Construction of a novel prognostic risk model based on m6A-related miRNAs for acute myeloid leukemia

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Abstract

N6-methyladenosine (m6A) modification and miRNAs were important in tumorigenesis and development. We aim to identify prognostic markers and construct a risk prediction model for AML patients. First, 17 prognostic m6A-related miRNAs were filtrated, whose expression profiles were included to cluster patients into 2 subtypes. The OS of cluster1 had worse prognosis. GSEA analysis showed cluster1 enriched in tumor-related pathways, including Toll like receptor signaling pathway, PPAR signaling pathway and apoptosis. Next, 10 miRNAs filtered by LASSO regression analysis were used to construct a risk model. Patients in high-risk groups had unfavorable prognosis and risk score might could act as the independent prognostic factors in AML. The expression of immune checkpoints (PD-L1, LAG-3 and CTLA4) were higher in high-risk group. Finally, we built a regulatory network of m6A regulators- m6A-related miRNAs-target mRNAs. The GO function analysis showed the target genes were enriched in the biological process related with leukemia, including tissue morphogenesis, regulation of leukocyte migration, positive regulation of cell adhesion and so on. The KEGG pathway analysis indicated that these genes were mainly enriched in Ras signaling pathway and signaling pathways regulating pluripotency of stem cells. The finding provided novel implication for efforts to improve the treatment of AML.

Introduction

Acute myeloid leukemia (AML) is a kind of malignant hematological cancer that originates from the malignant clonal proliferation of immature hematopoietic stem cells in bone marrow and peripheral blood. Because of the rapid progression, highly heterogenicity, extremely invasive and frequent mutation, AML has a high mortality which is the 10 causes of cancer-associated deaths[1]. Chemotherapy and stem-cell transplants remain the primary therapeutic treatment for AML patients. And current novel therapy regimens, including the use of novel kinase inhibitors, monoclonal antibodies and chimeric antigen receptor (CAR)-T cell therapy, have contributed to improve cure rate of AML[2, 3]. However, the overall outcomes still can't achieve satisfactory. Accordingly, our study is aimed to identify prognostic markers and construct a novel risk prediction model for improving treatment and prognosis of AML patients.

N6-methyladenosine (m6A) modification is the most common post-transcriptional epigenetic modification in eukaryotic non-coding RNAs (ncRNAs) and mRNAs, which can modulate RNA stability, translation, splicing, and degradation. It's a reversible and dynamic reaction controlled by methyltransferases (writers), binding proteins (readers) and demethylases (erasers) [4–6]. Several studies have indicated that m6A modification plays a critical role in the tumorigenesis and progression, including AML. For example, study showed that the m6A methyltransferase METTL3 could regulate myeloid differentiation of hematopoietic progenitor cells (HSPCs) and leukemia cells[7]. The m6A reader IGF2BP2 promoted AML progression and self-renewal of leukemia stem cells (LSCs), and high expression of IGF2BP2 were negatively correlated with survival[8]. The m6A reader YTHDF2 may acted as a therapeutic target by inhibiting selectively targets LSCs and promoting HSCs expansion[9]. The demethylase ALKBH5 was overexpressed in AML and associated with poor prognosis in AML patients [10].
miRNAs, one of the non-coding RNAs, can regulate gene expression through suppressing translation initiation and inducing mRNA degradation\cite{11, 12}. miRNA drives a distinct role in tumor biological behavior including cell differentiation, proliferation and metastasis. Previous investigations have found that miRNAs can act as prognostic and therapeutic biomarkers in various cancers\cite{13, 14}. Meanwhile, m6A modification is also widespread in miRNAs\cite{15}. The relationship between miRNAs and m6A modification has been proven to affect carcinogenesis\cite{16, 17}. However, m6A-modified miRNAs in the regulation of AML have rarely been investigated.

In this study, we identified miRNAs related to m6A regulatory genes and valued to prognosis of AML based on TCGA database. We analyzed the relationship of m6A-related miRNAs with clinicopathologic characteristics and tumor immune microenvironment. Moreover, a risk model was built based on m6A-related miRNAs for the prediction of overall survival (OS) in AML patients. Finally, we constructed a regulatory network of m6A regulators- m6A-related miRNAs- target genes.

