ORIGINAL RESEARCH

Identifying ribonucleotide reductase subunit genes as potential lung adenocarcinomas biomarkers using integrated bioinformatics analysis

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Abstract

Introduction

Dysregulation of ribonucleotide reductase (RR) subunit genes (RRM1, RRM2 and RRM2B) expression is reported to be involved in the occurrence of various human malignancies. However, the prognostic value of RR subunit genes expression in lung adenocarcinoma (LUAD) patients remains controversial.

Objective

This study aims to analyze the expression profiles, prognostic values, and immune infiltrating associations of RR subunit genes in LUAD to explore whether RR subunit gene expression has value in the prognosis of patients with lung adenocarcinoma (LUAD).

Methodology

We used multiple search engines to access multiple online bioinformatics databases, including Oncomine, TIMER, GEPIA, Kaplan–Meier Plotter, PrognoScan, the Human Protein Atlas, MD Anderson Cancer Center, UCSC Xena, cBioProtal, TCGA, GEO, DAVID, and STRING databases.

Results

The study found that RRM1 and RRM2 might be an attractive target for treating LUAD, while RRM2B were down-expressed in LUAD ($P < 0.05$). The study also found that high RRM1 or RRM2 expression, or low RRM2B expression suggested poor prognosis of LUAD patients in both TCGA and GEO databases ($P < 0.05$). Additionally, our results indicated that RR subunit genes expressions have different characteristics with immune infiltrating, RRM2B had a slight but significant positive correlation with almost every infiltrating immune cells except CD4+ T cells (all $P < 0.05$). Furthermore, by co-expression gene network analysis of RR subunit genes, we found that five new hub genes (PLK1, AURKA, CDCA8, TTK and CDC45) were significantly positively correlated with RRM1 and RRM2 expression whereas were negatively correlated with RRM2B expression, and these five hub genes were identified to be related with a poor prognosis in LUAD patients ($P < 0.05$).

Conclusion

The study findings demonstrate that RR subunit genes may be a prognostic marker and therapeutic target for LUAD patients.

Keywords: bioinformatics analysis; biomarkers; ribonucleotide reductase subunit genes; lung adenocarcinoma

Introduction

Lung cancer is the leading cause of cancer morbidity and mortality all over the world, which is classified into different histologic subtypes, including lung adenocarcinoma (LUAD), lung squamous carcinoma (LUSC), and large cell carcinoma (commonly referred as non–small cell lung cancer, NSCLC) and small cell lung cancer (SCLC)[1-3]. LUAD is the most common type of NSCLC, which exhibits distinct genetic drivers and divergent prognostic profiles compared to other types of lung cancer. In the past two decades, comprehensive molecular characterization of lung cancer has expanded our understanding of the cellular origins and molecular pathways affected in each of these sub-types. Many of these genetic alterations represent potential therapeutic targets for which drugs are constantly under development or have been used in clinical practice[4-6]. Therefore, further research on the pathogenesis, development and prognosis of LUAD will help to discover new targets and therapeutic drugs.

Ribonucleotide reductase (RR) has been identified as an important anticancer target and its inhibitors, alone or combined with other anticancer drugs, have been successfully used to treat multiple solid and hematological malignancies[7-10]. In fact, RR subunit genes have three types, including RRM1, RRM2 and RRM2B (also called p53R2), they are located in three different chromosomes and their expressions are varied and diverse in different types of cancers and their histologic variants[11]. Mammalian RR is a heterotetramer consisting of two large RRM1 subunits and two small RRM2 (RRM2 or RRM2B) subunits. In most cell types, expression of RRM1 is constant throughout all phases of cell cycle, while RRM2 expression is low in G1 phase, induced during G1/S transition, and degraded in G2/M and in G14 phase of the next cell cycle[12-14]. Although both RRM2 and RRM2B are highly homologous, the basal

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RRM2B expression is low under unstressed condition, and is profoundly induced by stress such as DNA damage or oxidative stress[15,16]. Consistent with the modes of regulation, RRM1/RRM2 complex controls progression of cells from G1 to S phase, whereas RRM1/RRM2B complex regulates DNA repair[17,18]. In this study, we analyzed multiple publicly available databases for the expression profiles of RR subunit genes and their correlations with survival information of LUAD patients. Similarly, we performed a gene co-expression network analysis to further define possible roles and explore the underlying mechanisms of the RR subunit genes and their co-expression networks in LUAD, which may promote precisive RR-targeting treatment for patients with LUAD in the future.

Methods

Oncomine database analysis

The expression level of RR subunit genes in various types of cancers were identified in the Oncomine database (https: //www. oncomine.org/resource/login.html). The threshold was determined according to the following values: P-value of 0.001, fold change of 1.5, gene ranking of all, and data type of mRNA[19].

TIMER database analysis

The database Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) includes more than 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA). The database was used to explore the mRNA transcriptional level of RR subunit genes in multiple cancer types including LUAD. Based on TIMER, we also evaluated the association between RR subunit genes and the abundance of 6 types of infiltrating immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) in LUAD patients, P-value Significant Codes: $0 \leq$ *** $< 0.001 \leq$ ** $< 0.01 \leq$ * $< 0.05 \leq < 0.1$[20].

Gene correlation analysis in GEPIA

GEPIA (29) is an interactive web that includes 9,736 tumors and 8,587 normal samples from TCGA and the GTEx projects, which analyses the RNA sequencing expression[21]. GEPIA was used to further analyze the expression and of RR subunit genes and partially interested co-expression genes in LUAD. Expression analysis of these genes performed by one-way ANOVA, and the filter criteria were as follows: FC > 1.4, P value < 0.05, and match TCGA normal data.

RR subunit genes and survival outcomes in public databases

To investigate the prognostic role of RR subunit genes mRNA in LUAD, the Kaplan-Meier plotter[22] (http:// www. kmplot.com; P-value < 0.05, FDR < 0.05), PrognoScan database[23] (http://dna00.bio.kyutech. ac.jp/PrognoScan/; the threshold was adjusted to a Cox P-value < 0.05), and the Human Protein Atlas database[24] (http://www.proteinatlas. org; P-value < 0.05) were used to determine the prognostic significance.

