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ORIGINAL RESEARCH

The prognostic value of ERCC1 and RRM1 gene expression in completely resected non-small cell lung cancer: tumor recurrence and overall survival

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Apichat Tantraworasin[1]
Somcharoen Saeteng[1]
Nirush Lertprasertsuke[2]
Nuttapon Arayawudhikul[3]
Choosak Kasemsarn[4]
Jayanton Patumanond[5]

[1]General Thoracic Unit, Department of Surgery, Faculty of Medicine, Chiang Mai University Hospital, Chiang Mai, Thailand; [2]Department of Pathology, Faculty of Medicine, Chiang Mai University Hospital, Chiang Mai, Thailand; [3]Cardiovascular Thoracic Unit, Department of Surgery, Lampang Hospital, Lampang, Thailand; [4]Cardiovascular Thoracic Unit, Department of Surgery, Chest Institute, Nonthaburi, Thailand; [5]Department of Community Medicine, Faculty of Medicine, Chiang Mai University Hospital, Chiang Mai, Thailand

Background: The roles of excision repair cross-complementing group 1 gene (*ERCC1*) expression and ribonucleotide reductase subunit M1 gene (*RRM1*) expression in completely resected non-small cell lung cancer (NSCLC) are still debatable. Previous studies have shown that both genes affected the overall survival and outcomes of patients who received platinum-based chemotherapy; however, some studies did not show this correlation. The aim of this study was to evaluate the prognostic values of *ERCC1* and *RRM1* gene expression in predicting tumor recurrence and overall survival in patients with completely resected NSCLC who received adjuvant chemotherapy and in those who did not.

Patients and methods: A retrospective cohort study was conducted in 247 patients with completely resected NSCLC. All patients had been treated with anatomic resection (lobectomy or pneumonectomy) with systematic mediastinal lymphadenectomy between January 2002 and December 2011 at Chiang Mai University Hospital, Chiang Mai, Thailand. They were divided into two groups: recurrence and no recurrence. Protein expression of ERCC1 and RRM1 was determined by immunohistochemistry. Correlations between clinicopathologic variables, including ERCC1 and RRM1 expression and tumor recurrence, were analyzed. Univariate and multivariate Cox proportional hazards regression analysis stratified by nodal involvement, tumor staging, intratumoral blood vessel invasion, intratumoral lymphatic invasion, and tumor necrosis was used to identify the prognostic roles of ERCC1 and RRM1.

Results: ERCC1 and RRM1 expression did not demonstrate prognostic value for tumor recurrence and overall survival in patients with completely resected NSCLC. In patients who did not receive adjuvant chemotherapy treatment, those with high ERCC1 and high RRM1 expression seemed to have greater potential for tumor recurrence and shorter overall survival than did those who had low ERCC1 and low RRM1 (hazard ratio [HR] =1.7, 95% confidence interval [CI] =0.6–4.3, P=0.292 and HR =1.6, 95% CI =0.5–4.5, P=0.411, respectively). In contrast, in patients who received adjuvant chemotherapy treatment, those with high ERCC1 and high RRM1 expression seemed to have benefited from adjuvant chemotherapy and showed good overall survival compared with those who had low ERCC1 and low RRM1 (HR =0.8, 95% CI =0.4–1.8, P=0.612 and HR =0.4, 95% CI =0.1–2.4, P=0.325, respectively). Subgroup analysis in patients whose first-line metastatic chemotherapy failed demonstrated that ERCC1 expression and RRM1 expression were not prognostic factors for tumor recurrence and overall survival; however, patients who had high ERCC1 and high RRM1 expression seemed to have benefited from first-line chemotherapy treatment (HR =0.7, 95% CI =0.3–1.8, P=0.458).

Conclusion: ERCC1 expression and RRM1 expression were not prognostic of tumor recurrence and overall survival in patients with completely resected NSCLC, either with or without adjuvant chemotherapy. Prospective studies that include a larger number of patients are needed for definite conclusions.

Keywords: ERCC1, RRM1, tumor recurrence, prognostic factor, NSCLC

Correspondence: Somcharoen Saeteng
General Thoracic Surgery Unit, Department of Surgery, Faculty of Medicine, 110 Intawaroroj Road, Chiang Mai University Hospital, Chiang Mai, Thailand 50200
Tel +66 815 954 549
Fax +66 539 461 39
Email tengearneae@gmail.com

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Introduction

Excision repair cross-complementing group 1 gene (*ERCC1*) is located on chromosome 19q13.2-q13.3 and contains 10 exons. This gene has many functions. First, it recognizes and incises branched double–single DNA structures and cuts the DNA strand carrying bulky lesions, platinum adducts, or ultraviolet-induced thymine dimers.[1] Second, it allows the repair of stalled DNA replication forks during the synthesis phase (S phase) of cell cycle. Third, it is linked to single-strand break repair.[2] Finally, it plays an important role in double-strand break repair, called single-strand annealing and microhomology-mediated end joining.[3] In the past 15 years, ERCC1 has become recognized for a predictive or prognostic role in advanced non-small cell lung cancer (NSCLC);[4–6] however, no conclusions have been reached about the relationship between ERCC1 expression and clinicopathologic variables, and ERCC1 has not been routinely tested in clinical practice in patients with completely resected NSCLC.

