An immunohistochemical study of EGFR expression in colorectal cancer and its correlation with lymph nodes status and tumor grade

Mojgan Mokhtari, Mina Memar Ardestani, Mohammad Movahedipour
Department of Pathology, Medical University of Isfahan, Isfahan, Iran

Background: Colorectal cancer (CRC) is a common human malignancy. Epidermal growth factor receptor (EGFR) is expressed in wide variety of human malignancies and is of some therapeutic and prognostic utility. The relationship between EGFR expression and regional lymph nodes involvement, and tumor grade in CRC has not been cleared, thus we decided to show it in a case-control study. **Material and Methods:** We chose paraffin-embedded tissue blocks of 46 CRCs with regional lymph nodes involvement as case group, and 46 CRCs without lymph nodes involvement as control group and then performed immunohistochemical staining for both groups. Moderate to strong, and complete staining of more than 10% of tumor cells was regarded as EGFR-positive. In analysis, *P*-value less than 0.05 was considered as significant. **Results:** EGFR expression was positive in 80.4% and 56.5% of patients in the case and the control groups, respectively, which the difference between them was statistically significant. EGFR was positive in 48% of grade I, 60% of grade II and 100% of grade III tumors. **Conclusions:** EGFR expression had relationship with lymph node involvement and tumor grade in CRC. Also, lymph node-involved CRCs showed higher scores of EGFR staining than control group. Thus, EGFR may be an additional factor to develop more aggressive CRCs and may predict the probability of lymph node involvement in these tumors.

Key words: Colorectal cancer, EGFR, epidermal growth factor receptor, immunohistochemical

INTRODUCTION

Colorectal cancer (CRC) is a common human malignancy and its incidence is increasing slowly. CRC is the second leading cause of cancer related death in both sex and often involves rectosigmoid. The most common histologic type of CRC is adenocarcinoma.[1-4]

Based on cell arrangement and amount of tubule formation, adenocarcinomas are divided in three grades: grade I or well differentiated, grade II or moderately differentiated, and grade III or poorly differentiated.[5,6]

The staging system for CRC is based on depth of invasion, number of involved regional lymph nodes and the presence or absence of distant metastasis. In stages I and II, lymph nodes involvement is not present, in stage III the tumor invades regional lymph nodes, and in stage IV the tumor shows distant metastasis

with or without lymph node involvement.[7,8] The treatment of CRC includes surgery, chemotherapy, and radiotherapy.[9,10]

Recently, the use of specific molecules, including epidermal growth factor receptor (EGFR), for treatment, and determination of prognosis of cancers, is being developed. EGFR---also known as HER_1---is a 170 KD transmembrane glycoprotein and is a member of the erbB family of receptor tyrosine kinase, including erbB1 (HER1), erbB2 (HER2), erbB3, and erbB4. EGFR is expressed in many human cancers, including 51%--85% of CRCs. Overexpression of EGFR correlates with disease progression, poor prognosis, metastatic spread, and drug resistance in many human cancers.[11-14]

Until now, the relationship between EGFR expression and lymph nodes status, and tumor grade in CRC, which may be of predictive value, has not been cleared.[14] The aim of this study was to determine this relationship by an immunohistochemical method in a case-control study.

MATERIALS AND METHODS

This research is a case-control study in which CRCs with lymph node involvement were designated as case and CRCs without lymph node involvement were considered as control group. The target population were paraffin

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blocks of primary CRCs diagnosed in Al-Zahra hospital (an Isfahan University of Medical Sciences-affiliated Hospital, Iran) during a period of three years (2006–2008). The sample size was calculated 46 cases for each group (according to P_1 = 60% and P_2 = 85% mentioned in previous articles, α = 5% and power = 80%),which were selected by convenient method among paraffin blocks (research number: 388312).

Inclusion criteria were defined as: (1) Paraffin blocks of colorectal adenocarcinoma in which a minimum of 14 lymph nodes had identified in the surrounding tissue of tumor. (2) Paraffin blocks which had adequate tumor tissue to section. Exclusion criteria were vice versa to the inclusion criteria.

One slide was prepared from each paraffin block then the sections were dewaxed (45 min in oven 60 °C). Proteinase K solution was employed to retrieve antigenic sites for 30 minutes. Endogenous peroxidas activity was blocked using 0.5% H_2O_2 for 10 minutes. The sections were incubated with primary antibody (Novocastra-RTU-EGFR) for 40 minutes. Subsequently, incubation with EnVision polymer was done for 30 minutes. Hematoxylin staining was performed for l minute then the slides were mounted. Intervening washing was done using phosphate buffered saline (PH = 7.2). Positive controls were normal oral mucosa and negative controls were provided by omitting the primary antibody. [14-17]

Finally, the slides were seen by two pathologists simultaneously and the results were recorded carefully and analyzed by SPSS-10 software. *P*-value less than 0.05 was considered as significant. The scoring system used in the evaluation of EGFR immunostaining was shown in Table 1. According to the cited references, the staining scores of 2+ and 3+ were regarded as EGFR-positive.

