M6a Regulator-Mediated RNA Methylation modification Patterns Correlated With Prognosis Value and Immune Response in Adenocarcinoma of lung

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Research Article

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Abstract

Background: Lung adenocarcinoma (LUAD) is one of the most common subtypes of non-small cell lung cancer (NSCLC) and the main cause of death of cancer patients worldwide. N6-methyladenosine (m6A) modification and long noncoding RNAs (lncRNAs) are of significance in the prognosis and immunotherapy response of LUAD. Therefore, it is important to identify lncRNAs related to m6A modification and construct a prognostic risk model in LUAD patients.

Method The expression of writers, readers and erasers of m6A related to LUAD were get from TCGA database. The m6A-related lncRNAs were identified from TCGA by the co-expression. Univariate and multivariate Cox regression analysis screened out the m6A-related IncRNAs which were valuable for prognosis in LUAD. LUAD patients were divided into different subgroups by consistent cluster analysis. The potential biological function mechanisms of different subgroups were analyzed by KEGG enrichment, and explored the differences of tumor immune microenvironment between subgroups. Finally, the prognostic risk model of prognosis-related m6A-related IncRNA was constructed by lasso regression, and detected its effect on immune microenvironment.

Results: We found that there were differences in the expression of writers, readers and erasers of m6A in LUAD tissues and adjacent normal tissues and identified abundant coexpressed IncRNAs. Thirty-two IncRNAs with prognostic value related to m6A were identified. Based on prognosis-related m6A-related IncRNAs, we divided LUAD patients into different subgroups by consistent cluster analysis, analyzed the difference in prognosis in the subgroups, and assessed the expression of m6A-related IncRNAs in different subgroups and the relationship between m6A-related IncRNAs and clinical factors. Tumor-related NOTCH, ERBB, cell cycle, MOTR, p53 and WNT signaling pathways were found to be enriched in Cluster 2 by KEGG. Moreover, we found that there was a significant correlation with immune checkpoint genes and the tumor immune microenvironment between the two clusters. A risk prognosis model was constructed by prognostic-related m6A-associated IncRNAs and further confirmed in the external cohort. Furthermore, the m6A-related risk model can effectively predict the prognosis and survival status of patients with LUAD. Finally, there are differences in risk models between immune checkpoint genes and the tumor immune microenvironment.

Conclusion: Our study constructed a prognostic risk model based on m6A-related IncRNAs with independent prognostic value and revealed the role of this model and IncRNAs in the tumor immune microenvironment. This finding suggests a new development prospect for immunotherapy strategies in LUAD.

Introduction

Lung adenocarcinoma (LUAD) is one of the most common subtypes of non-small cell lung cancer (NSCLC) (1, 2). It has attracted wide attention because of its high mortality rate and poor response to treatment (3). Currently, with the progress of surgery, radiotherapy and molecular therapy of lung cancer,
The prognosis of LUAD has been significantly improved. However, the 5-year survival rate of LUAD is still unsatisfactory (4). Numerous studies have demonstrated that reliable molecular markers are of significance in the prognosis of LUAD (5–7). Therefore, it is particularly important to construct a clinical prognosis model based on molecular markers.

Molecular epigenetics plays an important role in tumorigenesis, including RNA methylation and other modifications (8). RNA methylation accounts for more than 60% of all RNA modifications (9). m6A methylation mainly occurs on adenine in the "RRAH" sequence, and its function is determined by the "writer", "eraser" and "reader" (10, 11). m6A methylation is of significance in the regulation of gene expression, and abnormalities in its regulatory mechanism are closely associated with the occurrence and development of tumors (11) (12, 13). In addition, m6A modification is a reversible RNA epigenetic process that can affect various cellular processes (14). It is worth noting that the mechanism of m6A-regulated noncoding RNA (IncRNA) has a significance in malignant biological phenotypes such as proliferation and migration of tumor cells (12, 15), and these m6A-related IncRNAs are also of significance in the prognosis of tumors (16).

