ORIGINAL RESEARCH

RRM1 expression and the clinicopathological characteristics of patients with non-small cell lung cancer treated with gemcitabine

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Background: The usefulness of ribonucleotide reductase catalytic subunit M1 (RRM1) for predicting the therapeutic effects of gemcitabine-containing chemotherapy in patients with non-small cell lung cancer (NSCLC) remains controversial. RRM1-positive patients show unique clinicopathological features.

Methods: Here, we performed a meta-analysis to systematically evaluate the relationship between RRM1 expression and the clinicopathological characteristics of NSCLC patients treated with gemcitabine-containing regimens. A comprehensive electronic and manual search was performed to identify relevant articles. The pooled relative risk (RR) and 95% CI were used to estimate the relation between the clinicopathological characteristics of NSCLC patients and RRM1 expression.

Results: The study included 31 observational studies and 3,667 patients. The analysis showed no significant association between RRM1 expression and pathological type, stage, and smoking status; however, RRM1 positivity was significantly lower in women than in men (43.0% vs 51.7%, RR=0.84, 95% CI: 0.74–0.94, P=0.004).

Conclusion: The present pooled analyses demonstrated that RRM1 positivity in women with advanced NSCLC was associated with a higher rate of response to gemcitabine-containing regimens. Immunohistochemistry may be valuable to prescreen for RRM1 expression in clinical practice, whereas PCR can be routinely used as a verification method. These findings will help design suitable molecular-targeted therapies for NSCLC.

Keywords: RRM1, gemcitabine, meta-analysis, clinicopathological features, NSCLC

Introduction

Lung cancer is a common malignancy and the main cause of cancer-related mortality in the world. Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer patients worldwide.[1–3] Lung carcinoma is divided into two subtypes according to the response to conventional treatments and the histological features of tumors: small cell lung carcinoma and NSCLC. Despite advances in the therapeutic approaches to the treatment of lung cancer, including immunotherapy, chemotherapy, radiotherapy, and noninvasive surgery, the 5-year relative survival rate of patients with lung cancer is ~18% in the USA.[1]

First-line chemotherapy for NSCLC usually consists of a platinum-containing doublet regimen. Patients with advanced NSCLC are generally treated with chemotherapy drugs, including cisplatin and gemcitabine.[4] Gemcitabine, a pyrimidine nucleoside antimetabolite, is active in advanced NSCLC, especially in combination with a platinum derivative or a next-generation anticancer agent.[5,6] Pharmacoeconomic evaluation suggests that a gemcitabine-based regimen is the least costly regimen for the treatment of advanced NSCLC.[7] However, patients with advanced NSCLC may

develop resistance to gemcitabine, which is associated with a poor prognosis. Therefore, identification of the markers for predicting clinical outcomes and treatment sensitivity in patients receiving gemcitabine chemotherapy would be of great value.

Ribonucleotide reductase catalyzes the reduction of ribonucleotide 5-diphosphate to deoxyribonucleotide 5-diphosphate for DNA synthesis and damage repair. The ribonucleotide reductase catalytic subunit M1 (RRM1) gene is located on chromosome segment 11p15.5 and encodes a key enzyme involved in DNA synthesis that catalyzes the biosynthesis of deoxyribonucleotides.[8,9] Preclinical studies show that RRM1 is involved in gemcitabine sensitivity in NSCLC.[10,11] High levels of RRM1 are associated with a decreased response to gemcitabine-containing regimens, whereas RRM1 downregulation is associated with a high rate of response to gemcitabine-containing regimens.[12,13] Therefore, RRM1 may be a predictive biomarker for gemcitabine chemotherapy in NSCLC.

Reverse transcriptase PCR (RT-PCR) and immunohistochemistry (IHC) are currently used to detect RRM1. However, an effective algorithm for RRM1 gene screening in the clinical lung cancer population remains undetermined because these two approaches have both advantages and disadvantages. Thus, to increase the detection efficiency of the two methodologies, we studied whether combining the clinicopathological features of NSCLC with RRM1 detection would yield valuable information for the effective prescreening of patients in clinical practice. Although a large number of patients carrying the RRM1 gene show unique clinicopathological characteristics,[13–17] the detailed clinicopathological profiles remain unclear because of the small number of cases identified. Here, we performed a pooled analysis of a series of studies to assess the relationship between RRM1 gene expression in NSCLC and the clinicopathological characteristics of patients treated with gemcitabine-containing regimens. To the best of our knowledge, the present study is the first comprehensive and systematic analysis of the clinicopathological characteristics of NSCLC patients harboring the RRM1 gene.

Materials and methods
Literature search
EMBASE, PubMed, China National Knowledge Internet (CNKI), the WanFang database, and the Cochrane Library were searched for relevant studies up to September 2017. Electronic searches were performed using the terms "non-small cell lung cancer or NSCLC," "gemcitabine," and "ribonucleotide reductase M1 or RRM1." The detailed search strategies are showed in Table S1. This meta-analysis followed the PRISMA guidelines.

Eligibility criteria
Articles were included based on the following criteria: 1) included NSCLC patients, regardless of the pathological phenotypes; 2) provided information about the RRM1 gene detection method and the clinicopathological characteristics of lung carcinoma patients harboring the RRM1 gene, including gender, clinical stage, smoking habits, and pathological type; and 3) were published as full-text articles. Review articles, conference abstracts, case reports, and those lacking sufficient data (because of the limited data) to evaluate the relative risk (RR) and 95% CI were excluded.

