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ORIGINAL ARTICLE

Detection of EGFR expression in patients with colorectal cancer and the therapeutic effect of cetuximab

Jinbo Liu[1], Quanbo Zhou[1], Jizhong Xu[1], Jiaxiang Wang[2], Yi Zhang[3]

[1]Colorectal Department, [2]Pediatric Surgery Department, [3]Biological Treatment Center, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

Summary

Purpose: *This study was designed to detect the expression of epidermal growth factor receptor (EGFR) in tumor specimens of patients with colorectal cancer (CRC); moreover, the relationship between EGFR expression and clinical factors as well as prognosis were analyzed to provide a basis for individualized treatment of CRC.*

Methods: *This study used paraffin-embedded tumor specimens of 70 CRC patients who were treated with cetuximab. Immunohistochemistry (IHC) was used to detect the expression of EGFR in CRC tumor specimens. The patient clinical features and treatment administered were recorded and then, the therapeutic effect of cetuximab was evaluated. Progression-free survival (PFS) and overall survival (OS) were assessed.*

Results: *The positive expression rate of EGFR was 64% (45/70), while 18 patients had negative expression. Twenty-two cases had weak positive expression, 15 cases positive expression and another 15 strongly positive expression. Of 70 specimens, 27 (38.6%) had high EGFR expression belonging to 20 (50%) males and 7 (23%) females ($p<0.05$). However, age, Karnofsky performance status (KPS), tumor site, grade of differentiation and clinical stage showed no significant difference in relation to EGFR expression ($p>0.05$). No patient achieved complete remission (CR), 15 (21.4%) had partial remission (PR), 12 (17.1%) were in stable state (SD) and 40 (57.1%) patients had disease progression (PD). Disease control rate (DCR) was 39.02% (16/41) in the group with low EGFR expression and 48.28% (14/29) in the group with high EGFR expression ($p>0.05$).*

Conclusion: *EGFR expression in CRC tissue is correlated with patient gender. In the group with higher EGFR expression, the effectiveness of cetuximab was significantly higher than that in the low EGFR expression group, indicating correlation between the high expression of EGFR and the short-term effect of cetuximab.*

Key words: *cetuximab, colorectal cancer, EGFR*

Introduction

CRC, a common malignant tumor of the gastrointestinal tract, is characterized by high incidence, high mortality and low cure rate [1]. Most of the patients are already in middle and advanced stage of CRC at diagnosis due to the low early diagnosis rate. Chemotherapy is the main treatment method for metastatic or locally advanced CRC ; however, traditional chemotherapy drugs have toxic side effects, poor tolerance and unsatisfactory effectiveness [2]. The monoclonal antibody cetuximab, which acts on EGFR, is one of the most widely studied targeted drugs. Cetuximab shows remarkable therapeutic effects in numerous CRC clinical trials, used either alone or in combination with chemotherapy [3-6]. However, cetuximab evoked much debate in advanced CRC treatment in recent years. More than half of CRC patients will develop local recurrence or metastasis. Though the survival rate of CRC patients is nearly 90% at 5 years after early diagnosis, still more than half of the patients were found with infiltration or metastasis at the time of diagnosis

Correspondence to: Yi Zhang, MD. Biological Treatment Center, the First Affiliated Hospital of Zhengzhou University, No. 1 Jianshe Road, Zhengzhou, Henan, 450052, China. Tel and Fax: +86 0371 66913114, E-mail : zhangyi890523@163.com
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[7]. For these patients, their 5-year survival rate is only 10.8% [8]. In terms of the entire large intestine, rectum is the most frequent site of primary tumors. Carcinoma of the rectum accounts for about 40% of the total CRC incidence in Western countries [9]. In China, however, this figure is 60 to 70% [10]. Compared with colon cancer, rectal carcinoma has high local recurrence rate and low long-term survival most likely attributable to "incomplete" operation as the tumor is located deep in the pelvis [11]. Locally advanced rectal carcinoma has poorer prognosis, and its 5-year survival rate is only 20 to 40%.

In China, Zhou et al. [12] revealed that the effect of gefitinib in the treatment of advanced non-small cell lung cancer (NSCLC) could be predicted through EGFR mutations, and PFS in EGFR-mutated patients with high expression was clearly longer than in patients with low expression.

The correlation between EGFR expression and the therapeutic effect of cetuximab in CRC patients has not been studied yet in China. Therefore, this study evaluated the EGFR expression of 70 CRC tumor specimens with two-step HIC and analyzed its relationship with various clinical factors and disease prognosis, so as to provide a basis for individualized CRC treatment.

