GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

ERCC1 mRNA expression is associated with the clinical outcome of non-small cell lung cancer treated with platinum-based chemotherapy

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Genet. Mol. Res. 13 (4): 10215-10222 (2014)
Received January 8, 2014
Accepted May 26, 2014
Published December 4, 2014
DOI http://dx.doi.org/10.4238/2014.December.4.16

ABSTRACT. We conducted a prospective study to analyze the expression of the excision repair cross-complementing group 1 (*ERCC1*) and ribonucleotide reductase subunit M1 (*RRM1*) genes in 297 Chinese patients with advanced non-small cell lung cancer (NSCLC). The goal of this study was to evaluate these genes as potential biomarkers for prediction of tumor response and clinical outcome. Patients with unresectable, locally advanced or metastatic NSCLC were enrolled between September 2007 and September 2009, and they were followed up until September 2012. A fluorescence-based real-time detection method was used to quantify relative levels of *ERCC1* and *RRM1* cDNA. Relative amounts of *ERCC1* and *RRM1* cDNA were calculated by comparing to actin. By

the end of follow-up, 132 patients had died and 165 patients experienced progression. The median overall survival time was 18.7 months (range, 1-60 months). The median levels of *ERCC1* and *RRM1* were 2.46 x 10^{-2} and 0.97 x 10^{-2}, respectively. Patients with low *ERCC1* expression had a significantly higher rate of complete response to chemotherapy, with an OR (95%CI) of 1.56 (1.03-2.47). Moreover, individuals with low levels of *ERCC1* had longer overall survival than patients with high expression, with an adjusted hazard ratio (95%CI) of 0.57 (0.35-0.93). In summary, low *ERCC1* mRNA expression was associated with better response to chemotherapy and correlated with longer survival in advanced NSCLC patients treated with platinum-based chemotherapy.

Key words: Excision repair cross-complementing group 1; mRNA; Non-small cell lung cancer; Survival; Response to chemotherapy; Ribonucleotide reductase subunit M1

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 70% of all lung cancer cases (IARC, 2008). NSCLC is the leading cause of cancer-related deaths worldwide (IARC, 2008). A recent report from the World Health Organization (WHO) estimated that the age-standardized incidence of lung cancer is 33.5 cases per 100,000 people for the entire Chinese population. The overall incidence was higher in male patients (45.9 cases per 100,000) than female patients (21.3 cases per 100,000). NSCLC has a poor prognosis, and it has been reported that more than 50% of NSCLC patients present with stage III disease or metastatic disease at the time of diagnosis (Bartolucci et al., 2009; Su et al., 2011; Pesta et al., 2012). Recently, platinum-based doublet chemotherapy has become the most frequently used chemotherapy regimen for advanced NSCLC (Bidoli et al., 2007; Vilmar and Sorensen, 2009). Interestingly, patients with cancer of a similar clinical stage and histological type experience different clinical outcomes; therefore, genetic variants may be associated with platinum-based chemotherapy efficacy.

Platinum is cytotoxic because it disrupts double-stranded DNA in cells by forming platinum-DNA adducts. These adducts cause interstrand crosslinking, induce bulky distortion of DNA, and inhibit DNA replication (Johnson, 2001). These DNA lesions can be mended by DNA repair mechanisms; therefore, the activities of repair molecules can play a role in clinical outcomes, response to chemotherapy, and survival of patients treated with platinum-based chemotherapy (Johnson, 2001). Nucleotide excision repair (NER) is a central DNA repair pathway, and it is involved in eliminating both cisplatin-induced DNA adducts as well as nucleotides damaged by ultraviolet irradiation. The NER pathway involves the cooperation of around 20 enzymes to restore a segment of DNA containing a bulky adduct (Cai et al., 2012). The excision repair cross-complementing group 1 (*ERCC1*) gene, together with the xeroderma pigmentosum group F (*XPF*) gene, is responsible for making a 5-prime incision in DNA and is a rate-limiting step in the NER DNA repair pathway (Bepler et al., 2013). The ribonucleotide reductase subunit M1 gene (*RRM1*), located at the chromosomal locus 11p15.5, is involved in DNA synthesis. Specifically, *RRM1* catalyzes deoxyribonucleotide biosynthesis from cor-

responding ribonucleotides (Bepler et al., 2013).

