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Expression of ERCC1 and BRCA1 predict the clinical outcome of non-small cell lung cancer in patients receiving platinum-based chemotherapy

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ABSTRACT. We examined mRNA expression levels of ERCC1, BRCA1, RRM1, and human β-tubulin-III (TUBB3) in non-small-cell lung carcinoma (NSCLC) patients and investigated the association between expression of these genes and the clinical outcome of NSCLC treatment. A total of 366 patients who underwent surgery for NSCLC were included in this study. All patients received third-generation platinum-based chemotherapy as first-line treatment. The relative cDNA quantification for ERCC1, RRM1, BRCA1, and TUBB3 was determined using a fluorescence-based, real-time detection method. We found that low expression of ERCC1 and BRCA1 was associated with a good response to platinum-based chemotherapy, with an odds ratio [95% confidence interval (CI)] of 2.09 (1.33-3.27) and 2.92 (1.85-4.62), respectively. Multivariate Cox regression analysis indicated that patients with low expression of ERCC1 and BRCA1 attained a longer overall survival time than those with high expression, with a hazard ratio (95%CI) of 0.42 (0.23-0.77) and 0.39

(0.21-0.71), respectively. However, RMM1 and TUBB2 expressions were not correlated with clinical outcome of NSCLC. In conclusion, we found that low expression of ERCC1 and BRCA1 can be useful for selecting NSCLC patients who would benefit from chemotherapy and warrants further investigation in prospective studies.

Key words: Non-small cell lung cancer; BRCA1; RRM1; RRM2; Overall survival

INTRODUCTION

Lung cancer is a major cause of mortality from malignant disease because of its high incidence, malignant behavior, and lack of major advancements in treatment strategies (Ginsberg et al., 1993). Non-small cell lung cancer (NSCLC) is the most frequently observed lung cancer type and is expected to account for 80% of all lung cancer cases. More than 70% of NSCLC patients present locally advanced or metastatic disease at the time of diagnosis due to the asymptomatic nature of early disease and the lack of effective screening modalities (William et al., 2009). It is estimated that the 5-year survival rate for NSCLC is less than 15%, and NSCLC has been reported as the most fatal cancer in China in the past 10 years (IARC, 2008).

Since curative surgery is not an effective treatment for NSCLC, chemotherapy has become the primary treatment measure for advanced NSCLC patients. Currently, platinum-based doublets chemotherapy is the major treatment for advanced NSCLC and has been shown to improve overall survival time (Matakidou et al., 2007). Cisplatin plays an important role in the treatment of advanced NSCLC. However, the therapeutic effect of adjuvant chemotherapy after NSCLC complete excision is poor, primarily due to the drug resistance of tumor cells to anticarcinogens (Camps et al., 2007). Therefore, detection of molecular markers may facilitate the selection of drugs for postoperative adjuvant chemotherapy to increase the benefit to patients. A previous study indicated that bulky DNA adducts induced by cisplatin are mainly repaired through the action of nucleotide excision repair pathway proteins, including excision repair cross complementing 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1), and breast cancer susceptibility gene 1 (BRCA1) (Matakidou et al., 2007). Previous studies have reported an association between ERCC1, RRM1, and BRCA1 and NSCLC prognosis. However, the results are inconsistent. Therefore, we determined mRNA expression quantities of ERCC1, BRCA1, RRM1, and human β-tubulin-III (TUBB3) in NSCLC patients and investigated the association between expression of these genes and clinical outcome of NSCLC.

MATERIAL AND METHODS

Subjects

A total of 366 patients who underwent surgery for NSCLC between March 2009 and March 2010 were enrolled in our study. All biopsy samples were collected either from bronchoscopic or fine-needle aspiration biopsies. Informed consent was provided from each patient before beginning the study. Patients who had a prior history of malignancy, pregnancy, or a history of chemotherapy, radiotherapy, or surgery were excluded from the study.

Study design

Baseline assessment was conducted for all patients, including a physical examination and appropriate imaging studies, including X-rays and chest and abdomen computed tomography scan. All patients received third-generation platinum-based chemotherapy as first-line treatment, including gemcitabine, vinorelbine, or paclitaxel. The response to treatment was evaluated according to World Health Organization criteria (Miller et al., 1981). Complete remission (CR) and partial remission (PR) were considered to be responsive, while stable disease (SD) and progressive disease (PD) were considered to be non-responsive. Overall survival was defined from the start of therapy to the date of death.

RNA isolation and cDNA quantification

All patients were asked to provide 5 mL peripheral venous blood samples before receiving chemotherapy. Total RNA was extracted using an EZNA Blood RNA Mini Kit (Omega; Berkeley, CA, USA) and RNA dissolved in water according to manufacturer instructions.

