

**Research Article****Pyrimidine Metabolism-related gene RRM2 as a novel biomarker for Prognosis and immunotherapy in LUAD**Beibei Huang<sup>1,2\*</sup>, Jie Li<sup>1,2\*</sup>, Junru Feng<sup>3</sup>, Chen Xin<sup>4</sup>, Qing Ye<sup>1,2\*</sup>, Xin Kong<sup>1,2\*</sup><sup>1</sup>Department of Pathology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, China.<sup>2</sup>Intelligent Pathology Institute, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, Anhui, 230036, China.<sup>3</sup>Hefei National Laboratory for Physical Sciences at Microscale, The Chinese Academy of Sciences Key Laboratory of Innate Immunity and Chronic Disease, School of Basic Medical Sciences, Division of Life Science and Medicine, University of Science and Technology of China, Hefei 230027, China.<sup>4</sup>Hefei National Laboratory for Physical Sciences at the Microscale, CAS Key Laboratory of Mechanical Behavior and Design of Materials, Department of Precision Machinery and Precision Instrumentation, University of Science and Technology of China, Hefei 230026, China..**Abstract****Background:** Lung adenocarcinoma is the most common type of lung cancer. Although there have been many immunotherapy applications of immune checkpoint molecules, it is only effective for some patients.**Methods:** In this study, we downloaded the expression data, gene mutation data, and clinical characterization data of TCGA lung adenocarcinoma samples to identify metabolic markers associated with immune checkpoint molecules through bioinformatics methods. Enrichment analysis was performed using Gene Set Enrichment Analysis (GSEA). Correlations of gene expression with clinical characteristic were calculated using Fisher's exact test. Protein-protein interaction (PPI) networks were constructed and core genes were identified using the STRING database and Cytoscape. Kaplan-Meier survival curves were calculated by R language to evaluate the relationship between gene expression and prognosis. Finally, the CIBERSORT algorithm and linear regression model were used to assess the immune microenvironment infiltrating immune cells.**Results:** Through a comprehensive analysis of multiple data from 510 lung adenocarcinoma samples, we found that although patients with high pyrimidine metabolism lung adenocarcinoma have a worse prognosis, they may be a more suitable group of patients for immunotherapy. Among them, the pyrimidine metabolism-related

molecule RRM2 is highly expressed in patients with lung adenocarcinoma, and is related to the process of lymph node metastasis. Lung adenocarcinoma patients with high RRM2 expression corresponded to a shorter overall survival time and showed a significant positive correlation with multiple immune checkpoint molecules.

**Conclusion:** In conclusion, our study screened an immunotherapy-related metabolic molecule, RRM2, which can serve as a novel biomarker associated with prognosis in lung adenocarcinoma and provide a new target for targeted therapy of lung adenocarcinoma.**Introduction**

Lung adenocarcinoma (LUAD), which accounts for 40% of lung cancers, is usually diagnosed at an advanced stage and has a low average 5-year survival rate (about 20%) [1] [2] [3]. To promote tumor growth, tumor cells initiate a unique metabolic program [4] [5]. This is not only conducive to the unlimited proliferation of tumor cells, but also shapes a unique immune microenvironment, affecting the composition and biological functions of immune cells in the microenvironment [6] [7]. Tumor-specific metabolic programs form a unique tumor microenvironment, which is characterized by hypoxia, acidity, and nutrient deprivation, which becomes a huge obstacle to the body's anti-tumor immunity [8]. Over the past few years, it has become increasingly clear that targeting metabolism has the potential to enhance the effectiveness of immune checkpoint blockade therapies in NSCLC, melanoma, and other tumors [9]. For example, pemetrexed has begun immunotherapy for non-small cell lung cancer (NSCLC) [10]. In addition to directly affecting the therapeutic efficacy of immune checkpoint inhibitors, targeting metabolism is expected to alter the TME and promote immune cell infiltration, thereby transforming these resistant tumors into sensitive ones [11] [12].

Checkpoint inhibition is a novel approach to cancer immunotherapy and is rapidly showing progress in clinical and preclinical studies as

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an adjunct to and alternative to traditional cancer therapy [13]. Common immune checkpoints include PD-1, CTLA-4, LAG3, TIM-3, and VISTA [14]. The success of immune-checkpoint blockade shows that the immune system has excellent anti-tumor capabilities [15]. In many different types of tumors, checkpoint blockade has been shown to treat tumors through endogenous antitumor immune responses, such as PD-1 inhibitors [16]. Over the past 10 years, patients with many different types of cancer have been treated with immune checkpoint blockade with varying degrees of success [17]. PD-1 was originally identified as a gene that was upregulated in T cell hybridomas undergoing cell death, and was upregulated on T cells following activation by the T cell receptor [18]. PD-1 blockers have also achieved impressive clinical outcomes in patients with advanced NSCLC and are currently being studied in combination with CTLA-4 blockers [19]. Treatment with immune checkpoint inhibitors not only enhances immune cell activation signaling pathways [20], but also affects metabolic communication and competition between tumor and immune cells in the tumor microenvironment (TME) [21].

However, current studies combining targeting of metabolic pathways with immunotherapy are insufficient. In this study, a comprehensive analysis of transcriptomic data, mutation data, and Clinical Characteristics data of 510 lung adenocarcinoma tissue samples and 59 normal tissue samples from the TCGA dataset was performed to discover novel biomarkers and their prognostic value. To further explore the function and prognostic value of marker genes, we used protein interaction network analysis and immune cell composition analysis. Finally, correlation analysis of biomarker genes with immune checkpoints allowed us to identify potential targets with prognostic and therapeutic value.

## Materials and Methods

### Data Collection.

RNA-seq data, Mutation data and clinical characteristic data of 510 lung adenocarcinoma tissue samples and 59 normal tissue samples were collected on the cbiportal website [22], available through the link: [https://www.cbiportal.org/study/summary?id=luad\\_tcga\\_pan\\_can\\_atlas\\_2018](https://www.cbiportal.org/study/summary?id=luad_tcga_pan_can_atlas_2018), which was originated from TCGA database [23]. An unpaired Student's t-test was used to calculate the p value between tumor and normal samples.