Previous studies have suggested that *ERCC1* and *RRM1* may play a role in chemosensitivity and the clinical outcomes of NSCLC patients treated with platinum-based chemotherapy (Kotoula et al., 2012; Leng et al., 2012; Pesta et al., 2012; Mlak et al., 2013). However, few studies have investigated the effect of *ERCC1* and *RRM1* on the clinical outcome of NSCLC or the association between *ERCC1* and *RRM1* expression and clinical characteristics. Therefore, we conducted a prospective study to analyze the expression of *ERCC1* and *RRM1* in 297 Chinese patients with advanced NSCLC and examined the potential of these genes as biomarkers for predicting tumor response and clinical outcome.

MATERIAL AND METHODS

Patients and samples

This study included 323 patients with unresectable, locally advanced, or metastatic NSCLC who were enrolled between September 2007 and September 2009 at the Second Hospital of Jilin University. All patients had histologically confirmed inoperable stage IIIB or IV NSCLC. The inclusion criteria for this study specified that patients had to have been treated with at least 2 cycles of first-line platinum-based chemotherapy, had histologically confirmed inoperable stage IIIB or IV NSCLC, had a WHO performance status of 0 to 2, and had measurable tumor response. Clinical and demographic characteristics were obtained from medical records. The histological classification of NSCLC was determined according to WHO criteria (WHO, 1981). Patients were excluded from the study if they had a second primary tumor, were pregnant, had severe cardiopulmonary insufficiency, or were severely malnourished. All patients signed written informed consent forms. The ethics committees of the Second Hospital of Jilin University approved our study protocol.

Treatment

All patients received platinum-based doublet chemotherapy treatment. The regimens were 75 mg/m^2 cisplatin or carboplatin on day 1 and 1000 mg/m^2 gemcitabine on days 1 and 8, or 25 mg/m^2 vinorelbine on days 1 and 8, or 175 mg/m^2 paclitaxel on day 1. Chemotherapy treatment was stopped if individuals experienced disease progression or unacceptable toxicity.

Patients' responses to platinum-based doublet chemotherapy were evaluated, and patients were classified as either good or poor responders. Patients who had a complete response or partial response to chemotherapy were considered good responders, and patients with stable disease or progressive disease were defined as poor responders. Chest X-rays and computed tomography scans were used to examine patients for progressive disease. Overall survival (OS) was calculated from the date of chemotherapy initiation to the date of death or the date of last follow-up.

RNA isolation and cDNA synthesis

All patients were asked to provide 5 mL whole blood, and the whole blood samples were kept at -70ºC until use. Total RNA was extracted from blood with an EZNA Blood RNA

Mini Kit (Omega, Berkeley, CA, USA). A fluorescence-based real-time detection method was used to quantify relative cDNA levels of *ERCC1* and *RRM1*. The relative amounts of *ERCC1* and *RRM1* cDNA were calculated by comparing to the amount of β-actin. Polymerase chain reaction (PCR) was performed in a 20 μL reaction volume containing 50 ng genomic DNA, 200 μM dNTP, 2.5 U Taq DNA polymerase (Promega Corporation, Madison, WI, USA), and 200 μM primers. PCR conditions were as follows: 94°C for 2 min, 35 cycles of 94°C for 30 s, reduction to the annealing temperature (64°C) for 30 s, and then 72°C for 1 min. For quality control, different investigators performed genotyping on a random sample of 10% of all the cases and control subjects; the reproducibility was 100%.

