M6A REGULATORS RELATED TO MOLECULAR FEATURE, IMMUNE MICROENVIRONMENT AND PROGNOSIS IN LUNG ADENOCARCINOMA

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Background The dynamic changes of N6-methyladenosine controlled by m6A regulators are vital for cancer biology.1 However, the role of m6A regulators in lung adenocarcinoma (LUAD) remains unclear. This study aimed to gain a deeper insight into the roles of m6A regulators in LUAD by bioinformatics.

Methods 31 m6A regulators were retrieved from previous literature. The expression levels of the 31 regulators were investigated by TIMER2.0. The association between the 31 regulators and overall survival (OS) were explored via K-M plotter and Cox regression. Next, mutations status and copy number alterations of the 31 regulators were probed through cBioPortal. Moreover, the interaction between the 31 regulators were revealed via principal component analysis (PCA), protein-protein interaction (PPI), and Metascape enrichment by R programming, STRING 11.5, Cytoscape, and Metascape. Then, the immune microenvironment including immune score, specific immune cells infiltration, and expressions of immune-related gene families were probed via ESTIMATE, TIMER2.0, TIMER, and R programming. Next, LUAD samples were divided into 3 clusters based on the expression patterns of the 31 regulators by k-means method. The differences of OS, gene enrichment, immune microenvironment of the 3 clusters were compared by Log-rank test, GSVA, ESTIMATE, and CIBERSORTx. Moreover, a m6A Score Model was constructed using stepwise regression in this study. The predicting ability of the m6A Score Model was verified by GEO LUAD dataset and TCGA Pan-adenocarcinoma dataset.

Results 22 out of 31 regulators showed differential expression levels between LUAD and normal lung (figure 1). 28 out of 31 regulators were linked with OS (figure 2). The frequency of mutation and copy number alterations were 26.6% and 29.1% respectively (figure 3). PCA revealed the 31 m6A regulators displayed almost completely distinct distribution patterns between LUAD and normal lung (figure 4). PPI and Metascape enrichment indicated the 31 m6A regulators displayed close interrelation (figure 5). Between high and low expression groups of 20, 20 and 29 regulators, tumor purity, immune score, immune cells infiltrations exhibited statistical difference (figure 6). Moreover, 3 clusters displayed distinct OS, gene enrichment, and immune microenvironment (figure 7). Notably, a m6A Score Model was built up upon 15 m6A regulators (figure 8). The model was associated with OS for LUAD and pan-adenocarcinoma (figure 9).

Conclusions m6A regulators are associated with molecular features, immune microenvironment and prognosis in LUAD. m6A Score could be used as a reference to immunotherapy and a prognostic index for LUAD.

REFERENCE
Abstract 1424 Figure 4 Principal component analysis of 31 m6A regulators in 50 lung adenocarcinoma samples and 50 normal lung tissue samples from TCGA LUAD cohort.

Abstract 1424 Figure 5 (A) Protein-protein interaction network of 31 m6A regulators. (B) Metascape enrichment network of 31 m6A regulators.

Abstract 1424 Figure 6 (A) Stromal score in high and low expression level groups of each of 31 m6A regulators. (B) Immune score in high and low expression level groups of each of 31 m6A regulators. (C) ESTIMATE score in high and low expression level groups of each of 31 m6A regulators. *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05, ns: no significance.
Abstract 1424 Figure 7  (A) 3 clusters established according to the expression patterns of the 31 m6A regulators. (B) Overall survival plot of the 3 clusters. (C) Top 10 positively and top 10 negatively enriched hallmark gene sets of the 3 clusters. (D) Comparison of stromal score, immune score, and ESTIMATE score of the 3 clusters. (E) Comparison of specific immune cells infiltrations of the 3 clusters. (F) Comparison of PD-L1 and CTLA4 expression levels of the 3 clusters. *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05.

Abstract 1424 Figure 8  Nomogram of m6A to predict overall survival in lung adenocarcinoma.


Abstract 1424 Figure 9  (A) Overall survival of high and low m6A score groups in training set named TCGA LUAD dataset. (B) Comparison of stromal score, immune score, and ESTIMATE score in high and low m6A score groups. (C) Comparison of specific immune cells infiltrations in high and low m6A score groups. (D) Comparison of PD-L1 and CTLA4 expression level in high and low m6A score groups. (E) Overall survival of high and low m6A score groups in lung adenocarcinoma test set named GSE13213. (F) Overall survival of high and low m6A score groups in pan-adenocarcinoma patients test set named adenocarcinoma subset of TCGA Pan-Cancer dataset. *** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05.