Ribonucleotide reductase M1 gene (*RRM1*), located on chromosome 11p15.4, contains 10 axons that code for 792 amino acid proteins[7] and is a large catalytic subunit of ribonucleotide reductase, which is the main enzyme for de novo synthesis of most deoxyribonucleotides.[1] The vital functions of this gene are responsible for de novo DNA synthesis during the S phase of cell cycle (DNA replication) and DNA repair processes. The prognostic value of RRM1 has been mainly focused on advanced NSCLC treated with a gemcitabine-based regimen combined with platinum compounds.[8–10]

The roles of ERCC1 and RRM1 in completely resected NSCLC are still debatable. The aim of this study was to identify the correlations between ERCC1 and RRM1 expression and tumor recurrence and overall survival in patients with completely resected NSCLC with or without adjuvant chemotherapy, and to study the relationship between RRM1 and these outcomes in patients who were treated with a gemcitabine-based regimen.

Patients and methods

A retrospective cohort study was conducted in consecutive patients diagnosed with NSCLC and treated with anatomic resection and systematic mediastinal lymphadenectomy (labeled according to the *IASLC Staging Manual in Thoracic Oncology*)[11] between January 2002 and December 2011 at Chiang Mai University Hospital, Chiang Mai, Thailand. This study was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Patient characteristics, including signs and symptoms, tumor pathologic reports, and follow-up status, were reviewed from the medical recording system. Patients who had a single brain metastasis and underwent a craniectomy to remove their tumor before pulmonary resection (five patients) or had an evidence of residual tumor at the resection margin (five patients) or died within the first 30 days of the surgery (three patients) were excluded from this study.

All specimens were retrieved from formalin-fixed, paraffin-embedded tissue blocks and sliced at 10 mm intervals. The representative areas were marked and tissue microarrays were performed. Histopathologic examination was reevaluated by a single expert pathologist. Pathologic staging was determined according to the International Association for the Study of Lung Cancer TNM staging classification of NSCLC.[12] Histologic subtypes of lung cancer were determined according to World Health Organization classification[13] and the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma.[14] Presence of visceral pleural invasion, intratumoral blood vessel invasion (IVI), intratumoral lymphatic invasion, tumor necrosis, and perineural invasion were defined per a previous study.[15]

Regarding ERCC1 and RRM1 analysis, tissue microarrays were prepared from representative areas of the cancer from formalin-fixed, paraffin-embedded blocks. Sections of 4 micrometers thickness were cut from microarray blocks and mounted on adhesive-coated or charged glass slides. An indirect immunohistochemical (IHC) method was performed with an automated Benchmark XT detection system (Ventana Medical Systems, Tuscon, AZ, USA). For ERCC1 detection, the primary antibody was mouse monoclonal antibody clone SF1 (1:200 dilution) (Thermo Fisher Scientific, Waltham, MA, USA). For RRM1 detection, the primary antibody was rabbit polyclonal antibody (1:300 dilution) (Proteintech Group Inc, Chicago, IL, USA).

ERCC1 and RRM1 expression were classified in four levels[16] (0 referred to no expression, 1+ referred to 1%–10% expression, 2+ referred to 11%–50% expression, and 3+ referred to more than 50% expression, as shown in Figures 1 and 2) and further divided in two groups, high expression (2+ and 3+) and low expression (0 and 1+). In this study, we did not perform ERCC1 mRNA expression analysis.

All patients were actively followed postoperatively at 2 weeks, at 3–6 month intervals for the first 2 years, and yearly thereafter with a computed tomography scan of the chest and upper abdomen. Patients who had pathologic nodal

