SPANDIDOS PUBLICATIONS

MOLECULAR AND CLINICAL ONCOLOGY 3: 847-850, 2015

Epidermal growth factor receptor gene mutation status and its association with clinical characteristics and tumor markers in non-small-cell lung cancer patients in Northwest China

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Received January 16, 2015; Accepted March 24, 2015

DOI: 10.3892/mco.2015.564

Abstract. This study was conducted to investigate the mutation status of epidermal growth factor receptor (EGFR) and its association with clinical characteristics and tumor markers in non-small-cell lung cancer (NSCLC) patients from the Xinjiang Uygur Autonomous Region in China. We enrolled 51 cases of NSCLC patients who received radical surgical treatment in the First Affiliated Hospital of Xinjiang Medical University. Quantitative polymerase chain reaction was applied to detect exons 18, 19, 20 and 21 of the EGFR gene in tumor tissues. Multiple tumor markers, including carcinoembryonic antigen (CEA), were assessed preoperatively. The EGFR-positive rate was 49.02% (25/51), with a mutation rate of 8% (2/25) in exon 18, 52% (13/51) in exon 19, 40% (10/51) in exon 21 and no mutations in exon 20. The positive mutation rate in men and women was 37.5% (12/32) and 68.42%, respectively (13/19), with a statistically significantly higher rate in women (P<0.05). There were also statistically significant differences among adenocarcinoma, adenosquamous carcinoma and squamous cell carcinoma cases (P<0.05), while no statistically significant differences were observed in adenocarcinoma cases regarding degree of differentiation, lymph node metastasis and TNM stage (P>0.05). There was a statistically significant association between the EGFR gene mutation status and the preoperative serum CEA level (P<0.05). The mutation rate of the EGFR gene was 68.42% in female lung adenocarcinoma patients, which supports the application of targeted therapy in such cases. However, whether it is possible to obtain information regarding targeted therapy through measuring the level of serum CEA for NSCLC patients with unknown EGFR mutation status requires further investigation through related studies including a higher number of cases.

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Abbreviations: NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA199, carbohydrate antigen 199; CYFRA 21-1, cytokeratin-19-fragment; SCC, squamous cell carcinoma; ProGRP, progastrin-releasing peptide; AFP, α-fetoprotein

Key words: Xinjiang, non-small-cell lung cancer, epidermal growth factor receptor, targeted therapy, tumor marker

Introduction

Lung cancer is one of the most common malignant tumors in humans, with an equally high incidence in men and in women. Approximately 80% of lung cancer cases are non-small-cell lung cancer (NSCLC) (1). The majority of the patients present with advanced-stage lung cancer at diagnosis, when radical excision is no longer feasible (2) and the remaining treatment options include radiotherapy, chemotherapy and immune therapy. The rate of response to radiotherapy and chemotherapy is only 15-35%, which may not effectively improve the survival rate and life quality of the patients. Therefore, the total 5-year survival rate of lung cancer patients is only ~15% (2-4).

Over the last few years, genetic testing and targeted therapy based on epidermal growth factor receptor (EGFR) gene mutation status has attracted significant attention. Multiple clinical studies indicated that tyrosine kinase inhibitors (TKIs) have effectively extended the survival time and improved the life quality of NSCLC patients (5), with a total effectiveness rate of >70%. However, the effectiveness rate of TKI treatment for wild-type EGFR cancers is only 10-15% (6). Therefore, it is crucial to determine the mutation status of the EGFR gene in NSCLC patients prior to TKI treatment (6,7).

Tumor markers are specific molecules produced and released by tumor cells and they may be present in the tissues, body fluids and excreta of cancer patients. Tumor markers are mainly used in clinical practice to locate the primary tumor, screen high-risk populations, identify and diagnose benign and malignant tumors, determine tumor development, observe and evaluate the efficacy of tumor treatment and predict the

prognosis and recurrence of the tumor (8-10). The number of studies investigating the association of tumor markers with the EGFR gene is currently limited. In this study, multiple tumor markers were assessed preoperatively in patients with known EGFR gene mutations, to determine whether there is a correlation between EGFR mutation status and tumor markers.

