Association of EGFR mutations with low BRCA1 gene expression in non-small cell lung cancer

HUI LI, LING XIE and REN-SENG LAI

Department of Pathology, The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (TCM), Nanjing, P.R. China

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Abstract. Clinical studies suggest that the mRNA expression level of excision repair cross complementing group 1 gene (ERCC1) is associated with epidermal growth factor receptor (EGFR) mutation and breast cancer susceptibility 1 gene (BRCA1) mRNA expression in non-small cell lung cancer (NSCLC). In this study, the correlation between EGFR mutation status and ERCC1 and BRCA1 gene expression in Chinese NSCLC patients was examined. Real-time polymerase chain reaction (PCR) and direct sequencing were used to detect mRNA expression levels and EGFR mutation status, respectively in microdissected formalin-fixed paraffin-embedded non-small cell lung cancer tissues. EGFR mutations were detected in 27/103 patients (26.2%) and were found to be gender-related (P=0.001). The BRCA1 mRNA expression level was associated with histology, while there was no association with ERCC1. For the EGFR mutant-type, a high BRCA1 gene expression was detected in 2 cases (20.0%) and a low expression in 8 cases (80.0%), while for EGFR wild-type, a high BRCA1 gene expression was detected in 20 cases (43.5%) and a low expression in 26 cases (56.5%). There was no difference in the one-year survival period, according to results obtained for either the ERCC1 or BRCA1 mRNA expression levels. EGFR mutations in NSCLC samples are more likely to express low ERCC1 and BRCA1 mRNA levels. In these latter samples, a statistically significant difference was observed. However, to examine their correlation and clinical outcomes, additional studies are required.

Correspondence to: Professor Ren-Sheng Lai, Department of Pathology, The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (TCM), Nanjing 210029, P.R. China
E-mail: lairensheng@163.com

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Introduction

Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors. Platinum-based chemotherapy is the first-line treatment of advanced NSCLC. Studies showed that advanced NSCLC patients with a high expression of excision repair cross complementing group 1 gene (ERCC1) were resistant to cisplatin, resulting in the failure of chemotherapy treatment (1). By contrast, a low expression of ERCC1 mRNA levels indicated sensitivity to platinum (2). Moreover, preclinical and clinical studies have reported that breast cancer susceptibility 1 gene (BRCA1) mRNA expression was negatively correlated with cisplatin sensitivity (3,4). However, Iressa®, Tarceva® and other epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) are the most promising target treatment approach, while EGFR mutation is the indicator for the use of these drugs. Clinical studies also showed patients with EGFR mutations to have a better response to platinum (5). Additionally, recent studies have demonstrated that EGFR mutations in NSCLC samples were correlated with low ERCC1 mRNA levels (6). Therefore, a study on the correlation between EGFR mutations and ERCC1 and BRCA1 gene expression in a Chinese population was conducted.

Materials and methods

Subjects. A total of 103 Chinese patients were enrolled between March, 2007 and November, 2010. Of those, 62 were male and 41 female (median age, 41; range, 31-80 years). The patients were diagnosed based on the 2008 World Health Organization (WHO) classification. None of the patients received Iressa or chemotherapy prior to surgery. The patients were treated with platinum-based chemotherapy subsequent to surgery.

RNA extraction and real-time quantitative PCR (RT-qPCR) reaction for mRNA expression. Total RNA was isolated from paraffin-embedded NSCLC tissues using Tissue RNA kit (RNase-free FFPE kit; Qiagen, Valencia, CA, USA), after written informed consent was obtained from the participants. RT reaction (10 μl) was performed using: 1 μl gDNA, 6 μl RNA + DEPC H_2O, at 42˚C for 2 min, 0.5 μl primer (10 nmol/μl), 0.5 μl RTase and 2 μl buffer at 42˚C 30 min, then 95˚C for 5 min, carried out by SYBR-Green real-time PCR with an ABI 7900HT Fast Real-Time PCR system (Applied

Table I. Correlation between ERCC1 mRNA expression and the clinicopathological characteristics of patients with NSCLC.

Clinicopathological parameters	No. cases	High expression	Low expression	χ^2	P-value
Gender					
Male	62	34	27	1.998	0.157
Female	41	17	24		
Age (years)					
≥60	62	29	33	0.658	0.417
<60	41	22	18		
Pathological subtypes					
Adenocarcinoma	77	41	36		0.334
Squamous cell carcinoma	13	6	7		
Adenosquamous carcinoma	5	1	4		

ERCC1, excision repair cross complementing group 1 gene; NSCLC, non-small cell lung cancer.

Table II. Correlation between BRCA1 mRNA expression and the clinicopathological characteristics of patients with NSCLC.

Clinicopathological parameters	No. cases	High expression	Low expression	χ^2	P-value
Gender					
Male	36	20	16	1.244	0.265
Female	20	8	12		
Age (years)					
≥60	36	19	17	0.311	0.577
<60	20	9	11		
Pathological subtypes					
Adenocarcinoma	40	21	19		0.025
Squamous cell carcinoma	6	0	6		

BRCA1, breast cancer susceptibility 1 gene; NSCLC, non-small cell lung cancer.