Relative cDNA levels for ERCC1, RRM1, BRCA1, and TUBB3 were determined using a fluorescence-based, real-time detection method, and *β-actin* was used as a reference gene. The primers, probes, and reaction conditions are available upon request. Genotyping was conducted according to methods described in a previous report (Han et al., 2013). For quality control, we performed genotyping of 10% of the samples, and attained 100% consistency.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 (SPSS; Chicago, IL, USA). Continuous variables are reported as means ± standard deviation and the Student *t*-test was used to test differences between variables, whereas categorical variables are shown as frequencies and percentages and were determined using the chi-square test. Demographic characteristics were compared between cases and controls by using the χ^2 and Student *t*-tests. The roles of ERCC1, RRM1, BRCA1, and TUBB3 were evaluated through multivariate logistic regression analysis, with adjusted odd ratios (ORs) and their 95% confidence intervals (CI). Survival differences were compared using the log-rank test. Multivariate analysis of survival was conducted by using Cox regression analysis to identify independent prognostic variables, with hazard ratios (HRs) and 95%CIs. Survival distributions were estimated by using the Kaplan-Meier method and were assessed by using the log-rank test. Two-sided P values <0.05 were considered to be significant.

RESULTS

Patients

Patient characteristics are summarized in Table 1. The median age of the patients was 62.6 years (range = 25.5-86.4 years). Among these patients, 269 were men (73.5%) and 97 were women (26.5%), and 32.5% had a history of smoking. A total of 78.7% of patients had stage IV diseases and 49.2% had adenocarcinoma. All patients received platinum-based doublets chemotherapy. In addition, 47.8% of the patients achieved CR and PR to chemotherapy,

and 52.2% presented SD and PD. By March 2012, 13 patients had been lost, and 172 patients died during follow-up.

Table 1. Characteristics of patients included.

Characteristics	Number (N = 366)	Percentage (%)
Age		
Median age (years)	25.5-86.4	
≤60	171	46.7
>60	195	53.3
Gender		
Male	269	73.5
Female	97	26.5
Smoking status		
Never	247	67.5
Ever	119	32.5
Stage		
IIIB	78	21.3
IV	288	78.7
Histopathology		
Adenocarcinoma	180	49.2
Squamous	151	41.3
Mixed/other NSCLC	35	9.6
Response to chemotherapy		
CR or PR	175	47.8
SD or PD	191	52.2
ECOG performance status		
0	240	65.6
1	101	27.6
2	25	6.8

CR = complete remission; PR = partial remission; SD = stable disease; PD = progressive disease.

Quantification of mRNA expression

mRNA quantification was carried out using real-time polymerase chain reaction (PCR), and the expression levels of ERCC1, BRCA1, RRM1, and TUBB3 were evaluated and compared with *β-actin* as an internal reference gene. The standardized expression level was determined by dividing the target amount by the β-actin level. Cutoff points for ERCC1, BRCA1, RRM1, and TUBB3 were determined based on the median expression level. Median expression levels of ERCC1, BRCA1, RRM1, and TUBB3 were 0.535 ± 0.221, 0.067 ± 0.067, 0.186 ± 0.116, and 0.217 ± 0.113, respectively (Table 2). After determining a cut-off point for high and low expression, we found that low expressions of ERCC1 and BRCA1 were associated with a good response to platinum-based chemotherapy, with ORs (95%CI) of 2.09 (1.33-3.27) and 2.92 (1.85-4.62), respectively. However, low levels of RRM1 and TUBB3 did not influence the response to platinum-based chemotherapy.

Table 2. ERCC1, BRCA1, RRM1, and TUBB3 expression response to chemotherapy.

Expression level	Total expression quantities	Responders		Non-responders		P value	OR (95%CI)
		N = 148	%	N = 218	%		
High ERCC1	0.535 ± 0.221	58	39.19	125	57.34		1.0
Low ERCC1		90	60.81	93	42.66	<0.001	2.09 (1.33-3.27)
High BRCA1	0.067 ± 0.067	51	34.46	132	60.55		1.0
Low BRCA1		97	65.54	86	39.45	<0.001	2.92 (1.85-4.62)
High RRM1	0.186 ± 0.116	68	45.95	115	52.75		1.0
Low RRM1		80	54.05	103	47.25	0.21	1.32 (0.85-2.04)
High TUBB3	0.217 ± 0.113	70	47.30	113	51.83		1.0
Low TUBB3		78	52.70	105	48.17	0.77	1.06 (0.69-1.64)

Survival and mRNA expression

The median overall survival of patients treated with platinum-based chemotherapy was 18.6 months. Multivariate Cox regression analysis indicated that patients with low expression of ERCC1 attained a longer overall survival time than those with high expression, with an HR (95%CI) of 0.42 (0.23-0.77) (Table 3). We also observed that patients with low expression of BRCA1 had a longer overall survival time, with an HR (95%CI) of 0.39 (0.21-0.71). However, no association was observed between RMM1 and TUBB2 expression and prognosis of NSCLC.

