Research article

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Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer

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Abstract

Introduction: Many laboratories are currently evaluating the usefulness of determination of HER2, p53, and Ki67 proliferation indices using immunohistochemical techniques in cancer. Although the available studies suggest that these factors might indeed be helpful in making treatment decisions in cancer patients, their clinical usefulness is still controversial.

Methods: Expression of HER2, p53, and Ki67 was examined by immunohistochemistry in samples of breast tissue from 506 patients with invasive ductal carcinoma, obtained between 1981 and 1999 (median follow up period 82 months), and their significance for prognosis was analyzed.

Results: Of the 506 carcinoma tissue samples, 20.1%, 29.0%, and 53.6% were positive for HER2 over-expression, p53 protein accumulation, and Ki67 expression, respectively. Over-expression of HER2 significantly reduced disease free ($P=0.02$) and overall survival ($P=0.005$). Accumulation of p53 protein significantly decreased disease free ($P=0.01$) and overall survival ($P=0.01$). Patients with tumors that were positive for both HER2 and p53 relapsed and died within a significantly shorter period of time after surgery ($P=0.0001$ and $P<0.0001$, respectively). In multivariate analysis, patients with both HER2 and p53 positive tumors had considerably decreased overall survival ($P=0.04$), as did patients with larger tumor size and positive lymph node status.

Conclusion: The findings of the present study indicate that the coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer.

Keywords: HER2, immunohistochemistry, invasive ductal carcinoma, p53

Introduction

Prognostic biomarkers in a disease provide information regarding outcome irrespective of therapy. Candidate prognostic biomarkers in breast cancer include elevated levels of expression of proliferation indices such as Ki67 and proliferating cell nuclear antigen; expression of estrogen receptor (ER) and progesterone receptor; amplification and over-expression of HER2, cyclin D_1, and c-myc; p53 nuclear protein accumulation; bcl-2 expression; and alteration in angiogenesis proteins such as vascular endothelial growth factor [1–5]. In particular, review of the literature suggests that over-expression of HER2 and p53 may have prognostic significance in breast cancer. HER2 (c-erbB2) encodes a membrane protein (p185) that is tyrosine phosphorylated after interaction with its ligands. Over-expression of HER2 occurs through either amplification of the gene or mRNA over-expression. p53 is involved in regulating cell proliferation, inducing apoptosis, and in promoting chromosomal stability. Disruption of these functions appears to play an important role in carcinogenesis. There is evidence that over-expression of HER2 and p53 is involved in breast cancer progression [6]. This hypothe-

CMF = cyclophosphamide, methotrexate, and fluorouracil; ER = estrogen receptor.

sis is based on the high frequency of HER2 and p53 over-expression among invasive and noninvasive breast cancers and among benign breast diseases [7–9]. This suggests that HER2 and p53 play roles in the early stages of breast tumorigenesis.

In the present study we examined the expression of HER2, p53, and Ki67 in samples of breast tissue from 506 patients with invasive ductal carcinoma, obtained between 1981 and 1999, and analyzed their significance for prognosis. Our results indicate that the coexistence of HER2 over-expression and accumulation of p53 protein is a strong prognostic molecular marker in breast cancer.

Methods

Patients and breast cancer tissues

Breast tumor specimens from 506 female patients with primary invasive ductal carcinoma who were treated at Nagoya City University Hospital between 1981 and 1999 were included in the present study (Table 1). All patients had undergone mastectomy or lumpectomy. After surgery, 27% of patients received no additional therapy. Of the remaining patients, 20% received systemic adjuvant therapy consisting of endocrine therapy (tamoxifen) alone, 17% received chemotherapy alone, and 36% received combined endocrine therapy and chemotherapy. Patients who were positive for axillary lymph nodes received either oral administration of 5-fluorouracil derivatives for 2 years or a combination of cyclophosphamide, methotrexate, and fluorouracil (CMF). Patients were observed for disease recurrence and death at least once every 6 months for 5 years after surgery and yearly thereafter. The median follow-up period was 82 months (range 2–249 months).