Data collection
Data extraction was independently performed by two authors (Chao Wu and Qiu-Ping Leng) using a standardized form. Data such as first author, year of publication, gender, number of patients, pathological type of tumors, clinical stage, detection method, and RRM1 expression were collected. Discrepancies were settled by discussion, with disagreements resolved by consensus.

Statistical analysis
The pooled RR and 95% CI were used to estimate the strength of the clinicopathological characteristics of NSCLC patients harboring the RRM1 gene. The Cochran's Q-test and I^2 statistics were used to measure the statistical heterogeneity. The random-effects model was implemented if the heterogeneity was significant ($P<0.05$ or $I^2>50\%$)[18]; otherwise, the fixed-effects model was used.[19] Sensitivity analysis was performed by sequentially excluding studies from the current analysis to evaluate the stability of the pooled results. Begg's test was performed to evaluate the possible potential publication bias of the studies ($P<0.05$ was considered significant).[20] Meta-analyses were performed using Review Manager 5.3 (RevMan 5.3®; Nordic Cochrane Center, Copenhagen, Denmark). Categorical variables were analyzed by the χ^2 test using SPSS 24.0 (SPSS Inc., Chicago, IL, USA), and $P<0.05$ was considered statistically significant.

Results
Characteristics of included studies
As shown in the flow diagram for the study selection process (Figure 1), 395 potentially relevant studies were initially obtained from PubMed, EMBASE, CNKI, the WanFang
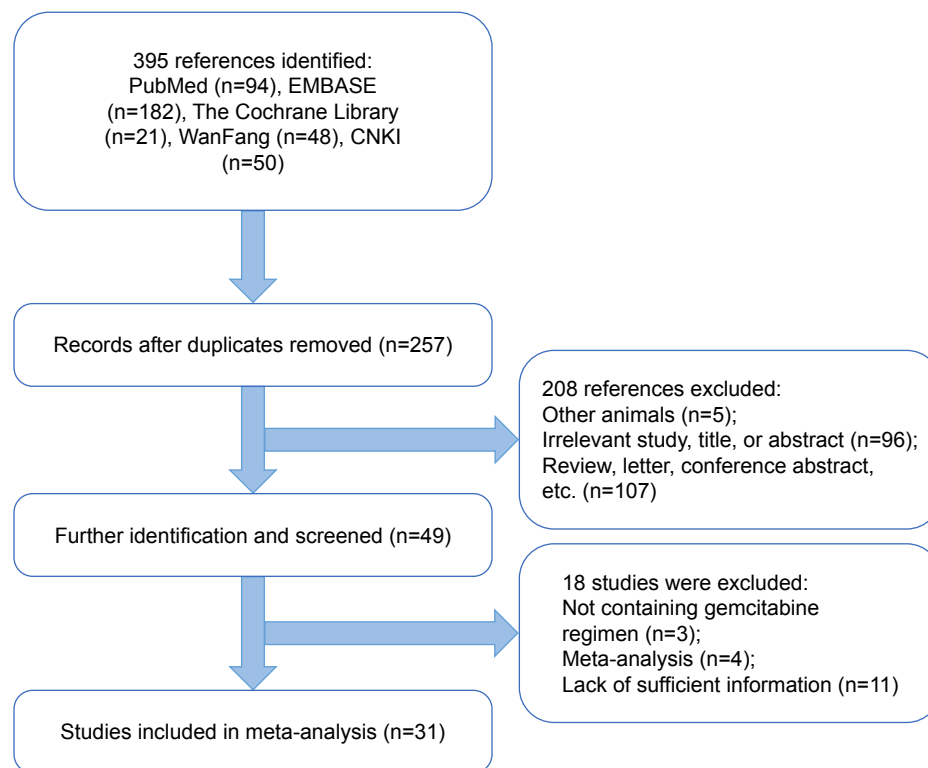
Figure 1 Flow diagram of study selection.

database, and the Cochrane Central Library. After screening the abstracts, titles, and contents, 31 observational studies including 3,667 patients were identified as eligible for the present study. Of the 31 studies, 16 (1,425 patients) reported on the relationship between RRM1 expression and gender in NSCLC patients treated with gemcitabine-containing chemotherapy, whereas the remaining 15 studies (1,242 patients) showed the relationship between RRM1 expression and pathological type. Three studies (288 patients) focused on the relationship between RRM1 expression and clinical stage, whereas eight studies (907 patients) focused on the association of RRM1 expression in NSCLC with smoking status. The baseline features of the selected studies are listed in Table 1.

Meta-analysis results

In total, 3,667 participants from 31 observational studies were included in the present meta-analysis; 1,772 patients (49.1%) harbored the RRM1 gene. Of the 31 studies, 16 studies demonstrated an association between RRM1 expression in NSCLC and gender. There was no significant heterogeneity among these studies ($P_{heterogeneity}$=0.74, I^2=0%); therefore, a fixed-effects model was used to analyze the data. The pooled findings are shown in Figure 2A. Women with positive RRM1 expression had a statistically

significantly lower risk of NSCLC than men (43.0% vs 51.7%, RR=0.84, 95% CI: 0.74–0.94, P=0.004). Fifteen studies assessed the role of RRM1 expression in the adenocarcinoma and non-adenocarcinoma groups. There was no significant heterogeneity among the studies ($P_{heterogeneity}$=0.68, I^2=0%). The results of the study showed no significant differences between the adenocarcinoma and non-adenocarcinoma groups (44.4% vs 44.3%, RR=0.97, 95% CI: 0.84–1.10, P=0.61) (Figure 2B). Three studies investigated the association between RRM1 expression and tumor staging. There was no significant heterogeneity among these studies ($P_{heterogeneity}$=0.53, I^2=0%); therefore, the data were analyzed using a fixed-effects model. The present meta-analysis demonstrated that there was no significant difference between stage I–II and stage III–IV (RR=0.88, P=0.07) (Figure 3A). In addition, eight studies evaluated the correlation between RRM1 gene expression and smoking history. There was no significant heterogeneity among these studies ($P_{heterogeneity}$=0.97, I^2=0%), and hence the data were analyzed using a fixed-effects model. The results demonstrated that there was no significant difference between smokers and nonsmokers (RR=0.88, P=0.07) (Figure 3B).