Currently, it has been found that the regulation of m6A-related genes is involved in the occurrence and development of LUAD (17). LCAT3, a novel m6A-regulated long noncoding RNA, plays an oncogenic role in lung cancer by binding with FUBP1 to activate c-MYC (18). Meanwhile, research revealed that as an m6A reader, YTHDF2 facilitates LUAD cell proliferation and metastasis by targeting AXIN1/Wnt/β-catenin signaling (19). Moreover, recent studies have shown that m6A regulation-related IncRNAs are associated with the prognosis of LUAD (20). However, the specific role of m6A modification and IncRNAs is unclear. Therefore, understanding the mechanism of m6A-related IncRNAs in LUAD and establishing a relevant clinical prognostic model are of significance for better predicting the survival of patients with LUAD.

In this study, we first extracted 14086 IncRNAs and 23 m6A regulatory gene expression data from TCGA datasets. Then, through Pearson's correlation analysis, we identified 2002 m6A-related IncRNAs. Furthermore, 36 m6A-related IncRNAs associated with the prognosis of LUAD were obtained by univariate and multivariate regression analyses. We analyzed these m6A-IncRNAs by clustering and risk assessment. A clinical prognostic risk model was constructed to predict the overall survival rate of patients with LUAD. In addition, we explored the relationship between these m6A-IncRNAs and the immune microenvironment and immune checkpoint inhibitor therapy. Overall, our study suggested that the prognostic risk model based on m6A-related IncRNAs could predict the prognosis and immunotherapy response of patients with LUAD.

Materials and Methods

3.1 Data collection

The expression and follow-up clinical data of all genes related to LAUD were obtained from TCGA (GDC (cancer.gov)), including 473 LAUD samples and 41 paired normal samples. We excluded patients
3.2 Identification of Prognostic Genes

We identified the expression of m6A-related genes in LUAD, including expression data on nine m6A writers, thirteen readers and two erasers. We found that there was a difference in the expression of writers METTL3, METTL14, METTL16, WTAP, ZC3H13, RBM15, RBM15B, and KIAA1429; readers YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC, LRPPRC, HNRNPA2B1, IGFBP2, IGFBP3, and RBMX; and erasers FTO and ALKBH5. Then, m6A-related genes related to prognosis were screened by univariate Cox regression analysis (P < 0.05). Subsequently, the R package “limma” (22) was used to filter the m6A-related lncRNAs (cor > 0.5, p < 0.001), and the R package “igraph” (23) was used to outline the coexpression network of m6A-related genes and lncRNAs. Univariate Cox regression analysis was used to identify prognostic m6A-related lncRNAs (p < 0.01). Differential expression of m6A-related lncRNAs in LUAD and adjacent tissues was detected by the Wilcoxon test(24).

3.3 Cluster analysis based on m6A-related IncRNAs

We used the R package “ConsensusClusterPlus” to cluster LUAD patients into different subtypes (iterations: 50, resampling: 0.8). When the k value = 2, the most stable cluster can be obtained. The Kaplan–Meier survival method(25) and log rank test were used to explore the clinicopathological factors between the two clusters.

3.4 Differences in immune checkpoint-related genes and the tumor microenvironment in different subgroups

Some scores, including immune, stromal and ESTIMATE scores, were analyzed by the “estimate” package of R(26). The expression of immune checkpoint molecules between the two clusters was estimated by the Wilcoxon test. We analyzed the correlation between m6A-related lncRNAs and immune checkpoint genes by the Pearson correlation test (p < 0.05).

To explore the difference in the influence on the signaling pathway between the two clusters, GSEA was performed (permutations: 1000 randomly, FDR < 0.05).

