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Prognostic significance of HER2/neu expression in gastric cancer

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Prognostische Bedeutung einer Expression von HER2/neu beim Magencarcinom

Zusammenfassung. *Hintergrund:* Das Magenkarzinom ist in vielen Ländern noch immer die häufigste Neoplasie. Deshalb werden neben den als Prognose Marker bekannten klinisch pathologischen Faktoren neue unabhängige Parameter untersucht. Immer mehr Daten deuten darauf hin, dass bei Patienten mit dieser Krebserkrankung eine Expression von HER2/neu eine Rolle spielt. Solide Untersuchungen haben gezeigt, dass diese Expression mit einem schlechten Ausgang und einem aggressiveren Erkrankungsverlauf korreliert ist.

Patienten und Methoden: Fünfzig Magenkarzinom Gewebsproben wurden mittels Immunhistochemie auf das Vorliegen von HER2/neu untersucht. Es wurde versucht zu erheben, ob ein Zusammenhang zwischen einer HER2/neu Expression und klinisch pathologischen Parametern der Patienten besteht. Außerdem wurde eine eventuelle prognostische Bedeutung einer HER2/neu Expression wurden erhoben.

Ergebnisse: Eine HER2/neu Membranfärbung wurde bei 7 (14 %) Fällen gefunden. Die Patienten mit einer Her2/neu Überexpression hatten im Vergleich zu den HER2/neu negativen Patienten eine schlechtere Prognose nach chirurgischer Therapie ($p = 0.001$, Log-rank test). Es wurde kein Zusammenhang zwischen einer HER2/neu Expression und klinisch-pathologischen Parametern gefunden.

Schlussfolgerungen: Unsere Ergebnisse zeigen, dass eine immunhistochemisch erhobene HER2/neu Überexpression mit der Überlebensrate der Patienten korreliert. Dies fassen wir als ein gutes indirektes Maß der Qualität unserer Untersuchungen auf. Die Methode könnte daher einen Wert als prognostischer Marker beim Magenkrebs haben.

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Summary. *Background:* Gastric cancer is still the most prevalent neoplasia in many countries. Therefore, besides the clinicopathological factors known to be prognostic markers, new independent parameters are being investigated. There is mounting evidence of the role of HER2/neu expression in patients with this type of cancer, and it has been solidly correlated to poor outcomes and a more aggressive disease.

Patients and methods: Fifty gastric cancer tissue specimens were examined for the presence of HER2/neu by immunohistochemistry. The correlation between HER2/neu expression and patient clinicopathological parameters was evaluated and the prognostic significance of HER2/neu expression was assessed.

Results: HER2/neu membrane staining was detectable in 7 (14.0%) cases. The patients with HER2/neu overexpression had worse prognosis after surgical therapy compared with those without expression of HER2/neu ($p = 0.001$, Log-rank test). No relationship was found between HER2/neu expression and other clinicopathological parameters.

Conclusions: Using immunohistochemistry, our data showed that the association between HER2/neu overexpression and patient survival provides a good indirect validation for quality of this investigation and it may act as a prognostic parameter in gastric cancer.

Key words: Gastric cancer, HER2/neu membrane staining, immunohistochemistry.

Introduction

Cancer of the stomach still remains a major health problem. It is one of the most commonly diagnosed malignancies and an important cause of mortality worldwide. The prognosis for patients with this type of cancer is poor, because of locoregionally advanced disease and distant metastases already present at the time of initial clinical manifestation [1]. The aggressive behavior of gastric cancer is reflected in an early spread to lymph nodes, even at

locations distant from the site of the primary tumor. That is why the survival of patients, who have advanced stage of cancer treated with palliative chemotherapy, remains low. A better understanding of the molecular basis of cancer has contributed to the development of rationally designed molecular targeted therapies, which interfere with the signaling cascades involved in cell differentiation, proliferation, and survival [2].

