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ORIGINAL ARTICLE

RRM1 and ERCC1 expression in peripheral blood versus tumor tissue in gemcitabine/carboplatin-treated advanced non-small cell lung cancer

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Abstract
Purpose To comparatively evaluate the prognostic or predictive value of ribonucleotide reductase M1 (RRM1) and excision repair cross-complementation 1 (ERCC1) gene expression in peripheral blood versus tumor tissue from patients with advanced non-small cell lung cancer (NSCLC) treated by gemcitabine/platinum chemotherapy.
Methods A total of 49 patients with advanced NSCLC receiving gemcitabine plus carboplatin chemotherapy were studied. RRM1 and ERCC1 mRNA levels in the peripheral blood and tumor tissue were determined by real-time fluorescent quantitative PCR. The relationships between gene expression and clinical and pathological factors, response to chemotherapy as well as prognosis, were evaluated.
Results RRM1 expression in peripheral blood and tumor tissue, but not ERCC1 expression, was found to be positively correlated ($r = 0.332$, 0.258; $P = 0.020$, 0.073; respectively). RRM1 and ERCC1 expression levels were nearly synchronous in both peripheral blood ($r = 0.351$; $P = 0.013$) and tumor tissue ($r = 0.634$; $P < 0.001$). Neither was correlated with clinical and pathological factors. Patients with low RRM1 expression in peripheral blood or low RRM1 or ERCC1 expression in tumor tissue experienced better response to chemotherapy (50.0 vs. 16.0%, 50.0 vs. 16.0%, and 54.2 vs. 12.0%; $P = 0.012$, 0.012, and 0.003; respectively), longer median survival (18.5 vs. 13.0 months, 18.5 vs. 12.0 months, and 19.8 vs. 12.5 months; $P = 0.043$, 0.014 and 0.007; respectively), and longer progression-free survival (6.0 vs. 4.0 months, 7.8 vs. 3.9 months, and 5.8 vs. 3.8 months; $P = 0.044$, 0.016, and 0.008; respectively). Cox multivariate regression analysis showed that ERCC1 expression in tumor tissue was independent indicator for overall survival.
Conclusions Advanced NSCLC patients with low RRM1 mRNA expression both in peripheral blood and in tumor tissue could benefit from gemcitabine/carboplatin chemotherapy. ERCC1 mRNA expression in tumor tissue may be a predictive and prognostic indicator in advanced NSCLC patients receiving gemcitabine/carboplatin chemotherapy.

Keywords RRM1 · ERCC1 · Gemcitabine · Non-small cell lung cancer · Real-time fluorescent quantitative PCR

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Introduction

Lung cancer is a high-incidence, high-mortality malignancy. Approximately 85% of all cases present with non-small cell lung cancer (NSCLC), most of which have

already reached an advanced stage when first diagnosed and thus cannot benefit from surgery. Although the widely used first-line chemotherapeutic regimens, which consist of a third-generation cytotoxic compound (e.g., gemcitabine, docetaxel, or vinorelbine) and a platinum agent (e.g., cisplatin or carboplatin), can improve the clinical outcomes of advanced NSCLC patients, only 30–40% patients have shown any response, and the overall 5-year survival rate is below 15% [1].

DNA is the molecular target of many anticancer drugs. Abnormal capacity to repair DNA is closely related to chemo-resistance, which is the main cause of ineffective chemotherapy. Ribonucleotide reductase (RR), which is the major rate-limiting enzyme of DNA synthesis, plays an important role in catalyzing the conversion of ribonucleoside diphosphates into deoxyribonucleoside diphosphates, which are the raw materials of DNA synthesis and repair. The M1 subunit of RR (RRM1) is a nucleotide-binding site. It controls the substrate specificity and overall activity of the enzyme. It is also a cellular target for gemcitabine [2]. Many previous reports have demonstrated that RRM1 may lead to gemcitabine resistance. Advanced NSCLC patients with low RRM1 expression have been found to benefit more from gemcitabine-containing chemotherapy than other patients [3–5]. Excision repair cross-complementation 1 (ERCC1) is one of the key enzymes in the nucleotide excision repair (NER) pathway, and it is involved in DNA damage recognition and DNA strand incision [6]. As shown by most preclinical and clinical studies, ERCC1 expression is negatively correlated with the efficacy of platinum. In this way, low levels of ERCC1 expression may predict increased treatment sensitivity and improved clinical outcomes to platinum-based chemotherapy [7–9].

At present, the predictive role of RRM1 and ERCC1 expression in the efficacy of gemcitabine plus platinum chemotherapy in advanced NSCLC patients remains unproven. Analyses of RRM1 and ERCC1 mRNA expression are mainly based on tumor tissue samples from operative resection, bronchofiberscopy, or percutaneous lung biopsy. However, for many patients with cytologically diagnosed advanced NSCLC who do not have enough tumor tissue samples, no effective method of gene expression detection is currently available.

This study compares the levels of RRM1 and ERCC1 mRNA expression in patients' peripheral blood to those in tumor tissue and evaluates the association between RRM1 and ERCC1 expression and the therapeutic efficacy of gemcitabine/carboplatin chemotherapy in patients with advanced NSCLC in terms of response and prognosis. In this way, our results show whether assessing the levels of RRM1 or ERCC1 mRNA expression, especially in the peripheral blood, would be suitable for use in clinical decision-making.

Materials and methods

Patients

Patients with histologically confirmed advanced NSCLC (stage III or IV), who were not candidates for surgery or radiotherapy with curative intent, were included in this study. Other eligibility criteria included no history of previous chemotherapy; the presence of at least one objectively measurable lesion; tumor tissue sample from bronchofiberscopy or percutaneous lung biopsy of sufficient size for the detection of gene expression; Eastern Cooperative Oncology Group performance status (ECOG-PS) $\leq$1; life expectancy $\geq$3 months; age (range 18–80 years); adequate bone marrow function (WBC count $\geq 4.0 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L); adequate renal and liver function (serum creatinine $\leq$1.5 times normal value; serum transaminase $\leq$2 times normal value). The exclusion criteria included pregnancy or lactation; serious infection or impairment of organ function; more than two metastases in other organs. All patients signed informed consent documents prior to entering the study.