Analysis of tumor microenvironment and survival outcomes in LUAD

To assess the tumor microenvironment and survival outcomes in TCGA of LUAD patients, the stromal score, immune score, and ESTIMATE score of LUAD dataset generated using the ESTIMATE algorithm was downloaded from https://bioinformatics.mdanderson.org/estimate. Meanwhile, clinical data of LUAD were also downloaded from UCSC Xena (https://xenabrowser.net/). After matching sample data, the Kaplan-Meier plots were further constructed to illuminate correlations between high and low levels of infiltration score and the overall survival (OS) of LUAD patients. The statistical significance of the correlation was tested by the T test. A P-value < 0.05 was set as the cutoff.

Screening of co-expression genes with RR subunit genes expression in LUAD

In order to further analyze the potential mechanism of the influence of RR subunit genes on LUAD, the co-expression genes of RR subunit genes in TCGA of LUAD transcriptome data were screened by cBioProtal data analysis platform (http://www.cbioportal.org).

Functional and pathway enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, http://david.ncifcrf.gov/) is an important program for the comprehensive gene function analysis, which aids the researchers to understand the biological significance of abundant genes[25]. Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis was performed for the obtained co-expression genes of RR subunit genes. A result with a P < 0.05 was considered statistically significant.

PPI network construction and module selection

Protein-protein interaction (PPI) analysis of co-expression genes of RR subunit genes was performed using an online software, The Search Tool for the Retrieval of Interacting Genes (STRING, https://string-db.org/)[26]. PPI network was visualized using Cytoscape (version 3.7.0)27, and the modules of the PPI network were further screened by cytoHubba to select the hub related genes.

Statistical analysis

Survival curves were produced by the PrognoScan and Kaplan-Meier plots. The results generated in Oncomine are displayed with P-values, fold changes, and ranks. The results of Kaplan-Meier plots, PrognoScan, and GEPIA are displayed with HR and P or Cox P-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation and statistical significance, and the strength of the correlation was determined using the following guide for the absolute value: 0.00–0.19 "very weak," 0.20–0.39 "weak," 0.40–0.59 "moderate," 0.60–0.79 "strong," 0.80–1.0 "very strong." P-values < 0.05 were considered statistically significant.

Results

The mRNA expression levels of RR subunit genes

The Oncomine database analysis revealed that RRM1 and RRM2 mRNA expression of lung cancer increased in 9 data sets and 11 data sets compared to the normal tissues, respectively. However, we found decreased in 4 data sets of RRM2B in lung cancer compared to normal tissues (Fig. 1a). Higher RRM1 and RRM2 expression was more likely to appear in multiple malignant neoplasms, such as bladder, brain and central nervous system (CNS), cervical, colorectal, esophageal, head and neck, and liver cancers in some data sets, while lower RRM2B expression was been found in esophageal, lymphoma as well as lung cancer. In addition, we also investigated RR subunit genes expression using the

RNA sequencing data of multiple malignancies in TCGA. As shown in Fig. 1b, RRM1, RRM2 and RRM2B expression were significantly higher in cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC) and stomach adenocarcinoma (STAD) compared with adjacent normal tissues. However, higher RRM1 and RRM2 expression has been found in LUAD, LUSC and uterine corpus endometrial carcinoma (UCEC), while lower RRM2B expression was shown in these cancers. Considering that LUAD is the most common type of lung cancer, we selected LUAD for further analysis and verification. As expecting, RRM1 and RRM2 expression was significantly elevated in LUAD by GEPIA. In contrast, the RRM2B gene showed a significantly lower expression in LUAD (Fig. 2a, b and c).

RR subunit genes mRNA levels predicts prognosis in LUAD

As shown in Fig. 3a and 3b, we found that RRM1 and RRM2 expression was associated with a poorer prognosis in LUAD patients in the Kaplan-Meier plotter database (RRM1, OS HR (95% CI) = 2.18 (1.7-2.8), P = 2.5E-10; FP HR (95% CI) = 1.68 (1.23-2.31), P = 0.0011; RRM2, OS HR (95% CI) = 1.96 (1.54-2.5), P = 2.6E-08; FP HR (95% CI) = 2.59 (1.86-3.61), P = 5.6E-09). However, low RRM2B expression could be a risk factor for a poor prognosis in LUAD patients (OS HR (95% CI) = 0.42 (0.32-0.54), P = 6.1E-12; FP HR (95% CI) = 0.58 (0.42-0.8), P = 0.00093, Fig. 3c).

In addition, we further used the Human Protein Atlas database, the RNA-sequencing data of which form TCGA databases, to analyze and verify the prognostic significance of RR subunit genes. The results were shown in Fig. 3d-3f (RRM1, 5-year survival high 35%, 5-year survival low 47%, P = 0.00089; RRM2, 5-year survival high 31%, 5-year survival low 49%, P = 0.0000016; RRM2B, 5-year survival high 50%, 5-year survival low 36%, P = 0.00054). Above all, these results suggest that high RRM1 and RRM2 expression or low RRM2B expression could be a risk factor for poor prognosis in LUAD patients.

Finally, the impact of RR subunit genes on survival rates was also evaluated utilizing the PrognoScan, which is mainly based on GEO databases (Table 1). For RRM1, two cohorts (GSE13213, GSE13210) included 177 samples and 204 samples of LUAD and showed that high RRM1 expression was marginally associated with poorer prognosis (OS HR = 1.83, 95% CI = 1.11 to 3.02, Cox P = 0.017; RFS HR = 4.86, 95%CI = 2.17 to 10.89, Cox P = 0.0001; RFS HR = 4.24, 95%CI = 1.85 to 9.72, Cox P = 0.0006; OS HR = 7.69, 95% CI = 2.71 to 21.82, Cox P = 0.0001). For RRM2, three cohorts (jacob-00182-MSK, GSE13213 and GSE13210) included 104, 177 and 204 samples of LUAD and showed that high RRM2 expression was also significantly associated with poorer prognosis (OS HR = 2.28, 95% CI = 1.44 to 3.60, Cox P = 0.0004; OS HR = 1.97, 95% CI = 1.47 to 2.63, Cox P = 4.70E-06; OS HR = 1.82, 95% CI = 1.40 to 2.36, Cox P = 8.11E-06; OS HR = 1.97, 95% CI = 1.36 to 2.86, Cox P = 0.00035; RFS HR=1.92, 95%CI=1.47 to 2.50, Cox P =1.94E-06). In contrast, high RRM2B expression was associated with a favorable prognosis (OS HR = 0.24, 95% CI = 0.10 to 0.56, Cox P = 0.001; RFS HR = 0.19, 95%CI = 0.10 to 0.37, Cox P = 1.56E-06).