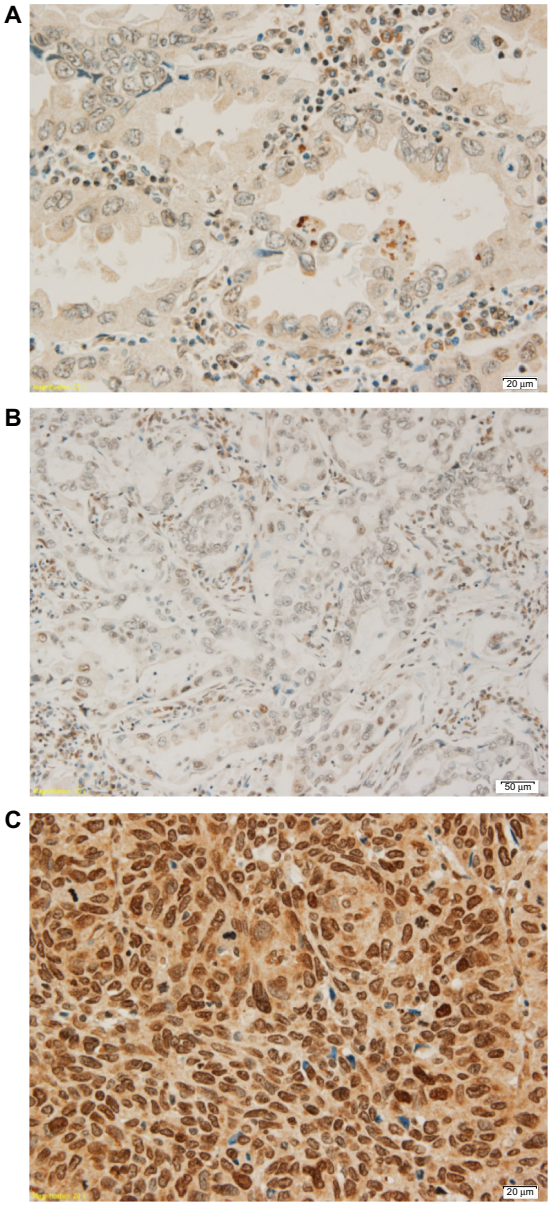

Figure 1 Three levels of ERCC1 gene expression.
Notes: (**A**) ERCC1 expression of adenocarcinoma 1+. (**B**) ERCC1 expression of adenocarcinoma 2+. (**C**) ERCC1 expression of squamous cell carcinoma 3+.
Abbreviation: ERCC1, excision repair cross-complementing group 1 gene.

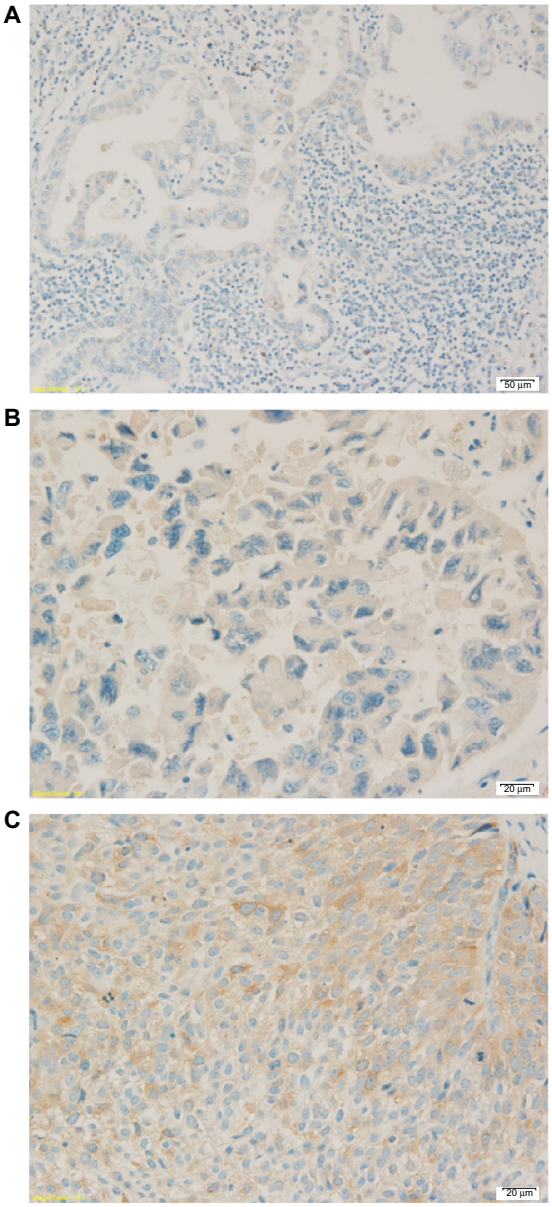

Figure 2 Three levels of RRM1 gene expression.
Notes: (**A**) RRM1 expression of adenocarcinoma 1+. (**B**) RRM1 expression of adenocarcinoma 2+. (**C**) RRM1 expression of squamous cell carcinoma 3+.
Abbreviation: RRM1, ribonucleotide reductase M1 gene.

involvement (stage IIA, IIB, IIIA and IIIB) received adjuvant platinum-based chemotherapy. If patients developed signs or symptoms that correlated with tumor recurrence or metastasis, they would be evaluated according to their signs or symptoms (ie, computed tomography brain or bone scan) and treated with first-line, second-line, and third-line of platinum-based chemotherapy according to usual clinical practice. Tumor recurrence was defined as evidence of a new lesion in the remaining lung, the hilum, the mediastinal lymph nodes, or elsewhere outside the hemithorax. The interval to recurrence was defined as the interval between the time of the operation and the discovery of the recurrence by means of either imaging or cytopathologic examination.

Patients were divided into two groups: recurrence and no recurrence. Categorical variables were expressed as count and percentage and analyzed by Fisher's exact test. Continuous variables were expressed as mean and standard deviation and analyzed by Student's *t*-test or Wilcoxon rank sum test. Tumor recurrence was expressed by using time zero as the date of surgery and recurrence as the end point. A Cox proportional hazards model adjusted for nodal involvement, pathologic staging, IVI, intratumoral lymphatic invasion, and

tumor necrosis was used to identify prognostic factors of tumor recurrence, including ERCC1 and RRM1. Subgroup analysis in patients whose chemotherapy failed (including first-line, second-line, or third-line) was used to identify the prognostic values of ERCC1 and RRM1 for predicting chemotherapy failure or progression of disease after a complete course of chemotherapy. All tests were two-tailed and performed with commercial statistical software, STATA version 11.0 (StataCorp LP, College Station, TX, USA).

