MUTATION ANALYSIS OF THE EGFR GENE IN PATIENTS WITH NON-SMALL CELL LUNG CANCER IN XINJIANG

Yi Shi*, Xue-lian Pang, Zhi-ping Ma, Wen-li Cui, Wei Zhang, Yu-qing Ma
Pathology, First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang Uygur Autonomous Region, China

ABSTRACT

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase (RTK) that links extracellular signals to control of cell survival, growth, proliferation, and differentiation. EGFR has been a therapeutic target for human malignancies, due to its frequent hyperactivation; therefore, it is necessary to investigate the characteristics of EGFR mutation and identify patients who are likely to benefit from therapeutics targeting specific EGFR mutations. In this study, we examined 766 non-small cell lung cancer (NSCLC) patients (675 tissue, 83 thoracic water precipitation, and 8 plasma samples) tested in the pathology department of the First Affiliated Hospital of Xinjiang Medical University from 2013 to 2017 using ARMS-PCR. The correlation between EGFR mutations and clinical-pathological features was further explored. Subgroup analyses according to ethnicity, histological type, sample type, and tumor grade were performed. These subgroup analyses showed the mutation rates in tumor tissue, thoracic water precipitation, and plasma samples were 30.5%, 37.3%, and 50.0% respectively. We found female ($p<0.0001$), non-smoker ($p<0.001$), adenocarcinoma ($p<0.0001$), and tissue specimens (tobacco use) were associated with a higher EGFR mutation rate. The most common mutations were exon 19 deletions (47.30%) and an L858R point (42.32%) mutation. We did not find any differences in EGFR mutations within ethnic groups. In addition, we did not find differences in common mutations and rare sensitive mutations in terms of survival rate after treatment with targeted therapies.

Keywords: *EGFR, ARMS-PCR, RTK, lung cancer.*

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide[1]. Platinum-based chemotherapies remain the main treatment choice for advanced non-small-cell lung cancer (NSCLC)[2]. However, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy has recently achieved promising successes in NSCLC patients harboring EGFR active mutations[3-5], significantly prolonging patient survival. Therefore, it is of great importance to determine the frequency of EGFR mutations in NSCLC patients. EGFR, as a driving gene, plays an important role in the treatment of advanced lung cancer, and EGFR-TKIs significantly prolong the progression-free survival (PFS) of patients with advanced NSCLC who are positive for EGFR mutations[6]; these have become a first-line standard treatment for patients with advanced EGFR mutant-positive NSCLC. For early NSCLC patients, there is still a high risk of disease recurrence after radical resection[7-9].

A large proportion of patients end up dying from a recurrence of lung cancer[10, 11]. With the rapid development of targeted therapy, more and more studies have begun to explore the application of EGFR-TKIs in the adjuvant therapy of early NSCLC following operations[12-14]. Research has shown

that EGFR patients with mutations who received EGFR-TKI targeted therapy after radical surgery for lung cancer and after completing adjuvant chemotherapy were less able to reduce the risk of postoperative recurrence than those who did not take EGFR-TKIs; there was also a trend in terms of extending overall survival (OS) (41.6 months vs 32. 6 months, P= 0.76)[15, 16]. EGFR has a mutation rate of about 30% in late-Asian NSCLC patients, but the mutation rate can be as high as 60% in patients who do not smoke, women, and pathological types of adenocarcinomas[17-19]. Based on this background, to investigate the difference of EGFR mutation rate and mutation spectrum in patients with NSCLS is necessary[20, 21].

Materials and methods

Data collection

We collected 766 non-small cell lung cancer (NSCLC) specimens from the list system of the pathology department of First Affiliated Hospital of Xinjiang Medical University from 2013 to 2017, including 675 tissue samples, 83 thoracic water precipitation samples, and 8 plasma samples. Patient information was collected such as gender, age, and smoking status. Disease data included the date of first NSCLC diagnosis, histological type, AJCC stage, nodal status, and distant metastases. Standardized case report forms were used to record the data in accordance with protocol instructions.

Smoking status was assessed using two methods. First, patients were classified according to their actual smoking status (never-smoked means that the subject smoked no cigarettes during his or her entire lifetime; ex-smoker means that the subject no longer smokes; occasional smoker means that the subject smokes, but not every day; and regular smoker means that the subject smokes every day). Second, smoking patients were classified according to their tobacco consumption, in pack-years.

