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Predictive role of *RRM1* and *BRCA1* mRNA expression on the clinical outcome of advanced non-small cell lung cancer

J.G. Liang[1,2], Z.Y. Jin[2], X.D. Gao[2], M.R. Te[2], L.H. Ge[3] and C.L. Wang[1]

[1]Department of Pulmonary Tumor,
Tianjin Medical University Cancer Institute and Hospital,
National Clinical Research Center for Cancer, Tianjin, China
[2]Department of Chest Surgery,
Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China
[3]Department of Radiology,
Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China

Corresponding author: C.L. Wang
E-mail: lihongge455@163.com

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ABSTRACT. This study aimed to evaluate the association between *RRM1* and *BRCA1* expressions and the therapeutic efficacy of platinum-based chemotherapy in non-small cell lung cancer patients in terms of their response and prognosis. In total, 377 patients agreed to participate in our study, and all of them received platinum-based combination chemotherapy between January 2008 and January 2009. The relative cDNA quantitation for *RRM1* and *BRCA1* was conducted using a fluorescence-based, real-time detection method, using *β-actin* as a reference gene. The average age of the 377 patients was 64.6 years (range: 25.5-86.4 years), including 269 men and 108 women. Patients with high *RRM1* expression benefited more from a platinum-containing regimen, and patients with high *BRCA1* expression showed a high response rate to a platinum-containing regimen and reduced disease progression. Patients with high *RRM1* expression were associated with

a longer progression-free survival (PFS) and overall survival (OS) than those with low expression, and the hazard ratios (HRs) (95% confidence interval (CI)) were 0.67 (0.32-0.91) and 0.54 (0.30-0.95), respectively. Patients with high *BRCA1* expression showed longer PFS and OS compared to those with low expression, and the HRs (95%CI) were 0.54 (0.30-0.95) and 0.62 (0.32-0.93), respectively. These results could be used in personalized chemotherapy decisions and to increase the response rate and prolonged survival, and could encourage exploration of the predictive value of other genes.

Key words: *RRM1*; *BRCA1*; Non-small cell lung cancer; Clinical outcome

INTRODUCTION

Lung cancer is a high incidence and high mortality malignancy, and is reported to be the highest fate cancer in China (IARC, 2008). Non-small cell lung cancer (NSCLC) accounts for almost 85% of all lung cancer cases, and most of these patients are at an advanced stage when they are diagnosed. Chemotherapy is one of the major treatment options for these patients. Despite the wide use of chemotherapy, such as platinum agents, the prognosis of advanced NSCLC patients is still poor (Molina et al., 2008). Almost 60-70% of patients do not show any response to chemotherapy, and the 5-year survival rate is always less than 15% (Jemal et al., 2002).

DNA is the molecular target of many anticancer drugs. An abnormal capacity to repair DNA is closely associated with chemo-resistance, which could greatly influence the effectiveness of chemotherapy. In order to improve the efficacy of chemotherapy and to reduce its toxicity, chemotherapy could be tailored according to the expression levels or polymorphisms of chemotherapy-related genes, such as those with lower *RRM1* tumor and mRNA levels tending to living longer (Rosell et al., 2004; Ceppi et al., 2006; Bepler et al., 2006; Boukovinas et al., 2008). Another study reported that the efficacy of gemcitabine could be greatly improved when specifically used according to the differences in mRNA expression of *BRCA1* and *RRM1* as well as *RRM2* (Simon et al., 2007). Lower *BRCA1* expression decreases the sensitivity to chemotherapy, such as paclitaxel or docetaxel, and reduces resistance to platinum. By contrast, over-expression of *BRCA1* could decrease cisplatin sensitivity and resistance to antimicrotubule agents (Rosell et al., 2007). However, the results of previous studies concerning the roles of *BRCA1* and *RRM1* expression on NSCLC prognosis are inconsistent. Therefore, the aim of our study was to evaluate the association between *RRM1* and *BRCA1* expression and the therapeutic efficacy of platinum-based chemotherapy in NSCLC patients in terms of their response and prognosis.