Results

Two hundred and forty-seven consecutive patients were enrolled in this study. The numbers of patients in the recurrence and nonrecurrence groups were 128 and 119, respectively. The most common cell type of tumor in both groups was adenocarcinoma (64.8% and 58%, respectively). The general characteristics of patients, including age, sex, smoking status, family history of malignancy, underlying disease, and symptoms, and of surgical procedures are shown by patient group in Tables 1 and 2, respectively; no statistically significant differences were found between groups. The numbers of patients who had a pathologic diagnosis of stage IIIA cancer, postive nodal involvement, or IVI were significantly higher in the recurrence group than in the nonrecurrence group (P=0.004, $P<0.001$, and P=0.004, respectively), as shown in Table 3. The lung was the most common tumor recurrence site (52.3%); the second was brain; and the third was bone, as shown in Table 4.

The univariate analysis of parameters for tumor recurrence demonstrated that stage IIIA cancer (hazard ratio [HR] =3.4, 95% confidence interval [CI] =1.8–6.4, $P<0.001$), positive nodal presence (HR =2.0, 95% CI =1.4–2.8, $P<0.001$), and presence of IVI (HR =1.8, 95% CI =1.3–2.5, P=0.001) were significant prognostic factors of tumor recurrence (Table 5), whereas high ERCC1 expression and high RRM1 expression were not prognostic factors of tumor recurrence. As shown in Table 6, multivariate analysis indicated that ERCC1 and RRM1 were not prognostic factors of tumor recurrence, either in patients who were treated with adjuvant chemotherapy or in those who were not. Moreover, ERCC1 and RRM1 expression did not affect overall survival in patients

Table 1 General characteristics of patients with and without tumor recurrence

Characteristics	Recurrence (n=128)	Nonrecurrence (n=119)	*P*-value
Age (years)	62.1 ± 10.4	62.9 ± 10.1	0.513
Sex			0.513
Male	75 (58.6)	69 (58.0)	
Female	53 (41.4)	50 (42.0)	
Smoking			0.259
Never smoked	27 (21.1)	31 (26.1)	
Stopped smoking	90 (70.3)	82 (68.9)	
Active smokers	7 (5.5)	6 (5.0)	
Passive smokers	4 (3.1)	0 (0.0)	
Packs per year	19.7 ± 16.1	20.7 ± 17.8	0.646
Family history of malignancy	7 (5.5)	10 (8.4)	0.255
Underlying diseases			
Chronic lung disease	21 (16.4)	21 (17.7)	0.464
Diabetic mellitus	14 (10.9)	16 (13.5)	0.341
Essential hypertension	51 (39.8)	40 (33.6)	0.189
Dyslipidemia	19 (14.8)	18 (15.1)	0.546
Symptoms			
Hemoptysis	45 (35.2)	45 (37.8)	0.381
Chronic cough	57 (44.5)	48 (40.3)	0.296
Poor appetite	12 (9.4)	15 (12.6)	0.271
Significant weight loss	27 (21.1)	35 (29.4)	0.087
Chest pain	10 (7.8)	10 (8.4)	0.524
Dyspnea	24 (18.8)	18 (15.1)	0.279
Asymptomatic	48 (37.5)	47 (39.5)	0.424

Note: Values are n (%) or mean ± standard deviation.
Abbreviation: n, number.

Table 2 Surgical procedure and chemotherapy of patients with and without tumor recurrence

Procedure and chemotherapy	Recurrence (n=128)	Nonrecurrence (n=119)	*P*-value
Surgical procedures			
Lobectomy	115 (89.8)	101 (84.9)	0.403
Bilobectomy (RUL and RML)	4 (3.1)	3 (2.5)	
Bilobectomy (RLL and RML)	8 (6.3)	11(9.2)	
Pneumonectomy	1 (0.8)	4 (3.4)	
Chemotherapy			
No chemotherapy	60 (46.9)	71 (59.7)	0.129
Adjuvant chemotherapy	60 (46.9)	43 (36.1)	
Neoadjuvant chemotherapy	8 (6.3)	5 (4.2)	
Chemotherapy regimens			
Paclitaxel plus platinum	31 (47.0)	27 (56.3)	0.914
Docetaxel plus platinum	10 (15.2)	5 (10.4)	
Navelbine plus platinum	11 (16.7)	7 (14.6)	
Gemcitabine plus platinum	3 (4.6)	2 (4.2)	
Other platinum-based regimens	11 (16.7)	7 (14.6)	

Note: Values are n (%).
Abbreviatons: n, number; RUL, right upper lobe; RML, right middle lobe.

