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Relationship between expression of EGFR in gastric cancer tissue and clinicopathological features

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ABSTRACT

Objective: To investigate the relationship between the expression of epidermal growth factor receptor (EGFR) in gastric cancer and the clinicopathological features and prognosis. **Methods:** A total of 78 paraffin specimens of gastric cancer operation were collected. The immunohistochemical method was used to detect the expression of EGFR in 78 cases of gastric cancer and 20 cases of adjacent normal tissue. The relationship between the high expression of EGFR and clinicopathological features was analyzed. **Results:** EGFR positive expression rate in the 78 cases of gastric cancer tissue was 57.7 %(45/78), while EGFR was not expressed in 20 cases of adjacent normal tissue. The high EGFR expression was positively correlated with the position of gastric cancer, tumor size, cell differentiation, invasive depth, lymph node metastasis and TNM staging, yet having no obvious relation with gender or age. **Conclusions:** EGFR expression level in gastric cancer is closely related to the incidence and development of gastric cancer, which can provide a theoretical basis for the targeted therapy for gastric cancer with EGFR as the target.

1. Introduction

Epidermal growth factor receptor (EGFR) belongs to receptor tyrosine kinase (RTK) and is the expression product of pro-oncogene ErbB1 (HER1). EGFR participates in the information control process in many cells, and its abnormal expression is closely related to many malignant tumors. After binding its ligand, EGFR gave priority to form heterodimer with HER2. After receptor dimerization, the EGFR dimer activates the PTK, and phosphorylation also occurs in its tyrosine residue. Subsequently, EGFR activates ERK/MAPK, phosphatidyl-inositol-3-kinase (PI3K), JAK/STAT pathway and the downstream effectors to regulate cell proliferation, migration, survival and tumor angiogenesis[1,2]. EGFR highly expresses in many malignant tumors and is related to the growth and invasion of tumors[3]. Lots of reports about EGFR can be seen in the gastric cancer associated literatures[4-6]. Recent studies about gastric cancer have indicated that EGFR highly expresses in gastric cancer and is closely related to the occurrence, development and biological behaviour of gastric cancer, and it is regarded as the ideal target in the treatment of tumors like gastric cancer. The biotherapy regarding EGFR as the target becomes the new research hotspot of gastric cancer[7-10]. This study adopted the immunohistochemical method to detect and analyze the EGFR expression in gastric adenocarcinoma tissues and adjacent normal gastric mucosa tissues in order to explore the clinical significance of using EGFR as the molecular target to guide the targeted therapy for gastric cancer.

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2. Materials and methods

2.1. Specimens

The paraffin specimens of 78 cases of gastric cancer tissue and 20 cases of adjacent normal gastric tissue were provided by Pathology Department, First Affiliated Hospital of Zhengzhou University which collected them during March 2000 and March 2007. Clinical stage was made according to the TNM staging criteria formulated by International Union against Cancer and American Joint Committee on Cancer.

2.2. Reagents and instruments

EGFR mouse anti-human monoclonal antibody (Product No. MAB-0196). The ready-to-use MaxVision detection kit and dimethylaminoazobenzene chromogenic reagent were purchased from Fuzhou Maixin Biotechnology Development Company.

Instruments: paraffin slicing machine, microscope, and electrothermal constant-temperature dry box. The images under microscope were captured and analyzed by Olympus Dp70 image analyzer.

2.3. Experimental method

The paraffin specimens were made into 3-5 μm sections. The sections were put into the 65 ℃ electrothermal constant-temperature dry box overnight and then deparaffinized. EGFR underwent enzymatic digestion and antigen retrieval by gastric enzyme. Then the product was incubated for 30 min in 37 ℃ water bath. It was washed by PBS solution for 3 min, and soaked by H_2O_2 100 mL+CH_3OH 900 mL solution for 10 min. After washed 3 times by PBS, the first antibody was added, overnight in the refrigerator at 4 ℃. After washed twice by PBS, the ready-to-use MaxVision detection kit was added at room temperature. 4 0min was given for full reaction. After washed three times by PBS, DAB coloration was conducted. It was counterstained by hematoxylin, and than wasdehydrated, mounted and observed under microscope.

