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Original Article

Prognostic value of ERCC1 and RRM1 in NSCLC patients with smoking history or not

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Abstract: Introduction: We aimed to assess the expression of *ERCC1* and *RRM1* in NSCLC patients with smoking history or not and investigate the therapeutic effect of biomarker-guided chemotherapy in those patients. Patients and methods: A total of 131 NSCLC patients at stage IIIb or IV were enrolled and divided into two groups based on their smoking history. Group A were smokers (n=67); Group B were non-smokers (n=64). The expression of *ERCC1* and *RRM1* were determined by real-time polymerase chain reaction analysis (RT-PCR). Clinicopathological characteristics, tumor response and patient survival were monitored for both groups. Results: In our study, the expression of *ERCC1* was significantly higher in smoking patients as compared with non-smoking group (*P*=0.028). Patients' characteristics in both groups were compared and there were no significant difference in patients age, tumor size or pathological types, but more male patients were occurred in Group A (*P*=0.001). Notably, the patients with low mRNA expression of *ERCC1* (χ^2=6.194, *P*=0.013) and *RRM1* (χ^2=5.012, *P*=0.025) were more sensitive to chemotherapy than patients with high expression levels. Compared with Group A, the patients in Group B displayed a higher response rate (59.3% vs. 32.8%; *P*=0.045), a lower 1-year recurrent rate (25.0% vs. 52.2%; *P*=0.001), a longer median progression-free survival (PFS) time (12.0 months vs. 14.1 months, *P*=0.008) and median overall survival (OS) time (22.9 months vs. 27.6 months; *P*=0.005). Conclusion: In summary, the expression levels of *ERCC1* and *RRM1* are mediated by smoking and correlated with the sensitivity to chemotherapy together with the clinical outcome of NSCLC patients. Our study suggests the adverse effect of smoking on the prognosis of NSCLC patients.

Keywords: Smoking, NSCLC, ERCC1, RRM1, clinical outcome and therapeutic effect

Introduction

Recently, primary lung cancer is one of the most common malignancies in the world. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of primary lung cancer cases [1]. Tobacco smoking is the predominant risk factor for the occurrence of lung cancer. There are many studies suggesting that smoking amount (pack-years) is a poor prognostic factor [2, 3]. However, the biomarker-based molecularly targeted therapeutic agents, such as gefitinib or erlotinib, are largely limited to non-smokers and in patients with adenocarcinoma histology. For those non-smoking NSCLC patients who are not sensitive to those molecular targeted drug as well as smoking NSCLC patients, treatment with platinum-based doublets is the standard therapeutic strategy. Platinum and gemcitabine represent the most important chemotherapeutic agents used to treat NSCLC patients.

Excision repair cross-complementing gene 1 (*ERCC1*) is involved in two critical DNA repair pathways: nucleotide excision repair and chain crosslink repair [4]. The DNA repair machinery allows cancer cell to repair the DNA damages caused by platinum compounds [5]. It has been reported that *ERCC1* expression levels were negatively correlated with cisplatin efficacy [6]. Another key gene in DNA synthesis and

Table 1. Patients' characteristics

Clinical features	Group A	Group B	χ^2	*P*-value
Gender			43.208	0.001
Male	61	23		
Female	6	41		
Age, years			0.901	0.343
<60	29	33		
≥60	38	31		
Histopathology			0.411	0.522
Adenocarcinoma	35	37		
No-adenocarcinoma	32	27		
Tumor size			1.747	0.186
<3 cm	31	37		
≥3 cm	36	27		
ERCC1 expression			4.802	0.028
Low	28	39		
High	39	25		
RRM1 expression			0.689	0.101
Low	23	31		
High	44	33		

repair is ribonucleotide reductase M1 (*RRM1*), which catalyzes the biosynthesis of deoxyribonucleotides from the corresponding ribonucleotides and repairs the nucleotide excision at later stage [7]. The overexpression of *RRM1* in lung tumors causes the resistance of tumor cells to platinum drugs and gemcitabine. Patients with low *RRM1* mRNA expression levels had significantly longer median survival time than those with high levels [8]. Therefore, the examination of *ERCC1* and *RRM1* levels may predict the prognosis of patients receiving chemotherapy. However, the relationship between smoking and the expression of *ERCC1* or *RRM1* remains unclear. In the present study, we aim to assess the expression of *ERCC1* and *RRM1* in NSCLC patients with smoking history or not and investigate the therapeutic effect of biomarker-guided chemotherapy in those patients.

