HER 2/neu protein expression in gastric cancer is associated with poor survival

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Received April 14, 2009; Accepted August 19, 2009

DOI: 10.3892/mmr_00000196

Abstract. The presence of HER 2/neu has been reported in gastric cancer, but its impact on patient survival remains unclear. The purpose of this study was to investigate the expression of HER 2/neu in gastric cancer. A total of 218 paired resected gastric cancer and corresponding normal specimens were collected. HER 2/neu protein expression was assessed by immunohistochemical staining. The correlation between HER 2/neu expression and patient clinicopathological parameters was evaluated and the prognostic significance of HER 2/neu expression was assessed by univariate and multivariate analyses. Forty-one out of 218 (18.8%) gastric cancer specimens showed HER 2/neu-positive expression. No relationship was found between membranous HER 2/neu expression and clinicopathological parameters. However, HER 2/neu expression was correlated with poorer overall survival ($p<0.001$). In multivariate analysis, HER 2/neu expression was a significant independent prognostic predictor of gastric cancer ($p<0.001$), and was associated with poor survival in gastric cancer patients. These data indicate that HER 2/neu may play a major role in the therapeutic management of gastric cancer.

Introduction

Gastric cancer is the second leading cause of cancer-related death worldwide (1). Surgery remains the mainstay of any curative treatment. Unfortunately, in advanced gastric cancer, currently available treatments including surgery, chemotherapy and radiotherapy have limited success. Since surgery is an insufficient treatment for most patients, developing active drugs against advanced gastric cancer is required (2,3).

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Key words: gastric cancer, HER 2/neu, prognosis

It has been reported that trastuzumab has significant anti-tumor activity related to HER 2/neu expression in gastric cancer cells and xenograft models (4-6). This finding has prompted investigation of the potential clinical benefits of trastuzumab in gastric cancer. However, conflicting results have been obtained in studies on HER 2/neu and its relationship with prognosis in gastric cancer. Some studies have reported that HER 2/neu overexpression is a poor prognostic factor for gastric cancer (7-10), while others have failed to find any association between HER 2/neu overexpression and prognosis (11,12). This discrepancy indicates that there may be factors othen than HER 2/neu expression levels influencing the effect of trastuzumab in gastric cancer. Accurate assessment of HER 2/neu status is therefore essential to determine which patients may benefit from trastuzumab treatment.

The purpose of the present study was to examine the expression status of HER 2/neu in gastric cancer and to evaluate whether HER 2/neu expression level is correlated with clinicopathological parameters and total prognosis or gender.

Materials and methods

Case selection. Specimens were obtained from 218 patients who underwent curative resection (R0) of gastric cancer at the Department of Surgical Oncology of Sir Run Run Shaw Hospital, Zhejiang University College of Medicine, between July 1999 and August 2006. Informed consent was obtained from all patients, and the study was conducted according to the guidelines of the Hospital Ethics Committee. The patients comprised 153 males and 65 females, aged 26 to 81 years (mean 56.8 years). No patient received anti-cancer treatment prior to surgery. The correlation between the expression of HER 2/neu and clinicopathologic parameters including age, gender, differentiation, location and pTNM pathological classification according to the International Union against Cancer (UICC) were evaluated.

Immunohistochemistry. Immunohistochemical analysis for HER 2/neu expression was performed on formalin-fixed paraffin-embedded sections of surgical specimens. The slides were deparaffinized in xylene and rehydrated in gradient ethanol solution. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 10 min. The slides were immersed

