Am J Transl Res 2023;15(8):5457-5468
www.ajtr.org /ISSN:1943-8141/AJTR0150974

Original Article

Low RPMB indicates better disease-free survival of adjuvant radiotherapy after radical surgery in thymoma

Ning An[1], Li Cui[2], Xue Yang[3]

[1]Department of Radiation Oncology, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China; [2]Department of Oncology, The People's Hospital of Pingyi County, Linyi 273399, Shandong, China; [3]Department of Medical Oncology, The Affiliated Hospital of Qingdao University, Qingdao 266003, Shandong, China

Received April 29, 2023; Accepted August 9, 2023; Epub August 15, 2023; Published August 30, 2023

Abstract: Background: The current use of adjuvant radiotherapy in thymoma (THYM) following radical surgery is primarily based on clinical factors and is a subject of ongoing debate. Methods: We developed a new biomarker, promotor methylation burden of Deoxyribonucleic acid repair genes (RPMB), to identify patients who may benefit from adjuvant radiotherapy after complete resection in THYM. RPMB quantitatively measures the promoter methylation level of Deoxyribonucleic acid (DNA) repair genes. Results: The methylation profile of 124 patients and corresponding clinical data were retrieved from The Cancer Genome Atlas (TCGA) database. The methylation level of DNA repair genes (DRGs) was found to be significantly hypomethylated juxtaposed to other genes across the whole human genome (all $P < 0.001$). THYM patients with higher RPMB tended to be female ($P = 1.114\times10^{-12}$) and have a more advanced Masaoka stage ($P = 0.034$). Kaplan-Meier analysis showed that high RPMB could significantly predict a poor disease-free survival (DFS) in THYM patients who received adjuvant radiotherapy after complete resection (HR = 5.750, 95% CI: 1.213-27.251, $P = 0.013$). Furthermore, Cox regression analysis indicated that RPMB was the only prognostic factor significantly associated with DFS after adjuvant radiotherapy ($P = 0.028$). Conclusions: Low RPMB may be a potential indicator to identify suitable patients who can benefit from adjuvant radiotherapy in THYM, sparing others from treatment toxicity.

Keywords: Thymoma, adjuvant radiotherapy, promotor methylation, disease-free survival

Introduction

Thymoma (THYM) is the most common primary tumor originating from the anterior mediastinum, despite its rarity (1.5 cases per million) [1-3]. While some patients are asymptomatic, a significant proportion present with symptoms such as chest pain, hoarseness, cough, or dyspnea. Additionally, approximately 30%-50% of patients suffer from myasthenia [4]. THYM can be locally invasive, for example, to the lung and pleura, but it rarely spreads to regional lymph nodes or extra-thoracic sites [5, 6]. Consequently, THYM is typically at a higher risk for local recurrence rather than distant relapse after radical resection [7, 8]. World Health Organization (WHO) histology and the Masaoka staging system are considered the best predictors of recurrence for THYM patients after radical resection, providing clinical guidance for adjuvant treatment modalities [9-11].

Radical surgery is the standard of care for all the resectable patients [12, 13], but the evidence for adjuvant radiotherapy is ambiguous and controversial. Due to its low morbidity, and prolonged disease course, extended follow-up is essential for THYM studies. Currently, no prospective studies have been reported for THYM adjuvant radiotherapy after complete resection, and most recommendations come from retrospective studies with limited patient numbers and considerable selection bias, which weakens the conclusions [14, 15]. The commonly accepted view is that adjuvant radiotherapy does not show additional benefit in Masaoka Stage I patients after radical resection [16, 17]. However, the role of adjuvant radiotherapy for locally invasive but nonmetastatic (Stages II and III) THYM is still controversial [16, 18, 19]. Some studies have reported that adjuvant radiotherapy could significantly decrease

the recurrence risk and potentially increase the 5-year overall survival (OS) [20, 21]. Conversely, other studies have failed to indicate a significant benefit in recurrence or survival for resected locally invasive THYM, especially for Stage II patients [16, 22, 23]. A recent meta-analysis of five studies reported that adjuvant radiotherapy of stage II/III THYM was significantly associated with an improvement in OS, but not in disease-free survival (DFS) [24]. However, this study excluded all reports published before 2013, and the majority of the included patients (n = 2,373) were not specifically staged as Masaoka stage II or III according to available data. Therefore, due to the incomplete poor-quality data, this study didn't provide more or better information than the previously published data [25].

The contradictory conclusions from different studies with poor quality indicate that the clinical pathological characteristics, like Masaoka stage and histology, alone might not be sufficient to guide the implementation of adjuvant radiotherapy. Unfortunately, so far, no molecular biomarker has been found in this clinical setting. Additionally, the therapeutic effects often vary drastically between patients, and the toxicity is a considerable issue for some patients who received adjuvant radiotherapy. In this study, we established a new biomarker, promotor methylation burden of Deoxyribonucleic acid repair genes (RPMB), to identify a specific patient group for whom adjuvant radiotherapy might confer a disease-free survival advantage.

Methods

Data collection and organization

Methylation profile and clinical data of THYM patients were obtained from the Bioconductor package "RTCGA" and "TCGAbiolinks" in December, 2021, allowing for access to methylation profiles and corresponding clinical data of 124 THYM patients. The methylation data was generated using the Illumina HumanMethylation450 chips system. β values, ranging from 0 to 1, represents the methylation level of CpG sites across the human genome. We defined the genomic region of a specific gene, in line with most studies, as the area between 1,000 base pair (bp) upstream of the transcription start site and 300 bp downstream of the promoter region of a particular gene [26]. If a single CpG site was located in the promoter region of a given gene, its β value was assigned as the methylation level of this gene. If multiple CpG sites were present in a promoter region, the mean of their β values was used as the methylation level of the given gene. This approach resulted in the methylation profile of 124 THYM tumors, comprising the methylation values of 20,275 genes [26]. For the clinical data, 111 THYM patients met the following criteria for further analysis: (a) no neo-adjuvant treatments of any type; (b) complete surgical resection; and (c) explicit DFS information.