Tumor microenvironment and survival outcomes in LUAD patients

To explore the correlation between tumor microenvironment and overall survival of LUAD patients, we first utilized the stromal score, immune score, and ESTIMATE score of LUAD from online software ESTIMATE of MD Aderson Cancer Center, which is a tool for predicting tumor purity, and the presence of infiltrating stromal/immune cells in tumor tissues using gene expression data based on TCGA datasets. As shown in Fig. 4a, the immune and ESTIMATE score was significantly increased in TCGA of LUAD patients (P < 0.0001), while the stromal score didn't be found noticeable changes. Meanwhile, clinical data of LUAD (641 patients, 13 of which had no survival data) were also downloaded from UCSC Xena. To determine the potentially clinical value of the immune score and the stromal score for LUAD patients, the Kaplan–Meier survival analysis was performed. Based on the median of immune score and stromal score, LUAD samples were divided into two groups, the high group (score ≥ median) and low group (score < median). We did not find significant differences of overall survival between high and low stromal score groups (Fig. 4b, P = 0.0646). In contrast, our results showed that patients in the high immune score and ESTIMATE score group had a significantly longer overall survival compared to patients in the low score group (Fig. 4c and 4d, P = 0.0114 and 0.007, respectively). These results suggested that the immune components in tumor microenvironment were more suitable for indicating the prognosis of LUAD patients.

Correlation analysis of RR subunit genes expression, 6 types of infiltrating immune cells and LUAD survival

To further determine whether the RR subunit genes involved in immune infiltration in LUAD patients, we analyzed the correlation between RR subunit genes expression and 6 types of infiltrating immune cells (B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells). RRM1, RRM2 and RRM2B gene expression had no significant correlations with tumor purity in LUAD (P = 2.34E-01, P = 8.72E-01, and P = 9.57E-01, respectively). Interestingly, RRM1 expression level had significant positive correlations with infiltrating levels of CD8+ T cells (r = 0.15, P = 8.66E-04), neutrophils (r = 0.17, P = 1.71E-04) and macrophages (r = 0.123, P = 6.33e-03) in LUAD (Fig. 5a). For RRM2, we found that there had significant negative correlations with infiltrating levels of B cells (r = -0.205, P = 5.74E-06) and CD4+ T cells (r = 0.117, P = 1.03E-02), but had significant positive correlations with neutrophils (r = 0.144, P = 1.56E-03) (Fig. 5b). Importantly, RRM2B had a slight but significant positive correlation with almost every infiltrating immune cells except CD4+ T cells, (B cells, r = 0.167, P = 2.33E-04, CD8+ T cells, r = 0.218, P = 1.17E-06, macrophages, r = 0.227, P = 4.21E-07, neutrophils, r = 0.11, P = 1.53E-02, and dendritic cells, r = 0.234, P = 1.67E-07, respectively). These results suggested that RR subunit genes plays a specific role in immune infiltration in LUAD. Since the tumor purity in RR subunit genes of LUAD were not significant, the correlations between RR subunit genes expression and the 6 types of infiltrating immune cells needs further study to confirm these results.

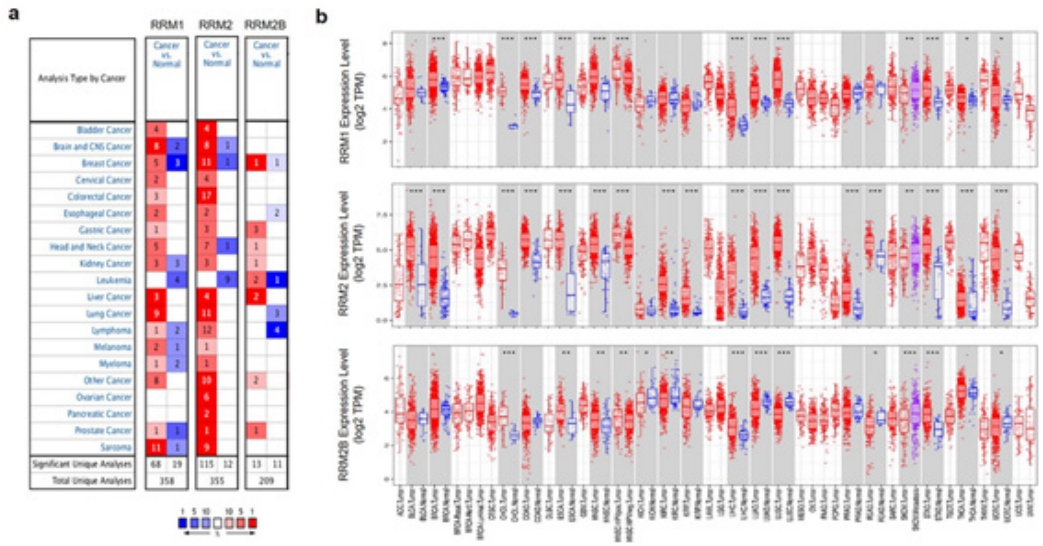

Fig 1 Ribonucleotide reductase (RR) subunit genes expression levels in different types of human cancers. (a) The significantly different levels of RRM1, RRM2, and RRM2B in data sets of different cancers compared with normal tissues in the Oncomine database. Red signifies the gene overexpression in the analyses, blue represents the gene underexpression. Intensity of color signifies the best rank of gene in those analyses. (b) Human RRM1, RRM2, and RRM2B expression levels in different tumor types from The Cancer Genome Atlas (TCGA) database were determined by database Tumor Immune Estimation Resource (TIMER) (0 ≤ * < 0.001 ≤ ** < 0.01 ≤ * < 0.05 ≤. < 0.1).**