Table 1

Clinicopathologic characteristics of patients with invasive ductal carcinoma

Parameter	Value (*n* [%])
n	506
Age at diagnosis (years)	
≤ 50	211 (42)
> 50	295 (58)
Age range (years)	22–91
Tumor size (cm)	
< 2	209 (41)
≥ 2	295 (59)
Number of positive lymph nodes	
0	276 (57)
1–3	115 (24)
> 3	93 (19)
Histological grade	
1	93 (17)
2	291 (59)
3	116 (24)
Adjuvant therapy	
None	137 (27)
Endocrine therapy	101 (20)
Chemotherapy	85 (17)
Combined	183 (36)
Follow up (months)	
Mean	91
Median	82
Range	2–249

Immunohistochemical analysis for estrogen receptor-α, HER2, p53, and Ki67

One 4-μm section from each submitted paraffin block was first stained with hematoxylin and eosin in order to verify that an adequate number of invasive ductal carcinoma cells were present and that quality of fixation was sufficient for immunohistochemical analysis. Serial sections (4-μm) were prepared from selected blocks and float mounted on adhesive coated glass slides for ER-α, HER2, p53, or Ki67 staining. Primary antibodies included monoclonal mouse antihuman estrogen receptor antibody (1D5; DAKO, Glostrup, Denmark) at 1 : 100 dilution for ER-α, rabbit antihuman c-erbB2 oncoprotein antibody (DAKO) at 1 : 200 dilution for HER2, monoclonal mouse antihuman p53 protein antibody (PAb1801; Novocastra, Newcastle, UK) at 1 : 50 dilution for p53, and monoclonal mouse antihuman Ki67 antibody (MIB-1; DAKO) at 1 : 100 dilution for Ki67. The DAKO EnVision system (DAKO EnVision labelled polymer, peroxidase) was used as the detection system for ER-α, HER2, and Ki67. The streptavidin-biotin system (SAB-PO kit; Nichirei Co., Inc., Tokyo, Japan) was applied for detection of the bound antibody of p53.

Immunohistochemical scoring

Immunostained slides were scored after the entire slide had been evaluated by light microscopy. The expression of ER-α was scored by assigning a proportion score and an intensity score according to Allred's procedure [10]. Any brown nuclear staining in invasive breast epithelium was counted toward the proportion score. Tumors with scores of 3 or greater were considered to be positive for ER-α expression. HER2 immunostaining was evaluated using the same method as is employed by the HercepTest (DAKO). To determine the score of HER2 expression the membrane staining pattern was estimated and scored on a scale of 0 to 3+. Tumors with scores of 2 or greater were considered to be positive for HER2 over-expression. The expression status of p53 and Ki67 was assessed according to the estimated proportion of nuclear staining

Table 2

Correlation between clinicopathologic factors and molecular markers

	HER2		p53		Ki67	
Parameter	Positive/total (*n* [%])	*P*	Positive/total (%)	*P*	Positive/total (%)	*P*
Total	104/503 (20.1)		145/500 (29.0)		268/500 (53.6)	
Tumor size (cm)						
<2.0	30/208 (14.4)	0.004	44/209 (21.1)	0.0009	110/209 (52.6)	NS
≥2.0	74/295 (25.1)		101/291 (34.7)		158/291 (54.3)	
Number of positive lymph nodes						
0	47/276 (17.0)	0.006	72/274 (26.3)	NS	145/274 (52.9)	NS
1–3	21/115 (18.3)		32/114 (28.1)		62/114 (54.4)	
>3	30/93 (32.3)		36/92 (39.1)		48/92 (52.2)	
Histological grade						
1	5/93 (5.4)	<0.0001	7/91 (7.7)	<0.0001	39/91 (42.9)	0.004
2	291/58 (19.9)		82/291 (28.2)		151/291 (51.9)	
3	40/116 (34.5)		55/116 (47.4)		76/116 (65.5)	
Estrogen receptor-α						
Negative	70/163 (42.9)	<0.0001	77/162 (47.5)	<0.0001	90/162 (55.6)	NS
Positive	34/339 (10.0)		68/340 (20.0)		179/340 (52.6)	