The epidermal growth factor receptor (EGFR) or HER/erbB family of tyrosine kinase receptors is widely recognized as a component of signal transduction network that is dysregulated in several major cancers [3,4]. EGFR/HER1, ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4 are members of this family and play a major role in the pathogenesis of many solid tumors including gastric cancer. HER2/neu is characterized by three distinct structural and functional domains, including an extracellular cysteine-rich section, a transmembrane portion, and an internal cytoplasmic tyrosine-kinase section. Signal transduction to the nucleus is mediated by ligand-binding homodimerization or heterodimerization of HER2/neu with other EGFR family members which is followed by receptor autophosphorylation and recruitment of specific SH-2 proteins [5]. Moreover, HER2-containing heterodimers generate intracellular signals that are significantly stronger than signals emanating from other HER combinations. In normal cells, few HER2/neu molecules exist at the cell surface, so few heterodimers are formed and growth signals are relatively weak and controllable. When HER2/neu is overexpressed multiple HER2/neu heterodimers are formed and cell signaling is stronger, resulting in enhanced responsiveness to growth factors and malignant growth [6]. This explains why HER2/neu overexpression is an indicator of poor prognosis in several tumors and may be predictive of response to treatment.

Immunohistochemistry was still the basic method for evaluating HER2/neu protein expression. There is several data that overexpression of HER2/neu has been associated with advanced disease, metastasis, and poor clinical outcome in breast, ovaria, lung and organs of digestive tract [7–10]. Overexpression of HER2 protein in gastric cancer, using immunohistochemistry, was first described in 1986 from Sakai [11]. Several immunohistochemical studies focused on gastric cancer have reported different frequencies of HER2/neu overexpression, over a wide range, from 8 to 91% [12, 13]. Some studies have reported that HER2/neu overexpression is a poor prognostic factor for gastric cancer, but the other has failed to find any association with prognosis whatsoever [14–17].

The purpose of this study was to examine the expression status of HER2/neu in gastric cancer and to evaluate whether HER2/neu expression is correlated with clinicopathological parameters and prognosis in patients with this kind of tumor.

Materials and methods

Patients

Specimens were obtained from 50 patients who underwent curative resection of gastric cancer at the Department of Surgery, University Hospital, Medical Faculty, Trakia University, Stara Zagora, between 1999 and 2009. The patients comprised 30 males and 20 females, aged 22–83 years (mean 64.58 years). No patient received anti-cancer treatment prior to surgery. Forty-one patients (82%) had the intestinal histologic type tumor and the other nine (18%) had diffuse type. Tumor staging was defined as 10.0% ($n=5$) for the I stage, 22.0% ($n=11$) for the II stage, 42.0% ($n=21$) for the III stage and 26,0% ($n=13$) for the IV stage. Tumor grading and staging was performed according to the TNM classification by UICC 2002 and Lauren histological classification [18, 19]. Tumor specimens were fixed in 10% buffered formalin and embedded in paraffin. Histological grading was performed on hematoxylin- and eosin-stained sections according to Kioshima et al. [20]. The main clinical and histological data are given in Table 1.

Informed consent was obtained from all patients.

Immunohistochemistry

For immunohistochemical staining, the paraffin blocks were prepared using tumor tissues from the periphery of the tumor adjacent to the normal tissues. Paraffin sections, 5-µm thick, were dewaxed in two xyllenes at 56°C for 1 h, and were rehydrated in ethanol. Later, they were washed in 0.1 M phosphate-buffered saline (PBS), pH 7.4, incubated in 1.2% hydrogen peroxide in methanol for 30 min, and rinsed in 0.1 M PBS, pH 7.4, for 15 min. Then the slides were incubated in a humid chamber for 60 min with antibody polyclonal rabbit anti-human c-erb-2 antibody, dilution 1:100 (DAKO, Denmark). After washing three times in PBS, the slides were incubated with DAKO-REAL™ En-Vision™ detection system (DAKO) for 60 min, then visualized with diaminobenzidine and counterstained with hematoxylin. We used breast cancer tissue known to exhibit high levels of marker for positive control and for negative control, the primary antibody was replaced with PBS.

All slices were evaluated without knowledge of the clinical outcome. Immunostaining was scored according to the consensus panel recommendations on HER2/neu scoring for gastric cancer: 0, no staining or in <10% of the tumor cells; 1+, faint/barely perceptible partial staining in >10% of tumor cells; 2+, weak to moderate complete or basolateral membranous staining in >10% of tumor cells; 3+, strong complete or basolateral membranous staining in >10% of tumor cells [21].