Comparing methylation level of Deoxyribonucleic acid repair genes (DRGs) with that of the others

The gene set of Deoxyribonucleic acid (DNA) repair (n = 552) was retrieved from Gene ontology (GO, http://www.geneontology.org) term "GO:0006281", with 531 DRGs found in the THYM methylation profile. The methylation level of DRGs was compared with that of the others. First, we randomly selected 531 genes (excluding 552 DRGs) for 1,000 times, and compared the methylation level of these random genes with that of the 531 DRGs using an unpaired t test. Second, we retrieved 10 gene sets from other GO terms, including secretion (GO:0046903), apoptotic process (GO:0006915), immune response (GO:0006955), cell development (GO:0048468), cell death (GO:0008219), angiogenesis (GO:0001525), morphogenesis (GO:0000902), cell proliferation (GO:0008283), cell adhesion (GO:0007155), and cell migration (GO:0016477). We compared their methylation levels with that of DRGs using an unpaired t test. The methylation values of different gene pools were calculated using the same method adopted by RPMB as mentioned below.

Calculating RPMB values

We binarized the methylation value of the 531 DRGs using a threshold of β value of 0.15. DRGs were classified as methylated if β the value was > 0.15, and as unmethylated if the value was ≤ 0.15. RPMB was defined as the ratio of methylated DRGs to the total number of all DRGs (n = 531).

Statistical analysis

All statistical analyses were performed using R programming software (Version 3.6.1). Gene

retrieval within different GO terms was conducted with the Bioconductor (Version 3.9) annotation package "org.Hs.eg.db" [27]. Propensity score matching was performed using the R package "MatchIt" to balance the included covariates. Kaplan-Meier survival analysis and log-rank test were used to confirm the survival difference between two patient groups.

Results

DRGs exhibit significant hypomethylation compared to other genes

The methylation level of 531 DRGs was compared to that of other randomly selected genes, using an unpaired t test, repeated 1,000 times. **Figure 1A** reveals that the median methylation value of DRGs was 0.195, significantly lower than all other random gene sets (10 random gene sets are presented in the boxplots, all with $P < 0.001$). Furthermore, the methylation values of DRGs were also compared with genes associated with 10 other GO terms, which play crucial roles in carcinogenesis. The results indicated that DRGs are significantly hypomethylated compared to genes involved in other biological processes (all $P < 0.001$, **Figure 1B**).

Baseline characteristics of THYM patients

Table 1 presents the demographics and baseline characteristics of 111 THYM patients. Patients were divided into two subgroups (low RPMB *vs*. high RPMB) based on the median RPMB value. We then investigated the correlations between RPMB and various baseline characteristics, including age (< 60 *vs*. ≥ 60 years), gender, ethnicity, histology (A/AB/B1 *vs*. B2/B3/C), Masaoka stage, and myasthenia gravis.

The χ^2 and Fisher's exact tests revealed significant correlations between RPMB value, gender and Masaoka stage (**Table 1**). High RPMB was significantly associated with females (χ^2 = 50.633, $P = 1.114\times10^{-12}$), and patients with more aggressive Masaoka stage (χ^2 = 6.737, P = 0.034). Other baseline characteristics, including age, ethnicity, histology, and myasthenia gravis, were balanced between the two RPMB-assigned subgroups (**Table 1**).

DFS analyses in overall cohort and subgroups

DFS analysis was conducted in 111 THYM patients after complete resection, and treatment effects within each subgroup of clinical factors were evaluated separately. The correlation between RPMB value and DFS was extensively explored across different variables, with results presented as forest plots in **Figure 2**.

In the DFS subgroup analysis, THYM patients within each factor were divided into two groups according to the median RPMB value. Cox regression analyses were performed to establish the association between RPMB and DFS in THYM. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated and are illustrated in **Figure 2**. The HR for the overall cohort was 1.668 (95% CI: 0.677-4.106, P = 0.266). Nearly all subgroups favored lower RPMB, except for three subgroups: female (HR = 0.762, 95% CI: 0.202-2.877, P = 0.688), favorable histology (HR = 0.743, 95% CI: 0.136-4.061, P = 0.731), and patients without adjuvant radiotherapy (HR = 0.512, 95% CI: 0.132-1.986, P = 0.333). All subgroups appeared non-significant in Cox analyses, except for adjuvant radiotherapy. High RPMB was significantly associated with poor DFS in patients who received adjuvant radiotherapy (HR = 5.750, 95% CI: 1.213-27.251, P = 0.028, **Figure 2**).

Difference in patient characteristics between patients with and without adjuvant radiotherapy

Figure 3 illustrates the differences in THYM pathological characteristics between patients with and without adjuvant radiotherapy. One hundred and ten patients were analyzed further, with detailed information on all the four clinical factors, including age (< 60 *vs*. ≥ 60 years), gender, histology (A/AB/B1 *vs*. B2/B3/C), and Masaoka stage (I *vs*. II-IVa). The distribution of these four characteristics is shown in **Figure 3A**; χ^2 and Fisher's exact tests were conducted for these four factors (**Figure 3B-E**). As typically observed in clinical practice, patients who received adjuvant radiotherapy tended to have more aggressive Masaoka stages (χ^2 = 27.168, $P = 1.865\times10^{-7}$, **Figure 3D**) and histology (χ^2 = 8.470, P = 0.004, **Figure 3E**). However, no significant differences were found between patients of different ages (**Figure 3B**) or genders (**Figure 3C**).

