

Research Article

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Detected EGFR mutation in cerebrospinal fluid of lung adenocarcinoma patients with meningeal metastasis

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Abstract: Objective: To discuss the application of ARMS method to detect EGFR gene mutation in cerebrospinal fluid of lung adenocarcinoma patients with meningeal metastasis. Methods: 5 cases of lung adenocarcinoma were identified with meningeal metastasis that were cleared EGFR gene mutation by gene sequencing method. From each patient 5ml cerebrospinal fluid was obtained by lumbar puncture. ARMS method was used to detect EGFR mutations in cerebrospinal fluid. Results: 5 samples of cerebrospinal fluid were successfully detected by ARMS method, 3 samples found that EGFR gene mutations, the mutations in line with direct sequencing method. Conclusion: ARMS method can be used to detect EGFR gene mutations of cerebrospinal fluid samples in lung adenocarcinoma with meningeal metastasis. But cerebrospinal fluid specimens from histological specimens, blood samples need to be confirmed by further comparative study whether there is advantage.

Keywords: Meningeal metastasis; Non-small cell lung cancer; ARMS method; Cerebrospinal fluid; EGFR gene mutations

1 Introduction

Lung cancer is one of the most common malignant tumors in the world. The leading causes of poor prognosis in patients with lung cancer are recurrence and distant metastasis. Among these, a small, but significant number (5%) of patients will develop meningeal metastasis (MM) [1]. Meningeal metastasis in lung cancer patients predicts poor prognosis, the median survival of untreated patients is only 4~6 weeks, even after comprehensive treatment, the median survival is only extended to 3~5 months [2].

Epidermal Growth Factor Receptor (EGFR) was over-expressed in 45%~70% non-small lung cancer (NSCLC) [3]. Tyrosine kinase inhibitor (TKI), an orally active small molecule that functions as a selective EGFR inhibitor, has showed better clinical effects in the therapy of NSCLC with EGFR mutation, also, the sensitivity of the patients with lung cancer of EGFR-TKI are closely associated with EGFR mutation status [4]. Based on the special property of EGFR-TKI, small molecular weight and the ease with which it passes through the blood brain barrier, previous studies have proved that gefitinib and erlotinib both have supportive treatments with meningeal metastasis in NSCLC with EGFR mutation resulting in clinical symptoms, changes of cerebrospinal fluid and imaging findings [5-6]. DNA of tumor tissues are the best resources for EGFR gene mutation analysis, however, most patients with advanced NSCLC cannot have the operation, bronchoscopy biopsy and puncture biopsy can't obtain enough tumor tissue for EGFR mutation testing. Thus, many studies focus on the amplification refractory mutation system (ARMS), which is performed to detect EGFR mutations in small specimens, specimens with lower content of tumor tissue and body fluids, showing great advantages in clinical research [7-10]. In our work, we performed ARMS to detect mutation status in exon 18-21 of EGFR gene from the cerebrospinal fluid of lung cancer meningeal metastasis patients, further investigated the feasibility of ARMS in detecting EGFR mutation, and finally provided evidence

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to EGFR-TKI type molecular drugs targeting therapy of lung cancer meningeal metastasis.

2 Materials and methods

2.1 Medical records

All participants were patients with non-small cell lung cancer with meningeal metastases undergoing tumor interventional therapy in the Tianjin Huanhu Hospital from March 2014 to February 2015, subject to the following conditions:

(1) patients with non-small cell lung cancer with meningeal metastases with definitive diagnosis in histology or cytology. (2) cancer patients with meningeal metastases diagnosed definitively in the cerebrospinal fluid by (CSF) cytology or radiography. (3) the EGFR mutation of the patients has been detected by the gene sequence of the tumor tissues. (4) the values of coagulation time (PT and APTT) and platelet of the patients are both in a normal range. (5) the intracranial hypertension of the patients can be controlled by dehydration therapy. (6) the patients can tolerate CSF collection by lumbar puncture. (7) the experiments about the patients has been approved by the Ethics Committee. (8) the patients have read and signed the informed consent form.

2.2 Research methods

2.2.1 CSF collection

All patients are within the scientific project of the Tianjin Health Bureau, (The Application Of The Detection Of CFS Circulating Tumor In The Diagnosis Of Lung Cancer With Meningeal Metastases), No.2014KZ042. CFS was collected from all patients by lumbar puncture. Our research collects 5ml CFS from every patient after this research project.