Table 3 Histopathologic reports of patients with and without tumor recurrence

Reports	Recurrence (n=128)	Nonrecurrence (n=119)	*P*-value
Histologic types			0.465
Adenocarcinoma	83 (64.8)	69 (58.0)	
Squamous cell carcinoma	31 (24.2)	37 (31.1)	
Others*	14 (10.9)	13 (10.9)	
Tumor grading			0.544
Well differentiated	48 (37.5)	34 (28.6)	
Moderately differentiated	52 (40.6)	55 (46.2)	
Poorly differentiated	20 (15.6)	19 (16.0)	
Undifferentiated	3 (2.3)	7 (5.9)	
Mucinous type of adenocarcinoma in situ	3 (2.3)	2 (1.7)	
Nonmucinous type of adenocarcinoma in situ	2 (1.6)	2 (1.7)	
Pathologic staging			0.004
IA	12 (9.4)	25 (21.0)	
IB	22 (17.2)	25 (21.0)	
IIA	24 (18.8)	21 (17.7)	
IIB	15 (11.7)	21 (17.7)	
IIIA	53 (41.4)	24 (20.2)	
IIIB	2 (1.6)	3 (2.5)	
Tumor diameter			0.340
≤5 cm	84 (65.6)	82 (68.9)	
>5 cm	44 (34.4)	37 (31.1)	
Nodal involvement			<0.001
Nodal negative	61 (47.7)	83 (69.8)	
Nodal positive†	67 (52.3)	36 (30.3)	
Tumor necrosis	55 (43.0)	46 (38.7)	0.288
Visceral pleural invasion	27 (21.1)	21 (17.7)	0.301
Neural invasion	7 (5.5)	3 (2.5)	0.198
Intratumoral lymphatic invasion	112 (87.5)	95 (79.8)	0.072
Intratumoral blood vessel invasion	63 (49.2)	38 (31.9)	0.004
ERCC1 expression			0.272
Low expression	77 (60.2)	77 (64.7)	
High expression	51 (39.8)	42 (35.3)	
RRM1 expression			0.375
Low expression	81 (63.3)	72 (60.5)	
High expression	47 (36.7)	47 (39.5)	

Notes: Values are n (%). *Other cell types included adenocarcinoma in situ, large cell carcinoma, adenoid cystic carcinoma,lymphoepithelioma-like carcinoma, and adenosquamous cell carcinoma; †nodal positive refers to the presenting of malignant cells in any node level (1–14).

Abbreviations: n, number; ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene.

with completely resected NSCLC with or without adjuvant chemotherapy, as shown in Table 7. There were no correlations between RRM1 expression and outcomes in patients who were treated with a gemcitabine-based regimen in terms of disease progression after receiving first-line treatment (HR =1.0, 95% CI =0.6–1.7, *P*=1.000).

Table 4 Sites of tumor recurrence (metastases)

Sites	Number of patients	Percent
Lung	67	52.3
Brain	38	29.7
Bone	22	17.2
Mediastinal lymph node	10	7.8
Pleura	8	6.3
Supraclavicular lymph node	7	5.5
Adrenal gland	6	4.7
Liver	4	3.1
Skin	4	3.1
Chest wall	3	2.3
Spleen	2	1.6
Kidney	1	0.8
Cervical lymph node	1	0.8
Stomach	1	0.8
Groin node	1	0.8

Subgroup analysis of the prognostic value of ERCC1 and RRM1 expression to predict chemotherapy failure or progression of disease after a complete course of chemotherapy demonstrated that ERCC1 expression and RRM1 expression were not prognostic factors of failed chemotherapy in univariate and multivariate models, as shown in Tables 8 and 9, respectively.

Discussion

ERCC1 is a DNA repair gene coding 5′ endonuclease in nucleotide excision repair pathway and has a vital rule in genomic stability, which is an important step in lung cancer pathogenesis.[17] For 15 years, ERCC1 has been recognized for its prognostic role in advanced NSCLC;[4–6] however, no conclusions have been reached regarding the relationship between ERCC1 expression and clinical outcomes (tumor recurrence and overall survival) in patients with completely resected NSCLC. In our study, univariate and multivariate analyses revealed no statistically significant differences between ERCC1 expression and tumor recurrence or overall survival in patients with completely resected NSCLC who received or did not receive adjuvant chemotherapy treatment. However, patients who had not received adjuvant chemotherapy and who had low ERCC1 expression tended to survive longer than those with high ERCC1 expression (HR =0.8, 95% CI =0.3–2.0, *P*=0.596), whereas patients who had received adjuvant chemotherapy and who had high ERCC1 expression tended to survive longer than those with low ERCC1 expression (HR =0.4, 95% CI =0.1–2.4, *P*=0.325). The trends in our results corresponded with several previous studies. Pesta et al[18] reported a group of 90 patients with NSCLC who underwent curative lung resection and concluded that patients who

Table 5 Univariate hazard ratios and 95% confidence intervals of tumor recurrence for parameters with clinical and statistical significance