Tumor tissue samples and peripheral venous blood samples predating the first dose of chemotherapy were collected and processed separately. Then, every patient received standard first-line chemotherapy: gemcitabine 1,200 mg/m^2 on days 1 and 8 and carboplatin AUC 5 on day 1 (21 days per cycle). Patient evaluations were carried out at baseline and after two cycles of chemotherapy [10]. The primary endpoint of this study was overall objective response rate, which was defined as confirmed complete and partial responses according to RECIST criteria [11]. The secondary endpoints included overall survival (OS) and progression-free survival (PFS). These were calculated from the start of therapy to the date of death in the case of OS or date of progression or death without progression in the case of PFS. This study was approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University.

Total RNA extraction and real-time fluorescent quantitative PCR

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 (PBMC) were isolated by centrifugation. Total RNA was extracted from PBMC immediately after collection using an EZNA® Blood RNA Mini Kit (Omega, Berkeley, CA, US) according to the manufacturer's instructions. Total isolated RNA sample was stored at −80°C, and complementary DNA (cDNA) was synthesized within 1 week for the total RNA

sample using a Reverse Transcription System (Promega, Madison, WI, US). The cDNA product was then stored at −20°C until use.

Tissue samples were from bronchofiberscopy or percutaneous lung biopsies. After pathologically confirmed by an experienced pathologist, the tumor tissue samples were grounded with mortar and pestle in liquid nitrogen and treated with Trizol-A+. Then, 0.2 mL chloroform was added and mixed with vigorous shaking followed by incubation for 5 min and centrifugation at 12,000*g* and 4°C for 15 min. The upper aqueous phase was transferred into a fresh tube, and 0.5 mL isopropyl alcohol was added for centrifugation. The RNA pellet was washed with 75% ethanol and vacuum-dried. RNA (in DEPC water) was then stored at −80°C. The cDNA was synthesized within 1 week and stored at −20°C until use.

Relative cDNA quantification of the gene expression of RRM1, ERCC1, and housekeeping gene *β*-actin (internal reference gene) was performed in a 96-well optical plate (Applied Biosystems) using real-time fluorescent quantitative PCR [RealMasterMix (SYBR Green) Kit; TIANGEN, Beijing, China] [12]. First, a primer pair for each gene was designed using online Primer 3.0 software based on the GenBank accessions AF107045 (RRM1 human genomic DNA), AF001925 (ERCC1 human genomic DNA); and AY582799 (*β*-actin human genomic DNA). All primers were purchased from Sangon Biotech (Shanghai) Co., Ltd. and the sequences were as follows: RRM1, 5′-ACT AAG CAC CCT GAC TAT GCT ATC C-3′ (sense), 5′-CTT CCA TCA CAT CAC TGA ACA CTT T-3′ (antisense); ERCC1, 5′-CTG GGA ATT TGG CGA CGT AA-3′ (sense) and 5′-ATG GAT GTA GTC TGG GTG CAG-3′ (antisense); *β*-actin, 5′-TGA GCG CGG CTA CAG CTT-3′ (sense), 5′-TCC TTA ATG TCA CGC ACG ATT T-3′ (antisense). Next, in each well, the following were combined: 22.5 μL Real Master Mix/SYBR Solution, 0.5 μL Rox reference dye, 20.5 μL DEPC-treated water, 1 μL of 50 μmol/L each primer pair, and 5 μL template cDNA. PCR conditions included an initial 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 threshold cycle (C_T) was the fractional cycle number at which the fluorescence generated by cleavage of the probe exceeded a fixed level above baseline. We had established a standard curve of the C_T values and the copy numbers of RRM1, ERCC1, and *β*-actin. We were able to get the number of copies for each gene based on their C_T values using this standard curve. Then, the target mRNA expression levels were quantified relative to the expression of *β*-actin, which were calculated as the ratio of the copy numbers of target mRNA to the copy numbers of *β*-actin mRNA.

Statistical analysis

All statistical analyses were carried out using SPSS software (Version 13.0; SPSS Inc., Chicago, IL, U.S.). We first checked the distribution of the RRM1 and ERCC1 expression levels using the Explore procedure in the SPSS software package. We found that the variances of the gene expressions were extremely high and not normally distributed. The cutoff point was selected according to the median value of RRM1 and ERCC1 expression levels to dichotomize patients into low-expression or high-expression RRM1 and ERCC1 subgroups.

The correlation between RRM1 and ERCC1 mRNA levels was evaluated as continuous variables by Spearman correlation coefficient. The Mann–Whitney *U* test was used to assess significant associations between the continuous variable gene expression and dichotomous variables (patient age, gender, tumor stage, response to chemotherapy, etc.). The differences in gene expression were also assessed as dichotomous variables (low vs. high) across all of the clinical and pathological factors using the chi-square test. Kaplan–Meier survival curves and Log-rank testing were used to analyze univariate distributions for OS and PFS. Cox's proportional hazards multivariate analysis was used to evaluate factors that had significant influences on OS and PFS at the univariate analysis. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 52 advanced NSCLC patients were recruited from the First Affiliated Hospital, School of Medicine, Zhejiang University, from February 2008 through May 2009. Three patients withdrew from the study due to side effects, and 49 patients' data were censored at the end of our follow-up period. The last day of follow-up was April 30, 2011. The median follow-up time was 20.0 months (ranging from 6.0 to 31.0 months). During this period, 36 patients died and 13 were still alive at the end of the study. Among the 49 remaining patients, 23 had stage III disease (9 IIIA and 14 IIIB) and 26 had stage IV disease. All 49 patients received a total of 201 cycles of gemcitabine/carboplatin chemotherapy, with a median of 4 cycles per patient. Nine patients received epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) as further lines of treatment, and four patients had significant tumor shrinkage and underwent radiotherapy with curative intent following chemotherapy. The median OS of all patients was 16.5 months (ranging from 4.1 to 31.0 months), and the median PFS was 4.5 months (ranging from 1.5 to