3.5 Construction and Calculation of the Risk Model and Its Correlation with Clinicopathological Factors

We further screened out m6A-related IncRNAs related to prognosis (OS) by the least absolute shrinkage and selection operator (LASSO) regression algorithm(27). Multivariate Cox regression was used to calculate the weighted sum of 18 m6A-related IncRNAs, and the risk score was calculated as a prognostic characteristic.(28) Then, we used survival analysis, ROC curve analysis and a risk plot to verify the possibility of the risk model. To determine whether the risk model has a certain predictive ability for OS by ROC and AUC curves(29), we performed univariate and multivariate Cox regression analyses to determine the independent prognostic role of the risk model. Finally, we analyzed the expression and prognosis of 18 m6A-related IncRNAs in LAUD through starBase (https://starbase.sysu.edu.cn)(30). The expression of
immune checkpoint molecules between the two risk groups was estimated by the Wilcoxon test. KEGG enrichment analysis (permutations: 1000 randomly, FDR < 0.05) was used to explore the role of risk models in tumor signaling pathways. The “estimate” package was used to explore differences in the tumor immune microenvironment in different risks.

3.6 Statistics

The data downloaded from TCGA were merged by Perl (version 5.24.3). In this study, all statistical analyses were performed by R software (version 4.0.2) and GraphPad Prism (version 8.2.0). A P value < 0.05 was considered to indicate statistical significance.

Results

4.1 Expression profile of m6A regulatory genes in LUAD

In this study, we obtained a TCGA dataset involving 473 LUAD tissues and 41 adjacent normal tissues. The expression of 23 m6A regulatory genes in LUAD was abstracted from the TCGA database, including 9 m6A writers, 13 readers and 2 erasers. We found that there was a difference in the expression of writers METTL3, METTL14, METTL16, WTAP, ZC3H13, RBM15, RBM15B, and KIAA1429; readers YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC, LRPPRC, HNRNPA2B1, IGFBP2, IGFBP3, and RBMX; and erasers FTO and ALKBH5 in LUAD and adjacent normal tissues (Fig. 1A-B). Furthermore, these differentially expressed m6A regulatory genes were associated with prognostic risk in patients with LUAD (Fig. 1C). Currently, it has been reported that the regulation of lncRNAs and m6A plays a significant role in LUAD. Thus, we extracted 2002 lncRNAs related to the expression of m6A regulatory genes from the TCGA database through code. The correlation between them is shown in Fig. 1D.

4.2 The m6A-lncRNAs related to prognosis in LUAD

Based on the aforementioned relationship between m6A regulators and lncRNAs in LUAD, lncRNAs play an important role in the tumorigenesis of lung cancer. Meanwhile, numerous lncRNAs have been demonstrated to be independent prognostic factors in lung cancer. Therefore, we continued to explore which m6A-related lncRNA was of significance in the prognosis of LUAD. The results of univariate Cox regression analysis showed that 36 of 2002 lncRNAs were significantly correlated with prognosis in LUAD (Fig. 2A). The clinical baseline information of 370 HCC cases in TCGA database is presented at (Table1) Meanwhile, a heatmap was used to show the expression of these prognosis-related m6A-lncRNAs in LUAD (Fig. 2B). Finally, through the TCGA database, we identified expression differences in 36 lncRNAs in almost all LUAD and adjacent normal tissues (Fig. 2C).
Table 1
Clinical Characteristics of the LUAD Cases in TCGA Database.

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4.3 Clustering study based on m6A-related IncRNAs with prognostic value

To obtain more m6A-related IncRNAs in LUAD, consensus clustering was used for cluster analysis of m6A-related IncRNAs. The results show that k = 2, which reveals that the interference between subgroups is minimal and that the distinction is significant (Fig. 3A). All of these results were based on the CDF
curve of the consistency matrix (Fig. 3B-C). Together, a total of 473 patients with LUAD were classified into two clusters. The results of the heatmap show that the expression of 36 m6A-related IncRNAs in Cluster 2 was higher than that in Cluster 1. Furthermore, we compared the clinicopathological characteristics between the two subgroups and found that there was a large proportion in Cluster 2 with more stages and associated with the late M stage. However, no significant differences in N and T stage were found between the two groups. Moreover, we observed that patients who experienced treatment were more concentrated in Cluster 2, but the significance of radiotherapy and chemotherapy alone or combined with radiotherapy and chemotherapy was not reflected between the two groups. Meanwhile, age, sex and smoking history were meaningless (Fig. 3E). Most importantly, the OS of LUAD in Cluster 2 was significantly shorter than that in Cluster 1 (Fig. 3D).

