An epidermal growth factor receptor exon 19 mutation in a mucin-producing gastric cancer sample from a Chinese patient

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Abstract

Objective: To determine whether a subgroup of gastric cancer patients might benefit from epidermal growth factor receptor-tyrosine kinase inhibitors.

Methods: A total of 103 gastric cancer samples were collected for this study. High-resolution melting and deoxyribonucleic acid sequencing were used to detect epidermal growth factor receptor mutations in exons 19 and 21.

Results: Polymerase chain reaction-high-resolution melting was successfully performed on all 103 samples. Aberrant melting curves were found in only one sample. Sanger sequencing revealed a 15 bp deletion (c.2235_2249del; p.Glu746_Ala750del) in epidermal growth factor receptor exon 19. The sample was from a male patient, and the pathological diagnosis was a mucin-producing gastric cancer with lymph node metastasis. To date, this is the first report on epidermal growth factor receptor exon 19 mutation in gastric cancer.

Conclusions: An epidermal growth factor receptor mutation in exon 19 was identified in mucin-producing gastric cancer sample from a male patient. This mutation indicates that the small subgroup of patients with mucin-producing gastric cancer might benefit from epidermal growth factor receptor-tyrosine kinase inhibitors.

Key words: gastric cancer, tyrosine kinase inhibitors, EGFR, gene mutation

Introduction

Gastric cancer is the second leading cause of cancer death worldwide, especially in eastern Asian countries (1). Chemotherapy has been long regarded as the cornerstone in treatment as the disease is usually diagnosed at an advanced stage, when the therapeutic efficacy of surgical intervention is significantly diminished. Unfortunately, chemotherapy has failed to yield a satisfactory therapeutic outcome to date. However, with increasing understanding of the molecular mechanisms driving gastric carcinogenesis, drugs targeting molecular changes specific to gastric carcinogenesis have been exploited for the treatment of gastric cancer (2,3). Trastuzumab, a monoclonal antibody directed against epidermal growth factor receptor 2 (HER2), has been approved by the United States Food and Drug Administration (FDA) for the treatment of gastric cancer (4). However, trastuzumab for gastric cancer (ToGA), a large Phase 3 clinical study, has demonstrated that only patients with HER2-positive tumors gain a significant benefit from trastuzumab (5). Therefore, other novel effective therapeutic strategies are desperately needed for the treatment of patients with gastric cancer. Motivated by the therapeutic success of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) in non-small...
cell lung cancer (NSCLC), investigators have recently begun to focus on their efficacy in the treatment of gastric cancer (6).

TKIs are specific inhibitors of the EGFR tyrosine kinase domain and exert dramatic anti-tumor activity in the treatment of NSCLC. EGFR mutations, particularly a deletion mutation in exon 19 and a substitution mutation in exon 21, are in general regarded as predictive biomarkers for high sensitivity of TKIs. These TKI-sensitive mutations have been found to occur more frequently in NSCLC patients with certain clinico-pathological features; patients are often Asian female non-smokers with adenocarcinoma (7–9).

Gastric cancer occurs with a higher frequency in Asia than in other areas of the world. This frequency intriguingly parallels the geographic distribution of NSCLC patients sensitive to TKIs. Furthermore, the pathology of adenocarcinoma accounts for >95% of gastric cancer (10). Although studies from countries including Japan, Korea, China, Portugal, Italy and Germany reported that EGFR mutations were absent in gastric cancer (11–16), three missense mutations in EGFR exon 21 (N842D, G858D and L858R) have been recently discovered in gastric cancers (17,18). Furthermore, G863D and L858R mutations have been demonstrated to be sensitive predictors for TKIs in NSCLC. Therefore, EGFR mutations might play a more important role in conferring sensitivity to TKIs in some gastric cancer patients.

In the present study, the EGFR gene was examined for mutations in exons 19 and 21 in a larger cohort of gastric cancers from Chinese patients. In addition, the clinico-pathological features of the patients harboring these mutations were reviewed to identify the patients who might benefit from therapeutic strategies which include EGFR-TKIs.

Patients and methods

Ethics statement

Patient samples were obtained from surgeries performed at The Third People’s Hospital of Dalian (Dalian, China). All protocols in the study were approved by the Ethics Committee of The Second Affiliated Hospital of Dalian Medical University (Dalian, China). Written informed consent was obtained from all patients, and the data were handled anonymously.

