Mutation status of epidermal growth factor receptor and clinical features of patients with combined small cell lung cancer who received surgical treatment

HONG-YANG LU\(^1,4\), WEI-MIN MAO\(^1\), QIAO-YUAN CHENG\(^2\), BO CHEN\(^3\), JU-FEN CAI\(^4\), XIAO-JIA WANG\(^1,4\), ZENG WANG\(^5\) and FA-JUN XIE\(^4\)

\(^1\)Key Laboratory Diagnosis and Treatment Technology on Thoracic Oncology (esophagus, lung), Zhejiang Province, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022; \(^2\)Department of Traditional Chinese Medicine, Zhejiang Institute for Food and Drug Control, Hangzhou, Zhejiang 310004; Departments of \(^3\)Pathology, \(^4\)Medical Oncology, and \(^5\)Pharmacology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022, P.R. China

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Abstract. The mutation status of epidermal growth factor receptor (EGFR) is correlated with the response of tumors to EGFR tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC), suggesting its usefulness as a biomarker in NSCLC. The incidence of EGFR mutation in NSCLC is higher in China than in the United States and European countries. There have been some case reports concerning cases of gefitinib-responsive small cell lung cancer (SCLC) with EGFR mutations. However, few large studies concerning the mutation status of SCLC patients have been performed. We detected EGFR mutations in exons 19 and 21 of 40 SCLC patients, three of whom had combined SCLC, from the Zhejiang Cancer Hospital using xTAG technology. Only two patients with combined SCLC had an EGFR mutation in exon 19. To determine the EGFR mutation status and clinical features of combined SCLC, we retrospectively analyzed the clinical features of seven patients with combined SCLC who had undergone surgical treatment in Zhejiang Cancer Hospital between 2007 and 2010. EGFR mutations in exons 19 and 21 were detected using the pyrosequencing assay. Of the seven patients with combined SCLCs, 71.4% were male, 71.4% were heavy smokers, most were over 60 years old and 71.4% of the cases were combined adenocarcinoma. Chemotherapy treatment and tumor stage were correlated with survival time. Of the seven cases, one had a mutation in exon 19 of EGFR in both the conventional SCLC and SCLC combined adenocarcinoma components. Combined SCLC commonly occurs in patients who are heavy smokers, male and over 60 years old, and most of the combined type cases are adenocarcinoma. The treatment of combined SCLC may be applied to cases of conventional SCLC. EGFR mutations may therefore occur in combined SCLCs, especially in SCLC combined adenocarcinoma in China.

Introduction

An epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) may be used as first-line therapy in patients with advanced non-small cell lung cancer (NSCLC) with EGFR mutations (1-5). The results of the INTEREST trial (6) suggest that gefitinib is able to provide similar overall survival to docetaxel in patients across a broad range of clinical subgroups and that EGFR biomarkers, including mutation status, may additionally identify which patients are likely to gain greatest progression-free survival (PFS) and overall response rate (ORR) benefit from treatment with gefitinib. Two hot spots of EGFR mutations are in-frame deletion at codons 747-749 (DEL) in exon 19 and a missense mutation at codon 858 (L858R) in exon 21. A case study concerning a Japanese patient with gefitinib-responsive small cell lung cancer (SCLC) reported that the patient had a deletion in exon 19 of EGFR (7). Another case study has reported that an American SCLC patient who had never smoked and who had an EGFR mutation responded to gefitinib (8). In China, there has also been a case report of a patient with SCLC who responded to gefitinib, but the status of the mutation is unknown (9). Therefore, the EGFR mutation status of SCLC is significant.

We detected EGFR exons 19 and 21 mutations in 40 SCLC patients, three of whom had combined SCLC, from the Zhejiang Cancer Hospital (Hangzhou, China) by xTAG technology. Only two of the combined SCLC patients showed an EGFR mutation in exon 19 (10). To identify the
clinical features and incidence of EGFR mutations in cases of combined SCLC in China, we retrospectively investigated seven cases of combined SCLC which were treated surgically and detected mutations in EGFR exons 19 and 21 using a pyrosequencing assay. The combined components of these patients were not only adenocarcinoma but also squamous cell carcinoma. Specimens obtained during surgery more accurately reflect the pathological status of the tumor and all our specimens were from surgically resected tumors.