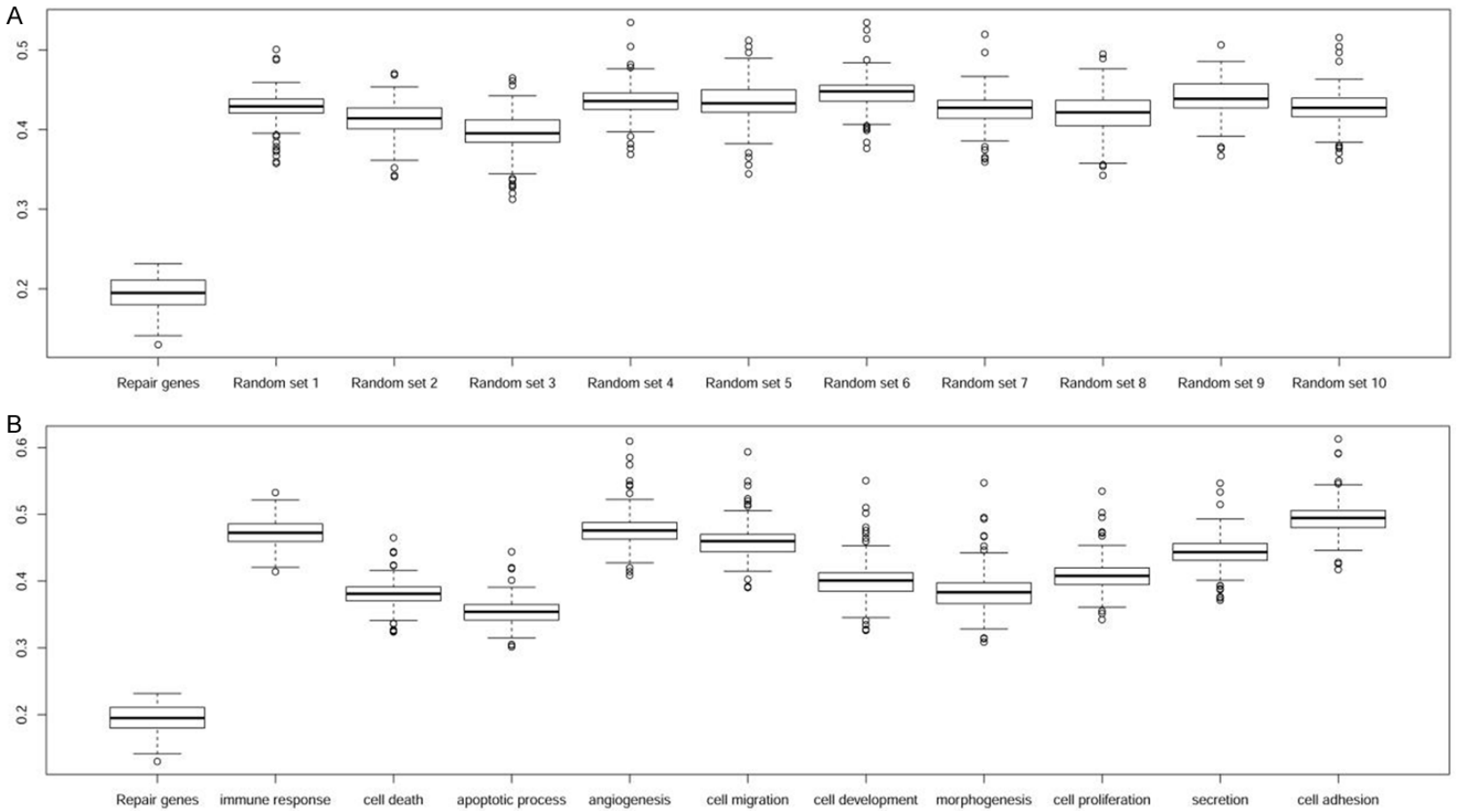

Figure 1. Comparisons of methylation level between Deoxyribonucleic acid repair genes (DRGs). A. Comparison between DRGs with other 10 groups of randomly selected genes. B. Comparison between DRGs with those within other 10 Gene ontology (GO) terms.

Table 1. Patient baseline characteristics

Characteristics	Low RPMB	High RPMB	χ^2	*p*
Age years (n = 111)				
< 60	29	27	0	1
≥ 60	29	26		
Gender (n = 111)				
Male	49	8	50.633	1.114×10^{-12}
Female	9	45		
Ethnicity (n = 109)				
Asian	6	4	0.850	0.654
Black	4	2		
White	47	46		
Histology (n = 111)				
A/AB/B1	34	27	0.386	0.535
B2/B3/C	24	26		
Masaoka stage (n = 111)				
I	22	15	6.737	0.034
II	32	25		
III/IVa	4	13		
Myasthenia Gravis (n = 109)				
Yes	16	17	0	1
No	36	40		

Abbreviations: RPMB, promotor methylation burden of Deoxyribonucleic acid repair genes.

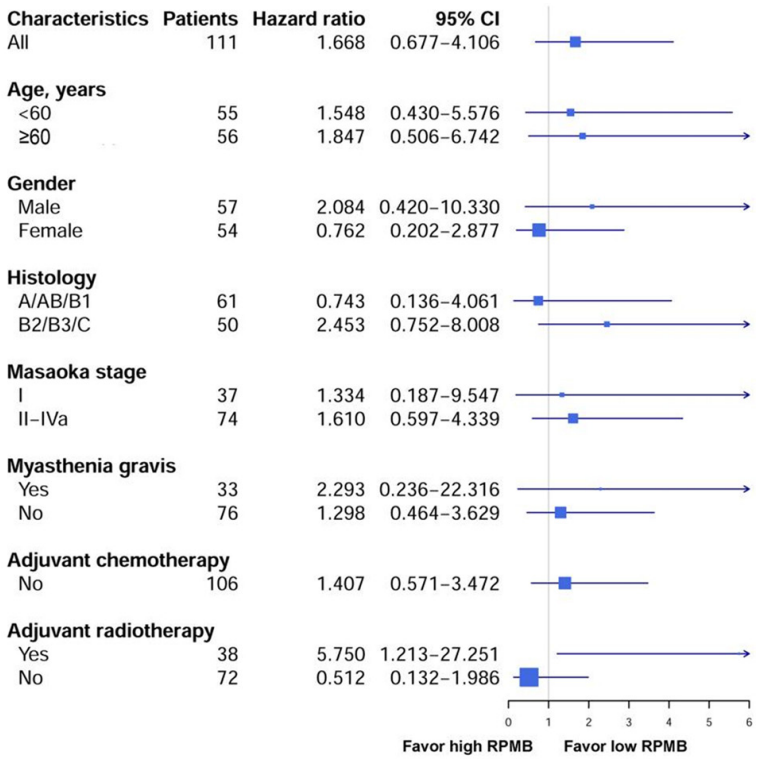

Figure 2. Forest plots of disease-free survival (DFS) in overall cohort and different subgroups.

