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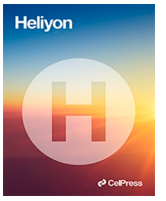

Prediction of disease-free survival of N1/2 non-small cell lung cancer after adjuvant chemotherapy by the biomarker RPMB

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ABSTRACT

No molecular biomarkers have been proven applicable in clinical practice to identify patients who can benefit from adjuvant chemotherapy in non-small cell lung cancer (NSCLC). In this study, we established a biomarker, RPMB, short for promotor methylation burden of DNA repair genes (DRGs), to identify the subgroup of patients who might benefit from adjuvant chemotherapy in NSCLC. Methylation profiles of 828 NSCLC primary tumors and their clinical information were downloaded from The Cancer Genome Atlas (TCGA) database. The RPMB for each patient after radical resection was calculated and its correlation with the prognosis of NSCLC was extensively investigated. DRGs of NSCLC were much more hypomethylated than the other genes (all $p < 0.001$). RPMB was defined as the ratio of methylated DRGs to the total number of all the DRGs. Patients with higher RPMB values tended to be nonsmokers, had adenocarcinoma, were female and had peripheral tumors. Subgroup analysis of forest plot among different clinical factors showed that high RPMB was significantly correlated to better disease-free survival (DFS) in pathologic N-positive patients after adjuvant chemotherapy (HR = 0.404, n = 62, $p = 0.034$). Notably, more superior DFS was exhibited in high RPMB NSCLCs with N1 nodal stage compared with those with low RPMB values (HR = 0.348, n = 47, $p = 0.043$). High RPMB might be used as a potential predictor to identify suitable N-positive NSCLC patients who can benefit from adjuvant chemotherapy after radical surgery.

1. Introduction

Adjuvant chemotherapy is the standard of treatment for non-small cell lung cancer (NSCLC) patients with stage II/III disease after R0 resection, especially for N-positive patients. The survival advantage of adjuvant chemotherapy in NSCLC has been reported in numerous studies. For example, the International Adjuvant Lung Cancer Trial (IALT) compared the clinical outcomes between cisplatin-based adjuvant chemotherapy and observation in completely resected NSCLCs. It was reported that the 5-year overall survival rate was 45% for cisplatin-based chemotherapy versus 40% for observation, and the 5-year disease-free survival (DFS) rate was 39% versus 34% after a median follow-up of 56 months [1]. A meta-analysis of 4,584 patients from the Lung Adjuvant Cisplatin

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Evaluation (LACE) collaborative group reported that adjuvant cisplatin-based chemotherapy increased the overall survival rate by up to 5.4%, and the advantage seemed more remarkable in patients with stage II/III disease and good performance score (PS) [2]. However, the evidence for the adjuvant chemotherapy in stage I NSCLC is quite controversial. The LACE meta-analysis showed a potential adverse effect of adjuvant chemotherapy for stage IA patients, and the no significant advantage was observed for stage IB NSCLCs [2]. The NCIC CTG JBR.10 trial also tried to test the efficacy of adjuvant chemotherapy in completely resected Stage IB/II NSCLCs [3]. It was reported that adjuvant chemotherapy significantly prolonged overall survival and DFS in stage II NSCLCs, while the benefit for stage IB disease was not found after 9 years of follow-up [4]. Moreover, the association between tumor size and the effect of adjuvant chemotherapy in NSCLCs is still controversial. The subgroup analyses of patients with stage IB NSCLC with tumors $\geq$4 cm or tumors $<$4 cm did not demonstrate a significant effect on overall survival [4], which was also demonstrated by a pooled exploratory analysis of the JBR.10 and CALGB9633 trials in node-negative NSCLCs [5]. Therefore, based on previous knowledge, lymph node metastasis is currently a decision-making factor for the usage of adjuvant chemotherapy.

However, the clinical outcomes vary drastically among different NSCLC patients, while the toxicity of adjuvant chemotherapy is still intolerable for a considerable portion of NSCLC patients. Therefore, there is an unmet need for identification of a biomarker that can be used to identify suitable patients who are more likely to benefit from chemotherapy and spare the rest from its associated toxicity. Some biomarkers, including EGFR, KRAS, and p53, have been evaluated retrospectively in NSCLCs as part of adjuvant therapy clinical trials. Unfortunately, none of these biomarkers showed predictive value in more than one study and no prospective studies have ever been conducted. Further studies in this area are urgently needed.

The Cancer Genome Atlas (TCGA) database is a publicly available dataset containing epigenetic information of a variety of cancer types. In this study, based on the methylation level of DNA repair genes (DRGs), we attempted to discover a new biomarker, promotor methylation burden of DRGs (RPMB), to identify suitable NSCLC patients who can benefit from adjuvant chemotherapy. In our previous study, RPMB was also demonstrated to be significantly associated with the prognosis of male N1 papillary thyroid cancer (PTC) after adjuvant radioiodine (RAI) therapy, indicating RPMB might be a potential predictor applicable in various cancer types and treatment modalities [6].