Quantitative polymerase chain reaction (qPCR) was used to detect mutations of the EGFR gene in NSCLC patients who received radical surgery in our hospital and the association of mutation status with clinicopathological characteristics and tumor markers was investigated, in order to establish a pathological basis for individual targeted therapy of postoperative NSCLC patients in Xinjiang.

Patients and methods

Patients. A total of 51 NSCLC patients who received radical surgery at the Department of Thoracic Surgery of the First Affiliated Hospital of Xinjiang Medical University between April, 2013 and July, 2014, were included in this study. The patients included 32 men and 19 women (male:female ratio, 1.68:1). None of the patients underwent radiotherapy, chemotherapy or other specialized therapy preoperatively and they all signed a consent form regarding the collection of samples from surgical tumor tissues.

Tumor histology and stage. There were 40 cases of adenocarcinoma, 7 of squamous cell carcinoma (SCC), 3 of adenosquamous carcinoma and 1 case of carcinoid tumor. As regards the degree of differentiation, 6 of the cases were well-differentiated, 28 were moderately differentiated and 17 were poorly differentiated. A total of 23 cases presented with lymph node metastasis. As regards postoperative pathological classification, 12 patients had stage IA, 8 had stage IB, 6 had stage IIA, 6 had stage IIB, 16 had stage IIIA, 2 had stage IIIB and 1 had stage IV disease.

Tumor marker determination. Tumor markers, including cytokeratin-19-fragment (CYFRA21-1), carbohydrate antigen 125 (CA125) and carbohydrate antigen 19-9 (CA19-9), SCC antigen, carcinoembryonic antigen (CEA), progastrin-releasing peptide (ProGRP) and α-fetoprotein (AFP) were measured preoperatively in the serum of all the patients.

Fasting blood was collected for analysis. CA125, CA19-9 and CEA were determined by the direct chemiluminescence method using an AFP determination kit [Siemens Healthcare Diagnostics (Shanghai) Co. Ltd., Shanghai, China]. CYFRA 21-1, SCC and ProGRP were determined by chemiluminescence particles immunoassays using the ARCHITECT CYFRA 21-1 Reagent kit [Abbott Laboratories Trading (Shanghai) Co., Ltd. Shanghai, China]. The results were interpreted as follows: CYFRA 21-1, negative ≤2.08 and positive >2.08 ng/ml; CA19-9, negative ≤37 and positive >37 U/ml; CA125, negative ≤32.4 and positive >32.4 U/ml; SCC antigen, negative ≤5 ng/ml and positive >5 ng/ml; AFP, negative ≤8.1 and positive >8.1 ng/ml; and ProGRP, negative ≤63 pg/ml and positive >63 pg/ml.

DNA extraction. The test samples were paraffin-coated tumor tissues. A total of 8-10 sections (8 μm) were placed in a 1.5-ml EP tube. DNA was extracted according to the instructions manual of the QIAamp DNA FFPE Tissue kit (Qiagen, Hilden, Germany). The concentration and purity of the DNA were determined with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Ottawa, ON, Canada). The OD260/OD280 ratio of the DNA sample was 1.8±0.2 and the OD260/OD230 ratio was ≥1.7; the concentration was 20-50 ng/μl.

qPCR. A probe that specifically recognizes the mutated EGFR and reference genes was designed. Fluorescence released by the probe was detected by qPCR. The gene mutation status was then determined. Mutations in exons 18, 19, 20 and 21 of the EGFR gene were qualitatively tested by the Human EGFR Gene Mutation Detection kit (Beijing ACCB Biotech Ltd., Beijing, China). There were positive and negative controls for every measurement. The reaction was usually completed within 1.5 h. The results of each reaction well were then read according to the amplification curve and interpreted as follows: For a specific locus of mutation in the sample, if there was amplification and the Ct value was ≤35, the sample was considered to be positive; if there was no amplification and the Ct value was >38, the sample was negative; in cases with 35<Ct value ≤38, sample positivity was suspected and the measurement was repeated.