Table 1 Baseline characteristics of 49 Chinese patients with advanced NSCLC

Characteristics	Number	Percentage (%)
Age (years)		
Median (range)	62 (24–80)	
Gender		
Male	33	67.3
Female	16	32.7
Smoking status		
Yes	18	36.7
No	31	63.3
Performance status		
0	19	38.8
1	30	61.2
Stage		
III	23	46.9
IV	26	53.1
Histology		
Squamous	14	28.6
Adeno	35	71.4
Response to chemotherapy		
Partial	16	32.7
Stable	21	42.8
Progressive	12	24.5
Metastasis sites		
Lymph Node	15	30.6
Lung	7	14.3
Bone	15	30.6
Brain	6	12.2
Liver	3	6.1
Others	3	6.1

13.5 months). The baseline characteristics of the 49 patients are shown in Table 1.

RRM1 and ERCC1 expression levels

The median RRM1 expression level in peripheral blood was 1.36×10^{-2} [range: 0.03×10^{-2}–32.86×10^{-2}; mean: 4.32×10^{-2}; standard deviation (SD): 6.18×10^{-2}], and the median ERCC1 expression level in peripheral blood was 14.67×10^{-2} (range: 0.18×10^{-2}–81.13×10^{-2}; mean: 22.09×10^{-2}; SD: 21.92×10^{-2}). In tumor tissue, the median RRM1 and ERCC1 expression levels were 12.40×10^{-2} (range: 0.07×10^{-2}–92.85×10^{-2}; mean: 16.51×10^{-2}; SD: 15.76×10^{-2}) and 23.05×10^{-2} (range: 0.59×10^{-2}–11.45×10^{-1}; mean: 32.15×10^{-2}; SD: 25.18×10^{-2}), respectively.

Among stage III patients, the gene expression levels of RRM1 in peripheral blood ranged from 0.21×10^{-2} to 12.97×10^{-2} (median 1.04×10^{-2}), and ERCC1 expression levels ranged from 0.18×10^{-2} to 52.35×10^{-2} (median 14.67×10^{-2}). In tumor tissue, RRM1 expression levels ranged from 0.33×10^{-2} to 39.49×10^{-2} (median 12.40×10^{-2}), and ERCC1 expression levels ranged from 0.59×10^{-2} to 62.47×10^{-2} (median 19.98×10^{-2}). Among stage IV patients, RRM1 expression levels in peripheral blood ranged from 0.03×10^{-2} to 32.86×10^{-2} (median 2.08×10^{-2}), and ERCC1 expression levels ranged from 0.30×10^{-2} to 81.13×10^{-2} (median 14.14×10^{-2}). RRM1 expression levels in tumor tissue ranged from 0.07×10^{-2} to 92.85×10^{-2} (median 13.01×10^{-2}), and ERCC1 expression levels ranged from 1.04×10^{-2} to 11.45×10^{-1} (median 23.63×10^{-2}), respectively.

Correlation between RRM1 and ERCC1 expression in tumor tissue and peripheral blood

The Spearman's correlation coefficient showed a positive correlation between RRM1 expression levels in peripheral blood and those in tumor tissue ($r = 0.332$, 95% CI: 0.062–0.602, $P = 0.020$, Fig. 1a), but no significant correlation was found in ERCC1 expression levels ($r = 0.258$, 95% CI: 0–0.534, $P = 0.073$, Fig. 1b). RRM1 expression was significantly correlated with ERCC1 expression in both peripheral blood ($r = 0.351$, 95% CI: 0.083–0.619, $P = 0.013$, Fig. 1c) and tumor tissue ($r = 0.634$, 95% CI: 0.413–0.855, $P < 0.001$, Fig. 1d).

Clinical and pathological factors related to RRM1 and ERCC1 expression

Using the Mann–Whitney *U* test, we observed no significant correlation between clinical and pathological factors such as age ($P = 0.904$, 0.222), gender ($P = 0.469$, 0.233), smoking status ($P = 0.787$, 0.885), performance status ($P = 0.652$, 0.277), histology ($P = 0.912$, 0.877), or tumor stage ($P = 0.245$, 0.749), and RRM1 expression levels in either peripheral blood or tumor tissues. Similarly, ERCC1 expression showed no correlation with age ($P = 0.857$, 0.389), gender ($P = 0.316$, 0.296), smoking status ($P = 0.097$, 0.709), performance status ($P = 1.000$, 0.580), histology ($P = 0.232$, 0.507), or tumor stage ($P = 0.317$, 0.298) in peripheral blood or tumor tissues. We also assessed the difference between the levels of gene expression as dichotomous variables (low vs. high) across all of the clinical and pathological factors using the chi-square test, and no significant differences were observed ($P > 0.05$) (Table 2).