4.4 Potential regulatory mechanisms of the two clusters

Many studies have shown that immune checkpoint blockade is significant in tumor immunotherapy and that m6A methylation is closely related to it, but the potential mechanism is still unclear. In this study, the immune checkpoint genes PD-L1, CTLA4, HAVCR2 and B7-H3, which are effective targets of immunotherapy, were selected. First, we determined the expression of these immune checkpoint genes in the two clusters and found that PD-L1 and B7-H3 were significant in Cluster 1 and Cluster 2 (p < 0.001) and normal and tumor tissues (p < 0.001) (Fig. 4A-H). Then, we explored the correlation between PD-L1, B7-H3 and m6A-related IncRNAs and found that most of them were positively correlated with PD-L1 and B7-H3 (p < 0.05) (Fig. 4J-K). These results implied the effect of m6A-related IncRNAs in regulating immunomodulators and provided a new strategy for LUAD immunotherapy. Furthermore, the immune function of m6A-related IncRNAs in LUAD was explored. T cells, NK cells and macrophages play an important role in the immune microenvironment. We recognize that M1 macrophages (p < 0.001), resting NK cells (p < 0.001), activated memory CD4 T cells (p < 0.001), and CD8 T cells (p < 0.05) were more highly expressed in Cluster 2 than in Cluster 1. However, activated mast cells (p < 0.05), NK cells (p < 0.001), resting mast cells (p < 0.001), regulatory T cells (p < 0.01), and plasma cells (p < 0.001) were more highly expressed in Cluster 1 than in Cluster 2 (Fig. 5A-I). These results suggest that m6A-related IncRNAs may regulate the tumor immune microenvironment in LUAD.

The biological mechanism of the heterogeneity of the two clusters was investigated by GSEA. The results showed that more tumor-related signaling pathways were enriched in Cluster 2, including the CELL CYCLE (NES: 2.5257452, FDR p value < 0.05), ERBB (NES: 1.7973282, FDR p value < 0.05), MTOR (NES: 1.7134794, FDR p value < 0.05), p53 (NES: 2.302949, FDR p value < 0.05) and WNT (NES: 1.6371493, FDR p value < 0.05) signaling pathways and the IN CANCER pathway (NES: 1.8742931, FDR p value < 0.05) (sFigure 1 A-G). All these differences between the two clusters indicated the role of m6A-related IncRNAs in the tumorigenicity of LUAD.

4.5 Construction and verification of a clinical prognosis model for LUAD based on m6A-related IncRNAs
We identified 36 differentially expressed m6A-related lncRNAs that were significantly associated with OS by univariate Cox regression analysis. Then, the 18 most significant m6A-related lncRNAs associated with prognosis were identified by LASSO regression analysis (Fig. 6A-C). Finally, through regression coefficients and expressions of the 18 m6A-related lncRNAs, a risk model for LUAD was constructed, as follows: risk score = (-0.24842) * expression (AC008763.1) +(-1.28861) * expression (AC010999.2) + 0.339972 * expression (AC008494.3) + 0.141112 * expression (AL606489.1) + (-0.27363) * expression (TSPOAP1-AS1) + 1.424991*expression (FRMD6-AS1) + (-1.45406) * expression(AL031600.2) + 0.033235* expression (LINC01876) +(-0.19802) * expression (AP002026.1)+ (0.022912)*expression(LINC02587)+(-0.10636)*expression(AC087752.3)+ -0.25166)*expression(AC090617.5) + 0.094322*expression(LINC02178)+ (-0.10619)*expression(AC026355.2)+(-1.20335)*expression(AC0185). Patients with LUAD were divided into high- and low-risk groups based on the median value of the risk score. The predictability of the risk model was evaluated by receiver operating characteristic (ROC) curve analysis, and our analysis showed that the areas under the ROC curve (AUCs) for OS in the training and testing cohorts were 0.779 and 0.758, respectively (Fig. 6D-E). We found that the higher the risk score was, the worse the survival status of patients in the training and testing cohorts (Fig. 6F-I). The heatmap indicates that 9 lncRNAs were highly expressed in the high-risk group in both the training and testing cohorts, and 9 lncRNAs were highly expressed in the low-risk group in both the training and testing cohorts (Fig. 6J-K). More significantly, we found that m6A-related lncRNAs expressed in high-risk groups were indeed highly expressed in LUAD compared with adjacent normal tissues, and each had a poor prognosis (sFigure 2, sFigure 3). However, m6A-related lncRNAs expressed in low-risk groups were highly expressed in adjacent normal tissues compared with LUAD tissues and had a better prognosis (sFigure 2, sFigure 3). Thus, our results suggested that the model based on the m6A-related lncRNA risk score can better predict the prognosis of patients.

