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Research Article

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The association between EGFR expression and clinical pathology characteristics in gastric cancer

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Abstract: *Objective:* The aim of this study was to investigate epidermal growth factor receptor (EGFR) expression in the gastric cancer tissues and the association of EGFR expression with clinical pathology characteristics. *Methods:* We firstly analyzed the copy number alteration of EGFR gene in gastric cancer by utilizing the online TCGA resources. We then gathered 71 cases of gastric cancer patients from February 2010 to February 2015 in our hospital. The cancer tissues and normal gastric tissues were tested for EGFR expression by immunohistochemical (IHC) staining and western blotting methods. The association of EGFR expression with clinical pathology characteristics and the prognosis value of EGFR expression were evaluated by the statistical software. *Results:* The copy number of EGFR gene was found to be increased in gastric cancer patients. Western blotting confirmed that EGFR protein was obviously upregulated in tested gastric cancer tissues. Additionally, our IHC results showed that the positive rates of EGFR expression in gastric carcinoid tumors (CTs) and distant normal gastric tissues were 45.1% (32/71) and 25.0% (9/36), respectively. The former was significantly higher than the latter ($P < 0.05$). The EGFR-positive expression in CTs was related to tumor size, invasion depth, and lymphatic metastasis. The median survival of the EGFR-positive patients was 15.6 months, which was less than that (23.0 months) of the EGFR-negative patients [HR = 2.12, 95%CI: 1.29–4.10 ($P < 0.05$)]. Also, online survival analysis showed that high expression of EGFR predicted shorter overall survival of gastric cancer patients. *Conclusion:* EGFR was highly expressed in gastric cancer tissues and associated with poor prognosis.

Keywords: epidermal growth factor receptor; gastric cancer; clinical characteristics; prognosis

1 Introduction

Gastric cancer, with high incidence and mortality, is a clinically common malignant solid tumor of the digestive tracts; in epidemiological studies, numerous new cases of gastric cancer worldwide are recorded every year [1]. And a high incidence of gastric cancer has been documented in China [2]. With improvements in living environment and anti-cancer options, the prognosis of gastric cancer has been significantly enhanced [3]. However, gastric cancer still remains characterized by poor prognosis and high recurrence rate. It is already known that epidermal growth factor receptor (EGFR) highly expressed in gastric carcinoid tumors (CTs) is related to clinicopathological features and prognosis in HER2-positive gastric cancer [4]. In our study, the expression levels of EGFR in pan gastric CTs and distant normal gastric tissues were detected through western blotting and immunohistochemistry staining. The relationship between EGFR expression and cancer prognosis was also investigated.

2 Materials and methods

2.1 Subjects investigated

71 patients with gastric cancer were admitted to the General Surgery Department in our hospital (the First Affiliated Hospital of Bengbu Medical College) from February 2010 to February 2015. Of these patients, 42 were males and 29 were females, with ages between 33–81 years old and at an average age of 55.8 ± 16.3 years. Thirty cases in Stage I+II and 41 cases in Stage III+IV (tumor, node, and metastasis staging) were enrolled, and their clinical data and histopathological specimens were retrospectively analyzed.

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2.2 Methods

2.2.1 Instruments and equipment

The instruments and equipment used for this study were as follows: (1) paraffin slicer (Leica Camera AG); (2) microscope (Olympus); (3) electric constant-temperature chamber; (4) microwave oven (Media); (5) centrifuge machine (Beckman Coulter); (6) micro pipette (Eppendorf Xplorer). (7) cataphoresis apparatus (Bio-rad); (8) microplate reader (Molecular Device); (9) and developing machine (Kodak)

2.2.2 Main reagents

MAB-0196 monoclonal anti-human EGFR monoclonal antibody was used as a primary antibody reagent. A ready-to-use EliVision™ two-step immunohistochemical staining kit and a 3,3'-diaminobenzidine (DAB) chromogenic kit (Solarbio) were also utilized.