**Materials and methods**

**Data collection and processing**

RNA sequencing data and corresponding clinical information on 148 AML patients were downloaded from The Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/). Through removing the genes with NA values greater than 50% and then using “impute” R package (number neighbors=10) to complete the missing values, 535 miRNAs were extracted from miRNA sequencing. Based on previous articles, expression data of 23 m6A regulatory genes (METTL3, METTL14, METTL16, WTAP, VIRMA, ZC3H13, RBM15, RBM15B, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, HNRNPC, FMR1, LRPPRC, HNRNPA2B1, IGFBP1, IGFBP2, IGFBP3, RBMX, FTO, and ALKBH5) were filtered from mRNA sequence.

**Identification of the prognostic m6A-related miRNAs**

Firstly, m6A-related miRNAs were filtered by Pearson correlation analysis with correlation coefficient 0.3 and \( p \) 0.05 of 535 miRNAs and 23 m6A-regulated genes. Then univariate Cox regression analysis was performed to identify the m6A-related miRNAs associated with AML prognosis at the criterion of \( p \) 0.01. The “igraph” R package was performed to plot the co-expression network graph.

**Analysis of m6A-related miRNAs clustering subgroups and clinicopathological factors by consensus clustering method**

Consensus cluster analysis was performed by the ConsensusClusterPlus package (50 iterations, sampling rate of 80%) on AML samples according to the expression of prognostic m6A-related miRNAs. The empirical cumulative distribution function plot was used to determine the optimal number of clusters (\( k=2 \)). Kaplan-Meier curves and log rank test were used to compare the overall survival (OS) of two cluster. The “pheatmap” package was used to visualize the expression levels of prognostic m6A-related miRNAs and describe the clinicopathological characteristics between different subgroups. Finally
we used GSEA to investigate the biological pathway associated with tumor progression between two clustered subgroups with random sampling of 1000 permutation and the criteria of $p \leq 0.05$.

**Tumor immune microenvironment evaluation**

To analyze the tumor microenvironment, the immune score, ESTIMATE score and stromal score were computed by the ESTIMATE algorithm and compared between 2 clusters by Wilcoxon test. The CIBERSORT algorithm was used to evaluate the relative proportions for 22 kinds of immune cells in the clustering subgroups. Spearman correlation analysis was conducted to perform correlation analysis of 22 immune cells, which was visualized by “ggcorrplot” package. The expression levels of 6 immune genes (PD-L1, LAG-3, NR4A1, IRF4, IRX3 and Tim-3) were compared between the subgroups by the “ggplot2” and “ggpubr” packages.

**Construction and validation of the risk model based on the prognostic m6A-related miRNAs**

The least absolute shrinkage and selection operator (LASSO) COX regression algorithm was used to further identify the optimized subset of prognostic m6A-related miRNAs. The gene expression levels and their corresponding coefficients were calculated and used to build the final risk model. Each sample's risk score calculating formula is: risk score= sum (each gene's expression × corresponding coefficient).

According to the median risk score, the patients were separated into high- and low-risk groups. And 148 AML patients was randomly split into a training set and a test set. Kaplan Meier survival analysis was implemented to evaluate the survival of patients within the two groups. Receiver operating characteristics (ROC) was applied to detect the prognosis prediction accuracy of the risk model. The “pheatmap” package was performed to construct heatmaps of risk score, patient survival time and expression of corresponding m6A-related miRNAs of the two groups in both training and testing cohorts.

**Independent prognostic analyses of the risk model**

We performed subgroup survival analysis of clinicopathological factors in different risk groups. Subsequently, Univariate and multivariate Cox regression analysis of risk score and clinical characteristics (age, gender and FAB classification) were performed to assess the independent prognostic value of the risk model. Prognostic-related factors were setting the criteria of $p \leq 0.05$.

**Correlation of risk model with clinicopathological features and immune infiltrating cells**

Wilcoxon test was used to analyze the distribution of risk score differences with respect to clinicopathological features, subgroup clustering of m6A-related prognostic miRNAs and immune genes. The “pheatmap” package was used to visualize the expression levels of prognostic m6A-related miRNAs and describe the clinicopathological characteristics in high- and low-risk groups. Pearson correlation test was conducted to evaluate the correlations between of risk scores and immune-cell infiltration. $p \leq 0.05$ was considered statistically significant.