NS, not significant.

of tumor cells that were positively stained. Scoring criteria for p53 were as follows (in the form proportion of nuclear staining=score): none=0, <1/10=1, 1/10–1/2=2, and >1/2=3. Scoring criteria for Ki67 were as follows (in the form proportion of nuclear staining=score): none=0, <1/100=1, 1/100–1/10=2, 1/10–1/2=3, and >1/2=4. Tumors with a score of 1 or greater for p53 were considered to be positive for p53 protein accumulation, and tumors with a score of 2 or greater for Ki67 were considered to be positive for Ki67 expression.

Statistical analysis

The χ^2 test was used to compare immunohistochemical results for molecular markers with clinicopathologic characteristics. Estimation of disease free and overall survival was performed using the Kaplan–Meier method, and differences between survival curves were assessed with the log-rank test. Cox's proportional hazards model was used for univariate and multivariate analyses of prognostic values.

Results

Relationship between HER2, p53, and Ki67 expression and clinicopathologic factors

Of the 506 primary invasive ductal carcinomas, 20.1%, 29.0% and 53.6% were positive for HER2 over-expression, p53 protein accumulation, and Ki67 expression, respectively (Table 2). HER2 over-expression was significantly correlated with tumor size ($P=0.004$), number of positive lymph nodes ($P=0.006$), and histological grade ($P<0.0001$), and an inverse association was found between HER2 over-expression and ER-α expression ($P<0.0001$). Significant associations were observed between p53 positivity and tumor size ($P=0.0009$), histological grade ($P<0.0001$), and negativity for ER-α ($P<0.0001$). Ki67 expression was significantly correlated with histological grade ($P=0.004$), whereas no association was found between Ki67 expression and tumor size, number of positive lymph nodes, or ER-α expression. There were 339 ER-α positive tumors (67.7%), and inverse associations were observed between ER-α expression and tumor size ($P=0.01$) and histological grade ($P<0.0001$).

Correlation between HER2 over-expression, p53 protein accumulation, and Ki67 expression

Over-expression of HER2 was significantly associated with p53 protein accumulation ($P<0.0001$), but not with Ki67 expression (Table 3). In contrast, a significant association was observed between expression status of p53 and that of Ki67 ($P<0.0001$).

Disease free and overall survival categorized by HER2, p53, and Ki67 expression

HER2 over-expression was associated with significantly reduced disease free ($P=0.02$) and overall survival ($P=0.005$; Fig. 1). Similarly, p53 protein accumulation

Table 3

Correlation between HER2, p53, and Ki67 expression

	p53		Ki67	
Parameter	Positive/total (%)	*P*	Positive/total (%)	*P*
HER2				
Negative	96/398 (24.1)	<0.0001	211/398 (53.0)	NS
Positive	49/103 (47.6)		57/103 (55.3)	
p53				
Negative			168/357 (47.1)	<0.0001
Positive			101/145 (69.7)	

was associated with significantly reduced disease free ($P=0.01$) and overall survival ($P=0.01$; Fig. 2). Both the disease free and overall survival curves were similar between the tumors positive for HER2 over-expression and the tumors positive for p53 protein accumulation (compare Fig. 1a with Fig. 2a, and Fig. 1b with Fig. 2b). In particular, significantly more patients with tumors that were negative for HER2 or p53 had been alive within 10 years after surgery (Figs 1b and 2b). On the other hand, there was no relation between Ki67 expression status and overall survival, whereas Ki67 expression was associated with significantly decreased disease free survival ($P=0.001$; Fig. 3). The tumors of 47 patients were positive for both HER2 over-expression and p53 protein accumulation. The disease free and overall survival curves demonstrate that these patients relapsed and died within a significantly shorter period of time after the surgery (Fig. 4; $P=0.0001$). Interestingly, the disease free and overall survival curves for patients with HER2 or p53 positive tumors were very similar to those for patients with tumors that were negative for both HER2 and p53 (Fig. 4).