2.4. Judgement of results

The positive and negative controls were set for all the experiments. Brownish yellow granular precipitation in the cell membrane and cytoplasm indicated the positive expression of EGFR, while there was no brownish yellow granular precipitation in the negative cells.

Staining grade: No positive cells in the whole section (-), the number of positive cells<10 %(+), the number of positive cells 10%-50 %(++), the number of positive cells ≥50 %(+++).

2.5. Statistical analysis

SPSS17.0 software was utilized to perform statistical analysis. A $P<0.05$ was taken to indicate a difference of statistical significance.

3. Results

3.1. EGFR expression in gastric cancer

The expression rate of EGFR in gastric cancer was 53.7% (45/78), but it did not express in normal gastric tissue. EGFR only expressed in cell membrane or cytoplasm without nucleus staining (Figure 1). EGFR protein expression had no significant correlation with patients' gender and age, but it was correlated to tumor position, tumor size, differentiation, invasive depth, lymph node metastasis, distant metastasis and clinical stage. The EGFR positive expression rates of patients with tumor diameter ≥5 cm, tumor located in the middle part, poor differentiation, infiltration into serous layer, lymph node metastasis, distant metastasis and Ⅲ+Ⅳ of TNM stage were 86.1%, 66.7%, 90%, 76.1%, 80.6%, 83.3% and 95%, respectively (Table 1).

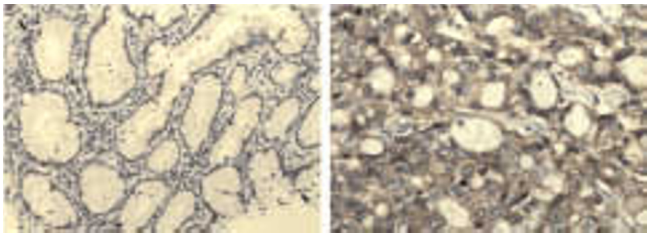

Figure 1. EGFR expression in normal gastric tissue and gastric cancer tissue.
A: Normal gastric tissue staining (SP×200); B: EGRF positive staining in gastric cancer tissue (SP×200).

3.2. Analysis of relationship between EGFR expression and survival rate of gastric cancer patients, tumor size

The one-year survival rate of patients with positive EGFR expression was 80% (36/45), while that of those with negative EGFR expression was 96.97% (32/33), with significant difference ($P<0.05$). The three-year survival rate of those with positive EGFR expression was 35.56% (16/45), while that of those with negative EGFR expression was 81.82% (27/33), with significant difference ($P<0.05$). The five-year survival rate of those with positive EGFR expression was 6.67% (3/45), while that of those with negative EGFR expression was 36.36% (12/33), with significant difference ($P<0.05$) (Table 2). The EGFR expression level was evaluated by the mean optical density value after immunohistochemical staining. The analysis of the correlation between EGFR expression level and life span was conducted, and they found to be negatively correlated. Using EGFR expression level as the independent variable and patients' life span as the

dependent variable, the acquired regression equation was: y=79.034 1-52.416 5x (Figure 2). The relationship between EGFR expression level and tumor size was analyzed, and the linear correlation between them was obtained by least square fitting method (Figure 3).

Table 1
Relationship between EGFR expression and clinicopathological parameters of gastric cancer.