In this study, nine patients received EGFR-TKIs as further lines of treatment. There were no significant differences between patients who received EGFR-TKIs and those who did not receive EGFR-TKIs with respect to RRM1 or ERCC1 mRNA expression in the peripheral

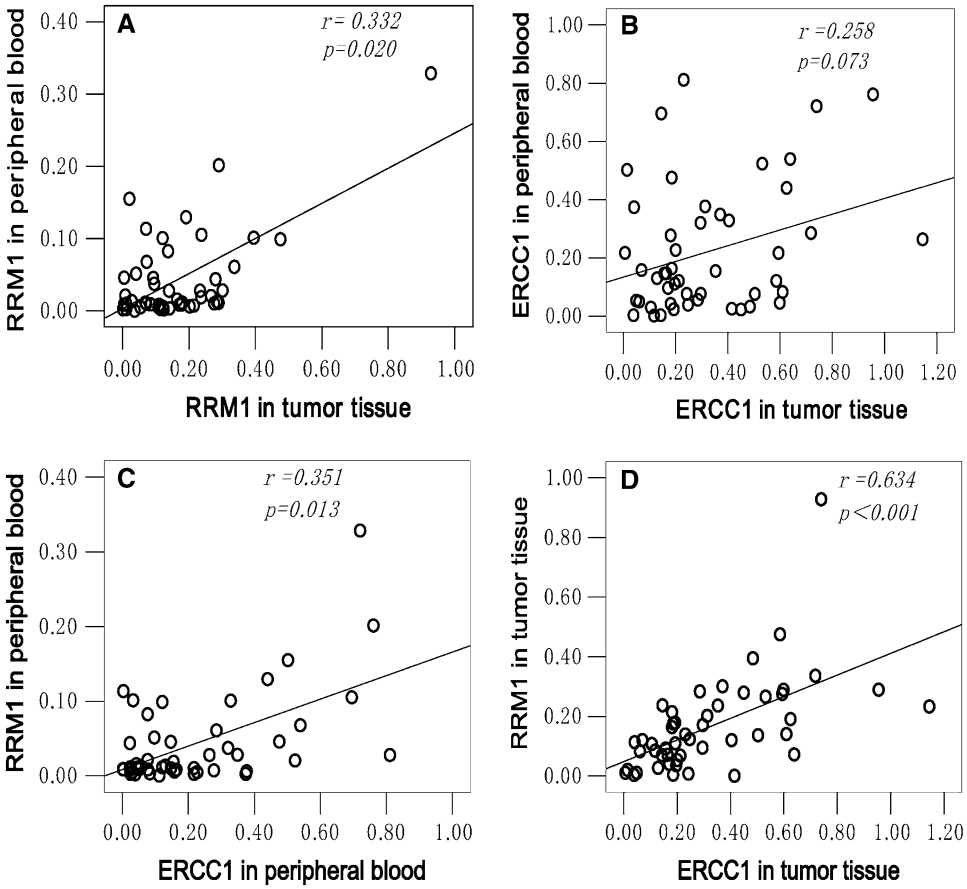

Fig. 1 Correlation between **a** ribonucleotide reductase M1 (RRM1) mRNA levels in peripheral blood and those in tumor tissue and **b** excision repair cross-complementation 1 (ERCC1) mRNA levels in peripheral blood and those in tumor tissue and **c** RRM1 and ERCC1 mRNA levels in peripheral blood and **d** RRM1 and ERCC1 mRNA levels in tumor tissue

blood or tumor tissues. We also included six patients with brain metastases because the levels of RRM1 and ERCC1 gene expression in patients with brain metastases did not significantly differ from those of patients without brain metastases ($P > 0.05$, all Mann–Whitney U test).

Correlation between RRM1 and ERCC1 expression and response to chemotherapy

Patients with low levels of RRM1 and ERCC1 expression in tumor tissue presented better responses to chemotherapy than those with high levels of RRM1 and ERCC1 expression (50.0 vs. 16.0%, $P = 0.012$; 54.2 vs. 12.0%, $P = 0.003$). Similarly, the response rate in patients with low levels of RRM1 expression in peripheral blood was higher than that of patients with high levels of RRM1 expression (50.0 vs. 16.0%, $P = 0.012$). No such correlation was observed for ERCC1 expression in peripheral blood (41.7 vs. 24.0%, $P > 0.05$).

OS and PFS in relation to RRM1 and ERCC1 expression

Patients with low levels of RRM1 expression in the peripheral blood had longer median OS (18.5 vs. 13.0 months, log-rank 4.083, $P = 0.043$, Fig. 2a) and prolonged median PFS (6.0 vs. 4.0 months, log-rank 4.062, $P = 0.044$, Fig. 3a) than those with high levels of expression. However, no statistically significant difference was observed in median OS (17.9 vs. 13.8 months, log-rank 1.235, $P = 0.266$, Fig. 2b) or median PFS (5.6 vs. 4.1 months, log-rank 1.650, $P = 0.199$, Fig. 3b) between patients with low or high levels of ERCC1 expression in the peripheral blood. In addition, patients with high levels of both RRM1 and ERCC1 expression in peripheral blood had shorter median OS (12.0 vs. 18.2 months, log-rank 4.602, $P = 0.032$, Fig. 2c) and median PFS (3.0 vs. 5.9 months, log-rank 9.457, $P = 0.002$, Fig. 3c) than those with at least one gene with low expression.

Patients with low levels of RRM1 or ERCC1 expression in tumor tissue experienced longer median OS (18.5 vs. 12.0 months, 19.8 vs. 12.5 months; log-rank 5.993, 7.215; $P = 0.014$, 0.007; respectively; Fig. 2d, e) and extended median PFS (7.8 vs. 3.9 months, 5.8 vs. 3.8 months; log-rank 5.857, 7.069; $P = 0.016$, 0.008; respectively; Fig. 3d, e). It was found that either median OS (24.6 vs. 12.0 months, log-rank 10.584, $P = 0.001$, Fig. 2f) or PFS (10.3 vs. 4.0 months, log-rank 8.627, $P = 0.003$, Fig. 3f) became significantly increased among patients with low expression

Table 2 Expression of RRM1 and ERCC1 in the groups with different clinical and pathological factors (*n*, %)