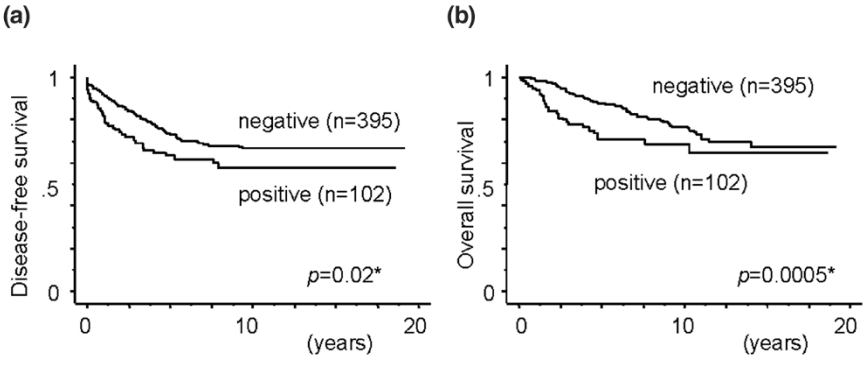
Prognostic analysis of disease free survival

In the univariate analysis (Table 4), combined HER2 and p53 status ($P<0.0001$) and Ki67 status ($P=0.001$), as well as tumor size ($P<0.0001$), number of positive lymph nodes ($P<0.0001$), histological grade ($P<0.0001$), ER-α expression ($P=0.03$), and the type of adjuvant therapy ($P<0.0001$) were strongly able to predict disease free survival. Multivariate analysis showed reduced disease free survival with increasing tumor size ($P=0.02$), increasing number of positive lymph nodes ($P<0.0001$), and positive Ki67 status ($P=0.003$). There was no significant relation between disease free survival and combined HER2 and p53 status, histological grade, ER-α expression, and the type of adjuvant therapy in multivariate analysis (Table 4).

Prognostic analysis of overall survival

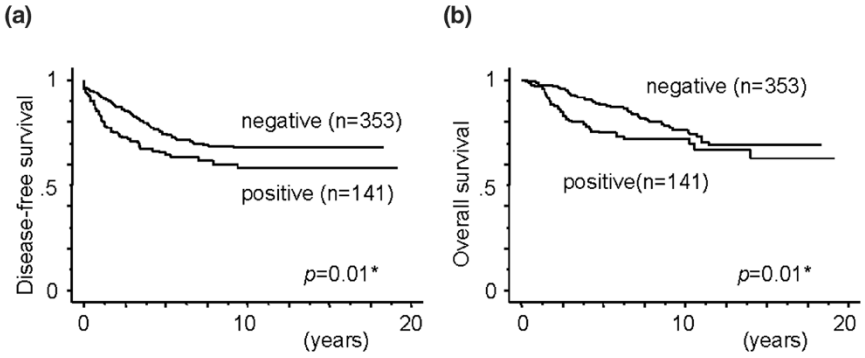
Univariate analysis (Table 5) demonstrated significant associations between overall survival and combined HER2 and p53 status ($P<0.0001$), as well as tumor size ($P<0.0001$), number of positive lymph nodes ($P<0.0001$), histological grade ($P<0.0001$), ER-α expression ($P=0.002$), and the type of adjuvant therapy ($P=0.0001$). There was no significant relation between Ki67 expression status and overall survival. In multivariate analysis, patients with HER2 and p53 positive tumors ($P=0.04$), as well as patients with larger tumor size ($P=0.008$) and positive lymph node status ($P<0.0001$), had significantly reduced overall survival (Table 5). There was no significant relation between overall survival and histological grade, ER-α expression, and the type of adjuvant therapy in multivariate analysis.