Materials and methods

Specimen collection

A total of 131 patients at stage IIIB or IV were enrolled between August 2010 and August 2012 in our hospital (The First Affiliated Hospital of Henan University of Science and Technology). The median age of the enrolled patients was 64.1 (25.3-82.1) year(s). Among these patients, 69 (52.7%) were above the age of 60 years old, 84 (64.1%) were male patients. A total of 60 (45.8%) patients were diagnosed as stage IIIB and 72 (54.9%) patients had adenocarcinoma. There were 67 (51.1%) patients with smoking history (least one cigarette per day for 1 year). Patients who had a history of chemotherapy, radiotherapy, predicted survival time <3 months or pregnancy were excluded from the study. Until August 2015, we found that 8 patients were lost and 86 patients died during follow-up. The study protocol was approved by the ethics committee of the First Affiliated Hospital of Henan University of Science and Technology.

Reagents and instruments

The mRNA was extracted from lung tumors with mRNA extraction kits that were purchased from Qiagen (Hilden, Germany). The gene expression relative quantification detection kit (*ERCC1* and *RRM1*) was purchased from Amoy Diagnostics Company Ltd. (Xiamen, China). The ABI 7500 Sequence Detection System was obtained from Applied Biosystems (Foster City, Calif, USA).

Real-time polymerase chain reaction analysis for ERCC1 and RRM1

The gene expression relative quantification detection kits for *ERCC1* (ADx-ER01) and *RRM1* (ADx-RR01) were used to perform quantitative real-time PCR. All of the experimental procedures were based on the manufacturer's instructions. The PCR program was initiated with a 15 min denaturation step at 95°C, followed by 40 cycles of 95°C for 10 sec, 60°C for 32 sec. The relative mRNA levels of *ERCC1* and *RRM1* were normalized to β-actin.

Study design

The patients' gender, age, pathological type and other factors were recorded for analysis. Subsequently, the patients in each group received different chemotherapy regimens according to the expression levels of *ERCC1* and *RRM1*. Chemotherapy was formulated as following: the patients with low expression of *ERCC1* were given platinum-based chemotherapy drugs or platinum-based doublets (plati-

Table 2. Group A: the expression of *ERCC1* and *RRM1* response to chemotherapy

Expression level	Responders	Non-responders	χ^2	*P*-value
ERCC1 expression			4.605	0.032
High	10	21		
Low	20	14		
RRM1 expression			4.091	0.045
High	14	24		
Low	14	8		

Responders: CR + PR; *Non-responders:* SD + PD.

Table 3. Group B: the expression of *ERCC1* and *RRM1* response to chemotherapy

Expression level	Responders	Non-responders	χ^2	*P*-value
ERCC1 expression			6.194	0.013
High	12	19		
Low	23	10		
RRM1 expression			5.012	0.025
High	12	19		
Low	19	9		

Responders: CR + PR; *Non-responders:* SD + PD.

Table 4. Comparison of the therapeutic effect and survival

Outcome	Non-smoking group	Smoking group	χ^2	*P*-value
Response rate	59.3%	32.8%	4.019	0.045
1-year recurrent rate	25.0%	52.2%	10.215	0.001
Median PFS time month(s)	14.1	12.0	7.087	0.008
Median OS time month(s)	27.6	22.9	7.850	0.005

Responders: CR + PR; *Non-responders:* SD + PD.

num-based chemotherapy in combination with other first-line drugs including gemcitabine, vinorelbine or paclitaxel) while the patients with low expression of *RRM1* were given gemcitabine single-agent or gemcitabine-based doublets. Those patients with high expression of *ERCC1* and *RRM1* were given chemotherapy regimens by clinicians' experience. All patients received chemotherapy 4-6 cycles (21 days per cycle). The chemotherapy regimens were adjusted when patients showed disease progression or severe adverse effects. In order to assess whether patients showed progressive disease, a series of medical examinations were required, including X-rays, chest and abdomen computed tomography scan. The sensitivity of patients to treatment was assessed according to World Health Organization criteria [9]. 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. OS and PFS were observed by follow-up. OS was calculated from the date of assignment to either the date of death or last clinical follow-up. PFS was the time interval between the dates of first treatment and either disease progression or death.

