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Vascular endothelial growth factor expression is an independent poor prognostic factor for human epidermal growth factor receptor 2 positive gastric cancer

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ABSTRACT

Background: The positive expression of human epidermal growth factor receptor 2 (HER-2) is phenotypically associated with differentiated gastric cancer (GC) and is a prognostic factor for resectable GC. The aim of this study was to explore the clinical significance of vascular endothelial growth factor (VEGF), protein kinase B, and p38 mitogen-activated protein kinase (MAPK) regarding outcome in patients with HER-2 positive GC, and to analyze the relationship between these molecules and clinicopathologic parameters.

Materials and methods: Between January 2001 and December 2012, radical-intent gastrectomy specimens from GC patients were evaluated for HER-2 expression by immunohistochemistry (IHC); and with HER-2 expression levels of 2+ were further subjected to fluorescence *in situ* hybridization analysis. HER-2 positivity was defined by a HER-2 3+ score on IHC or a HER-2 2+ score on IHC and HER-2 amplification by fluorescence *in situ* hybridization. The expression of VEGF, phosphorylated protein kinase B, and phosphorylated p38 MAPK in HER-2 positive specimens was scored using IHC.

Results: Fifty-four patients with HER-2 positive stage I-III GCs were identified. Univariate analysis of prognostic factors showed that tumor differentiation, the presence of vascular invasion, and overexpression of p-p38 MAPK, and VEGF significantly affected prognosis. Multivariate analysis identified vascular invasion (hazard ratio = 2.704; 95% confidence interval = 1.074-7.088; $P < 0.033$) and the overexpression of VEGF (hazard ratio = 2.760; 95% confidence interval = 1.083-6.753; $P < 0.035$) to be independent prognostic predictors of HER-2 positive GC.

Conclusions: VEGF overexpression and the presence of vascular invasion were independent poor prognostic factors for HER-2 positive GCs.

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Introduction

Despite the global decrease in the overall incidence of gastric adenocarcinoma (gastric cancer [GC]), it remains the third leading cause of cancer-related deaths worldwide.[1] Radical surgery plays the most important role in the curative treatment of GC patients. However, most patients present with advanced disease, with 5-y survival rates of approximately 10%.[2,3] Human epidermal growth factor receptor 2 (HER-2) is involved in tumor cell proliferation, adhesion, migration, apoptosis, and differentiation, and it is believed to be a new prognostic factor and a novel therapeutic target.[4] Our previous studies indicated that HER-2 positivity did not affect survival in stage I-IV GC patients undergoing tumor resection.[5] In a subpopulation study, univariate analysis identified HER-2 positivity to be a favorable prognostic factor for stage III/IV GC; however, the multivariate analysis did not confirm HER-2 positivity as an independent prognostic factor.[6] Begnami *et al*.[6] reported in a small-scale study ($n = 11$) that the survival in patients with HER-2 positive GC was poorer than that in patients with HER-2 negative GC (17 *versus* 40 mo; $P = 0.023$). Furthermore, the ToGA trial showed that HER-2 positive unresectable or metastatic GC patients treated with chemotherapy alone had a median survival of 11.8 mo,[7] which was longer than that reported in previous studies, ranging from 8 to 11 mo.[8,9] However, studies on the molecular signaling pathway in HER-2 positive GC are still inadequate in terms of explaining these conflicting results.