Parameters	HR	95% CI	*P*-value
Age >70 years	1.0	0.7–1.5	0.965
Male	1.1	0.8–1.5	0.654
Smoking			
Never smoked	Reference		
Stopped smoking	1.4	0.9–2.2	0.107
Active smokers	1.4	0.6–3.1	0.470
Passive smokers	1.8	0.6–5.2	0.259
Histologic types			
Others*	Reference		
Adenocarcinoma	1.0	0.6–1.8	0.962
Squamous cell carcinoma	0.8	0.4–1.5	0.507
Tumor grading			
Well differentiated	Reference		
Moderately differentiated	0.9	0.6–1.3	0.432
Poorly differentiated	0.9	0.5–1.5	0.671
Undifferentiated	0.4	0.1–1.4	0.160
Mucinous type of adenocarcinoma in situ	0.9	0.3–2.7	0.785
Nonmucinous type of adenocarcinoma in situ	0.9	0.2–3.9	0.941
Pathologic staging			
IA	Reference		
IB	1.7	0.8–3.5	0.136
IIA	2.1	1.1–4.2	0.039
IIB	1.9	0.9–4.0	0.102
IIIA	3.4	1.8–6.4	<0.001
IIIB	2.3	0.5–10.1	0.284
Tumor diameter >5 cm	1.3	0.9–1.9	0.146
Nodal positive†	2.0	1.4–2.8	<0.001
Tumor necrosis	1.3	0.9–1.9	0.103
Visceral pleural invasion	1.2	0.8–1.9	0.376
Neural invasion	1.6	0.8–3.5	0.214
Intratumoral lymphatic invasion	1.6	0.9–2.6	0.099
Intratumoral blood vessel invasion	1.8	1.3–2.5	0.001
High ERCC1 expression	1.2	0.8–1.7	0.295
High RRM1 expression	0.9	0.6–1.3	0.673
Chemotherapy			
No chemotherapy	Reference		
Adjuvant chemotherapy	1.4	0.9–1.9	0.086
Neoadjuvant chemotherapy	1.4	0.7–3.0	0.344

Notes: *Other cell types included adenocarcinoma in situ, large cell carcinoma, adenoid cystic carcinoma, lymphoepithelioma-like carcinoma, and adenosquamous cell carcinoma; †nodal positive refers to the presenting of malignant cells in any node level (1–14).
Abbreviations: HR, hazard ratio; CI, confidence interval; ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene.

had been treated with adjuvant chemotherapy and had shown low expression of ERCC1 had adverse prognoses. Many previous studies reported converse results. In 2008, a large retrospective cohort study of the International Adjuvant Lung Trial-bio study enrolled 867 patients with resected NSCLC;[19] using IHC, the researchers found that ERCC1-negative tumor produced a significantly prolonged survival in patients who had received adjuvant chemotherapy (adjusted HR =0.65, 95% CI =0.50–0.86, *P*=0.002), whereas patients who did not received adjuvant chemotherapy and had ERCC1-positive tumors survived longer than those with ERCC1-negative tumors (adjusted HR =0.66, 95% CI =0.49–0.90, *P*=0.009). They concluded that patients with completely resected NSCLC and ERCC1-negative tumors appeared to benefit from adjuvant cisplatin-based chemotherapy, whereas patients with ERCC1-positive tumors did not. Lee et al[20] demonstrated that patients with a positive ERCC1 expression without adjuvant chemotherapy survived longer than ERCC1-negative patients (median overall survival 7.6 years for ERCC1-positive versus 4.0 years for ERCC1-negative, *P*=0.046) and concluded that ERCC1 expression was a positive prognostic marker in resected NSCLC. Bepler et al[21] reported that low ERCC1 indicated a significant benefit for adjuvant chemotherapy (HR =0.73 for chemotherapy versus control, *P*=0.02).

Table 6 Multivariate hazard ratios and 95% confidence intervals of tumor recurrence for ERCC1 and RRM1 expression

ERCC1 expression	RRM1 expression	HR	95% CI	*P*-value
Without adjuvant chemotherapy				
Low	Low	Reference		
Low	High	0.6	0.3–1.6	0.360
High	Low	1.4	0.5–3.5	0.496
High	High	1.7	0.6–4.3	0.292
With adjuvant chemotherapy				
Low	Low	Reference		
Low	High	1.1	0.5–2.6	0.818
High	Low	1.4	0.6–3.2	0.471
High	High	0.8	0.4–1.8	0.612

Note: All analyses adjusted for nodal involvement, pathologic staging, intratumoral blood vessel invasion, intratumoral lymphatic invasion, and tumor necrosis.
Abbreviations: ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene; HR, hazard ratio; CI, confidence interval.

Table 7 Multivariate hazard ratios and 95% confidence intervals of death for ERCC1 and RRM1 expression

ERCC1 expression	RRM1 expression	HR	95% CI	*P*-value
Without adjuvant chemotherapy				
Low	Low	Reference		
Low	High	0.8	0.3–2.0	0.596
High	Low	1.6	0.5–4.5	0.411
High	High	1.1	0.4–2.8	0.876
With adjuvant chemotherapy				
Low	Low	Reference		
Low	High	1.6	0.1–18.5	0.696
High	Low	2.3	0.6–9.2	0.222
High	High	0.4	0.1–2.4	0.325

Note: All analyses adjusted for nodal involvement, pathologic staging, intratumoral blood vessel invasion, intratumoral lymphatic invasion, and tumor necrosis.
Abbreviations: ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene; HR, hazard ratio; CI, confidence interval.