Table 3. Univariate analysis for the prediction of clinical outcome.

Gene	Death		Alive		Overall survival	
	N = 172	%	N = 194	%	P value	HR (95%CI)[1]
High ERCC1	97	56.40	86	44.33		1.0
Low ERCC1	75	43.60	108	55.67	0.002	0.42 (0.23-0.77)
High BRCA1	98	56.98	85	43.81		1.0
Low BRCA1	74	43.02	109	56.19	<0.001	0.39 (0.21-0.71)
High RRM1	88	51.16	95	48.97		1.0
Low RRM1	84	48.84	99	51.03	0.58	0.86 (0.48-1.53)
High TUBB3	89	51.74	94	48.45		1.0
Low TUBB3	83	48.26	100	51.55	0.41	0.80 (0.45-1.42)

[1]Adjusted for gender, age and smoking status.

DISCUSSION

With the development of novel treatments for resected NSCLC, platinum agents have become the standard first-line treatment (Crinò et al., 2010). However, treatment response rate and overall survival differ in NSCLC patients, since some of these patients show toxicity and drug resistance. This indicates that the chemoresistance of certain genes plays a critical role in the treatment response to chemotherapy. Therefore, detection of some genetic markers may be valuable for individualizing patient treatment and enable discrimination of patients most likely to respond to combination therapy from those likely to be non-responsive. We observed an inverse correlation between ERCC1 and BRCA1 mRNA expression and response to platinum-based chemotherapy in advanced NSCLC patients. Patients with lower expression of ERCC1 and BRCA1 showed a significantly higher response to platinum-based chemotherapy and a longer overall survival time than those with high expression levels. Moreover, ERCC1 and BRCA1 expression may be independent predictive factors for predicting clinical outcome.

The *ERCC1* gene, which contains 10 exons and encodes a 297-acetaldehyde ammonia product, has been mapped to chromosome 19q13.32. This chromosome is involved in correcting excision repair deficiencies of the nucleotide excision repair pathway (van Duin et al., 1986; Reed, 1998). Previously published studies have indicated a close relationship between the expression of *ERCC1* and response to chemotherapy in various cancers, including gastric cancer, extrahepatic bile duct cancer, and colorectal cancer (De Dosso et al., 2013; Park et al., 2013; Yuanming et al., 2013). For NSCLC, previous studies indicated that the ERCC1 protein may play an important role in the prognosis of NSCLC patients treated with chemotherapy (Yan et al., 2013; Tiseo et al., 2013). A study conducted in China indicated that negative

ERCC1 protein expression was associated with NSCLC prognosis (Yan et al., 2013). Another study conducted in Italy showed that ERCC1-negative patients had better response rates, highlighting it as a potential prognostic factor for NSCLC (Tiseo et al., 2013). However, several studies reported no association between ERCC1 expression and clinical outcome of NSCLC (Sodja et al., 2012; Han et al., 2013). Inconsistencies in these studies may be explained by differences in population background, and further studies with different populations are needed to confirm our findings.

The *BRCA1* gene, a 100-kb sequence with a locus at chromosome segment 17q12-21, plays an important role in repairing double-stranded DNA rupture and DNA damage mediated by cisplatin resistance (Tassone et al., 2003). Previous studies reported a significant correlation between the top tertile of BRCA1 mRNA expression and increased response to adjuvant chemotherapy (Matakidou et al., 2007). A meta-analysis reported a significant relationship between BRCA1 expression and the clinical outcome of chemotherapy in NSCLC patients, and showed that low/negative BRCA1 expression indicated a better objective response rate and longer overall survival (Yang et al., 2013). Our study also found that low expression of BRCA1 was correlated with a better response to chemotherapy, which agrees with the results of previous reports.

There are two limitations to our study. First, since our study was only conducted in one location, selection bias was inevitable, and the results may be limited for extraction to other populations. Second, the sample size in our study was relatively small, reducing the statistical power to identify the differences in RRM1 and TUBB3 between groups. Therefore, further large sample multicenter studies are needed.

In conclusion, this study reports that low expression of ERCC1 and BRCA1 can be used as a predictor of response to platinum-based chemotherapy in NSCLC patients. This observation suggests that ERCC1 and BRCA1 may substantially contribute to the future design of individualized cancer treatment in NSCLC patients. Therefore, further multicenter studies including various populations are required to confirm the results of our study.

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