EVALUATION OF HER-2 EXPRESSION IN DIGESTIVE SYSTEM CANCER

MIHAELA TICA[1*], LILIANA DUMITRU[2], ADRIANA DUMITRU[2], MIHAELA POPARDA[1], CRENGUTA SERBOIU[3], ANCA FLORESCU[4], ELENA IONICA[4], VALERIA TICA[4]

Keywords: digestive system cancer, HER-2, immunofluorescence
Abstract: Cancer can occur in various areas of the digestive system. Diagnosis is by endoscopy with biopsy (EDS-for esophagus and stomach, colonoscopy for colon and rectum) and by surgery (resection of stomach, colon and rectum) with exam HP extemporaneous of the tumoral segments, followed sometimes by CT, RMN, endoscopic ultrasound (esophagus) for staging. Treatment varies with localization on the digestive tract and, generally includes surgery with or without chemotherapy and radiotherapy. Our aim was to evaluate the human epidermal growth factor receptor 2 (HER-2), which overexpression has been shown to be a significant negative prognostic variable. We performed immunofluorescence of HER-2 in 27 colorectal cancer samples, 2 esophagus cancer samples and 5 gastric cancers, respectively, by using antibodies against this antigen. Positive staining of HER-2 was observed when comparing with the normal (a safe margin of healthy tissue around it). The method used will enable accurate diagnosis of a digestive tract tumor and will be useful for selecting appropriate therapeutic strategies.

INTRODUCTION

The human epidermal growth factor receptor-2 (HER-2) is a member of the tyrosine kinase receptor superfamily. HER-2 protein is also known as c-erb-2 or *neu*. HER-2/neu gene is located on chromosome 17q21 and encodes a 185-kDa transmembrane protein which exhibits tyrosine kinase activity. HER-2/neu protein is extensively homologous and related to the epidermal growth factor receptor (EGFR), both being involved in normal cell proliferation and tissue growth.

First, HER-2/neu was investigated in breast cancer, but recently HER-2/neu overexpression has also been detected in other solid tumors as ovary, lung, stomach, and colon. HER-2/neu role as a prognostic factor in cancer has been controversial because some of the initial studies failed to find an association with prognosis. In other studies, researchers found a direct correlation between HER-2 expression and poorer survival.

Colorectal cancer (CCR) is the third prevalent cancer in men and women and there are many researches about molecular and epidemiologic aspects of this disease. In regard with CCR are few articles about the HER-2/neu expression in colorectal adenocarcinoma.

In one study, scientists reviewed the HER-2/neu in gastrointestinal tumors. According to this, HER-2/neu protein overexpression or gene amplification is associated with approximately one-fourth of all gastrointestinal tract malignancies (Ross J. S., 2001).

The low prevalence of HER-2/neu gene amplification and protein overexpression suggests that this oncogene plays an infrequent role in the development and progression of colon cancer and that the primary mechanism of dysregulated HER- 2/neu expression in colon cancer, as in breast cancer, is gene amplification (Nathanson D. R., 2003).

Gastric cancer (GC) is one of the most common tumors and remains the second leading cause of cancer mortality in the world (Kelley J.R., 2003). Barrett's esophagus and dysplasia are associated with the development of esophageal adenocarcinoma (Montgomery E., 2001) while helicobacter pylori infection, atrophic gastritis, intestinal metaplasia, and dysplasia are related with gastric adenocarcinoma (Correa P., 1996; Gravalos C., 2008).

HER-2 overexpression in patients with gastric cancer has been correlated to poor outcomes and a more aggressive disease. More, preclinical data are showing significant antitumor efficacy of anti-HER-2 therapies (particularly monoclonal antibodies directed towards the protein) in *in vitro* and *in vivo* models of gastric cancer. As a result, several clinical trials are exploring in different settings and with diverse designs the potential of anti-HER-2 therapies in gastric cancer patients. The review summarizes the rationale, preclinical evidence, retrospective clinical analyses, and the interim clinical data pertaining HER-2 therapies in gastric cancer (Gravalos C., 2008).

Esophageal carcinoma (EC) is ranking ninth in the list of most common cancers in the world. Esophageal carcinoma is a disease of the mid to late adulthood. Its late mortality is high with only 8% of patients surviving more than five years with a median survival of nine months. There are no differences in survival chances according to sex, racial background and histological type (Toni Lerut, 2001).

Adenocarcinoma of the gastroesophageal junction (GEJ) remains a significant clinical problem that is increasing in incidence and is associated with a poor prognosis. The majority of patients present with advanced disease and less than 50% undergo curative treatment.