### Identify Differentially Expressed Genes (DEGs).

Before performing differential expression analysis on RNA-seq data, we took the logarithm ( $\log_2$ ) of the RSEM-normalized expression data and compared gene expression values between tumor samples and normal samples. Significantly differentially expressed genes were filtered with  $|\log_2 \text{ fold change}| > 2$ ,  $p \text{ value} < 1e-10$  and  $FDR < 0.05$  across all samples. An unpaired Student's t-test and Benjamini-Hochberg multiple test were used to calculate the p value and FDR value between tumor samples and normal samples.

### Gene Set Enrichment Analysis (GSEA).

We first sorted the  $\log_2$  fold change gene expression matrix (tumor vs normal) in descending order, and then used the R package "clusterProfiler" to calculate the normalized enrichment scores (NES) and p values in the gene expression matrix for the metabolism-related gene set downloaded from the MSigDB database [24]. Gene sets with p-values less than 0.05 were considered significantly enriched, with a positive NES value indicating that the pathway was

2.4. Kaplan-Meier Survival Analysis. We used Kaplan-Meier (K-M) survival analysis via the "survminer" and "survival" packages of R to examine the associations between subgroup and overall survival. Comparison of two survival curves was used a statistical hypothesis test called the log rank test. The p values less than 0.05 was considered statistically significant.

### Calculation of Pyrimidine Metabolism Gene Set Score.

The "pyrimidine metabolism" gene set was downloaded from MSigDB with Systematic name: M5109, that are publicly accessible at [https://www.gsea-msigdb.org/gsea/msigdb/cards/KEGG\\_PYRIMIDINE\\_METABOLISM](https://www.gsea-msigdb.org/gsea/msigdb/cards/KEGG_PYRIMIDINE_METABOLISM). For gene expression data, we first summed the Z-scores of the expression values of all genes contained within the gene set in all samples, and then normalized them to a value from 0 to 1 across samples as gene set signature score. The tumor samples were divided into high pyrimidine metabolism and low pyrimidine metabolism groups according to the median expression level of gene set feature score.

### Correlation Analysis of Gene Expression and Clinical Characteristics.

First, the samples were divided into high and low expression groups according to the median expression of gene among all tumor samples, and then Fisher's exact test was used to estimate the correlation analysis between clinical characteristics and gene expression. P value  $< 0.05$  were considered as significant.

### Protein Interaction Network Analysis.

We used GeneMANIA [25] to predict gene function and association of RRM2 with other proteins, including protein and genetic interactions, pathways, co-expression, co-localization, and Shared protein domains.

### Assessing the Association of RRM2 with Immune Cell Types.

First, we applied the CIBERSORT [26] analysis tool to gene expression data to estimate the abundance of various immune cells in mixed tumor samples. The signature file used was LM25, which consists of 547 genes that accurately distinguish 22 mature human hematopoietic populations and activation states, including seven T cell types, naive and memory B cells, plasma cells, NK cells, and myeloid subtypes. Then, two methods were used to assess the association of RRM2 with immune cell subsets. The first method: we divided the samples into high expression RRM2 group and low RRM2 group according to the gene expression level of RRM2, and then calculated the  $\log_2$  (od ratio) of the proportion of immune cell subsets in the two groups. The second method: The "OLS" function in the statistical model Python package was used to perform linear regression as an approach to measure the correlation of RRM2 gene expression and proportion of immune cell types.

### Correlation Analysis of RRM2 and immune checkpoint molecules.

The "OLS" function in the statsmodels Python package was used to perform linear regression as an approach to measure the correlation of RRM2 with immune checkpoint molecules including CD276, LAG3, PD1, PD-L1, TIM3, TIGIT, CTLA4, SIGLEC15 and HAVCR2. R-squared is a goodness-of-fit measure for linear regression models, and p characterizes the probability that two variables are significantly linearly related.

### Statistical Analysis.

Data analysis were accomplished by R software (version 3.6.1), Python software (version 3.8.2) and Prism (version 8.4.3). The levels of significance were indicated as follows: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ .