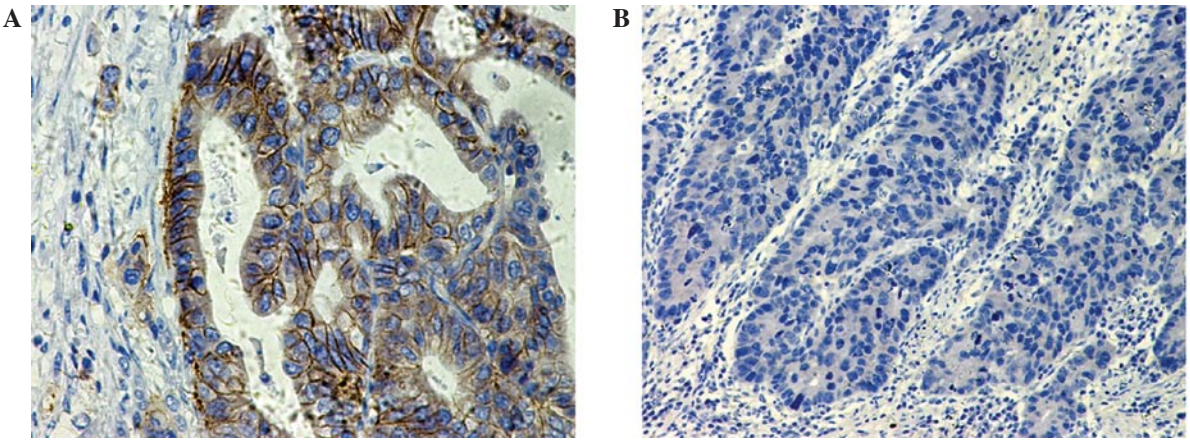

Figure 1. Immunohistochemical analysis of HER 2/neu expression in gastric cancer. (A) Strong complete cell membrane staining was observed in >10% of tumor cells. (B) No cells were stained in the cell membranes of the gastric cancer tissue.

in 10 mM citric buffer (pH 6.0) with heating for 15 min for antigen retrieval, then cooled at room temperature for 20 min and washed with phosphate-buffered saline (PBS). Non-specific binding was blocked by pre-incubation with 10% fetal calf serum in PBS with 0.01% sodium azide, then the slides were incubated in a humid chamber for 1 h with antibody against HER 2/neu HercepTest Kit (Dako Cytomation, Denmark). After washing three times in PBS, the slides were incubated with the EnVision-HRP complex (undiluted; Dako) for 60 min, then visualized with diaminobenzidine (Dako) and counterstained with hematoxylin. For substitute negative controls, the primary antibody was replaced with PBS. Positive controls included breast cancer tissue known to exhibit high levels of marker.

The slides were examined and scored independently to avoid subjective bias by two experienced pathologists. Evaluation of the results was performed according to the criteria recommended by the manufacturer by assigning a score of 0 to 3+. Scores were defined as follows: 0, no staining or membrane staining in <10% of the tumor cells; 1+, faint/barely perceptible membrane staining in >10% of the tumor cells, cells stained in part of the membrane; 2+, weak to moderate staining of the entire membrane in >10% of the tumor cells; 3+, strong staining of the entire membrane in >10% of the tumor cells. Specimens with scores of 0 and 1+ were regarded as being HER 2/neu-negative, while scores of 2+ and 3+ indicated positive expression of HER 2/neu.

Follow-up. Patients underwent follow-up until death or until the date of the last follow-up on November 30, 2007. During the follow-up period, 52 patients (23.9%) succumbed to the disease. The median follow-up interval was 51.1 months (range 6-127 months).

Statistical analysis. Statistical analysis was conducted using the statistical program SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). Pre-treatment characteristics were analyzed using the 2-tailed χ^2 test. The 2-tailed t-test was used to evaluate the correlation between HER 2/neu expression and clinicopathologic parameters. Univariate analysis of patient survival was performed using the Kaplan-Meier method. Survival curves were compared using the log-rank test. Multivariate analysis was performed using logistic regression and Cox's proportional hazard model. The accepted level of significance was set at $p<0.05$.

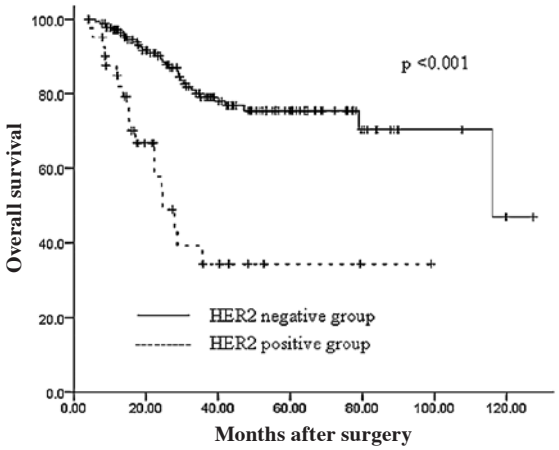

Figure 2. Overall survival. The 5-year overall survival rates were 70.6% for the HER 2/neu-negative expression group and 34.3% for the HER 2/neu-positive expression group ($p<0.001$).

