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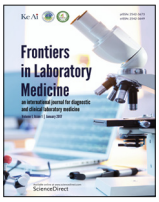

Evaluation of EGF, EGFR, and E-cadherin as potential biomarkers for gastrointestinal cancers

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SUMMARY

Biomarkers for early diagnosis and prognostic evaluation of cancers are still unsatisfactory in clinical management. The seeking of new biomarkers with better performance is still a challenge. EGF, EGFR, and E-cad are soluble biomarkers with clinical potential. ELISA was used to analyze serum EGF, EGFR, and E-cad from 58 gastrointestinal cancer patients and 20 control subjects. Immunohistochemistry was used to analyze Ki-67 expression in tumors. The results were correlated with clinical features. This study found that the preoperative and postoperative serum EGF and EGFR levels were significantly higher than in controls. The preoperative EGF and E-cad levels were significantly higher than postoperative levels. There was no correlation between EGF, EGFR, and E-cad levels and serum CEA or tumor Ki-67 scores. The serum EGF level was significantly higher in high TNM stage, with lymph node involvement patients than in low TNM stage, no lymph node involvement patients. Serum EGF and EGFR are potential biomarkers for the diagnosis and screening of gastrointestinal cancers. Inter-combination analysis of three biomarkers has improved the performance of the assays.

Introduction

Cancers of the gastrointestinal tract are common worldwide and especially in China.[1] It is known that surviving these cancers is closely related to early diagnosis and appropriate clinical management during the course of the disease.

Tumor biomarkers serve as an index for early detection, therapeutic response monitoring, and prediction of the outcomes of gastrointestinal cancers, and are critical for clinical administration. Biomarkers currently being used in the diagnosis, therapeutic response monitoring, and prognosis prediction of gastrointestinal cancers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are rather low in either sensitivity or specificity.[2] Thus, the seeking of more effective biomarkers is still a challenging task.

While seeking new tumor biomarkers for the detection and monitoring of gastrointestinal cancers, we performed literature research to screen newly emerged markers with potential clinical values in scientific reports. A certain number of markers have been selected for further evaluation.

Epidermal growth factor (EGF) and its receptor EGFR play an important role in various biological signaling pathways. The EGFR is a member of the tyrosine kinase transmembrane receptor family and is activated upon the binding of its ligand EGF.[3]

Studies found that soluble (serum) EGF and EGFR were associated with certain types of cancers including gastric cancer and hepatocellular carcinoma, thus they are potential biomarkers for prognostication.[4–7] The soluble EGFR is believed to be released by ectodomain shedding of its extracellular domain at the cellular membrane, and the excretion of the full length of EGFR is believed to occur through the biological process of exosomes alongside other constituents.[8]

E-cadherin (E-cad) is a member of the cadherin family and plays an important role in cell–cell adhesion and signaling. Soluble E-cad is a product of the proteolytic cleavage of E-cad, and altered serum levels of E-cad have been reported in certain types of cancers, thus it may be a potential tumor biomarker that can be used in diagnosis, therapeutic response monitoring, and prognostication.[9–18]

Although the soluble form of E-cad has been found in serum over two decades ago,[19] and its clinical value has been studied

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broadly, it has been inconclusive up to now whether it may be used as a biomarker in clinical practice.

Despite a number of reports on soluble EGF, EGFR, and E-cadherin in the different types of cancers as mentioned above, the results are still inconclusive or contradictory. One reason for this may have been that a variety of test kits have been used in the studies. Thus, further evaluation is needed to confirm the clinical significance which may lead to clinical usage in the future, in addition to the development of more sensitive and automated analysis.

In this report, we showed the serum EGF, EGFR, and E-cad concentrations from 58 patients with gastrointestinal cancers, including esophageal, stomach, and colorectal cancers. Among them, 53 were paired analysis cases with both preoperative and postoperative parameters. We also correlated the serum levels of EGF, EGFR, and E-cad with serum CEA, cell proliferation index-Ki-67 in tumors, and clinical features of patients. The performances of these three biomarkers, either alone or in combination, have also been analyzed.