**Construction of a network of m6A regulators- m6A-related miRNAs- target genes**
Above 10 miRNAs related with the m6A regulators and risk model was filtered by previous Pearson correlation analysis and LASSO regression analysis. The target genes of these miRNAs were predicted using the TargetScan database. We chose the top 20 genes with the highest score as the target genes for construction of a regulatory network. Totally 183 target mRNAs were further analyzed and annotated by Gene Ontology (GO) function and KEGG pathway enrichment analyses through Metascape.

Results

Identification of the prognostic m6A-related miRNAs

The study flowchart was shown in Fig 1. The expression data of 23 m6A-regulatory genes based on previous publications and miRNAs were obtained from the TCGA database. Through removing the genes with NA values greater than 50% and then using “impute” R package (number neighbors=10) to complete the missing values. 535 miRNAs were extracted. Subsequently, the result showed that there were 120 m6A-related miRNAs by Pearson correlation analysis with correlation coefficient 0.3 and \( p < 0.05 \). Co-expression network was visualized was showed in Fig 2A. Then we used univariate Cox regression analysis to screen out the prognostic m6A-related miRNAs in 148 AML samples. The forest plot demonstrated that 17 m6A-related miRNAs were related to survival \( (p < 0.01, \text{Fig 2B}) \).

Consensus clustering analysis of m6A-related miRNAs with prognosis

To explore the biological characteristics of the m6A-related miRNAs of AML, the 148 tumor samples were divided into cluster1 (n=83) and cluster2 (n=65) by consensus clustering analysis. The CDF curves of consensus matrix showed that the subgroups had the highest stability when k=2 (Fig 3A-C). The survival analysis indicated that the overall survival (OS) of cluster2 was longer than that of the cluster1 (Fig 3D). Further we compared the OS of the two clusters in different clinical subgroups. As shown in Fig.S1, the OS of two clusters had significant differences in age\( \leq 60 \) \( (p < 0.001) \), female \( (p=0.004) \), male \( (p=0.025) \), and FAB M3-7 \( (p=0.001) \). The distribution of clinical characteristics between two clusters was showed in the heatmap (Fig 3E). GSEA was used to investigate the biological mechanism of contributing the heterogeneity of the two clusters. The result displayed that several pathways related to tumor progression pathways enriched in the cluster1 compared with cluster2, including Toll like receptor signaling pathway \( (\text{NES}=1.64, \ p=0.014) \), PPAR signaling pathway \( (\text{NES}=1.45, \ p=0.048) \) and apoptosis pathway \( (\text{NES}=1.53, \ p=0.031) \) (Fig.2 F-H).

The tumor immune microenvironment of two clusters

Tumor immune microenvironment played a crucial impact on tumor progression. The immune score and estimate score calculated by ESTIMATE algorithm were significantly different between two clusters \( (p \leq 0.01, \text{Fig 4A}) \). Moreover, the infiltration of 22 immune cell types in AML microenvironment were analyzed by CIBERSORT algorithm. Compared to cluster2, we found that T cells CD4 memory activated \( (p < 0.05) \) and monocytes \( (p < 0.01) \) covered bigger fraction in cluster1, while dendritic cells resting \( (p < 0.05) \), mast cells activated \( (p < 0.001) \) and mast cells resting \( (p < 0.05) \) covered a small fraction (Fig 4B). The bar
chart showed the percentage of various immune cells per samples (Fig 4C). Correlation analysis of immune cells was displayed in Fig 4D.

In addition, we analyzed the correlations between the expression of the immune checkpoint molecules (PD-L1, LAG-3, Tim-3, CTLA4, CD47 and TIGIT) and the m6A-related prognostic miRNAs. Cluster1 exhibited higher expression level of LAG-3 ($p=0.03$) and CTLA4 ($p=0.013$), while lower expression level of CD47 ($p=0.007$) than cluster2 (Fig 4E-J). These results suggested that m6A-related miRNAs were importantly correlated with immune activity in AML tumorigenesis.

