RESEARCH ARTICLE

Differences in Epidermal Growth Factor Receptor Gene Mutations and Relationship with Clinicopathological Features in NSCLC Between Uygur and Han Ethnic Groups

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Abstract

Objective: To investigate differences in mutations of epidermal growth factor receptor (EGFR) gene and relationships with clinicopathological features in patients with non-small cell lung cancer (NSCLC) between Uygur and Han ethnic groups. Methods: The Scorpions amplification refractory mutation system (Scorpions ARMS) was used to measure mutations in exons 18, 19, 20 and 21 of the EGFR gene in paraffin-embedded tumor tissue from NSCLC cases, and statistical analysis was performed to investigate links with clinicopathological features in different histological types of NSCLC. Results: Results from ARMS testing showed EGFR mutations in tumor tissues from six (6) of 50 NSCLC patients of Uygur ethnic group, with a positive rate of 12.0%; four of them (4) had exon 19 deletion in EGFR, and two (2) had L858R point mutation in exon 21 of EGFR. Statistically significant difference was noted in EGFR genetic mutation between adenocarcinoma and non-adenocarcinoma ($P < 0.05$), but no differences with gender, age group, smoking status, or stage ($P > 0.05$). EGFR mutations were detected in tumor tissues from 27 of 49 NSCLC patients of Han ethnic group , with a positive rate of 55.1%; 19 of them had exon 19 deletions, seven (7) had L858R point mutations in exon 21 of EGFR and one (1) had mutations in both exon 18 G719X and exon 20 T790M of EGFR. Statistically significant differences were noted in EGFR genetic mutations between genders and between adenocarcinoma and non-adenocarcinoma ($P<0.05$), but not with age group, smoking status, or stage ($P > 0.05$). Conclusion: Statistically significant differences were noted in the positive rates of EGFR genetic mutations in NSCLC patients between Uygur and Han ethnic groups, with lower positive rates for the Uygur cases.

Keywords: Non-small cell lung cancer - epidermal growth factor receptor - genetic mutation

Asian Pacific J Cancer Prev, **14 (5)**, 2879-2883

Introduction

Lung cancer is the most lethal malignant tumor worldwide, and is associated with extremely poor prognosis. Its 5-year overall survival is 15% in the United States of America (US), and as low as 10% in China. In addition, incidence of lung cancer is still on the rise in most countries. In 2010, an estimated population of 222,500 patients were newly diagnosed with lung cancer and bronchiolar carcinoma (116,750 males and 105,770 females), with 157,300 deaths (86,200 males and 71,100 females) (Jemal et al., 2010). Its treatment also gains much attention, and surgery, radiotherapy and chemotherapy have always been the mainstay in the treatment of non-small cell lung cancer (NSCLC). However, since traditional therapies are not specific, substantial side effects are resulted in the patients while achieving efficacy. Therefore, with ever-increasing research into pathogenesis and bio-behaviors of the tumor, more attention is transferred to molecular targeting therapy, which is characterized by better specificity and mild adverse effects. In the past few years, much investigation has focused on targets including EGFR, K-RAS and VEGF in the field of molecular targeting therapy; and a good number of molecular targeting agents against these targets have emerged. During these years, molecular targeting therapies against EGFR have gained increasing reputation in the treatment of NSCLC. Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) has high selectivity for populations. Multiple clinical studies (Pao et al., 2004; Marchetti et al., 2005; Shigematsu et al., 2005). suggested female Asian patients with adenocarcinoma who had never smoked had superior efficacy with Gefitinib and Erlotinib, particularly those with bronchioloalveolar carcinoma (BAC). By selection of eligible population, efficacy was improved by more than 40% with TKIs agents. Over these years, selection of EGFR-TKIs benefiting population based on molecular marker stratification has become an important part of optimized EGFR-TKIs therapies. Lynch et al. (2004) and