2.2.3 EGFR expression

An EliVision™ two-step immunohistochemical staining kit was used to detect EGFR. The paraffin section was oven-dried for 3 h. After de-waxing was performed, the section was rinsed with distilled water for 2 min. The antigen was retrieved. Peroxidase activity was eliminated. The section was rinsed twice with phosphate-buffered saline (PBS), incubated for 1 h at 37°C after the primary antibody was added, and rinsed again with PBS. The section was incubated for 20 min at 37°C after a polymer helper was added, incubated for 0.5 h at 37°C after poly peroxidase anti-mouse/rabbit immunoglobulin G was added, and rinsed for 5 min with PBS before color was developed with the DAB chromogenic kit.

2.2.4 Western blot

After the total protein was extracted from 20 pairs of gastric cancer patients by RIPA buffer containing protease inhibitors, they were quantified by a BCA quantification kit (Solarbio). Equal amounts of protein were loaded into the SDS-PAGE and transferred to the PVDF membrane. After that, the membrane was incubated with desired antibodies (EGFR and GAPDH) overnight, followed by the incubation of the appropriate HRP-conjugated secondary antibodies for 1 h at RT. Finally, all blots were visualized with ECL and analyzed by ImageJ software.

2.3 Statistical analysis

Data were statistically analyzed in Stata12.0. The measured data were expressed by $\bar{x} \pm s$. The *t*-test was adopted in the between-group comparison, and the counted data were expressed in terms of rate. χ^2 -test or Fisher's exact test were also employed in the between-group comparison. A proportional hazard model for log-rank test was selected for survival analysis. Statistically significant difference was determined at $P < 0.05$.

3 Results

3.1 EGFR expression in tissues

We firstly considered whether copy number of EGFR gene was altered in gastric cancer. By utilizing the online TCGA database, a total of 263 gastric cancer samples were analyzed. The results shown in **Figure 1** illustrated the gain and amplification of EGFR gene copy number truly existed in gastric cancer. Next we performed western blotting to detect the protein expression of EGFR protein. We found that EGFR was significantly upregulated in these tested samples (**Figure 2**). Afterwards, IHC staining results concluded that the positive rates of EGFR in gastric CTs and distant normal gastric tissues were 45.1% (32/71) and 25.0% (9/36), respectively. The former was significantly higher than the latter ($P < 0.05$).

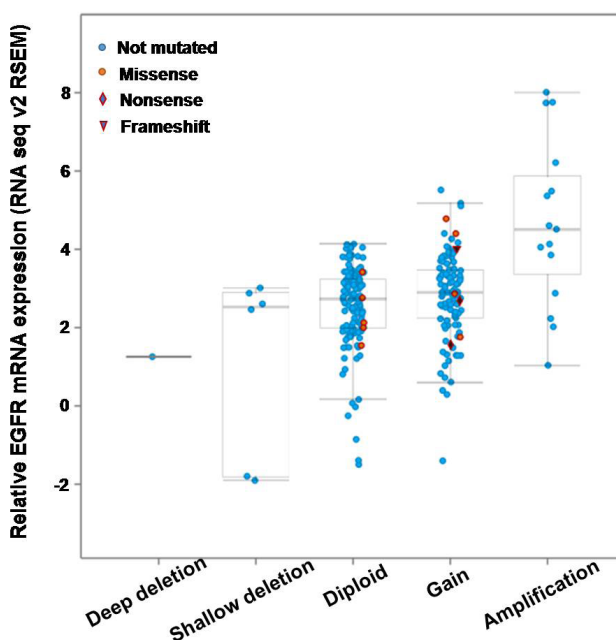


Figure 1. Copy number alteration of EGFR gene in gastric cancer. The copy number of EGFR gene was analyzed by the TCGA database. Gain and Amplification were considered as increased copy number.