**Materials and methods**

**Patient characteristics.** Seven cases of combined SCLC from the Zhejiang Cancer Hospital (Hangzhou, China) between 2007 and 2010 were investigated. Of the patients with combined SCLC, 71.4% were male, 71.4% were heavy smokers, most were >60 years old and 71.4% of the cases were combined adenocarcinoma. None of the patients were clearly pathologically diagnosed prior to surgery. The specimens were obtained from surgically resected tumors. The histological diagnosis of combined SCLC was based on the standard criteria defined by the WHO classification. The patients were aged 47-74 years (median, 62), two were female and five were male. Two cases were stage IB, one was IIB and four were II IA according to the seventh edition of the TNM classification for lung cancer. Two of the patients were non-smokers and five were heavy smokers. There were five cases of SCLC combined adenocarcinoma and two cases of SCLC combined squamous cell carcinoma (Fig. 1A). Most of the patients underwent lobectomy and lymph node dissection. Five patients received chemotherapy and two patients received no chemotherapy. Only one patient received thoracic radiotherapy (Table I). This study was approved by the ethics committee of the Zhejiang Cancer Hospital.

**Pyrosequencing assay for gene mutation.** We detected two examples of adenocarcinoma and SCLC combined adenocarcinoma components in case 6. We also detected two examples of conventional SCLC and SCLC combined adenocarcinoma components in case 7. In the other five patients, only one sample for each patient was detected. Genomic DNA was isolated and purified from formalin-fixed paraffin-embedded tissues using a GPure FFPE Tissue DNA Extraction kit (GeneTech, Shanghai, China). For the amplification of fragments of exons 19 and 21 of the EGFR gene from isolated genomic DNA, we designed PCR amplification primers for pyrosequencing: EGFR-19, forward: 5’-GGATCCCAGAAGGTGAGAAAGTT-3’; EGFR-19, reverse biotinylated primer: 5’-GAGAAAAGGTGGGCCTGAGGT-3’; and EGFR-21, forward: 5’-GGGCATGAACATTGGAGG-3’. EGFR-21, reverse biotinylated primer: 5’-TCCCTGGTGTCAGGAAAATG-3’. Each PCR assay contained forward and reverse primers (each 4 pmol), 2 µl template DNA solution and 2 units hotstart Taq DNA Polymerase (Takara, Shiga, Japan) in a 40 μl volume. The PCR conditions consisted of initial denaturation at 95°C for 3 min; 50 cycles of 95°C for 15 sec, annealing at 56°C for 30 sec and 72°C for 30 sec; and final extension at 72°C for 5 min. The PCR products were sequenced using the Pyrosequencing PyroMark ID system (Qiagen, Hilden, Germany) following the manufacturer’s instructions, using the two pyrosequencing
Primers (5'-3' orientation): EGFR-19, TCCCGTCGCTATCAA; EGFR-21, AAGATCACAGATTTTGG. Pyrosequencing was performed using PyroMark Gold Q96 Reagents (Qiagen) containing enzyme and substrate mixture, dATP-S, dCTP, dGTP and dTTP.

Statistical analysis. The statistical significance of the mean values was determined using SPSS 13.0 (Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant result.

Results

The majority of the patients (71.4%) received chemotherapy and most underwent more than 4 cycles. Cases 4 and 6 were alive at the end of their follow-up periods and cases 2 and 5 could not be followed up for longer than 31 and 18 months, respectively. Cases 3 and 6 did not receive chemotherapy and the survival time of case 6 (stage IB) was longer than that of case 3 (stage IIIA). The survival time of case 3 was shorter than that of the other stage IIIA patients who received chemotherapy (Table II). No mutations of exons 19 and 21 were observed, with the exception of case 7. Case 7 was found to have a mutation in exon 19 (codon 746-754) of EGFR both in the conventional SCLC and the SCLC combined adenocarcinoma components. H&E, hematoxylin and eosin; SCLC, small cell lung cancer; EGFR, epidermal growth factor receptor; staging according to the seventh edition of the TNM classification for lung cancer.

Discussion

Combined SCLC is defined as SCLC combined with an additional component that consists of any of the histological types of NSCLC, usually adenocarcinoma, squamous cell carcinoma or large cell carcinoma. SCLC accounts for approximately 15% of lung cancers (11). Combined SCLC has been reported to account for 1-3.2% of all SCLC cases (12,13). However, 28% of SCLC patients who undergo surgical resection exhibit combined SCLC, with SCLC with large cell carcinoma being the most common, followed by adenocarcinoma and squamous cell carcinoma (14). Specimens obtained during surgery more accurately reflect the pathological features of

<table>
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<th>Patient no.</th>
<th>Survival (months)</th>
<th>EGFR 19 mutation</th>
<th>EGFR 21 mutation</th>
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<tbody>
<tr>
<td>1</td>
<td>26</td>
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<td>No</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>&gt;18</td>
<td>No</td>
<td>No</td>
</tr>
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<td>No</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>Yes</td>
<td>No</td>
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EGFR, epidermal growth factor receptor; SCLC, small cell lung cancer.