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Paez et al. (2004). first reported that mutation in tyrosine kinase encoding region of EGFR gene was an essential prerequisite for positive response to targeting agents in NSCLC. Study results demonstrated that EGFR tyrosine kinase inhibitor Gefitinib (Brand name Iressa®) achieved a response rate of more than 80% in multant tumors , but was basically inefficacious in wild-type tumors without mutations. These results were confirmed by scientists from other countries after they were published. Therefore, guiding EGFR-TKIs therapy with EGFR gene status as predictive molecular marker is an important and practical strategy. It opens a new path to NSCLC therapy for obtaining a preliminary understanding of the sensitive molecular mechanism of NSCLC against Gefitinib or Erlotinib and providing effective predictive marker for efficacy evaluation.

According to Chinese literatures, EGFR gene mutations vastly varied from region to region. For example, exon 21 mutation predominated in Taiwan, and exon 19 in Guangdong region, but no significant difference was noted between these two exons in Beijing (Huang et al., 2004; Mu et al., 2005; Qin et al., 2005). Through these studies, it was noticeable that studies were mostly from Europe and East Asia, but rarely from Middle East and Middle Asia. China is vast in territory and consisted of many ethnic groups. In Xinjiang, which is approximately one sixth of the territory and inhabited by minorities, the incidence of lung cancer is increasing year by year. Limited clinical experience suggests NSCLC patients of Uygur ethnic group are less responsive to TKIs compared to patients of Han ethnic group, but there are no further detailed clinical data and genetic mutation information. To further understand EGFR mutation status in Uygur and Han ethnic groups of NSCLC patients, we have conducted this study to analyze the differences in mutations of epidermal growth factor receptor gene and their relationships with clinicopathological features in non-small cell lung cancer between Uygur and Han ethnic groups.

Materials and Methods

Materials Samples were obtained from 50 patients of Uygur ethnic group with NSCLC confirmed by pathology after surgery or bronchial or lung biopsy from 2003 to 2012 in the Cancer Hospital affiliated to Xinjiang Medical University. These included 39 males and 11 females; age ranged from 21 to 75 years, with a median age of 56.6 years; 19 had history of smoking. According to the UICC TNM staging criteria 1997, the disease was rated stage I in 12 patients, stage II in 14 patients, stage III in 23 patients, and stage IV in one (1) patient. Tissue typing was performed according to WHO histology criteria for lung cancer with squamous carcinoma in 23 patients, adenocarcinoma in 18 patients, large cell cancer in three (3) patients, carcinoid in four (4) patients, and mucoepidermoid carcinoma in two (2) patients. Samples were obtained from 49 NSCLC patients of Han ethnic group, including 29 males and 20 females. Age ranged from 33 to 77 years, with a median age of 57.6 years; 24 patients had history of smoking. According to the UICC TNM staging criteria 1997, the disease was rated stage I in four (4) patients, stage II in two (2) patients, stage III in 12 patients, and stage IV in 31 patients. Tissue typing was performed according to WHO histological classification criteria for lung cancer with squamous carcinoma nine (9) patients, adenocarcinoma in 36 patients, large cell cancer in two (2) patients, and poorly differentiated cancer in two (2) patients.

In the meantime, Uygur and Han ethnic groups were compared, and were found comparable in terms of age, gender, TNM staging and pathological types ($p > 0.05$) without significant difference. Basic characteristics of these patients could be found in table 9:

Sampling All pathological samples were fixed in 4% neutral formalin and paraffin-embedded, and underwent conventional HE staining. Tissue sections with > 80% tumor tissue slice were selected.

Scorpions amplification refractory mutation system (Scorpions ARMS) was used to measure mutations in exons 18, 19, 20 and 21 of EGFR gene in paraffin-embedded NSCLC tissue.

Statistis All clinicopathological data, including gender, age, smoking history, pTNM staging, histological typing and EGFR genetic mutation results were entered into a computer, and tested with Chi-square test using SPSS 15.0 software. $P < 0.05$ indicated statistically significant difference.