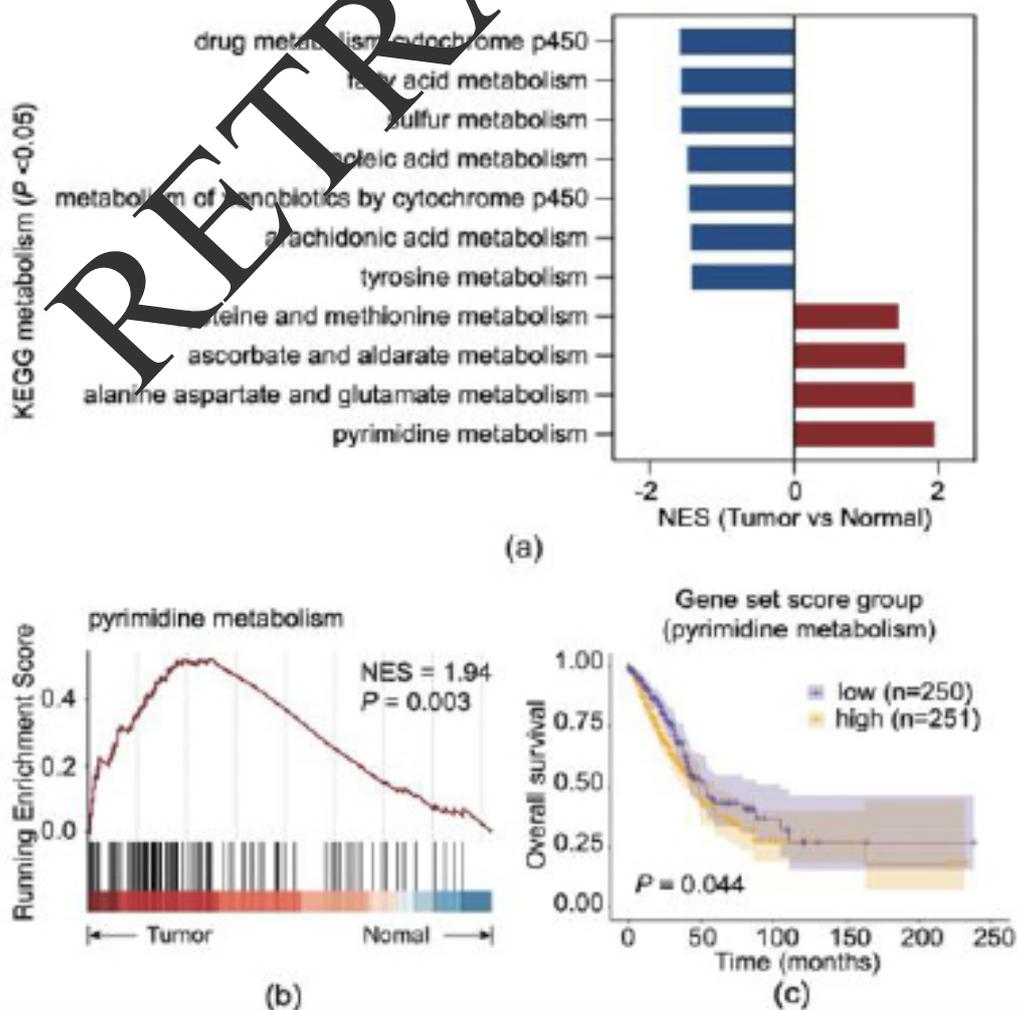
## Results

### Identification of Metabolic Pathways Significantly Enriched in Lung Adenocarcinoma Samples.

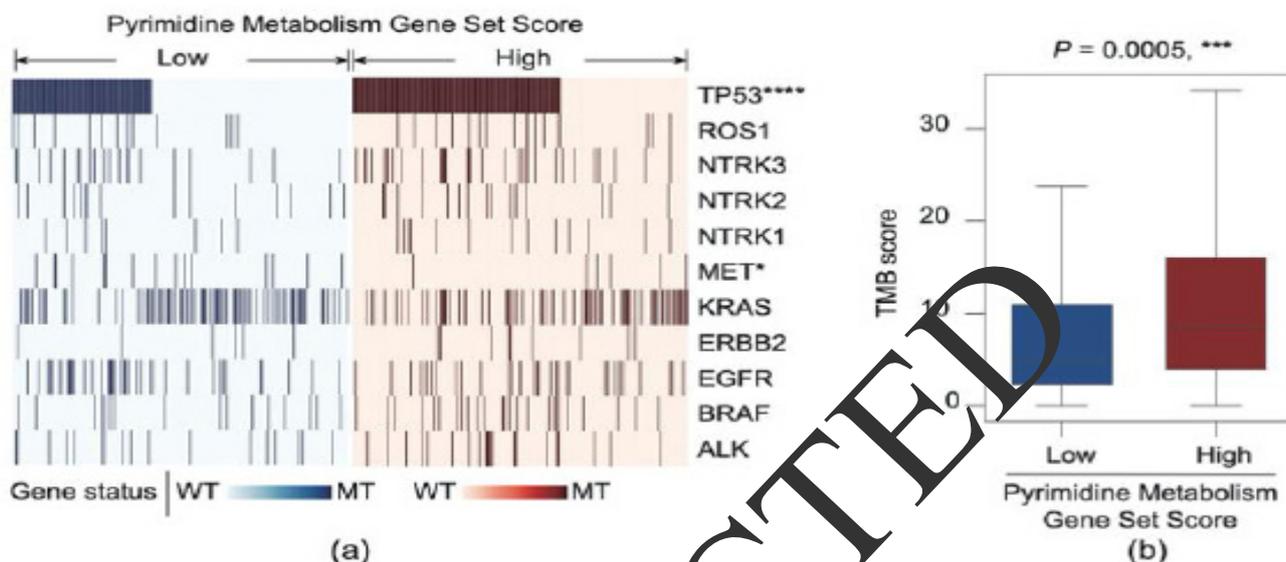
We collected a total of 41 metabolism-related KEGG pathways from the MSigDB database (Supplementary Table S1). To investigate the differences in metabolic pathways between lung adenocarcinoma samples and normal samples, we used GSEA enrichment analysis, resulting in a significant differential enrichment between 510 lung adenocarcinoma samples and 59 normal samples. We found that "pyrimidine metabolism", "alanine aspartate and glutamate metabolism", "cysteine and methionine metabolism" and "ascorbate and aldarate metabolism" were significantly enriched in tumor samples (Figure