**Construction of a risk score model based on m6A-relation miRNAs for AML patients**

We used LASSO-penalized Cox analysis to optimize the prognostic model based on m6A-related miRNAs in AML patients. Firstly, 10 of previous 17 miRNAs were identified as the most powerful prognostic m6A-related miRNAs (hsa-let-7a-2-3p, hsa-miR-181b-3p, hsa-let-7b-5p, hsa-miR-196b-5p, hsa-miR-151a-5p, hsa-miR-181c-5p, hsa-miR-155-3p, hsa-miR-511-5p, hsa-let-7a-3p and hsa-miR-3913-5p). A risk model was constructed according to the regression coefficients and expression values of these 10 miRNAs (Fig 5A-B). Risk core=$-0.0792\times hsa$-let-7a-2-3p$-0.1242\times hsa$-miR-181b-3p$+0.1607\times hsa$-let-7b-5p$+0.0221\times hsa$-miR-196b-5p$+0.0790\times hsa$-miR-151a-5p$-0.0224\times hsa$-miR-181c-5p$+0.1419\times hsa$-miR-155-3p$+0.0499\times hsa$-miR-511-5p$+0.0027\times hsa$-let-7a-3p$-0.0147\times hsa$-miR-3913-5p$. We calculated the risk score of all samples and divided them into high- and low-risk groups based on the median value. To better validate the predictive efficacy of the risk model, the 148 patients were randomly categorized into training group (n=74) and testing group (n=74) for following analyses. The result confirmed the OS of the low-risk groups were longer than the high-risk group in both the training and test sets ($p \leq 0.01$, Fig 5C-E). And ROC curves (AUCs) analysis was used to assess to the sensitivity and specificity of the prediction of prognostic model. The AUC at 1-year was 0.74 in training group and 0.77 in testing group (Fig 5D-F). The discrepancies of risk scores and survival statuses of patients in the training and testing sets were displayed in Fig 4G-H.

**Independent prognostic factor of risk score based on the m6A-related miRNAs for AML patients**

Subgroup analyses were performed from different subgroups stratified by age, gender and FAB types in AML patients. The result showed most subgroups had significant survival differences in different risk groups (Fig S2 A-F). Meanwhile, we conducted univariate and multivariate Cox regression analysis to validated the predictive efficacy of the risk model based on the m6A-related miRNAs. Our results showed that OS was evidently related with age and risk score in both training set and testing set by the univariate analyses (Fig S3 A C). The multivariate Cox regression analysis suggested that age ($p=0.001$, HR 3.427, 1.679-6.995) and risk score ($p=0.005$, HR 3.161, 1.406-7.110) acted as the independent prognostic factors for AML patients in training set (Fig S3 B), while risk score ($p=0.001$, HR 3.314, 1.959-5.605) acted as the independent prognostic factors in testing set (Fig S3 D).

**Association of prognostic risk score with clinicopathological factors and immune infiltrating levels**
The differentiation of risk score stratified by age, gender, FAB classifications and cluster were evaluated in
the entire 148 samples. The elderly group \( (p=0.006) \) and cluster1 \( (p=0.001) \) had higher risk scores (Fig 6A-D). The heatmap showed that age, gender and cluster groups were significant factors between the high-risk and the low-risk group and displayed that 10 me6A-related miRNAs had relatively different expression 
levels in different risk groups (Fig 6E). Further we analyzed the relationship between risk score with 
immune checkpoints and various types of immune cells. The results demonstrated that expression levels 
of PD-L1 \( (p=0.027) \), LAG-3 \( (p=0.021) \) and CTLA4 \( (p=0.022) \) were higher in high-risk groups, while 
expression levels of CD47 \( (p=0.007) \) was higher in low-risk group (Fig 7A-D). And risk scores were 
significantly associated with resting mast cells \( (R=-0.37, p=0.001) \) and monocytes \( (R=0.28, p=0.001) \) (Fig 7D-E).

**Construction of a network of m6A regulators- m6A-related miRNAs- target genes**

10 most powerful prognostic m6A-related miRNAs and corresponding m6A regulators were filtered by 
previous LASSO regression analysis and Pearson correlation analysis. We chose the top 20 genes with 
the highest score as the target genes by the TargetScan database. Finally, a regulatory network was built, 
comprised of 10 m6A regulators, 10 m6A-related miRNAs and 185 target mRNAs (Fig 8A). Through GO 
function analysis, the target genes were enriched in the biological process related with leukemia, 
including tissue morphogenesis, regulation of leukocyte migration, positive regulation of cell adhesion 
and so on (Fig 8C). And the KEGG pathway analysis indicated that these genes were mainly enriched in 
Ras signaling pathway and signaling pathways regulating pluripotency of stem cells (Fig 8B).