Patients and samples

Formalin-fixed, paraffin-embedded (FFPE) gastric cancer samples (n = 103) not previously analyzed for EGFR mutations were collected from patients who underwent surgical resection at The Third People’s Hospital of Dalian. Histological examination was performed by two experienced pathologists (see in Supplementary Data). According to the histological classification of gastric cancer proposed by the World Health Organization, all samples were diagnosed as advanced gastric adenocarcinoma (n = 103) and included diagnoses of mucin-producing gastric adenocarcinoma (n = 11). The cohort consisted of males (n = 76) and females (n = 27) with an average age of 64.7 years (age range: 36–93 years). No patient had received preoperative radio- or chemotherapy.

Genomic DNA isolation and high-resolution melting analysis

One section (5 μm) from each biopsy was chosen for hematoxylin and eosin staining, and an experienced pathologist identified the areas containing at least 70% tumor cells. Genomic DNA was extracted from the tumor cell-enriched areas of the sections with the FFPE DNA Extraction Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) according to the manufacturer’s protocols. PCR-high-resolution melting (PCR-HRM) analysis was performed to detect EGFR mutations in exons 19 and 21. Reaction mixtures for PCR were performed in a final volume of 10 μl containing 1x PCR Buffer, 200 μM dNTPs, 0.25 U HotStart Taq (Takara Bio Inc., Dalian, China), 5 ng genomic DNA, 1x LC Green Plus (Biofire Diagnostics, Salt Lake City, UT, USA), and 2.5 mM MgCl2 and 0.5 μM of each primer, or 1.5 mM MgCl2 and 1 μM of each primer for EGFR exons 19 and 21, respectively. PCR primers for EGFR were designed as previously described (19). Cycling parameters for PCR were as follows: (i) EGFR exon 19: 95°C for 5 min; 45 cycles of 95°C for 15 s, 60°C for 1 min; (ii) EGFR exon 21: 95°C for 10 min; 45 cycles of 95°C for 30 s, 54°C for 10 s and 72°C for 1 min. A wild-type sample was used as the control for HRM analysis. The shifted melting curves and the difference curves were used to identify samples with mutations. In the difference curves, the melting curves of the control sample were converted to a horizontal baseline. Experiments were repeated in their entirety for DNA samples where the HRM curves deviated from the baseline. Results were consistent in repeated experiments.

DNA sequencing

The PCR product was purified using the GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific, Rockford, IL, USA) according to the manufacturer’s protocols, and sequenced on an ABI Prism 3730 sequence detection system (Takara Bio, Inc.). Primers for sequencing EGFR exon 19 were as follows: forward, 5′-GTGGCATCCTGCTGGTAA CATCC-3′ and reverse, 5′-TTGAGATGACCCAGGTCTCTC-3′. The cycling parameters were the following: 95°C for 10 min; 45 cycles of 95°C for 30 s, 54°C for 1 min and 72°C for 1 min.

Results

PCR-HRM analysis for EGFR 19 and 21 exon mutations was successfully performed on all 103 gastric cancer samples. Shift of the sample melting curves from the wild-type melting curves highlighted by significant deviation from the horizontal baseline in the difference curves were regarded as potentially HRM mutation positive. Shifted melting curves were observed in only one gastric cancer sample (Fig. 1). Sanger sequencing was performed on DNA from this sample and a wild-type sample to determine the exact EGFR mutation. An in-frame deletion of 15 bp, c.2235 to c.2249, was found when sequences were compared (Fig. 1). The deletion was absent in the corresponding adjacent normal mucosa indicating that the mutation was somatic. The tumor sample with EGFR exon 19 mutation was histologically diagnosed as a gastric cancer with intracellular mucin (Fig. 2). Finally, the tumor sample with EGFR exon 19 mutation exhibited both perineural and vascular invasion.