Statistical analysis. The SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) was used for data analysis. A percentage was used as an evaluation index of positive EGFR mutation status. Within-group differences were analyzed using the non-parametric independent t-test. Between-group differences were analyzed using the Chi-square test. $P<0.05$ was considered to indicate statistically significant differences.

Results

EGFR mutation status by exon and gender. The overall positive mutation rate of EGFR was 49.02% (25/51); the mutation rate of exon 18 was 8% (2/25), of exon 19 52% (13/25) and of exon 21 40% (10/25), whereas no mutations were detected in exon 20. The positive rate in men and women was 37.5% (12/32) and 68.42% (13/19), respectively. The difference between men and women was statistically significant ($P<0.05$; Table I).

EGFR mutation status by histology and stage. The positive rate in adenocarcinoma and adenosquamous carcinoma patients was 55% (22/40) and 100% (3/3), respectively. All 7 cases of SCC and the single case of carcinoid tumor were negative for EGFR mutations. The positive rate in adenocarcinoma patients was statistically significantly higher compared with that in SCC patients ($P<0.05$) (Table II). The positive rate in patients with well-differentiated tumors was 83.33% (5/6), moderately differentiated tumors 46.43% (13/28) and poorly differentiated tumors 41.18% (7/17); there were no statistically significant differences observed ($P>0.05$). The positive EGFR mutation rate in patients with and in those without lymph node metastasis was 52.17% (12/23) and 46.43% (13/28), respectively; the difference was not statistically significant ($P>0.05$). The positive rate was 41.67% (5/12) and 62.5% (5/8) in patients with stage IA and IB disease, respectively; 66.67% (4/6)

Table I. Association between gender and epidermal growth factor receptor (EGFR) gene mutation status.

Gender	Total cases (n=51)	EGFR mutations		P-value
		Negative (n=26)	Positive (n=25)	
Male	32	20	12	0.03271
Female	19	6	13	

Table II. Association between tumor histology and epidermal growth factor receptor (EGFR) gene mutation status.

Histology	Total cases (n=51)	EGFR mutations		P-value
		Negative (n=26)	Positive (%) (n=25)	
AdenoCa	40	18	22 (55.0)	0.002
SCC	7	7	0 (0.0)	
AdenoSCC	3	0	3 (100.0)	
Carcinoid tumor	1	1	0 (0.0)	

Ca, carcinoma; SCC, squamous cell carcinoma.

Table III. Association between serum tumor markers and epidermal growth factor receptor (EGFR) gene mutation status.

Markers	EGFR mutations		P-value
	Positive (n=25)	Negative (n=26)	
CYFRA 21-1			0.346
Positive	15	18	
Negative	10	8	
CA19-9			1.000
Positive	2	3	
Negative	23	23	
CA125			1.000
Positive	7	7	
Negative	18	19	
SCC antigen			0.610
Positive	2	1	
Negative	23	25	
CEA			0.025
Positive	20	13	
Negative	5	13	
AFP			0.510
Positive	0	1	
Negative	25	25	
ProGRP			0.235
Positive	0	3	
Negative	25	23	

CYFRA 21-1, cytokeratin-19-fragment; CA19-9, carbohydrate antigen 19-9; CA125, carbohydrate antigen 125; SCC, squamous cell carcinoma; CEA, carcinoembryonic antigen; AFP, α-fetoprotein; ProGRP, progastrin-releasing peptide.

and 16.67% (1/6) in patients with stage IIA and IIB disease, respectively; 50% (8/16) and 50% (1/2) in patients with stage IIIA and IIIB disease, respectively; and 100% (1/1) in stage IV, without statistically significant differences (P>0.05).

Association between EGFR mutation status and serum tumor markers. The overall positive rate of CEA was 64.71% (33/51), of CYFRA 21-1 64.71% (33/51), of CA125 27.45% (14/51), of CA19-9 9.80% (5/51), of SCC and ProGRP 5.88% (3/51 each) and of AFP 2.38% (1/51).