Figure 1



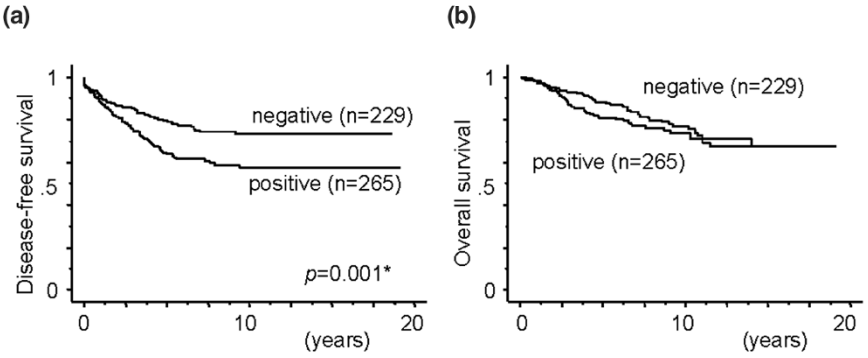
Effect of HER2 over-expression on **(a)** disease free and **(b)** overall survival among 497 patients with invasive ductal carcinoma. Disease free and overall survival were significantly better in patients with HER2 negative tumors than in patients with HER2 positive tumors ($P=0.02$ and $P=0.0005$, respectively).

Figure 2



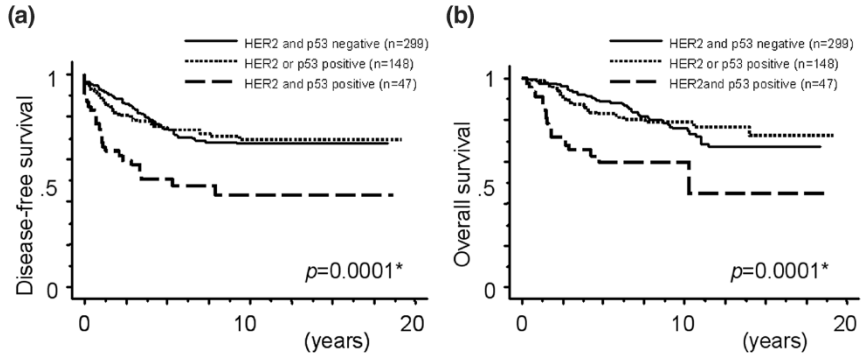
Effect of p53 protein accumulation on **(a)** disease free and **(b)** overall survival among 494 patients with invasive ductal carcinoma. Disease free and overall survival were significantly better in patients with p53 negative tumors than in patients with p53 positive tumors ($P=0.01$ for both).

Figure 3



Effect of Ki67 expression on **(a)** disease free and **(b)** overall survival among 494 patients with invasive ductal carcinoma. Disease free survival was significantly better in patients with Ki67 negative tumors than in patients with Ki67 positive tumors ($P=0.001$).

Figure 4



Effect of coexistence of HER2 over-expression and p53 protein accumulation on **(a)** disease free and **(b)** overall survival among 494 patients with invasive ductal carcinoma. Disease free and overall survival were significantly worse in patients with HER2 and p53 positive tumors than in patients with HER2 and p53 negative tumors and HER2 or p53 positive tumors ($P=0.0001$ for both).

Table 4

Prognostic factors in 470 invasive ductal carcinomas compared with disease free survival

	Univariate	Multivariate	
Parameter	*P*	*P*	RR (95% CI)
Tumor size	<0.0001	0.02	0.626 (0.418–0.938)
Number of positive lymph nodes	<0.0001	<0.0001	0.227 (0.144–0.359)
Histological grade	<0.0001	0.09	0.712 (0.479–1.057)
Estrogen receptor-α	0.03	0.42	1.176 (0.787–1.756)
HER2/p53	<0.0001	0.06	0.630 (0.391–1.017)
Ki67	0.001	0.003	0.589 (0.416–0.833)
Adjuvant therapy	<0.0001	0.76	1.098 (0.606–1.988)

CI, confidence interval; RR, relative risk.