Factor	Cases (*n*)	EGFR	
		Positive	*P* value
Total number of positive		40(57.7%)	
Gender			
Male	40	25(62.5%)	χ^2=0.778
Female	38	20(52.6%)	*P*=0.378
Age			
<50	25	18(72%)	χ^2=3.086
≥50	53	27(50.9%)	*P*=0.079
Tumor size			
<5 cm	42	14(33.3%)	χ^2=22.122
≥5 cm	36	31(86.1%)	*P*=0.000
Tumor position			
Superior part	22	5(22.7%)	χ^2=11.369
Middle part	18	12(66.7%)	*P*=0.003
Inferior part	38	28(73.7%)	
Differentiation			
Well-differentiated	26	5(19.2%)	χ^2=19.562
Moderately differentiated	22	13(59.1%)	*P*=0.000
Poorly differentiated	30	27(90%)	
Depth of invasion			
Not into serous layer	32	10(31.3%)	χ^2=15.543
Into serous layer	46	35(76.1%)	*P*=0.000
Lymph node metastasis			
No	42	16(38.1%)	χ^2=14.318
Yes	36	29(80.6%)	*P*=0.000
Distant metastasis			
No	60	30(50%)	χ^2=6.303
Yes	18	15(83.3%)	*P*=0.012
TNM stage			
Ⅰ+Ⅱ	58	26(44.8%)	χ^2=15.338
Ⅲ+Ⅳ	20	19(95%)	*P*=0.000

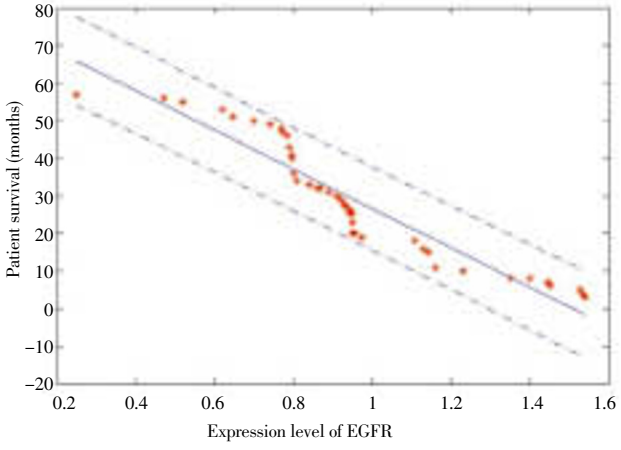

Figure 2. Relationship between EGFR expression level and life span of patients.

Table 2
Relationship between EGFR expression and the life span of gastric.

cancer patients	Negative	Positive
Total cases	33	45
1-year survival	32	36
3- year survival	27	16
5- year survival	12	3
Median survival time(month)	49	28

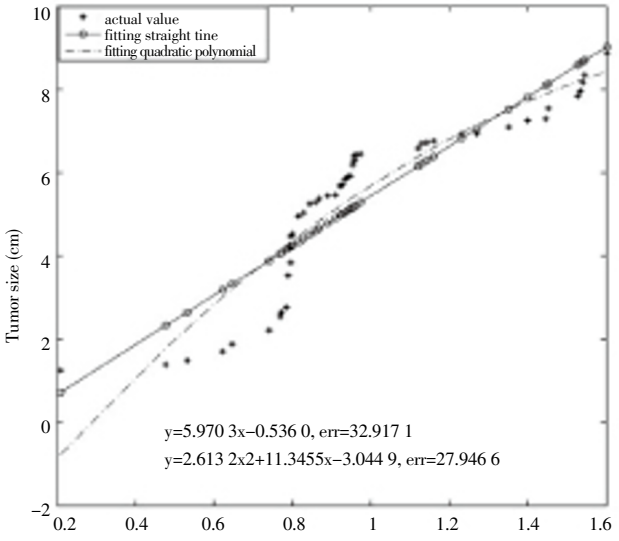

Figure 3. Relationship between EGFR expression level and tumor size

3.3. Analysis of the survival curve of gastric cancer patients

The 5-year life span data of 78 gastric cancer patients were collected. Kaplan-Meier method was adopted for single factor survival analysis. The survival curve of gastric cancer patients was drawn, and the log-rank test was conducted (Figure 4). It was clear shown in Figure 3 that the survival rate of gastric cancer patients with negative EGFR expression was higher than that of gastric cancer patients with positive EGFR expression.