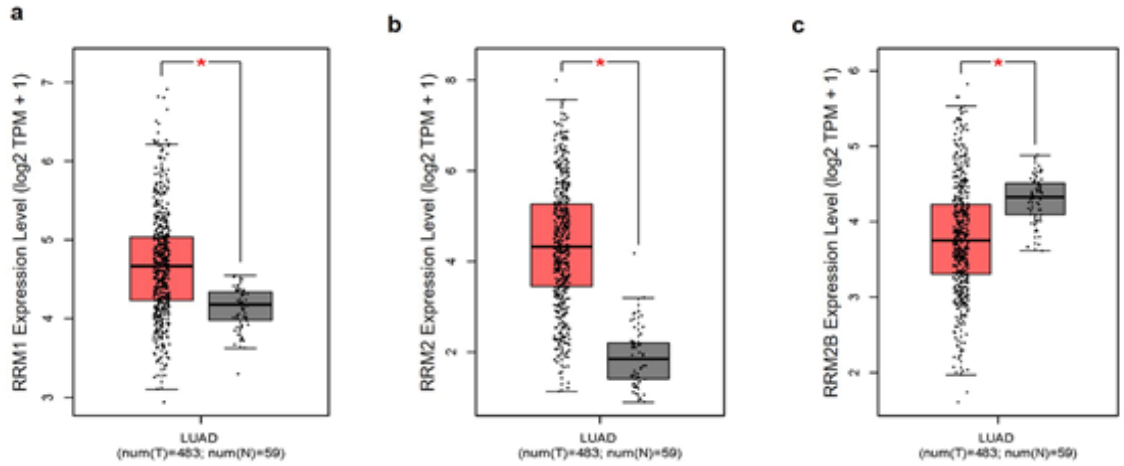

Fig 2 The mRNA expression of ribonucleotide reductase (RR) subunit genes, RRM1 (a), RRM2 (b), and RRM2B (c) between tumor and non-tumor samples in lung adenocarcinomas (LUAD) patients in the Gene Expression Profiling Interactive Analysis (GEPIA) database (* P < 0.05).

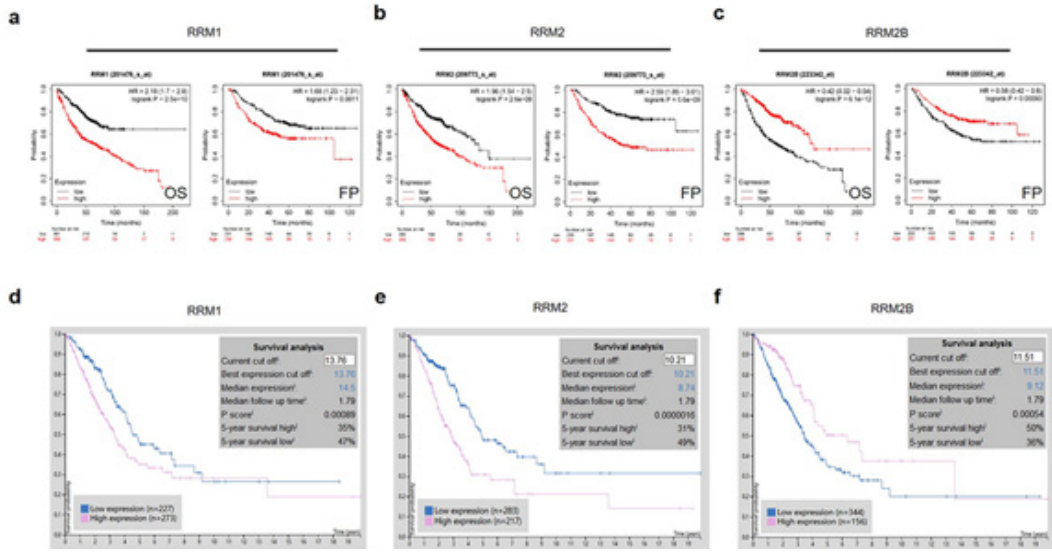

Fig 3 Kaplan-Meier survival curves compare the high and low expression of ribonucleotide reductase (RR) subunit genes in lung adenocarcinomas (LUAD). In the Kaplan-Meier plotter database, (a) high RRM1 expression was correlated with a worse overall survival (OS) and progression-free survival (PF). (b) High RRM2 expression was also correlated with a worse OS and PF. (c) High RRM2B expression was correlated with better OS and PF in LUAD patient. In TCGA data, high RRM1 (d) and RRM2 (E) expression were also correlated with worse OS, while high RRM2B was correlated with better OS (F).

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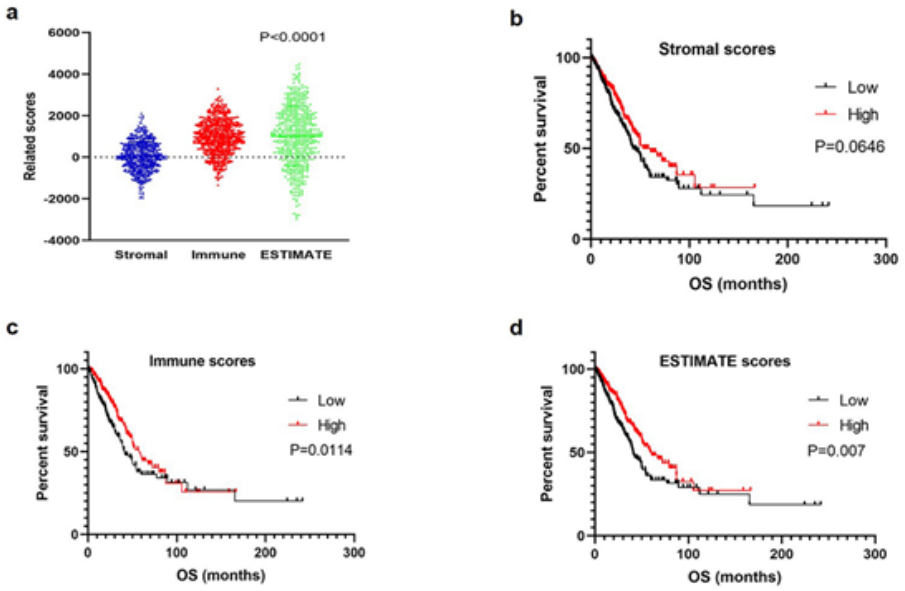

Fig 4 Tumor microenvironment and survival condition of lung adenocarcinomas (LUAD) patients. (a) The stromal score, immune score, and ESTIMATE scores of LUAD patients. (b) Stromal score was not associated with outcome of LUAD (P = 0.0646). (c–d) Both immune score and ESTIMATE scores were positively associated with LUAD patients (P = 0.0114 and 0.007, respectively).

