J BUON 2013; 18(1): 98-104
ISSN: 1107-0625 www.bu-on.org/jbuon
E-mail: jbuon@ath.forthnet.gr

ORIGINAL ARTICLE

Clinical role of HER-2/neu expression in colorectal cancer

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Summary

Purpose: To evaluate the HER-2/neu expression and its relationship with clinicopathological parameters and prognosis in colorectal cancer patients.

Methods: A total of 51 colorectal cancer patients who underwent resection with curative intent from January 2005 to March 2006 were included in this study. Patients were regularly followed up and survival data were obtained as of as April 2011. HER-2/neu protein expression was evaluated from tissue samples from the primary tumor using a semiquantitative standardized immunohistochemical staining kit. Staining intensity was scored as faint (1+), weak to moderate (2+) and moderate to strong (3+).

Results: Forty-nine (96.1%) patients showed 1+ staining, 2 (3.9%) 2+, while no case was strongly positive (3+) for HER-2/neu. No apparent association was noted between HER-2/neu expression and patients' age, gender, tumor location, tumor grade, stage and survival.

Conclusion: Moderate (2+) overexpression of HER-2/neu was detected in a small proportion of colorectal cancer patients. Considering the low rate of HER-2/neu overexpression in colorectal cancer, studies with larger sample sizes using standardized tests are essential to understanding the biologic role of HER-2/neu in this disease.

Key words: colorectal cancer, HER-2/neu, immunostaining

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Received: 27/05/2012; Accepted: 24/06/2012

Introduction

Colorectal cancer is the third most common cancer after lung and breast cancers. According to the GLOBOCAN project estimates, 1,235,108 new colorectal cancer cases were registered in 2008 and accounted for 609,051 deaths worldwide [1]. Earlier diagnosis and the use of new treatment modalities have increased survival rates over the past decades, nonetheless mortality remains high [2]. In the quest for new ways to improve colorectal cancer patients' prognosis, investigators have focused on biological markers that could serve as prognostic and predictive factors as well as targets for therapy.

The proto-oncogene HER-2/neu is localized to chromosome 17q and encodes a transmembrane tyrosine kinase growth factor receptor; a component of a four-member family receptors including epidermal growth factor receptor (EGFR), HER-3 and HER-4 [3]. In normal cells, activation of this receptor controls normal cell growth, differentiation and motility [4]. In cancer cells dysregulation of these pathways and increased expression of HER-2/neu promotes tumor cell growth and migration [5,6]. In breast cancer, HER-2/neu overexpression has been documented in up to 34% of invasive cancer cases and has proved to be an important prognostic and predictive factor [7]. Furthermore, HER-2/neu has been used as an ideal target for monoclonal antibody therapy. Trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of HER-2/neu, has shown as a single agent or in combination with standard chemotherapeutics to significantly prolong the survival in women with HER-2/neu –overexpressing breast cancer [8,9].

The proven importance of HER-2/neu in breast carcinoma pathology and the obvious survival benefit from the use of trastuzumab therapy has led to evaluation of HER-2/neu expression in various tumor types. The expression of HER-2/neu has been studied in prostate, ovarian and lung cancers as well as in several forms of gastrointestinal malignancies including colorectal cancer [10-13]. The latter has a reported HER-2/neu overexpression rate ranging from 4 to 83%, and to date data regarding its prognostic value remain inconclusive [13].

The aim of this study was to evaluate the HER-2/neu expression and its relationship with clinicopathological parameters and prognosis in colorectal cancer patients.

Methods

Patients

A total of 51 colorectal cancer patients who underwent resection with curative intent at the first Department of Propaedeutic Surgery, Hippocrateion Hospital, Athens Medical School, from January 2005 to March 2006 were included in this study. Included patients were 18 years old or older. Excluded were patients with nonepithelial colorectal lesions, those with prior malignancies and those who had received chemotherapy or radiation therapy for colorectal cancer prior to surgery or had died within 45 days after the operation.

Data regarding patients' sex, age, tumor location, tumor grade and stage were collected from the medical records, the pathologists' reports and the operation data. The tumors were staged according to the Astler-Coller staging system. Patients were regularly followed up, and survival data were registered as of April 2011. The median duration of follow-up was 60 months (range 35–64) and included information regarding disease recurrence, overall survival (OS) and disease-free survival (DFS). Recurrent disease was defined as appearance of locoregional and/or distant metastatic lesions after curative resection. OS and DFS were defined as the time period between the date of the primary treatment to the date of death or the date of appearance of locoregional or metastatic disease, respectively. The study protocol was approved by the hospital's Research Ethics Committee and all patients provided oral informed consent before study enrollment.