**TABLE 1:** Correlations with clinical characteristics in LUAD samples with high and low pyrimidine metabolic pathways

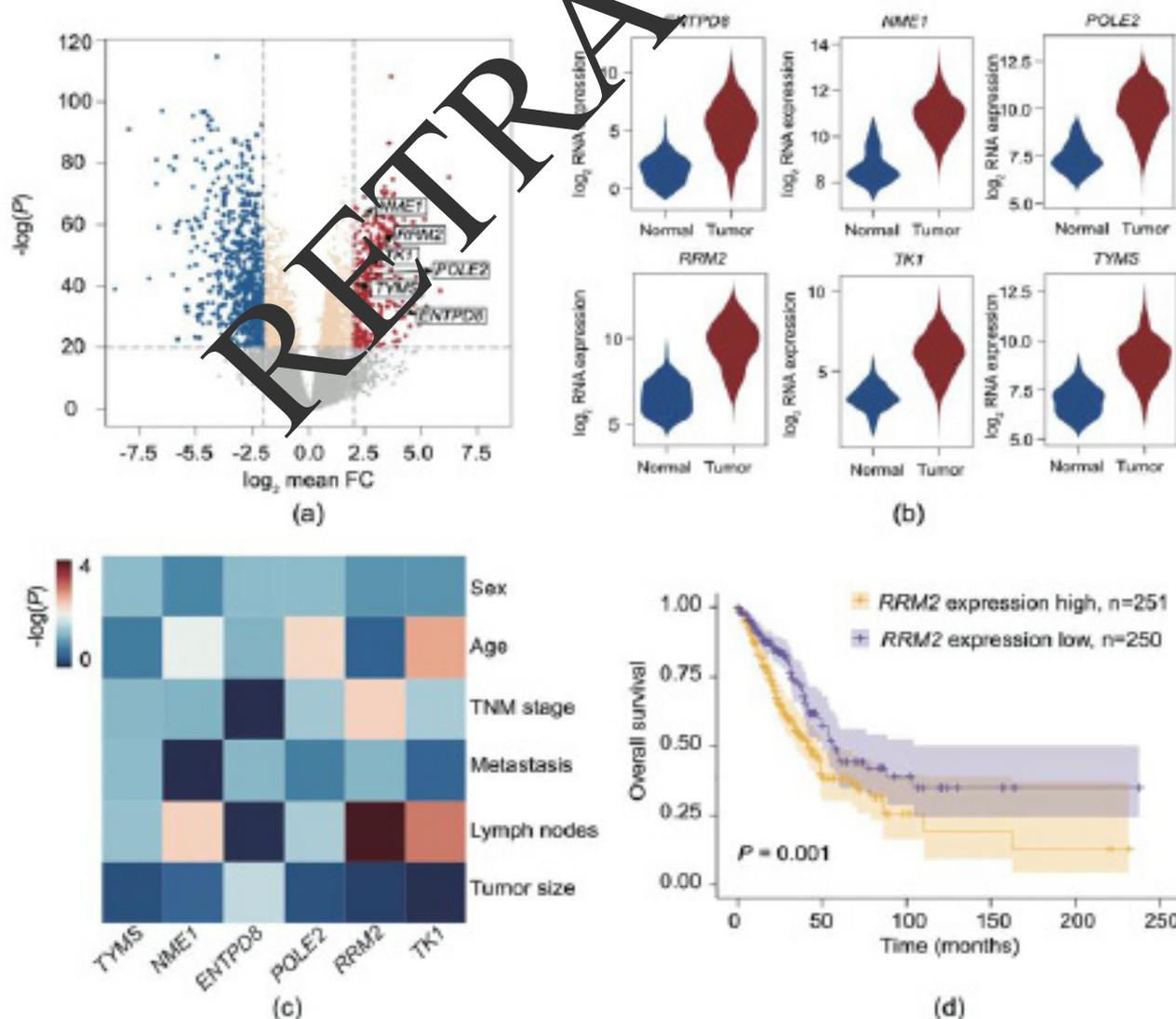
clinical characteristics	number	Gene set scores for pyrimidine metabolic		p value
		High(n=251)	Low(n=250)	
Sex				0.504
male	233	113	120	
female	268	138	130	
Age(year)				0.250
>60	334	158	176	
<=60	157	83	74	
TNM stage				0.150
I	270	144	127	
II	121	65	56	
III	80	29	51	
IV	27	13	14	
Distant metastasis				0.807
Yes	25	13	12	
No	332	181	151	
Lymph node metastasis				0.266
Yes	167	78	89	
No	323	168	155	
Tumor size				0.074
T1+T2	427	221	206	
T3+T4	63	29	38	



**FIGURE 1:** Pyrimidine metabolic pathway is highly enriched in lung adenocarcinoma. (a) Histograms show metabolic pathways with significant enrichment differences between lung adenocarcinoma and normal samples in GSEA analysis results. Red indicates that this pathway is highly enriched in lung adenocarcinoma, and blue indicates that this pathway is highly enriched in normal samples. (b) GSEA enrichment plots highlighting RNA-seq signals for pyrimidine metabolism. (c) Kaplan-Meier (K-M) overall survival curve of lung adenocarcinoma patients grouped according to sample pyrimidine metabolism score. NES, Normalized enrichment scores. \* $P < 0.05$ , \*\* $P < 0.01$ .



**FIGURE 2:** TMB was positively correlated with the level of pyrimidine metabolism in lung adenocarcinoma samples. (a) Mutation status of driver genes in samples with high and low pyrimidine metabolism. (b) Boxplot showing TMB scores in samples with high and low pyrimidine metabolism.



**FIGURE 3:** RRM2 was significantly overexpressed in lung adenocarcinoma samples and correlated with prognosis. (a) Volcano plot showing differentially expressed genes in lung adenocarcinoma samples compared to normal samples. Dark red dots indicate genes that are highly expressed in lung adenocarcinoma samples, and dark blue dots indicate genes that are under-expressed in lung adenocarcinoma samples. Genes marked with text indicate differentially expressed genes located in the pyrimidine metabolic pathway. (b) Violin plot showing the gene expression of 6 differentially expressed genes in lung adenocarcinoma and normal samples. (c) Correlations between genes and clinical characteristics. Fisher's exact test was used to estimate the correlation analysis between clinical characteristics and gene expression. (d) K-M overall survival curve of lung adenocarcinoma samples by median RRM2 expression.

1(a)). Meanwhile, pathways such as “drug metabolism cytochrome p450” and “fatty acid metabolism” were significantly enriched in normal samples compared with tumor samples (Figure 1(a)). Among the 11 enriched metabolic pathways, the “pyrimidine metabolism” showed the most significant enrichment in tumor samples (Figure 1(b), NES = 1.94, p value = 0.03).