Materials and methods

Patients

A total of 58 cases of gastrointestinal cancer (40 colorectal, 16 stomach, and 2 esophageal) were enrolled consecutively in this study in a period from February 2016 to August 2016, of which 39 were male and 19 were female, with ages ranging from 36 to 83 years old. The sample size was determined by the analytical power of samples in statistics. Patients diagnosed upon pathological examinations were involved regardless of gender, age, and ethnic backgrounds. Patients who failed for surgical treatment (because of distant metastasis, elderly status, or refusal of surgical treatment by family members), and thus lacked sampling after treatment, or had more than one type of cancer were excluded from the study. The tumor staging was made according to the TNM staging system, which combines UICC, AJCC, and WHO standards. Twenty routinely healthy individuals with no known cancer or history of cancer were selected as controls, with age and gender matched to those of the patient group (33–77 years old, 10 males and 10 females). The study was carried out with the permission of the Institute Review Board of the People's Hospital of Guangxi Zhuang Autonomous Region in regards to the use of human materials. Among the 58 patients, 53 had paired preoperative and postoperative blood samples. The requirement of a consent agreement from the patients was waived upon the hospital's policy for this study.

Blood sample collection

Fasting blood samples were obtained the day before surgical operation or other treatments (baseline) and on day 5 after surgery using the serum separation tube (BD healthcare, Franklin Lakes, NJ, USA). The blood samples were transported to laboratory within 30 min of collection and were centrifuged at 3,000 rpm at 4 °C for 10 min; the serum was collected into a 2 ml centrifuge tube and stored at −80 °C until required.

Enzyme-linked immunosorbent assay (ELISA)

The quantikine ELISA human EGF, EGFR, and E-cadherin immunoassay (catalog number DEG00, DEGFR0, and DCADE0) kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA) following the manufacturer's instructions. All required reagents, working standards, and sample dilutions were prepared and stored properly prior to assay sampling. The OD 450 nm was measured immediately on a microplate reader. Results were represented as pg/mL (EGF) and ng/mL (EGFR and E-cad).

Chemiluminescence analysis

Serum CEA levels in patients were measured utilizing the chemiluminescence method (Access CEA reagent kit) on a Beckman Coulter Unicel DXI 800 automated immuno-analyzer (Beckman Coulter Inc. Brea, CA, USA) following the manufacturer's instructions. Results were represented as ng/mL.

Immunohistochemistry results of Ki-67

The Ki-67 immunohistochemistry results were obtained from the medical records.

Statistical analysis

The statistical analysis was performed using the SPSS version 18.0, from which the Mann–Whitney *U*-test was performed. A p value of less than 0.05 was considered significant. The Receiver Operating Characteristic (ROC) was analyzed using the MedCal software, and the Area Under roc Curve (AUC) was obtained.

Results

The basic characteristics of patient collectives and the summary of analytical results are shown in Tables 1 and 2.

The scatter plots and the performance of serum EGF, EGFR, and E-cad analysis

Fig. 1 shows the scatter plots of serum EGF, EGFR, and E-cad from gastrointestinal cancer patients and control subjects.

The Receiver Operating Characteristic (ROC) curve analysis was performed. The results showed that for EGF, an area under the curve (AUC) of 0.800 was obtained with the Youden index of 0.519; the specificity and sensibility under this index were 95.0% and 56.9%, respectively. While for EGFR, an AUC of 0.700 was obtained with the Youden index of 0.374; the specificity and sensibility under this index were 65.0% and 72.41%, respectively. The AUC for E-cad alone was 0.540 with the Youden index of 0.193; the specificity and sensibility were 90.0% and 29.31%, respectively. The performances of inter-combination analysis were as follow: AUC: 0.804, Youden index: 0.572, specificity: 90.0%, sensitivity: 67.24% (EGF+EGFR); AUC: 0.816, Youden index: 0.560, specificity:

Table 1
Basic characteristic of patient collectives.

Characteristic	n (%)	Median (range)
Gender		
Male	39 (67.2%)	
Female	19 (32.8%)	
Age		59 (36–83)
<50 years	14	
≥50 years	44	
No. of patients who had surgery	53	
No. of patients who had no surgery	5	
Histological type		
Adenocarcinoma	56	
Squamous cell carcinoma	2	
Tumor types		
Colorectal cancer	40	
Esophagus cancer	2	
Gastric cancer	16	

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