2. Materials and methods

2.1. Collection of clinical data and methylation profile

In December 2020, we collected the DNA methylation data of 828 NSCLCs and their clinical information through Bioconductor package "RTCGA". The β value from 0 (unmethylated) to 1 (fully methylated) was used to represent the methylation value of each CpG site. The promoter region was defined as the genomic region between 1,000 bp upstream to 300 bp downstream of the transcription start site [7]. Methylation value of a given gene was defined as the mean β value of all the CpG sites mapping to this gene's promoter region. Patient selection was conducted according to the following criteria: (1) none of the patients received any kind of neo-adjuvant therapies; (2) all patients received complete resection of primary tumor and regional lymph nodes; and (3) detailed clinical and survival information were available.

2.2. Collection of DRGs

We retrieved the gene list of DRGs from Gene Ontology (GO) database with the GO term "GO:0006281". Thus, 552 DRGs were collected, and 528 DRGs were available in the methylation profile of NSCLC in TCGA.

2.3. Comparison of DRGs' methylation level with others

First, 528 genes were sampled randomly 1,000 times among non-DRGs, and methylation value of DRGs were then compared with each randomly selected gene set using unpaired *t*-test. Moreover, we also compared the methylation level of DRGs with the genes in other 10 GO terms, including cell proliferation, cell development, angiogenesis, cell death, secretion, cell migration, morphogenesis, apoptotic process, immune response, and cell adhesion. We chose these GO terms due to their significance in the biological process of carcinogenesis.

2.4. Determination of RPMB values

First, these 528 DRGs were categorized into "methylated" and "unmethylated" groups based on the cutoff of β value 0.2. Second, the ratio of methylated DRGs (β value $>$ 0.2) among all the 528 DRGs were designated as the RPMB of this particular patient.

2.5. Statistical analysis

Bioconductor R packages and R programming software were used to perform statistical analysis in present study. Annotation R package "org.Hs.eg.db" was used to retrieve the gene lists of GO terms [8]. Kaplan-Meier survival analysis was carried out to demonstrate the survival difference between two groups of patients divided based on the median value of RPMB. Cox regression analysis was also conducted between different RPMB groups based on the median value.

3. Results

The schematic diagram is illustrated in Fig. 1.

3.1. Promoter methylation levels of DRGs were much lower than the others

Our analysis of the TGCA database revealed that the median value of DRG methylation was 0.157, which was much lower than that of all 1,000 randomly selected non-DRG sets based on the *t*-test (Fig. 2A displayed the boxplot of 10 random gene groups, and all $p < 0.001$). The methylation level of DRGs was also lower compared to any of the other 10 gene sets closely related to the processes of carcinogenesis (Fig. 2B, with all $p < 0.001$).

3.2. Clinical characteristics

The baseline clinical characteristics of NSCLCs were displayed in Table 1. Median RPMB values were used as the cutoff to divide the patients into two groups, and then the correlation between RPMB and patient clinical variables was investigated, including age, sex, ethnicity, location (central or peripheral), laterality (left or right), histology (adenocarcinoma or squamous carcinoma), distant metastasis, tumor size (pT), pathological lymph node (pN), and smoking index (>400 or ≤400). The Fisher's exact test indicated that RPMB was significantly related to sex, location, histology, pT stage and smoking index (Table 1). NSCLCs with higher RPMB tended to be female (Wald $\chi^2 = 226.99$, $p < 2.2 \times 10^{-16}$), located in the peripheral region (Wald $\chi^2 = 7.030$, $p = 0.008$), adenocarcinoma (Wald $\chi^2 = 320.56$, $p < 2.2 \times 10^{-16}$), T1 (Wald $\chi^2 = 10.097$, $p = 0.006$) and smoking index ≤400 (Wald $\chi^2 = 19.312$, $p = 1.11 \times 10^{-5}$). Other clinical variables were all balanced in baseline analysis.

