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Establishment of a new molecular subtyping and prognostic signature with m6A/m5C/m1A/m7G regulatory genes for hepatocellular carcinoma

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ABSTRACT

Background: RNA modification, including m6A, m5C, m1A, and m7G, participated in tumor progress. Therefore, the purpose of the present study was to explore the role of m6A/m5C/m1A/m7G regulatory genes in the prognosis and tumor microenvironment (TME) for hepatocellular carcinoma (HCC).

Methods: 71 m6A/m5C/m1A/m7G regulatory genes expression for HCC was detected, differentially expressed genes were screened, and molecular forms were classified by unsupervised consensus clustering. Cox regression and the Least Absolute Shrinkage and Selection Operator (LASSO) analysis were applied to establish a prognostic signature. Time-dependent receiver operating characteristic (ROC) curves were evaluated for clinical effectiveness and accuracy of the prognostic hazard model. In cluster subtypes and risk models, the differences in prognosis, immune cell infiltration, immune checkpoint, immunotherapy, and drug sensitivity between different subtypes were evaluated.

Results: HCC patients were classified into two clusters (cluster 1 and cluster 2) according to the expression of 71 m6A/m5C/m1A/m7G regulatory genes. Cluster 1 had a poor prognosis and different immune cell infiltration. Cluster 1 had higher immune checkpoint expression and TIDE score than cluster 2. Subsequently, we construct a five-gene prognostic model of m6A/m5C/m1A/m7G regulatory genes (YTHDF2, YTHDF1,YBX1, TRMT61A, TRMT10C). The Kaplan-Meier and ROC curve analysis showed that the prognostic signature exhibited good predictability. The risk score was considered an independent poor prognostic index. The high-risk group had higher immune checkpoint expression and higher TIDE scores. 5-Fluorouracil, docetaxel, doxorubicin, etoposide, gemcitabine, paclitaxel, sorafenib, and vinblastine were more suitable for high-risk patients. ECM receptor interaction, cell cycle, and Leishmania infection were enriched in the high-risk group.

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Conclusion: The clustering subgroups and prognostic model of m6A/m5C/m1A/m7G regulatory genes were linked with bad prognosis and TME for HCC, and had the potential to be a novel tool to evaluate the outcomes of HCC patients.

1. Introduction

Hepatocellular carcinoma (HCC) has become a common solid malignancy and the major cause of death worldwide [1]. The pathophysiology of HCC is a complex multi-step process [2]. Treatment is difficult and the prognosis is poor. Although there are various methods such as surgery, interventional therapy, and targeted therapy, the therapeutic effect and prognosis of liver cancer are still poor. Hence, it is of important clinical significance to look for potential biomarkers to evaluate HCC prognosis and offer guidelines for the cure of HCC.

RNA modification is a significant pathway of epigenetic regulation and plays a vital part in oncogenesis, progression, and prognosis. With advances in RNA sequencing technology, many RNA modifications have been discovered, including N6-methyladenosine (m6A), 5-methylcytosine (m5C), N1-methyladenosine (m1A), and N7-methylguanosine (m7G) modification [3]. M6A is one of the most common forms of methylation modification, and a lot of evidence show that m6A plays a leading part in cancer development, including cervical cancer, HCC, non-small cell lung cancer, thyroid cancer, esophageal cancer, gastric cancer, breast cancer, prostate cancer, colorectal cancer, and endometrial cancer [4–9]. For example, ALBH5 can inhibit the degradation of lncRNA PVT1, thereby promoting the progression of osteosarcoma [10]. M5C methylation modification mainly occurs in tRNA and rRNA and is usually related to the translation process of proteins, which partakes in tumor progression, invasion, metastasis, and tumor resistance. The prognosis of patients with high expression of NSUN2, YBX1, and HDGF is poor, indicating that m5C methylation mediated by NSUN2 promotes the occurrence of bladder cancer [11]. Overexpression of NSUN4 and ALYREF are closely related to adverse outcomes of liver cancer [12]. M1A methylation modification is a reversible modification of tRNA and mRNA, which is positively correlated with protein production, and research in tumors has mainly focused on HCC, gastrointestinal tumors, and bladder cancer. ALKBH3 can reduce the level of m1A methylation in liver cancer cells to stimulate carcinogenesis and the progress of HCC and may become a new target point of HCC therapy in the future [13]. The m7G methylation modification is also a methylation modification that occurs on tRNA, which helps to maintain the stability of tRNA [14,15]. Several examples of evidence confirmed that m7G modification participates in the biological processes of various tumors [16–18]. At present, the part of m6A/m5C/m1A/m7G regulatory genes in the progress of liver cancer is not completely clear. As a consequence, our work utilized data derived from The Cancer Genome Atlas (TCGA) database to establish the risk model for m6A/m5C/m1A/m7G regulatory genes to confirm HCC prognosis.