Survival analysis of THYM patients after adjuvant radiotherapy

Among the 110 patients who underwent complete resection, 38 received adjuvant radiotherapy, while 72 did not. DFS analysis revealed no significant difference between these two groups (HR = 1.179, n = 110, *P* = 0.72, **Figure 4A**). To reduce the potential imbalance, propensity score matching with a 1:1 ratio (**Figures 5** and **6**) was conducted using four clinical factors: age (< 60 *vs.* ≥ 60 years), gender, histology (A/AB/B1 *vs.* B2/B3/C), and Masaoka stage (I *vs.* II-IVa). Even after matching, no significance difference in DFS was observed between the two groups (HR = 1.115, n = 76, *P* = 0.83, **Figure 4B**). However, among the 38 patients who received adjuvant radiotherapy after complete resection, high RPMB was significantly associated with poor DFS (HR = 5.750, n = 38, *P* = 0.013, **Figure 4C**).

Furthermore, Cox analysis, considering RPMB and the aforementioned four clinical factors, was conducted among patients who received adjuvant radiotherapy, and RPMB and aforementioned four clinical factors were taken into consideration. Univariate Cox analysis indicated that RPMB was the only significant prognostic factor for THYM patients who received adjuvant radiotherapy after complete resection (HR: 5.750, 95% CI: 1.213-27.251, *P* = 0.028, **Table 2**).

Discussion

While radical resection is considered as the primary treatment modality for thymoma

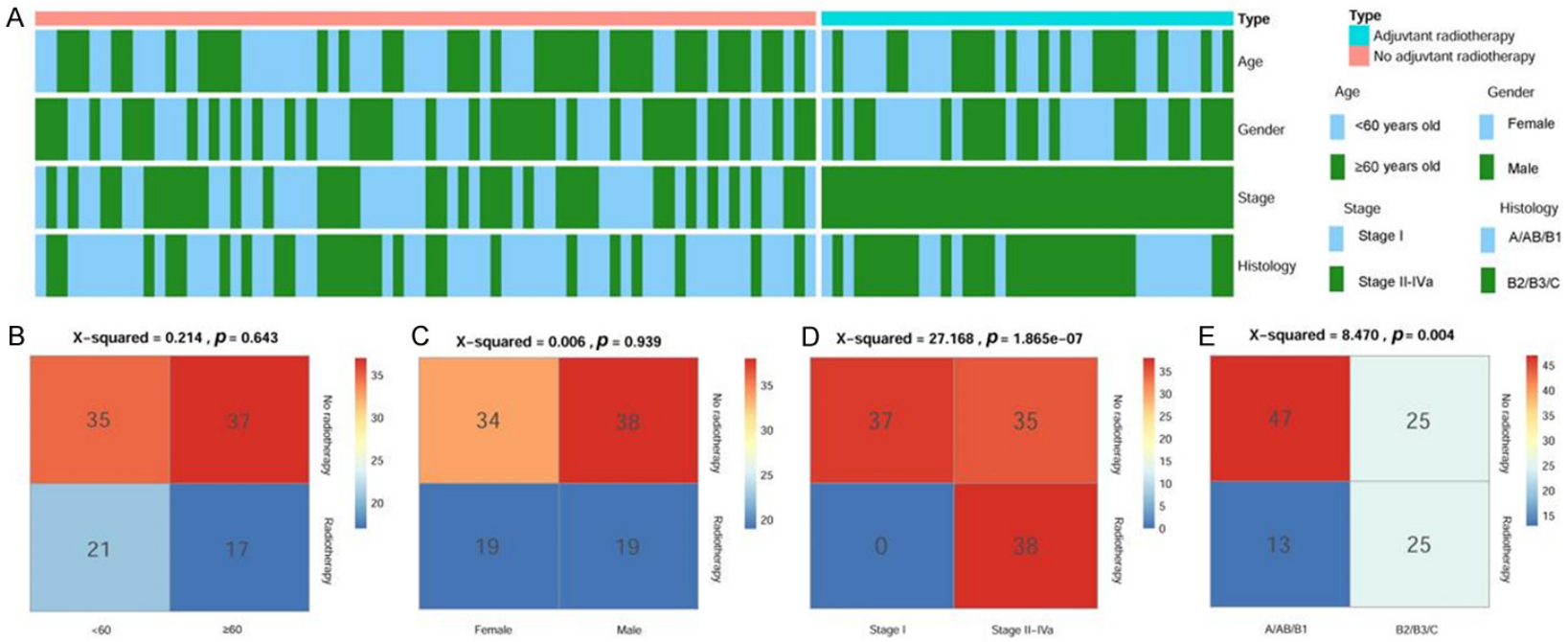

Figure 3. Difference in patient characteristics between patients with and without adjuvant radiotherapy. A. The distribution of these four clinical factors, including age (< 60 *vs.* ≥ 60 years old), gender, histology (A/AB/B1 *vs.* B2/B3/C), and Masaoka stage (I *vs.* II-IVa). B. χ^2 and Fisher's exact tests between whether adjuvant radiotherapy was used and age. C. χ^2 and Fisher's exact tests between whether adjuvant radiotherapy was used and gender. D. χ^2 and Fisher's exact tests between whether adjuvant radiotherapy was used and Masaoka stage. E. χ^2 and Fisher's exact tests between whether adjuvant radiotherapy was used and histology.

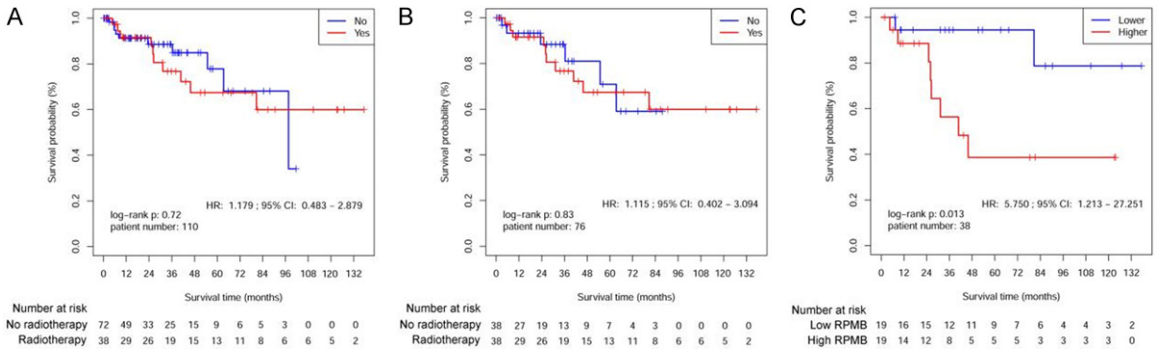

Figure 4. DFS analysis. A. DFS analysis between the patients with and without adjuvant radiotherapy. B. DFS analysis between the patients with and without adjuvant radiotherapy after matching. C. DFS analysis of all the patients after adjuvant radiotherapy by RPMB level.