**Discussion**

Although great progress has been in therapy, the survival rate of AML patients still remains low. Hence, 
increasing studies focus on further molecular and pathway mechanisms to explore prognostic and 
therapeutic biomarkers for AML patients. Previous findings have found that m6A methylation was the 
most important RNA modifications, while could affect gene expression and regulate disease onset and 
development. Except regulating in mRNAs, m6A modification is also commonly enriched in non-coding 
RNAs, such as miRNAs. METTL3 could promote cell proliferation in bladder cancer by regulating pri-
miR221/222 process[18]. METTL14 played a positively role on survival of hepatocellular carcinoma. It 
suppressed the tumor metastasis through modulating the process of pri-miR126 in an m6A-dependent 
manner[19].miRNA-1266 was lowly expressed in colorectal cancer tissues and could inhibit the cancer 
cell proliferation by targeting the demethylase enzyme FTO[20]. The study showed that YTHDC1 could 
recognize m6A-modified pri-miR30d to facilitate its mature. As the target for YTHDC1, miR-30d repressed 
pancreatic tumorigenesis by suppressing the aerobic glycolysis signaling[21]. In ovarian cancer, miR-74-
5p overexpression indued cell apoptosis by directly targeting HNRNPC and NFIX[22]. However, the role of 
m6A modification and miRNAs in AML have rarely been examined.

In this study, we identified 120 miRNAs correlated to 23 m6A regulatory genes using TCGA-AML data set. 
By Cox regression analysis, 17 m6A related miRNAs were filtrated to have a prognostic value for AML
patients. 9 miRNAs were associated with favorable prognosis (let-7a-2-3p, miR-181b-3p, miR-100-5p, miR-181a-3p, miR-181b-5p, miR-181a-5p, miR-181c-5p, miR125b-5p and miR-3913-5p), while 8 miRNAs were related with poor prognosis (let-7b-5p, miR-196b-5p, miR-151a-5p, let-7b-3p, miR-511-5p, miR-10a-5p and let-7a-3p). The prognostic results of most genes were consisted with many studies [23–29]. To further evaluate the biological characteristics of the m6A-related miRNAs of AML, the tumor samples were divided into cluster1 and cluster2 by consensus clustering analysis. For clinical features, there were significant difference in age between two clusters. The OS of cluster2 was longer compared with that of the cluster1. Subgroup survival analyses shows the obvious differences in age ≤ 60, female, male and FAB M3-7. Compared with cluster2, the signaling pathways related to tumor progression enriched in the cluster1 included Toll like receptor signaling pathway, PPAR signaling pathway and apoptosis pathway. The TME comprises a complicated network of extracellular matrix, stromal cells and immune cells and was proved to be important in tumor progression. To better understand the AML immune microenvironment was helpful for revealing new therapeutic targets[30, 31]. The immune score and estimate score calculated by ESTIMATE algorithm were significantly different between two clusters. Meanwhile, we found an increased number of T cells CD4 memory activated and monocytes in cluster1, while a bigger fraction of mast cells activated and mast cells resting activated in cluster2. What's more, the expression of immune checkpoint, including LAG-3, CTLA4 and CD47 were obviously different between two clusters. It is suggested that the m6A-related mRNAs and tumor immune microenvironment were closely associated to AML.

To better understand the prognostic effect of these miRNAs, a risk model was constructed by LASSO regression analysis based on 10 miRNAs to predict prognosis in AML patents. The OS of patients in low-risk group were higher than those of patients in high-risk group. And the multivariate Cox regression analyses indicated that risk score could act as the independent prognostic factors in AML. And the results showed that prognostic risk score was related with clinicopathological factors, including patient age and cluster groups. The cluster 1 predicted poor prognosis with highly risk score, suggesting that our risk model based on m6A-related miRNAs was robust and reliable in predicting the OS Of AML patients. Immune infiltration analysis indicated that monocytes were positively related with risk score, which resting mast cells were negatively related with risk score. The expression levels of immune checkpoint genes (PD-L1, LAG-3 and CTLA4) were higher in high-risk group comparing to low-risk group. Recent studies have found the remarkable progress of immune checkpoints blockade provided a novel treatment strategy for AML. Earlier clinical studies with PD-1/PD-L1 blockade have shown promising outcome in AML patients[32, 33]. These findings supported that our risk model might have important implications for clinical application.

