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, phosphatidylinositol-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.
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 °C 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 °C water bath. It was washed by PBS solution for 3 min, and soaked by H2O2, 100 mL+CH3OH 900 mL solution for 10 min. After washed 3 times by PBS, the first antibody was added, overnight in the refrigerator at 4 °C. After washed twice by PBS, the ready-to-use MaxVision detection kit was added at room temperature. 40min 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%)     | χ²=0.778|
| Female                 | 38        | 20(52.6%)     | χ²=0.378|
| Age                    |           |               |         |
| <50                    | 25        | 18(72%)       | χ²=3.086|
| ≥50                    | 53        | 27(50.9%)     | P=0.079 |
| Tumor size             |           |               |         |
| <5 cm                  | 42        | 14(33.3%)     | χ²=22.122|
| ≥5 cm                  | 36        | 31(86.1%)     | P=0.000 |
| Tumor position         |           |               |         |
| Superior part          | 22        | 5(22.7%)      | χ²=11.369|
| Middle part            | 18        | 12(66.7%)     | P=0.003 |
| Inferior part          | 38        | 28(73.7%)     |         |
| Differentiation        |           |               |         |
| Well–differentiated    | 26        | 5(19.2%)      | χ²=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%)     | χ²=15.543|
| Into serous layer      | 46        | 35(76.1%)     | P=0.000 |
| Lymph node metastasis  |           |               |         |
| No                     | 42        | 16(38.1%)     | χ²=14.318|
| Yes                    | 36        | 29(80.6%)     | P=0.000 |
| Distant metastasis     |           |               |         |
| No                     | 60        | 30(50%)       | χ²=6.303 |
| Yes                    | 18        | 15(83.3%)     | P=0.012 |
| TNM stage              |           |               |         |
| I+II                   | 58        | 26(44.8%)     | χ²=15.338|
| III+IV                 | 20        | 19(95%)       | P=0.000 |

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 abnormality 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, Phosphatidylinositol 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 metastasis 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 metastasis and distant metastasis, and TNM stage. All these indicated that EGFR took part in the biological behaviors like proliferation, invasion and metastasis 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 gastric 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, Mitsu 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 mutation 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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