Discussion

One of the success stories in the treatment of human cancers is targeted therapy against receptor kinases. EGFR and HER2, for example, have become a focus of drug development, as receptor activity often becomes unlinked in many epithelial cancers including gastric cancer. Based on our previous findings of EGFR mutations specifically in mucin-producing gastric cancers, a larger cohort of gastric cancers was examined with the inclusion of 11 tumors of this subtype. In our previous study, shifted melting curves were observed in two gastric cancer samples (18). Mutation types of the two gastric cancer samples were identified to be missense mutations in EGFR exon 21 (G863D and L858R). Interestingly, the patient in the present study had several
Figure 1. High-resolution melting analysis and sequencing chromatograms of a wild-type sample and the mutated gastric cancer sample. (A) Melting curves of the wild-type sample (gray) and the mutated gastric cancer sample (red). Evidence of a mutation is on the basis of deviation of the red curve from the wild-type gray curve. (B) Difference curves of the two samples depicted in A. The wild-type sample is represented by the horizontal baseline in gray. The mutated gastric cancer sample is represented by the red curve, deviates from the gray horizontal baseline. (C) Sequencing chromatogram of the wild-type sample. (D) Sequencing chromatogram of the gastric cancer sample with the c.2235_2249del; p.Glu746_Ala750del mutation in EGFR exon 19.

Figure 2. A hematoxylin and eosin (H&E) stain and an Alcian blue-PAS stain of the gastric cancer sample harboring EGFR mutation in exon 19. (A) An H&E stain of a histological section of the sample harboring EGFR mutation in exon 19. (B) An Alcian blue-PAS stain of the same sample. (SP: 20 × 10)
clinical characteristics in common with the two patients, where EGFR exon 21 mutations were previously detected (Table 1). All three patients were men aged over 50 years. Their diagnoses were all advanced invasion into the gastric wall with lymph node metastasis. The tumor sample in the present study was histologically diagnosed as gastric cancer with intracellular mucin. The two tumor samples in our previous study were histologically diagnosed as gastric cancer with extracellular mucin and gastric cancer with intracellular mucin. Tumor samples with both extracellular and intracellular mucin belong to mucin-producing gastric cancers. These results indicated that although EGFR exon 19 and 21 mutations were rare in gastric cancer, they might be the characteristic of the subgroup of mucin-producing gastric cancers. Aberrant activation of EGFR attributable to gene mutation, gene amplification or protein over-expression contributes to the development of various epithelial cancers including gastric cancer. Since the 1980s when the receptor was proposed to be a potential therapeutic target for the treatment of human epithelial cancers, many drugs have been widely developed which target the receptor, and some of them have been successfully used in clinic, such as TKIs (20–22). Gefitinib, one of the most developed TKIs, was approved by the FDA for the treatment of NSCLC in 2003 (23). Furthermore, investigators have demonstrated that EGFR mutations, especially the deletion mutation delE746-A750 in exon 19 and the substitution mutation (L858R) in exon 21, were associated with gefitinib sensitivity of NSCLC patients (24). In 170 NSCLC patients with different EGFR mutations, those patients with EGFR mutations in exon 19 exhibited significantly longer progression-free survival (12.8 months) than those with mutations in exon 21 (10.6 months) (25). The finding supported the hypothesis that EGFR mutations in exon 19, especially delE746-A750, was more effective in predicting TKI sensitivity (26, 27). Furthermore, delE746-A750 was identified in an ovarian cancer patient who was sensitive to the treatment of gefitinib in a Phase II study (28). These results indicated that EGFR mutations in exon 19 might underlie sensitivity to TKIs in cancer patients of diverse tumor types. Thus, the patient harboring the EGFR mutation in exon 19 in the present study might have benefited from treatment with gefitinib, although we can only speculate as the patient was not actually treated with the TKI. Following its successful use in the treatment of NSCLC, gefitinib was entered into clinical trials for many other human cancers including gastric cancer. In a multi-center trial from Japan involving 73 gastric cancer patients, one patient was reported to have had a partial lysis, gefitinib sensitivity of the gastric cancer patient was not definitive associated with EGFR mutations in exons 19 or 21. In conclusion, this retrospective study indicated that mucin-producing gastric cancer sustained EGFR mutations not only in exon 21 but also in exon 19. Regardless of the rare incidence of the mutation overall in gastric cancers, the small subgroup of patients with mucin-producing gastric cancer might benefit from treatment with EGFR-TKIs.

### Supplementary data


### Funding

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### Conflict of interest statement

None declared.

### References


### Table 1. Mutations and clinico-pathological features

<table>
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<th>Histological type</th>
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<td>76</td>
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<td>III</td>
</tr>
</tbody>
</table>

MAC, mucinous adenocarcinoma; SRC, signet-ring cell carcinoma; LN, lymph node.