Clinical and pathological factors	RRM1 in tumor tissue		RRM1 in peripheral blood		ERCC1 in tumor tissue		ERCC1 in peripheral blood	
	Low ($n = 24$)	High ($n = 25$)	Low ($n = 24$)	High ($n = 25$)	Low ($n = 24$)	High ($n = 25$)	Low ($n = 24$)	High ($n = 25$)
Age (years)								
≤60	12 (50.0)	11 (44.0)	10 (41.7)	13 (52.0)	13 (54.2)	10 (40.0)	11 (45.8)	12 (48.0)
>60	12 (50.0)	14 (56.0)	14 (58.3)	12 (48.0)	11 (45.8)	15 (60.0)	13 (54.2)	13 (52.0)
Gender								
Male	15 (62.5)	18 (72.0)	18 (75.0)	15 (60.0)	18 (75.0)	15 (60.0)	16 (66.7)	17 (68.0)
Female	9 (37.5)	7 (28.0)	6 (25.0)	10 (40.0)	6 (25.0)	10 (40.0)	8 (33.3)	8 (32.0)
Smoking status								
No	15 (62.5)	16 (64.0)	14 (58.3)	17 (68.0)	14 (58.3)	17 (68.0)	18 (75.0)	13 (52.0)
Yes	9 (37.5)	9 (36.0)	10 (41.7)	8 (32.0)	10 (41.7)	8 (32.0)	6 (25.0)	12 (48.0)
Performance status								
0	11 (45.8)	8 (32.0)	10 (41.7)	9 (36.0)	10 (41.7)	9 (36.0)	10 (41.7)	9 (36.0)
1	13 (54.2)	17 (68.0)	14 (58.3)	16 (64.0)	14 (58.3)	16 (64.0)	14 (58.3)	16 (64.0)
Histology								
Adeno	18 (75.0)	17 (68.0)	17 (70.8)	18 (72.0)	17 (70.8)	18 (72.0)	19 (79.2)	16 (64.0)
Squamous	6 (25.0)	8 (32.0)	7 (29.2)	7 (28.0)	7 (29.2)	7 (28.0)	5 (20.8)	9 (36.0)
Tumor stage								
III	11 (45.8)	12 (48.0)	14 (58.3)	9 (36.0)	12 (50.0)	11 (44.0)	11 (45.8)	12 (48.0)
IV	13 (54.2)	13 (52.0)	10 (41.7)	16 (64.0)	12 (50.0)	14 (56.0)	13 (54.2)	13 (52.0)

No significant differences were observed between the levels of gene expression as dichotomous variables (low vs. high) across all of the clinical and pathological factors using the chi-square test ($P > 0.05$)

RRM1 ribonucleotide reductase M1; *ERCC1* excision repair cross-complementation 1

levels of both RRMI and ERCC1 in comparison with those who had high levels of one or both genes in tumor tissue.

Clinical and pathological factors related to OS and PFS

Tumor stage, performance status, and response to chemotherapy were all found to have great impact on OS (log-rank 4.309, 5.358, and 6.875; $P = 0.038$, 0.021 and 0.009, respectively, Table 3). However, only response to chemotherapy significantly affected PFS (log-rank 17.176, $P < 0.001$, Table 4). In addition, the 9 patients who received EGFR-TKIs had slightly but not significantly better prognoses than patients who did not receive EGFR-TKIs (18.5 vs. 14.0 months, log-rank 0.210, $P = 0.647$). The six patients with brain metastases had slightly but not significantly shorter OS values than patients without brain metastases (12.0 vs. 16.5 months, log-rank 0.350, $P = 0.852$).

Cox multivariate regression analysis of potential prognostic factors of advanced NSCLC

Based on the Cox multivariate regression analysis, low levels of ERCC1 expression in tumor tissue [Hazard ratio (HR): 4.361, 95% CI: 1.165–16.327, $P = 0.029$], but not low levels of RRM1 expression, together with concomitant low levels of expression of both RRM1 and ERCC1 in tumor tissue (HR: 12.947, 95% CI: 2.292–73.438, $P = 0.004$) and performance status (HR: 2.253, 95% CI: 1.002–5.064, $P = 0.049$), emerged as independent prognostic factors for OS (Table 3). The response to chemotherapy was found to be a unique and independent prognostic factor for PFS (HR: 5.519; 95% CI: 1.722–17.684; $P = 0.004$; Table 4).

Discussion

Chemotherapy customized according to reliable molecular prognostic and predictive markers may be of great benefit to patients with cancer. Many preclinical and clinical studies have extensively investigated the association between RRM1 and ERCC1 expression levels and chemotherapy resistance in NSCLC. The available dates suggest that, in advanced NSCLC, RRM1 and ERCC1 may be among the most promising predictive markers.

Rosell et al. [4] detected the RRM1 mRNA expression in tumor tissues of 20 NSCLC patients receiving gemcitabine and cisplatin by means of quantitative RT-PCR and found there was a significant increase in OS (13.7 vs. 3.6 months,