Results

HER 2/neu expression is correlated with clinicopathologic parameters. The 218 gastric cancer tissue specimens were examined for the presence of HER 2/neu, determined by immunohistochemistry. Of these specimens, 41 (18.8%) exhibited HER 2/neu-positive expression (Fig. 1). HER 2/neu expression was correlated with tumor differentiation and location. There were no differences between the groups in terms of gender, tumor size, differentiation and location.

Survival analysis. According to univariate analysis, HER 2/neu-positive expression was associated with poorer overall survival in gastric cancer. The 5-year overall survival rates were 70.6% in the HER 2/neu-negative expression group and 34.3% in the HER 2/neu-positive expression group ($p<0.001$; Fig. 2). In multivariate analysis, HER 2/neu expression was the independent predictor (hazard ratio 6.923; $p<0.001$) (Table II).

Table I. Comparison of clinicopathologic features in gastric cancer.

	Negative (n=177)	Positive (n=41)	p-value
Age	55.8	60.8	0.019
Gender			0.131
Male	120 (67.8)	33 (80.5)	
Female	57 (32.2)	8 (19.5)	
Location			0.001
Upper body	55 (31.1)	12 (29.3)	
Lower or middle body	122 (68.9)	29 (70.7)	
Tumor differentiation			0.476
Differentiated	65 (36.7)	18 (43.9)	
Undifferentiated	112 (63.3)	23 (56.1)	
Tumor size (mm)	4.9	5.1	0.624
Serosal invasion			0.851
No	55 (31.1)	13 (31.7)	
Yes	122 (68.9)	28 (68.3)	
Nodal involvement			0.482
No	72 (40.7)	14 (34.2)	
Yes	105 (59.3)	27 (65.8)	

Table II. Results of stepwise multivariate analysis for prognostic factors (n=104).

	Overall survival		
Variable	Risk ratio	95% CI	p-value[a]
Her 2/neu (positive vs. negative)	6.923	(2.125-22.552)	<0.001
Serosal invasion (yes vs. no)	8.124	(2.459-26.833)	0.001
Node involvement	1.124	(1.074-1.176)	<0.001
Age	1.031	(1.005-1.057)	0.021
Gender (male vs. female)			0.814
Location (middle vs. upper body)			0.624
Tumor (differentiated vs. undifferentiated)			0.596
Tumor size			0.477

[a]Cox proportional hazards model. HR, hazards ratio; 95% CI, 95% confidence interval; mLNs, metastatic lymph nodes; serosal invasion, pathologic tumor classification; node involvement, lymph node status. Variables entered into the regression model were HER 2/neu, serosal invasion, mLNS, age, tumor size, histologic type, gender and tumor location. Age, tumor size and number of mLNs were consecutive variables; the remaining variables were categorized.

Discussion

The HER 2/neu protein is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptor (EGFR) family (13,14). Overexpression of HER 2/neu protein in gastric cancer determined using immunohistochemistry was first described in 1986 (15). Overexpression of the HER 2/neu receptor is detected in 9-38% of human gastric cancer patients (16,17). In our data, overexpression of the HER 2/neu protein occurred in 41 of 218 (18.8%) gastric cancer specimens as determined by immunohistochemistry, which is concordant with previous reports (18,19).

The role of HER 2/neu as a prognostic factor in gastric cancer is controversial (8,9). Reports have suggested that HER 2/neu expression is an important prognostic factor (20,21). In this study, HER 2/neu expression was examined in 218 curative resection (R0) gastric cancer cases. HER 2/neu was revealed to have a powerful adverse prognostic effect, and was an independent significant predictive factor for survival. This suggests that HER 2/neu may act as a prognostic parameter in gastric cancer.

At present, there are few therapeutic options available for gastric cancer. Our findings suggest that trastuzumab has significant anti-tumor activity in gastric cancer cases positive for HER 2/neu expression.

In conclusion, the expression of HER 2/neu was associated with poor prognosis in gastric cancer. Consequently, HER 2/neu is a potential molecular marker for predicting patient outcome in gastric cancer patients.

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