To further evaluate the regulatory mechanism of miRNAs associated with risk model, we built a regulatory network of m6A regulators- m6A-related miRNAs- target genes. Using the TargetScan database to find the target gene of m6A-related miRNAs. The regulatory network comprised of 10 m6A regulators, 10 m6A-related miRNAs and 185 target mRNAs. The GO function analysis showed the target genes were enriched in the biological process related with leukemia, including tissue morphogenesis, regulation of leukocyte migration, positive regulation of cell adhesion and so on. And the KEGG pathway analysis
indicated that these genes were mainly enriched in Ras signaling pathway and signaling pathways regulating pluripotency of stem cells. The molecular regulation of miRNAs and m6A modification together affected the development of acute myeloid leukemia.

However, our study also has several limitations. The prognostic model based on m6A-miRNAs needs to be further validated the efficacy using the external cohort with larger patient numbers. And further functional experiments are also need to elucidate the regulatory mechanisms of m6A-related miRNAs in the occurrence and progression of AML.

Conclusion

In summary, our study showed that m6A-related miRNAs were closely related to clinical factors and tumor immune microenvironment in AML. The risk model based on m6A-related miRNAs were helpful for predicting AML patient outcome. The expression levels of immune checkpoints were different in tow risk sets. These results provide new insights in therapeutic strategy for AML.

Declarations

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author Contributions

YZ and LX designed the study. SC, XX and JM analyzed the data and composed the manuscript and literature review. YC, DY, LH, AL and HZ provided figures and pathology review. All authors have read and approved of the final version of the manuscript.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Statement

The patient has provided their written informed consent for the publication of this manuscript and any identifying images or data.
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References


**Figures**

![Study flow chart of this study.](image-url)

**Figure 1**

Study flow chart of this study.
Figure 2

Identification of the prognostic m6A-related miRNAs. **A** Co-expression network of m6A regulators and m6A-related miRNAs. **B** The forest plot of the prognostic roles of the 17 m6A-related miRNAs with $p < 0.01$. 
Figure 3

Consensus clustering analysis based on 17 m6A-related miRNAs. A Consensus matrix for k=2. B The CDF for k=2 to 10. C Relative change in area under CDF area for k=2 to 9. D Kaplan-Meier curve for OS of cluster 1/2 subtypes. E The heatmap of clinicopathological feature between tow clusters. F-H GSEA showed that Toll like receptor signaling pathway, PPAR signaling pathway and apoptosis pathway were differentially enriched in cluster1. NES, normalized enrichment score; CDF, cumulative distribution functions.
Figure 4

Analyses of tumor immune microenvironment between two clusters. **A** Comparison of immune score, ESTIMATE score and stromal score. **B** The fraction of 22 types of infiltrating immune cells in two clusters. **C** The bar chart showed the percentage of 22 types of immune cells per samples. **D** Correlation analysis of 22 types of immune cells. **E-J** The expression levels of immune checkpoint (PD-L1, LAG-3, Tim-3, CtvLA4, CD47 and TIGIT) in two clusters. *p<0.05, **p < 0.01 and ***p < 0.001.
Figure 5
Construction of a risk score model based on m6A-relation miRNAs. A B LASSO Cox regression algorithm for 17 m6A-related miRNAs. C Kaplan Meier analysis in the training set. D Receiver operation characteristic (ROC) curves of risk model predicting survival in the training set. E Kaplan Meier analysis in the testing set. F ROC curves of risk model predicting survival in the testing set. G Distribution of risk score survival statuses of AML patients and expression levels of the 10 m6A-related miRNAs in different...
risk group in the training set. Distribution of risk score survival statuses of AML patients and expression levels of the 10 m6A-related miRNAs in different risk group in the testing set.

Figure 6

Association of prognostic risk score and clinicopathological factors. A-D Comparison of risk score in different subgroup (age, gender, FAB types, cluster groups). E Heatmap of clinicopathological features and expression levels of 10 m6A-related miRNAs between different risk groups. *p < 0.05, **p < 0.01 and ***p < 0.001.
Figure 7

Association of prognostic risk score and immune infiltrating levels. A-D Comparison of the expression levels of immune checkpoints (PD-L1, LAG-3, CTLA4 and CD4) between high-risk and low-risk groups. E-F Pearson correlation analysis of risk score and immune cells (resting mast cells and monocytes).
Figure 8

Supplementary Files
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- Supplementary.rar