The research was extended to the analysis of two detection methods (RT-PCR and IHC) for direct comparison of sensitivity and specificity. Of 1,231 patients analyzed by

Table I Baseline characteristics of the included studies

Author	Year	Total	Gender	Smoking	Pathological type	TNM (stage)		RRM1 overall response		RRM1 detection
			M (F)	Y (N)	AD (NAD)	I–II	III–IV	+(H)	−(L)	
Bepler et al[12]	2006	35	18 (17)	33 (2)	11 (24)	1	34	4/16	11/19	PCR
Rosell et al[13]	2004	75	62 (13)	NR	33 (42)	NR	75	1/4	6/12	PCR
Rosell et al[14]	2004	67	NR	NR	NR	NR	NR	11/15	16/17	PCR
Souglakos et al[15]	2008	53	45 (8)	NR	NR	NR	53	6/25	7/17	PCR
Guo et al[29]	2015	52	38 (14)	33 (19)	28 (24)	15	28	4/25	3/13	IHC
Xing et al[30]	2014	138	82 (56)	72 (66)	40 (98)	NR	138	7/32	105/209	IHC
Zeng and Shan[31]	2009	51	23 (28)	21 (30)	33 (18)	NR	51	3/17	16/34	IHC
Zhao et al[32]	2014	158	115 (43)	36 (45)	38 (43)	NR	158	19/86	14/72	PCR
Xian-Jun et al[33]	2014	208	158 (50)	69 (139)	92 (115)	NR	208	40/104	49/104	PCR
Wang et al[34]	2014	418	316 (102)	154 (264)	181 (237)	NR	418	68/209	105/209	PCR
Liang et al[35]	2014	377	269 (108)	130 (247)	118 (259)	NR	377	114/236	50/114	PCR
Dong et al[36]	2014	229	45 (36)	36 (45)	38 (43)	NR	81	7/29	19/52	IHC
Jian-Wei et al[37]	2013	294	202 (92)	97 (197)	101 (193)	NR	294	101/185	51/109	PCR
Su et al[38]	2011	85	67 (18)	22 (63)	44 (41)	NR	85	0/4	8/18	PCR
Wu et al[39]	2014	50	23 (27)	NR	NR	18	32	NR	NR	PCR
Wang et al[40]	2010	124	80 (44)	NR	83 (41)	NR	124	18/50	40/74	IHC
Guo et al[41]	2010	142	82 (60)	NR	75 (67)	NR	142	22/71	27/71	PCR
Lee et al[42]	2010	40	31 (9)	NR	20 (20)	NR	40	1/13	6/25	IHC
Ma et al[43]	2014	68	47 (21)	41 (27)	31 (37)	44	24	NR	NR	IHC
Jiang et al[44]	2013	60	44 (16)	NR	30 (30)	NR	60	10/34	15/26	IHC
Lin et al[45]	2016	40	24 (16)	21 (19)	30 (10)	NR	NR	11/19	14/21	IHC
Vilmar et al[46]	2013	140	83 (57)	NR	61 (79)	NR	140	NR	NR	IHC
Xu et al[47]	2015	257	138 (119)	99 (158)	NR	NR	NR	NR	NR	PCR
Liu et al[48]	2017	66	46 (20)	NR	49 (17)	36	30	NR	NR	IHC
Liu et al[49]	2009	61	43 (18)	NR	29 (32)	NR	61	5/26	16/35	IHC
Li and Liu[50]	2010	71	48 (23)	NR	46 (25)	NR	71	3/40	11/31	IHC
Yang et al[51]	2009	30	24 (6)	NR	5 (25)	NR	30	2/16	11/14	IHC
Zhang et al[52]	2012	49	33 (16)	18 (31)	35 (14)	NR	49	4/25	12/24	PCR
Bepler et al[53]	2008	52	26 (26)	50 (2)	NR	34	18	2/17	10/18	PCR
Boukovinas et al[54]	2008	102	87 (9)	NR	NR	NR	96	8/31	21/64	PCR
Gao et al[55]	2011	75	50 (25)	47 (28)	49 (26)	NR	75	9/29	19/46	IHC

Abbreviations: AD, adenocarcinoma; F, female; H, high; IHC, immunohistochemistry; L, low; M, male; NAD, non-adenocarcinoma; NR, no report; RRM1, ribonucleotide reductase catalytic subunit M1; RT-PCR, real-time reverse transcriptase PCR.

IHC in 15 studies, 539 (43.8%) had high-level expression of RRM1; 2,265 patients had successful RT-PCR detection of RRM1 expression in 16 studies, of which 1,256 (55.5%) expressed the RRM1 gene (Table 2).

