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Original Article

Clinical Significance of HER2 and EGFR Expression in Colorectal Cancer Patients with Ovarian Metastasis at a Tertiary Care Centre

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ABSTRACT

Background: *EGFR and HER2 overexpression has been reported to play important roles in colorectal cancer (CRC) development and metastasis. The purpose of the study is to compare the expression of HER2 and EGFR in primary tumours and ovarian metastases in CRC patients with ovarian metastases and their influence on prognosis.*

Methods: *The present study included 100 patients with CRC, Clinical examination, liver ultrasonography (US) or computed tomography (CT) scan were performed for each patient. The average age was 45.0 ± 12.0 years.*

Results: *There was no significant correlation in the expression of HER2 and EGFR with age, tumour site, tumour differentiation, tumour diameter, number of positive mesenteric lymph nodes or vascular cancer embolus (P > 0.05). There was no significant correlation between the expression of HER2 and the clinicopathological features in CRC patients with non-ovarian metastasis (P>0.05)*

Conclusions*: The present study revealed that HER2 and EGFR are highly expressed in ovarian metastases and primary tumours of CRC patients with ovarian metastases. Some preclinical and clinical studies have already demonstrated the efficacy of EGFR inhibitors in advanced colorectal carcinomas and their potential synergistic effects with chemotherapy and radiation therapy.*

Keywords: *Colorectal Cancer, HER2, EGFR, Prognosis.*

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INTRODUCTION

Colorectal malignancies remain one of the leading causes of cancer deaths, worldwide, in men and women.[1] Earlier diagnosis of colorectal cancer (CRC), better knowledge of its clinicohistological prognostic factors and its surgical treatment combined or not with chemotherapy or radiation therapy have contributed to the improved outcome of affected patients. The most significant and independent prognostic factors accepted to date in CRC remain the TNM (tumor–node–metastasis) stage and 'potential' residual disease after initial surgery.[2]

EGFR is expressed in a variety of human tumors, including gliomas and carcinomas of the lung, colon, head and neck, pancreas, breast, ovary, bladder, and kidney. Mutations, gene amplification, and protein overexpression of various elements of this pathway not only contribute to carcinogenesis but also impact prognosis and provide specific targets for therapeutic intervention. The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein and receptor tyrosine kinase (TK) that is encoded by the c-erbB-1 proto-oncogene.[3]

The human epidermal growth factor receptor 2 (HER-2) signaling pathway plays an important role in tumor proliferation, angiogenesis, differentiation and metastasis in CRC.[4] HER-2-targeted drugs, including Herceptin, have been developed and widely applied for the treatment of breast cancer presenting with membranous HER-2 overexpression.[5,6]

EGFR is positively expressed in 59 to 85% of CRC specimens, and its overexpression is closely related to clinical stage, lymph node metastasis, disease-free survival, poor overall survival, and 5-year recurrence rate.[7,8] HER2 is

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an emerging therapeutic target and prognostic factor for metastatic CRC.[9] The positive expression rate of HER2 protein in CRC tumours varied from 2 to 11%, and this rate increased in more advanced diseases. The purpose of the study is to compare the expression of HER2 and EGFR in primary tumours and ovarian metastases in CRC patients with ovarian metastases and their influence on prognosis.

METHODS

The present study included 100 patients with CRC, Clinical examination, liver ultrasonography (US) or computed tomography (CT) scan were performed for each patient. The average age was 45.0 ± 12.0 years. The inclusion criteria were as follows: (1) postoperative diagnosis of primary CRC based on histopathology; and (2) ovarian metastases confirmed by postoperative pathology. The exclusion criterion was the presence of primary ovarian cancer or ovarian metastases derived from other cancers.

At the same time, third-stage patients with non-ovarian metastases were selected as controls for EGFR or HER2. The inclusion criteria for the controls were (1) female patients with primary colorectal tumours confirmed by postoperative histopathology; (2) no ovarian metastases detected by computed tomography; and (3) previous confirmation of the HER2 or EGFR expression status with immunohistochemistry (IHC).

Immunohistochemical Analysis

All resected CRCs were received fresh, fixed in 10% pH-neutral formalin, embedded in paraffin. Serial sections from the same blocks were used for HER2 and EGFR analysis. Specimen sections were dewaxed by xylene, rehydrated with a standard ethanol gradient, and subjected to high temperature and high pressure for antigen retrieval. The slices were incubated in H2O2 for 10 min at room temperature, subjected to dropwise addition of the corresponding primary antibody followed by incubation at 4 °C overnight, rinsed with phosphate buffered saline (PBS), and subjected to dropwise addition of secondary antibody. Characteristics studied included age, sex, tumor site, tumor size, degree of histological differentiation (well/moderate/poor), number of invaded lymph nodes counted during the slide review and classified as N1 or N2, based on UICC/TNM staging system, perineural invasion and/or venous emboli (classified as present or absent), the presence of synchronous metastases and the occurrence of metastases during the follow-up period.

Scoring System

HER2 is mostly localized to the cell membrane, while EGFR is expressed in the cell membrane and cytoplasm. HER2 expression was scored as described in the VENTANA study[10]: (−), no tumour cell staining or less than 10% cell membrane staining; (+), > 10% cells had weak or almost undetectable cell membrane staining; (++), > 10% cells were weak to moderate cell membrane staining; and (+++), > 10% of cells have strong cell membrane staining. EGFR expression was determined according to the staining intensity and the number of positive cells. The staining intensity was scored as follows: no staining, 0 points; staining faintly visible, 1 point; weak-medium staining, 2 points; and strong staining, 3 points. The number of positively stained cells was scored as follows: ≤ 10% positive cells, 1 point; 11 to 50%, 2 points; 51 to 80%, 3 points, and ≥ 80%, 4 points.