MATERIAL AND METHODS

Subjects

In total, 415 patients who were histologically confirmed with advanced NSCLC were

eligible for inclusion in our study. All biopsy samples were collected either bronchoscopically or by fine-needle aspiration biopsies. In total, 377 patients agreed to participate in our study, and all of them received platinum-based combination chemotherapy at the Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer between January 2008 and January 2009. Patients who did not receive previous chemotherapy and radiotherapy, had a history of other malignant cancer within 5 years, were pregnant or lactating, had serious infections, or impairment of organ function were excluded.

Study design

The present study was designed to analyze the predictive value of *RRM1* and *BRCA1* mRNA expression in patients with NSCLC. All patients received platinum-based chemotherapy as first-line chemotherapy, such as gemcitabine, vinorelbine, or paclitaxel. The intravenous dosages were 75 mg/m^2 cisplatin, 1000 mg/m^2 carboplatin and gemcitabine, and 25 mg/m^2 vinorelbine on day 1 and 8 for a maximum of four cycles, and the treatments were suspended until disease progression or unacceptable toxicity. If patients showed three grades of non-hematology toxicity, four grades of hematology toxicity, febrile neutropenia, or infection, the chemotherapeutic drug dosage was reduced by 25% for the next cycle. When the chemotherapy was finished, the patients were followed up every month by telephone up to the time of death or the end of the study. The response to chemotherapy was classified by using the Response Evaluation Criteria In Solid Tumors (RECIST) (Therasse et al., 2000). We also evaluated the overall survival (OS) and progression-free survival (PFS) of all patients. The OS was defined from the start of therapy to the date of death, and PFS was defined from the start of therapy to the date of progression or the date of death without progression. All patients signed informed consent before enrolling in the study, and our study was approved by the Ethics Committee of the Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer.

RNA isolation and gene expression analysis

Peripheral venous blood samples were collected from each patient before they received their first cycle of chemotherapy. Samples were collected in 2-mL EDTA anticoagulant tubes, and peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation. The total RNA was extracted from PBMCs immediately after collection using an EZNA Blood RNA Mini Kit (Omega, Berkeley, CA, USA) according to manufacturer instructions. Isolated total RNA samples were stored at -70°C, and complementary DNA (cDNA) was synthesized over 1 week for the total RNA sample using a Reverse Transcription System (Promega, Madison, WI, USA). The cDNA product was stored at -20°C.

The relative cDNA quantitation for *RRM1* and *BRCA1* was conducted using a fluorescence-based, real-time detection method, using *β-actin* as a reference gene. Primers and probes for the gene analysis are shown in Table 1. Amplification of genes was conducted in 25-μL volumes with 0.25 μM of each primer pair, 0.02 mM dNTPs, 25 mM $MgCl_2$, 1.25 U Taq polymerase, and 5X polymerase chain reaction (PCR) buffer. The PCR conditions started with denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 20 s, and annealing at 60°C for 60 s. The relative amount of cRNA was determined by comparing the threshold cycle with the standard curve, and their standardized concentrations were determined by reference to the *β-actin* concentration.

Table 1. Primes and probes of *RRM1* and *BRCA1*.

Gene	Probe	Forward primer	Reverse primer
RRM1	5'-AAGGTGCACACAAGCGTCCTGGG-3'	5'-AAGCTGGAAAAGACCCTGCC-3'	5'-CTCGGGTGAGGAACAGTCCA-3'
BRCA1	5'-GACTGGGTCACCTGGAAATC-3'	5'-GACTGGCTCGCCTGGGCT-3'	5'-AAGTGCCTTGGTGCTCAC-3'

Statistical analysis

Continuous variables are reported as means ± SD and were analyzed using the independent sample Student *t*-test. Categorical variables are reported as the percentage (%) of subjects (N), and were analyzed by using the χ^2 test. The Hardy-Weinberg equilibrium and between group comparisons of genotype distributions were analyzed using the χ^2 test. Patients achieving complete response or partial response were defined as "responders", and patients with stable disease or progressive disease were defined as "non-responders". Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were used to assess the association between gene expression and response. The association between mRNA expression levels and survival was estimated using hazard ratios (HRs) and its 95%CIs from multivariate Cox regression models.