Sensitivity analysis and publication bias

Sensitivity analysis was performed by sequentially excluding individual studies to examine the impact of each study on the summarized findings. This revealed that the findings were statistically robust and credible (data not shown). Begg's test was performed to evaluate the potential publication bias of the studies.[20] The shape of the funnel plot was symmetrical, suggesting there was no obvious publication bias (Figure 4).

Discussion

Gemcitabine (2′,2′-difluorodeoxycytidine), which is active against NSCLC, is a deoxycytidine analog that is incorporated into DNA and competitively inhibits DNA synthesis.[21] Resistance to gemcitabine is associated with RRM1 overexpression.[22–24] RRM1 catalyzes the biosynthesis of deoxyribonucleotide and participates in DNA synthesis and damage repair. Preclinical studies show that RRM1 expression is associated with chemosensitivity to gemcitabine-containing therapies and that a low level of RRM1 expression is associated with a better response to gemcitabine-containing chemotherapy.[10,11]

RRM1, which is located on chromosome segment 11p15.5, is involved in the regulation of tumor proliferation, invasiveness, and metastasis. This region, also known as LOH11A, shows frequent loss of heterozygosity in NSCLC.[8,9] Previous clinical studies demonstrated a close relationship between RRM1 expression and the response to gemcitabine-containing regimens in different types of cancer, including pancreatic cancer, nasopharyngeal carcinoma, and NSCLC.[8,12,15,17,25–56]
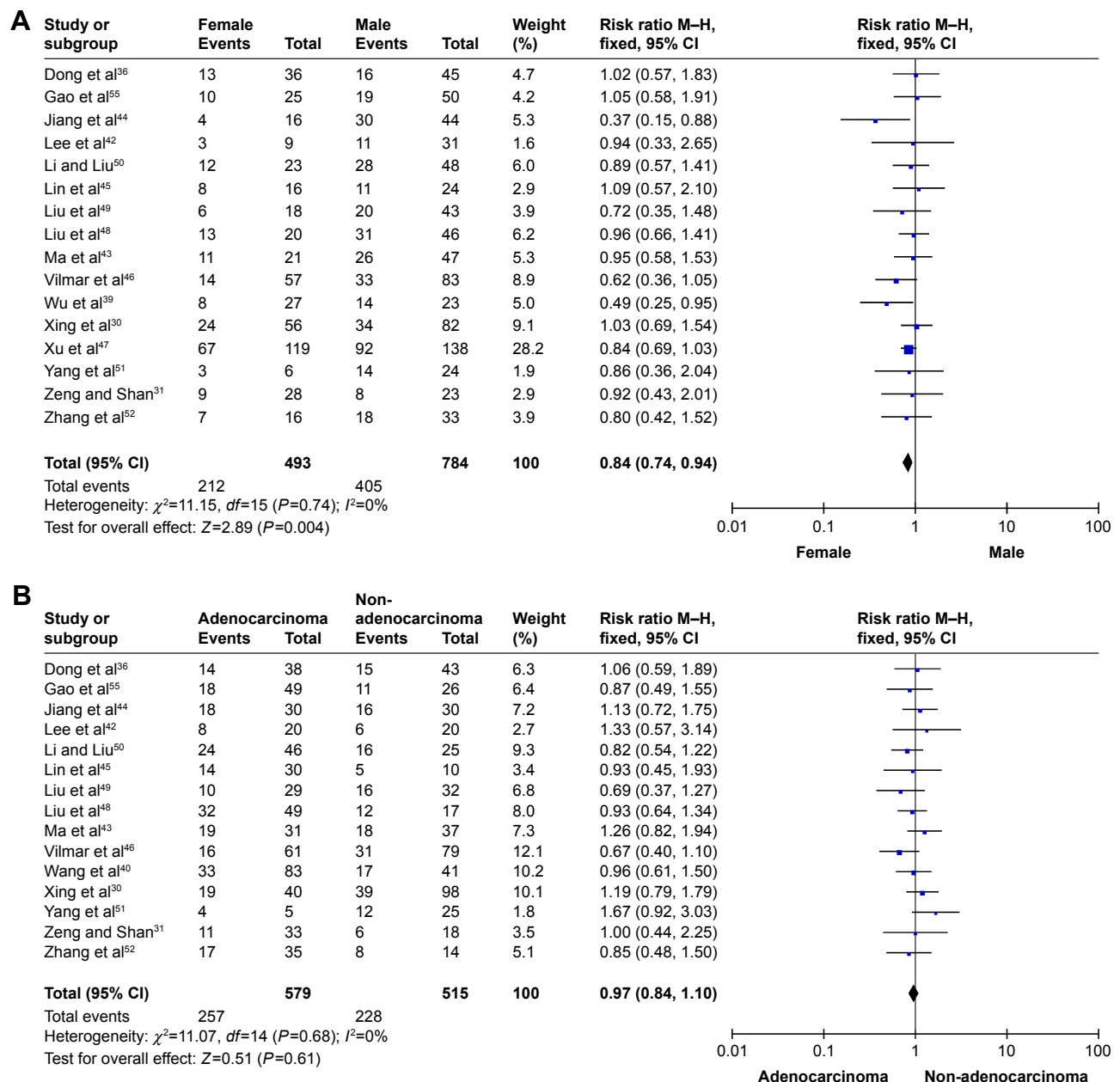
A

