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Identifying EGFR Mutations from SCLC Patient Plasma by Mutant-Enriched Liquidchip Technology*

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) such as erlotinib and gefitinib are targeted drugs for the kinase domain of EGFR. They are widely used for the treatment of non-small cell lung cancer (NSCLC). The EGFR exon 19 deletion mutation and the L858R mutation in exon 21 comprise approximately 90% of the somatic mutations in NSCLC patients that respond to EGFR TKI. Several recent studies have also reported that small cell lung cancer (SCLC) patients with EGFR mutations responded to gefitinib. Further study, however, has been limited due to the difficulty obtaining tumor specimens from SCLC patients.
Objectives. The aim of this study was to explore the EGFR mutation status in SCLC patients by plasma analysis.
Material and Methods. Plasma samples from SCLC patients were collected for mutant-enriched liquidchip (MEL) analysis to identify the EGFR mutations in exon 19 and 21.
Results. The exon 19 deletion mutation was detected in one out of 35 patients (a female non-smoker). No exon 21 mutations were found.
Conclusions. A prevalence of EGFR mutations in SCLC is rare, and occurs most frequently in females and non-smokers (**Adv Clin Exp Med 2014, 23, 2, 191–195**).

Key words: small cell lung cancer, epidermal growth factor receptor, gene.

It is well known that epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) such as erlotinib and gefitinib are targeted drugs used in the treatment of non-small cell lung cancer (NSCLC) [1–5]. The presence of EGFR mutations is a biomarker predicting the response of NSCLC patients who will receive EGFR TKI treatment. It has been reported that the frequency of EGFR mutations in Asia is higher than in Europe and North America [6, 7]. Interestingly, several recent studies have reported that small cell lung cancer (SCLC) patients with EGFR mutations responded to gefitinib [8, 9]. The response of Chinese SCLC patients to gefitinib has also been observed with the status of EGFR mutations unknown [10].

Fewer than 5% of SCLC patients are staged T1-2N0M0, and most SCLC patients do not receive

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surgical resection. Therefore very few studies about SCLC patients' mutation status are available, due to the difficulty in obtaining tumor tissues. The most recent report is a prospective study from Taiwan showing that EGFR exon 19 mutations were detected in only 2.6% (2 of 76) SCLC patients [11]. Tatematsu et al also reported low EGFR mutation frequency among Japanese SCLC patients: 5 out of 122 cases (4.1%) [12]. The authors of the current study previously reported two combined SCLC patients with EGFR exon 19 deletion mutation out of 40 SCLC patients undergoing surgical resection [13]. To further explore the EGFR mutation incidence in SCLC patients without surgery, a novel mutant-enriched liquidchip (MEL) technology was used to identify EGFR mutation in SCLC patient plasma.

Material and Methods

Patient Characteristics

Thirty-five SCLC patients were recruited in this study between 2011 and 2012. Their clinical information is shown in Table 1. Plasma samples were acquired along with the cancer diagnosis. DNA was extracted by standard means. All the samples were included in a mutant-enriched liquidchip polymerase chain reaction (MEL-PCR) analysis. The study was approved by the Ethical Review Committee of Zhejiang Cancer Hospital.

Mutant-Enriched Liquidchip (MEL)

The MEL-PCR protocol was adapted and modified from Asano et al. [14]. It consists of two-step PCR procedures and one restriction enzyme digestion. In brief, there was a uniquely designed restriction site in each of the wild-type EGFR genes of exons 19 and 21: Mse I (TTAA) for exon 19 and Msc I (TGGCCA) for exon 21. After the first PCR amplification, restriction digestion was performed to selectively remove the wild-type EGFR. In the second PCR, the mutated EGFR was then enriched. Following PCR amplification, the PCR product was hybridized to a mixture of probe-bearing microsphere populations in a hybridization buffer. The probes were designed against the sequence of wild-type and mutant of EGFR exon 19 deletions and L858R. The mixtures were then heated to 96°C for 2 min, followed by 15 min incubation at 56°C. Then 25 uL of streptavidin-conjugated phycoerythrin (SA-PE) in hybridization buffer was added to the mixture and incubated for 5 min at 56°C. The reactions were analyzed on a Luminex 200 system.