Statistical analysis

The SPSS 16.0 program for Windows was used for statistical analysis. The chi-squared test was used to compare the immunohistochemical staining and the clinicopathological parameters. Survival was compared by the Kaplan–Meier log-rank test. The accepted level of significance was set at $p<0.05$.

Results

Correlation between HER2/neu overexpression and clinicopathological parameters

Fifty gastric cancer tissue specimens were examined for the presence of HER2/neu by immunohistochemistry. HER2/neu membrane staining (1+, 2+, 3+) was detectable in 7 (14.0%) cases: one case with 3+, four cases with 2+, and two cases with 1+ staining (Fig. 1a–d).

HER2/neu expression was correlated with clinicopathological parameters. There was no significant difference in gender and age with respect to HER2/neu expression. No statistically significant associations were observed be-

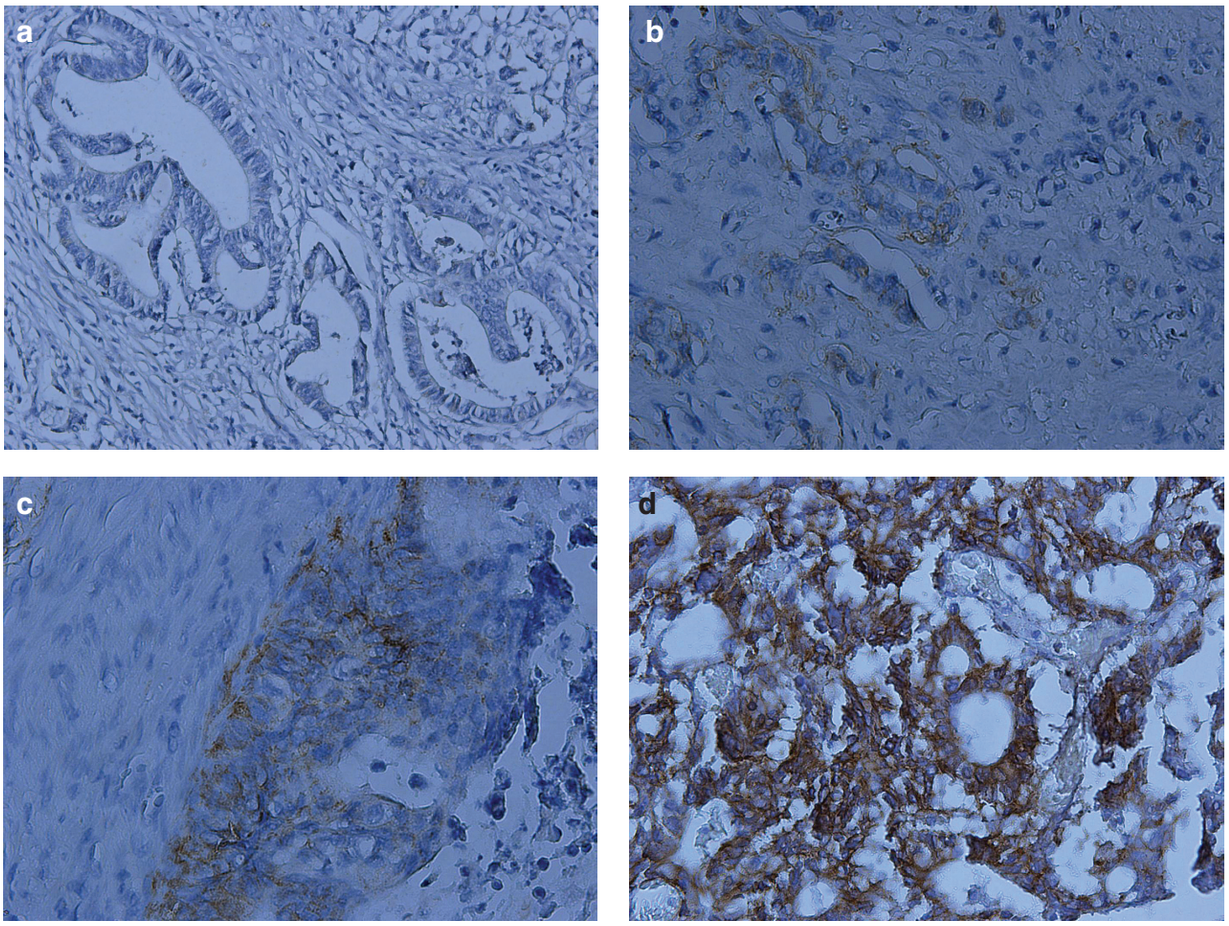

Fig. 1. Tumor cells HER 2/neu expression in gastric cancer: **a.** no staining; **b.** 1+ faint partial staining; **c.** 2+ moderate membranous staining; **d.** 3+ strong membranous staining (Magnification **a, d** × 200; **b, c** × 400)