Methods

Clinical materials and treatment methods

Seventy advanced CRC patients who were treated with cetuximab from 2010 to 2014 were included in this study. The inclusion criteria were as follows: positive pathological diagnosis; the patients developed metastasis or recurrence during cetuximab treatment which lasted for no less than 2 weeks; the included patients should have assessable lesion, complete clinicopathological data (age, gender, tumor grade of differentiation, stage, etc.) and long-term survival follow-up information. Postoperative staging was based on the TNM system (7th edition) and the patients' physical condition was scored according to KPS. All patients signed informed consent and the study was approved by the Ethics Committee of our hospital.

Two kinds of treatment were applied: the first one was cetuximab (Merck Serono Co., Ltd, China), first dose was 380 mg/m^2 with 5 mg dexamethasone (for pretreatment), followed by 230 mg/m^2 every week after taking antihistamine drugs. The second one was to administer combination chemotherapy regimens, containing oxaliplatin (XELOX), oxaliplatin+calcium folinate+fluorouracil (FOLFOX4) and irinotecan (FOLFIRI).

Experimental procedures

Known positive EGFR expression tissue, normal tissue and phosphate buffered saline (PBS) were taken as positive control, negative control and blank control, respectively.

Firstly, paraffin blocks were cut into 5 μm slices and placed on glass slide; then, all the slices were put into oven at 60 °C. Two hrs later, slices were washed with appropriately concentrated PBS three times (3 min each time) after Xylene I, II and III were added for dewaxing, each for 10 min, and gradient alcohol (100, 95, 85 and 60%) was added for rehydrating, each for 1 min. Afterwards, tissue slices were treated with 0.15 ml of enzyme digestive fluid (Gino Biomedical Technology Co., Ltd., Hangzhou, China) and put into oven at 37°C for 8 min, and then they were washed with PBS twice, 3 min each time.

Secondly, at room temperature, each slice was exposed to 0.05 ml of 3% H_2O_2 to inactivate the endogenous peroxidase activity, and 5 min later it was washed with PBS 3 times, 3 min each time. Then, 0.05 ml of cetuximab (Zhongshan Gold Bridge Co., Ltd, serial number 31G7) was added on every tissue slice, and slices were put at 4°C overnight and washed with PBS 3 times (3 min each time) after cetuximab was completely combined with the antigen.

Thirdly, slices were washed with PBS 3 times (5 min each time) after adding universal immunoglobulin G (IgG) antibody-horse raddish peroxidase (HRP) polymer and left at room temperature for 15 min. Then, each tissue slice was treated with 0.05 ml of fresh diaminobenzidine (DAB) in the dark.

Fourthly, tissue slices were stained with hematoxylin & eosin and differentiated with 0.1% hydrochloric acid after being washed with distilled water; then, slices turned blue after being washed with tap water. Afterwards, slices were dehydrated with differently graded ethyl alcohols, and finally sealed with gum for observation.

Fifthly, slices were reviewed by two pathologists in a double-blind way, and EGFR expression was evaluated according to immunostaining intensity. Meaningfully stained cells of each case were observed randomly from 5 different microscopic fields (x400) and scored in a proper order based on their percentages. The percentage intervals included <5%, 5-25%, 25-50%, 50-75% and >75%, involving 0-4 points. EGFR positive cells showing yellow, pale brown and brown color were scored for 1, 2 and 3 points, respectively.

Statistics

SPSS 17.0 software (SPSS Inc., Chicago, Ill, USA) was used for statistical analyses, and the relationship between EGFR protein expression and gender, age, KPS score, tumor site, grade of differentiation and stage were analyzed with chi-square test. Kaplan-Meier method with log rank test were used for survival analysis. Cox prognostic model was applied in the analysis of related factors that influence the therapeutic effect. Statistically significant difference was set at $p<0.05$.

Table 1. Patient and tumor characteristics

Characteristics	*Gender*		*KPS score*			*Tumor location*		*Grade of differentiation*		*Stage*	
	Male	*Female*	90	80	70	*Rectum*	*Colon*	*Low*	*Moderate*	*III*	*IV*
Cases	40	30	28	30	12	34	36	40	30	26	44

Table 2. Relationship between EGFR expression and clinical factors

Clinical information	*Low expression*	*High expression*	*X*	*p value*
Gender			4	0.03
Male	20	20		
Female	23	7		
Age, years			3	0.08
<60	24	22		
≥60	18	6		
90	15	13		
KPS score			0.02	0.99
80	18	12		
70	7	5		
Tumor location			0.02	0.88
Rectum	20	15		
Colon	18	17		
Grade of differentiation			0.2	0.63
Low	16	14		
Moderate	25	15		
Stage			0.02	0.89
II	15	11		
IV	24	20		

Results

Patients' clinical data

The study included 70 patients (40 male and 30 female), with age ranging from 24 to 74 years. The KPS scoring results showed that there were 28 cases of patients scored 90, 30 cases of patients scored 80 and 12 cases of patients scored 70. Thirty-six out of 70 patients suffered from colonic adenocarcinoma and 34 had rectal adenocarcinoma. Of 70 cases, 40 (57.1%) had moderately differentiated tumors and the remaining 30 (42.9%) had low differentiated tumors; 26 (37.1%) cases had stage III and 44 (62.7%) stage IV disease. Detailed data are shown in Table 1.