Results

Results from EGFR genetic mutation testing Overall positive rate of EGFR mutation was 12.0% (6/50) in 50 NSCLC patients of Uygur ethnic group; four of them (4)

Table 1. EGFR Genetic Mutation Rates in NSCLC Patients of Uygur and Han Ethnic Groups

Ethnic groups	No. of patients	EGFR gene		
		No. of patients with no mutation (%)	No. of patients with mutation (%)	*P* value
Uygur ethic group	50	44(88)	6(12)	*P*=0.000
Han ethnic group	49	22 (44.9)	27(55.1)	

Table 2. Relationship Between EGFR Genetic Mutation and Clinicopathological Features in NSCLC Patients of Uygur Ethnic Group

Clinical groups	No. of patients	EGFR gene		
		No. of patients with no mutation (%)	No. of patients with mutation (%)	*P* value
Gender	50			
Male	39	35(89.7)	4(10.3)	0.475
Female	11	9(81.8)	2(18.2)	
Age	50			
≤60	24	22(91.7)	2(8.3)	0.443
>60	26	22(84.6)	4(15.4)	
History of smoking	50			
No	31	28(90.3)	3(9.7)	0.519
Yes	19	16(84.2)	3(15.8)	
pTNM staging	50			
Stage I	12	9(75.0)	3(25.0)	0.112
Stages II - IV	38	35(92.1)	3(7.9)	
Histological types	50			
Adenocarcinoma	18	13(72.2)	5(27.8)	0.01
Non-adenocarcinoma	32	31(96.9)	1(3.1)	

Table 3. Relationship Between EGFR Genetic Mutation and Clinicopathological Features in NSCLC Patients of Han Ethnic Group

Clinical groups	No. of patients	EGFR gene		
		No. of patients with no mutation (%)	No. of patients with mutation (%)	*P* value
Gender	49			
Male	29	17(58.6)	12(41.4)	0.02
Female	20	5(25)	15(75)	
Age	49			
≤60	29	14(48.3)	15(51.7)	0.567
>60	20	8(40)	12(60)	
History of smoking	49			
No	25	8(32)	17(68)	0.064
Yes	24	14(58.3)	10(41.7)	
pTNM staging	49			
Stage I	4	0(0)	4(100)	0.117
Stages II-IV	45	22(48.9)	23(51.1)	
Histological types	49			
Adenocarcinoma	36	11(30.6)	25(69.4)	0.001
Non-adenocarcinoma	13	11(84.6)	2(15.4)	

had exon 19 deletion of EGFR, and two (2) had L858R point mutation in exon 21 of EGFR. Overall positive rate of EGFR mutation was 55.1% (27/49) in 49 NSCLC patients of Han ethnic group; 19 had exon 19 deletion of EGFR, seven (7) had L858R point mutation in exon 21 of EGFR and one (1) patient had mutations in both exon-18 G719x and exon-20 T790M. Statistically significant difference was found in EGFR genetic mutation rate of NSCLC patients between Uygur and Han ethnic groups (Table 1).

Relationship between EGFR genetic mutation and clinicopathological features in NSCLC patients of Uygur ethnic group Positive rates of EGFR genetic mutation were 27.8% (5/18), 4.3% (1/23), 0% (0/3), 0% (0/4) and 0% (0/2), respectively, in patients with adenocarcinoma, squamous carcinoma, large cell cancer, carcinoid and mucoepidermoid carcinoma. Patients with adenocarcinoma had significantly higher EGFR genetic mutation rate as compared to those with non-adenocarcinoma, with statistically significant difference between them ($P < 0.05$). Analyzing the relationship between EGFR genetic mutations and clinicopathological features in NSCLC, no differences were found in EGFR genetic mutations in terms of genders, age groups, smoking statuses, and staging ($P > 0.05$) (Table 2).