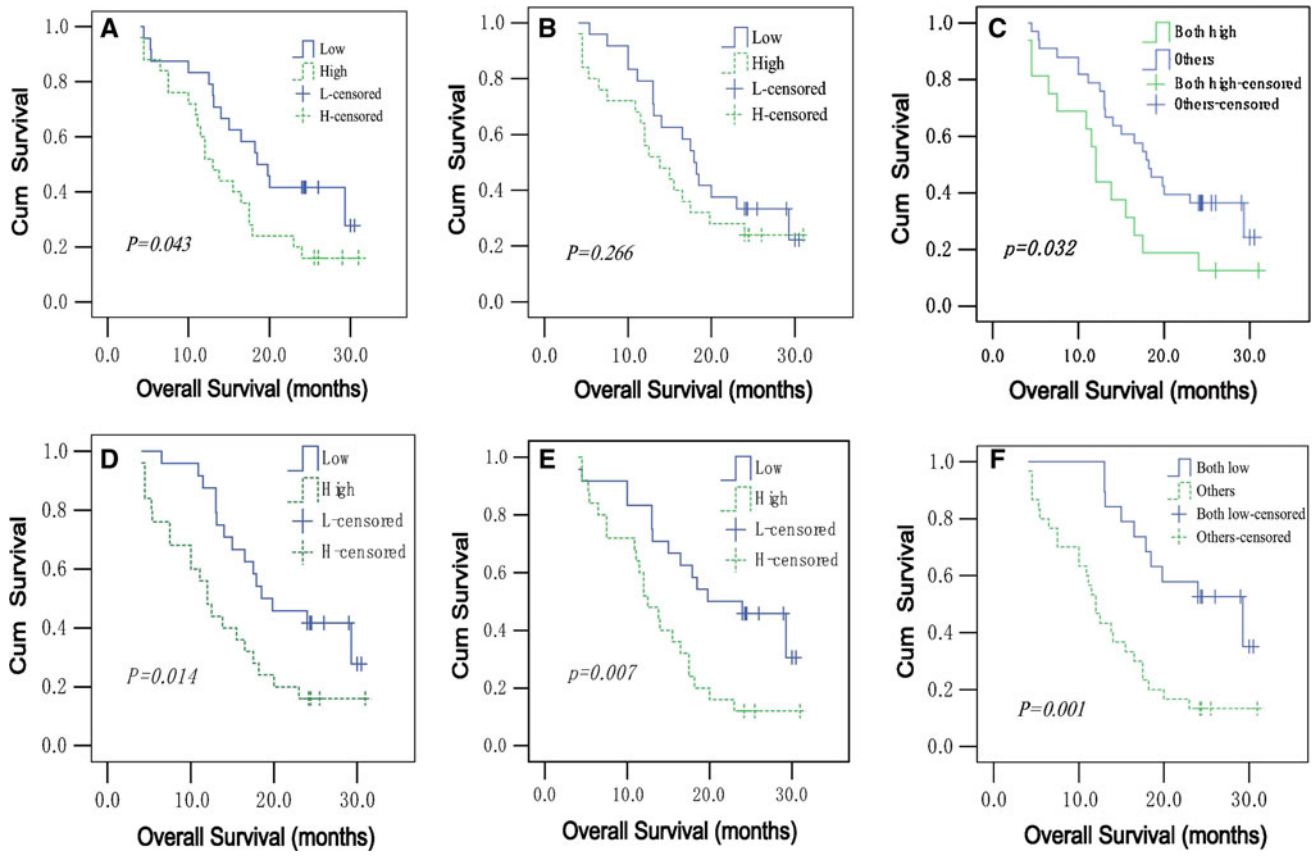

Fig. 2 Overall survival curves for total study population by ribonucleotide reductase M1 (RRM1) and excision repair cross-complementation 1 (ERCC1) expression: **a** RRM1 in peripheral blood **b** ERCC1 in peripheral blood **c** concomitant high expression of RRM1 and ERCC1 in peripheral blood, "Others" refers to patients with at least one gene with low expression **d** RRM1 in tumor tissue **e** ERCC1 in tumor tissue **f** concomitant low expression of RRM1 and ERCC1 in tumor tissue, "Others" refers to patients with at least one gene with high expression

$P = 0.009$) and PFS (8.4 vs. 2.7 months, $P = 0.02$) in patients with low RRM1 mRNA expression levels compared with those with high expression. In a prospective phase II clinical trial, Bepler et al. [3] studied 35 patients with locally advanced NSCLC. They showed that RRM1 expression detected by quantitative RT-PCR was significantly ($P = 0.002$) and inversely correlated ($r = -0.498$) with disease response. Recently, the results of a meta-analysis ($n = 1{,}243$) showed that advanced NSCLC patients with low or negative RRM1 expression experienced a higher response rate than those with high or positive expression [Odds ratio (OR): 0.31, 95% CI: 0.21–0.45, $P < 0.00001$], with up to a 3.94-month increase in OS (95% CI: 2.15–5.73, $P < 0.0001$) and a 2.64-month delay in progression (95% CI: 0.39–4.89, $P = 0.02$), when treated with gemcitabine or gemcitabine plus platinum chemotherapy [13].

In addition, Chen et al. carried out a meta-analysis ($n = 836$) to evaluate correlations between ERCC1 expression and the efficacy of platinum-based chemotherapy in advanced NSCLC patients. The results demonstrated a significantly better response (28.4% vs. 46.7, OR: 0.48, 95% CI: 0.35–0.64, $P < 0.00001$) and longer OS [73.7 vs. 45.1 weeks, Median ratio (MR): 0.77, 95% CI: 0.47–1.07, $P < 0.00001$] in patients with low or negative ERCC1 expression than in patients with high or positive ERCC1 expression [14]. Advanced NSCLC patients with high levels of ERCC1 expression also experienced significantly worse response to chemotherapy [Risk ratio (RR): 0.80, 95% CI: 0.66–0.98] and a higher risk of death (HR: 2.04; 95% CI: 1.48–2.80) than those with low levels of ERCC1 expression [15].

Despite this, the predictive role of RRM1 and ERCC1 expression in the efficacy of gemcitabine plus platinum chemotherapy remains disputable. More evidence must be collected before these two genes can be widely used as markers in clinical practice to provide NSCLC patients with tailored treatment.