4.6 The risk model of m6A-related lncRNAs as an independent prognostic factor for LUAD

We found that the risk model based on m6A-related lncRNAs could be used as an independent prognostic factor by univariate and multivariate regression analyses (Fig. 7A-D). The results of these analyses showed that OS was obviously related to stage (P < 0.001) and riskScore (P < 0.001) in the testing cohort and related to stage (P < 0.001) and riskScore (P < 0.001) in the training cohort (Fig. 7E-F). Then, through survival analysis, we revealed that high-risk patients with LUAD exhibited worse OS than low-risk patients in the testing (p < 0.001) and training cohorts (p < 0.001). Furthermore, the high- and low-risk groups showed heterogeneity in different clinical subgroups. We compared the OS of the two groups in different clinical subgroups, indicating that the survival rates of the two clusters had significant differences in the incidences of stage I + stage II (P < 0.001), stage + stage (P = 0.007), M0 (P < 0.001), M1b (P < 0.039), T1-2 (P < 0.001), T3-4 (P < 0.001), N0 (P < 0.001), age ≤ 65 (P < 0.001), age ≤ 30 (P < 0.001), and age > 60 (P = 0.015). All these results showed that the prognosis of high-risk patients was worse than that of low-risk patients.
4.7 Prognostic risk model was associated with tumorigenesis mechanism

We explored the relationship between the risk prognosis model and tumor immunity. The results showed that the expression of B7-H3 was higher in the high-risk group than in the low-risk group (Fig. 8B). However, the expression of PD-L1 was not significantly different between the two risk groups (Fig. 8A). Then, we found that the risk model was associated with immune cells, and the results showed that the higher the risk score was, the greater the number of M0 macrophages (R = 0.15, p = 0.0019), CD4 memory activated T cells (R = 0.16, p = 0.00054), M1 macrophages (R = 0.21, p = 0.000056), and gamma T cells (R = 0.13, p = 0.0081) present. However, M2 macrophages (R =-0.094, p = 0.48), plasma cells (R =-0.11, p = 0.017), and monocytes (R =-0.15, p = 0.0023) were more common when the risk score was low (Fig. 8C-J). Furthermore, through KEGG enrichment analysis, it was found that the high-risk group was mainly concentrated in the tumor-related cell cycle and p53 signaling pathways, which are closely related to tumor immunity. (Fig. 8K-M). Collectively, the risk score established based on m6A-related lncRNAs is of great significance to immunotherapy and tumorigenesis in patients with LUAD.

Discussion

The prognosis of LUAD is very poor. Currently, the effect of chemotherapy, radiotherapy, targeted therapy and even immunotherapy for LUAD is not satisfactory(32). Therefore, it is particularly important to find new prognostic targets for the treatment of LAUD. Increasing attention has been given to the role of m6A modification-related lncRNAs in tumors(33, 34). Among RNA modifications, m6A modification accounts for the vast majority(20). The regulatory network of m6A-lncRNAs affects the biological function of most tumor cells and predicts the prognosis of various tumors(34). Some studies have demonstrated that m6A-related lncRNAs are biomarkers of prognosis in colon adenocarcinoma(35), hepatocellular carcinoma(36), breast cancer(37), cervical cancer(38), prostate cancer(39) and gastric cancer(40) patients. In this study, we mainly focused on the expression, prognosis and mechanism of m6A in LAUD, which provides more options for the research and treatment of LAUD.