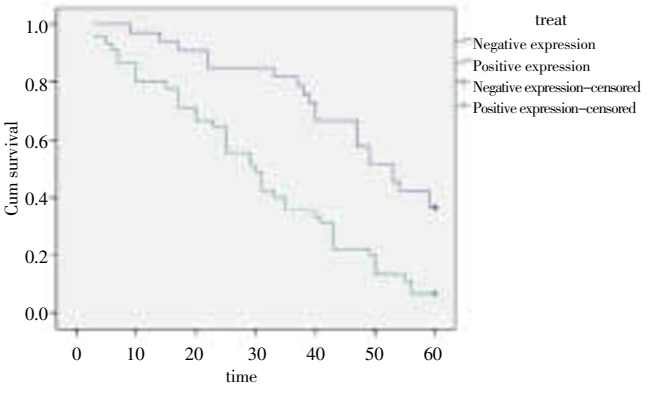

Figure 4. Kaplan-Meier analysis of the life span of gastric cancer patients.

4. Discussion

Worldwide, gastric cancer is the fourth most commonly diagnosed cancer and the second most common cause of cancer death[11], accounting for almost 10% of all cancer deaths in 2008[12]. EGFR expresses in 43% of the gastric cancer patients and highly expresses in 11% of them[13]. EGFR signal abnormity plays an important role in the development of many human tumors. EGFR combines with its ligand to form homo- or hetero- dimers in the cell surface, and thus activates three main signal pathways in the downstream: Ras/Raf/MAPK pathway, Phosphatidyl inositol triphosphate (P13K) and AKT pathway, JAK and STAT pathway[14]. These signal transduction pathways finally mediate a series of processes including cell differentiation, survival, migration, invasion, adhesion and cell damage repair. The EGFR targeting therapy can block the activation of signal transduction pathway, thus achieving the goals of treatment[15]. It has been found that the EGFR overexpression participates in the oncogenesis and proliferation of tumor cells. EGFR involves in the tumor cell metabasis through various mechanisms like reconstruction, adherence, transference and expression of cytoskeleton, and activation of protein lipase[16]. EGFR overexpression is also associated with the poor prognosis of resectable gastric cancer[17]. We found that the EGFR positive expression rate in gastric cancer tissue was 57.7%, and the expression of EGFR was also closely related to tumor size, position, differentiation, invasive depth, whether having lymph node metabasis and distant metabasis, and TNM stage. All these indicated that EGFR took part in the biological behaviours like proliferation, invasion and metabasis of gastric cancer tumor cells. At the same time, the survival rate of patients with positive EGFR expression was obviously lower than that of patients with negative EGFR expression. EGFR expression level had quantitative relationship with the patients' life span and tumor size. All these results indicated that EGFR may influence the process of tumor and finally influence the prognosis of patients.

There are various reports about EGFR expression in gatric cancer, and about 9%-62.7% of the reported expression differences may be caused by different sample sizes, detection methods or standards for evaluation. Some reported that EGFR did not express in normal gastric mucosa but highly expressed in gastric cancer tissues, and the EGFR expression may be related to the amplification and mutation of EGFR gene, the continuous activation of EGFR and the activation of abnormal signal transduction pathway. However, it is controversial about whether the high EGFR expression in gastric cancer is caused by gene amplification or gene mutation. Kimura *et al*[18] found high EGFR expression but did not found gene amplification. However, Mitsui *et al*[19] found high EGFR expression and 4% gene amplification rate. Some reported the EGFR gene mutation in the kinase area. Moutinho *et al*[20] found the EGFR gene mutaion in 6 of the 77 gastric cancer patients. Kimura *et al*[21] found mutation in one of the six gastric cancer cell lines in the *in vitro* mutation research. The mechanism of high EGFR expression in gastric cancer is not clear because the amplification and mutation rates of EGFR gene are low. The results of our experiment indicated that EGFR did not express in normal tissue, which was consistent with the previous reports. EGFR specific expression exists in gastric cancer, the survival rate and life span of patients with positive EGFR expression are obviously lower than those with negative EGFR expression, and therefore, EGFR can be the ideal target for gastric cancer treatment. With the in-depth studies, people can design the more effective EGFR monoclonal antibody against gastric cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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