Statistical analysis

The data were analyzed using the statistical software SPSS (version 19.0; IBM SPSS, Armonk, NY, USA). The survival distribution was plotted using Kaplan-Meier methods and the significance was analyzed by log-rank test. Categorical variables were compared using the χ^2 and Fisher's exact test with a test level α=0.05. The *P*-value was set to bilateral distribution and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients' characteristics

Patients' characteristics in both groups were compared, and there were no significant difference in age, histopathology, or tumor size of patients ($P>0.05$), but more male patients were occurred in Group A ($P=0.001$). The expression of *ERCC1* was strikingly higher in smoking patients ($\chi^2=4.802$, $P=0.028$) than non-smokers. However, we did not find any correlation between the expression of *RRM1* and with smoking ($\chi^2=2.689$, $P=0.101$; **Table 1**).

The role of ERCC1 and RRM1 on patients' response to chemotherapy

In Group A, those patients with low levels of *ERCC1* and *RRM1* were associated with high sensitivity of tumor cells to platinum-based chemotherapy (**Table 2**).

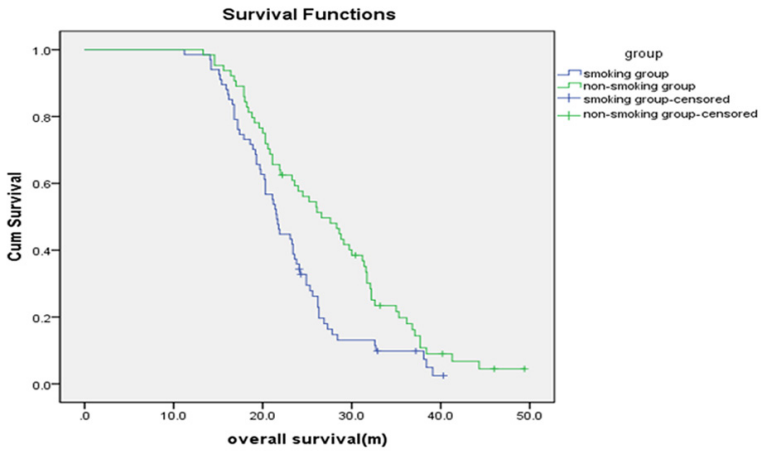

Figure 1. Kaplan-Meier curve for overall survival time.

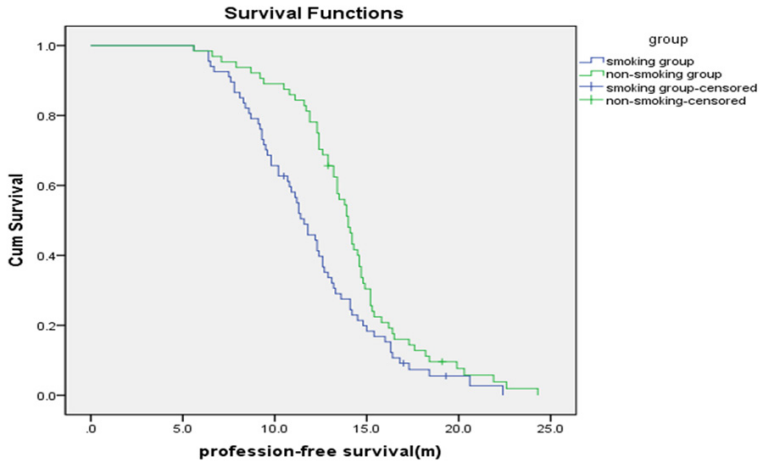

Figure 2. Kaplan-Meier curve for profession-free survival time.

In Group B, we found that low expression levels of *ERCC1* and *RRM1* were also associated with a sensitive response to platinum-based chemotherapy (**Table 3**).