HER-2/neu evaluation process

Tissue samples from the primary tumor were fixed in neutral buffered formalin, and then dehydrated in a series of alcohols and xylene, followed by infiltration by melted paraffin. Sections of 4 μm in thickness were cut and mounted on silanized slides and air-dried at 37 °C overnight. Immunohistochemical staining for

Table 1. Patient and tumor characteristics.

Characteristics	*N (%)*
Age (years, mean±SD)	70.9±9.3
Gender	
Male	18 (35.3)
Female	33 (64.7)
Tumor location	
Colon	22 (43.1)
Rectal	29 (56.9)
Astler-Coller stage	
A	2 (3.9)
B1	12 (23.5)
B2	19 (37.3)
C1	4 (7.8)
C2	14 (27.5)
Grade of differentiation	
Well	4 (7.8)
Moderate	43 (84.3)
Poor	4 (7.8)
HER-2/neu stain	
2+	2 (3.9)
1+	49 (96.1)

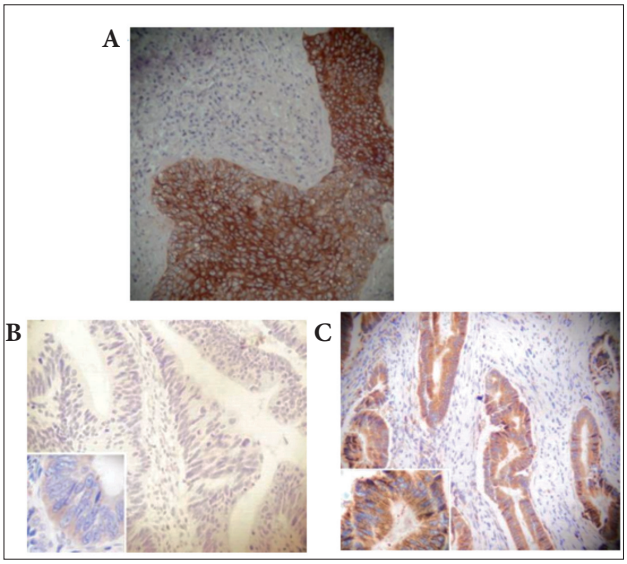

Figure 1. Immunohistochemical staining for HER-2/neu. **A:** 3+ in breast cancer cells. **B:** 1+ and **C:** 2+ staining of tumor cells.

HER-2/neu was determined with the Dako Herceptest assay (Dako Corp., Carpenteria, CA), according to the manufacturer's recommendations. Deparaffinized tissue sections were boiled in 10 mmol/l citrate buffer for antigen retrieval and then immersed in 3% hydrogen peroxide containing 15 mmol/L sodium azide (NaN3). Following incubation with the primary rabbit antibody to human HER-2/neu protein a visualization reagent, consisting of both secondary goat anti-rabbit immunoglobulin molecules and horseradish peroxidase molecules linked to a common dextran polymer backbone, was applied. Bound antibody was visualized using a peroxidase chromogen substrate. The sections were then counterstained with hematoxylin and coverslipped. Results were interpreted using a light microscope by two independent pathologists blinded to each other's findings and to the patients' data. Negative controls were created by omission of the primary antibody and replacement with phosphate buffered saline (PBS). Invasive breast cancer specimens were used as positive controls. The 4-tiered scoring system suggested by the manufacturer for use in breast cancer was utilized. Score zero was defined as undetectable staining or membrane staining in <10% of the tumor cells. Score 1+ was defined as faint membrane staining in >10% of the tumor cells, 2+ as weak to moderate complete membrane staining in >10% of the tumor cells, and 3+ as a moderate to strong complete membrane staining observed in >10% of the tumor cells. HER-2/neu protein expression was defined as negative (scores 0 and 1+) or positive (scores 2+ and 3+). This cutoff point was based on studies in breast cancer.

Statistical analysis

A standard statistical software package (SPSS 11.5 for Windows, SPSS Inc, Chicago, IL) was used in the analysis. Descriptive statistics were calculated for all variables. Continuous variables were normally distributed and presented as mean±standard deviation (SD) and categorical variables were presented as percentages. Fisher's exact test was used to compare categorical variables. Means were compared with the Student's t-test. Survival curves were generated according to the Kaplan-Meier method and differences in survival were assessed using the log-rank test. P values less than 0.05 were considered statistically significant.

