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ORIGINAL ARTICLE

Detection of epidermal growth factor receptor mutation in non-small-cell lung carcinoma using cytological and histological specimens

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Summary

Purpose: *Epidermal growth factor receptor (EGFR) mutations are prerequisites for the targeted therapy with anti-EGFR tyrosine kinase inhibitors (TKIs) in non-small-cell lung carcinomas (NSCLCs). In patients with advanced-stage NSCLC, sometimes cytological specimens, including those from fine-needle aspiration cytology (FNAC) and pleural effusion, are the only materials for mutation analysis. The purpose of this study was to compare the results of EGFR mutation detection from cytological specimens and histological samples and to evaluate the difference between them, therefore to assess if cell block is a valid source for detection of EGFR mutation.*

Methods: *Forty-seven samples from advanced-stage NSCLCs were obtained with individually matched cell blocks (CBs) from FNAC (29 cases) or pleural fluid (18 cases), and formalin-fixed paraffin-embedded (FFPE) blocks from biopsy (34 cases) or surgical excision (13 cases). CBs and FFPE blocks were simultaneously tested for EGFR hot mutations in exons 18, 19, 20 and 21 by polymerase chain reaction (PCR)-direct sequencing and amplification refractory mutation system (ARMS)-PCR.*

Results: *EGFR mutations were identified in 18/47 (38.3%) or 21/47 (44.7%) cases using CBs and 16/47 (34.0%) or 19/47 (40.4%) using FPPE blocks by PCR-direct sequencing or ARMS-PCR, respectively. The incidence of EGFR mutation was not statistically significant between CBs and FFPE blocks using PCR-direct sequencing or ARMS-PCR ($p=0.668$ or $p=0.677$, respectively).*

Conclusion: *Our study suggests that cytological specimens are optimal for advanced NSCLC. The successful use of these non-invasive specimens in molecular pathology is beneficial for patients requiring targeted therapy.*

Key words: *cytology, EGFR, histology, lung carcinomas, non-small-cell lung carcinomas*

Introduction

Over the past decades, progress has been achieved in the diagnosis and treatment of NSCLCs, particularly with the wide application of TKIs, such as gefitinib and erlotinib [1-9]. Thus, detection of EGFR mutations has become a routine methodology for predicting the effectiveness of TKIs in NSCLCs [10-16]. IASLC, ATS and ERS published the international multidisciplinary new classification theme of lung adenocarcinoma in 2011, which emphasises the requirement of EGFR mutation analysis in TKI targeted therapy [17].

Currently, the detection of EGFR mutation predominantly uses formalin-fixed, FFPE tissue blocks derived from biopsy or surgical excision [1,2,4-9]. In a considerable proportion of lung cancer patients, particularly those with advanced disease, it is easy to obtain tumor cells in different types of cytology samples because of their widespread distribution. Cytology materials, such as CBs from pleural effusion, and FNAC may be an alternative for the detection of EGFR mutation, particularly when FFPE material is unavailable in NSCLC patients with advanced tumor stage [18,19].

This study aimed to compare the consistency of EGFR detection results between histological samples and corresponding cytological samples, and evaluate whether CB is a valid sample source

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for the detection of EGFR mutation. PCR-direct sequencing and ARMS-PCR were applied for EGFR mutation analysis in this study [20].

Methods

Patients and tumor tissues

Forty-seven consecutive NSCLC patients treated at the Zhejiang Cancer Hospital (Zhejiang, China) between January 2010 and June 2013 were included in this study. This study was conducted in accordance with the declaration of Helsinki and after approval from the Ethics Committee of Zhejiang Cancer Hospital. Written informed consent was obtained from all participants.

Cytological and histological samples were obtained from cases. CBs were also available in all cases after smear preparation. The time intervals between the acquisition of all the cytological samples and the corresponding histological samples were less than 4 weeks. The patient and diagnostic material acquisition method are summarised in Table 1.

Twelve cases among the 29 FNAC cases had FNAC of supraclavicular lymph nodes, and then underwent further biopsy and tissue collection with fiberoptic bronchoscopy. The remaining 17 FNAC cases underwent percutaneous lung lesion puncture, of which 13 cases underwent surgical resection (8 with thoracotomy and 5 via thoracoscopy) and 4 cases underwent FNAC and core biopsy. The other 18 cases had pleural effusions, and underwent biopsy via a fiberoptic bronchsoscope.

Table 1. Clinicopathological characteristics of 47 NSCLCs

Parameters	*Frequency N (%)*
Gender	
Male	27 (57.4)
Female	20 (42.6)
Age (years)	
≤39	1 (2.1)
40~59	23 (48.9)
≥60	23 (48.9)
TNM stage	
3	19 (40.4)
4	28 (59.6)
Cytological samples	
Pleural effusion	18 (38.3)
FNAC	29 (61.7)
Lung lesion	17 (36.2)
Supraclavicular lymph node	12 (25.5)
Histological samples	
Small biopsy	34 (72.3)
Core biopsy	4 (8.5)
Bronchofibroscope lung biopsy	30 (63.8)
Resection	13 (27.7)
Thoracotomy	8 (17.0)
Thoracoscopy	5 (10.7)

DNA extraction

Ten 4 μm haematoxylin and eosin-stained sections were obtained after a pathologist carefully evaluated whether the slides contained sufficient number of cancer cells for molecular analysis. Genomic DNA was extracted according to standard procedures (Qiagen, Valencia, CA, USA).