We divided the patients into high pyrimidine metabolism group and low pyrimidine metabolism group according to the score of pyrimidine metabolism in the sample. By analyzing the association between the pyrimidine metabolism level of lung adenocarcinoma patients and various clinical characteristics, we found that there was no significant correlation between the overall pyrimidine metabolism level of the sample and the clinical characteristics of the patients (Table 1). Therefore, then we evaluated the impact of differences in pyrimidine metabolism between samples on prognosis. To assess the impact of differences in pyrimidine metabolism between samples on prognosis, we performed an overall survival (OS) analysis of samples from the high and low pyrimidine groups and found that lung adenocarcinoma samples with high pyrimidine metabolism showed worse prognostic outcomes (Figure 1(c), p value = 0.04).

**Mutation and TMB and in LUAD Samples with High and Low Pyrimidine Metabolism.**

Next, we examined whether the two groups of lung adenocarcinoma samples with different levels of pyrimidine metabolism differed at the level of driver mutations. In addition to TP53, we found that a critical mutation driven gene MET [27] had more mutations in the low-pyrimidine metabolome (Figure 2(a)). Although the remaining lung cancer driver genes did not differ significantly in the number of mutations between the two pyrimidine metabolism groups, we observed

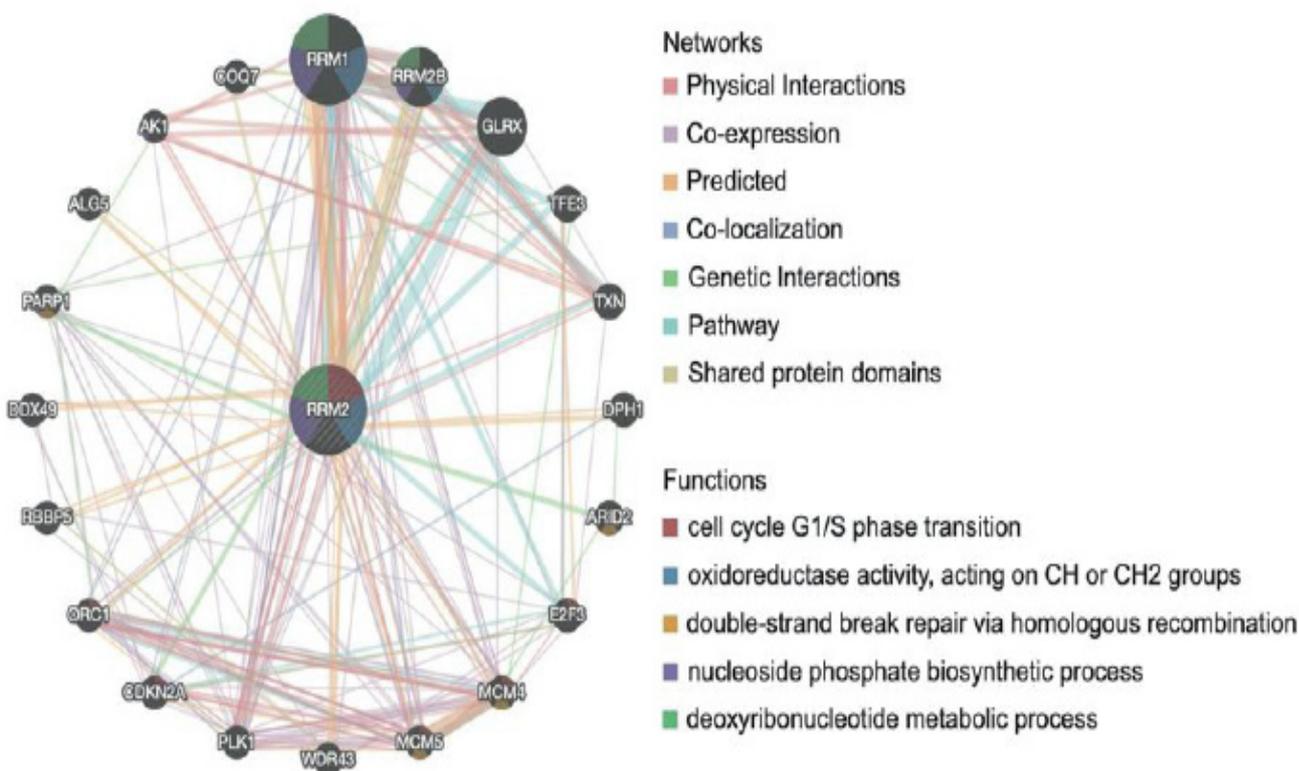
that lung adenocarcinoma patients with high pyrimidine metabolism had higher TMB scores (Figure 2(b), p value = 0.0005). This suggests that the higher the degree of pyrimidine metabolism in the tumor sample, the higher TMB scores, and the worse the cancer status.

**Six genes in the pyrimidine metabolic pathway are highly expressed in lung adenocarcinoma samples compared to normal samples.**

The pyrimidine metabolism pathway contains a total of 98 genes. To screen out differentially expressed pyrimidine metabolism-related genes in lung adenocarcinoma, we performed differential gene expression analysis on 510 lung adenocarcinoma samples and 59 normal samples. The volcano plot showed that lung adenocarcinoma samples significantly up-regulated 355 genes and significantly down-regulated 740 genes compared with normal samples (Figure 3(a), Supplementary Table S2). Among all the differential genes, 6 are from the pyrimidine metabolism pathway, namely ENTPD8, NME1, POLE2, RRM2, TK1 and TYMS (Figure 3(b)).