Study or subgroup	Female Events	Total	Male Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Dong et al[36]	13	36	16	45	4.7	1.02 (0.57, 1.83)
Gao et al[55]	10	25	19	50	4.2	1.05 (0.58, 1.91)
Jiang et al[44]	4	16	30	44	5.3	0.37 (0.15, 0.88)
Lee et al[42]	3	9	11	31	1.6	0.94 (0.33, 2.65)
Li and Liu[50]	12	23	28	48	6.0	0.89 (0.57, 1.41)
Lin et al[45]	8	16	11	24	2.9	1.09 (0.57, 2.10)
Liu et al[49]	6	18	20	43	3.9	0.72 (0.35, 1.48)
Liu et al[48]	13	20	31	46	6.2	0.96 (0.66, 1.41)
Ma et al[43]	11	21	26	47	5.3	0.95 (0.58, 1.53)
Vilmar et al[46]	14	57	33	83	8.9	0.62 (0.36, 1.05)
Wu et al[39]	8	27	14	23	5.0	0.49 (0.25, 0.95)
Xing et al[30]	24	56	34	82	9.1	1.03 (0.69, 1.54)
Xu et al[47]	67	119	92	138	28.2	0.84 (0.69, 1.03)
Yang et al[51]	3	6	14	24	1.9	0.86 (0.36, 2.04)
Zeng and Shan[31]	9	28	8	23	2.9	0.92 (0.43, 2.01)
Zhang et al[52]	7	16	18	33	3.9	0.80 (0.42, 1.52)
Total (95% CI)		493		784	100	0.84 (0.74, 0.94)

Total events 212 405
Heterogeneity: χ^2=11.15, df=15 (P=0.74); I^2=0%
Test for overall effect: Z=2.89 (P=0.004)

B

Study or subgroup	Adenocarcinoma Events	Total	Non-adenocarcinoma Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI
Dong et al[36]	14	38	15	43	6.3	1.06 (0.59, 1.89)
Gao et al[55]	18	49	11	26	6.4	0.87 (0.49, 1.55)
Jiang et al[44]	18	30	16	30	7.2	1.13 (0.72, 1.75)
Lee et al[42]	8	20	6	20	2.7	1.33 (0.57, 3.14)
Li and Liu[50]	24	46	16	25	9.3	0.82 (0.54, 1.22)
Lin et al[45]	14	30	5	10	3.4	0.93 (0.45, 1.93)
Liu et al[49]	10	29	16	32	6.8	0.69 (0.37, 1.27)
Liu et al[48]	32	49	12	17	8.0	0.93 (0.64, 1.34)
Ma et al[43]	19	31	18	37	7.3	1.26 (0.82, 1.94)
Vilmar et al[46]	16	61	31	79	12.1	0.67 (0.40, 1.10)
Wang et al[40]	33	83	17	41	10.2	0.96 (0.61, 1.50)
Xing et al[30]	19	40	39	98	10.1	1.19 (0.79, 1.79)
Yang et al[51]	4	5	12	25	1.8	1.67 (0.92, 3.03)
Zeng and Shan[31]	11	33	6	18	3.5	1.00 (0.44, 2.25)
Zhang et al[52]	17	35	8	14	5.1	0.85 (0.48, 1.50)
Total (95% CI)		579		515	100	0.97 (0.84, 1.10)

Total events 257 228
Heterogeneity: χ^2=11.07, df=14 (P=0.68); I^2=0%
Test for overall effect: Z=0.51 (P=0.61)

Figure 2 Meta-analysis of data for RRM1.
Notes: (**A**) Female vs male. (**B**) Adenocarcinomas vs non-adenocarcinomas.
Abbreviations: RRM1, ribonucleotide reductase catalytic subunit M1c; M–H, Mantel–Haenszel.

Here, we reviewed almost all available published articles and conducted the present meta-analysis to examine the correlation between RRM1 expression and the clinicopathological features of NSCLC. Analysis of 3,667 participants from 31 studies indicated a low rate (49.1%) of RRM1 expression in NSCLC patients. Therefore, we examined the clinicopathological characteristics of RRM1-positive lung cancer patients to improve the screening efficacy. The present analysis demonstrated that RRM1 positivity in lung cancer patients was more prevalent in men than in women (43.0% vs 51.7%, RR=0.84, 95% CI: 0.74–0.94, P=0.004).

The proportion of RRM1-positive patients in adenocarcinoma was slightly lower than that in non-adenocarcinoma, although the relation was not statistically significant (RR=0.97, 95% CI: 0.84–1.10, P=0.61). The proportion of RRM1-positive patients was slightly lower in nonsmokers than in smokers with NSCLC (47.0% vs 51.5%, RR=0.88, 95% CI: 0.76–1.01, P=0.07). Similarly, RRM1 gene positivity was not strongly correlated with clinical stage in NSCLC patients (RR=1.12, 95% CI: 0.86–1.46, P=0.40).