EGFR Mutation analysis

Tumor samples were obtained from primary or metastatic lesions and were handled and stored following the laboratories' quality control requirements. Biopsy site and technique used were recorded. Cytological samples were accepted only when histological material was unavailable. After tumor DNA extraction, EGFR mutation was analyzed at the laboratory of the First Affiliated Hospital, Xinjiang Medical University, tested by an Amplification Refractory Mutation System (ARMS)-based EGFR mutation detection kit (EGFR 18-21 exon PCR kit, Yakangbo, Beijing, China). This kit allows the detection of 32 mutations in the EGFR gene.

Statistical analyses

Statistical analysis was performed by the SPSS software program. The per-protocol analysis (PPS) set was used for all statistical analyses. Mutation prevalence and corresponding 95% confidence intervals (95% CI) were calculated using the Wilson score method. Associations between mutations and demographic and clinical characteristics were analyzed by χ^2 tests or Fisher's exact tests, as appropriate. Characteristics associated with mutations with a P-value<0.05 were included in a multivariate logistic model. All analyses were two-sided and a P-value<0.05 was considered significant. In the multivariate analysis, a P-value<0.01 was considered significant.

Results

Baseline characteristics of the study population

The clinical baseline characteristics of the 766 patients in this study are shown in Table 1. Seven clinical characteristics of patients were analyzed in this study, including age, sex, smoking, ethnicity, histological type, sample type, and tumor grade. In terms of age, according to the median, the patients were divided into three groups: <65 years, 65-74.9, and >75 years old. Because of the diversity of ethnic minorities in Xinjiang, and the study covered 10 ethnic groups of patients; therefore, the results of the study have a strong individualized guiding role in the treatment of lung cancer among ethnic minorities in Xinjiang.

Correlation between EGFR mutation and clinicopathological features

A total of 766 patients were enrolled in the study group, and the correlation between EGFR mutation and clinicopathological features is shown in Table 2. Of the 766 cases, 241 (31.5%, 241/766) were EGFR mutation-positive. Among them, 129 (46.2%, 129/279) were female, 112 (23.0%, 112/487) were male, 130 (45.6%, 130/285) were non-smoking, 111 (23.1%, 111/481) were smokers, 5 (50%, 5/10) had adenosquamous cell carcinoma (ADSC), 214 (41.9%, 214/511) had adenocarcinoma (AD), 18 (7.9%, 18/227)

had squamous cell carcinoma (SCC), and 4 (22.2%, 4/18) small cell lung cancer (SCLC).

Statistical analysis showed that the distribution of EGFR gene mutation-positive cases in terms of gender, smoking status, and histological type was statistically significant ($p<0.01$), while the distribution of age, ethnicity, sample type, and tumor grade were not statistically significant ($p>0.05$).

Baseline Characteristic			EGFR gene mutation Positive		EGFR gene mutation Negative	
		Total	Number	%	Number	%
		766	241	31.5%	525	68.5%
Age group	<65	426	144	33.8%	282	66.2%
	65–74.9	238	68	28.6%	170	71.4%
	>75	102	29	28.4%	73	71.6%
Gender	Male	487	112	23.0%	375	77.0%
	Female	279	129	46.2%	150	53.8%
Smoking	Yes	481	111	23.1%	370	76.9%
	No	285	130	45.6%	155	54.4%
Ethnicity	Han	647	203	31.4%	444	68.6%
	Uygur	61	20	32.8%	41	67.2%
	Kazak	19	5	26.3%	14	73.7%
	Hui	23	10	43.5%	13	56.5%
	Mongolian	8	1	12.5%	7	87.5%
	Kirgiz	2	0	0.0%	2	100.0%
	Manchu	2	0	0.0%	2	100.0%
	Tujia	2	0	0.0%	2	100.0%
	Tatar	1	1	100.0%	0	0.0%
	Xibe	1	1	100.0%	0	0.0%
Histology	AD	511	214	41.9%	297	58.1%
	SCC	227	18	7.9%	209	92.1%
	ADSC	10	5	50.0%	5	50.0%
	SCLC	18	4	22.2%	14	77.8%
Sample type	Tissue	675	206	30.5%	469	69.5%
	Thoracic water Precipitation	83	31	37.3%	52	62.7%
	Plasma	8	4	50.0%	4	50.0%
Tumor grade	I	108	29	26.9%	79	73.1%
	II	136	33	24.3%	103	75.7%
	III	223	73	32.7%	150	67.3%
	IV	285	92	32.3%	193	67.7%

Table 1: Baseline characteristics of the study population. *(ADSC: adenosquamous cell carcinoma; AD: adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung cancer).*