Surgical resection is the mainstay of treatment and can cure patients with early stage cancer. The survival rate of patients with advanced resectable gastric or gastroesophageal junction (GEJ) cancers (Cunningham D., 2006; Macdonald J.S., 2001), remains poor despite new treatment strategies, such as perioperative chemotherapy or adjuvant chemo- radiation therapy (C. Gravalos, 2008).

The aim of our study was to evaluate the human epidermal growth factor receptor 2 (HER-2) in patients with gastrointestinal cancers. HER-2 overexpression has been shown to be a significant negative prognostic variable. Clinical and experimental evidence suggest that aberrant HER-2 signaling contributes to tumor initiation and disease progression.

MATERIALS AND METHODS

Patient information

This retrospective study involved 34 specimens of malignant digestive system lesions of patients who underwent surgery at the Emergency University Hospital of Bucharest, and the Emergency Clinical Hospital of Bucharest.

All patients gave informed consent according to institutional guidelines prior to surgery.

Diagnosis was by endoscopy with biopsy (EDS-for esophagus and stomach, colonoscopy for colon and rectum) and by surgery (resection of colon and rectum) with exam HP extemporaneous of the tumoral segments, followed sometimes by CT, RMN, endoscopic ultrasound (esophagus) for staging.

Tumors were collected in the Biochemistry and Molecular Biology Center and staged according to TNM system classification by the pathologists in the Pathological Department of Hospitals involved in the study. This classification system establishes the stage depending on the depth of invasion (T), the involvement of lymph nodes (N) and the presence of distant metastasis (M).

Hematoxylin & eosin staining

This method was performed on cryo-conserved tumoral tissue of 5-µm thick sections. After 10 minutes immersion in hematoxylin solution, slides with the tissue sections were rinsed and prepared for Blue Reagent application. Afterwards slides were washed and incubated one minute with eosin solution, followed by ethanol application, one minute, air dried and cover slipped with water/glycerol. Slides were visualized with a conventional light microscope (Fig.1).

We used hematoxylin and eosin (H&E) stains in order to recognize the morphologic changes that form the basis of cancer diagnosis. Hematoxylin has a deep blue-purple color and stains nucleic acids, whereas eosin is pink and stains proteins nonspecifically. In a typical tissue, nuclei are stained blue, whereas the cytoplasm and extracellular matrix have varying degrees of pink staining.

Immunofluorescence

In order to perform the immunofluorescence for HER-2/neu, serial fragment tissues of 5-µm, previously conserved at 80^0C were air-dried for 30 min at 37^0C, and fixed in acetone for 10 min. Nonspecific binding was blocked by the application of normal goat serum in a humidity chamber, at a 1:10 dilution, for 60 min, and 4^0C. Afterwards slides were rinsed and incubated with the primary mouse monoclonal IgG_1 against human HER-2/neu protein (Santa Cruz) at 1:150 dilution, for 60 min at room temperature. After washing, secondary goat anti-mouse IgG-FITC (Santa Cruz) diluted to a titer 1:150 was applied for 1 h at room temperature. Slides were rinsed with PBS, de-hydrated, cover slipped, and visualized under the microscope.

RESULTS AND DISCUSSION

Our study contained specimens from 27 colorectal, 2 esophagus and 5 gastric cancer patients. The mean age for the CCR patients was 66.6; the mean age for the EC patients was 67.5 years; the mean age of the GC patients was 66.4 years.

To determine the score of HER-2 expression the membrane staining pattern was estimated and scored on a scale of 0 to 3+, where 0 corresponded to tumour cells that were completely negative, 1+ corresponded to faint perceptible staining of the tumour cell membranes, 2+ corresponded to moderate staining of the entire tumour cell membranes and 3+ was strong circumferential staining of the entire tumour cell membranes.

Out of 34 cases, 4 cases (11.7%) were positive, and 30 (88.3%) negative. Positive staining of HER-2 was observed in 3 samples of CCR (11.1%): x2 (3+ staining), x1 (2+ staining) and 1 with GC (20%): 1x (2+ staining) – (Fig.2-4). An example of the absence of HER-2/neu expression can be seen in Fig. 5-6.

These rates of HER-2-/neu positivity might be due by the small number of cases (34 specimens). However, the results are in agreement with some other larger patient series.

Nathanson et al. studied HER-2/neu expression in CCR by immunohistochemistry (IHC) and found that among 139 cases, evaluated HER-2/neu overexpression was seen in 5 cases (3.6%) (Nathanson D.R., 2003).