Biosystems, Carlsbad, CA, USA). PCR reactions were performed using 2.5 μl SYBR-Green Master mix (Applied Biosystems), 0.25 μl primer (20 pmol/μl), 1 μl DNA (25 ng/μl), and adding DEPC H_2O to the total volume of 5 μl. Reaction without template was used as the negative control and β-actin as the endogenous control.

DNA extraction and direct sequencing for EGFR mutation status. Genomic DNA was isolated from 103 cases of paraffin-embedded NSCLC tissues using a tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA), after obtaining written informed consent from the participants. The quality of DNA was determined by electrophoresis. Primers for EGFR were designed based on the sequence from NCBI GenBank and constructed by Sangon Biotech Co., Ltd., (Shanghai, China). PCR reactions (20 μl) were performed as follows: 10X PCR buffer 2 μl, HotStar TaqDNA Polymerase (Qiagen) 0.25 μl, 5X Q-solution 4 μl, dNTP 2 μl, each Primer 1 μl, DNA template 1 μl and distilled water 8.75 μl. Reactions without a template were used as the negative control. The PCR products were purified using an AxyPrep™ PCR clean-up kit (Axygen Biosciences, Union City, CA, USA). A cycle sequencing reaction was performed using BigDye® Terminator v.3.1 Cycle Sequencing kit (Applied Biosystems) 0.8 μl, BigDye Sequence buffer 1.6 μl, forward/reverse primer 0.3 μl and purified PCR product 1 μl, adding ddH_2O to 10 μl. The electrophoresis of the purified sequencing product was performed by the ABI 3100 capillary sequencer and the DNA sequence was analyzed by DNA Sequencing Analysis Software v.5.1 (Applied Biosystems).

Statistical analysis. mRNA expression was expressed as the mean ± standard error of the mean (SEM). Pearson's χ^2 or Fisher's exact test was used to examine the correlation between biological markers and clinicopathological characteristics. Pearson's correlation coefficient analysis was used to analyze the expression correlation. The Mann-Whitney U test was used to determine significant associations between gene expression and EGFR mutation status. A two tailed P-value <0.05 was considered as significant. The statistical analysis was performed by SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

Table III. Correlation between EGFR mutation status and the clinicopathological characteristics of patients with NSCLC.

Clinicopathological characteristics	EGFR mutant	EGFR wild-type	Mutation rate (%)	P-value
Pathological subtypes				0.462
All	27	76	26.2	
Adenocarcinoma	24	53	31.2	
Squamous cell carcinoma	2	11	15.4	
Adenosquamous carcinoma	1	4	20.0	
Gender				
Male	8	54	12.90	0.001
Female	19	22	46.34	
Age (years)				
≥60	19	41	31.67	0.137
<60	8	35	18.60	

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

Approval. The study was approved by the Ethics Committee of Nanjing University of Traditional Chinese Medicine (TCM), Nanjing, China. A statement of informed consent was signed by the participants.

Results

Pathological characteristics. The histological subtypes were: 77 adenocarcinomas, 5 adenosquamous carcinomas, 13 squamous cell carcinomas and 8 others. The slides were reviewed by two pathologists.

ERCC1 gene mRNA expression. ERCC1 gene expression levels were detected in specimens from 103 patients with NSCLC. The median ERCC1 expression value of 4.33 was set as the cut-off value (7), ERCC1 $\Delta Ct<4.33$ was considered a low, while $\Delta Ct>4.33$ was considered a high expression. ERCC1 expression was divided into several groups, based on the pathological classification. The results showed that ERCC1 gene expression was not associated with gender, age or histology (Table I).

BRCA1 gene mRNA expression. BRCA1 gene expression was detected among 56 cases. The median BRCA1 expression value of 6.88 was used as the cut-off value to define low/high mRNA expression. BRCA1 expression was divided into several groups, based on the pathological classification. As shown in Table II, the mRNA expression of BRCA1 was independent of gender or age, but associated with histology (P=0.025).

EGFR mutation status. EGFR mutation testing showed that: 27 in 103 (26.2%) patients were EGFR mutant-type, of which 24 cases were adenocarcinoma (24/77, 31.2%) and 2 were squamous cell carcinoma (2/13, 15.4%) (Table III). The mutation rate of the adenocarcinoma was 31.2%, which was slightly higher compared with other subtypes of NSCLC, although no statistically significant difference was evident. Results also showed that EGFR mutation was significantly higher in females (19/41, 46.34%) compared with males (8/62, 12.90%) (P=0.001). Mutations occurred mainly in exon 19 deletion (E19del) and exon 21 L858R mutation, accounting for 85.2% (23/27) of the total mutations.

Table IV. Correlation between ERCC1, BRCA1 mRNA expression and EGFR mutation status.

Expression levels	EGFR mutant	EGFR wild-type
ERCC1 high	10	41
ERCC1 low	17	35
BRCA1 high	2	20
BRCA1 low	8	26

ERCC1, excision repair cross complementing group 1 gene; BRCA1, breast cancer susceptibility 1 gene; EGFR, epidermal growth factor receptor.