2.2.2 Detection of the EGFR mutation in cerebrospinal fluid by ARMS

The 18-21 exons of EGFR gene in cerebrospinal fluid samples were amplified with specific primers by polymerase chain reaction, PCR. The 29 mutations in EGFR 18-21 exons were detected by ABI 7500 PCR instrument. The PCR kit was purchased from Xiamen Aide Biological

Medicine Technology. The main steps including: 1. DNA extraction: (1). Centrifuge 5mL cerebrospinal fluid, collect 200ul fluid in the bottom into the 1.5mL EP tube, add 20ul protease K, mix uniformly. (2). Add 200ul buffer AL, vortex to mix uniformly, incubate in 56°C for 10min. (3). Add 200ul ethanol (conc. 96%-100%), vortex to mix, centrifuge rapidly. (4). Transfer the mixture from Step (3) onto the Spin Column, put the Spin Column on the 2ml collection tube, 12000 rpm centrifuge for 1min, remove the supernatant. (5). Add 500ul buffer AW1, 12000rpm centrifuge for 1min, remove the supernatant. (6). Add 500ul buffer AW2, 12000rpm centrifuge for 3 min, remove the supernatant. (7). Centrifuge for 1min, remove the supernatant. Put the Spin Column to a new collection tube. (8). Add 50 ul buffer AW (or distilled water) onto the adsorbed film, incubating in room temperature for 5min, 12000rpm centrifuge for 1min. (9). Repeat Step (8). (10). Measure the DNA content, store in -20°C. 2. ARMS-PCR detection. (1). Thaw the positive control materials, vortex rapidly, centrifuge shortly, wait to use. (2). DNA preparation from unknown sample (conc. 0.4-1.0ng/ul), add 2.25 ul Taq enzyme, vortex rapidly, centrifuge shortly, wait to use. (3). Prepared 45 ul positive control materials, add 2.25 ul Taq enzyme, vortex rapidly, centrifuge shortly, wait to use. (4). Prepared 45 ul ultrapure water (NTC), add 2.25 ul Taq enzyme, vortex rapidly, centrifuge shortly, wait to use. (5). Add 5 ul mix DNA samples, positive control materials, NTC to PCR reaction tube, cover the tubes tightly, centrifuge shortly, put them into ABI7500 PCR instrument. (6). Set the PCR program: Phase I 95°C, 5 min; Phase II 95°C, 25s; 64°C, 20s; 72°C, 20s, 15 cycles; Phase III, 93°C, 25s; 60°C, 35s; 72°C, 20s, 31 cycles, detect the fluorescence signal in 60°C.

3 Results

5 patients of non-small cell lung cancer with meningeal metastases who met the group criteria participated in our experiment. They all belong to the science fund project of the Tianjin Health Bureau, (The Application Of The Detection Of CFS Circulating Tumor In The Diagnosis Of Lung Cancer With Meningeal Metastases), No.2014KZ042. All 5 patients were female with a median age of 60 years old (from 46 to 64 years old). The pathology of primary tumor was lung adenocarcinoma. 2 of the patients presented with mutations of 19 exon deletion, and 3 presented with L858R site mutation. The result about position of the site mutation detected by ARMS is the same as the result detected by gene sequence. Also, the numbers

of the circulating tumor cells (CTCs) in the CFS of these 3 cases are higher than the CTCs numbers of negative results detected by ARMS. All results are shown in Table 1.

4 Discussion

Meningeal metastasis carcinoma refers to metastasis and diffuse infiltration of leptomeninges and subarachnoid brain by primary tumour cells, causing the symptoms and signs of brain tissue, cranial nerve and spinal cord injuries [10]. Early diagnosis and early treatment can effectively slow nerve function defect caused by the disease progression. Traditionally, meningeal metastasis of lung cancer is mainly treated with conservative treatment, radiotherapy, intrathecal chemotherapy and systemic chemotherapy. The main aim of the treatment is to improve or stabilise the central nervous function of the patients, improve the quality of life and prolong the survival periods, but the whole curative effect is barely satisfactory [2]. With the emergence of EGFR-TKI type molecular targeted drugs, such as gefitinib and Erlotinib. For EGFR gene mutation of meningeal metastasis from lung cancer, the application of EGFR-TKI type molecular target drug treatment can significantly prolong patient survival and improve the patient's quality of life. Direct sequencing method is currently the gold standard for detection of EGFR mutations. This method is impeded by volume of specimens, time-consuming, and tedious operation [11]. The ARMS method uses the Taq DNA polymerase probe for different known mutations to design appropriate primers to detect the mutation gene. The amplification products are analyzed by real-time fluorescent quantitative PCR technology. This

method has the advantages of high sensitivity, simple, quick operation and so on. However, the price is expensive. For the samples with lower content of tumor tissue and body fluid specimens, The ARMS method has a significant advantage over the direct sequencing method.

