.3)	7 (46.7)	1.00	7 (46.7)	4 (26.7)	4 (26.7)	0.313
>50	21 (80.8)	5 (19.2)		14 (53.8)	12 (46.2)		18 (69.2)	3 (11.5)	5 (19.3)	
Tumor size (cm)										
<2	3 (100)	0 (0)	1.00	2 (66.7)	1 (33.3)	0.586	1 (33.3)	1 (33.3)	1 (33.3)	0.521
>2	14 (77.8)	4 (22.2)		8 (44.4)	10 (55.6)		12 (70.6)	3 (17.6)	2 (12.8)	

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor 2

significant ($P = 0.002$). There were 10 (45.5%) cases whose primary tumors were positive for PR expression while their liver metastases were negative [Table 5].

Of the patients with available data on the primary tumor, only 1 was triple negative (3.8%). Meanwhile, liver metastases tissues revealed 4 triple-negative cases (9.7%). The liver metastasis specimen of the case with a triple-negative primary breast tumor was also evaluated as triple negative, and thus, this patient did not show tumor receptor status conversions. However, one triple-negative liver metastasis sample had originated from a primary breast tumor of the luminal A molecular subtype (ER positive, PR positive, and HER2 positive), and this case manifested tumor receptor status conversions for all three markers.

Discussion

Breast cancer is the most prevalent cancer among females and possesses the second highest mortality rate after lung cancer (1). 30%–40% of women with breast cancer develop metastatic disease. Breast cancer mainly metastasizes to bone, lungs, liver, and to brain. The liver is a common site for the metastasis of solid cancers and is the third most common site for breast cancer metastasis.[22]

ErbB receptors influence cell reproduction, differentiation, and migration. Their overexpression was implicated in various breast cancers and found to be associated with a high incidence of metastasis.[23] EGFR activation facilitates the degradation of extracellular matrix barriers in response to tumor invasion, and thus, increases activities of the matrix that promote *in vitro* cell invasion.[24]

EGFR mutations are uncommon in breast carcinomas. However, studies have reported higher mutation rates in certain molecular subtypes. Rates of EGFR immunoreactivity in triple negative tumors are known to vary between 45% and 75%. It is thought that these tumors would benefit from therapies that include agents targeting EGFR.[25,26]

The literature contains many studies on the detection of EGFR in breast tumors and metastases that have used methods such as immunohistochemistry, fluorescence *in situ* hybridization, and PCR. Using immunohistochemistry, Burness *et al*. determined high EGFR expression in triple negative primary breast tumors, regardless of amplification.[27] Weber and colleagues conducted EGFR mutation analysis by using PCR in 48 sporadic breast carcinomas and 24 hereditary breast carcinomas. They detected EGFR mutations in 14.6% of sporadic breast carcinomas and 45.8% of hereditary breast carcinomas.[28] Teng *et al*. determined EGFR mutations in 11.8% of triple-negative breast cancers using PCR, reporting exon 19 deletions and exon 21 missense mutations in particular.[29] On the other hand, Generali *et al*. inspected 42 sporadic breast tumors without further classification and did not determine any EGFR mutations.[30] In our study, liver metastases of 41 patients were examined for EGFR gene mutations using PCR. However, we did not determine EGFR gene mutations in any of our cases similar to Generali's study in terms of the number of cases, inclusion of all subtypes without classification. Considering that our study included a relatively low number of cases and that EGFR gene mutations are uncommon across breast tumors and are usually encountered in triple-negative breast tumors, one reason for this result may be the inclusion of all molecular subtypes.

There are studies in the literature that have conducted EGFR analysis on patients with brain metastasis from primary breast carcinoma. Using immunohistochemistry, Grupka *et al*. determined EGFR expression in 39% of brain metastases in triple-negative tumors.[31] Gaedcke *et al*. conducted an immunohistochemical EGFR analysis and determined EGFR expression in 16% of primary breast tumors and 40% of brain metastasis tissues originating from breast tumors.[18] Shao *et al*., on the other hand, investigated samples obtained from primary breast cancer patients with no known brain metastases after 10 years and found EGFR expression in only 7%.[8]

Liver is a common metastasis site for breast cancer patients, and liver metastasis is a prognostic factor with a negative effect on survival. Metastatic breast cancer patients with liver metastases were shown to have significantly lower overall survival times than those without liver metastases. Sihto *et al*. demonstrated that the liver was one of the

Table 3: Primary tumor/liver metastasis human epidermal growth factor 2 receptor status conversions

Primary tumor HER2 (n=23)	Liver metastasis (n=23)			
	Positive	Equivocal	Negative	*P*
Positive	2 (8.7)	1 (4.3)	0 (0.0)	0.197
Suspected positive	1 (4.3)	1 (4.3)	2 (8.7)	
Negative	2 (8.7)	3 (13.0)	11 (47.8)	

Table 4: Primary tumor/liver metastasis estrogen receptor status conversions

Primary tumor ER (n=23)	Liver metastasis ER (n=23)		
	Positive	Negative	*P*
Positive	17 (73.9)	2 (8.7)	1.000
Negative	2 (8.7)	2 (8.7)	