Table II. Survival and EGFR mutation of combined SCLC patients.
the tumor than biopsy and malignant pleural effusion. In the study by Tatematsu et al., most of the specimens were obtained from biopsy (15). The specimens reported by Shiao et al. include 10 computed tomography-guided biopsy specimens, 17 echo-guided aspiration specimens, 37 echo-guided biopsy specimens, one surgical lobectomy specimen and 11 malignant pleural effusion specimens (16), whereas in the present study, all the specimens were obtained during surgery.

The incidence of the EGFR mutation in NSCLC is higher in China than in the United States and European countries (17,18). Few large studies concerning the mutations status of patients with SCLC have been performed. EGFR mutations have been detected in five (4%) of 122 Japanese patients with SCLCs, of which 15 cases were combined SCLCs. The patients with EGFR mutations were mainly in the light smoker and histological combined subtype. In three cases of the combined SCLC subtype, both components of adenoscarcinoma and SCLC harbored an EGFR mutation (15). Fukui et al. retrospectively investigated six resected cases of combined SCLC with an adenocarcinoma component in Japan to elucidate the clinicopathological parameters and detect the EGFR mutation status (19). With regard to EGFR, no specific mutation was detected in five of the six patients, whereas only one female patient who had never smoked had the same mutation in exon 21 (L858R) in both the SCLC and adenocarcinoma components (19). A prospective study on 76 specimens from patients with SCLC conducted between 2004 and 2009 at the National Taiwan University Hospital reported that two cases (2.6%) tested positive for the EGFR mutation with reverse transcription polymerase chain reaction (RT-PCR) and direct sequencing and both cases were deletions in exon 19. Additionally, three patients were diagnosed with combined SCLC but showed no EGFR mutation in exon 19 or 21 (19). In our previous study, we detected EGFR mutations in exons 19 and 21 of 40 cases and two combined SCLCs with EGFR mutation in exon 19 (10). However, more combined SCLCs cases are required to identify clinical features and detect EGFR exon 19 and 21 mutations in China. EGFR mutations were predictive of the response of single-agent TKIs, and EGFR gene copy number was also associated with response to TKIs, albeit with lower sensitivity and specificity (20). Therefore, in this study, we detected EGFR mutations instead of EGFR copy number. Compared with other genotyping and genetic detection methods, pyrosequencing technology is unique. Unlike hybridization-based assays, which may yield false-negative results, pyrosequencing produces a correct sequence even in the presence of a novel mutation. This is significant for microbiological applications. Another benefit of pyrosequencing is that the data is quantitative, thus it is possible to measure the relative amounts of alleles.

The seventh edition of the TNM classification was also cited in the National Comprehensive Cancer Network (NCCN) guidelines for SCLC (2011 version 1). Surgery may be used to treat NSCLC patients with stages IA, IB, IIA, IIB and IIA disease, but only T1-2N0M0 SCLC patients may be considered for surgical treatment. Cases of T1-2N0M0 SCLC have been reported to account for less than 5% of all SCLCs (21), thus few SCLCs can be treated surgically. It is difficult to diagnose combined SCLC by biopsy and the rarity of patients with combined SCLC makes it difficult to determine the optimal management and biological characteristics of this tumor. The treatment of combined SCLC was managed according to NCCN guidelines (Version 1.2011). There have been few studies concerning combined SCLC and more studies should be conducted to identify the clinical features of these patients. This study demonstrates that combined SCLC frequently occurs in patients who are heavy smokers, male and aged over 60 years and that most cases are combined adenocarcinoma. Case 3 did not receive chemotherapy and the survival time of this patient was shorter than that of the other stage IIIA patients who received chemotherapy. We suggest that chemotherapy is significant for combined SCLC and most of the patients in the present study received chemotherapy. Cases 3 and 6 did not receive chemotherapy, but the survival time of case 6 was longer than that of case 3. The cause of the difference in survival time may be the different stages of case 3 (IIIA) and case 6 (IB). Thus, stage may affect survival time. Concurrent chemotherapy combined with mediastinal radiotherapy should be used to treat SCLC patients with lymph node metastasis following surgery and adjuvant chemotherapy and radiotherapy may be considered for stage IIIA NSCLC patients. Ideally, if the combined SCLC with lymph node metastasis is present after surgery, radiotherapy should be used in conjunction with chemotherapy. However, in our study, only one patient received radiotherapy.

In conclusion, EGFR mutations may occur in combined SCLCs, particularly in SCLC combined with adenocarcinoma in China.

Acknowledgements

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