Expression of EGFR in colorectal cancer

Membranes in different tissues showed different levels of brown staining (Figure 1). Of 70 tumor tissues, 52 (64%) showed positive expression of EGFR, including 22 cases of weakly positive expression, 15 cases of positive expression and 15 cases of strongly positive expression; the remaining 18 cases showed negative expression.

Relationship between EGFR expression and clinical factors, short-term results and survival

Of 70 specimens, 27 (38.6%) belonging to 20 males and 7 females, had high EGFR expression, and the difference of expression between the high and low expressing groups was signifi-

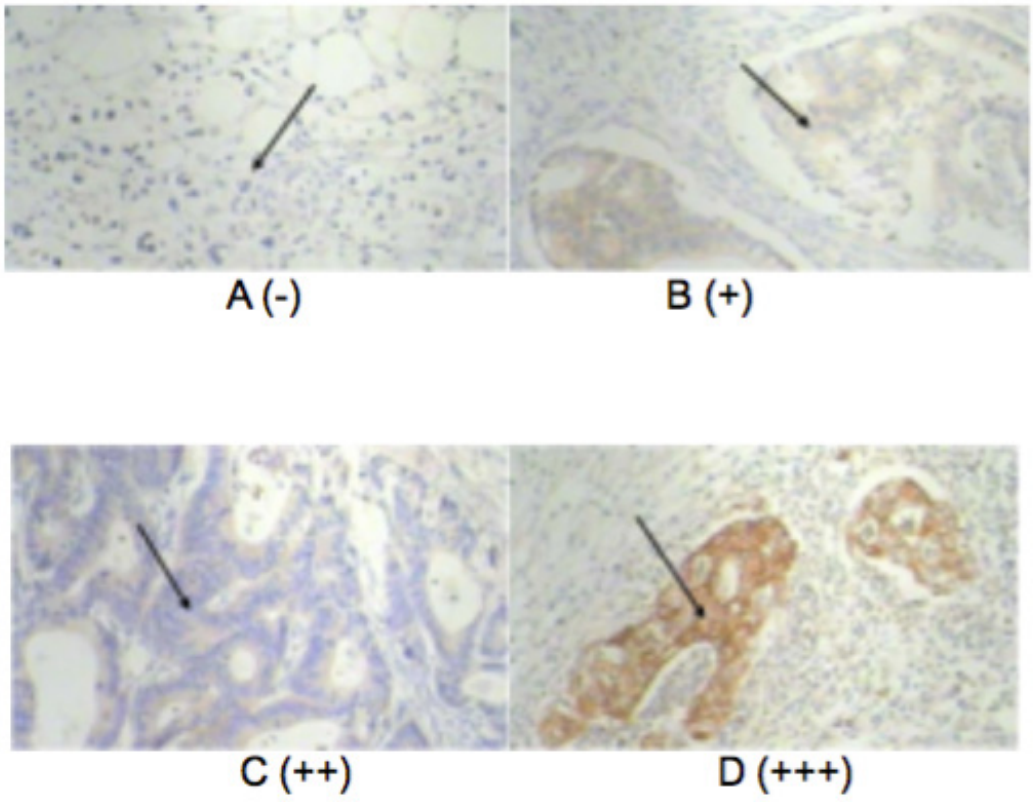

Figure 1. EGFR expression in colorectal cancer. **A** (-) negative expression (arrow); **B** (+) weakly positive expression (arrow); **C** (++) positive expression (arrow); **D** (+++) strongly positive expression (arrow) (streptavidin-peroxidase x 200).

cant ($p<0.05$). However, EGFR expression showed no statistical significance in relation to age, KPS, tumor site, grade of differentiation and clinical stage ($p>0.05$) (Table 2).

No patient achieved CR, and 15 patients achieved PR, for a total objective response rate of 21.4%; 12 (17.1%) patients had SD and 40 (57.1%) developed PD. DCR in low and high expression groups was 39.02% (16/41) and 48.28% (14/29) respectively, without significant difference ($p>0.05$). Median OS in high and low EGFR expression groups was 12 and 11 months, respectively, with 48% and 47.4% one-year survival rate, respectively, the difference being not statistically significant ($p>0.05$). In multivariate analysis

PFS in low and highly expressing EGFR groups was 6 and 5 months respectively, again without significant difference ($p>0.05$). The relationship between of EGFR expression and OS/PFS is shown in Figures 2 and 3.