Relationship between EGFR genetic mutation and clinicopathological features in NSCLC patients of Han ethnic group Positive rates of EGFR genetic mutation were 69.4% (25/36), 11.1% (1/9), 50% (1/2), and 0% (0/2), respectively, in patients with adenocarcinoma, squamous carcinoma, large cell cancer and poorly differentiated cancer. Patients with adenocarcinoma had significantly higher EGFR genetic mutation rate as compared to those with non-adenocarcinoma, with statistically significant difference between them ($P < 0.05$). Analyzing the relationship between EGFR genetic mutations and other clinicopathological features in NSCLC, no differences were noted in EGFR genetic mutations in terms of age, smoking status, or staging ($P > 0.05$) (Table 3).

Discussion

EGFR gene is located in 7p12-14 region in the short arm of chromosome 7, consisting of 28 exons. The tyrosine kinase function is encoded by exons 18 - 24. So far, more than 90% of EGFR genetic mutations have been discovered in exons 19 - 21, with the highest mutation rate in exon 19, accounting for more than 50% of overall mutations in EGFR gene (Qin et al., 2005; Tokumo et al., 2005). By competitively binding to EGFR at the highly conservative ATP binding site in EGFR structural domain, they block tyrosine residue phosphorylation and thus prevent signaling through EGFR pathway, eventuating inhibition on tumor cell proliferation, invasion, metastasis and vascularization, and promotion on tumor cell apoptosis. Results from clinical trials have suggested EGFR-TKIs are associated with high bioavailability, strong selectivity, good tolerability, mild adverse effects, and significant anti-tumor activity.

Study OPTIMAL (Xiao et al., 2011; Zhou et al., 2011).was a multi-center phase III randomly controlled study initiated by the Chinese Thoracic Oncology Group (C-TONG). For patients with advanced NSCLC carrying EGFR mutations, first-line Erlotinib therapy significantly improved progression-free survival compared to standard first-line regimen (Gemcitabine + Carboplatin, GC) (E vs. GC,13.7 months vs. 4.6 months, HR 0.164, 95% CI 0.10 - 0.26, $P < 0.0001$). Objective response rate (ORR) and tolerability were also remarkably better than Gemcitabine + Carboplatin. Results from study EURTAC (MILLER et al., 2009; Inal et al., 2012). suggested median PFSs were 9.7 and 5.2 months, respectively, with Erlotinib and standard first-line therapy for in western NSCLC patients with EGFR sensitive mutations. These results were similar to those of Asians, further illustrated the association between EGFR genetic mutations and EGFR-TKI efficacy. But ethnic group was not the definitive factor for efficacy with these agents. It was the incidence of EGFR genetic mutation in different ethnic groups that determined how much they benefited from these agents. By EGFR genetic testing, the most eligible treatment candidates could be identified among patients with advanced lung cancer and received targeting therapy, in order to improve efficacy of these agents. By using EGFR testing, response rate to TKIs could be increased to 80% or more. By now many major medical centers have been using EGFR testing to select patients for TKI therapies. In literatures, mostly reported were trial data from Asians and Europeans, but hardly any data on EGFR mutation detection in Middle Asia and Middle East populations. The Uygur ethnic group accounted for about 45% of the overall population in Xinjiang Province. And lung cancer, with sustained high prevalence, represented one of the malignant tumors that inflicted the most threat to human well-being. Niyaz et al. (1999). compared primary lung cancers among different ethnic groups in Xinjiang, and found higher constituent ratio for adenocarcinoma in Uygur and Hui ethnic groups (25% in Hui, 17.28% in Uygur, 15.61% in Han, and 11.77% in Kazak ethnic groups), and higher constituent ratio for small cell lung cancer in Han ethnic

