Original Research Article

Analysis of the EGFR gene mutation in patients with non-small cell lung cancer in a Chinese population

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Abstract

Purpose: To investigate the epidermal growth factor receptor (EGFR) gene mutations and analyze their clinical significance in patients with non-small cell lung cancer (NSCLC) in Hubei province of China.
Methods: A total of 138 paraffin embedded tissues were taken from patients with NSCLC who were treated at Hubei Hospital from January 2014 to June 2015. The tissue DNA was extracted and EGFR mutation was evaluated by polymerase chain reaction (PCR) sequencing analysis of exons 18, 19, 20, and 21.
Results: The overall mutation rate of EGFR gene was 30.43 % (42/138) in 138 NSCLC patients. The mutation rates of EGFR gene at exon 18, 19, 20, 21 were 0 % （0/138), 13.8 % （19/138), 0.7 % (1/138) and 15.9 % (22/138), respectively. The mutation rate of EGFR gene was higher in female patients than that in males (62.2 % (28/45) vs 15.1 % (14/93), $p < 0.01$), and higher in non-smoking patients than in smoking ones ($p < 0.05$), but had no correlation with age in NSCLC patients ($p > 0.05$). EGFR mutation frequency in adenocarcinoma was higher than that in squamous cell carcinoma: 33.9 % (41/121) vs. 5.9 % (1/17, $p < 0.05$).
Conclusion: EGFR mutations in NSCLC patients mainly exist in exons 19 and 21, and the mutation rate of exon 21 is higher than that of exon 19, which is more commonly found in female, adenocarcinoma and non-smoking patients.

Keywords: Non-small cell lung cancer, Epidermal growth factor receptor (EGFR), Targeted therapy, Sequencing

INTRODUCTION

Lung cancer is one of the most common cancers in the world and is responsible for one third of all cancer-related death [1], NSCLC comprising 80 % of lung cancers is now a disease that can be classified into clinically relevant molecular subgroups, each with distinct clinicopathologic features and potential for targeted therapies [2]. Among these molecular subsets, the identification of EGFR tyrosine kinase domain mutations that predict sensitivity to tyrosine kinase inhibitors (TKIs) is the most prominent event [3-5]. Patients with EGFR mutations, however, are also a heterogeneous group with different sensitivity to TKIs, such as erlotinib or gefitinib. EGFR kinase domain mutations occur primarily in exons 18-21 which encode part of the tyrosine kinase domain [6-8].

The presence of EGFR mutations has also been associated with treatment in East Asian countries, female sex, bronchioloalveolar carcinoma subtype of adenocarcinoma, and

Trop J Pharm Res, August 2016; 15(8): 1637

improved survival [9]. One factor with a very strong association with these sensitivity mutations was a history of never smoking cigarettes [10]. Patients with a high likelihood of response to treatment with tyrosine kinase inhibitors can be identified by molecular analysis of lung tumor tissue to detect these sensitivity mutations. The personalization of cancer care aims to predict effective therapy regimes according to the molecular profiles of individual patients and their cancers and only patients who carried EGFR mutation can benefit from targeted therapies [4,11].

In this study, we performed multiplex amplification of exons 18-21 of EGFR using pyrosequencing to detect base changes or deletions in codons 746-753 to analyze the mutational frequency in 138 cases of lung cancer and provide theoretical basis for individualized diagnosis and treatment of lung cancer patients of Hubei province in China.

METHODS

Tissue procurement

Tumor specimens were collected from 138 patients with NSCLC at the time of surgical resection before systemic treatment. All specimens were frozen immediately and stored in liquid nitrogen until DNA was extracted. Demographic characteristics and clinical data, including age, sex, smoking status, date of diagnosis, tumor stage, treatment, progression were obtained. Our institutional review board approved this study, and written informed consent was obtained from all patients. This study was approved by the Ethics Committee of the Hubei Hospital (approval ref. no. 20140101) and followed the guidelines of Helsinki Declaration [12].