Statistical Analysis

SPSS 13.0 software was used for the statistical analysis.

Results

The EGFR exon 19 mutation was detected in one out of 35 samples (2.8%) (Fig. 1). No patients with the EGFR exon 21 mutation were found (Fig. 2). The only patient with an EGFR mutation is a 57-year-old female non-smoker carrying the c.2236-2250 del 15 (p.E746-A750 del) mutation. Chest CT revealed a tumor in the left lower lung with mediastinal lymph node metastasis. Bone and brain metastasis occurred before treatment. Figure 3 presents the histological type of the tumor, based upon which the patient was diagnosed with SCLC. The stage of this patient was IV according to the TNM classification system. The patient received 6 cycles of chemotherapy with etoposide plus cisplatin, and a partial response was observed after four cycles of chemotherapy.

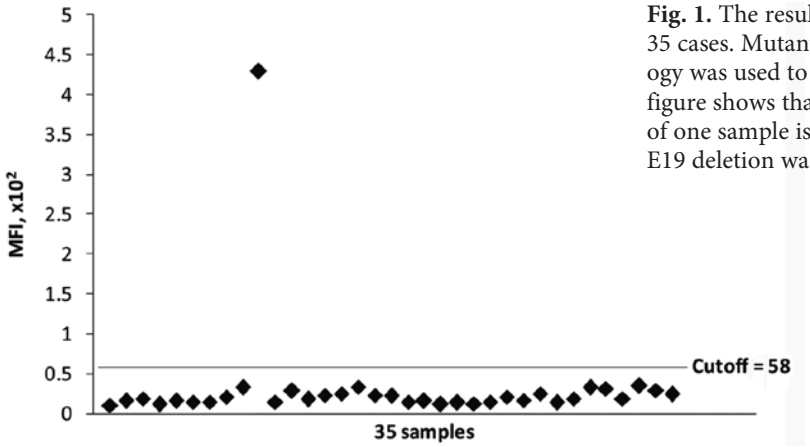

Fig. 1. The results for the EGFR E19 mutation in 35 cases. Mutant-enriched liquidchip (MEL) technology was used to detect the EGFR E19 mutation. The figure shows that the median fluorescence intensity of one sample is above the cut-off value, which means E19 deletion was detected

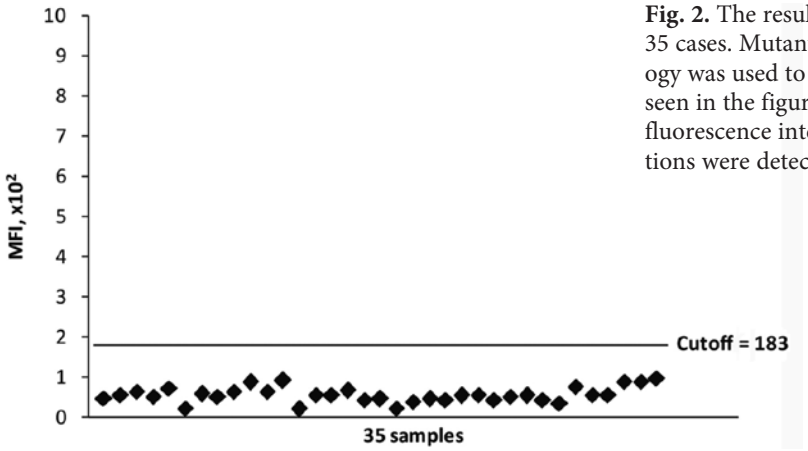

Fig. 2. The results for the EGFR E21 mutation in 35 cases. Mutant-enriched liquidchip (MEL) technology was used to detect the EGFR E21 mutation. As seen in the figure, no sample has a value of median fluorescence intensity above the cut-off, so no mutations were detected