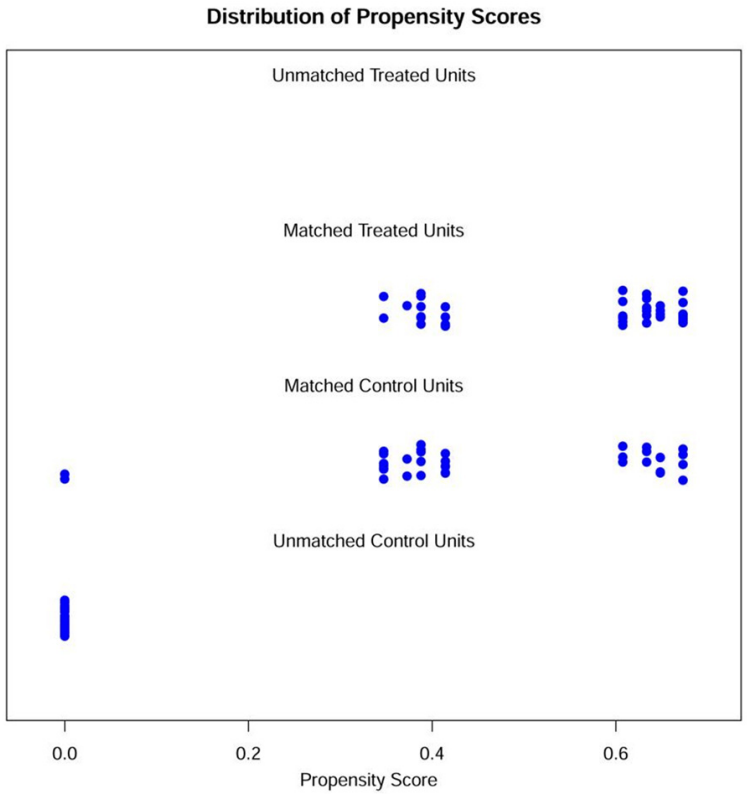

Figure 5. Jitter plot for distance values before and after matching. Individual's values for matched and unmatched treatment and control units arranged horizontally by their propensity scores.

(THYM), its effectiveness diminishes with advancing Masaoka stages. The disease-free survival (DFS) rate is nearly 100% in patients with Masaoka stage I, where the tumors does not invade beyond the capsule [28]. However, the risk of recurrence is relatively high in patients at advanced stages, even after complete resection. Consequently, adjuvant radiotherapy is theoretically suggested to reduce local recurrence in advanced-stage THYMs [29]. However, the role of adjuvant radiotherapy in THYM has been mired in controversy due to small-sized and poor-quality retrospective studies. Currently, the Masaoka stage is undoubtedly the most crucial factor considered for adjuvant radiotherapy in THYMs [30-32]. However, conflicting results make it challenging to draw definitive conclusions about the clinical benefits of adjuvant radiation in patients with locally invasive disease [33, 34]. The limited performance of clinic-pathological factors suggests a pressing need for a molecular biomarker to guide clinical decision-making in adjuvant radiotherapy for THYM patients after complete resection. Unfortunately, no such biomarker with predictive ability for the prognostic benefit of adjuvant radiotherapy has been discovered to date.

The essential functions of DNA methylation have been extensively studied in embryo development [35], aging [36], and most importantly, cancer [37-40]. Dysregulation of promoter methylation, which disrupts chromatin and DNA bio-structures [41], plays a significant role in carcinogenesis, leading to the discovery of numerous methylation-related biomarkers in other cancer types [42-45]. Our consideration