3.3. Overall survival and DFS analysis in overall patients

Cox analysis was conducted to assess the association between RPMB and patients' clinical outcome in each aspect of clinico-pathological variable (Fig. 3A for overall survival, and Fig. 3B for DFS). The hazard ratio (HR) for overall survival in overall NSCLCs was 1.175 [95% confidence interval (CI): 0.892–1.548, n = 828, $p = 0.251$, Fig. 3A). The forest plot for overall survival showed that the majority of the subgroups favored lower RPMB, except for female (HR = 0.887, 95% CI: 0.531–1.479, $p = 0.645$), central location (HR = 0.890, 95% CI: 0.460–1.724, $p = 0.730$), squamous carcinoma (HR = 0.934, 95% CI: 0.547–1.597, $p = 0.804$), and N negative (HR = 0.956, 95% CI: 0.653–1.400, $p = 0.817$). None of these subgroup analyses was significant, except for N-positive patients, in whom poor overall survival was significantly associated with higher RPMB (HR = 1.620, 95% CI: 1.061–2.474, $p = 0.025$). With respect to DFS, 317 NSCLCs were collected according to the aforementioned criteria. The hazard ratio of DFS in overall NSCLCs was 1.168 (95% CI: 0.837–1.630, $p = 0.360$, Fig. 3B). The HRs of DFS favored lower RPMB in almost all of the subgroups, except for female (HR = 0.860, 95% CI: 0.453–1.636, $p = 0.647$), squamous carcinoma (HR = 0.716, 95% CI: 0.374–1.373, $p = 0.315$), T3-4 (HR = 0.901, 95% CI: 0.324–2.508, $p = 0.842$), and N-positive (HR = 0.790, 95% CI: 0.473–1.322, $p = 0.370$). The results of all the subgroups were nonsignificant, except for N-negative patients, in whom poor DFS significantly associated with higher RPMB (HR = 1.662, 95% CI: 1.060–2.607, $p = 0.027$).

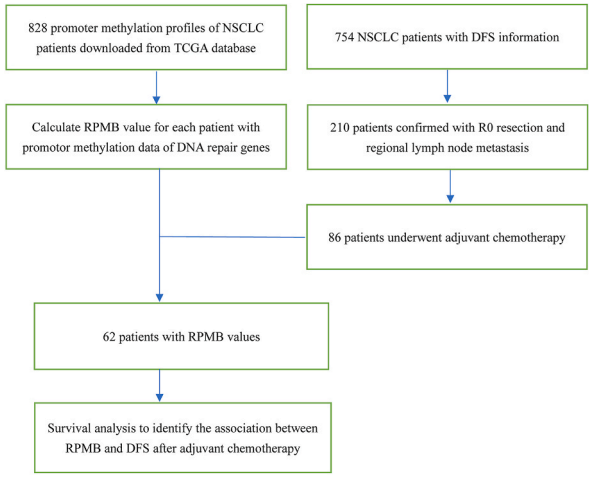

Fig. 1. Schematic diagram of this study. Clinical information from the TCGA dataset contains many missing values. Although 828 NSCLC patients contained methylation data, only 210 patients with DFS information could be confirmed with R0 resection and regional lymph node metastasis. Ultimately, only 62 patients with RPMB values after adjuvant chemotherapy could be used in this study. Abbreviations: NSCLC, non-small cell lung cancer; DFS, disease-free survival; TCGA, The Cancer Genome Atlas; RPMB, promotor methylation burden of DNA repair genes.