In this study, advanced NSCLC patients with low levels of RRM1 expression in tumor tissue presented a higher response rate (50.0 vs. 16.0%, $P = 0.012$) and a noticeable benefit with respect to OS and PFS (18.5 vs. 12.0 months, 7.8 vs. 3.9 months; $P = 0.014$, 0.016;

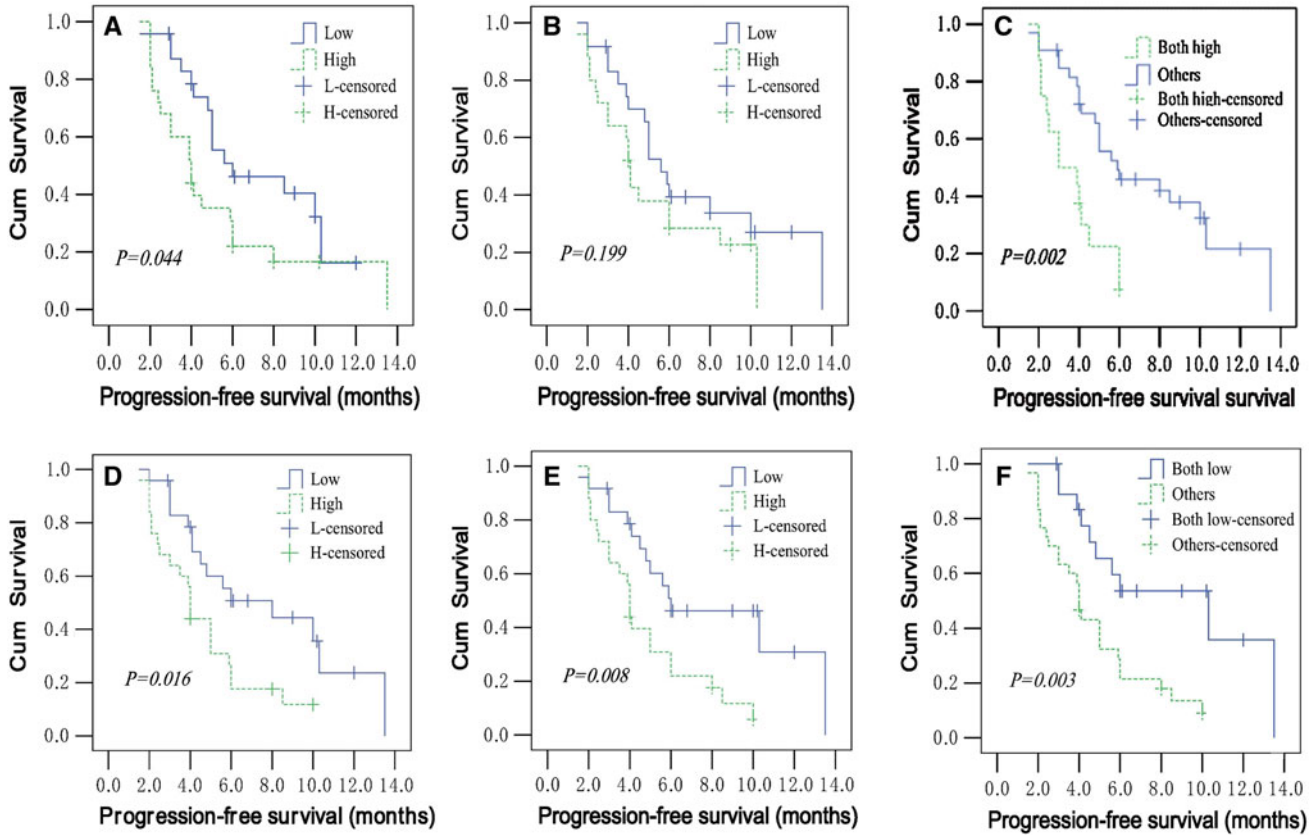

Fig. 3 Progression-free survival curves for total study population by ribonucleotide reductase M1 (RRM1) and excision repair cross-complementation 1 (ERCC1) expression: **a** RRM1 in peripheral blood **b** ERCC1 in peripheral blood **c** concomitant high expression of RRM1 and ERCC1 in peripheral blood, "Others" refers to patients with at least one gene with low expression **d** RRM1 in tumor tissue **e** ERCC1 in tumor tissue **f** concomitant low expression of RRM1 and ERCC1 in tumor tissue, "Others" refers to patients with at least one gene with high expression

respectively) over those with high levels of RRM1 expression. Significant differences were also seen in response to gemcitabine and carboplatin chemotherapy (54.2 vs. 12.0%, $P = 0.003$), median OS (19.8 vs. 12.5 months, $P = 0.007$), and median PFS (5.8 vs. 3.8 months, $P = 0.008$) between patients with low and high levels of ERCC1 expression in tumor tissue. In addition, a strong significant correlation between RRM1 and ERCC1 mRNA expression in tumor tissue was observed in this study ($r = 0.634$, 95% CI: 0.413–0.855, $P < 0.001$). These were consistent with the results of previous studies [16–19].

At present, the quantitative analyses of RRM1 and ERCC1 mRNA expression levels are mainly based on tumor tissue from an operative resection, bronchofiberscopy, or percutaneous lung biopsy. However, most advanced NSCLC patients are diagnosed and confirmed by clinical cellular pathology, and no enough tumor tissue is available for such detection. For this reason, clinical practices require a simpler and more convenient method of detection before individualized treatment can be realized for patients with advanced NSCLC.