Table 5

Prognostic factors in 470 invasive ductal carcinomas compared with overall survival

	Univariate	Multivariate	
Parameter	*P*	*P*	RR (95% CI)
Tumor size	<0.0001	0.008	0.470 (0.273–0.809)
Number of positive lymph nodes	<0.0001	<0.0001	0.275 (0.157–0.482)
Histological grade	<0.0001	0.09	0.665 (0.419–1.056)
Estrogen receptor-α	0.002	0.16	1.389 (0.866–2.229)
HER2/p53	<0.0001	0.04	0.565 (0.323–0.990)
Ki67	0.28		
Adjuvant therapy	0.0001	0.75	0.879 (0.400–1.930)

CI, confidence interval; RR, relative risk.

Discussion

The present study indicates that the coexistence of HER2 over-expression and p53 protein accumulation has strong prognostic significance in invasive ductal carcinoma of the breast after a median follow-up period of 82 months.

HER2 encodes a 185-kDa transmembrane glycoprotein with intracellular tyrosine kinase activity that belongs to the epidermal growth factor receptor family [11]. Although no ligand has been identified for HER2, several peptide growth factors bind to the other members of the family. Amplification or over-expression of HER2 is observed in 20–40% of human breast cancers. The prognostic significance of HER2 over-expression was first reported in 1987 [12]. Subsequently, over 200 studies have been reported in which the role of amplification/over-expression of HER2 was investigated as a prognostic marker in breast cancer. Also, we previously reported that HER2 amplification was strongly associated with both disease free and overall survival in breast cancer [5]. In the present study we extended our analysis of HER2 over-expression to more than 500 invasive ductal tumors, and showed that over-expression of HER2 was associated with poor prognosis.

Nearly one-third of breast cancers have mutations in the p53 gene, which are associated with high histological grade and clinical aggressiveness [3]. Immunohistochemical assays generally detect nuclear accumulation of the protein, which is often related to conformational alterations and a prolonged half-life of the encoded protein [13,14]. Accumulation of p53 protein was significantly associated with poor prognosis in our study and in other studies of patients with breast cancer [15,16]. These studies suggest both a prognostic and a predictive role for p53 [1].

Tumors with both HER2 over-expression and p53 protein accumulation were reported in several studies, and patients with such tumors were found to have poor prognosis [17–22]; the findings reported here also indicate that both HER2 over-expression and p53 protein accumulation are associated with markedly poorer disease free and overall survival. On the other hand, some studies have shown a better prognosis in patients with breast cancers with HER2 overexpression and p53 protein accumulation [23]. These differences may reflect the effect of various therapeutic regimens.

Most patients with early breast cancer receive adjuvant treatment, and the identification of predictive factors may help in selecting the optimal therapeutic strategy for individual patients. HER2 over-expression may be associated with reduced efficacy of adjuvant endocrine therapy with tamoxifen [24–26]. The role of p53 mutations in the efficacy of endocrine therapy is still under evaluation. On the other hand, previous data suggested that HER2 positive tumors might be resistant to adjuvant treatment with CMF [27,28]. There is evidence that women whose tumors over-express HER2 are likely to derive greater benefit from therapy with anthracycline-containing regimens than from alkylating agents [25,29–31]. It was also reported that patients with both HER2 and p53 positive tumors had an improved 10-year survival when treated with a high dose FAC (fluorouracil, doxorubicin, cyclophosphamide) regimen [29]. The patients included in the present study were treated with tamoxifen, fluorouracil, or a CMF regimen, and anthracycline based chemotherapy was not used. Further studies are needed to determine which endocrine or chemotherapeutic agents should be used in breast cancers with different expression profiles, especially in patients with poor prognosis.

Conclusion

We examined the expression of HER2, p53, and Ki67 in 506 invasive ductal carcinoma tissue samples. The results indicate that the coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer.

Competing interests

None declared.

Acknowledgements

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