Multivariate Cox regression analysis

The variables which were assessed with multivariate Cox regression analysis for possible impact on OS, such as gender, age, KPS score, tumor site, grade of differentiation, clinical stage and EGFR expression, showed that only clinical stage (as a prognostic factor) was found to have independent statistical significance ($p<0.05$). As for PFS, no factor was found to express independent statistical significance.

Discussion

In Western countries the incidence of CRC is high and this disease is also common in China. In recent years, the incidence of CRC in China is rising each year in large and medium-sized cities as the people's lifestyle and dietary habits change. Positive EGFR expression has been documented in many malignant tumors in different degrees. Researches demonstrate that the excessive expression and abnormal activation of EGFR gene are related to CRC prognosis [13,14]. Sixty to 80% of CRC patients have upregulated EGFR gene expression [15-17]. With the development of chemotherapeutic drugs, CRC treatment has made a great progress in the last 10 years, and fluorouracil was and still is a significant agent for a beneficial outcome. Applications of irinote-

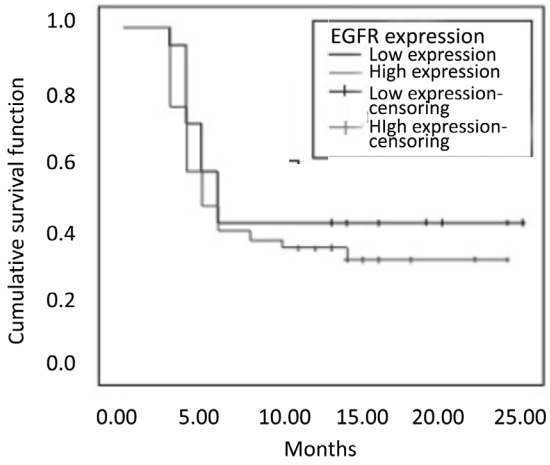

Figure 2. Relationship between overall survival and positive EGFR expression (p>0.05).

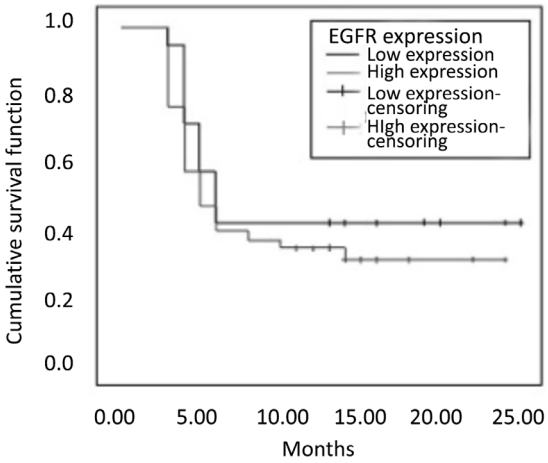

Figure 3. Relationship between progression free survival and EGFR expression (p>0.05).

can or oxaliplatin in combinatorial regimens like FOLFOX and FOLFIRI are used widely at present, achieving improved objective response rates and prolonging OS. Although cetuximab can produce various side effects, these are relatively mild compared with the routine chemotherapy.

This study detected the EGFR expression of CRC patients with IHC and evaluated the correlation of EGFR expression with clinical factors. EGFR expression plays important roles in a variety of tumors concerning cell growth, proliferation, differentiation, malignant transformation, angiogenesis and apoptosis. As clinical stage is still a critical prognostic factor in CRC research, the present study also indicated that clinical stage was a main factor independently influencing OS.

CRC is a disease with obvious individual differences. For CRC patients in T3,4N0M0 stage, postoperative recurrence and metastasis rates were 20 to 30%, while most of the rest could achieve clinical cure [18,19]. TNM staging is an important basis for clinicians to select the best treatment method and evaluate its therapeutic effect. TNM staging should be renewed and perfected continuously in order to guide the clinical treatment effectively.

In recent years, EGFR has turned into a key component in CRC targeted therapy, and the application of cetuximab in clinical practice further speaks for the important role of EGFR in the occurrence and development of CRC. Due to the limited sample size of this study, there might be grouping deviations. Future research should expand the sample size, compare the differences of diverse effects and discuss the factors influencing long-term outcome. In the meantime, hypotheses on a few different experimental results require further research in order to better illustrate EGFR expression in CRC patients as well as the clinical effect of cetuximab.

Conclusion

Positive expression of EGFR in CRC tissues was related with gender. However, no obvious correlation was noticed between EGFR expression and patient age, KPS score, tumor location, grade of differentiation or stage. The efficiency rate of cetuximab was obviously higher in the group with high EGFR expression than in the group with low EGFR expression, indicating that expressive abundance of EGFR was correlated to some degree with beneficial short-term effect of cetuximab.

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