group (18.28% in Han, 12.35% in Uygur, 5.88% in Kazak, and 12.5% in Hui ethnic groups). Such histological types distribution was different from those reported by Chinese Academy of Medical Science (405 patients) and Shanghai Chest Hospital (2013 patients), suggesting histological types distribution in lung cancer might vary due to data source, sample size, and geographic region. In a way, this finding also reflected huge impact of genetic background and environmental factors on the occurrence of lung cancer. Xinjiang is inhabited by minorities, and those ethic groups' life-styles, growing backgrounds, diets and living conditions vary from one to another. To further increase clinical data on EGFR gene, especially such data from minorities, we used tissue samples from NSCLC patients of Uygur and Han ethnic groups to measure EGF genetic mutations in China. The results showed an overall positive rate of 12.0% (6/50) in 50 NSCLC patients of Uygur ethnic group, and an overall positive rate of 55.1% (27/49) in 49 NSCLC patients of Han ethnic group. Statistically significant difference was noted in EGFR genetic mutation rate between Uygur and Han ethnic groups. Positive rates of EGFR genetic mutation were 27.8% (5/18), 4.3% (1/23), 0% (0/3), 0% (0/4) and 0% (0/2), respectively, in patients with adenocarcinoma, squamous carcinoma, large cell cancer, carcinoid tumor and mucoepidermoid carcinoma in Uygur ethnic group. Patients with adenocarcinoma had significantly higher EGFR mutation rate as compared to those with non-adenocarcinoma, with statistically significant difference between them ($P<0.05$). No differences were found in EGFR genetic mutations in terms of genders, age groups, smoking statuses, or staging. Positive rates of EGFR genetic mutation were 69.4% (25/36), 11.1% (1/9), 50% (1/2), and 0% (0/2), respectively, in patients with adenocarcinoma, squamous carcinoma, large cell cancer and poorly differentiated cancer. Female patients with NSCLC had significantly higher EGFR genetic mutation rate than male patients with NSCLC, with statistically significant difference ($P < 0.05$). Analyzing the relationship between EGFR genetic mutations and other clinicopathological features in NSCLC, no differences were noted in terms of genders, age groups, smoking statuses, or staging ($P > 0.05$).

EGFR mutation rate was lower in Caucasians (less than 10%), but higher in Asians (30% to 50%). In this study, we obtained a preliminary understanding on the EGFR mutation status in NSCLC in Uygru and Han ethnic groups of Xinjiang. Genetic mutation rate was relatively lower in Uygur than of Xinjiang than that in Han ethnic group , but similar to Caucasian population. This also explained why NSCLC patients of Uygur ethnic group had less favorable efficacy than counterparts of Han ethnic group when we selected target treatment population based on clinical features due to the absence of the knowledge on EGFR genetic status. No gender difference was seen in genetic mutation rate in NSCLC patients of Uygur ethnic group, but such difference was established in NSCLC patients of Han ethnic group. Nonetheless, the present study had a small sample size. We looked forward to conducting more studies in the future, so as to guide targeting therapy on molecular level and to provide rationale for medical centers in remote regions where EGFR genetic detection was not possible at the moment.

EGFR expression has important clinical implication in NSCLC, and studies on EGFR genetic mutation promote the development of molecular targeting therapies against lung cancer. Efficacy of targeting agents varies hugely from individual to individual, but they have unique mechanism of action, and act on tumor cells specifically without damaging normal tissue. Therefore, they are very promising in improving treatment outcome in tumors. But there are still important issues to be solved, including how to select patients who might best respond to targeting agents in a readily feasible way, how to use genetic mutation in targeting therapy and how to avoid the development of resistant mutation in targeting therapy. With gradual elucidation of tumor pathogenesis and increased understanding on the effect of EGFR mutations on tumors, molecular targeting therapy will draw more and more attention in the treatment of tumors. Therefore, by using proper genetic testing, we hope to identify eligible population for targeting therapy, and to achieve individualized therapy while reducing financial burden for the population and saving medical resource.

Acknowledgements

The author(s) declare that they have no competing interests.

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