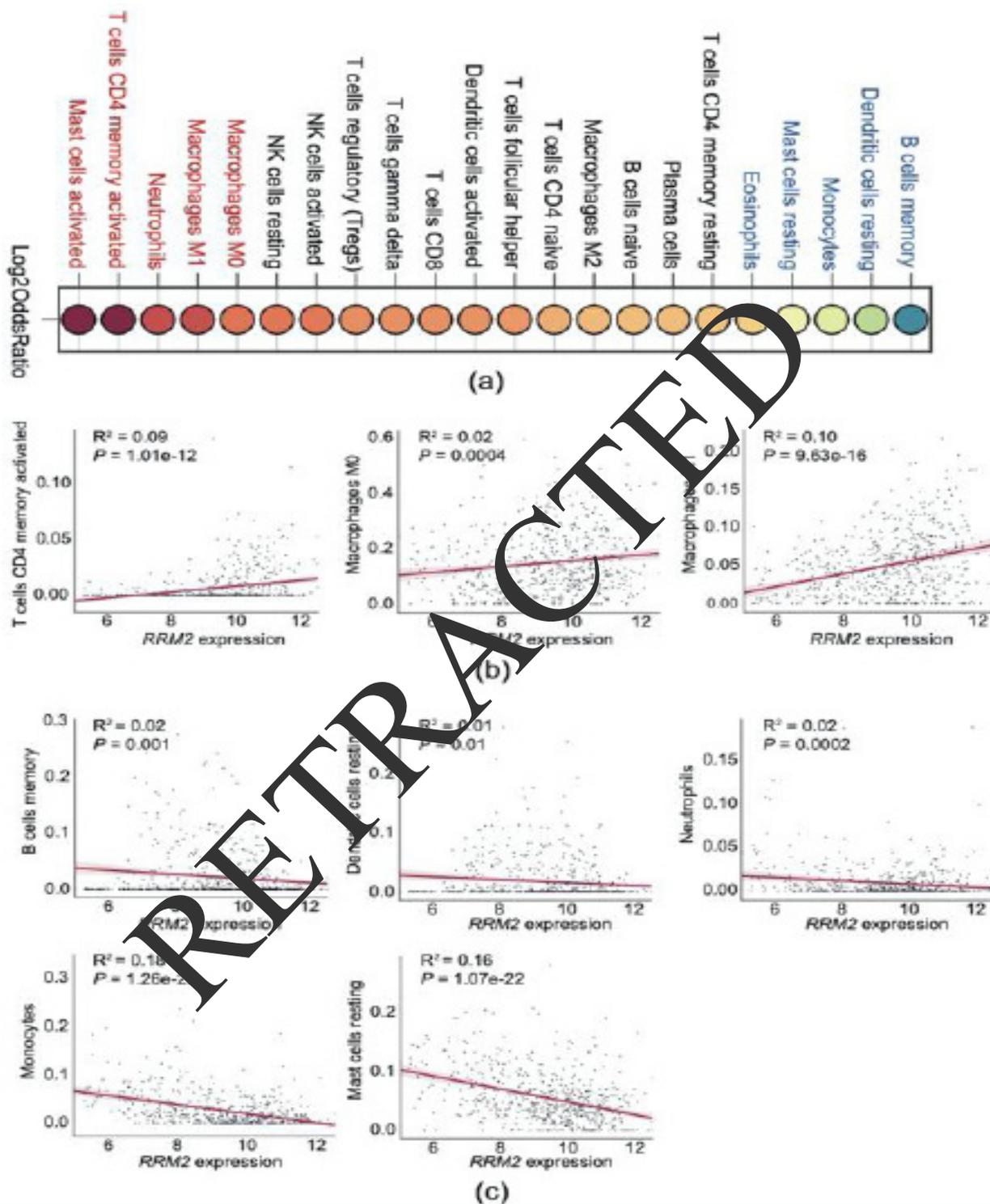
**The correlation between Pyrimidine metabolism related genes and clinical characteristics.**

We analyzed the correlation between the 6 pyrimidine metabolism-related genes that were significantly highly expressed in lung adenocarcinoma samples and the clinical characteristics of the samples, and found that RRM2 showed the strongest correlation with lymph node metastasis (Figure 3(c)). Considering that there is a clear correlation between the expression of RRM2 gene and the clinical characteristics of patients, we then divided samples into high and low groups according to the expression of RRM2, and calculated the difference overall survival between the two groups. The results of the K-M survival curve showed that lung adenocarcinoma samples with high expression of RRM2 tended to have a worse prognostic status (Figure 3(d)).



(a)

**FIGURE 4:** PPI network generated using GeneMANIA. The network edges refer to the types of interactions between gene pairs, and the colors filled in the nodes refer to the paths the genes are involved in.



**FIGURE 5:** Correlation of RRM2 expression in LUAD with immune infiltrating cells. (a) Dot plot showing the log<sub>2</sub> (odds ratio) of the comparison between the cell proportions of immune cell types in the tumor and normal samples. (b) Linear regression analysis was used to compare the relationship between RRM2 expression and the proportion of immune cells.