Several immunohistochemical studies in colorectal carcinomas have reported different frequencies of HER-2/neu overexpression, in a wide range from 0% to 30% (Kapitanovic S., 1997). There have been only a limited number of studies that employed fluorescence in situ hybridization (FISH) on HER-2/neu gene amplification in colorectal carcinomas, and amplification has been detected in 0–30% of cases (Osako T., 1998).

The roll of HER-2/neu oncogene in tumors of the gastrointestinal tract was also investigated by Ross et al. that found wide range of HER-2/neu expression in esophageal, gastric and colon carcinoma (Ross J. S., 2001).

In the last 10 years, some new relevant studies have been reported. In one of this, researchers found a very high rate of membranous or cytoplasmatic HER-2 expression, by IHC in a prospective series of 203 gastric cancer patients (Allgayer H., 2000). They used a monoclonal antibody against HER-2 together with a highly sensitive streptavidin-biotin-elite kit, and evaluate the membrane and cytoplasmatic staining, also. By this study was observed a significant association of increasing expression of HER-2 on IHC with shorter disease-free survival and overall survival.

A literature review shows an obvious controversy regarding HER2 expression in esophageal carcinoma. Overexpression (2+/3+) of HER2 was found only in 7.7% of the primary esophageal carcinoma (essentially of squamous carcinoma type, ESCC) and only in 1 of the 6 cases of esophageal adenocarcinoma (Qichun Wei, 2007).

HER-2 expression in esophageal carcinoma has been reported to vary from 0 up to about 65% (Dreilich M., 2006; Reichelt U., 2007). The humanized monoclonal antibody trastuzumab (Herceptin), which specifically targets the extracellular domain of HER2, has of high interest for physicians in targeting therapy, as therapeutic benefit was proved in patients with HER2-positive metastatic breast cancer (Barnes D.M., 2000).

We used a mouse monoclonal antibody raised against NIH/3T3 cells transfected with Neu of human origin and looked for the membrane staining of HER-2/neu in digestive system tumors. Activation of Neu potentiates tumor cell motility and protease secretion and invasion, and also modulates cell cycle checkpoint function, DNA repair and apoptotic responses. Measurement of increased Neu expression can be a predictor of disease prognosis. Neu may also prove to be a promising target for therapeutic agents.

In regard with the HER-2/neu relationship to prognosis in colorectal cancer patients, there are conflicting results. Some studies have reported an association between HER-2/neu overexpression and advanced stage, decreased survival, or both (Saeki T., 1995; Sun X.F., 1995), and other has failed to find any association with prognosis (Nathanson D.R., 2003; Ochs A.M., 2004).

The clinical significance of HER-2/neu expression in colon cancer was studied, also. This was done by IHC and researchers found overexpression of HER-2/neu in 12.5% of investigated patients. Tumors with positive HER-2/neu showed higher rates of nodal metastases and poor mean survival (Park D.I., 2004).

In a large cohort of colorectal tumors and lymph node metastases study, c-erbB-2 was expressed in 81.8% of analysed tumors. They concluded that there are any correlation between c-erbB2 staining and lymph node metastases (McKay J.A., 2002).

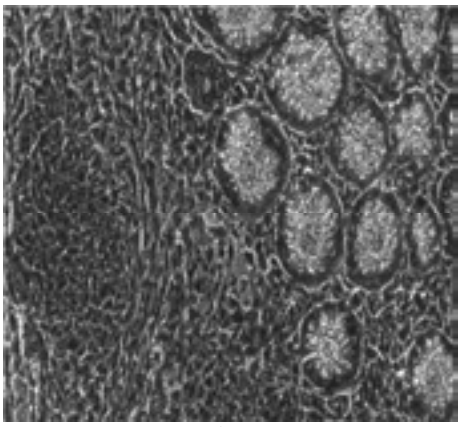

Fig. 1. H&E staining of tissue section (magnification x 10).

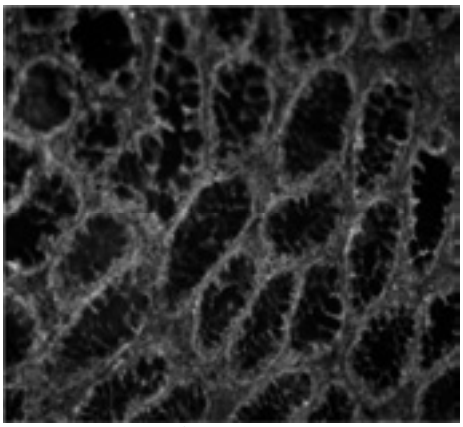

Fig. 2. IF staining for HER-2/neu in CCR. Strong staining of the entire membrane (3+).