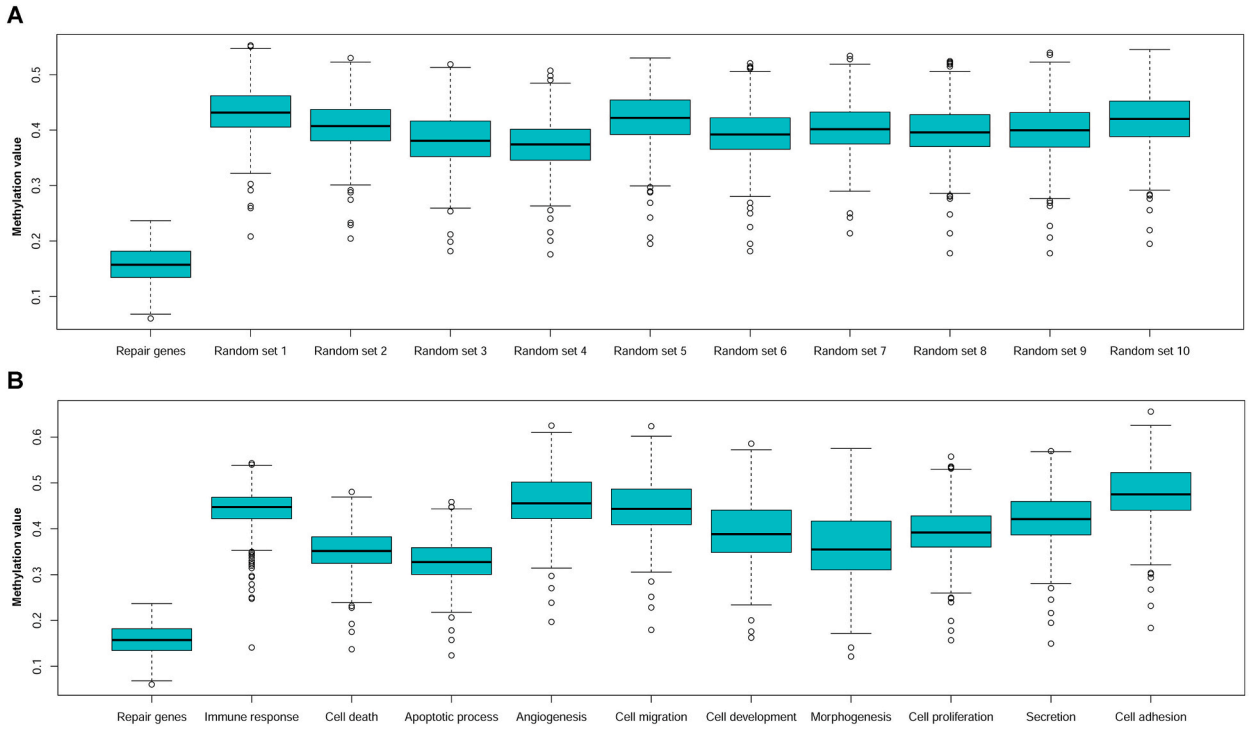

Fig. 2. Comparison of the promoter methylation values between DRGs and the others. A. Comparison of methylation values between DRGs and 10 other randomly selected gene sets. B. Comparison of methylation values between DRGs and those within other 10 GO terms. Abbreviations: DRGs, DNA repair genes; GO, Gene Ontology.

3.4. Survival analysis of N1/2 patients after adjuvant chemotherapy

Two-hundred and ten NSCLC patients with regional disease that received radical R0 surgery without any kind of neoadjuvant therapy were collected, and only 62 N1/2 patients received adjuvant chemotherapy. The baseline characteristics of these patients are shown in Table S1. Kaplan-Meier analysis indicated that NSCLC patients with lower RPMB levels had significantly poorer DFS (HR = 0.404, n = 62, $p = 0.034$, Fig. 4A). We further conducted DFS analysis in adenocarcinoma and squamous cell carcinoma, respectively (Fig. S1). The significance of the DFS difference was not reached for either histology group, but the consistent tendency was quite obvious in adenocarcinoma (HR = 0.478, n = 29, $p = 0.25$, Fig. S1A).

Among these 62 N-positive patients, 47 were found to have pathologic N1 disease, and DFS analysis indicated that RPMB was significantly associated with the DFS of N1 patients (HR = 0.348, $p = 0.043$, Fig. 4B). However, probably due to limited patient number, the significance was not reached for N2 patients (HR = 0.503, n = 15, $p = 0.4$, Fig. S2), but the tendency was very straightforward that N2 patients with lower RMPB showed a poor DFS. Moreover, significance was also reached for DFS differences among NSCLCs with ≥ T2 tumors (HR = 0.352, n = 50, $p = 0.03$, Fig. 4C). Eventually, we collected 37 NSCLCs with both ≥ T2 disease and pathologic N1. Surprisingly, the HR value was also decreased, despite of the reduced patient number due to added-in constraints (HR = 0.281, n = 37, $p = 0.051$, Fig. 4D). Furthermore, DFS analysis was also conducted with O^6-methylguanine-DNA methyltransferase (MGMT) methylation values, and the results indicated that MGMT methylation was not significantly correlated with DFS after radical surgery and adjuvant chemotherapy (HR = 1.180, n = 62, $p = 0.69$, Fig. 5A). For the overall survival of N1/2 patients who received adjuvant chemotherapy, 65 patients with detailed overall survival information were eventually collected in order to conduct overall survival analysis. Although a significant difference was not reached, a consistent trend of overall survival benefit was clearly observed among these patients (HR = 0.667, n = 65, $p = 0.45$, Fig. 5B). We also conducted overall survival analyses in N1 patients (Fig. S3A), patients with tumor size ≥ T2 (Fig. S3B) and those with N1 and tumor size ≥ T2 as well (Fig. S3C). Unfortunately, none of these analyses showed a significant difference.

3.5. Cox analysis of DFS among 62 N1/2 patients

Nine factors were taken into consideration, including age, sex, histology, laterality, location, tumor size, regional lymph node (N1 or N2), smoking index, and RPMB. The results indicated that RPMB was the only potential predictor for N positive patients who had undergone adjuvant chemotherapy after R0 resection (HR: 0.404, 95% CI: 0.170–0.959, $p = 0.040$, Table 2).

Table 1
Patient baseline characteristics.

Characteristics	Low RPMB	High RPMB	*p*
Age years (n = 789)			
<70	228	223	0.399
≥70	182	156	
Sex (n = 828)			
Male	357	131	**$<2.2 \times 10^{-16}$**
Female	67	273	
Ethnicity (n = 714)			
Asian	6	7	0.810
Black	39	35	
White	307	320	
Location (n = 365)			
Central	113	60	**0.008**
Peripheral	98	94	
Laterality (n = 791)			
Left	165	163	1
Right	233	230	
Histology (n = 828)			
Adenocarcinoma	106	352	**$<2.2 \times 10^{-16}$**
Squamous	318	52	
pT status (n = 825)			
T1	107	138	**0.006**
T2	238	212	
T3-4	78	52	
pN status (n = 812)			
N0	274	261	0.971
N+	143	134	
Metastasis (n = 605)			
M0	318	264	1
M1	13	10	
Smoking index (n = 705)			
>400	284	205	**1.11×10^{-5}**
≤400	86	130	

Abbreviations: RPMB, promotor methylation burden of DNA repair genes; pT, pathologic tumor size; pN, pathologic lymph node.