Statistical analysis

The SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables are expressed as the means ± SD and categorical variables are expressed as the frequency and percentage. The associations between *ERCC1* and *RRM1* expression and chemotherapy response were assessed by ORs and their 95%CIs. The associations between *ERCC1* and *RRM1* expression and OS of NSCLC patients were assessed by Cox regression models with hazards ratios and 95%CIs. The OS times of all patients were plotted using the Kaplan-Meier method. All tests were 2-sided and $P < 0.05$ was considered to be statistically significant.

RESULTS

The clinical characteristics of all patients are shown in Table 1. Of the 323 patients, 297 patients agreed to participate in our study, for a 91.95% participation rate. The median age of the patients included was 61.5 years (range, 34.6-74.1 years), and 69.1% of the patients were male. In this study, 57.5% of the patients were TNM stage IV. Adenocarcinoma and squamous cell carcinoma were the histological types of 56.7 and 37.4% of the NSCLC patients, respectively. There were 54.5% of the patients with an Eastern Cooperative Oncology Group performance status of 0 or 1.

During the follow-up period, 5 patients were dropped from the study because of loss of contact. All patients were followed up until September 2012. At the end of the follow-up period, 132 patients had died and 165 patients had experienced progressive disease. The median OS time was 18.7 months (range, 1-60 months).

ERCC1 and *RRM1* mRNA expression levels were calculated in comparison to β-actin levels. The median levels of *ERCC1* and *RRM1* were 2.46×10^{-2} and 0.97×10^{-2}, respectively. Expression levels of *ERCC1* and *RRM1* mRNA were classified as either high or low expression by comparing to the median level. Of the 297 patients in the study, 146 patients had a complete response to chemotherapy and 151 patients had a partial response to chemotherapy. Patients with low *ERCC1* expression had a significantly higher rate of complete response to chemotherapy, with an OR (95%CI) of 1.56 (1.03-2.47) (Table 2).

The associations between *ERCC1* and *RRM1* mRNA expression and OS were analyzed using a Cox regression analysis. We found that low levels of *ERCC1* expression were associated with longer OS times compared to high expression, with an adjusted HR (95%CI) of 0.57 (0.35-0.93) (Table 3).

Table 1. Demographic and clinical characteristics of patients included in the study.

Characteristic	No. N = 297	%
Mean age (range), years	61.5 (34.6-74.1)	
Gender		
Female	92	30.98
Male	205	69.02
Smoker		
Never	161	54.21
Ever	136	45.79
Histology		
Adenocarcinoma	168	56.57
Squamous cell carcinoma	111	37.37
Mixed NSCLC	18	6.06
Stage		
IIIB	126	42.42
IV	171	57.58
ECOG performance stage		
0	135	45.45
1-2	162	54.55

ECOG = Eastern Cooperative Oncology Group; NSCLC = non-small cell lung cancer.

Table 2. *ERCC1* and *RRM1* mRNA expression and response to chemotherapy.

	Complete response	Partial response	OR (95%CI)	P value	Adjusted OR (95%CI)	P value
	N = 146	N = 151				
ERCC1						
High	66	82	1.0 (Ref.)	-	1.0 (Ref.)	-
Low	80	69	1.44 (0.89-2.33)	0.12	1.56 (1.03-2.47)	0.03
RRM1						
High	71	78	1.0 (Ref.)	-	1.0 (Ref.)	-
Low	75	73	1.13 (0.70-1.83)	0.60	1.32 (0.84-1.97)	0.11

ERCC1 = excision repair cross-complementing group 1; RRM1 = ribonucleotide reductase subunit M1.

Table 3. *ERCC1* and *RRM1* mRNA expression and OS of NSCLC patients.

	Deaths N = 116	Median OS (months)	HR (95%CI)	P value	Adjusted HR (95%CI)	P value
ERCC1						
High	49	20.6	1.0 (Ref.)	-	1.0 (Ref.)	-
Low	67	27.7	0.64 (0.39-1.04)	0.05	0.57 (0.35-0.93)	0.02
RRM1						
High	55	23.1	1.0 (Ref.)	-	1.0 (Ref.)	-
Low	61	24.4	0.86 (0.53-1.40)	0.51	0.85 (0.50-1.35)	0.34

CI = confidence interval; ERCC1 = excision repair cross-complementing group 1; HR = hazard ratio; NSCLC = non-small cell lung cancer; OS = overall survival; RRM1 = ribonucleotide reductase subunit M1.