In our research, we first analyzed the expression of 23 m6A-related regulatory genes in LUAD. Through the analysis of the TCGA-LAUD dataset, we found that there was a significant difference in the expression of 22 m6A-related regulatory genes between LUAD and adjacent normal tissues. Furthermore, we found that there was a relationship between m6A-related regulatory genes and the prognosis of LUAD. Therefore, we explored the factors by which m6A methylation modification regulation affects the prognosis of LUAD. lncRNAs related to m6A modification play an important role in LUAD (41). First, we screened differentially expressed lncRNAs from the TCGA-LUAD dataset, and 2002 lncRNAs were found to be associated with m6A regulators. Then, we found that 36 of these lncRNAs were associated with the prognosis of LUAD in univariate and multivariate Cox analyses. We explored the differential expression of the 36 lncRNAs in LUAD and adjacent normal tissues. All these results demonstrated that m6A-related lncRNAs were closely related to the prognosis of LAUD.
Furthermore, the potential biological functions and mechanisms of these m6A-related lncRNAs in LUAD were explored. We used consensus clustering analysis to divide patients into two clusters and study potential biological functions and mechanisms. Many studies have demonstrated the importance of tumor immune checkpoint genes in targeted therapy, such as PD-L1 CTLA4, HAVCR2, TIGIT and B7-H3(42). The role of the immune microenvironment in tumors has attracted increasing attention. Thus, we focused mainly on the correlations of m6A-related lncRNAs with these two factors. We found that the expression of PD-L1 and B7-H3 in Cluster 2 was higher than that in Cluster 1, and m6A-related lncRNAs were closely related to the expression of PD-L1 and B7-H3. Moreover, Cluster 2 had a greater effect on immune cells in the tumor microenvironment than Cluster 1. To further explore the relationship between the prognosis of LUAD and the tumor immune microenvironment, GSEA was performed. The results showed that the cell cycle, Erbb signaling pathway, MTOR signaling pathway, p53 signaling pathway, and Wnt signaling pathway were enriched in Cluster 2, corresponding to a worse prognosis. Importantly, abnormalities in these pathways play an important role in tumor immunity. Previous studies suggested that the EGFR signaling pathway is involved in the downregulation of MHC I and MHC II, upregulation of PD-L1 expression, increased number and activity of Tregs, and inhibited activity of CTLs(43), indicating that m6A-related lncRNAs may regulate apoptosis in LUAD. Generally, we speculated that m6A-related lncRNAs regulated tumor immunity and prognosis of LUAD patients by these pathways.

The prognostic risk model was constructed by 18 prognosis-associated m6A-related lncRNAs, and the risk scores of LUAD patients were estimated by the median risk score. Patients with LUAD were divided into high- and low-risk groups, and the high-risk group had a worse prognosis. All analyses, including ROC curves and univariate and multivariate Cox regression, were performed to confirm that the score could be used as an independent prognostic factor. Meanwhile, there was a significant relationship between the score and the clinical-pathological features of LUAD patients. Notably, our prognostic risk model had AUC values > 0.75 and was more accurate in assessing prognosis than previous research. More importantly, we explored the mechanisms between the prognosis of LUAD and the risk model. GSEA revealed that the Erbb, MTOR, and p53 signaling pathways were enriched in the high-risk group. Significantly, these pathways were also enriched in Cluster 2, associated with a poor prognosis. Thus, we were further convinced that m6A-related lncRNAs regulated tumor immunity and affected the prognosis of LUAD.

In conclusion, we comprehensively identified and analyzed lncRNAs related to m6A in LUAD. These m6A-related lncRNAs were closely related to tumor immunity in LUAD. We found m6A-related lncRNAs with prognostic value in LUAD and constructed a novel clinical prognostic risk model based on these lncRNAs, and this risk model had reliable predictive behavior for prognosis. There was a significant relationship between the risk score and the malignancy of LUAD. Our research provides new insights into the prognosis and immunotherapy of LUAD.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors’ Contributions

Li Yang and Shuliang Guo designed the subject direction. Junhao Mu, Yuezhou Zhang, Jing Huang, Weiyi Li, Min Ao, Haiyun Dai, Jing Liu collected the data. Yishi Li and Li Xu analyzed the data, Junhao Mu and Yuezhou Zhang wrote the draft manuscript. All authors contributed to the article and approved the submitted version. Junhao Mu and Yuezhou Zhang contributed equally to this work.