4. Discussion

Cisplatin-based chemotherapy is the cornerstone regimen of adjuvant chemotherapy in NSCLC, and the long-term side effects cannot be ignored. The long-term follow-up of the IALT trial reported that there were more deaths found in the chemotherapy group after 7.5 years of follow-up, and the benefit brought about by chemotherapy decreased dramatically over time [9]. The long-term side effects of cisplatin-based chemotherapy could cause coronary artery vasospasm resulting from hypomagnesemia or elevated serum cholesterol levels, leading to direct damage to the endothelium [10]. Additionally, up to 25% of patients treated with cisplatin-based chemotherapy exhibit a persistent decrease in glomerular filtration rate [11]. The decreasing efficacy of adjuvant chemotherapy is probably due to the long-term adverse effects of cisplatin-based regimens. Therefore, the investigation of molecular biomarkers is essential to identify NSCLC patients suitable for this treatment modality [12]. Unfortunately, no such a prognostic biomarker has ever been identified and proven to be applicable in this clinical setting. In the present study, RPMB was established as a promising indicator to cope with problems.

It has been widely reported that DNA methylation plays a very important role in accelerating embryonic development [13], aging [14], and tumorigenesis [15–18], by disarranging the bio-structures of genetic materials [19]. The promoter dysregulation also plays a greatly essential part in the process of carcinogenesis and biomarker discoveries [20–23]. Additionally, the dysregulation of methylation in DRGs has been reported to be related to the carcinogenesis and clinical outcomes of many cancer types [24–27]. RPMB was hypothesized as a potential predictor for adjuvant chemotherapy was due to the inspiration of the notable predictive ability of MGMT. MGMT is one of the DRGs closely associated with drug resistance against alkylator-based chemotherapy [28,29], and its dysregulation of promoter methylation has been frequently observed in various types of cancer [30–33]. The methylation of MGMT was proven as a molecular biomarker to predict the efficacy of chemotherapy, for instance, nitrosoureas [34] and temozolomide [35] in glioma. In addition, the hypermethylation of MGMT was also demonstrated as a strong favorable indicator in glioma patients who received concurrent radio-chemotherapy in two RCTs [36,37]. Notably, we first reported that high RPMB was significantly associated with better DFS in patients who received adjuvant radiotherapy in gastric cancer [38]. The rationale of using RPMB as an indictor of adjuvant chemotherapy in NSCLC is listed below: (1) The biological theory of cisplatin-based chemotherapy aims to compromise vital macromolecules, directly or indirectly, primarily by targeting DNA biostructures. Hypermethylation-induced inactivation of DRGs could bring a more favorable prognosis probably due to intensifying the DNA damaging bio-effects raised by chemotherapy; (2) DRGs as a whole must cooperate closely with each other to ensure that the highly complicated process goes smoothly. Promotor methylation of all DRGs should be taken into consideration collectively, rather than MGMT alone, to predict the prognosis of adjuvant