Table 8 Univariate hazard ratios and 95% confidence intervals of failed chemotherapy after tumor recurrence for parameters with clinical and statistical significance

Parameters	HR	95% CI	*P*-value
Age >70 years	0.9	0.4–1.8	0.684
Male	1.0	0.6–1.7	0.907
Smoking			
Never smoked	Reference		
Stopped smoking	0.9	0.5–1.6	0.738
Active smokers	0.5	0.1–2.1	0.354
Passive smokers	0.6	0.1–4.3	0.591
Histologic types			
Others*	Reference		
Adenocarcinoma	1.0	0.4–2.3	0.980
Squamous cell carcinoma	0.8	0.3–2.2	0.686
Tumor grading			
Well differentiated	Reference		
Moderately differentiated	1.1	0.6–1.9	0.757
Poorly differentiated	0.5	0.1–2.0	0.316
Undifferentiated	–	–	–
Mucinous type of adenocarcinoma in situ	1.8	0.4–8.0	0.392
Nonmucinous type of adenocarcinoma in situ	1.1	0.4–3.2	0.874
Pathologic staging			
IA	Reference		
IB	0.8	0.3–2.2	0.638
IIA	1.4	0.5–3.9	0.472
IIB	1.7	0.6–4.8	0.315
IIIA	1.0	0.4–2.4	0.910
IIIB	1.8	0.4–7.3	0.411
Tumor diameter >5 cm	1.7	0.9–2.9	0.083
Nodal positive†	1.0	0.6–1.6	0.835
Tumor necrosis	0.7	0.4–1.3	0.307
Visceral pleural invasion	1.0	0.5–1.9	0.927
Neural invasion	1.2	0.3–4.8	0.841
Intratumoral lymphatic invasion	0.8	0.4–1.7	0.532
Intratumoral blood vessel invasion	1.4	0.8–2.4	0.208
High ERCC1 expression	0.8	0.5–1.5	0.509
High RRM1 expression	0.8	0.5–1.3	0.381
Regimens of chemotherapy			
Platinum plus other drug	Reference		
Platinum plus gemcitabine	0.8	0.5–1.5	0.509

Notes: *Other cell types include: adenocarcinoma in situ, large cell carcinoma, adenoid cystic carcinoma, lymphoepithelioma-like carcinoma and adenosquamous cell carcinoma; †nodal positive refers to the presenting of malignant cells in any node level (1–14).
Abbreviations: HR, hazard ratio; CI, confidence interval; ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene.

Custodio et al[22] and Olaussen et al[19] also reported that patients with ERCC1-negative tumors derived a substantial benefit from adjuvant chemotherapy, when compared with patients with ERCC1-positive tumors.

Patients with advanced disease or tumor recurrence were treated with platinum-based chemotherapy. The results from univariate and multivariate analysis in this study demonstrated that ERCC1 expression was not a predictive

Table 9 Multivariate hazard ratios and 95% confidence intervals of failed chemotherapy after tumor recurrence for ERCC1 and RRM1 expression

ERCC1 expression	RRM1 expression	HR	95% CI	*P*-value
Low	Low	Reference		
Low	High	1.2	0.4–3.2	0.782
High	Low	1.8	0.5–6.7	0.397
High	High	0.7	0.3–1.8	0.458

Note: All analyses adjusted for nodal involvement, pathologic staging, intratumoral blood vessel invasion, intratumoral lymphatic invasion, and tumor necrosis.
Abbreviations: ERCC1, excision repair cross-complementing group 1 gene; RRM1, ribonucleotide reductase M1 gene; HR, hazard ratio; CI, confidence interval.

value for failed chemotherapy; however, there was a trend toward increased risk for failed chemotherapy if patients had high ERCC1 expression but had low RRM1 expression (HR =1.8, 95% CI =0.5–6.7, *P*=0.397). Patients who had a high expression of ERCC1 and RRM1 trended to have a good response to chemotherapy (HR =0.7, 95% CI =0.3–1.8, *P*=0.458). Reynolds et al[10] also reported no predictive value for ERCC1 expression when assessed by IHC. Booton et al[23] demonstrated that ERCC1 expression did not favor a prognostically better outcome after platinum-based chemotherapy in advanced NSCLC; however, this study identified ERCC1 by mRNA analysis, which was different from our study. Wang et al[4] retrospectively reviewed 124 patients with advanced NSCLC and reported that patients with ERCC1-negative tumors but RRM1-positive tumors had a longer median survival time than those with ERCC1-positive tumors (13.4 months versus 9.1 months, *P*=0.007). Roth et al[24] performed a systematic review and meta-analysis of 11 studies on the prognostic role of ERCC1 in advanced NSCLC and reported that patients who had high ERCC1 expression had a significantly lower response (risk ratio =0.8, 95% CI =0.66–0.98) and a significantly higher risk for death (HR =2.04, 95% CI =1.48–2.80). However, a recent study by Friboulet et al[25] reported using the 8F1 antibody to measure the level of ERCC1 expression by means of IHC analysis in a validation set of samples obtained from 494 patients in two independent Phase III trials (the National Cancer Institute of Canada Clinical Trials Group JBR.10[26] and the Cancer and Leukemia Group B 9633 Trial from the Lung Adjuvant Cisplatin Evaluation Biology Project).[27] Both trials studied the correlation between the absence of ERCC1 expression and platinum response. They mapped the epitope recognized by 16 commercially available ERCC1 antibodies and investigated the capacity of the different ERCC1 isoforms to repair platinum-induced DNA damage. The results of their study showed that there was low validity for detecting the predictive

effect of immunostaining for ERCC1 protein. None of the 16 antibodies could distinguish among the four ERCC1 protein isoforms, whereas only one isoform produced a protein that had full capacities for nucleotide excision repair and cisplatin resistance. They concluded that IHC analysis with the use of currently available ERCC1 antibodies did not specifically detect the unique functional ERCC1 isoform; therefore, the usefulness of IHC for ERCC1 in guiding therapeutic decision making was limited.