tween HER2/neu expression and tumor size, lymph node and distant metastases, tumor stage and histology type (data not shown). HER2/neu expression was detected in 27.7% of high/moderately differentiated tumors and in only 6.3% of poorly differentiated tumors, although the difference did not reach statistical significance ($\chi^2 = 2.83$, $p = 0.093$).

Survival analysis

Clinical data obtained from the archival records were available for 39 patients. All of them were followed-up until the 1st of March 2010. At the end of the follow-up, 11 (32.35%) of the patients survived, with median survival period of 54.2 months (range from 1.68 to 60.08 months). Twenty-eight patients died with median survival period of 7.1 months (range 0.3 to 43.6 months). For analyzing the impact of HER2/neu on the survival after surgery, we determined survival rates for 39 patients, five of them had HER2/new overexpressing tumors (moderately and strongly positive) and 34 had HER2/neu negative tumors. The patients with HER2/neu overexpression had worse prognosis after surgical therapy compared to those without expression of HER2/neu. The median overall survival

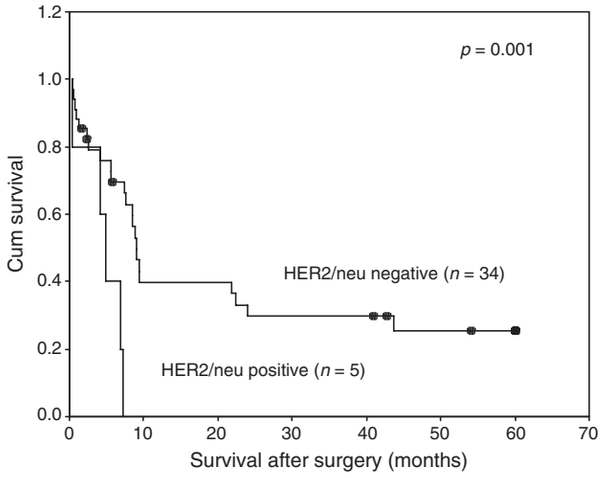

Fig. 2. Median overall survival of 4.87 months of patients with HER2/neu overexpression *vs.* 8.94 months of patients without HER2/neu expression ($p = 0.001$; log-rank test)

was 4.87 months (95% CI; 3.44–6.30) for HER2/neu overexpressing patients and 8.94 (95% CI; 7.86–10.03) for HER2/neu-negative patients ($p = 0.001$, Log-rank test) (Fig. 2).

Discussion

The HER2/neu protein is a 185-kDa transmembrane tyrosine kinase receptor and a member of EGFR family. It is encoded by a gene located on chromosome 17q21 near to the topoisomerase IIa genes [7, 22]. Under normal conditions, the function of HER2/neu and EGFR is tightly regulated in a cell-cycle-dependent manner. The tyrosine kinase activity of these receptors is active in G0/G1 phase, and is suppressed in G2/M phase likely due to hyperphosphorylation of serine and/or threonine residues [20]. Overexpression or mutation of HER2/neu receptor, which commonly occurs in cancer cells, may disrupt this regulation and keep tyrosine kinases highly active through the cell cycle [23]. Indeed, several studies indicate a role of HER2/neu in the development of numerous types of human cancer.