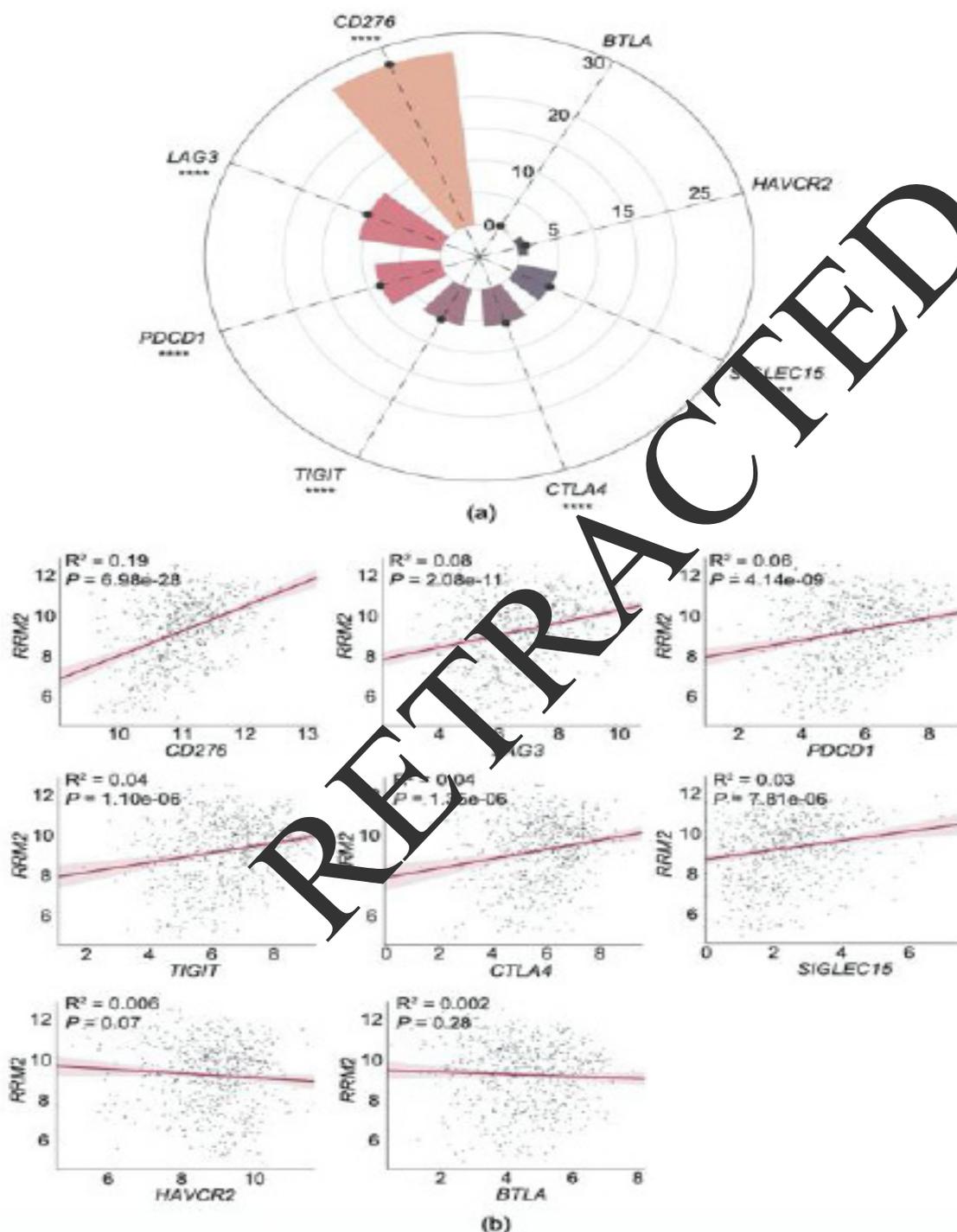
**PPI-protein interaction network and functional enrichment of RRM2.**

To further explore the biology of RRM2 and its interactions with other proteins, we used PPI analysis tool: GeneMANIA (version 3.6.0). RRM1, the large subunit of human ribonucleotide reductase, which is involved in the regulation of cell proliferation, cell migration, tumour and metastasis development, and the synthesis of deoxyribonucleotides for DNA synthesis [28]. We noticed the strongest protein interaction association between RRM1 and RRM2(Figure 4(a)). In addition, proteins such as RRM2B, GLRX, and PLK1 also have certain interactions with RRM2, including physical connection, co-expression, and co-localization (Figure 4(a)).

The results of gene function analysis showed that RRM2 may be involved in and play a role in biological processes such as “cell cycle G1/S phase transition”, “oxidoreductase activity, acting on CH or CH<sub>2</sub> groups” and “double-strand break repair via homologous recombination” (Figure 4(a)).

**Correlation between RRM2 expression and proportion of immune cell subsets.**

The immune microenvironment of lung adenocarcinoma affects tumor progression and prognosis. Previous studies have revealed the heterogeneity of the immune microenvironment in tumors and their possible functions in lung cancer [29] [30]. Therefore, to reveal the differences in immune cell composition among lung adenocarcinoma



**FIGURE 6:** Correlation of RRM2 expression in LUAD with immune checkpoint molecules. (a) The radar plot shows the correlation between immune checkpoint molecules and RRM2 expression, and the level of bar represents  $-\log_{10}(p \text{ value})$ . (b) Linear regression analysis were used to compare the relationship between RRM2 expression and the expression of CD276, LAG3, PDCD1, TIGIT, CTLA4, SIGLEC15 and HAVCR2.

patients with differential RRM2 expression, we used the software CIBERSORT to compare the proportions of 22 immune cell subsets in two groups of lung adenocarcinoma samples. “Mast cells activated”, “T cells CD4 memory activated”, “Neutrophils”, “Macrophages M1” and “Macrophages M0” were more represented in lung adenocarcinoma samples with high RRM2 expression than in lung adenocarcinoma samples with low RRM2 expression (Figure 5(a)). Conversely, “B cells memory”, “Dendritic cells resting”, “Monocytes”, “Mast cells resting” and “Eosinophils” decreased in the samples with high expression of RRM2 (Figure 5(a)).

In order to more accurately evaluate the correlation between RRM2 and immune cell subsets, we used a linear regression model to calculate the relationship between RRM2 expression and the proportion of immune cell subsets in each sample. We found that the RRM2-associ-

ated cell types calculated by this method overlapped with the above 10 cell types. For example, M1 macrophages showed the strongest positive correlation among the subpopulations in which the proportion of cells increased with increasing RRM2 expression (Figure 5(b)). The top 5 cell subsets shown in Figure 5(a) with the most significant reduction in the proportion of high-expressing RRM2 samples all showed a significant negative correlation with the expression of RRM2 in the second method (Figure 5(b)), that is, RRM2 the higher the expression level, the lower the proportion of these cell subsets.

