Clinics and Research in Hepatology and Gastroenterology (2015) 39, 121—126

Available online at ScienceDirect www.sciencedirect.com

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ORIGINAL ARTICLE

HER2/neu over-expression predicts poor outcome in early gastric cancer without lymph node metastasis

Yongjia Yan[a], Li Lu[b], Chunna Liu[b], Weidong Li[a], Tong Liu[a], Weihua Fu[a,*]

[a] *Department of General Surgery, Tianjin Medical University General Hospital, Tianjin 300052, China*
[b] *Tianjin Medical University, Tianjin 300070, China*

Available online 28 August 2014

Summary
Purpose: Human epidermal growth factor receptor 2 (HER2/neu) is involved in the pathogenesis of several types of cancer, including gastric cancer. However, there remains a paucity of data regarding the prognostic relevance of HER2/neu in early gastric cancer without lymph node metastasis (pN0 EGC). The aim of our study was to analyze whether the over-expression of HER2/neu significantly predicts poor outcomes of pN0 EGC.
Patients and methods: Sixty-seven patients who underwent operative resection for pN0 EGC was enrolled. The HER2/neu status was examined by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).
Results: The HER2/neu-positive rate was 16.4%. HER2/neu over-expression showed a significant correlation with histological type ($P \leq 0.001$), tumor location ($P = 0.022$) and Lauren grade ($P = 0.012$). Multivariate analysis showed HER2/neu serves as a good prognostic marker to predict the risk of poor outcome for pN0 EGC. (HR = 1.384, 95.0% CI: 1.142—1.897 $P = 0.005$)
Conclusion: Considering HER2/neu over-expression significantly predicts poor outcome in pN0 EGC, accurate HER2/neu assessment would be done before endoscopic therapy. For HER2/neu-positive patients, radical surgery should be performed.

Introduction

Gastric cancer (GC) is still a problem of human health all over the world. Despite its incidence rate decreases globally, GC is the fourth most common cancer [1,2]. Early gastric cancer (EGC) is defined as gastric cancer confined to the mucosa and submucosa of the stomach, with/without lymph node metastasis. In recent years, a high participation rate

* Corresponding author. Department of General Surgery, Tianjin Medical University General Hospital, Tianjin 300052, China. Tel.: +86 1382 1661125; fax: +86 2260 362365.
E-mail address: drfuweihua@gmail.com (W. Fu).

http://dx.doi.org/10.1016/j.clinre.2014.06.019
2210-7401/

for endoscopic screening has shown that EGC accounts for almost half of all GC [3,4]. The high cure rate of EGC and the low frequency of perigastric lymph node metastases have allowed the development of more limited modified procedure that improve patient quality of life without compromising cure rate [5,6].

Human epidermal growth factor receptor 2 (HER2/neu) is a proto-oncogene encoded by ERBB2 on chromosome 17. The role of HER2/neu is to inhibit cell apoptosis and promote proliferation. This may lead to excessive or uncontrolled cell growth and may cause tumorigenesis [7,8]. Advances in the molecular biology, genomics and proteomics have led to better understanding of the role for HER2/neu. HER2/neu is closely associated with several malignant forms of cancer, notably GC. Anti-HER2/neu antibody trastuzumab has been approved for the treatment of gastric cancer after a successful phase III trial (ToGA) which demonstrated improved survival for HER2/neu-positive advanced GC patients [9]. HER2/neu were connected to both poor outcomes and higher recurrence in breast cancer [10—12]. Studies even have demonstrated trastuzumab was effective against early breast cancer [13—15]. However, the value of HER2/neu serve as a prognosis factor in EGC remains unclear. Therefore, the aim of this study was to analyze whether the over-expression of HER2/neu amplification significantly predicts poor outcomes of EGC without lymph node metastasis.

Patients and methods

Patients

We chose all patients with EGC who were confirmed between January 2000 and December 2008 by postoperative pathology to have no lymph node metastasis at Tianjin Medical University General Hospital, Tianjin, China. Only patients with gastric cancer confined to the mucosa and submucosa were finally included. Oncological assessment was performed every six months to each patient, and these patients were followed up by phone calls or mail for the status of survival. There were a total of 67 successfully followed up. Formalin-fixed surgical specimens were used. The patients' clinicopathological characteristics were collected with the help of hospital information system. The pathologic diagnosis was made by two pathologists individually based on Lauren's classification, and histological differentiated types.

HER2/neu status was tested by cutting the samples into 4 μm sections then using immunohistochemistry (IHC). An IHC HER2/neu 3+ breast cancer sample was used as a positive control. Ethical and methodological aspects of this study were approved by the Ethical Committee of Tianjin Medical University.

IHC

Anti-HER2/neu (4b5) antibody (Ventana Medical Systems, Inc. Tucson, Arizona) was used to carry IHC staining as the primary antibody against HER2/neu on an automatic immmunostainer (Benchmark XT, Ventana Medical Systems Inc. Tucson, Arizona) according to the manufacturer's instructions.

By following the National Comprehensive Cancer Network (NCCN) guideline, a semiquantitative approach was used to generate a score for each case as follows: no membrane staining or membrane staining in less than 10% of the tumor cells (score 0), faint/barely perceptible partial membrane staining in more than 10% of the tumor cells (1+), weak to moderate staining of the entire membrane in more than 10% of the tumor cells (2+), and strong staining of the entire membrane in more than 10% of the tumor cells (3+). IHC 0, 1+ was considered negative for HER2/neu over-expression, and IHC 3+ were considered positive. In cases with IHC 2+, fluorescence in situ hybridization (FISH) was used to detect HER2/neu amplification levels. IHC scoring was checked by two independent pathologists who were not familiar with the clinical data.

FISH

In IHC 2+ patients, The PathVysion®HER2 DNA Probe kit (LSI®HER2/neu Spectrum OrangeTM/CEP®17 Spectrum GreenTM) was used to carry out FISH analysis according to the manufacturer's protocol to detect HER2/neu amplification levels. FISH positive was defined as HER2/neu: CEP17 ratio≥2. IHC 2+ patients would be considered HER2/neu-positive only if they are FISH+.

Statistical analysis methods

The Chi^2 test and Krushal-Wallis test were carried out to compare the relationship between the HER2/neu status and clinicopathological factors. Overall survival was calculated using the Kaplan-Meier method, and differences between survival rates were analyzed with the long-rank test. The Cox proportional hazard model was used for multivariate analysis. Results were considered significant when *P* values were less than 0.05. Statistical analyses were performed using SPSS-19 (IBM SPSS Statistics, Chicago, IL).

Result

Patients and tumor characteristics

Between January 2000 and December 2008 a total of 67 patients underwent curative-intent surgery for EGC and were successfully followed up. The patient characteristics are summarized in Table 1. The patients had an average age of 57.3 years and 43 of them were male, with follow numbers of different Lauren's tumor types (intestinal: 61.2%; diffuse: 17.9%; mixed: 20.9%). In terms of tumor location, 32.8% of the 67 tumors located at the gastro-esophageal junction, 13.4%, 7.5%, 46.3% located at cadia, fundus and body/antrum respectively. The morphological type of patients was type I (16.4%) type II (68.7%) and type III (14.9%). All 67 patients' specimens were adequate for IHC evaluation of HER2/neu status. Of the 13 IHC 2 + specimens selected for FISH evaluation, and 4 of which were positive. According to the definition, there were 11 patients (16.4%) were HER2/neu-positive. The result of IHC and FISH was shown in Fig. 1.