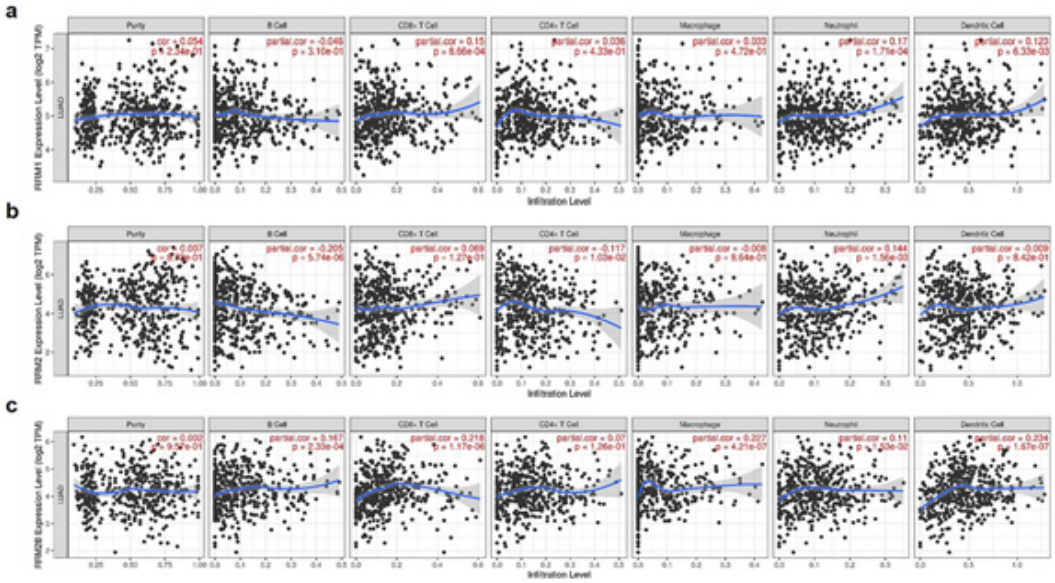

Fig 5 Correlation analysis between ribonucleotide reductase (RR) subunit genes and immune infiltration levels. The correlations between (a) RRM1, (b) RRM2, (c) RRM2B and tumor purity and infiltration levels of six types of infiltrating immune cells (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells) were analyzed respectively by TIMER in LUAD.

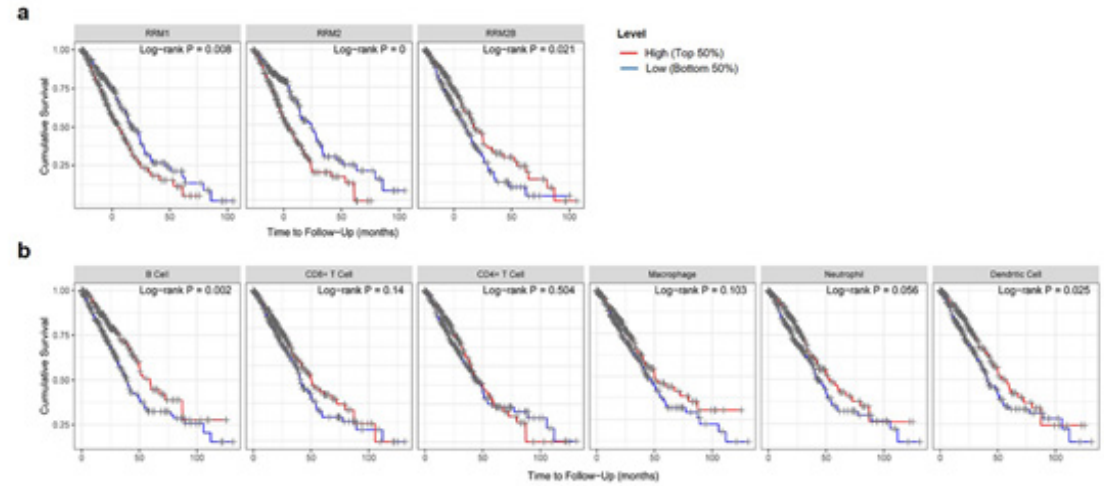

Fig 6 Ribonucleotide reductase (RR) subunit genes expression (a) and infiltrating immune cells stats (b) with LUAD patient's prognosis

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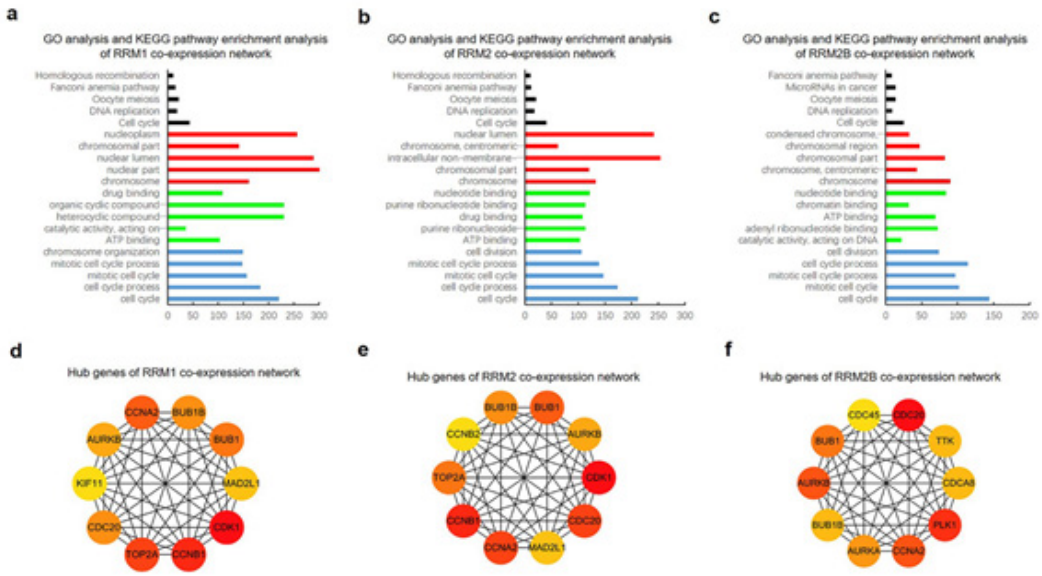

Fig 7 The co-expression genes network of ribonucleotide reductase (RR) subunit genes in lung adenocarcinomas (LUAD) based on The Cancer Genome Atlas (TCGA) data. Gene Ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of RRM1 (a), RRM2 (b) and RRM2B (c) co-expression network. Hub genes of RRM1 (d), RRM2 (e) and RRM2B (f) co-expression network, respectively.