Data Availability

The datasets used and analyzed during the study are available from the corresponding authors on reasonable request. The datasets generated and analysed during the current study are available in the TCGA database (GDC (cancer.gov)), GSEA(GSEA (gsea-msigdb.org)) and Starbase(https://starbase.sysu.edu.cn/).

Ethics approval

This is an observational study. The Research Ethics Committee has confirmed that no ethical approval is required. We didn’t use any human participant in our study.

Consent to publish

Not applicable.

References


Figures
Figure 1

The m6A-related regulatory gene significantly correlated with LUAD. (A - B) Heatmap (A) and expression value (B) of the 23 m6A regulatory genes in 41 adjacent normal tissues and 473 tumor tissues. *** $P<0.001$. (C) Forest plot: the prognostic ability of the 23 m6A regulatory genes in LUAD. (D) Co-expression network of m6A-related regulatory genes and IncRNAs.
Figure 2

The significance of m6A-related IncRNAs in LUAD. (A) Forest plot: the prognostic ability of the 36 m6A-related IncRNAs in LUAD. (B-C) Heatmap (B) and expression value (C) of the 36 m6A-related IncRNAs in 41 adjacent normal tissues and 473 tumor tissues. *** $P<0.001$. 
Figure 3

Consensus clustering of the patients with LUAD based on 36 prognostic m6A-related IncRNAs. (A) Consensus clustering matrix for k = 2. (B) The CDF for k = 2 to 9. (C) Relative change in area under the CDF curve for k = 2 to 9. (D) Survival analysis (OS) for two clusters in LUAD. (E) Heat map of the relationship between m6A-related IncRNAs from two clusters and clinicopathological features of patients with LUAD. ** P<0.01.
Figure 4

The difference expression level of immune checkpoints genes between cluster1 and cluster2. (A-H) The expression level of PD-L1, B7-H3, CTLA4, HAVCR2 in two clusters, adjacent normal and tumor tissues. *** P<0.001. (J-K) Correlation of the 36 prognostic m6A-related IncRNAs with PD-L1 and B7-H3. **P<0.01, ***P<0.001.
Figure 5

The different expression level of immune cells between cluster1 and cluster2. M1 macrophages, resting NK cells, activated memory CD4 T cells, and CD8 T cells were highly expressed in Cluster 2 than in Cluster 1, activated mast cells, NK cells, resting mast cells, regulatory T cells and plasma cells were highly expressed in Cluster 1 than in Cluster 2 (A-I). **P<0.01, ***P<0.001.
Figure 6

Construction of a risk model based on 18 prognostic m6A-related IncRNAs. (A) Barplot of the IncRNAs related to prognosis. (B-C) The m6A-related IncRNAs are filtered by LASSO regression. (D-E) ROC curves of risk model for predicting survival in training and testing cohort. (F-I) Distribution of OS and risk scores in patients with LUAD, in training and testing cohort. (J-K) The expression heat map of 18 m6A-related IncRNAs in the high and low risk group, in training and testing cohort.
Figure 7

Risk score model is an independent prognostic factor. (A-D) Univariate and multivariate Cox analysis in training and testing cohort. (E-F) Kaplan–Meier showed that the high-risk group exhibited worse OS than the low-risk group in training and testing cohort. (G-P) Kaplan–Meier: Subgroup analysis of LAUD patients in the stage I–II group, III–IV group, M0 group, M1b group, T1-T2 group, T3-T4 group, N0 group, age<=60 group and age >60 group.
Figure 8

The relationship between risk score and expression of immune checkpoint genes and its mechanism. (A-B) relationship between PD-L1, B7-H3 and high /low risk group. (C-J) Relationship between immune cells and high /low risk group. (K-M) KEGG analysis showed the signal pathways involved in the high-risk group.

Supplementary Files

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