PCR-direct sequencing

The EGFR exons 18, 19, 20 and 21 were amplified by PCR. Primers used for amplification were as follows: exon 18, 5′-GAGGTGACCCTTGTCTCTGTGT-3′ (forward) and ′-CCCAAACACTCAGTGAAACAAA-3′ (reverse); exon 19, 5′-TGCCAGTTAACGTCTTCCTTCT-3′ (forward) and 5′-TGAACATTTAGGATGTGGAGAT-3′ (reverse); exon 20, 5′-ACTTCACAGCCCTGCGTAAAC-3′ (forward) and 5'- ATGGGACAGGCACTGATTTGT-3′ (reverse); and exon 21, 5′-GAGCTTCTTCCCATGATGATCT-3′ (forward) and 5′-GAAAATGCTGGCTGACCTAAAG-3′ (reverse).

The PCR reaction procedure consisted of 6 min of annealing at 95 °C, 35 cycles of 95 °C for 40 sec, 55 °C for 30 sec and 72 °C for 45 sec and a final extension at 72 °C for 7 min. The PCR amplicons were purified and subjected to bidirectional sequencing by an ABI Prism 3500 DNA Analyser (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. EGFR mutations were identified by an NCBI BLAST database.

ARMS-PCR

Amplifications were performed using an ADx EGFR mutation kit (Amoy Biotech, Xiamen, China). A total of 29 EGFR mutations in exons 18 to 21 were detected by this method. Reactions were conducted in RT PCR MX3000 (Agilent Technologies, Inc., Santa Clara, CA, USA). DNA was amplified at 95 °C for 5 min. The initial 15 cycles consisted of 95 °C for 25 sec, 4 °C for 20 sec and 72 °C for 20 sec, followed by a second round of 15 cycles of 93 °C for 25 sec and 60 °C for 35 sec and final elongation at 72 °C for 20 sec. EGFR mutations were interpreted according to the manufacturer's instructions.

Statistics

Data were analysed using SPSS 16.0 software

Table 2. Incidence of EGFR mutations in CB and FFPE blocks by PCR-direct sequencing and PCR-ARMS

	N	*PCR- direct sequencing N (%)*	*ARMS-PCR N (%)*
CB			
Pleural effusion	18	7 (38.9)	8 (44.4)
FNA	29	11 (37.9)	13 (44.8)
FFPE blocks			
Small biopsy	34	10 (29.4)	12 (35.3)
Surgery	13	6 (46.2)	7 (53.8)
Total			
FFPE blocks	47	18 (38.3)	21 (44.7)
CBs	47	16 (34.0)	19 (40.4)

For abbreviations see text

package for Windows (SPSS, Inc., Chicago, IL, USA). Correlations on the categorical variables between the different groups were analysed by Fisher's exact test (x^2 test). The threshold was established at 0.05 (two-tailed).

Results

The EGFR mutation results are summarised in Table 2. The incidence rates of EGFR mutation in CBs or FFPE blocks were 38.3% (18/47) and 44.7% (21/47) or 34.0% (16/47) and 40.4% (19/47) by PCR-direct sequencing or ARMS-PCR, respectively. No statistically significant difference was observed in the detection efficiency of EGFR mutation between CB and FFPE blocks using PCR-direct sequencing (p=0.668) and ARMS-PCR (p=0.677).

The differences in the detection rates of EGFR mutation in CBs were not statistically different between pleural fluids and FNAC samples using either method (PCR-direct sequencing: pleural 7/18 vs FNAC 11/29, p=0.948; ARMS-PCR: pleural 8/18 vs FNAC 13/29, p=0.980). The EGFR mutations in FPPE blocks between the small biopsy and surgical samples were not significantly different regardless of the method used (PCR-direct sequencing: small biopsy 10/34 vs surgical samples 6/13, p=0.279; ARMS-PCR: small biopsy 12/34 vs surgical sample 7/13, p=0.246).

All EGFR mutations in 47 NSCLCs are detailed in Table 3. Mutations in exons 19 and 21 accounted for 90.9% (20/22) of the total mutations in CBs and 89.5% (17/19) of the total mutations in FFPE blocks. The distribution of EGFR mutations between the CBs and FFPE blocks was not statistically significant (p>0.05).

Table 3. The distribution of EGFR mutations in NSCLCs

EGFR mutations	*Cytological samples N (%)*	*Histological samples N (%)*	*p value*
Exon18	0	0	-
Exon19	12 (25.5)	10 (21.3)	0.626
Exon20	2 (4.2)	2 (4.2)	-
Exon21	8 (17.0)	7 (14.9)	0.778
Total	22 (46.8)	19 (40.4)	0.533

Discussion

Chemotherapy has limited effects on TNM stages III and IV NSCLC patients, and the prognosis of these patients is very poor [21]. However, a substantial proportion of these patients may benefit from EGFR-TKIs [1-9]. The effectiveness of EGFR-TKIs is related to the presence of functional EGFR mutations in NSCLCs. Therefore, evaluation of EGFR mutation status is indicated in NSCLC patients [6,7]. Most EGFR mutation studies in NSCLC patients were conducted using FFPE tissues. However, FFPE tissues are not always available because approximately 70% of stages III and IV NSCLCs are diagnosed by cytopathology, including endoscopy brushing and/or FNAC on neck lymph nodes. Successful detection of EGFR mutations in cytological specimens is pivotal for further treatment in NSCLC patients.

Our study demonstrated similar detection rates and patterns of EGFR mutations between the CB and FFPE blocks. We also noticed that the detection of EGFR mutation between pleural effusion and FNAC was not significantly different. Collectively, our results suggest that cytological samples were suitable in accurately detecting EGFR mutations in NSCLCs. Compared with surgical resections, cytology has several advantages, such as easy accessibility, low cost and minimal complications.

In summary, CB derived from cytological samples is appropriate for EGFR mutation studies, and allows easy sampling in patients with stages III/IV NSCLCs who may benefit from EGFR-TKI treatment.

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