No significant association was observed between EGFR gene mutation and the level of preoperative serum CYFRA 21-1, CA125, CA19-9, SCC, ProGRP and AFP (all P-values >0.05). However, when the paired sample Chi-square test was used, a statistically significant association was observed between the expression of preoperative serum CEA and EGFR gene mutation status (P<0.05) (Table III).

Discussion

Lung cancer is one of most common malignant tumors. Although there have been significant advances in the comprehensive treatment of lung cancer, the 5-year survival rate and life quality of the patients remain very low (11). The traditional radiotherapy and chemotherapy are associated with significant toxicity and side effects due to the lack of specificity (1). Customized targeted therapy was a major breakthrough in the treatment of NSCLC patients and the EGFR gene is an important target (3). As one of the members of ErbB family, the EGFR gene is located on the short arm of chromosome 7 and consists of 28 exons (2). EGFR is a transmembrane glycoprotein receptor with tyrosine kinase activity and is highly expressed in 45-70% of NSCLC patients (12,13). EGFR acts on the cell signal transduction pathway, promotes the differentiation and proliferation of tumor cells, promotes tumor angiogenesis and metastasis and inhibits apoptosis (14-16). Multiple studies have proven that customized therapy significantly improved the survival rate and life quality of EGFR-positive lung cancer patients (17,18). With the improved understanding of the process of tumor pathogenesis and of the effects of EGFR gene mutation on tumor characteristics, targeted therapy has become increasingly more important, perfected and normalized (15). Molecular-targeted therapy using EGFR as the target is currently used in the clinical setting for the treatment of lung cancer (19,20).

It was reported that, in China, lung cancer patients mainly exhibit EGFR mutations in exons 19 and 21, accounting for 54.5 and 40.3% of the total mutation rate, respectively, while mutations are rare in exons 18 and 20 (15). In this study of 51 NSCLC patients, the mutation rate in exons 19 and 21 was 52 and 40%, respectively, while that in exon 18 was only 8%, which is consistent with the majority of the literature reports (2,14,16).

According to Wu *et al* (21) the mutation rate of the EGFR gene in women and men was 42.9 and 23.1%, respectively. In this study, the mutation rate in female patients was 68.42%, which was higher compared with that in male patients (37.5%), with a statistically significant difference (P<0.05) (10,18,19,21). The degree of differentiation of the tumor was an important index for assessing malignant potential and prognosis. According to this study, the mutation rate in patients with well-differentiated tumors was 83.33%, in patients with moderately differentiated tumors 46.43% and in patients with poorly differentiated tumors 41.18%, which was inconsistent with previous findings (3). This inconsistency may be attributed to the lack of well-differentiated cases in this study; therefore a larger sample size is required to confirm this result (11,22). In addition, no statistical association was observed between lymph node metastasis, TNM stage and EGFR mutation (P>0.05), which was in agreement with the findings of Li *et al* (1).

According to Xu *et al* (23) and Shoji *et al* (24), the positive mutation rate of the EGFR gene was found to be increased when the level of serum CEA was positive preoperatively. In this study, of the 51 cases, 33 were positive for CEA, including 20 EFGR-positive cases, with a mutation rate of EGFR for CEA-positive patients of 60.6% (20/33). According to the statistical analysis, there was a significant association between EGFR mutation and the level of preoperative serum CEA (P<0.05). Therefore, the level of preoperative CEA expression is likely to be an independent predictor of EGFR mutations.

In this study, the mutation rate of EGFR in female lung adenocarcinoma patients in the Xinjiang region was ~70% and the high estimated level of CEA was associated with EGFR mutation status. It remains to be elucidated whether the determination of serum CEA can replace EGFR testing for NSCLC patients by increasing sample size.

Acknowledgements

This study was supported by Returned Overseas Students to Science and Technology Activities Fund (2012-111) and the National Natural Science Foundation of China (no. 30960383). The authors would also like to express their gratitude to the laboratory staff for helping with the measurement of the tumor markers and the Pathology Department staff for assisting in the determination of EGFR gene mutation.

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