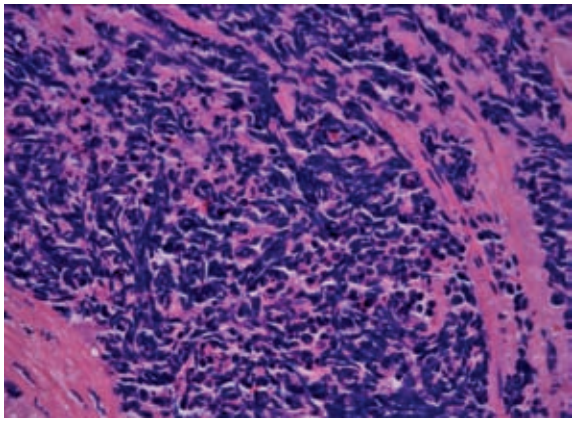

Fig. 3. The pathology of the SCLC patient with the EGFR 19 mutation. The patient was a 57-year-old female non-smoker. The sample was obtained by bronchoscope. The patient's pathological results were detected using hematoxylin and eosin staining. The tumor cells are dense and of small size, with a lack of cytoplasm, disappearance of nucleolus and finely granular nuclear chromatin

Table 1. Clinical characteristics of the study population

Clinical characteristic	Cases
No. of patients (total)	35
Sex (female/male)	7/28
Stage	
I	0
II	0
IIIA	2
IIIB	6
IV	27
Smoking history	
Nonsmoker	10
Light smoker	0
Moderate smoker	3
Heavy smoker	22

Discussion

EGFR mutations are rare in SCLC patients. The mutations reported so far include G719A, exon 19 deletions and L858R, which covered most of the mutation sites in NSCLC. SCLC patients with EGFR mutations most frequently occur in females, nonsmokers and in cases of SCLC combined with adenocarcinoma [13]. To avoid the difficulty of obtaining SCLC tumor specimens, the authors of the current study explored the use of MEL-PCR technology to analyze EGFR mutations in SCLC patient plasma samples.

Analysis of circulating DNA or RNA in plasma requires only a small blood sample, which makes it a promising non-invasive diagnostic method [15]. Many technologies are available for analyzing circulating nucleic acid [16, 17]. MEL technology was developed by Wu [18] to detect KRAS mutations using serum or formalin-fixed and paraffin-embedded samples. The technique is a method of high sensitivity, high reliablity and high throughput [18]. In the current study, a very low EGFR mutation rate was found (1/35, 2.8%), which was consistent with reports in literatures [11–12]. Due to the fact that tumor specimens of SCLC patients are always obtained from biopsy or needle puncture, few specimens are available for further analysis. Plasma can serve as an alternative option for mutation detection and make MEL technology more useful in molecular diagnosis.

It is well known that EGFR mutations in NSCLC are prevalent in females, nonsmokers, Asians and adenocarcinoma [7, 19, 20]. The SCLC patient with EGFR mutation in the current study is a female and non-smoker, which is consistent with previous studies [11–13]. This consistence leads to the hypotheses that EGFR mutations in SCLC may also be relatively prevalent in females and nonsmokers. As the presence of EGFR mutations is the most reliable biomarker to predict tumor

responses to EGFR TKI in NSCLC patients, the authors hypothesize that gefitinib can treat SCLC patients with EGFR mutations. A partial response to gefitinib has actually been reported in SCLC patients with EGFR mutations before [8, 9]. Further study is proposed to investigate the role of EGFR mutations in TKI treatments for SCLC patients.

The authors conclude that EGFR mutations are rare in SCLC patients, and might be relatively prevalent in females and non-smokers. Circulating DNA is an alternative target for mutation analysis, especially for those patients who cannot undergo surgery. MEL technology can be used to detect low-frequency mutations in plasma.

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