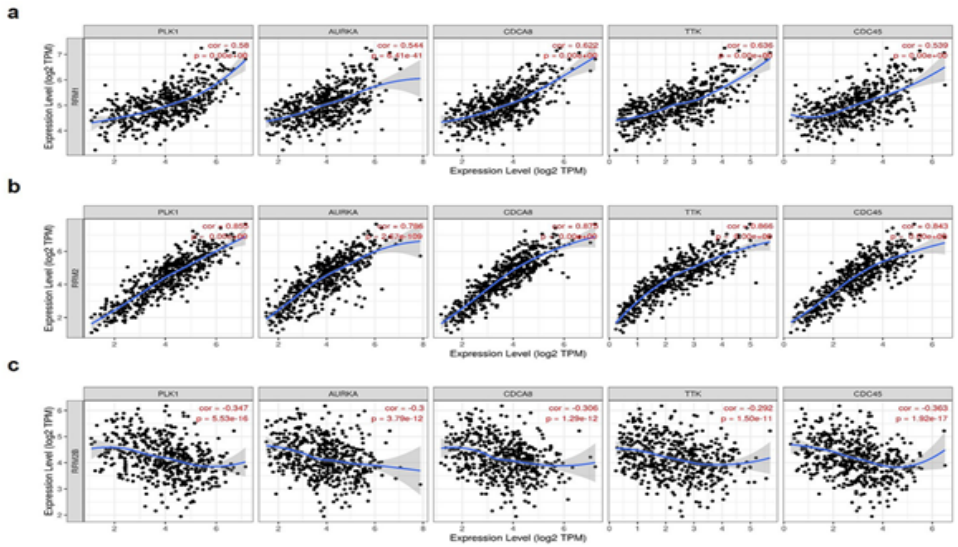

Fig 8 Correlation analysis of ribonucleotide reductase (RR) subunit genes and top five hub genes (PLK1, AURKA, CDCA8, TTK and CDC45) in co-expression genes network. RRM1 (a), RRM2 (b) and RRM2B (c), respectively.

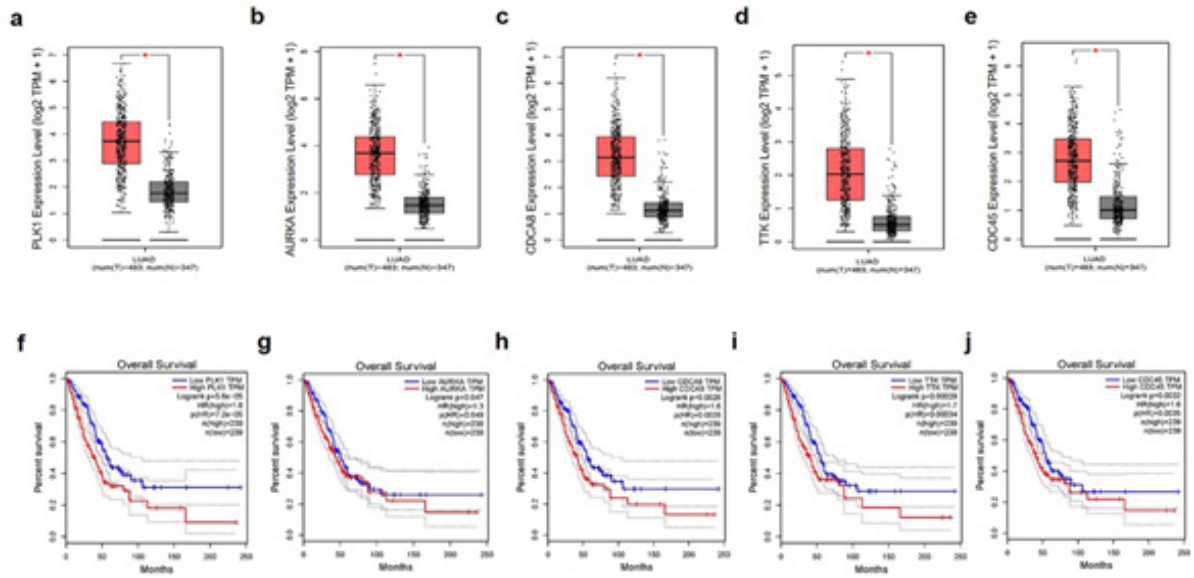

Fig 9 The mRNA expression of PLK1 (a), AURKA (b), CDCA8 (c), TTK (d) and CDC45 (e) between tumor and non-tumor samples in LUAD patients. * P < 0.05. The Kaplan-Meier survival curves comparing the high and low expression of PLK1 (f), AURKA (g), CDCA8 (h), TTK (i) and CDC45 (j) in LUAD patients.