DNA extraction, PCR and direct sequencing of EGFR gene

DNA of the tumor tissue was successfully extracted by TIANamp Blood/cell/tissue genomic DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer's instruction. Four separate PCR reactions, each with the corresponding pair of primers, were used to amplify exons 18-21 of the EGFR genes. The designed primers were as follows. Exon 18: Forward: 5'-CAA GTG CCG TGT CCT GG-3'; Reverse: 5'-AAA TGC CTT TGG TCT GTG AA-3', Exon 19: Forward: 5'-ATA TCA GCC TTA GGT GCG G-3', Reverse: 5'-GGG AAA GAC ATA GAA AGT GAA CA-3', Exon 20: Forward: 5'-TTC ACA GCC CTG CGT AAA C-3', Reverse: 5'-TTG AAT CCA AAA TAA AGG AAT GT-3'; Exon 21: Forward: 5'-TGG TCA GCA GCG GGT TAC-3', Reverse: 5'-TCA TTC ACT GTC CCA GCA AG-3'. PCR amplification was carried out on ABI 9700 PCR thermal cycler (Applied Biosystems, USA). The PCR amplification was performed with a denaturation step at 94 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 52 °C for 10 s, 72 °C for 30 s, and final extension step at 72 °C for 2 min. The PCR products were analyzed using a PyroMark Q24 system (Qiagen) according to standard protocols. The order of nucleotide dispensation was chosen based on suggestions provided by the PyroMark Assay Design Software 2.0.

Statistical analysis

The association between two categorical variables was assessed using the Chi-square test. Allele frequency was determined via direct counting and a *p*-value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Patient characteristics

There were 93 men and 45 women. Sixty six patients declared their smoking history. The clinical and demographic characteristics of the 138 NSCLC patients are shown in Table 1.

Table 1: The clinical and demographic characteristics of NSCLC patients

Variable	General data		Cases (N)	Percentage (%)
Gender	Male		93	67.4 (93/138)
	Female		45	32.6 (45/138)
Age	≤ 60 years		103	74.6 (103/138)
	> 60 years		35	25.4 (35/138)
Smoking or not	Smoking	Male	61	44.2 (61/138)
		Female	5	3.6 (5/138)
	Non-Smoking	Male	32	23.2 (32/138)
		Female	40	29.0 (40/138)

Distribution of EGFR mutations in 138 NSCLC patients

The overall mutation rate in EGFR was 30.43 % (42/138). With respect to the EGFR mutation status, we found 19 patients (13.8 %) with an in-frame deletion in exon 19, 21 patients (15.2 %) with L858R mutations and 1 patient (0.7 %) with L861Q mutation in exon 21 and 1 patient (0.7 %) with T790M mutation in exon 20 in the 138 NSCLC patients, as shown in Table 2.

Association between EGFR mutation and clinical characteristics in 138 NSCLC patients

As shown in Table 3, the mutation rate of EGFR gene had no correlation with age in NSCLC patients (30.1 % vs 31.4 %, $p > 0.05$), it was higher in female patients than that in males (62.2 % (28/45) vs 15.1% (14/93), $p < 0.01$), and higher in non-smoking patients than in smoking ones (28.1 % vs 8.2 % in males, 71.8 % vs 16.7 % in females, $p < 0.05$). EGFR mutation frequency in adenocarcinoma was higher than that in squamous cell carcinoma (33.9 % vs 5.9 %, $p < 0.05$).

As shown in Table 4, in both adenocarcinoma and squamous cell carcinoma, the mutation rate of EGFR gene was higher in female patients than that in males (65.8 % vs 15.7%, 14.3 % vs 0 %).

DISCUSSION

Gefitinib and erlotinib are orally available EGFR-TKIs frequently used to treat NSCLC patients with EGFR mutations [13,14]. EGFR proteins control essential signaling pathways that regulate cell proliferation [15]. Increased levels of EGFR gene expression are observed in NSCLC, and its expression is correlated with an adverse prognosis [16].

