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Original Article

Epidermal growth factor receptor gene mutation status of non-small cell lung cancer in Han and ethnic minorities in the Guangxi province of China

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Abstract: The epidermal growth factor receptor (EGFR) mutation status of non-small cell lung cancer (NSCLC) have been studied in Caucasian and Asia, but the status in minority populations is still unknown. This study aims to investigate the status of EGFR mutation in the Chinese population focusing on the difference between Han and ethnic minorities in the Guangxi region of China. All the specimens were collected from formalin fixed paraffin embedded tissue section or fresh tissue. The EGFR mutation was assessed by amplification refractory mutation system-polymerase chain reaction. Chi-square test or Fisher's exact test were used to compare any difference. The overall rate of EGFR mutation was 39.4%. The rate on Han, Zhuang, Yao, Mulao and other minorities were 38.3%, 43.3%, 27.8%, 66.7% and 33.3%, respectively. The rate of EGFR mutation show no statistical difference on the different races studied ($P = 0.211$). However, there were 6 cases seen in 9 Mulao patients in this cohort. The EGFR mutation was more frequent on older (>65 years) males of Zhuang origin but no significant difference between age and EGFR mutation on gender was evident in the Han population. The EGFR mutation subtypes show no significant distribution difference on Han and Zhuang populations ($P = 0.088$). These multi-ethnic comparative data from clinical routine tests in Guangxi, China will contribution to the knowledge of clinical characteristics and race factors associated with EGFR mutational status in NSCLC.

Keywords: Epidermal growth factor receptor, mutation, non-small cell lung cancer, Chinese ethnic minorities

Introduction

Worldwide, lung cancer is the leading cause of cancer death in males and it has been upgraded to be the leading cause of cancer death in females in developed countries [1]. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of primary lung cancer cases [2]. According to the NSCLC guidelines published in 2015 [3] and surgery is still the first treatment selected for NSCLC, or in the case of advance lung cancer this is combined with chemoradiotherapy. However, if the prospective patient is epidermal growth factor receptor (EGFR) mutation-positive, then a tyrosine kinase inhibitor (TKI) is the first choice for postoperative adjuvant therapy even if sensitivity is detected during the chemotherapy period.

However, there are varying EGFR mutation frequency distributions in the different regions of the Guangxi Province. Activating EGFR mutations range from 31% to 66% in the Asian populations [4-6] and 7.5% to 40% in Caucasians [7, 8]. The proximate cause of the difference maybe gene polymorphisms in the different areas as well as their presence in different races. In previous studies on Asian patients, mostly adenocarcinoma patients were studied, and no analyses were performed on the different nationalities. When considering the multiracial characteristic of the Chinese population, it is important to study the mutation status in the different nationalities. This study was therefore designed to investigate the relationship between mutation status and the associated clinical characteristics among the various Chinese nationalities, such as Han and Zhuang which are the largest and second largest races in China. Every minority lives in their own area but the Han ethnic group has the widest distribution. In this study we performed a retrospec-

Table 1. The frequency of the EGFR mutation

Subtypes	N	Percent (%)	% of mutated cases
Wild type	644	60.6	
19-del	214	20.2	51.2
L858R	168	15.8	40.2
20-ins	2	0.2	0.5
T790M	2	0.2	0.5
G719X	3	0.3	0.7
L861Q	4	0.4	1.0
19-del/L858R	4	0.4	1.0
19-del/T790M	3	0.3	0.7
19-del/20-ins	1	0.1	0.2
19-del/G719X	2	0.2	0.5
L858R/T790M	3	0.3	0.7
L858R/G719X	1	0.1	0.2
L858R/S768I	1	0.1	0.2
L858R/L861Q/S768I	2	0.2	0.5
T790M/G719X	1	0.1	0.2
G719X/S768I	2	0.2	0.5
L861Q/S768I	5	0.5	1.2
Total	1062	100.0	39.4

tive analysis where NSCLCs were tested for EGFR mutation status in the daily clinical routine tests performed in the Central Laboratory of the Guangxi Tumor Hospital.

Patients and methods

Clinical samples

This study was approved by the local ethics committee in Affiliated Tumor Hospital of Guangxi Medical University, China. 1062 lung cancer patients were collected consecutively from August 2010 to April 2015. The median age was 57 years and ranged from 19 to 86 years. (male 678, female 384; 809 with lung adenocarcinoma, 140 with squamous cell lung carcinoma, 56 with adenosquamous carcinoma and 57 others who could not be classified into these 3 groups). All the patients were tested for the presence of EGFR mutation in exons 18-21. Among the intake of patients, there are 799 Han, 224 Zhuang, 18 Yao, 9 Mulao and 12 cases of other smaller minorities. All these patients have been diagnosed previously by the gold standard pathologic diagnosis and have not been subjected to TKI therapy. Smoking status was classified as non-smokers (<100 lifetime cigarettes), former smokers (quit >1 year prior to diagnosis), or current smokers (still smoking, or quit <1 year prior to diagnosis).