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Table 1 Survival analysis of ribonucleotide reductase (RR) subunit genes in lung adenocarcinomas (LUAD) patients

Gene	Dataset	Endpoint	Number	In (HR-high / HR-low)	Cox P-value	In (HR)	HR [95% CI-low CI-up]
RRM1	GSE13213	Overall Survival	117	1.00857	0.0172905	0.605508	1.83 [1.11 - 3.02]
	GSE31210	Relapse Free Survival	204	1.17718	0.000120899	1.58127	4.86 [2.17 - 10.89]
	GSE31210	Relapse Free Survival	204	0.948709	0.000623419	1.44564	4.24 [1.85 - 9.72]
	GSE31210	Overall Survival	204	1.65171	0.00012536	2.04017	7.69 [2.71 - 21.82]
RRM2	jacob-00182-MSK	Overall Survival	104	1.20921	0.000422838	0.823733	2.28 [1.44 - 3.60]
	GSE13213	Overall Survival	117	1.26224	4.70E-06	0.677312	1.97 [1.47 - 2.63]
	GSE13213	Overall Survival	117	1.17854	8.11E-06	0.597736	1.82 [1.40 - 2.36]
	GSE31210	Overall Survival	204	1.85906	0.000352093	0.678209	1.97 [1.36 - 2.86]
	GSE31210	Relapse Free Survival	204	1.53064	1.94E-06	0.650149	1.92 [1.47 - 2.50]
RRM2B	GSE31210	Overall Survival	204	-1.37967	0.00116781	-1.44298	0.24 [0.10 - 0.56]
	GSE31210	Relapse Free Survival	204	-1.65533	1.56E-06	-1.66774	0.19 [0.10 - 0.37]

Considering that the purity of cancer or different infiltrating immune cells stats may also influence the prognostic analysis, we further evaluated RR subunit genes expression and infiltrating immune cells stats for LUAD patient's prognosis after adjustment by tumor purity from TIMER[28]. As shown in Fig. 6a, high RRM1 and RRM2 expression was significantly associated with poor prognosis in LUAD patients (P = 0.008 and 0.00017, respectively), while high RRM2B expression was significantly associated with a favorable prognosis (P = 0.0205). These results were consistent with the above prognostic analysis. Similarly, we also found that higher infiltrating levels of B cell or dendritic cells were associated with increased survival in LUAD patients (P = 0.001868947 and 0.025205621, respectively) (Fig. 6b).

Screening and analysis of co-expression genes with RR subunit genes in LUAD

In order to further analyze the potential mechanism of the influence of RR subunit genes on LUAD, the co-expression genes of RR subunit genes in TCGA LUAD transcriptome data were screened by cBioProtal data analysis platform. When absolute value of Spearman score was more than 0.3, we found that 1955, 3670 and 1337 co-expression genes with RRM1, RRM2 and RRM2B, respectively. The top 10 co-expression genes of RRM1, RRM2 and RRM2B were presented in Table 2. As shown in Fig. 7a, b and c, GO analysis and KEGG pathway enrichment analysis were performed for the obtained top 500 co-expression genes with RR subunit genes. The top terms of biological process (BP) included cell cycle, cell cycle process, mitotic cell cycle, mitotic cell cycle process and cell division. Molecular function (MF) indicated enrichment predominantly involved in ATP binding, catalytic activity and drug binding. As for cell component (CC), these genes showed significant enrichment in chromosome and chromosomal part. Additionally, KEGG pathway analysis revealed that these genes were significantly enriched in the cell cycle, DNA replication, oocyte meiosis and fanconi anemia pathway.

Next, we used the obtained top 500 co-expression genes to integrate the PPI network by STRING online software, and then carried out using cytoHubba to select the hub related genes, and the top 10 significant related genes were selected based on the degree of importance, top 10 hub genes of RRM1 and RRM2 were very similar, such as CDK1, CCNB1, TOP2A, CCNA2, BUB1, BUB1B, CDC20, AURKB and MAD2L1 (Fig. 7d and e). For RRM2B, we found five new hub genes comparable with RRM1 and RRM2, including PLK1, AURKA, CDCA8, TTK and CDC45 (Fig. 7f).

Furthermore, we found that these five new hub genes significantly positive correlated with RRM1 and RRM2 expression (both Spearman's correlation > 0.5) but were negatively correlated with RRM2B expression (Fig. 8 a, b and c). Finally, the online tool GEPIA database was used to compare mRNA expression levels of these five new hub genes in tumor and non-tumor samples in LUAD patients, and significantly increasing expression of PLK1, AURKA, CDCA8, TTK and CDC45 were found in LUAD patients compared to control (Fig. 9a-9e, all P < 0.05). The prognostic value of these five hub genes was also analyzed. As shown in Fig. 9f-9j, the high expression of PLK1, AURKA, CDCA8, TTK and CDC45 was associated with poor OS in LUAD patients (PLK1, HR = 1.8, Logrank P = 7.2E-05; AURKA, HR = 1.3, Logrank P = 0.047; CDCA8, HR = 1.6, Logrank P = 0.0026; TTK, HR = 1.7, Logrank P = 0.00029 and CDC45, HR = 1.6, Logrank P = 0.00032, respectively).

Table 2 cBioPortal analysis of the 10 genes most closely related to ribonucleotide reductase (RR) subunit genes

	Correlated Gene	Cytoband	Spearman's Correlation	P-Value	Q-Value
RRM1	KIF18A	11p14.1	0.698001886	1.03E-76	2.07E-72
	FAM111B	11q12.1	0.693762037	1.97E-75	1.98E-71
	CLSPN	1p34.3	0.68926361	4.26E-74	2.86E-70
	SMC2	9q31.1	0.687361417	1.54E-73	7.75E-70
	WDHD1	14q22.2	0.674410008	7.47E-70	3.01E-66
	FEN1	11q12.2	0.673995442	9.73E-70	3.26E-66
	DTL	1q32.3	0.671944148	3.58E-69	1.03E-65
	CKAP2L	2q14.1	0.668892998	2.44E-68	6.13E-65
	HELLS	10q23.33	0.662470872	1.28E-66	2.87E-63
	E2F8	11p15.1	0.660312594	4.75E-66	9.57E-63
RRM2	NCAPG	4p15.31	0.897566	2.61E-185	3.37E-181
	CCNA2	4q27	0.897463	3.35E-185	3.37E-181
	BUB1	2q13	0.892723	1.99E-180	1.34E-176
	CCNB2	15q22.2	0.885582	1.22E-173	6.16E-170
	CENPA	2p23.3	0.883024	2.57E-171	1.03E-167
	CKAP2L	2q14.1	0.880323	6.37E-169	2.14E-165
	SGO1	3p24.3	0.88003	1.15E-168	3.30E-165
	NUSAP1	15q15.1	0.878449	2.70E-167	6.27E-164
	SPC25	2q24.3	0.878429	2.80E-167	6.27E-164
	CDC6	17q21.2	0.87369	2.78E-163	5.59E-160
RRM2B	EDA2R	Xq12	0.553944	6.50E-43	1.31E-38
	FCHO2	5q13.2	0.5323	3.63E-39	2.30E-35
	PKMYT1	16p13.3	-0.53192	4.20E-39	2.30E-35
	CD302	2q24.2	0.531706	4.56E-39	2.30E-35
	CPNE3	8q21.3	0.528915	1.32E-38	5.33E-35
	TACC3	4p16.3	-0.524	8.44E-38	2.83E-34
	TROAP	12q13.12	-0.52348	1.02E-37	2.95E-34
	TEDC1	14q32.33	-0.52254	1.45E-37	3.66E-34
	TEDC2	16p13.3	-0.52113	2.46E-37	5.50E-34
	KIFC1	6p21.32	-0.5193	4.84E-37	9.74E-34