Table 2. Patient clinicopathologic and survival data stratified by HER-2/neu immunoreactivity

Characteristics	*HER-2/neu (-)* *N (%)*	*HER-2/neu(+)* *N (%)*	*p-value*
Age, years (mean±SD)	71±9.4	68±2.1	0.71
Gender			0.65
Male	17 (34.7)	1 (50.0)	
Female	32 (65.3)	1 (50.0)	
Tumor location			0.84
Colon	21 (42.9)	1 (50.0)	
Rectal	28 (57.1)	1 (50.0)	
Astler-Coller stage			0.16
A	2 (4.1)	0 (0.0)	
B1	12 (24.5)	0 (0.0)	
B2	19 (38.8)	0 (0.0)	
C1	3 (6.1)	1 (50.0)	
C2	13 (26.5)	1 (50.0)	
Grade of differentiation			0.82
Well	4 (8.2)	0 (0.0)	
Moderate	41 (83.7)	2 (100.0)	
Poor	4 (8.2)	0 (0.0)	
Lymph node metastases	16 (32.7)	2 (100.0)	0.12

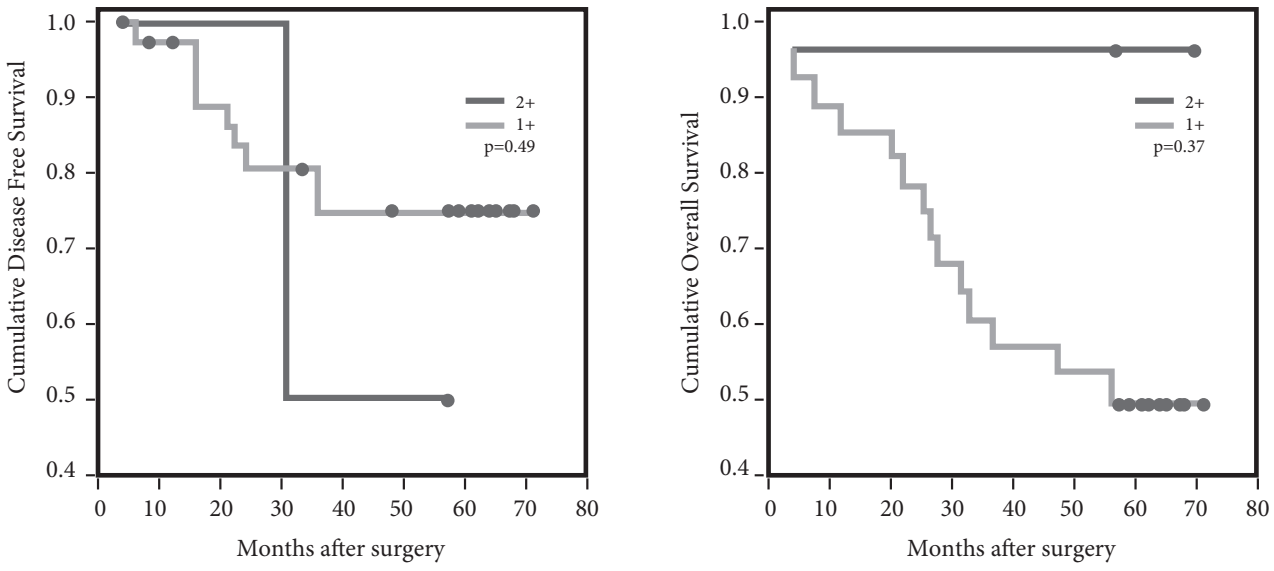

Figure 2. Kaplan-Meier disease-free and overall survival curves with regard to HER-2/neu expression.

Results

Patient and tumor characteristics are presented in Table 1. Study analysis included 18 male and 33 female patients with a mean age of 70.9±9.3 years. Twenty-two (43%) patients presented with colon and 29 (57%) with rectal tumors. Astler-Coller stage A had 2 (3.9%) patients, 12 (23.5%) had stage B1, 19 (37.3%) stage B2, 4 (7.8%) stage C1 and 14 (27.5%) stage C2. There was a clear predominance of patients with grade II tumors (84.3%) and an equal percentage of patients with grade I and III tumors. Forty-nine patients (96.1%) showed 1+ immunostaining, 2 (3.9%) 2+,

while no patient showed 3+ expression for HER-2/neu (Figure 1). Both patients with 2+ HER-2/neu expression had moderately differentiated tumors with lymph node metastases (stage C1 and C2). Although the majority of HER-2/neu negative tumors had no lymph node involvement (67.3%), this correlation was not significant (p=0.12). There was no apparent association between HER-2/neu expression and patients' age, gender, tumor location, tumor grade, OS and DFS (Table 2, Figure 2).