Characteristic			Positive		Negative			
	Total	Total	Number	%	Number	%	95% CI	p-value
		766	241	31.5%	525	68.5%		
Gender	Men	487	112	23.0%	375	77.0%	-1.902–0.143	0.0001**
	Women	279	129	46.2%	150	53.8%		
Smoking	Yes	481	111	23.1%	370	76.9%	-0.625–1.439	0.001**
	No	285	130	45.6%	155	54.4%		
Histology	AD	511	214	41.9%	297	58.1%	-1.029–-0.159	0.0001**
	SCC	227	18	7.9%	209	92.1%		
	ADSC	10	5	50.0%	5	50.0%		
	SCLC	18	4	22.2%	14	77.8%		
Sample type	Tissue	675	206	30.5%	469	69.5%	-0.500–0.706	0.236
	Thoracic water Precipitation	83	31	37.3%	52	62.7%		
	Blood	8	4	50.0%	4	50.0%		
Tumor grade	I	108	29	26.9%	79	73.1%	-0.360–-0.003	0.228
	II	136	33	24.3%	103	75.7%		
	III	223	73	32.7%	150	67.3%		
	IV	285	92	32.3%	193	67.7%		

Table 2: Correlation between EGFR mutation and clinicopathological features.
(ADSC: adenosquamous cell carcinoma; AD: adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung cancer).

Differences in survival between different mutation types

There were 241 EGFR mutation-positive cases among the 766 patients in this study. The mutation types were classified into eight types: L858R, 19 DEL, L861Q, G719X, S768I, 20INS, T790M, and combined mutations. The mutations L858R (42.32%, 102/241) at exon 21 and 19DEL (47.30%, 114/241) at exon 19 were found significantly more frequently than the other types of mutations. We assessed the association between different EGFR mutation types and patient survival. The results showed that different types of EGFR mutations had no significant effect on the survival of patients ($p>0.05$) (Table 3). Patients with combined mutations (median survival, 158) and G719X (median survival, 155) mutations had a longer survival time, while patients with 20 INS (median survival, 90) had a shorter survival time (Figure 1), but the difference was not significant.

The relationship between mutation types of EGFR gene and patient mortality was also studied. The results showed that 96 patients died and 81 patients were still alive, regardless of the number of lost patients. A Chi-square test showed that different mutation types of the EGFR gene had no significant effect on patient mortality ($p>0.05$) (Table 4). The Cox proportional hazard model (Figure 2) showed

that different types of EGFR did not affect the survival of patients in our study: the regression coefficient was 0.106 (p>0.05).

EGFR mutation type	Number	%	Survival median/weeks	z-value	p-value
L858R	102	42.32%	125		
19 DEL	114	47.30%	149		
L861Q	3	1.24%	146		
G719X	8	3.32%	155		
S768I	3	1.24%	Lost contact	-0.042	0.966
20 INS	5	2.07%	90		
T790M	1	0.41%	129		
Combined mutations	5	2.07%	158		
None	525		96		
Total	766				
*p<0.05 **p<0.01					

Table 3: Correlation analysis between EGFR gene mutation type and patient survival.
(ADSC: adenosquamous cell carcinoma; AD: adenocarcinoma; SCC: squamous cell carcinoma; SCLC: small cell lung cancer).

Discussion

Xinjiang Uyghur Autonomous Region is a provincial-level autonomous region of China. It is the largest Chinese administrative division and is home to a number of ethnic groups. Whether the difference of life reflects the difference of mechanism or genetic background has to be studied in depth and detail, especially in Xinjiang where a few ethnic minorities are numerous. It is necessary to study EGFR fusion according to local characteristics of gene incidence for future selection of inhibitors of EGFR genes. In China, the domestic study of EGFR mutations to guide individualized treatment has mostly focused on the Han ethnicity. Research on Xinjiang Uygur populations is less frequent.

In this paper, NSCLC specimens from 766 initially diagnosed NSCLC patients (675 tissue, 83 thoracic water precipitation, and 8 plasma samples), in which the Han ethnicity comprised 647 cases, and Uygur 61 cases. Basic information of patients such as sex, pathology, age, and smoking or not, the baseline level was consistent. All the 766 samples were assessed by the ARMS-PCR method; the results showed that 241 cases had EGFR mutations, for a mutation rate of 31.5%. Of these, 203 cases were Han, i.e., with a mutation rate of 31.4%, and 20 cases were Uygur, with a mutation rate of 32.8%; there were no statistical differences between rates for these ethnicities. EGFR mutation hotspots are mainly concentrated on exon 19th and 21st[22], The EGFR mutations detected in this paper were mainly the 19 DEL and 21 L858R mutations. Some reports have shown that the EGFR mutation rate of lung cancer has ethnic differences. In Japan, EGFR mutations in 94 patients with NSCLC were studied and analyzed[23]; the total mutation rate was found to be 33%, and the exon 19-missing and 21 exon L858R were the mutations detected. Another study in Europe on EGFR mutations in 162 patients with NSCLC showed a mutation rate of EGFR of 24.7%. The mutation rate in Chinese patients with NSCLC