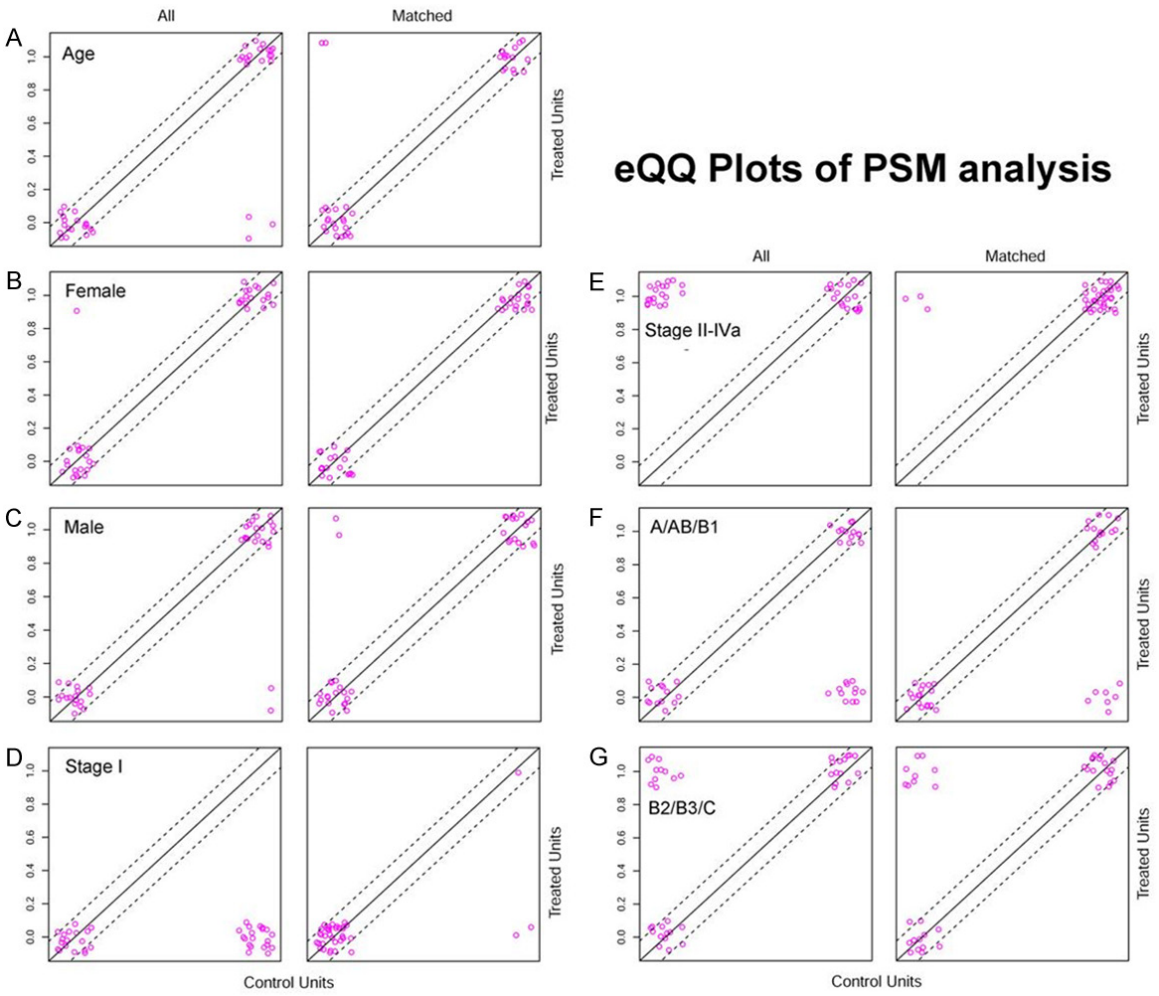

Figure 6. Empirical quantile-quantile (eQQ) plots for each covariate before and after matching. Interpolating points in the smaller group based on the weighted quantiles of the other group. When points are approximately on the 45-degree line, the distributions in the treatment and control groups are approximately equal. A. eQQ plots for age. B. eQQ plots for female. C. eQQ plots for male. D. eQQ plots for stage I. E. eQQ plots for stage II-IVa. F. eQQ plots for histology A/AB/B1. G. eQQ plots for histology B2/B3/C.

of RPMB as a predictor of adjuvant radiotherapy in THYM, was inspired by the intimate interaction between genomic instability and DNA repair. Defects in the DNA repair system, a fundamental characteristics of cancer, cause large-scale genomic instability [46]. Conversely, molecular events related to DNA damage repair might provide opportunities for biomarker discovery and potential clinical interventions [47, 48]. Genomic instability can accelerate spontaneous mutation, leading to aggressive biological phenotypes of cancer cells [49, 50]. Therefore, we hypothesized that the hypermethylation of DNA repair genes (DRGs) might promote large-scale genomic instability, leading to aggressive phenotypes of THYM cells, thereby counteracting the therapeutic efficacy of adjuvant radiotherapy. Consistent with our hypothesis, our study demonstrated that high RPMB was significantly associated with poor DFS in THYM patients who received adjuvant radiotherapy, suggesting RPMB might be a potential biomarker to identify patients who can benefit from adjuvant radiotherapy.

Interestingly, the methylation pattern in THYM aligns closely with our previous study on gastric adenocarcinoma [51]. Primarily, DRGs were significantly hypomethylated compared to other genes across the entire human genome. This low methylation level of DRGs could be a protective mechanism to keep the DNA repair sys-

Table 2. Univariate analysis of disease-free survival (DFS) after adjuvant radiotherapy

Factors	Univariate Cox regression	
	HR (95% *CI*)	*p*
Age (years)		
< 60	Reference	-
≥ 60	1.757 (0.494-6.244)	0.384
Gender		
Female	Reference	-
Male	0.330 (0.085-1.285)	0.110
Histology		
A/AB/B1	Reference	-
B2/B3/C	1.298 (0.273-6.178)	0.743
Masaoka stage		
II	Reference	-
III-IVa	2.573 (0.709-9.339)	0.151
RPMB		
Lower	Reference	-
Higher	5.750 (1.213-27.251)	**0.028**

Significant *p* values are in bold ($P < 0.05$). Abbreviations: *HR*, hazard ratio; *CI*, confidence interval.

tem vigilant against potential genomic damage, and to redirect transformed tumor cells back towards a normal state. Furthermore, our baseline characteristic analysis revealed that DRGs were significantly more hypermethylated in females than in males in both cancers (**Table 1**). Although gender disparity in clinical outcomes has not been established in THYM adjuvant radiotherapy, the difference in DRG methylation might offer a potential explanation for the observed variations in molecular characteristics and therapeutic responses between the two genders. Certainly, further research on RPMB in other cancer types is required to confirm that this pattern of DRG methylation is not merely coincidental.

As mentioned earlier, the only consensus is that adjuvant radiotherapy should not be used in Masaoka stage I disease after radical surgery. **Figure 3** illustrates the four clinical factors considered by radiation oncologists when deciding on adjuvant radiotherapy. Among the 110 patients who underwent complete resection, none at Masaoka stage I received adjuvant radiotherapy (**Figure 3D**), consistent with standard clinical protocol. Chi-square and Fisher's exact tests indicated that only Masaoka stage and WHO histology were significantly associated with the clinical use of adjuvant radiotherapy. Radiation oncologists tended to use adjuvant radiotherapy in patients with more advanced Masaoka stages and more aggressive histology (**Figure 3**). Thus, survival analysis demonstrated that with the intervention of adjuvant radiotherapy, no significant DFS difference was observed between the two groups of patients with different treatment modalities, implying that adjuvant radiotherapy can bring survival advantage for patients with unfavorable clinic-pathologic factors (**Figure 4A**, **4B**). RPMB was proven significantly associated with DFS after adjuvant radiotherapy by both survival (**Figure 4C**) and Cox analysis (**Table 2**), outperforming all other clinical factors available in The Cancer Genome Atlas (TCGA) data. In the Cox analysis, gender was not significantly associated with DFS after adjuvant radiotherapy, thereby eliminating potential gender bias (**Table 2**). To date, no molecular biomarker has been discovered to predict the efficacy of adjuvant radiotherapy in THYM.

Conclusions

Low RPMB may be a potential indicator to identify suitable patients who can benefit from adjuvant radiotherapy in THYM, sparing others from the toxic effects caused during treatment.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81801734 to X.Y., 81802271 to N.A.), the Natural Science Foundation of Shandong Province, China (ZR2019QH003 to X.Y.) and by Qilu Health Outstanding Young Talents Training Project (to N.A. No. 3843).