RESULTS

The patient characteristics are shown in Table 2. The average age of the 377 patients was 64.6 years (range: 25.5 to 86.4 years), including 269 men and 108 women. One hundred and thirty patients (34.6%) were smokers, 195 patients (51.6%) had stage IV NSCLC, and 118 patients (31.4%) had adenocarcinoma. All 377 patients were followed up until January 2011, and 256 patients (67.9%) died during the follow-up period.

Table 2. Characteristics of the patients included.

Characteristics	Number	Percentage (%)
Median age (years)	64.6 (25.5-86.4)	
Gender		
Male	269	71.3
Female	108	28.7
Smoking status		
No	247	65.4
Yes	130	34.6
Stage		
IIIB	182	48.4
IV	195	51.6
Histopathology		
Adenocarcinoma	118	31.4
Squamous	259	68.6
Response to chemotherapy		
Complete or partial response	164	43.5
Stable disease	213	53.2
ECOG Performance status		
0	156	41.4
1	221	58.6

The mRNA quantification was carried out using real-time PCR, and the results were evaluated using *β-actin* as an internal reference gene. The cut-off points of the *RRM1* and

BRCA1 expression levels were determined according to the median expression levels of all 377 patients. The median levels of *RRM1* and *BRCA1* expression were 2.48 x 10^{-2} and 0.12 x 10^{-2} according to the expression of the internal reference gene *β-actin*. The samples were further divided into high and low *RRM1* and *BRCA1* expression groups. The associations between *RRM1* and *BRCA1* expressions and response to chemotherapy are shown in Table 3. Patients with high *RRM1* expression benefited more from a platinum-containing regimen, and patients with high *BRCA1* expression showed a higher response rate to a platinum-containing regimen and less disease progression (Table 3). The multivariate logistic regression model showed that *RRM1* and *BRCA1* were the predictive factors of the response rate, and the ORs (95%CI) were 1.70 (1.08-2.68) and 1.68 (1.07-2.66), respectively.

Table 3. Association between *RRM1* and *BRCA1* and response to chemotherapy.

Expression level	N	%	Responders	%	Non-responders	%	OR (95%CI)	P value
Low *RRM1*	141	37.5	50	35.4	91	64.6	-	-
High *RRM1*	236	62.5	114	48.4	122	51.6	1.70 (1.08-2.68)	0.015
Low *BRCA1*	138	36.6	49	35.5	89	64.5	-	-
High *BRCA1*	239	63.4	115	48.1	124	51.9	1.68 (1.07-2.66)	0.017
Total	377		164	43.5	213	56.5		

A significant relationship was found between the expression levels of *RRM1* and *BRCA1* and PFS and OS (Table 4). Patients with high *RRM1* expression were associated with a longer PFS and OS than those with low expression (6.1 *vs* 8.3 months, P = 0.027 for PFS; 10.5 *vs* 12.4 months, P = 0.011 for OS), and the HRs (95%CI) were 0.67 (0.32-0.91) and 0.54 (0.30-0.95), respectively. Patients with high *BRCA1* expression had longer PFS and OS compared to those with low expression (6.2 *vs* 8.4 months, P = 0.011 for PFS; 9.8 *vs* 13.2 months, P = 0.028 for OS), and a significant HR (95%CI) was found (HR = 0.54, 95%CI = 0.30-0.95 for PFS; HR = 0.62, 95%CI = 0.32-0.93 for OS).

Table 4. Association between *RRM1* and *BRCA1* expression and survival of NSCLC.