In the present study, we evaluated HER2/neu protein overexpression in 50 cases of gastric cancer using immunohistochemistry and polyclonal antibody against HER2/neu protein. Our data demonstrated HER2/neu protein membrane expression in 14% of gastric cancers, whereas moderately and strongly positive HER2/neu staining was demonstrated in 10% of tumors, which is consistent with previously reported observations. More of the studies, which determined HER2/neu expression by IHC using monoclonal antibody report that HER2/neu receptor is detected in 9–38% of human gastric cancer patients [2, 21]. Allgayer et al. found a very high rate of HER2/neu expression using IHC in a prospective series of 203 gastric cancer patients [24]. Two major explanations for this considerable discrepancy in HER2/neu expression between their series (91% positive cases) and other studies (up to 38% positive cases) could be drawn: first, a monoclonal antibody against HER2/neu together with a highly sensitive streptavidin–biotin detection system have been used, and second, authors did not restrict their evaluation to membrane staining, but also considered cytoplasmic staining as positive. In general, the possible reasons for the large variability in reported HER2/neu positive rates might be the use of different methodology, the different scoring systems being employed, and the subjectivity of the pathologists' interpretations [21].

High frequency of HER2/neu expression has also been noted in other types of cancers including breast, lung, and oral cancers [7, 25], suggesting that HER2/neu expression is likely to contribute to the development of human neoplasm. In addition, clinical data reveal that the HER2/neu is involved in the pathogenesis and progression of many types of human malignancies. Expression of HER2/neu has been shown to correlate with invasive and poor prognostic features of breast, ovarian, and other common human cancers, including shorter survival, early relapse, and an increased number of lymph node metastases [9, 26].

Nowadays, there is mounting evidence of the role of HER2/neu expression in patients with gastric cancer, and it has been solidly correlated to poor outcomes and a more aggressive disease. Patients with HER2/neu overexpression exhibited significantly decreased overall survival rates compared to HER2/neu-negative patients. Earlier, a significant association of increasing expression of HER2/neu on IHC with shorter disease-free survival and overall survival was observed [14, 27, 28]. In recent years, some new relevant studies have been reported. In 2009, Zhang et al. reported in a series of 102 gastric cancer patients that HER2/neu overexpression correlate with decreased survival time [29]. In the same year Yu, et al. demonstrated in a study comprising 1143 gastric cancer patients that HER2 expression could not predict poor patient outcome [30]. Again, in a cohort of 841 Korean gastric cancer patients HER2/neu positive status was not related to prognosis of gastric cancer [31]. Interest represented the study of Marx et al. Again they detected 19% positive specimens of 166 gastric cancers, correlation between patient survival and HER2/neu expression was not observed [32]. However, most of the publications in the literature confirm the strong association between HER2/neu overexpression and patient survival [2, 9]. This suggests that HER2/neu may act as a prognostic parameter in gastric cancer. It is very important as regard to the efficacy of conventional therapies, which can be greatly enhanced by anti-HER2/neu agents [33].

Regarding pathologic variables, a higher rate of HER2/neu expression in intestinal histological type than in diffuse-type has consistently been reported [28, 29]. Jaehne et al. found that the membrane staining was significantly greater in well and moderately differentiated tumors of the intestinal type when compared with poorly differentiated lesions and carcinomas of the diffuse type [28]. We could not find any correlation between HER2/neu expression and histology of the tumor, due to the relatively small percentage of HER2/neu positive cases (14%), which can attenuate the statistical power of the study and could be a reason for false–negative results. We acknowledge also the limitation of our small sample size in determining the clinical implications of HER2/neu positivity in gastric cancer. Although, there were no statistically significant clinicopathological associations with HER2/neu expression, HER2/neu expression in our series differed according to the tumor differentiation grade. Among the 18 high/moderately differentiated tumors, we found 27.7% of them to be HER2/neu positive compared with 6.3% among 32 poorly differentiated tumors. Another published study on HER2/neu in 841 Korean gastric cancer patients reported similar results for the tumor differentiation and HER2/neu expression (26.4% for differentiated tumors *vs.*, 9.0% for undifferentiated tumor, $p < 0.05$) [31]. Our result coincided with the above mentioned data. However, there are little known data for the relation between HER2/neu overexpression and cancer differentiation. The reason for the selective overexpression of HER2/neu in high/moderately differentiated gastric cancers are unclear and might be related to selective expression of HER2/neu in intestinal-type gastric carcinomas.

Using immunohistochemistry, our data showed that expression of the HER2/neu protein occurred in 7 of 50 (14.0%) of the gastric cancer specimens. The patients with this find had worse survival after surgical therapy. This association between HER2/neu expression and patient survival provides a good indirect validation for quality of this

investigation and it may act as a prognostic parameter in gastric cancer.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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