Written informed consent was obtained from all the patients in TCGA database by NCI.

Disclosure of conflict of interest

None.

Abbreviations

DNA, Deoxyribonucleic acid; RPMB, promotor methylation burden of DNA repair genes; THYM, thymoma; DRGs, DNA repair genes; TCGA, The Cancer Genome Atlas (TCGA) database; DFS,

disease-free survival; OS, overall survival; GO, Gene ontology; HR, Hazard ratio; CI, confidence interval; bp, base pair.

Address correspondence to: Xue Yang, Department of Medical Oncology, The Affiliated Hospital of Qingdao University, No. 16 Jiangsu Road, Shinan Distrct, Qingdao 266003, Shandong, China. Tel: +86-0532-82912757; E-mail: yxue0409@qdu.edu.cn

References

[1] Engels EA and Pfeiffer RM. Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies. Int J Cancer 2003; 105: 546-551.

[2] Engels EA. Epidemiology of thymoma and associated malignancies. J Thorac Oncol 2010; 5 Suppl 4: S260-265.

[3] Proceedings of the first international conference on thymic malignancies. August 20-21, 2009. Bethesda, Maryland, USA. J Thorac Oncol 2010; 5 Suppl 4: S259-370.

[4] Bernard C, Frih H, Pasquet F, Kerever S, Jamilloux Y, Tronc F, Guibert B, Isaac S, Devouassoux M, Chalabreysse L, Broussolle C, Petiot P, Girard N and Seve P. Thymoma associated with autoimmune diseases: 85 cases and literature review. Autoimmun Rev 2016; 15: 82-92.

[5] Hwang Y, Park IK, Park S, Kim ER, Kang CH and Kim YT. Lymph node dissection in thymic malignancies: implication of the ITMIG lymph node map, TNM stage classification, and recommendations. J Thorac Oncol 2016; 11: 108-114.

[6] Benveniste MF, Korst RJ, Rajan A, Detterbeck FC and Marom EM; International Thymic Malignancy Interest Group. A practical guide from the International Thymic Malignancy Interest Group (ITMIG) regarding the radiographic assessment of treatment response of thymic epithelial tumors using modified RECIST criteria. J Thorac Oncol 2014; 9 Suppl 2: S119-124.

[7] Ogawa K, Uno T, Toita T, Onishi H, Yoshida H, Kakinohana Y, Adachi G, Itami J, Ito H and Murayama S. Postoperative radiotherapy for patients with completely resected thymoma: a multi-institutional, retrospective review of 103 patients. Cancer 2002; 94: 1405-1413.

[8] Monden Y, Nakahara K, Iioka S, Nanjo S, Ohno K, Fujii Y, Hashimoto J, Kitagawa Y, Masaoka A and Kawashima Y. Recurrence of thymoma: clinicopathological features, therapy, and prognosis. Ann Thorac Surg 1985; 39: 165-169.

[9] Quintanilla-Martinez L, Wilkins EW Jr, Choi N, Efird J, Hug E and Harris NL. Thymoma. Histologic subclassification is an independent prognostic factor. Cancer 1994; 74: 606-617.

[10] Pescarmona E, Rendina EA, Venuta F, D'Arcangelo E, Pagani M, Ricci C, Ruco LP and Baroni CD. Analysis of prognostic factors and clinicopathological staging of thymoma. Ann Thorac Surg 1990; 50: 534-538.

[11] Oncological significance of WHO histological thymoma classification. A clinical study based on 286 patients. Jpn J Thorac Cardiovasc Surg 2003; 51: 1 p preceding table of contents.

[12] Ried M, Potzger T, Sziklavari Z, Diez C, Neu R, Schalke B and Hofmann HS. Extended surgical resections of advanced thymoma Masaoka stages III and IVa facilitate outcome. Thorac Cardiovasc Surg 2014; 62: 161-168.

[13] Bretti S, Berruti A, Loddo C, Sperone P, Casadio C, Tessa M, Ardissone F, Gorzegno G, Sacco M, Manzin E, Borasio P, Sannazzari GL, Maggi G and Dogliotti L; Piemonte Oncology Network. Multimodal management of stages III-IVa malignant thymoma. Lung Cancer 2004; 44: 69-77.

[14] Forquer JA, Rong N, Fakiris AJ, Loehrer PJ Sr and Johnstone PA. Postoperative radiotherapy after surgical resection of thymoma: differing roles in localized and regional disease. Int J Radiat Oncol Biol Phys 2010; 76: 440-445.

[15] Chang JH, Kim HJ, Wu HG, Kim JH and Kim YT. Postoperative radiotherapy for completely resected stage II or III thymoma. J Thorac Oncol 2011; 6: 1282-1286.

[16] Utsumi T, Shiono H, Kadota Y, Matsumura A, Maeda H, Ohta M, Yoshioka Y, Koizumi M, Inoue T and Okumura M. Postoperative radiation therapy after complete resection of thymoma has little impact on survival. Cancer 2009; 115: 5413-5420.

[17] Korst RJ, Kansler AL, Christos PJ and Mandal S. Adjuvant radiotherapy for thymic epithelial tumors: a systematic review and meta-analysis. Ann Thorac Surg 2009; 87: 1641-1647.

[18] Mariano C, Ionescu DN, Cheung WY, Ali RH, Laskin J, Evans K, Carolan H and Murray N. Thymoma: a population-based study of the management and outcomes for the province of British Columbia. J Thorac Oncol 2013; 8: 109-117.

[19] Harnath T, Marx A, Strobel P, Bolke E, Willers R and Gripp S. Thymoma-a clinico-pathological long-term study with emphasis on histology and adjuvant radiotherapy dose. J Thorac Oncol 2012; 7: 1867-1871.

[20] Pollack A, Komaki R, Cox JD, Ro JY, Oswald MJ, Shin DM and Putnam JB Jr. Thymoma: treatment and prognosis. Int J Radiat Oncol Biol Phys 1992; 23: 1037-1043.