Signaling molecules downstream of HER-2, such as protein kinase B (Akt) and mitogen-activated protein kinase (MAPK), are reported to drive cell proliferation, survival, differentiation, angiogenesis, and invasion.[10,11] In addition, one of the downstream effects of Akt or MAPK is the production of vascular endothelial growth factor (VEGF).[11,12] Uncontrolled angiogenesis is a critical step in the growth, invasion, recurrence, and metastasis of tumors.[13] Studies have indicated that tumor angiogenesis is a useful predictor for recurrence in patients with GC.[14] VEGF, a powerful potential tumor angiogenic growth factor, has been shown to be strongly associated with a poor outcome and unfavorable clinicopathologic features, including lymph node metastasis and vascular invasion in patients with GC.[12,15-18] For HER-2 positive GC, the expression of these proteins in surgical specimens was unclear, and the association between the clinicopathologic factors and those targeted proteins was not investigated. Furthermore, whether VEGF, phosphorylated Akt (p-Akt), and phosphorylated p38 MAPK (p-p38 MAPK) expression status could be prognostic factors in patients with HER-2 positive GC undergoing radical resection remains uncertain. The aims of this study were to examine the degree of VEGF, p-Akt, and p-p38 MAPK expression, to elucidate the correlation between the clinicopathologic characteristics and status of VEGF, p-Akt, and p-p38 MAPK expression, and to evaluate the prognostic role of VEGF, p-Akt, and p-p38 MAPK expression for survival in patients with HER-2 positive GC undergoing radical resection.

Materials and methods

This study was approved by the institutional review board of the Chang Gung Memorial Hospital (no. 100-4279B). In total, 54 patients with stage I-III, HER-2 positive GC who underwent radical-intent gastrectomy (R0 and R1) at Chang Gung Memorial Hospital, Taoyuan, Taiwan, between January 2001 and June 2012 were enrolled. The standard procedure included a spleen-sparing and/or pancreas-sparing subtotal or total gastrectomy plus D1 or D2 lymphadenectomy depending on the degree of tumor invasion and lymph node metastasis. Resection of involved adjacent organs was performed with clear surgical margins.[2] Tumor staging was performed according to the 7th edition of the American Joint Committee on Cancer Staging System.[19] Postoperative adjuvant chemotherapy with fluoropyrimidine-based or platinum-based regimens was indicated for patients with stage II-III disease. HER-2 positivity was defined by a HER-2 3+ score on immunohistochemistry or a HER-2 2+ score on immunohistochemistry and HER-2 amplification by fluorescence *in situ* hybridization (FISH).

Immunohistochemical analysis of HER-2 expression

Formalin-fixed paraffin-embedded tissue samples were prepared using an automated tissue-arraying instrument (BEECHER ATA-27, Beecher Instruments, Sun Prairie, WI). Three representative areas were selected for each tumor and marked on the tissue slide on which hematoxylin-eosin staining was performed. The corresponding tissue block for each tumor was sampled. The designated area from each donor block was punched with a tissue cylinder of 1 mm in diameter, and the sample was transferred to a recipient block. The arrayed tissue sections were used for the immunohistochemical analysis of HER-2 expression (1:200, A485, Dako). Tissue sections 3 μm in thickness were deparaffinized in xylene and rehydrated in a graded ethanol series. The immunohistochemical analysis was performed using an automated immunostainer (BOND-MAX, Leica, Wetzlar, Germany). The following scoring system was used: score 0, no membrane staining or staining of <10% of the cells; 1+, incomplete membrane staining in >10% of the cells; 2+, weak-to-moderate complete membrane staining of >10% of the cells; and 3+, strong and complete membrane or laterobasal staining in >10% of the cells.[20] An appropriate positive control (breast carcinoma with HER-2 overexpression) was included in each run, and the HER-2 stains were analyzed by two senior pathologists.

Fluorescence in situ hybridization

GC specimens with HER-2 expression levels of 2+ were further subjected to FISH analysis. FISH was performed for HER-2 amplification using the PathVysion HER-2 DNA Probe Kit (Abbott Molecular, Des Plaines, IL). Tissue sections (4-μm thickness) were placed onto coated slides, air dried, and baked overnight at 56°C. Sections were deparaffinized in xylene thrice for 10 min each, and immersed in 100% ethanol twice for 5 min. After air drying, the slides were treated in paraffin pretreatment solution (Paraffin Pretreatment Kit II, Abbott Molecular/Vysis, Des Plaines, IL) for 10 min at 80°C, washed with distilled water for 3 min at room temperature, and treated with protease solution for 15 min at 37°C. Slides were