Detailed information about the application of ARMS testing CSF has been not reported. 5 CSF samples with EGFR mutation from lung cancer patients with meningeal metastasis were obtained from 2014KZ042 study. The patients informed and the ethics committee of hospital consented. 5 ml of cerebrospinal fluid specimen remaining after 2014KZ042 study were collected from each patient for EGFR mutation detection using ARMS testing. 1 patient was observed with L858R point mutation and 2 patients with exon 19 deletion, which was consistent with the results of direct sequencing. No EGFR mutation was observed in the rest 2 patients. The study results suggest the feasibility of using ARMS for detection of EGFR mutation on cerebrospinal fluid specimens. However due to the limited sample size, it remains to be confirmed in further comparative studies whether cerebrospinal fluid specimen used in ARMS could provide any superiority over tissue or blood specimen analysis. Moreover, EGFR mutation was detectable using ARMS in the 3 cerebrospinal fluid specimens with abundant CTCs, necessitating further investigation on the correlation between CTC count and ctDNA. The data was considered to be not conclusive enough due to the small sample size and absence of control samples. Results of this study are expected to provide reference for the design of further clinical studies.

Conflict of interest statement: Authors state no conflict of interest.

Table 1: Basic information of 5 patients and results of ARMS.

No.	Gender	Age (Y)	Pathological types	EGFR mutation	ARMS results	CTCs/7.5ml
1	Female	64	Adenocarcinoma	Exon19 deletion	Exon19 deletion	720
2	Female	63	Adenocarcinoma	L858R	No	3
3	Female	52	Adenocarcinoma	L858R	L858R	246
4	Female	46	Adenocarcinoma	L858R	No	77
5	Female	60	Adenocarcinoma	Exon19 deletion	Exon19 deletion	148

References

- [1] Nagpal S, Riess J, Wakelee H. Treatment of leptomeningeal spread of NSCLC: a continuing challenge[J]. *Curr Treat Options Oncol*, 2012, 13(4): 491-504
- [2] Ma C, Jiang R, Li JD, et al. Research progress of lung cancer with leptomeningeal metastasis[J]. *Chinese Journal of Lung Cancer*, 2014, 17(9): 695-700
- [3] Grunwald V, Hidalgo M. Developing inhibitors of the epidermal growth factor receptor for cancer treatment[J]. *J Natl Cancer Inst*, 2003, 95(3): 851-867
- [4] Tamura K, Okamoto I, Kashii T, et al. Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403)[J]. *Br J Cancer*, 2008, 98(5): 907-914
- [5] Togashi Y, Masago K, Masuda S, Mizuno T, Fukudo M, Ikemi Y, Sakamori Y, Nagai H, Kim YH, Katsura T, Mishima M. Cerebrospinal fluid concentration of gefitinib and erlotinib in patients with non-small cell lung cancer[J]. *Cancer Chemother Pharmacol*, 2012, 70(3): 399-405
- [6] Kuiper JL, Smit EF. High-dose, pulsatile erlotinib in two NSCLC patients with leptomeningeal metastases—one with a remarkable thoracic response as well[J]. *Lung Cancer*, 2013, 80(1): 102-105
- [7] Zhang X, Zhao Y, Wang M, et al. Detection and comparison of epidermal growth factor receptor mutations in cells and uid of malignant pleural effusion in non-small cell lung cancer[J]. *Lung Cancer*, 2008, 60(2): 175-182
- [8] Brevet M, Johnson ML, Azzoli CG, et al. Detection of EGFR mutations in plasma DNA from lung cancer patients by mass spectrometry genotyping is predictive of tumor EGFR status and response to EGFR inhibitors[J]. *Lung Cancer*, 2011, 73(1): 96-102
- [9] Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer[J]. *J Clin Oncol*, 2009, 27(16): 2653-2659
- [10] Jiang R, Ma CH, Zhu ZL, et al. Application of circulating tumor cell detection in cerebrospinal fluid in the diagnosis of meningeal metastasis from non-small cell lung cancer[J]. *Chinese Journal of Contemporary Neurology and Neurosurgery*, 2014, 14(7): 698-701
- [11] Li C, Wu J, Wang Z, et al. A comparison of direct sequencing and ARMS assay performance in EGFR mutation analysis of non-small cell lung cancer patients[J]. *Zhongguo Fei Ai Za Zhi*. 2014, 17(8): 606-611