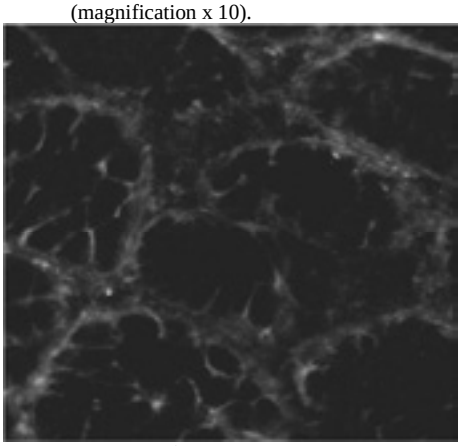

Fig. 3. IF staining for HER-2/neu in GC. Weak to moderate staining of the membrane (2+).

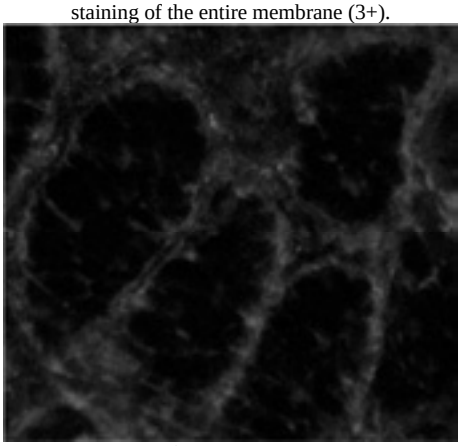

Fig. 4. IF staining for HER-2/neu in CCR. Weak to moderate staining of the membrane (2+).

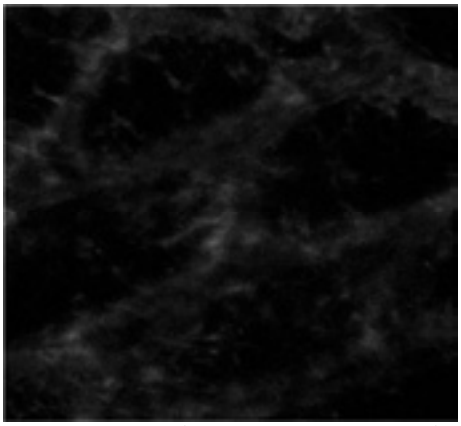

Fig. 5. IF staining for HER-2/neu in EC. Lack of expression

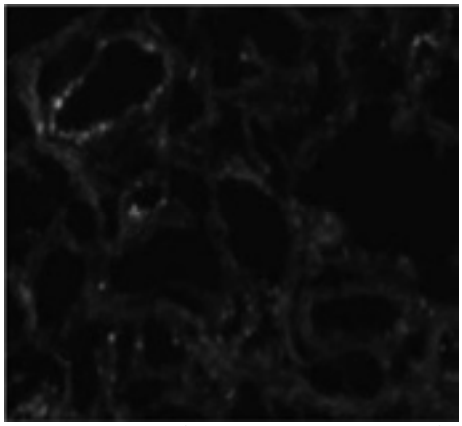

Fig. 6. IF staining for HER-2/neu in GC. Lack of expression.

CONCLUSIONS

Positive staining of HER-2/neu was observed when comparing with the normal (a safe margin of healthy tissue around it).

HER-2/neu overexpression (11.7%) may constitute an independent prognostic factor in digestive system cancer patients; those patients that have HER-2/neu overexpression might be potential candidates for new adjuvant therapy which involve the use of humanized monoclonal antibodies.

The fluorescence method used will enable accurate diagnosis of a gastrointestinal tract tumor and will be useful for selecting appropriate therapeutic strategies.

REFERENCES

Allgayer H, Babic R, Gruetzner KU, et al., 2000. C-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor associated protease systems. *J Clin Oncol* 18:2201-2209.

Barnes D.M. and Miles D.W., 2000. Response of metastatic breast cancer to trastuzumab? *Lancet* 355: 160-161.

Correa P., 1996. *Helicobacter pylori* and gastric cancer: state of the art. *Cancer Epidemiol Biomarkers Prev* 5:477-481.

Cunningham D, Allum WH, Stenning SP, et al., 2006. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *New Eng J Med*, 355(1):11-22.

Dreilich M., Wanders A., Brattstrom D., Bergstrom S., Hesselius P., Wagenius G. and Bergqvist M., 2006. .HER-2 overexpression (3+) in patients with squamous cell esophageal carcinoma correlates with poorer survival, *Dis Esophagus* 19: 224-231.