DNA preparation

Genomic DNA was extracted from fresh tissue or formalin fixed paraffin embedded (FFPE) tissue by using TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China) according to the manufacturer's instructions.

EGFR mutation analysis

In this study, we used the AmoyDx EGFR Mutations Detection Kit (Amoy Diagnostics, Xiamen, Fujian, China) by Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) to detect the EGFR mutations that included 29 hotspots mutation in exons 18, 19, 20 and 21. Positive quality controls and negative controls were included with each sample test performed. All mutations were detected by Light Cycler 480 II (Roche diagnostics) real time PCR system and data were analyzed by LightCycler Adapt software (LightCycler 480 Software, v1.5). The PCR program consisted of three stages. The first stage, 95°C for 5 minutes; the second stage, 95°C 25 seconds, 64°C 20 seconds, 72°C 20 seconds, 15 cycles; the third stage, 93°C 25 seconds, 60°C 35 seconds, 72°C 20 seconds, 31 cycles. FAM and HEX signals were collected during the third stage at 60°C. Determine the sample test results base on the different mutations Ct value. All above assays followed the manufacturer's protocol.

Statistical analysis

The comparison in different groups or the differences in a group were performed using the chi-square test or Fisher's exact test as needed. A two-sided *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

Frequency of EGFR mutations and clinical characteristics

The distributions of the 1062 patients' EGFR mutations are shown in **Table 1**. Among the 1062 patients, there were 418 cases of EGFR mutations and the overall mutation rate was

Table 2. Clinical characteristics in association to the EGFR mutation status

	N total	Wild type N (%)	EGFR mutation N (%)	*p*-value
Age (y)				
≤65	842	515 (61.2)	327 (38.8)	0.494
>65	220	129 (58.6)	91 (41.4)	
Gender				
Male	678	468 (69.0)	210 (31.0)	0.000
Female	384	176 (45.8)	208 (54.2)	
Age and Gender				
Male				
≤65 years	515	357 (69.3)	157 (30.5)	0.669
>65 years	164	111 (67.7)	53 (32.3)	
Female				
≤65 years	328	158 (48.2)	170 (51.8)	0.026
>65 years	56	18 (32.1)	38 (67.9)	
Histology				
ADC	808	429 (53.1)	379 (46.9)	0.000
ADSC	56	39 (69.6)	17 (30.4)	
SQC	140	124 (88.6)	16 (11.4)	
Smoking status				
Smoker	345	259 (75.1)	86 (24.9)	0.000
Former smoker	98	70 (71.4)	28 (28.6)	
Never smoker	619	315 (50.9)	304 (49.1)	

ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; SQC, squamous cell lung carcinoma.

Table 3. Frequency of EGFR mutation on different racial groups

	Han (%)	Zhuang (%)	Yao (%)	Mulao (%)	Others (%)
Wild type	493 (61.7)	127 (56.7)	13 (72.2)	3 (33.3)	8 (66.7)
19-del	151 (18.9)	54 (24.1)	4 (22.2)	3 (33.3)	2 (16.7)
L858R	132 (16.5)	31 (13.8)	1 (5.6)	2 (22.2)	2 (16.7)
20-ins	1 (0.1)	0 (0.0)	0	1 (11.1)	0
T790M	2 (0.3)	0 (0.0)	0	0	0
G719X	3 (0.4)	0 (0.0)	0	0	0
L861Q	3 (0.4)	1 (0.4)	0	0	0
19-del/L858R	1 (0.1)	3 (0.1)	0	0	0
19-del/T790M	2 (0.3)	1 (0.4)	0	0	0
19-del/20-ins	1 (0.1)	0 (0.0)	0	0	0
19-del/G719X	1 (0.1)	1 (0.4)	0	0	0
L858R/T790M	1 (0.1)	2 (0.9)	0	0	0
L858R/G719X	1 (0.1)	0 (0.0)	0	0	0
L858R/S768I	1 (0.1)	0 (0.0)	0	0	0
L858R/L861Q/S768I	0 (0.0)	2 (0.9)	0	0	0
T790M/G719X	1 (0.1)	0 (0.0)	0	0	0
G719X/S768I	1 (0.1)	1 (0.4)	0	0	0
L861Q/S768I	4 (0.5)	1 (0.4)	0	0	0
Total	799 (100)	224 (100)	18 (100)	9 (100)	12 (100)

39.4%. The rate of EGFR mutation was significantly different depending on the gender ($P<0.001$), histological typing ($P<0.001$) and smoking status ($P<0.001$; **Table 2**). A pairwise comparison showed no difference between the rate of EGFR mutation and current as well as former smokers ($P = 0.467$). However, non-smokers showed a significantly higher rate when compared to current and former smokers ($P<0.001$).