Item	Mark	Molecular diagnosis							Total	CS	p
		19 DEL	20 INS	CM	G719X	L858R	L861Q	T790M			
D	0	41 (51.90%)	2 (66.67%)	1 (33.33%)	2 (28.57%)	48 (59.26%)	1 (33.33%)	1 (100.00%)	96 (54.24%)	4.942	0.551
	1	38 (48.10%)	1 (33.33%)	2 (66.67%)	5 (71.43%)	33 (40.74%)	2 (66.67%)	0 (0.00%)	81 (45.76%)		
Total		79	3	3	7	81	3	1	177		
* p<0.05 ** p<0.01											

Table 4: Chi-square test for EGFR mutation type vs. mortality.
(CM: Combined mutations; D: Death or not; CS: Chi-squared statistic).

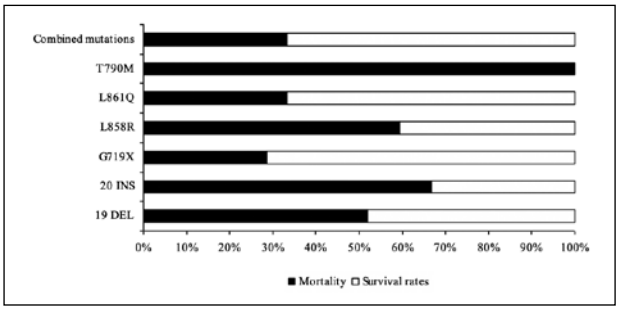

Figure 1: Survival function curve.

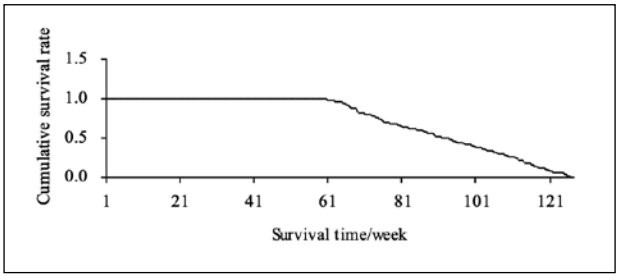

Figure 2: Survival function curve.

was higher than that in Europe and the United States[24], but similar to the rest of Asia and Japan.

Lung cancer in the Uygur population in Xinjiang has its own pathogenic characteristics. Shenhongli and others in 2013 noted that in the case of male and Han EGFR mutations[25], the Uygur mutation rate was 10.8%. With a Han mutation rate of 44%. Guo and others[26] detected 76 cases of Uygur NSCLC in Xinjiang in 2014; from their research, the mutation rate of EGFR was 15.79%. In our study, the mutation rate of EGFR in the Uygur population was much higher compared with previous research, but the mutation rate was still low compared with Han. Uygur belongs to the Caucasian race, but Han belongs to the Mongolian race. In addition, the two ethnic groups differ in terms of their social culture, living areas, and lifestyle. However, molecular genetics and epidemiological investigations are still needed for further verification.

All in all, this paper compares Han and Uygur NSCLC. In the case of EGFR mutations, it is concluded that the mutation rate of EGFR in Han was a little lower than that of Uygur, but there was no statistical difference; the distribution of age, ethnicity, sample type, and tumor grade were not statistically significant ($P>0.05$), while the distribution of EGFR gene mutation-positive cases in patients with gender, smoking status, and histological type was statistically significant ($P<0.05$). In this study, the specimens of EGFR mutations in NSCLC were collected from the list system of the pathology department of First Affiliated Hospital of Xinjiang Medical University from 2013 to 2017. There were no other criteria for the selection of samples, and there were only 61 Uygur cases in the total specimens, so there may be some limits of the study that may affect its outcome. We need more detailed research to reveal the differences of races in Xinjiang. Identifying which patients may benefit from the research into EGFR mutations is our final goal.

Conclusion

In conclusion, our study characterized the distributions of EGFR mutations in NSCLC patients in Xinjiang and investigated the relevance of gender, smoking status, histology type, sample type, tumor grade, race, and mutation type. Statistical analysis showed that gender, smoking status, and histological type significantly affected the distribution of EGFR gene mutation-positive cases ($P<0.01$). Although these outcomes have some statistical significance, they cannot perfectly elucidate the EGFR-mediated molecular basis of tumorigenesis, development, and therapeutic resistance and identify potential therapeutic targets. More extensive studies are therefore needed.

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Corresponding Author:
Yi Shi
Email: her78e@163.com
(China)