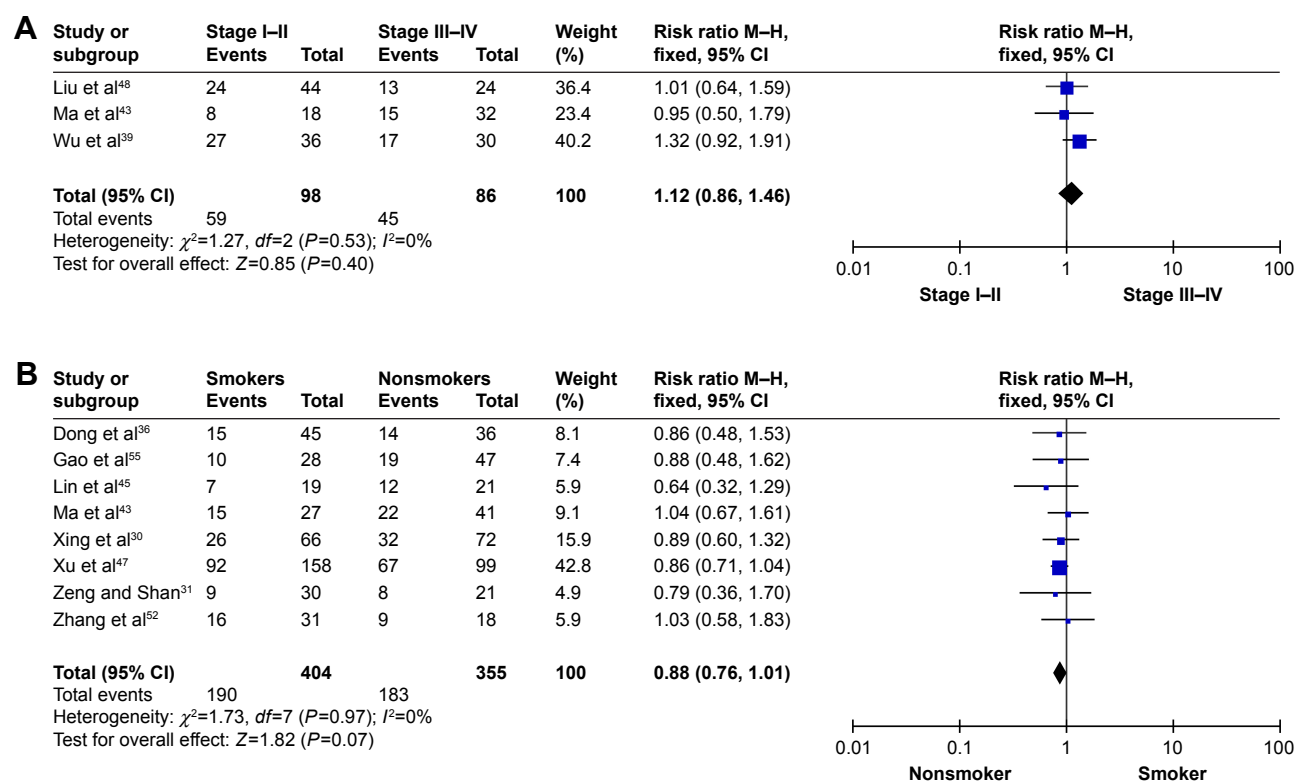The present study provided evidence to guide the pre-screening of NSCLC patients for selecting a population likely

A

Study or subgroup	Stage I–II Events	Total	Stage III–IV Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI	Risk ratio M–H, fixed, 95% CI
Liu et al[48]	24	44	13	24	36.4	1.01 (0.64, 1.59)	
Ma et al[43]	8	18	15	32	23.4	0.95 (0.50, 1.79)	
Wu et al[39]	27	36	17	30	40.2	1.32 (0.92, 1.91)	
Total (95% CI)		**98**		**86**	**100**	**1.12 (0.86, 1.46)**	
Total events	59		45				

Heterogeneity: χ^2=1.27, df=2 (P=0.53); I^2=0%
Test for overall effect: Z=0.85 (P=0.40)

B

Study or subgroup	Smokers Events	Total	Nonsmokers Events	Total	Weight (%)	Risk ratio M–H, fixed, 95% CI	Risk ratio M–H, fixed, 95% CI
Dong et al[36]	15	45	14	36	8.1	0.86 (0.48, 1.53)	
Gao et al[55]	10	28	19	47	7.4	0.88 (0.48, 1.62)	
Lin et al[45]	7	19	12	21	5.9	0.64 (0.32, 1.29)	
Ma et al[43]	15	27	22	41	9.1	1.04 (0.67, 1.61)	
Xing et al[30]	26	66	32	72	15.9	0.89 (0.60, 1.32)	
Xu et al[47]	92	158	67	99	42.8	0.86 (0.71, 1.04)	
Zeng and Shan[31]	9	30	8	21	4.9	0.79 (0.36, 1.70)	
Zhang et al[52]	16	31	9	18	5.9	1.03 (0.58, 1.83)	
Total (95% CI)		**404**		**355**	**100**	**0.88 (0.76, 1.01)**	
Total events	190		183				

Heterogeneity: χ^2=1.73, df=7 (P=0.97); I^2=0%
Test for overall effect: Z=1.82 (P=0.07)

Figure 3 Meta-analysis of data for RRM1.
Notes: (**A**) Stage I–II vs stage III–IV. (**B**) Nonsmokers vs smokers.
Abbreviations: M–H, Mantel–Haenszel; RRM1, ribonucleotide reductase catalytic subunit M1.

to harbor this specific gene. Dong et al[36] reported that RRM1 expression in tumor tissues could be a predictive biomarker in patients with advanced NSCLC receiving gemcitabine-containing chemotherapy. These authors showed that 83 of 229 (36.20%) NSCLC tumors were RRM1-positive, and 13 RRM1-positive tumors were detected in women (36.11% [13/36]). Vilmar et al[46] reported that 47 of 140 (33.57%) NSCLC patients were RRM1-positive, and 14 RRM1-positive tumors were found in women (24.56% [14/57]). These results are consistent with those of the present meta-analysis showing a prevalence of 43.00% in women. As the incidence of RRM1 is low in NSCLC patients, investigating the clinicopathological characteristics of RRM1-positive lung cancer patients is necessary to improve the screening efficacy and reduce