Frequency of EGFR mutations and the clinical characteristics in the different races

The EGFR mutation rates of Han, Zhuang, Yao, Mulao and other races were 38.3%, 43.3%, 27.8%, 66.7% and 33.3%, respectively (**Table 3**).

The rate of EGFR mutations showed no statistical difference between different racial groups ($P = 0.211$). The rate of EGFR mutation significantly different in the two genders ($P<0.001$), histological typing ($P<0.001$) and smoking status ($P<0.001$) on the Han and Zhuang populations (**Tables 4**, **5**). From the data shown in **Table 6**, we can demonstrate that the clinical characteristics of age, gender and histological subtypes show no significant dif-

Table 4. Clinical characteristics in association to the EGFR mutation status on the Han population

	N total	Wild type N (%)	EGFR mutation N (%)	*p*-value
Age (y)				
≤65	615	379 (61.6)	236 (38.4)	0.936
>65	184	114 (62.0)	70 (38.0)	
Gender				
Male	515	359 (69.7)	156 (30.3)	0.000
Female	284	134 (47.2)	150 (52.8)	
Age and Gender				
Male				
≤65 years	381	263 (69.0)	118 (31.0)	0.571
>65 years	134	96 (71.6)	38 (28.4)	
Female				
≤65 years	234	116 (49.6)	118 (50.4)	0.081
>65 years	50	18 (36.0)	32 (64.0)	
Histology				
ADC	600	319 (53.2)	281 (46.8)	0.000
ADSC	43	33 (76.7)	10 (23.3)	
SQC	112	100 (89.3)	12 (10.7)	
Smoking status				
Smoker	275	204 (74.2)	71 (25.8)	0.000
Former smoker	69	51 (74.0)	18 (26.0)	
Never smoker	455	238 (52.3)	217 (47.7)	

ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; SQC, squamous cell lung carcinoma.

Table 5. Clinical characteristics in association to the EGFR mutation status on the Zhuang population

	N total	Wild type N (%)	EGFR mutation N (%)	*p*-value
Age (y)				
≤65	192	115 (59.9)	77 (40.1)	0.018
>65	32	12 (37.5)	20 (62.5)	
Gender				
Male	136	93 (68.4)	43 (31.6)	0.000
Female	88	34 (38.6)	54 (61.4)	
Age and Gender				
Male				
≤65 years	110	81 (73.6)	29 (26.4)	0.007
>65 years	26	12 (46.2)	14 (53.8)	
Female				
≤65 years	82	34 (41.5)	48 (58.5)	0.078
>65 years	6	0 (0.00)	6 (100.0)	
Histological types				
ADC	179	95 (53.1)	84 (46.9)	0.015
ADSC	11	5 (45.5)	6 (54.5)	
SQC	23	19 (82.6)	4 (17.4)	
Smoking status				
Smoker	58	46 (79.3)	12 (20.7)	0.000
Former smoker	26	17 (65.4)	9 (34.6)	
Never smoker	140	64 (45.7)	76 (54.3)	

ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; SQC, squamous cell lung carcinoma.

ference between the Han and Zhuang populations. But there was a statistical difference between Han and Zhuang on smoking status (P = 0.049). However, the rate of EGFR mutation showed no significant difference between current and former smokers on Han and Zhuang (P = 0.964, P = 0.173, respectively). Only the current smokers showed a significantly lower rate when compared to the non-smokers (P<0.001, P<0.001, respectively) on Han and Zhuang populations. However, there was a marked significant difference between former smokers compared to the non-smokers (P<0.001) in Han, but no difference in Zhuang (P = 0.065). This comparison data between any two means are not shown in the tables.

Characteristics of the EGFR mutation subtypes

In the EGFR mutation subgroups, we can find that 19-del and L858R were the most popular mutations in those selected cases. The corresponding rates in the EGFR mutated cases were 51.2% and 40.2%, respectively. The frequency of 19-del was significantly more than the L858R mutation (P = 0.001). The frequency of 19-del also showed a significantly higher rate than L858R mutation (P = 0.001) on Zhuang but not significantly difference on Han (P = 0.123; **Table 7**). Beyond that, we can find a number of non-single-site mutations in this study,

Table 6. The comparison between the Han and Zhuang populations on the clinical characteristics associated with EGFR mutations