3.2 Higher EGFR expression was correlated to specific clinicopathological features

Through our statistic χ^2 -test or Fisher's exact test, we found that EGFR-positive expression in CTs was related to tumor size, invasion depth, and lymphatic metastasis ($P < 0.05$; Table 1).

3.3 EGFR and prognosis

According to the IHC results, the median survival of the EGFR-positive patients was 15.6 months, which was less than that (23.0 months) of the EGFR-negative patients [$HR = 2.12$, 95%CI: 1.29–4.10 ($P < 0.05$)] (Figure 3A). Next, we applied an independent resource (<http://kmplot.com/analysis/>) to analyze the correlation between EGFR expression and prognosis of gastric cancer patients. As shown in Figure 3B, we found that high expression of EGFR predicted shorter overall survival.

4 Discussion

Worldwide authoritative studies on cancer epidemiology have shown that the number of new cases with gastric cancer is 0.6 million worldwide every year; of these cases, the number of deaths is up to 0.40 million [5]. Gastric cancer has been considered as a major threat to public health [6,7]. Their 5-year survival is low, and they frequently suffer from recurrence and metastasis [8]. Thus, long-term treatment efficacy is poor.

With the recent progress in molecular biology research, some gastric cancer-associated markers, including

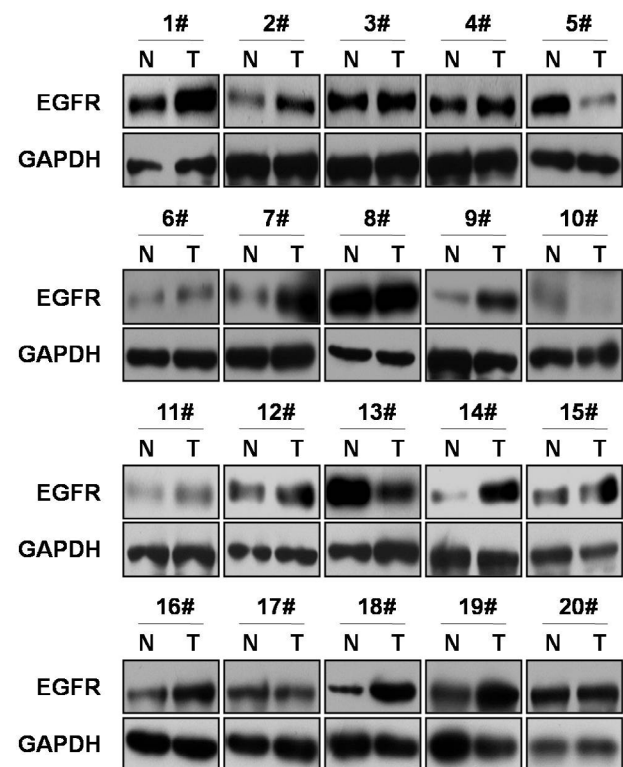


Figure 2. EGFR protein expression in gastric cancer samples. A total of 20 pairs of gastric CTs (including N: normal gastric tissue, T: tumoral CT) were gathered and protein expression of EGFR was assessed by western blotting. GAPDH was used as the internal control.

prognosis and diagnostic criteria, have been developed. EGFR belongs to tyrosine kinase I-type receptor family. Highly expressed EGFR is related to poor prognosis [9]. Most of the tumors with a highly expressed EGFR often exhibit evident malignant biological behavior, and the highly expressed EGFR is an independent risk factor and marker

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

Clinicopathological features	n = 71	EGFR		P
		Positive (n = 32)	Negative (n = 39)	
Age (year)				> 0.05
> 50	36	17	19	
≤ 50	35	15	20	
Gender				> 0.05
male	44	20	24	
female	27	12	15	
Tumor diameter (cm)				< 0.05
> 5	33	20	13	
≤ 5	38	12	26	
Depth of infiltration				< 0.05
not invasion of serous layer	22	4	18	
invasion of serous layer	49	28	21	
Lymph node metastasis				< 0.05
yes	26	7	19	
no	45	25	20	