The role of ERCC1 and RRM1 on the prognosis of patients

By comparing the patients' therapeutic effect and survival in both groups (**Table 4**), we found that Group B displayed higher response rate (59.3% vs. 32.8%; *P*=0.045). We also found that 1-year recurrent rate in Group A was higher than Group B (52.2% vs. 25.0%; *P*=0.001). Median PFS time was 12.0 months in the Group A versus 14.1 months in the Group B. The statistical analysis shows significant differences between two groups (*P*=0.008). Moreover, a longer median OS was observed in the Group B (27.6 months vs. 22.9 months; *P*=0.005 **Figures 1** and **2**).

Discussion

Nowadays, a wide range of molecular markers are being tested and several candidates such as ERCC1 and RRM1 have been found to present prognostic value in advanced NSCLC patients [1, 10]. However, conclusive data are lacking regarding the relationship between smoking and the expression of *ERCC1* and *RRM1*. Our study aimed to assess the expression of *ERCC1* and *RRM1* in NSCLC patients with smoking history or not and investigate the therapeutic effect of biomarker-guided chemotherapy in those patients.

The results of our study show that the expression of *ERCC1* was significantly higher in smoking patients as compared with non-smoking group (*P*=0.028), which is inconsistent with data presented by Xu et al. [11]. We speculate that the difference may be caused by study design, sample sizes, factors used to define smoking. We also compared patients' characteristics in both groups and there were no significant difference in patients' age, tumor size or pathological types.

In the present study, we also found that patients with low expressions of *ERCC1* and *RRM1* were associated with a good response to platinum-based chemotherapy in both groups. Several studies have reported that ERCC1 and/or RRM1 expression-based chemotherapy could improve clinical outcomes in advanced NSCLC. Gong et al. [12] reported that Low/negative RRM1 expression in advanced NSCLC was associated with higher response rate to gemcitabine-containing regimen and better prognosis. Reynolds C et al. [13] found that the expression of ERCC1 and RRM1 levels are predic-

tive of response to therapy. Lord RV [14] et al. reported that association between lower *ERCC1* mRNA expression levels and improved survival after treatment with a combination Gem/CDDP regimen for patients with advanced stage NSCLC. However, we noticed that a recent study by Toffart et al. [15] did not observe a chemotherapy response or survival from individualized therapy using ERCC1, BRCA1 and TUBB3, expression as molecular signatures. We analysis that the difference may be used a different approaches to detect the expression of *ERCC1* and *RRM1.* In their study, Toffart et al. used the tumor tissue samples for an immunohistochemical (IHC) method evaluation of eight biomarkers. IHC is semiquantitative, subjective, and may be affected by a range of poorly controlled variables and it is difficult to identify an objective cutoff value for using IHC in prospective trials [16]. IHC detects ERCC1 and RRM1 at protein level, while RT-PCR assays at mRNA level. Compared to IHC, RT-PCR assays the biomarkers need small tumor sample, which is feasible and applicable in clinical application [17].

After comparing the therapeutic effect and survival in both groups, we found that non-smoking patients had a higher response rate, a lower 1-year recurrent rate, a longer median PFS and OS than those smoking patients. Toh et al. found that overall survival of non-smokers was significantly better than smokers in 975 advanced NSCLC [18]. Nordquist et al. also reported that there was a significant difference in survival between non-smokers and current smokers with mixed surgical and nonsurgical advanced NSCLC, and that non-smoking status was a statistically significant independent predictor of survival based on multivariate analysis [19]. Tokujiro Yano found that both vascular and lymphatic permeation in the primary tumor were less frequently found in never-smoking NSCLC patients than smoking NSCLC patients [20]. The better outcomes of NSCLC in never-smokers were supported by our results.

Our study has two limitations. Firstly, all patients were selected from one hospital, which may cause selection bias. Secondly, smoking histories were mainly obtained in a personal interview in the hospital, which may not reflect the true smoking status of patients. In conclusion, our study has demonstrated that the expression of *ERCC1* was significantly higher in the smokers and the patients with high expression of *ERCC1* were resistant to platinum drugs. Our data suggest that smoking patients may be less responsive to chemotherapy and show earlier metastasis and higher recurrence. Encouragingly, much attention has been focused on the relationship between the gene expression and cigarette consumption in recent years. Xu Y et al. has provided a web server for observing the effects of smoking on gene expression in human lung [21]. Therefore both therapeutic innovations and prevention strategies should be developed in the future to target against smokers with cancer.

Disclosure of conflict of interest

None.

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