[21] Curran WJ Jr, Kornstein MJ, Brooks JJ and Turrisi AT 3rd. Invasive thymoma: the role of medi-

astinal irradiation following complete or incomplete surgical resection. J Clin Oncol 1988; 6: 1722-1727.
[22] Singhal S, Shrager JB, Rosenthal DI, LiVolsi VA and Kaiser LR. Comparison of stages I-II thymoma treated by complete resection with or without adjuvant radiation. Ann Thorac Surg 2003; 76: 1635-1641; discussion 1641-1632.
[23] Blumberg D, Port JL, Weksler B, Delgado R, Rosai J, Bains MS, Ginsberg RJ, Martini N, McCormack PM, Rusch V, et al. Thymoma: a multivariate analysis of factors predicting survival. Ann Thorac Surg 1995; 60: 908-913; discussion 914.
[24] Tateishi Y, Horita N, Namkoong H, Enomoto T, Takeda A and Kaneko T. Postoperative radiotherapy for completely resected Masaoka/Masaoka-Koga stage II/III thymoma improves overall survival: an updated meta-analysis of 4746 patients. J Thorac Oncol 2021; 16: 677-685.
[25] Falkson CB. Postoperative radiation for completely resected stage II/III thymoma: what do we know in 2021? J Thorac Oncol 2021; 16: 515.
[26] An N, Yang X, Cheng S, Wang G and Zhang K. Developmental genes significantly afflicted by aberrant promoter methylation and somatic mutation predict overall survival of late-stage colorectal cancer. Sci Rep 2015; 5: 18616.
[27] Carlson M. org. Hs. eg. db: Genome wide annotation for Human. In.: R package version; 2013.
[28] Ahmad U and Huang J. Current readings: the most influential and recent studies involving surgical management of thymoma. Semin Thorac Cardiovasc Surg 2013; 25: 144-149.
[29] Venuta F, Anile M, Diso D, Vitolo D, Rendina EA, De Giacomo T, Francioni F and Coloni GF. Thymoma and thymic carcinoma. Eur J Cardiothorac Surg 2010; 37: 13-25.
[30] Zhu G, He S, Fu X, Jiang G and Liu T. Radiotherapy and prognostic factors for thymoma: a retrospective study of 175 patients. Int J Radiat Oncol Biol Phys 2004; 60: 1113-1119.
[31] Regnard JF, Magdeleinat P, Dromer C, Dulmet E, de Montpreville V, Levi JF and Levasseur P. Prognostic factors and long-term results after thymoma resection: a series of 307 patients. J Thorac Cardiovasc Surg 1996; 112: 376-384.
[32] Masaoka A. Staging system of thymoma. J Thorac Oncol 2010; 5 Suppl 4: S304-312.
[33] Wright CD, Wain JC, Wong DR, Donahue DM, Gaissert HA, Grillo HC and Mathisen DJ. Predictors of recurrence in thymic tumors: importance of invasion, World Health Organization histology, and size. J Thorac Cardiovasc Surg 2005; 130: 1413-1421.
[34] Rena O, Papalia E, Oliaro A, Ruffini E, Filosso P, Novero D, Maggi G and Casadio C. Does adjuvant radiation therapy improve disease-free survival in completely resected Masaoka stage II thymoma? Eur J Cardiothorac Surg 2007; 31: 109-113.
[35] Law JA and Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 2010; 11: 204-220.
[36] Yuan T, Jiao Y, de Jong S, Ophoff RA, Beck S and Teschendorff AE. An integrative multiscale analysis of the dynamic DNA methylation landscape in aging. PLoS Genet 2015; 11: e1004996.
[37] Docherty SJ, Davis OS, Haworth CM, Plomin R and Mill J. DNA methylation profiling using bisulfite-based epityping of pooled genomic DNA. Methods 2010; 52: 255-258.
[38] Laird PW. The power and the promise of DNA methylation markers. Nat Rev Cancer 2003; 3: 253-266.
[39] Costello JF and Plass C. Methylation matters. J Med Genet 2001; 38: 285-303.
[40] Baylin SB. Tying it all together: epigenetics, genetics, cell cycle, and cancer. Science 1997; 277: 1948-1949.
[41] Akhavan-Niaki H and Samadani AA. DNA methylation and cancer development: molecular mechanism. Cell Biochem Biophys 2013; 67: 501-513.
[42] De Carvalho DD, Sharma S, You JS, Su SF, Taberlay PC, Kelly TK, Yang X, Liang G and Jones PA. DNA methylation screening identifies driver epigenetic events of cancer cell survival. Cancer Cell 2012; 21: 655-667.
[43] Deckers IA, Schouten LJ, Van Neste L, van Vlodrop IJ, Soetekouw PM, Baldewijns MM, Jeschke J, Ahuja N, Herman JG, van den Brandt PA and van Engeland M. Promoter methylation of CDO1 identifies clear-cell renal cell cancer patients with poor survival outcome. Clin Cancer Res 2015; 21: 3492-500.
[44] Busche S, Ge B, Vidal R, Spinella JF, Saillour V, Richer C, Healy J, Chen SH, Droit A, Sinnett D and Pastinen T. Integration of high-resolution methylome and transcriptome analyses to dissect epigenomic changes in childhood acute lymphoblastic leukemia. Cancer Res 2013; 73: 4323-4336.
[45] Choudhury JH and Ghosh SK. Promoter hypermethylation profiling identifies subtypes of head and neck cancer with distinct viral, environmental, genetic and survival characteristics. PLoS One 2015; 10: e0129808.
[46] Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov 2022; 12: 31-46.

[47] Pearl LH, Schierz AC, Ward SE, Al-Lazikani B and Pearl FM. Therapeutic opportunities within the DNA damage response. Nat Rev Cancer 2015; 15: 166-180.
[48] O'Connor MJ. Targeting the DNA damage response in cancer. Mol Cell 2015; 60: 547-560.
[49] Nowell PC. The clonal evolution of tumor cell populations. Science 1976; 194: 23-28.
[50] Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991; 51: 3075-3079.
[51] An N, Yu Z, He XJ, Zhao YY, Yu L, Zhang YC, Lu HJ and Yang X. Promoter methylation of DNA repair genes predicts disease-free survival of gastric adenocarcinoma after adjuvant radiotherapy. Mol Ther Oncolytics 2020; 18: 109-117.