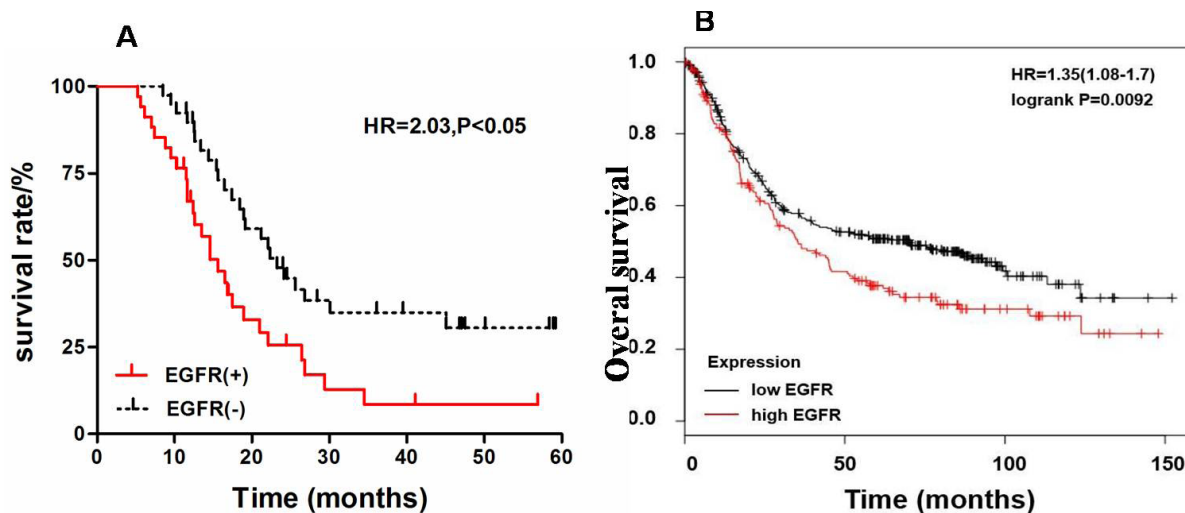


Figure 3. EGFR Expression–Prognosis Survival Curve. (A) Survival curve was plotted according to the IHC-based EGFR expression level. A proportional hazard model for log-rank test was selected for survival analysis (B) Prognosis survival curve was given by the online resource (<http://kmplot.com/analysis/>) and the correlation between EGFR expression and prognosis of gastric cancer patients was analyzed as like in (A).

of poor prognosis [10]. By detecting the EGFR expression in gastric CTs, Mizukami et al. [11] found that the positive rate is 46%, and the positive expression is related to tumor size, differentiation grade, and lymphatic metastasis. In our study, we firstly analyzed the copy number alteration of the EGFR gene. Then the EGFR protein expression levels in CTs of 71 patients with gastric cancer and in distant normal gastric tissues were detected through immunohistochemistry SP and western blotting. The results showed that the positive rate of EGFR in CTs was significantly higher than that in distant normal gastric tissues. This finding indicated that EGFR plays major roles in the occurrence and development of gastric cancer. Gastric cancer is characterized by a large tumor, deep invasion, and high positive EGFR rate in patients with lymphatic metastasis. Hence, the EGFR-positive expression is a marker of malignant biological behavior, which is related to tumor size, invasion depth, and lymphatic metastasis. The EGFR-positive expression is likely related to poor prognosis. The relationship between EGFR expression and survival is assessed on the basis of survival curve. The median survival time of the EGFR-positive patients is 15.6 months, which is less than that (23.0 months) of the EGFR-negative patients ($P < 0.05$). Also, an independent website conducted survival analysis which also reflected the critical prognosis importance of EGFR in gastric cancer.

The positive rate of EGFR in gastric CTs is relatively high and significantly related to prognosis. Therefore, EGFR can be used as an important indicator to assess the prognosis of gastric cancer clinically.

Conflict of interest: Authors declare nothing to disclose.

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