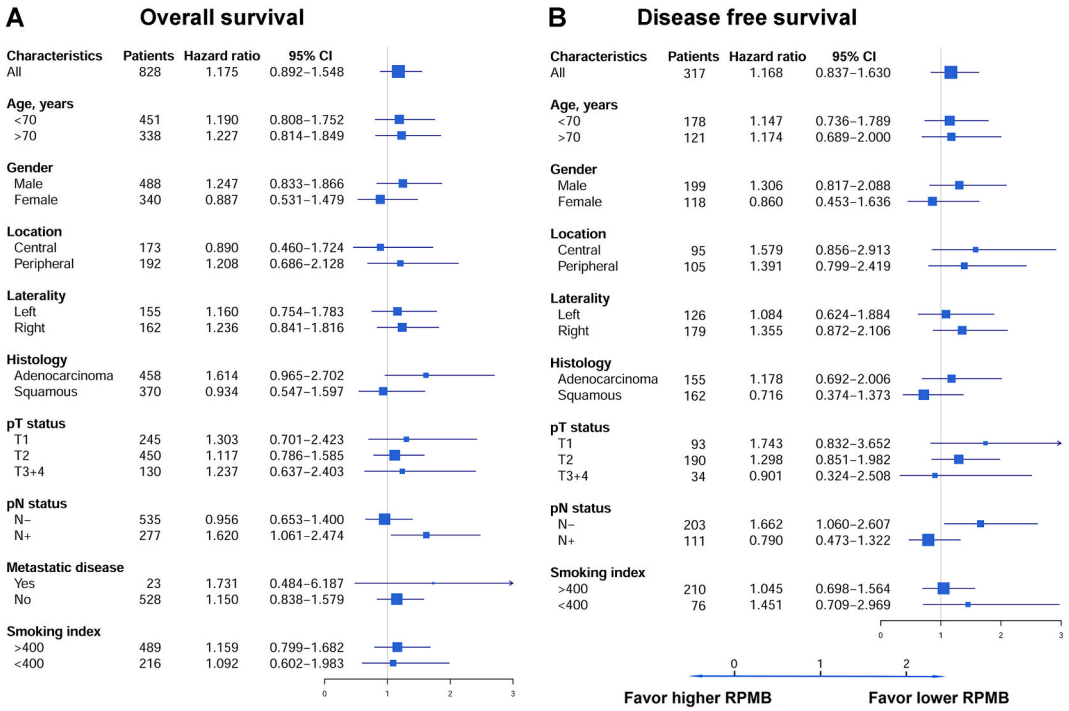

Fig. 3. Subgroup analysis of overall survival and DFS in NSCLC patients after radical surgery. A. Forest plots were used to show the prognostic association in different subgroups for both overall survival. B. Forest plots were used to show the prognostic association in different subgroups for DFS. Patient numbers, hazard ratios and 95% CIs are shown in these two forest plots. Abbreviations: DFS, disease-free survival; pT, pathologic tumor size; pN, pathologic lymph node; CI, confidence interval.

chemotherapy. It is also proven in this study that MGMT methylation alone could not significantly discriminate DFS between low and high RPMB groups (Fig. 5A), while RPMB, the measurement of promoter methylation for all DRGs, was proven successful in fulfilling the mission.

The methylation patterns in NSCLC were greatly consistent with our previous studies in gastric cancer and PTC. DRGs were all highly hypomethylated in comparison to the other genes in all three cancer types [6,38]. The theory is very plausible that hypomethylation of DRGs might be a safeguarding mechanism to rescue the genomic crisis caused by various cancer treatments, including radiotherapy and chemotherapy. DRG inactivation through hypermethylation might render malignant tumors exposed to the full-scale attack of radiotherapy or chemotherapy in high RPMB patients, by reducing DRG's self-protecting bioeffects, thereby leading to a better clinical outcome.

Nevertheless, in our previous research on RPMB in PTC, high RPMB predicted poor DFS in male PTCs who received adjuvant radioiodine (RAI) therapy [6]. However, RAI therapy in PTCs possesses distinct radio-biological effects from that of chemotherapy in NSCLC or radiotherapy in gastric cancer and glioma. In adjuvant RAI therapy, a single dose of ^{131}I radioiodine is administered that concentrates in the remnant tumors and thyroid tissues [39]. Thus, the major bio-effect of RAI is lethal damage by radiation, instead of sublethal damage of regular fractionation in other cancer types. Therefore, the discussion of the predictive ability of a certain biomarker must account for different diseases and different clinical contexts. Further investigations of RPMB in other cancer types and treatment modalities are greatly needed to shed light upon the underlying molecular mechanism of RPMB.

Table 1 shows that female patients tended to have high RPMB levels, which was also consistent with our previous study in gastric cancer [38]. Being female is a favorable factor for adjuvant chemotherapy, as tested in both a previous meta-analysis [40] and our research (Table 2, although nonsignificant). Other clinical factors significantly associated with RPMB, including histology, location and smoking index, seem able to be well explained by sex. Female patients tend to be nonsmokers and afflicted with adenocarcinoma, whose primary tumors are primarily peripheral. RPMB seems to be a sensible channel to link all these clinical factors together with the outcome of adjuvant chemotherapy, since RPMB was the only significant prognostic indictor of DFS (Table 2). Further studies are greatly needed to focus on the RPMB difference between different sexes.

The 62 N1/2 NSCLC patients who received adjuvant chemotherapy contained 12 T1 patients and 50 patients with ≥T2 tumors. Survival analysis indicated that DFS was significantly associated with RPMB in NSCLCs with ≥T2 tumors (Fig. 4C). Furthermore, we examined data from 33 patients with N1 nodal metastasis and ≥T2 tumors as well, to conduct DFS analysis. Although the patient number was quite limited, a remarkable survival difference could also be observed between different RPMB groups, with a p value very close to significance (Fig. 4D), suggesting that patients with larger primary tumor size and N1 nodal disease might increase the predictive value of RPMB in NSCLCs. Unfortunately, RPMB was not shown significantly associated with overall survival in patients who underwent adjuvant chemotherapy, although high RPMB seemed to be a favorable factor (Fig. 5B). As we also mentioned before,