ERCC1, BRCA1 mRNA expression and EGFR mutation. A positive correlation was found between ERCC1 and BRCA1 mRNA expression (r=0.318, P=0.017). Of 27 EGFR mutant-type NSCLC, a high ERCC1 gene expression was detected in 10 cases (37.0%), while a low expression was found in 17 cases (63.0%). Of 10 EGFR mutant-type NSCLC, a high BRCA1 gene expression was detected in 2 cases (20.0%), while a low BRCA1 expression was found in 8 cases (80.0%). As shown in Table IV, the EGFR mutant-type was more likely to be categorized as ERCC1-low (P=0.313) and BRCA1-low (P=0.018), while the association of EGFR mutation and BRCA1 expression reached statistically significant levels.

Clinical outcome based on biological markers. The follow-up result showed that one-year survival was 82.6, 76.2 and 84.6% in the low ERCC1, low BRCA1 and EGFR mutant groups, respectively vs. 76.3, 80.0 and 71.7% in the high ERCC1, high BRCA1 and EGFR wild-type groups, respectively. No difference was detected in the one-year survival rate, based on either

ERCC1, BRCA1 mRNA expression levels or EGFR mutation status (P>0.05).

Discussion

Predictive biomarkers were used to select suitable patients for molecular-targeted therapy to improve the outcome. Several biomarkers including ERCC1, BRCA1, RRM1 and EGFR have been characterized in retrospective analyses of individualized therapy. The ERCC1 gene encodes a protein with a nucleotide excision repair (NER), an important member in NER systems (8). Previous studies have shown that patients with a low ERCC1 expression are likely to benefit from platinum-based chemotherapy, whereas this is not the case for patients with a high expression, suggesting that ERCC1 may be a predictor of chemotherapy efficacy (9). The association between ERCC1 expression and patient demographics has also been examined, with conflicting results (6,10,11). Such results may be explained by differences in trial design and methods. In this study, ERCC1 expression was detected in the paraffin-embedded NSCLC tissues and findings showed that this expression was not associated with histology, gender and age.

BRCA1 is important in multiple DNA damage repair pathways and is considered to be a differential modulator of survival with cisplatin and paclitaxel (12-14). Decreased BRCA1 mRNA expression enhances cisplatin sensitivity but leads to resistance to antimicrotubule agent such as paclitaxel. To examine the distribution of BRCA1 expression in NSCLC, we quantified BRCA1 mRNA levels in 56 specimens with NSCLC. The results show that the mRNA is expression of BRCA1 was independent of gender and age but is associated with histology and ERCC1 expression. The positive correlation between ERCC1 and BRCA1 expression suggests that a high intratumoral BRCA1 expression is accompanied by an elevated ERCC1 expression, which may be explained by the fact that ERCC1 and BRCA1 are both involved in the pathway of NER to repair DNA adducts.

EGFR, whose overexpression and/or mutation is able to control tumor growth through the signal transduction pathway, has become a potent target gene for tumor therapy (15). However, EGFR-targeted drugs, such as TKI had no significant effect on patients without EGFR mutations (16). Studies have found that the EGFR mutation rate was ~33% in the Asian population and that EGFR mutant-type patients benefited from platinum chemotherapy (17,18). In the present study, 103 patients with NSCLC were examined for EGFR mutations. Results show a total of 27 cases (26.2%) had EGFR mutations, consistent with previous reports (17,18). Mutations occur mainly in E19del and exon 21 L858R mutation, accounting for 85.2% (23/27) of the total mutations. Of the 27 mutations, 13 cases of E19del were female. In addition, EGFR mutations are more likely to occur in females, especially for E19del.

IPASS and other studies have shown patients with EGFR mutations to be more sensitive to platinum-based chemotherapy in NSCLC (19-21). EGFR and DNA repair pathways have been linked in preclinical studies, possibly through BRCA1, with implications for platinum-based therapy (22-24). A multi-center trial has shown that EGFR mutation was highly associated with a low mRNA expression level of ERCC1 in NSCLC (6). In this study, the results show that in EGFR mutant patients ERCC1 and BRCA1 were more likely to exhibit a low expression (63.0 and 80.0%, respectively), although the first was not statistically significant, which may be due to the relatively small patient population or ethnic differences.

Certain studies suggest that a high ERCC1 and BRCA1 mRNA expression is correlated with poor survival in NSCLC (25,26). In the present study, due to the short follow-up period, available data are limited regarding the long-term survival of patients. As yet, none of these three markers are potential independent prognostic factors for clinical outcome in NSCLC.

In summary, the correlation between EGFR mutations, ERCC1 and BRCA1 expression in NSCLC was investigated, and a trend for a low expression of ERCC1 and BRCA1 in EGFR mutant patients was detected, possibly accounting for the high sensitivity of platinum-based chemotherapy in NSCLC. In addition, data on biomarkers showed controversial results in different laboratories, therefore, laboratory tests assessing biomarkers need to be standardized.

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