Table 2: Mutations status in exons 18 - 21 of EGFR in 138 NSCLC patients

Exon	Mutations type	Mutation cases	Mutation rate (%)
18	G719S	0	0 (0/41)
19	Del E746-A750	19	13.8 (19/41)
20	T790M	1	0.7 (1/41)
21	L861Q	1	0.7 (1/41)
	L858R	21	15.2 (21/41)
Total		42	30.43 (42/138)

Table 3: Association between EGFR mutation and clinical characteristics in 138 NSCLC patients

Clinical characteristics		Detected cases	EGFR		Mutation rate (%)	*P*-value
			Mutation	No mutation		
Age						
≤ 60 years		103	31	72	30.1	0.882
> 60 years		35	11	24	31.4	
Gender						
Male		93	14	79	15.1	0.000
Female		45	28	17	62.2	
Smoking or not						
Male	Smoking	61	5	56	8.2	0.011
	No smoking	32	9	23	28.1	
Female	Smoking	6	1	5	16.7	0.030
	No smoking	39	28	11	71.8	
Histological type						
Adenocarcinoma		121	41	80	33.9	0.039
squamous cell carcinoma		17	1	16	5.9	

Table 4: Association between EGFR mutation, gender and histologic type in 138 NSCLC patients

Gender	Adenocarcinoma (n=121)		Mutation rate (%)	Squamous cell carcinoma (n=17)		Mutation rate (%)
	Mutant-type	*Wild-type*		*Mutant-type*	*Wild-type*	
Male	13	70	15.7	0	10	0
Female	25	13	65.8	1	6	14.3

EGFR mutations have been found more frequently in non-smoking East Asian women with adenocarcinoma with NSCLC [17,18].

The ethnic and region differences in the incidence of EGFR mutations in NSCLC remain incompletely understood. In this study, we chose a population of Hubei patients to study the incidence rate of EGFR mutations. The overall mutation rate of EGFR gene was 30.43 % in Hubei NSCLC patients, consistent with data reported for other East Asian populations [19]. The mutation rates of EGFR gene at exon 18, 19, 20, 21 were 0, 13.8, 0.7 and 15.9 %, respectively. The mutation rate of EGFR gene was higher in female patients than that in males ($p < 0.01$), and higher in non-smoking patients than in smoking ones, this finding suggests that endogenous agents from female hormone-derived mutagenic metabolites may play a role in EGFR mutagenesis.

Furthermore, it has been shown that females are more susceptible to environmental carcinogens because DNA mutations were more frequently found in never-smoking female than never-smoking male lung cancer patients [20]. But the mutation rate of EGFR gene had no correlation with age in NSCLC patients ($p > 0.05$); EGFR mutation frequency in adenocarcinoma was higher than that in squamous cell carcinoma ($p < 0.05$), and the mutation rate of EGFR gene was also higher in female patients than that in males.

In our analysis, the frequency of EGFR mutations in patients who had never-smoking was 48.6 % (35 of 72 patients). The frequency of EGFR mutations has been observed to vary from 26 % to 68 % based on how the mutational analysis was performed, geography and other clinical characteristics of the patients investigated [21-23]. Shigematsu and Pham identified EGFR mutations in 51 % of those who have never smoked (85 of 166, 34 of 67 patients), and so the results similar to ours [19,24].

Clinical parameters and molecular characteristics are very useful to the therapy for lung cancer. The study demonstrate that a universally available and verifiable clinical characteristic can estimate the incidence of mutations in exons 18, 19, 20 and 21 of EGFR, and assist physicians in choosing appropriate therapy for each individual patient.

Limitation of the study

In this study, the sample size of participants was small; thus, the results may be biased. Therefore, a study using a larger sample is required.

CONCLUSION

EGFR mutations in NSCLC patients are mainly found in exons 19 and 21, and the mutation rate of exon 21 is higher than that of exon 19, which is the one more commonly found in female, adenocarcinoma and non-smoking patients.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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