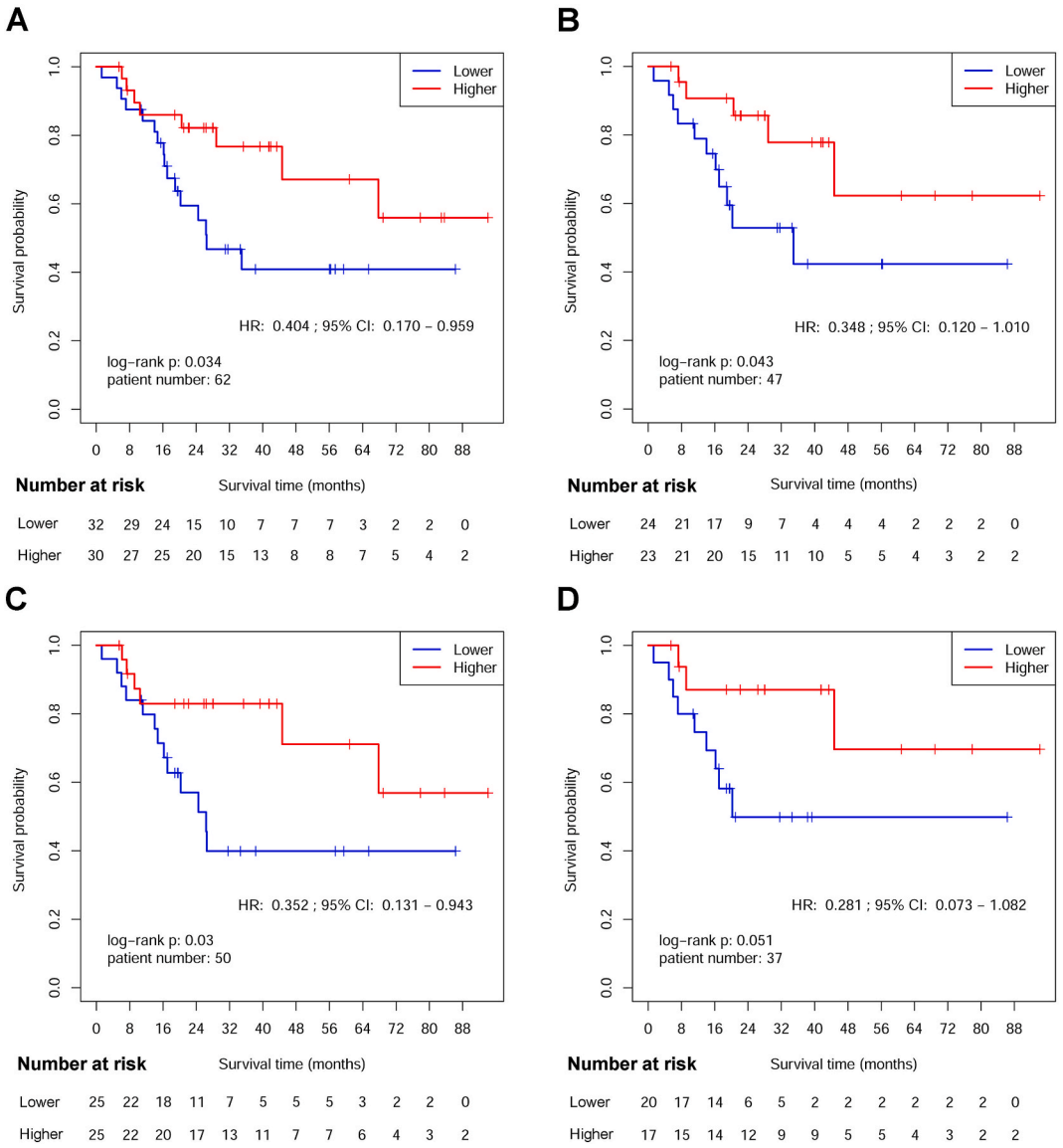

Fig. 4. Kaplan-Meier estimates of DFS after adjuvant chemotherapy by RPMB level. A. Survival analysis of DFS in N1/2 NSCLC patients. B. Survival analysis of DFS in NSCLCs with N1 disease. C. Survival analysis of DFS in patients with tumor size ≥ T2. D. Survival analysis in patients with pathological N1 disease and tumor size ≥ T2. Abbreviations: NSCLC, non-small cell lung cancer; DFS, disease-free survival; RPMB, promotor methylation burden of DNA repair genes.

overall survival is certainly not a sensible measurement to assess the efficacy of adjuvant chemotherapy, since after new tumors recur, a variety of treatment modalities, including radiotherapy, targeted therapy, and immunotherapy, would be administered in different sequences and combinations, leading to a different overall survival of the patients. This is also the reason why DFS was the primary focus to assess the efficacy of adjuvant chemotherapy in this study.