Discussion

Overexpression of the transmembrane HER-2/neu protein has proved to be a significant ally in breast cancer treatment [8,9]. The role of this biological marker has also been examined in several solid tumors with variable results [10-13]. In the present study HER-2/neu overexpression was detected in 3.9% of patients with colorectal cancer. No correlation could be found between HER-2/neu overexpression and any clinical or prognostic variable.

In concordance with our results a retrospective study by Schuell et al. involving 77 specimens of colorectal cancer lesions, and using the Hercep-Test Kit showed scores of 2+ and 3+ membrane staining in only 1% and 3% of the patients, respectively. No relationship was found between membranous HER-2/neu expression and patients' clinicopathological data or survival [14]. Similarly, according to Nathanson et al. among 139 cases HER-2/neu overexpression was seen in 5 cases (3.6%) and HER-2/neu gene amplification was observed in 4 (2.4%) out of 169 tumor specimens. Neither HER-2/neu overexpression nor gene amplification were correlated with any clinicopathologic features or patients' survival [15]. A study by Kavanagh et al. in 132 colorectal cancer patients showed moderate membranous staining in 9 (8%) patients and strongly positive in 2 (2%); there was no correlation with gender, age, grade, Dukes stage, TNM stage, time to recurrence and 5-year survival [16].

In disagreement with the aforementioned data a study by Park et al. reported that 65 (47.4%) out of 137 colorectal cancer patients overexpressed HER-2/neu protein; although no relationship was found with tumor grade or stage it was independently related to survival by multivariate analysis [17]. Osako et al. in a study involving 146 colorectal cancer patients showed that 100 (68.5%) revealed cytoplasmic staining and only 3 of the 100 showed membranous staining for HER-2/neu. Cytoplasmic overexpression correlated with tumor stage and was an independent prognostic factor [18]. A study by Kay et al. in 164 patients with Dukes B disease although failed to show membranous staining in any case, revealed cytoplasmic staining in 33.5% of the patients which was correlated independently with survival [19]. Zhou et al. studied 173 colorectal carcinoma patients and reported that HER-2/neu expression was found in the cytoplasm and membrane in 52% of the cases and was an independent prognostic factor of survival [20].

The role of HER-2/neu protein in colorectal cancer is still questionable; the most likely explanation for the discrepancy among published data lies in technical issues regarding immunohistochemistry. The detection of HER-2/neu protein is highly dependent from tissue fixation and choice of primary antibody [21]. Furthermore, the duration of antigen retrieval, the dilution of the antigen and the duration of the peroxidase reaction are also critical steps in HER-2/neu staining [22]. Prolonged storage can be a major problem, especially when specimens are stored as unstained slides [23]. In the present study the Herceptest was used - a validated assay for HER-2/neu staining in breast cancer - with strict adherence to the manufacturer's recommendations. Another important issue is the lack of agreement regarding whether only membranous or cytoplasmic or both stainings should be considered as an indication for the overexpression of HER-2/neu. In contrast to membranous, cytoplasmic localization of HER-2/neu is more frequently encountered, yet its prognostic value also remains elusive [18,19,24,25]. A study by Half et al. regarding HER-2/neu localization in colorectal tumors found that only membranous HER-2/neu was associated with gene amplification and high levels of HER-2 mRNA, features suggestive of a possible oncogenic role [25]. In the present study cytoplasmic staining may have been present but was not included in the determination of positivity.

The limitations of the present study should be noted. The number of participating subjects was small; nevertheless, the results were comparable to those of larger series [14-16,25]. The present study focused only in the immunohistochemical overexpression of HER-2/neu .Given the vulnerability of immunohistochemistry to various technical issues, fluorescence *in situ* hybridization (FISH) has been proposed as an alternative or adjunct method for the evaluation of HER-2/neu [26]. In breast cancer HER-2/neu gene amplification by FISH has been highly correlated to immunohistochemical overexpression and is a validated prognostic factor [27,28]. On the contrary, in colorectal cancer HER-2/neu overexpression is greater than predicted by gene amplification data, suggesting that overexpression in these tumors may not be due to gene amplification [13,15,17,25]. Furthermore, FISH is not commonly found in many pathology laboratories, and requires a fluorescence microscope and significant interpreter' expertise [29].

Overexpression of HER-2/neu was detected in a small proportion of colorectal cancer patients; there was no apparent association with age, gender, clinical stage or patients' survival. It appears that HER-2/neu expression rarely presents within the therapeutic range (2+ and 3+), a finding supported by previous researchers including a multi-institutional phase II trial regarding the combination of trastuzumab with standard chemotherapeutics in patients with advanced disease [30]. Although these data undermine the importance of HER-2/neu in colorectal cancer, the lack of sufficient sample size and consensus on immunohistochemical staining interpretation among published studies, highlights the need for further investigations.

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