Table 2 Methods for the detection of RRM1 expression

Method	RRM1 expression		No of studies (cases)
	High	**Low**	
IHC	539 (43.8%)	692 (56.2%)	15 (1,231)
PCR	1,256 (55.5%)	1,009 (44.5%)	16 (2,265)
Total	1,795 (51.3%)	1,701 (48.7%)	31 (3,496)

Note: χ^2 test indicated that there was significant difference between the two methods in the detection rate of RRM1 expression (χ^2=43.46, P=0.000).
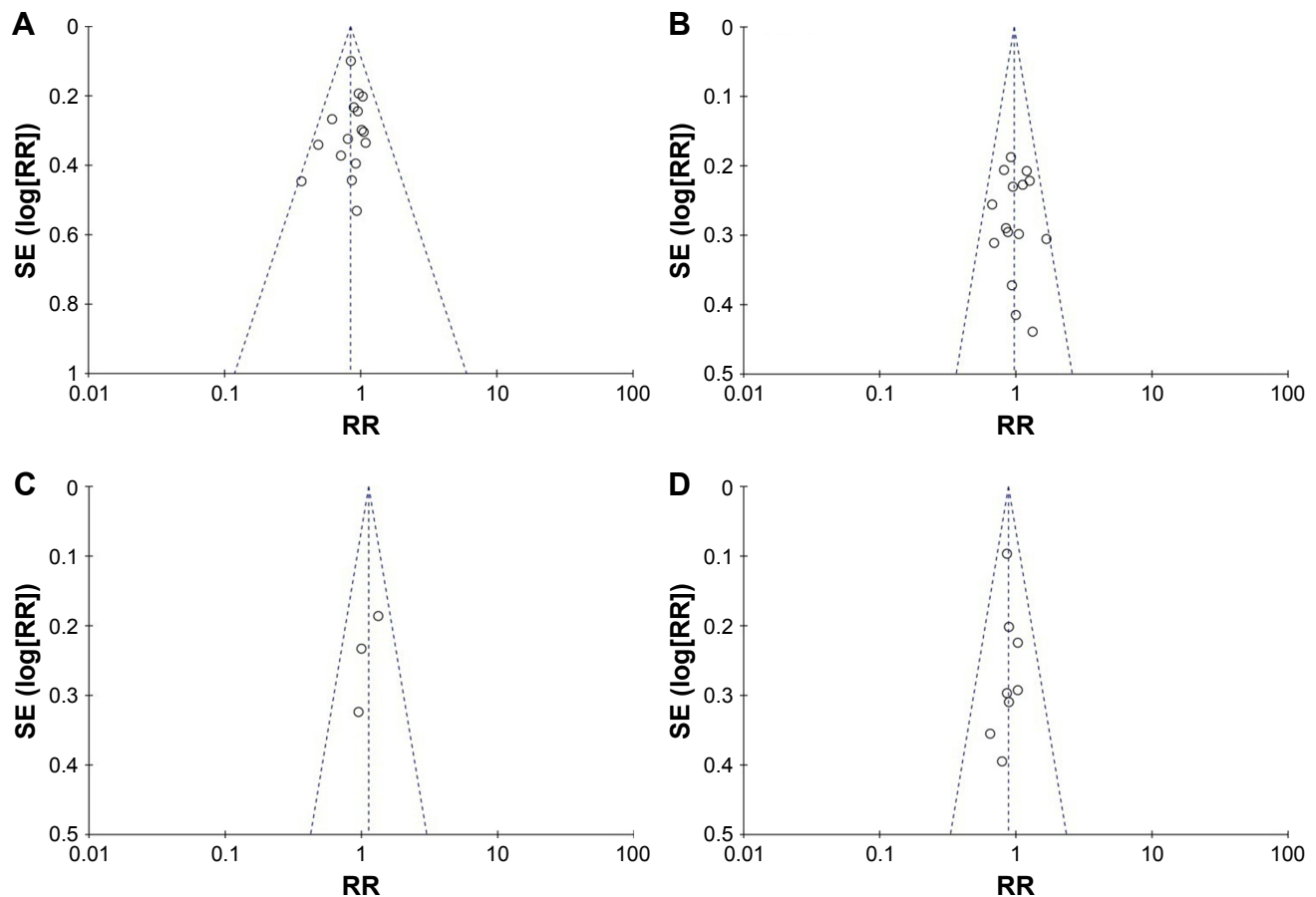**Abbreviations:** IHC, immunohistochemistry; RRM1, ribonucleotide reductase catalytic subunit M1.

medical costs. Evaluation of the clinicopathological characteristics of NSCLC patients was the first prescreening step.

RT-PCR and IHC are the most reliable methodologies to detect the RRM1 gene. Reynolds et al used a fluorescence-based IHC method combined with automated quantitative analysis, but failed to observe important differences in survival in NSCLC patients with different levels of RRM1 gene expression who received gemcitabine-containing chemotherapy.[57,58] Zheng et al[59] used these two methods simultaneously and found that the mRNA expression levels were closely related to protein expression levels. In the present study, the positive rates of IHC and RT-PCR were 43.8% and 55.5%, respectively, indicating that these methods are reliable for detecting RRM1 expression. IHC, which is a well-established method for detecting RRM1 expression, is a convenient and cost-effective technique for detecting RRM1 expression in patients with NSCLC.[60] Currently, RT-PCR is the most sensitive method for detecting and quantifying mRNA. Large-sample prospective studies are necessary to further evaluate the efficacy of RT-PCR detection for the prediction of chemosensitivity to gemcitabine associated with RRM1 expression.