Statistical Analysis

All data were statistically analysed using SPSS 22. Comparisons of EGFR and HER2 expression between groups were using the Wilcoxon rank sum test. Comparisons of data according to HER2 or EGFR expression were performed by Fisher's exact probability method. The correlation of HER2 and EGFR expression was performed by Spearman's ranked correlation coefficient.

Survival analysis was established according to the Kaplan-Meier method. Comparison of survival times was carried out using log-rank test. A P-value less than 0.05 was considered statistically significant.

RESULTS

The clinical and histological characteristics of the 148 patients are reported in Table 1. There was no significant correlation in the expression of HER2 and EGFR with age, tumour site, tumour differentiation, tumour diameter, number of positive mesenteric lymph nodes or vascular cancer embolus (P > 0.05). There was no significant correlation between the expression of HER2 and the clinicopathological features in CRC patients with non-ovarian metastasis (P>0.05)

Table 1: Demographic characteristics of patients

	HER2 (n=100)	EGFR (n=100)
Sex		
Male	60	65
Female	40	35
Mean Age	60.0 ± 15.6	65.0 ± 12.8

Table 2. Clinicopathological variables and their correlation with immunohistochemical expression of HER2 and EGFR in primary tumours

Characteristics	HER2 (n=100)	EGFR (n=100)	
Primary tumour location			0.500
Colon	60	65	
Rectum	40	35	
Differentiation			0.399
High	55	45	
Mid low	45	55	
Positive lymph node			0.040
0	30	35	
1-3	40	40	
>4	30	25	
Tumour diameters			0.99
<5 cm	60	70	
>5 cm	40	30	
Vascular cancer embolus			0.065
Yes	35	45	
No	65	55	

DISCUSSION

The purpose of the study is to compare the expression of HER2 and EGFR in primary tumours and ovarian metastases in CRC patients with ovarian metastases and their influence on prognosis. EGFR and HER2 are all reported to be involved in the development and progress of CRC.[7-9,11] But

their role in CRC ovarian metastases had not been clarified. In this respect, we analysed their expression and investigated their clinical importance in CRC patients with ovarian metastases. Although EGFR expression has been studied in colorectal cancers only limited and inconsistent data are available concerning the EGFR activation status in this malignancy.[12-14]

Park et al[15] revealed HER-2 overexpression in 47.4% of 137 patients with CRC, whereas Antonacopoulou et al[16] observed overexpression in 24.7% of 124 patients using IHC performed on whole sections. Demirbas et al[17] demonstrated HER-2 overexpression in 9.6% of 104 patients with CRC using tissue microarray (TMA). The results of these studies indicate that the expression of HER-2 in CRC is associated with the prognosis and may constitute a potential candidate for novel adjuvant therapies involving humanized monoclonal antibodies, such as Herceptin. However, other studies have demonstrated that the expression of HER-2 in CRC was not associated with the prognosis, based on a subjunctive scoring system of IHC. Kruszewski et al[18] reported HER-2 overexpression in 27% of 202 CRC patients, while Kavanagh et al[19] observed overexpression in 11% of 132 patients using IHC performed on whole sections. Kim et al[20] reported HER-2 overexpression in 0.5% of 185 patients with CRC.

Recent studies showed that distal carcinomas were more likely to be HER2 or EGFR positive than proximal carcinomas despite the tests used.[21] Higher frequencies of HER2 overexpression were found in rectal cancer than in descending colon or right colon cancers.[22]

CRC involves changes in multiple oncogenes, tumor suppressor genes and signal transduction pathways. Almost all tumors with more than one locus are involved in tumorigenesis. EGFR inhibitors have been widely used in oncotherapy. The identification of the mutant kirsten rat sarcoma viral oncogene homolog (KRAS) as a predictor of resistance to EGFR monoclonal antibodies created a major change in the treatment of CRC.[23,24]

However, whether HER2 overexpression could predict a higher risk of ovarian metastasis remains unknown. In addition, HER2 was reported to be a negative biomarker for EGFR-targeted treatments such as cetuximab and panitumumab in CRC.[25,26] Thus, HER2 status should be considered when developing treatment strategies for those patients. The role of EGFR was an important player in initiation and progression in CRC. EGFR overexpression was detected in 80–90% of colorectal tumours. Another study showed that 65% of the primary CRC tumours, 66% of the metastases, and 43% of the matched primary CRC metastases were EGFR positive.[27]

The metastatic lesions may also be suitable for anti-HER-2 therapy due to the homogenicity of HER-2 expression in CRC. These results indicate that HER-2 may be a promising target as an adjuvant therapy for patients with CRC.

CONCLUSION

The present study revealed that HER2 and EGFR are highly expressed in ovarian metastases and primary tumours of CRC patients with ovarian metastases. Some preclinical and clinical studies have already demonstrated the efficacy of EGFR inhibitors in advanced colorectal carcinomas and their potential synergistic effects with chemotherapy and radiation therapy. However, further studies are required to confirm these results.

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