Ghaffarzadegan K., N. Sharifi, H. Vosooghynia, T. Shakeri, T. Ghiasi Moghadam, Sh. Ghanad Kafi, S. Lari, G. Nassiri, 2006. HER2/neu expression in colon adenocarcinoma and its correlation with clinicopathologic variables. IJBMS, Vol. 9, No. 1.

Gravalos C., Jimeno A., HER2 in Gastric Cancer: A New Prognostic Factor and a Novel Therapeutic Target, *Annals of Oncology*, 2008. 19(9):1523-1529.

Kapitanovic S., Radosevic S., Kapitanovic M. et al., 1997. The expression of p185(HER-2/neu) correlates with the stage of disease and survival in colorectal cancer. *Gastroenterology*, 112:1103–1113.

Kelley JR, Duggan JM, 2003. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol*, 56:1-9.

Macdonald J.S, Smalley S.R, Benedetti J., et al., 2001. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Eng J Med*, 345(10):725-730.

McKay J.A., Loane J.F., Ross V.G., *et al.*, 2002. C-erbB2 is not a major factor in the development of colorectal cancer, *Br. J. Cancer*, 86: 568-73.

Montgomery E., Goldblum J.R, Greenson J.K., et al., 2001. Dysplasia as a predictive marker for invasive carcinoma in Barrett's esophagus. A follow-up study based on 138 cases from a diagnostic variability study. *Hum Pathol* 32:379-388.

Nathanson D.R., Culliford A. T., Shia J., *et al.*, 2003. HER 2/neu expression and gene amplification in colon cancer, *Int. J. Cancer*, 105: 796-802.
Nathanson D.R., Culliford A.T. 4th, Shia J. et al, 2003. HER 2/neu expression and gene amplification in colon cancer. *Int J Cancer* 105:796–802
Ochs A.M., Wong L., Kakani V. et al, 2004. Expression of vascular endothelial growth factor and HER2/neu in stage II colon cancer and correlation with survival. *Clin Colorectal Cancer* 4:262–267.
Osako T., Miyahara M., Uchino S., Inomata M., Kitano S., Kobayashi M., 1998. Immunohistochemical study of c-erbB-2
Park D.I., Oh S.J., Park S.H., Yun J.W., *et al.*, 2004. Clinical significance of HER-2/neu expression in colon cancer. *Korean J. Gastroenterol*., 44:147-52.
protein in colorectal cancer and the correlation with patient survival. *Oncology*, 55:548–555.
Qichun Wei, Lirong Chen, Liming Sheng, Hans Nordgren, Kenneth Wester,Jorgen Carlsson, 2007. EGFR, HER2 and HER3 expression in esophageal primary tumours and corresponding metastases, International Journal of Oncology 31: 493-499.
Reichelt U., Duesedau P., Tsourlakis MCh., Quaas A., Link B.C., Schurr P.G., Kaifi J.T., Gros S.J., Yekebas E.F., Marx A., Simon R., Izbicki J.R. and Sauter G., 2007.Frequent homogeneous HER-2 amplification in primary and metastatic adenocarcinoma of the esophagus. Mod Pathol 20: 120-129.
Ross J. S., McKenna, 2001. The HER-2/neu oncogene in tumors of the gastrointestinal tract, *Cancer Invest*., 19: 554-68.
Saeki T., Salomon D.S., Johnson G.R., Gullick W.J., Mandai K., Yamagami K., Moriwaki S., Tanada M., Takashima S., Tahara E., 1995. Association of epidermal growth factor-related peptides and type I receptor tyrosine kinase receptors with prognosis of human colorectal carcinomas. *Jpn J Clin Oncol* 25:240–249.
Sun X.F., Carstensen J.M., Stal O., Zhang H., Nordenskjold B., 1995. C-erbB-2 oncoprotein in relation to DNA ploidy and prognosis in colorectal adenocarcinoma. *APMIS* 103:309–315.
Toni Lerut, 2001. Carcinoma of the esophagus and gastro-esophageal junction, *Surgical Treatment, Part I Esophagus*.

1- Emergency University Hospital of Bucharest
2- Emergency Clinical Hospital of Bucharest
3- University of Medicine and Pharmacy "Carol Davila"
4- University of Bucharest, Faculty of Biology, Department of Biochemistry and Molecular Biology
*- Correspondent Author: Mihaela Tica; Phone: +40-213180561; Fax: +40-213180561; 169 Splaiul Independentei, Bucharest, Romania; mihaella_roro@yahoo.com