RESULTS

EGFR immunoreactivity was evaluated in 46 CRCs without regional lymph node involvement as control group and 46 CRCs with regional lymph node metastasis as case group and the following results were observed:

Twenty-six of the 46 CRCs in the control group (56.5%) and 37 of the 46 CRCs in the case group (80.4%) were EGFR-positive.

Chi-Square test showed EGFR expression was significantly higher in the case than the control group (*P*-value = 0.01). Also, the results of EGFR staining score in both groups were summarized in Table 2. Mann–Whitney test revealed the case group significantly showed higher EGFR staining scores than the control group (*P*-value = 0.001).

Of the total CRCs in the both groups, 25 cases were grade I, 40 cases were grade II and 27 cases were grade III. EGFR was positive in 48% of grade I, 60% of grade II, and 100% of grade III tumors. Mann-Whitney test showed EGFR expression had relationship with CRC grade, thus increase in tumor grade associated with increase in EGFR-positivity (*P*-value < 0.001).

The number of involved lymph nodes in the case group range from 1 to 8. Thirty-six patients in the case group had less than 4 involved nodes (stage IIIa or IIIb) of which 77.8% were EGFR-positive, and 10 patients had 4 or more involved nodes (at least stage IIIc) of which 90% were EGFR-positive. Fisher's Exact test demonstrated no significant difference between the two groups in EGFR expression (*P* = 0.36).

DISCUSSION

This case-control study demonstrated significant difference in EGFR expression rate between CRCs with lymph node involvement (at least stage III) compared with CRCs without lymph node involvement (stages I and II), also, the results showed EGFR staining scores were higher in the lymph node-involved group. This research also revealed EGFR expression Correlates with tumor grade and increase in tumor grade accompanies with rise in EGFR expression rate. No significant difference was seen between stage IIIc and stage IIIa/IIIb tumors in EGFR expression.

The usual approach to the colorectal cancer is biopsy obtaining before doing surgery. The routine histological examination of biopsy alone can determine diagnosis but cannot predict the probability of synchronous lymph nodes involvement. On the other hand, in some colectomy specimens no or a few lymph nodes are found and this issue complicates the staging of tumor. In addition, having the knowledge about some characteristics of tumor can help

Table 1: The scoring system used in the evaluation of EGFR immunostaining[18-20]

Score	Quality and quantity of staining
0	No staining or nonspecific staining of tumor cells
1+	Weak and incomplete staining of more than 10% of tumor cells
2+	Moderate and complete staining of more than 10% of tumor cells
3+	Strong and complete staining of more than 10% of tumor cells

Table 2: Frequency distribution of EGFR staining scores in both groups

Groups	Case group		Control group	
EGFR score	Number	(%)	Number	(%)
0	2	4.4	5	10.9
1+	7	15.2	15	32.6
2+	18	39.1	21	45.6
3+	19	41.3	5	10.9
Total	46	100	46	100

clinician in the approach to patient before doing operation. The main purpose of this study was to determine probable role of EGFR in the prediction of lymph node involvement and estimation of tumor stage. The concluded results from this study show the application of EGFR immunostaining may help in these circumstances. For example, EGFR-expressing tumors more likely seem to invade lymph nodes than their EGFR-negative counterparts. The observed relationships between EGFR expression and lymph node involvement, and tumor grade suggest a negative prognostic role for EGFR in CRCs.

EGFR is expressed in many human malignancies and seems to characterize worse prognosis and tumor progression in at least some of them. Previous studies of CRC and EGFR were more about the prevalence of expression of this receptor in CRC,[11-14] while its value in the prediction of the tumor behavior has not been cleared. Furthermore, the use of anti-EGFR drugs in CRCs is now limited to metastatic tumors (stage IV) while nonmetastatic tumors may also benefit from it as well. The relationship between EGFR status and CRC stage varies in the literature and the relationship of this receptor and CRC grade has not been cleared.[14,21]

One study showed EGFR expression associated with higher-stages in CRC.[22] Another study performed in 2010 revealed increase in EGFR intensity accompanies with poor prognosis in CRC.[23] On the contrary, Porschen in 1993 and McKay in 2002 did not find any relationship between EGFR expression and stage and prognosis of CRC.[24,25]

The discrepancy observed between previous studies may be due to application of different scoring system, doing different IHC method, and variation between observers. For evaluation of staining intensity we used the scoring system recommended in the same studies thus this caused, though does not completely remove, low interobserver variability. Finally, it is recommended that the use of EGFR immunostaining to be considered as a routine method in the assessment of CRCs biopsies to predict the probability of lymph node involvement, if possible, and also the effect of anti-EGFR drugs on nonmetastatic CRC to be evaluated by designing additional clinical trial studies.

CONCLUSIONS

EGFR expression has relationship with lymph node involvement and tumor grade in CRC. Also, lymph node-involved CRCs express higher levels of EGFR in comparison with non lymph node-involved counterparts. Thus, EGFR may be an additional factor to develop more aggressive CRCs and may predict the probability of lymph node involvement in these tumors.

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