Discussion

In this study, we found that each RR-related gene was associated differentially with OS of LUAD patients. Elevated expression levels of RRM1 and RRM2 were associated with worse prognosis, while higher RRM2B expression was found to be associated with better prognosis in the whole cohort of LUAD patients. Moreover, RRM2B mRNA levels correlated with the abundance of tumor-infiltrated immune cells compared with RRM1 or RRM2. Importantly, we also found five new hub genes (PLK1, AURKA, CDCA8, TTK and CDC45), both significantly increasing expression and with poor prognosis in LUAD prognosis, were significantly positive correlated with RRM1 and RRM2 expression whereas were negatively correlated with RRM2B expression.

RR plays a critical role in DNA synthesis and thus is essential for cell proliferation and the development of malignancy[29]. In normal human cells, three RR subunit proteins from two-types of holoenzymes (RRM1- RRM2 and RRM1 - RRM2B) and their genes are separately distributed in three different chromosomes. Although RRM2 and RRM2B are highly homologous in their gene sequences, their expressional levels and subcellular localizations are differently regulated in cells. Some studies showed that high RRM1 expression was associated with better survival in early stage NSCLC[30] or had poor prognosis in LUAD[31], while another study showed that RRM1 protein expression had no significant predictive value for early NSCLC patients[32]. The controversial results were also found in RRM2, some studies suggested that high expression of RRM2 correlated with a shorter overall survival for NSCLC patients[33,34], while others did not find any predictive value[30,35]. Previously studies have shown that high expression of RRM2B protein was a favorable prognostic factor in early NSCLC patients[36]. However, other authors reported that RRM2B expression did not play a prognostic role in NSCLC patients with resected TNM stages I–III tumors[32,37]. These results implied the different roles of RR subunits in LUSC and LUAD, suggesting that it is necessary to separate tumors by their histological or pathological subtypes during research and clinical evaluation of molecular biomarkers in NSCLC. In our study, we focus on the relationship between RR subunit genes and LUAD. After combining RNA-seq and clinicopathologic data from several public databases, we found that expression of

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RRM1 and RRM2 genes was both significantly increased and correlated with a worse prognosis in LUAD patients and demonstrated a tumor-promoting role for the RRM1-RRM2 holoenzyme. In contrast, the expression of RRM2B was significantly decreased in LUAD by analyzing data from Oncomine, TIMER, and GEPIA. However, our results demonstrate that high expression of RRM2B protein was a favorable prognostic factor in LUAD patients.

In this study, the relationship between the expression of RR subunit genes and immune infiltrating in LUAD was also analyzed using the TIMER database. Expressions of RRM2B were significantly associated with various immune infiltrating cells compared with RRM1 or RRM2, suggesting that RRM2B might play prominent roles in regulating tumor immune cell infiltrating other than RRM1 and RRM2, this might partly explain why high RRM2B expression patients always have a favorable prognosis. In addition, we explored the potential mechanisms that RR subunit genes are involved in the carcinogenesis of LUAD, and constructed a PPI network and performed GO and KEGG analyses for co-expression genes with RR subunit genes in LUAD by cBioProtal data analysis platform. The results showed that RRM1 and RRM2 top co-expression genes were very similar. These genes mainly participate in cell cycle process, the mitotic cell cycle, mitotic cell cycle process and cell division. MF indicated enrichment predominantly involved in ATP binding, catalytic activity and drug binding. KEGG pathway analysis revealed that these genes were significantly enriched in the cell cycle, DNA replication, oocyte meiosis and fanconi anemia pathway. Our results also suggested that high RRM1 and RRM2 expression were significantly associated with poor prognosis, while high RRM2B expression with a favorable prognosis of LUAD patients. The co-expression gene network of RR subunit genes further shown genes with poor prognosis (such as PLK1, AURKA, CDCA8, TTK and CDC45) tends to be highly expressed at the same time. Therefore, clinical relevance of RR subunit genes and their co-expression gene network in LUAD should also be further illustrated in the future.

This study provided potential biomarkers for clinical prognosis of LUAD. However, there were some limitations. Firstly, although the dysregulation of RR subunit genes expression has been found closely related to prognosis of LUAD patients, the related molecular regulatory mechanisms should be further tested. Secondly, clinical validations and functional studies were needed to reveal the inner mechanisms of how RR subunit genes and their co-expression gene network correlated with the clinical outcomes of lung cancer patients.

Conclusions

In conclusion, high RRM1 and RRM2 expression and low RRM2B expression suggested poor prognosis in patients with LUAD. Meanwhile, by co-expression gene network analysis of RR subunit genes, we found that five new hub genes (PLK1, AURKA, CDCA8, TTK and CDC45) tend to be highly expressed and associated with a poor prognosis in LUAD patients at the same time. These findings demonstrate that RR subunit genes may be a prognostic marker and therapeutic target for LUAD.

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Conflicts of 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.

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Abbreviations

LUAD, lung adenocarcinoma; LUSC, lung squamous carcinoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; RR, ribonucleotide reductase; TIMER, tumor immune estimation resource; TCGA, the cancer genome atlas; GO, gene ontology; KEGG, kyoto encyclopedia of genes and genome; PPI, protein-protein interaction; STRING, search tool for the retrieval of interacting genes; GEPIA, gene expression profiling interactive analysis; OS, overall survival.

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