DISCUSSION

Individualized chemotherapy based on molecular prognostic markers can improve the prognosis of cancer patients. Our study showed that *ERCC1* expression was predictive of tumor response to chemotherapy and identified *ERCC1* expression as an independent prognostic factor for Chinese patients with NSCLC treated with platinum-based chemotherapy. Patients with high expression of *ERCC1* achieved significantly longer survival times than patients

with low *ERCC1* expression. The results of our study are in agreement with previous findings in a small cohort of NSCLC patients treated with platinum-based chemotherapy (Vassalou et al., 2013; Yamashita et al., 2013). However, another study found no association between *ERCC1* expression and treatment response or survival (Ozdemir et al., 2012; Tantraworasin et al., 2013). Our study suggests that *ERCC1* expression may predict the prognosis of advanced NSCLC patients.

ERCC1 plays a crucial role in NER and has been reported to influence the effectiveness of platinum-based chemotherapy in various cancers including gastric cancer, colorectal cancer, and bladder cancer, as well as NSCLC (Huang et al., 2013; Yamashita et al., 2013; Li et al., 2013, 2014). Huang et al. (2013) reported that overexpression of *ERCC1* is an important predictor of early treatment failure in patients with stage III colorectal cancer treated with FOLFOX adjuvant chemotherapy (leucovorin, 5-fluorouracil, and oxaliplatin), and *ERCC1* expression could be used to identify patients who would benefit from intensive follow-up and enhanced therapeutic programs. Li et al. (2013) reported a study conducted in an American population that indicated that combined *ERCC1* and *ERCC2* functional single nucleotide polymorphisms might influence the OS of gastric cancer patients. For NSCLC, the association between *ERCC1* expression and prognosis is inconsistent among studies. Tantraworasin et al. (2013) conducted a retrospective cohort study of 247 patients with completely resected advanced NSCLC and found that *ERCC1* and *RRM1* expression were not prognostic factors for tumor recurrence and overall survival. Another study found no association between *ERCC1* expression and survival or treatment response (Ozdemir et al., 2012). However, a study conducted in Greece indicated that *ERCC1* could be used to refine prognosis and thus individualize chemotherapy for advanced-stage NSCLC patients (Vassalou et al., 2013). Moreover, a recent meta-analysis found a significantly better therapy response and longer OS in patients with low or negative *ERCC1* expression compared to patients with high or positive *ERCC1* expression (Chen et al., 2010). The discrepancies among the reports may be explained by differences in ethnicities of the patients studied, the source of control subjects, the sample sizes, and also by chance. Future studies are needed to confirm the association between *ERCC1* polymorphisms and NSCLC prognosis.

The expression of *RRM1* protein by immunostaining has been reported to predict the clinical outcome of human cancers including breast cancer, pancreatic cancer, and gastric cancer (Jorgensen et al., 2013; Wang et al., 2013; Zhang et al., 2013). For NSCLC, some studies have reported that there is no association between *RRM1* expression and clinical outcomes (Tantraworasin et al., 2013). In our study, we found that *RRM1* expression was not a prognostic factor for advanced NSCLC patients who did or did not receive adjuvant chemotherapy.

In conclusion, our results indicate that low *ERCC1* mRNA expression is associated with better treatment response in advanced NSCLC patients who receive platinum-based chemotherapy and that *ERCC1* expression correlates with longer survival. Therefore, we suggest that *ERCC1* mRNA expression could be used as a surrogate marker to help individualize NSCLC treatment strategies.

ACKNOWLEDGMENTS

Research supported by the Natural Science Foundation of China (#81272472).

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