RRM1 is a region with a frequent loss of heterozygosity in NSCLC[28] and is involved in tumor invasiveness and metastasis.[29] The important mediator that affects RRM1 to regulate cellular signaling, survival, and migration is phosphatase and tensin homologue.[22] In our study, RRM1 expression had no prognostic value for tumor recurrence or overall survival in patients with completely resected NSCLC, whether they had received adjuvant chemotherapy or not. Moreover, there was no correlation between RRM1 expression and disease progression in patients treated with a gemcitabine-based regimen (HR =1.0, 95% CI =0.6–1.7, P=1.000). However, patients who did not receive adjuvant chemotherapy with high RRM1 and ERCC1 expression tended to have more potential for tumor recurrence than those with low RRM1 and ERCC1 expression (HR =1.7, 95% CI =0.6–4.3, P=0.292), whereas patients who received adjuvant chemotherapy and who had high RRM1 and ERCC1 expression tended to benefit from adjuvant chemotherapy (HR =0.8, 95% CI =0.4–1.8, P=0.612). About overall survival, patients with high RRM1 and ERCC1 expression who received adjuvant chemotherapy tended to have a longer survival than those with low RRM1 and ERCC1 expression (HR =0.4, 95% CI =0.1–2.4, P=0.325). Subgroup analysis of failed chemotherapy after treatment with first-line, second-line, or third-line chemotherapy demonstrated that patients with high RRM1 and ERCC11 expression tend to benefit from chemotherapy (HR =0.7, 95% CI =0.3–1.8, P=0.458). RRM1 expression did not affect outcomes of patients who were treated with a gemcitabine-based regimen; however, in this study, only 14 patients (12%) received a gemcitabine-based regimen (ten patients for first-line and two patients for second-line chemotherapy). In previous studies, high RRM1 expression was associated with prolonged survival in NSCLC patients.[30] Recently, Bepler et al[31] performed randomized international Phase III trials of ERCC1 and RRM1 expression-based chemotherapy versus gemcitabine/carboplatin in advanced NSCLC and demonstrated that patients in both treatment arms with low ERCC1 and RRM1 expression who received the same treatment (the control arm was gemcitabine/carboplatin and the experimental arm was docetaxel/carboplatin) had a statistically better progression-free survival (8.1 months in the control arm and 5.0 months in experimental arm, P=0.02). Gong et al[32] reported a meta-analysis of 18 studies of the correlation between RRM1 expression and clinical outcomes of gemcitabine-containing regimen in advanced NSCLC (n=1,243) and found that patients with low/negative RRM1 expression had a significantly higher response rate to a gemcitabine-containing regimen (odds ratio =0.31, 95% CI =21–0.45, P<0.001). Patients with low/negative RRM1 who were treated with a gemcitabine-containing regimen survived 3.94 months longer (95% CI =2.15–5.73, P<0.001) and had a longer time of progression (2.64 months; 95% CI =0.39–4.89, P=0.02) than those with high/positive RRM1. They concluded that low/negative RRM1 expression was associated with better response to a gemcitabine-containing regimen and better prognosis in patients with advanced NSCLC. A few previous studies focused on patients with early-stage NSCLC who were treated with surgery only. Zheng et al[33] retrospectively reviewed 187 patients with completely resected NSCLC without adjuvant chemotherapy and reported that a low RRM1 expression was associated with a median overall survival of more than 60 months, and high RRM1 expression with more than 120 months.

Limitations of this study include the retrospective nature of the study, a small number of patients who received a gemcitabine-containing regimen, and the small sample size.

Conclusion

Our study demonstrated that ERCC and RRM1 expression have no prognostic value for tumor recurrence and overall survival, either in the setting of patients with completely resected NSCLC with or without platinum-based adjuvant chemotherapy or patients with failed first-line, second-line, or third-line chemotherapy. Furthermore, ERCC1 and RRM1 expression do not have prognostic value for the benefits of gemcitabine-containing regimen. Our results contrast with previous reports that high ERCC1 and RRM1 expression tend to be associated with long survival among patients with completely resected NSCLC and are also associated with an adverse response to chemotherapy. The relationship between ERCC1 expression, RRM1 expression, and clinical outcomes of chemotherapy treatment in completely resected NSCLC and advanced NSCLC still needs to be clarified. There are some ongoing clinical trials based on ERCC1 and/or RRM1 expression in NSCLC.

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Disclosure

The authors report no conflict of interest in this work.

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