	Han N (%)	Zhuang N (%)	*p*-value
Age (y)			
≤65	236 (38.4)	77 (40.1)	0.642
>65	70 (38.0)	20 (62.5)	
Gender			
Male	156 (30.3)	43 (31.6)	0.254
Female	150 (52.8)	54 (61.4)	
Age and Gender			
Male			
≤65 years	118 (31.0)	29 (26.4)	0.279
>65 years	38 (28.4)	14 (53.8)	
Female			
≤65 years	118 (50.4)	48 (58.5)	0.098
>65 years	32 (64.0)	6 (100.0)	
Histological types			
ADC	281 (46.8)	84 (46.9)	0.443
ADSC	10 (23.3)	6 (54.5)	
SQC	12 (10.7)	4 (17.4)	
Smoking status			
Smoker	71 (25.8)	12 (20.7)	0.049
Former smoker	18 (26.0)	9 (34.6)	
Never smoker	217 (47.7)	76 (54.3)	

ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; SQC, squamous cell lung carcinoma.

Table 7. The distribution of EGFR mutation subtypes in the Han and Zhuang populations

	Han N (%)	Zhuang N (%)	*p*-value
19-del	151 (18.9)	54 (24.1)	0.088
L858R	132 (16.5)	31 (13.8)	
Others	23 (2.9)	12 (5.4)	
p-value*	0.123	0.001	

*This is the comparison distribution of 19-del and L858R in Han and Zhuang respectively.

such as the two sensitive site mutations 19-del coupled with L858R or the sensitive combined resistant mutation 19-del couple with T790M. All of the EGFR mutation subtypes have no distribution difference between the Han and Zhuang populations (**Table 7**).

Discussion

In the 1062 lung cancer patients from the Affiliated Tumor Hospital of Guangxi Medical University, we could demonstrate an overall rate of 39.4% EGFR mutations. The findings that EGFR mutation frequently occurred in females, non-smokers, adenocarcinoma and NSCLC patients were in accordance with other previous studies on different populations [4-7]. With regards to the females, the finding that the higher rate of EGFR mutation at an older age (≥65 years) when compared to a younger age group (<65 years) was also accordance with the study in a cohort of Caucasians [7]. 19-del was the most frequently observed mutation followed by L858R, and this characteristic was in line with previous studies in a cohort of Japanese, Chinese and Caucasian populations [4, 5, 7].

In this study, we conducted a comparison between different ethnic groups in China. We can conclude that there was no significant statistical difference on the different racial groups (Han, Zhuang, Yao, Mulao and other minorities). However, the EGFR mutation rate seen in a national minority, Mulao, with 9 cases where 6 harbored EGFR mutations (3 19-del, 2 L858R, 1 20-ins), was 66.7% and this was much higher than any other ethnic groups in previous studies [9-15] except Gao et al. [5]. The majority of the Mulao population lived in Luocheng Mulao Autonomous County. Therefore, it is possible that this region and this race are the reasons leading to differences seen.

In this study, we found the relationship between EGFR mutation and age, gender and histological subtypes are not different between the Han and Zhuang populations. Gahr et al. reported that the rate of advanced age (>65 years) EGFR mutation were significantly higher than in younger females [7]. In this study, we can demonstrate that the older patients (>65 years) have a higher rate of EGFR mutation in Zhuang. In addition, the older age group (>65 years) have a higher mutation rate in male Zhuang but there was no age difference in the Han.

The distribution of EGFR mutation subtypes showed no statistical difference in the different ethnic backgrounds in this study. 19-del and L858R were still the most popular mutations and the distribution of the rate was no significantly different between Han and Zhuang. In

previous studies, it was demonstrated that 19-del was the most frequently occurring mutation followed by L858R [4, 7, 16]. Marchetti A et al. reported that L858R was the most frequent mutation in their study [17]. However, when the data relating to the Han and Zhuang were analyzed separately, in this study we find that 19-del have a significantly higher rate than L858R in Zhuang but is not significantly difference in Han populations.

In previous studies, we can see few co-existing mutations. Tanaka et al. have a study in a cohort of Japanese people and found 7 cases (0.6%) harbored co-existing mutations [4]. 25 cases (2.4%) harbored co-existing mutations in this study (**Table 1**). However, the clinical benefit of the patients that harbored co-existing mutations remains to be determined.

In summary, the result of our general data analysis is mostly in accordance with other researchers. However, when we divided data according to different races, there were some intriguing differences between the Han and Zhuang Chinese populations. EGFR mutation was more frequent in an older age group (>65 years) when compared to a younger age group (<65 years) among females in the Zhuang but no difference was seen in the Han population. The subtypes of EGFR mutations 19-del showed a significantly higher rate than L858R but no significant difference was seen in the Han population.

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Disclosure of conflict of interest

None.

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