ER: Estrogen receptor

Table 5: Primary tumor/liver metastasis progesterone receptor status conversions

Primary tumor PR (n=22)	Liver metastasis PR (n=22)		
	Positive	Negative	*P*
Positive	11 (50.0)	10 (45.5)	0.002
Negative	0 (0.0)	1 (4.5)	

PR: Progesterone receptor

most common distant metastasis sites at the first relapse following adjuvant therapy in HER2-positive tumors.[32]

The treatment of breast cancer includes surgery, hormonotherapy, or adjuvant and neoadjuvant chemotherapy depending on ER and PR statuses, and radiotherapy. In addition, targeted therapies have been effective in the treatment of HER2-positive patients in the recent years. Since most patients with metastatic breast cancer cannot be cured, the treatment methods that will offer the most effective palliation and increase the quality of life and survival are adopted, and the general approach favors systemic therapies. Metastatic breast cancers can also be treated with chemotherapy, surgery, hormonotherapy, and targeted therapy agents.[33] In this sense, statuses of ER, PR, and HER2 become important for both the primary and the metastatic tumor. Many studies have shown discordances between ER, PR, HER2 statuses of primary breast cancers and metastatic tumors.[16,34]

In this study, we also analyzed the ER, PR, HER2 immunochemical statuses of 41 liver metastasis tissues originating from primary breast carcinomas. In our study, 17.4% of the cases demonstrated discordant ER statuses and 45.5% discordant PR statuses, with a greater difference in PR statuses in accordance with the results obtained by Lower *et al*.[34] Brunn Rasmussen and Kamby compared ER statuses of primary breast tumors and metastatic lymph node, bone marrow, and liver tumors using immunohistochemistry. They found that 41% of primary tumors were ER-positive, while rates of ER positivity for lymph node, bone marrow, and liver metastases were, respectively, 35%, 20%, and 17%. Discordant ER statuses between the primary tumor and distant metastasis sites were determined in 41% of bone marrow metastases and 44% of liver metastases, with the majority of the patients manifesting a change from ER-positive primary tumors to ER-negative metastases.[16] On the other hand, our study determined ER positivity in 82.6% of primary tumors and 82.9% of liver metastases. However, we were able to acquire the ER status of the primary breast cancer in only 23 cases. In our study, 8.7% of the cases had ER-positive primary tumors and ER-negative metastases. Hoefnagel and colleagues compared 233 distant breast cancer metastases at various recurrence sites (76 skin, 63 liver, 43 lung, 44 brain, and 7 gastrointestinal) with their primary tumors in terms of ER, PR, and HER2 statuses. Considering the threshold value as 1% in immunohistochemical evaluations, they determined the rates of discordance in ER and PR statuses between primary breast tumors and metastases as 15.1% and 32.6%, respectively. Immunohistochemistry determined HER2 status conversions at a rate of 5.2%.[19]

In 2018, Schrijver *et al*. conducted a meta-analysis of 39 studies that evaluated receptor status conversions between primary breast tumors and breast cancer metastases. Rates of status conversion from positive to negative were found as 22.5%, 49.4%, and 21.3%, respectively, for ER, PR, and HER2, while rates of status conversion from negativity to positivity were found respectively as 21.5%, 15.9%, and 9.5%. Moreover, ER status conversions were significantly more common in central nervous system and bone metastases in comparison to liver metastases and PR status conversions were more common in bone metastases.[35] In the present study, we determined positive to negative status conversion rates, respectively, as 8.7%, 45.5%, 0% for ER, PR, HER2, and negative to positive status conversion rates respectively as 8.7%, 0%, and 8.7%. Our results are in accordance with the results of the cited study as PR-positive primary tumor/PR-negative metastasis status conversions showed a higher rate.

Studies in the literature report higher ER and PR negativity rates in distant metastases compared to the primary tumor, while HER2 positivity is higher. These observations have important clinical implications, because they determine whether or not certain patients receive the appropriate systemic therapy for their metastases.

Our study used PCR to conduct EGFR gene mutation analysis on biopsies of liver metastasis from breast carcinoma and the ER, PR, and HER2 statuses of the patients were evaluated immunohistochemically.

In PCR analyses, none of the liver metastasis from breast carcinoma cases were detected to have EGFR gene mutations. Considering that the study included a low number of cases and that EGFR gene mutations are uncommon across breast tumors and are usually encountered in triple-negative breast tumors, one reason for this result might be the inclusion of a single case with triple negative characteristics.

When our study compared the immunohistochemical ER, PR, and HER2 analyses of liver metastases with the primary breast tumor, liver metastases of certain cases were found to have different receptor statuses. These results are consistent with the results of many studies done on primary breast carcinoma/distant organ metastasis receptor status conversions.

Conclusions

The detection of EGFR mutations, HER2 status and hormone receptor conversions are important so that primary breast tumor and metastasis patients can benefit from the recently developed targeted therapies. We did not find any studies in the literature that have conducted EGFR gene mutation analyses on liver metastasis from breast carcinoma cases. Therefore, studies on EGFR gene mutation are warranted that include a larger number of patients, and particularly, a patient group that contains triple-negative breast tumors.

Financial support and sponsorship

This study was financially supported by Inonu University Scientific Research Projects Coordination Unit (BAP).

Conflicts of interest

There are no conflicts of interest.

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