This study had several limitations. First, the conclusions are mainly based on observational studies. A meta-analysis

Figure 4 Funnel plot of the outcome of clinicopathological feature and the RRM1 gene.
Notes: (**A**) Female vs male; (**B**) adenocarcinomas vs non-adenocarcinomas; (**C**) stage I–II vs stage III–IV; (**D**) nonsmokers vs smokers.
Abbreviation: RRM1, ribonucleotide reductase catalytic subunit M1.

of well-designed nonrandomized studies can be as accurate as that of randomized-controlled trials.[61] Second, only studies written in English or Chinese were included in the meta-analysis. This means that eligible studies published in other languages may have been overlooked, which may have introduced selection bias. Third, most of the participants of the current pooled analysis were Asian. Therefore, future studies should include subjects from diverse ethnicities. The present survey also lacked sufficient individual patient data. Therefore, additional details and subgroup data such as sex, smoking status, pathologic classification, and clinical stages are needed for further analysis.

Conclusion

Our meta-analysis suggests that RRM1 positivity is related to a higher response rate to gemcitabine-based regimens in women with advanced NSCLC. RRM1 may be used as a biomarker to predict the response rate to gemcitabine-based chemotherapy in NSCLC. IHC could be used to prescreen for RRM1 expression in the clinic, and RT-PCR could be

used for confirmation. Further large-scale, well-designed prospective studies are necessary to determine the potential correlation between RRM1 expression in NSCLC and the clinicopathological characteristics of patients treated with gemcitabine-containing chemotherapy.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Search strategy

PubMed via the NCBI Entrez system from inception to 1 September 2017		
#1	"Carcinoma, Non-Small-Cell Lung" [Mesh]	43491
#2	NSCLC [Title/Abstract]	31187
#3	"Lung cancer*" [Title/Abstract]	123251
#4	"Lung carcinoma*" [Title/Abstract]	16074
#5	"Lung neoplasm*" [Title/Abstract]	370
#6	"Lung tumor*" [Title/Abstract]	5242
#7	"Lung tumour*" [Title/Abstract]	833
#8	"Non-small-cell*" [Title/Abstract]	48315
#9	"Non-small cell*" [Title/Abstract]	48315
#10	"Non small-cell*" [Title/Abstract]	48315
#11	"Non small cell*" [Title/Abstract]	48315
#12	(#3 OR #4 OR #5 OR #6 OR #7) AND (#8 OR #9 OR #10 OR #11)	46076
#13	#1 OR #2 OR #12	58288
#14	"Gemcitabine" [Supplementary Concept] OR "gemcitabine" [All Fields]	14018
#15	"RRM1" [All Fields] OR "ribonucleotide reductase M1" [All Fields] OR "ribonucleotide reductase subunit M1" [All Fields] OR "ribonucleotide reductase large subunit" [All Fields]	675
#16	#13 AND #14 AND #15	94
EMBASE (via Elsevier) Search Strategy from 1980 to 1 September 2017		
#1	"Lung cancer"/exp OR "lung cancer"	345530
#2	"Non small cell lung cancer"/exp	119859
#3	"Nonsmall cell":tn,lnk,ab,ti	4485
#4	"Lung tumor"/exp	370444
#5	"Lung carcinoma"/exp	171987
#6	"Lung neoplasm":tn,lnk,ab,ti	379
#7	"Lung tumour"/exp	370444
#8	"Thoracic cancer":tn,lnk,ab,ti	297
#9	"Nsclc":tn,lnk,ab,ti	60146
#10	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9	393353
#11	"Gemcitabine":tn,lnk,ab,ti	23161
#12	"RRM1":tn,lnk,ab,ti	974
#13	"Ribonucleotide reductase m1"/exp	113
#14	"Ribonucleotide reductase subunit m1"/exp	30
#15	"Ribonucleotide reductase large subunit"/exp	57
#16	#12 OR #13 OR #14 OR #15	1060
#17	#10 AND #11 AND #16	182
Cochrane Library (from inception to 01 September 2017) Search Strategy		
#1	MeSH descriptor: [Lung Neoplasms] explode all trees	6056
#2	MeSH descriptor: [Carcinoma, Non-Small-Cell Lung] explode all trees	3099
#3	"Lung cancer*":ti,ab,kw	11984
#4	"Non-small cell*":ti,ab,kw	7740
#5	"Non small cell*":ti,ab,kw	7740
#6	"Nonsmall cell*":ti,ab,kw	277
#7	"Nsclc":ti,ab,kw	5108
#8	#1 or #2 or #3 or #4 or #5 or #6 or #7	13862
#9	Gemcitabine*	3393
#10	"Ribonucleotide reductase subunit m1"	7
#11	"Ribonucleotide reductase large subunit"	1
#12	"Ribonucleotide reductase m1"	9
#13	"RRM1"	38
#14	#10 or #11 or #12 or #13	40
#15	#8 or #9 or #14	21
CNKI Search Strategy from inception to 1 September 2017		
#1	肺癌 AND RRM1 AND 吉西他滨	50
WanFang Database Search Strategy from inception to 1 September 2017		
#1	肺癌 AND RRM1 AND 吉西他滨	48