RRM1 and ERCC1 are commonly expressed in all types of cells. Vogel et al. [20] found ERCC1 expression in peripheral blood to be associated with DNA repair capacity in NSCLC patients. Dong et al. [21] found there no significant difference in RRM1 expression between peripheral blood mononuclear cells and tumor tissue samples ($P = 0.345$). Recently, Wang et al. [22] conducted a large and impressive study of 591 NSCLC patients treated with first-line platinum-based chemotherapy. They found that patients with NSCLC in the high tertile of DNA repair capacity (DRC) in peripheral lymphocytes had significantly worse overall and 3-year survival than those in the low tertile of DRC (HR: 1.33, 95% CI: 1.04–1.71, $P = 0.023$; and HR: 1.35, 95% CI: 1.04–1.76, $P = 0.025$; respectively). It is promising to use DRC in peripheral lymphocytes as a prognostic factor to guide tailored individual therapeutics for patients with NSCLC. In our study, a positive correlation was observed between tumor tissue and peripheral blood samples with respect to RRM1 mRNA expression ($r = 0.332$, 95% CI: 0.062–0.602, $P = 0.020$). This result must be further confirmed by studies with larger sample sizes. Our result suggests that RRM1 expression in peripheral

Table 3 Factors associated with overall survival

	Number of patients	Median OS (month)	Univariate analysis		Multivariate analysis	
			Log-rank	*P*	HR (95% CI)	*P*
Stage						
III	23	20.0 (13.0–27.0)	4.309	0.038	1.667 (0.730–3.801)	0.225
IV	26	13.0 (11.4–14.6)				
Performance status						
0	19	19.8 (10.3–29.3)	5.358	0.021	2.253 (1.002–5.064)	0.049
1	30	13.0 (11.0–15.0)				
Response						
Partial	16	24.0 (12.4–31.3)	6.875	0.009	2.558 (0.993–6.591)	0.052
Stable/progressive	33	13.1 (9.3–16.9)				
In peripheral blood						
RRM1						
Low	24	18.5 (14.3–22.7)	4.083	0.043	1.276 (0.452–3.608)	0.645
High	25	13.0 (10.2–15.8)				
ERCC1						
Low	24	17.9 (15.5–20.3)	1.235	0.266		
High	25	13.8 (8.9–18.7)				
RRM1 and ERCC1						
Both high	16	12.0 (11.0–13.0)	4.602	0.032	0.545 (0.212–1.401)	0.208
Low level of one or both genes	33	18.2 (14.5–21.9)				
In tumor tissue						
RRM1						
Low	24	18.5 (10.7–26.3)	5.993	0.014	2.179 (0.723–6.567)	0.167
High	25	12.0 (9.7–14.3)				
ERCC1						
Low	24	19.8 (11.6–28.0)	7.215	0.007	4.361 (1.165–16.327)	0.029
High	25	12.5 (9.7–15.3)				
RRM1/ERCC1						
Both low	19	24.6 (16.1–33.5)	10.584	0.001	12.947 (2.292–73.438)	0.004
High level of one or both genes	30	12.0 (10.1–13.9)				

Only the factors that showed significance in univariate analysis were included in the multivariate analysis

RRM1 ribonucleotide reductase M1; *ERCC1* excision repair cross-complementation 1; *OS* overall survival; *CI* confidence interval; *HR* hazard ratio

blood may indirectly predict the efficiency of gemcitabine–platinum combination chemotherapy, but the exact mechanism underlying this is still unknown. We found that patients with low levels of RRM1 expression in peripheral blood experienced more effective chemotherapy (50.0 vs. 16.0%, $P = 0.012$), prolonged median OS (18.5 vs. 13.0 months, $P = 0.043$), and PFS (6.0 vs. 4.0 months, $P = 0.044$) than those with high levels of RRM1 expression. However, no such correlation was found for ERCC1 expression in peripheral blood. The same conclusion was drawn in a previous study conducted by Isla et al. [23].

In conclusion, low levels of RRM1 expression not only in tumor tissue but also in peripheral blood may be associated with better response to treatment and longer median OS and PFS in advanced NSCLC treated by gemcitabine plus carboplatin. ERCC1 expression in tumor tissue may serve as an independent marker of prognosis and sensitivity to gemcitabine plus carboplatin chemotherapy. However, only a few samples could be evaluated in this study, and most of them were stage III NSCLC. In addition, nine patients who received EGFR-TKIs and six patients with brain metastases were included in this study. For these reasons, more prospective random studies, with larger sample sizes, are needed to further evaluate the prognostic and predictive value of RRM1 and ERCC1 expression, especially in peripheral blood.

Table 4 Factors associated with progression-free survival

	N	Median PFS (month)	Univariate analysis		Multivariate analysis	
			Log-rank	*P*	HR (95% CI)	*P*
Response						
Partial	16	10.3 (9.3–10.9)	17.176	<0.001	5.519 (1.722–17.684)	0.004
Stable/progressive	33	4.0 (3.5–4.5)				
In peripheral blood						
RRM1						
Low	24	6.0 (1.1–10.9)	4.062	0.044	1.250 (0.443–3.524)	0.674
High	25	4.0 (2.8–5.2)				
ERCC1						
Low	24	5.6 (4.6–6.6)	1.650	0.199		
High	25	4.1 (3.9–4.3)				
RRM1/ERCC1						
Both high	16	3.0 (1.2–4.8)	9.457	0.002	0.566 (0.205–1.563)	0.272
Low level of one or both genes	33	5.9 (2.0–9.8)				
In tumor tissue						
RRM1						
Low	24	7.8 (3.3–12.7)	5.857	0.016	1.143 (0.407–3.209)	0.800
High	25	3.9 (3.8–4.2)				
ERCC1						
Low	24	5.8 (2.0–9.9)	7.069	0.008	1.699 (0.522–5.533)	0.379
High	25	3.8 (3.7–4.1)				
RRM1/ERCC1						
Both low	19	10.3 (3.9–16.7)	8.627	0.003	3.505 (0.710–17.307)	0.124
High level of one or two genes	30	4.0 (3.7–4.3)				

Only the factors that showed significance in univariate analysis were included in the multivariate analysis

RRM1 ribonucleotide reductase M1; *ERCC1* excision repair cross-complementation 1; *PFS* progression-free survival; *CI* confidence interval; *HR* hazard ratio

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Conflicts of interest None of the authors have any conflicts of interest.

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