**The expression of RRM2 in lung adenocarcinoma is co-expressed with various immune checkpoint molecules.**

The treatment of lung cancer has changed dramatically in recent years due to the advent of immune checkpoint inhibitors (ICIs). The successful use of PD-1 blockers in anticancer therapy eventually led to the

development of contemporary ICIs [31]. Besides PD-1, other immune checkpoints have also been shown to play a role in anti-tumor, such as CTLA-4, TIGIT, etc. [32]. Therefore, we examined the co-expression between RRM2 gene and various immune checkpoint molecules. We found that the expression level of the XXX molecule CD276 in lung adenocarcinoma samples exhibited a strong positive correlation with RRM2 (Figure 6(a,b),  $p$  value =  $6.98e-28$ ). We observed that the expression level of RRM2 was significantly positively correlated with multiple immunosuppressive molecules B7-H3 (CD276), SIGLEC15, CTLA4, TIGIT, PD-1 (PDCD1) and LAG3 (Figure 6(a,b)). Meanwhile, we found no correlation between RRM2 expression and BTLA or Tim-3 (HAVCR2) molecules (Figure 6b).

## Discussion

Over the past decade, significant progress has been made in the therapeutic application of immune-checkpoint inhibitors (ICIs) in cancer [33]. PD-1, PD-L1 blockers have been shown to improve overall survival in advanced metastatic non-small cell lung cancer [34]. In some patients, improved 5-year survival has been achieved. Nivolumab, atezolizumab, pembrolizumab are approved for the treatment of lung cancer [35]. There is increasing evidence that immune checkpoint inhibitors affect the metabolic state of immune cells or tumor cells [36]. Programmed cell death protein 1 (PD-1) is one of the most attractive therapeutic targets in immunotherapy, and its interaction with ligands impairs the metabolic reprogramming of T cells [37]. Besides PD-1, other immune checkpoint molecules such as CTLA-4 and LAG-3 have also been reported to affect the metabolic program of immune cells [38] [39]. A PFKFB3 inhibitor, a glycolysis inhibitor, in combination with the immune checkpoint molecule CTLA-4 has been shown to enhance the effect of single immunotherapy in a B16 mouse model [40] [41]. Although immunotherapy has brought good news to some patients, most patients do not benefit from immunotherapy [42] [43]. These studies suggest that the effects of immunotherapy vary according to metabolic responses and responses. However, effective prognostic biomarkers obtained by integrating metabolic and immune are insufficient.

In this study, we downloaded a TCGA lung adenocarcinoma dataset, including gene expression data, mutation data, and clinical characteristic data for 510 LUAD samples. First, we found a significant enrichment of pyrimidine metabolic pathways in lung adenocarcinoma using GSEA analysis of all metabolic pathways. Lung adenocarcinoma samples with high pyrimidine metabolism had worse prognosis and higher TMB scores. This suggests that although this group of patients has a worse prognosis, it may be more suitable for immunotherapy. Then, combined with the gene expression data, we screened out 6 pyrimidine metabolism genes whose expression levels in lung adenocarcinoma samples were significantly higher than those in normal samples. Finally, combined with the clinical characteristics of the samples, we used Fisher's exact test and overall survival analysis to find that RRM2 is a novel biomarker that can predict the prognosis of lung adenocarcinoma, which may be associated with lymph node metastasis in lung adenocarcinoma.

To further understand the function of RRM2 in lung adenocarcinoma and its impact on prognosis, we analyzed the protein interaction network of RRM2 using Genemania. It was found that it may participate in the biological role of the cell cycle together with proteins such as RRM1. We grouped lung adenocarcinoma samples according to the level of RRM2 expression. We noticed that the expression level of RRM2 correlated with the proportion of various immune cells infiltrating, suggesting that the pyrimidine metabolism in our lung adenocarcinoma affects the immune composition of the microenvironment. Therefore, we examined the association of therapeutically valuable immune checkpoint molecules with RRM2. Various inhibitory im-

mune checkpoints, such as PD-1, TIGIT, and CTLA-4, etc., showed a significant positive correlation with the metabolism-related molecule RRM2.

However, this study has some shortcomings. The bulk RNA-seq data used in the study included not only tumor cells, but also other cells of the tumor microenvironment, such as immune cells and stromal cells. Therefore, the identification and differentiation of molecularly derived cell subsets may lead to some new insights. Overall, our study shows that the pyrimidine metabolism-related molecule RRM2 can serve as a metabolic marker associated with immunotherapy and help advance research on targeted therapy and prognostic detection in LUAD.

## Conclusion

In this study, we combined expression data, mutation data, and clinical characterization of samples from lung adenocarcinoma and normal tissue, focusing on genes involved in pyrimidine metabolism that play a role in lung adenocarcinoma. Our results identify the association of the gene RRM2 in the pyrimidine metabolic pathway with disease progression in lung adenocarcinoma. Our results not only show that the expression level of RRM2 is closely related to lymph node metastasis and prognosis in patients with lung adenocarcinoma, but also further elucidate the impact of RRM2 on immune microenvironment composition and immune checkpoint genes. These results suggest that RRM2 can be used as a biomarker for lung adenocarcinoma prognosis and immunotherapy. These results provide important insights that RRM2 can serve as a biomarker for lung adenocarcinoma prognosis and immunotherapy, and help advance research on the treatment and prognosis of cancer patients.

## Data Availability

RNA-seq data, Mutation data and clinical characteristic data of 510 lung adenocarcinoma tissue samples and 59 normal tissue samples were collected on the cBioportal website ([https://www.cbioportal.org/study/summary?id=luad\\_tcga\\_pan\\_can\\_atlas\\_2018](https://www.cbioportal.org/study/summary?id=luad_tcga_pan_can_atlas_2018)), which was originated from TCGA database. The code used for data analysis in this article can be found in the following GitHub repository: [https://github.com/cathy1994xixi/LUAD\\_RRM2](https://github.com/cathy1994xixi/LUAD_RRM2).

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Beibei Huang conceived and conducted the project. Beibei Huang collected data and performed the data analysis in the article with the help of other authors. Junru Feng, Li Jie and Qing Ye put forward many constructive comments on the project. Beibei Huang and Xin Kong wrote the paper with the help of Qing Ye and all the other authors.

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