Expression level	Progression-free survival			Overall survival		
	Median survival (95%CI, months)	Log-rank P	HR (95%CI)	Median survival (95%CI, months)	Log-rank P	HR (95%CI)
Low *RRM1*	6.1 (3.2-11.7)	-	-	10.5 (3.2-16.4)	-	-
High *RRM1*	8.3 (3.6-12.2)	0.027	0.67 (0.32-0.91)	12.4 (3.6-18.4)	0.031	0.54 (0.30-0.95)
Low *BRCA1*	6.2 (3.8-11.6)	-	-	9.8 (3.8-17.5)	-	-
High *BRCA1*	8.4 (4.2-12.8)	0.011	0.77 (0.40-0.93)	13.2 (4.2-18.9)	0.028	0.62 (0.32-0.93)

DISCUSSION

Standard first-line chemotherapy, such as paclitaxel, gemcitabine, docetaxel, or vinorelbine, is usually used in combination with a platinum chemotherapy compound, and the treatment has become the mainstay of treatment for several cancers, such as advanced NSCLC, bladder cancer, and pancreatic cancer (Toschi et al., 2005). The present study found an association between the expression levels of *RRM1* and *BRCA1* and response to chemotherapy in advanced NSCLC patients. Patients with high *RRM1* and *BRCA1* expression ben-

efitted from a significantly higher response rate and better PFS and OS compared to those with low expression. These results provide important information for individualized chemotherapy based on patients' molecular biomarkers, which could help to increase the response rate and prolong survival (Rosell et al., 2004; Cobo et al., 2007; Simon et al., 2007).

RRM1, which is located on chromosome segment 11p15.5, usually shows a frequent loss of heterozygosis in NSCLC, and its differential expression results in different responses to gemcitabine chemotherapy. Previous studies have indicated that high *RRM1* expression levels could indicate significant benefits of cisplatin or gemcitabine in resected lung cancer treatment (Ren et al., 2012; Leng et al., 2012; Zhang et al., 2012). Ren et al. (2012) reported that chemotherapy that is customized in terms of *RRM1* expression levels was associated with a higher response rate and longer PFS and OS in patients who received chemotherapy. Another study, which was also conducted in China, showed that the expression of *RRM1* in tumor tissues and peripheral blood lymphocytes was closely correlated with the response to chemotherapy and prognosis of patients with advanced NSCLC treated with adjuvant chemotherapy. In our study, we found that patients with high *RRM1* expression had a significantly higher response rate, PFS, and OS than those with low expression, which is in line with previous studies. However, a study conducted in China with 85 tumor tissues and 34 adjacent tissue samples reported that *RRM1* expression did not affect the response to platinum-based adjuvant chemotherapy (Leng et al., 2012). Another study conducted in Denmark with 443 patients reported that RRM1 protein expression had no predictive impact in patients with cisplatin, paclitaxel, and gemcitabine (Vilmar et al., 2013). Further large sample studies are warranted to clarify this association.

BRCA1, a 100 kb region located at chromosome segment 17q12-21, plays an important role in repairing double-stranded DNA rupture and DNA damages mediated by cisplatin resistance (Tassone et al., 2003). Previous studies reported that a significant correlation was found between the top tercile of *BRCA1* mRNA expression and increased response to adjuvant chemotherapy (Matsuoka et al., 2007). Low *BRCA1* expression was associated with better sensitivity to cisplatin and etoposide (Husain et al., 1998; Lafarge et al., 2001; Quinn et al., 2003, 2007). However, the low expression was related to antimicrotubule drugs, such as paclitaxel, docetaxel, and vinorelbine (Quinn et al., 2003, 2007). In the present study, patients with high *BRCA1* mRNA expression showed a better response and longer time to progression when receiving platinum-based chemotherapy, which is in line with previous studies (Quinn et al., 2003, 2007).

The current study has several limitations. First, the study was conducted in a single hospital in China, and the cohort might not be representative of China as a whole. Second, prognosis of NSCLC is influenced by multiple genes, and further studies including more genes are warranted.

In conclusion, the present study showed that the expressions of *RRM1* and *BRCA1* are associated with the prognosis of NSCLC patients receiving chemotherapy. This observation could be used in personalized chemotherapy decisions and to increase the response rate and prolonged survival, and could encourage further explorations of the predictive value of other genes.

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