As a variety of clinical information is missing in TCGA database, the limited number of samples is one of the limitations in this study. Only 62 N positive patients who received both complete resection and adjuvant chemotherapy were collected for further analysis. However, as far as we know, TCGA is the only data resources where both clinical information and methylation dataset are available for such an analysis of NSCLCs. Currently, it is still impossible to validate the RPMB's predicting power in another independent dataset. In the future, we will carry out a prospective study to further consolidate our previous results in terms of RPMB's potential clinical application in NSCLC.

5. Conclusions

Our analysis provides evidence for RPMB as a useful biomarker in identifying N positive NSCLC patients suitable for the usage of

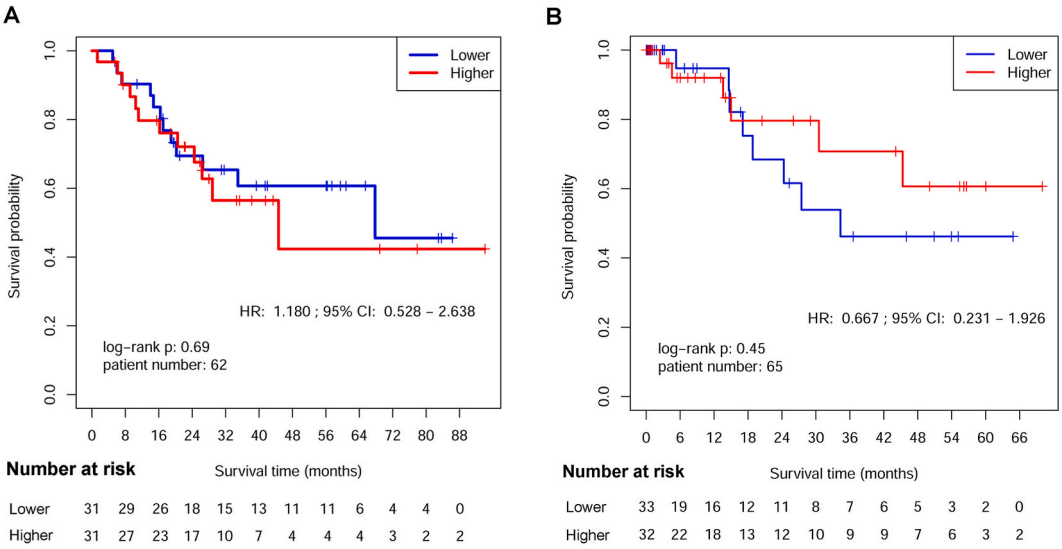

Fig. 5. DFS analysis with MGMT methylation and overall survival analysis after adjuvant chemotherapy by RPMB level. A. DFS analysis with MGMT methylation of N-positive patients after adjuvant chemotherapy. B. Overall survival analysis of all patients after adjuvant chemotherapy by RPMB level. Abbreviations: DFS, disease-free survival; RPMB, promotor methylation burden of DNA repair genes.

Table 2
Cox analyses of disease-free survival after adjuvant chemotherapy of N + NSCLC.

Factors	Cox regression		
	HR	95% *CI*	*p*
Age (years)			
< 70 (n = 43)	Reference	–	–
≥70 (n = 15)	0.615	0.209–1.809	0.377
Sex			
Female (n = 19)	Reference	–	–
Male (n = 43)	1.444	0.594–3.508	0.418
Histology			
Adenocarcinoma (n = 29)	Reference	-	–
Squamous (n = 33)	0.931	0.416–2.085	0.862
Laterality			
Left (n = 28)	Reference	-	–
Right (n = 34)	0.949	0.425–2.119	0.899
Location			
Central (n = 26)	Reference	-	–
Peripheral (n = 15)	1.295	0.458–3.667	0.626
pT			
T1 (n = 12)	Reference	–	–
T2+3 (n = 50)	1.091	0.407–2.926	0.863
pN			
N1 (n = 47)	Reference	–	–
N2 (n = 15)	1.278	0.544–3.001	0.574
Smoking index			
≤400 (n = 17)	Reference	–	–
> 400 (n = 39)	0.666	0.291–1.528	0.338
RPMB			
Lower (n = 32)	Reference	–	–
Higher (n = 30)	0.404	0.170–0.959	**0.040**

* Significant *p* values were in bold ($p < 0.05$). Abbreviations: *HR*, hazard ratio; *CI*, confidence interval; NSCLC, non-small cell lung cancer; RPMB, promotor methylation burden of DNA repair genes; pT, pathologic tumor size; pN, pathologic lymph node.

adjuvant chemotherapy after R0 resection.

Author contribution statement

Ning An: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed

reagents, materials, analysis tools or data; Wrote the paper.

Xue Yang: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at TCGA LUSC and LUAD.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18266.

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