2. Materials and methods

2.1. The differential expression of m6A/m5C/m1A/m7G regulatory genes

Gene expression data for 374 HCC samples were acquired from the TCGA database (http://cancergenome.nih.gov/) as a training cohort. 23 m6A regulated genes, 14 m5C regulated genes, 10 m1A regulated genes, and 29 m7G regulated genes were obtained from the literature, and the duplicated genes were removed, and finally, we got 71 m6A/m5C/m1A/m7G regulated genes to study [19–23]. Subsequently, the differential expression of m6A/m5C/m1A/m7G regulatory genes was detected through the Wilcoxon test.

2.2. GO and KEGG enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) methods are generally utilized for evaluating the biologic functions and signal pathways involved in 71 m6A/m5C/m1A/m7G regulatory genes [24].

2.3. Consensus clustering analysis

The k-mean approach was utilized to discover different patient patterns associated with m6A/m5C/m1A/m7G regulatory genes using the ConsensusClusterPlus" package. Principal component analysis (PCA), UMAP, and tSNE were utilized to investigate the regional distribution between subgroups. Kaplan-Meier survival analysis and logistic regression were applied to evaluate the correlation between overall survival (OS) and clinical features for HCC patients in subtypes [25]. We also analyzed differences in immune cell infiltration, immune checkpoints, and immunotherapy response between both subgroups.

2.4. Establishment and validation of the prognostic model

The differentially expressed m6A//m5C/m1A/m7G regulatory genes were analyzed by single-factor regression to screen the regulatory genes significantly linked with the outcomes of HCC. To reduce the fitting degree of the model, we adopted the Least Absolute Shrinkage and Selection Operator (LASSO) regression analysis to establish the prognostic hazard signature for m6A/m5C/m1A/m7G regulatory genes. Patients were divided into high-risk group and low-risk group based on the median risk score. Kaplan-Meier analysis was to determine the prognosis of HCC in the two risk groups. We used "ggplot 2" package for PCA analysis and the

timers package for the ROC analysis of the model and then calculated the Area Under Curve (AUC) values for 1-, 3-, and 5-years. Finally, further multiple analysis was employed to confirm risk score as an independent prognostic index. Moreover, we randomly selected 186 patients from the TCGA database as an internal testing cohort and LIRI-JP from the ICGC database as an external testing cohort to validate the availability of prognosis signature.

2.5. Risk signature association with immune features

Single sample gene set enrichment analysis (ssGSEA) is an algorithm for assessing tumor-related immunocyte infiltration. We calculated the infiltration degree of 24 immune cells in HCC by ssGSEA algorithm and explored the difference in immune cell expression between the high-risk group and low-risk group [26]. Present studies indicate that immune checkpoints are closely linked to proliferation, metastasis of tumors, and patient prognosis evaluation. We studied differential expression between immune checkpoints in risk subgroups to assist in immunotherapy. Tumor Immune Dysfunction and Exclusion (TIDE) is applied to evaluate the possibility of immunological escape in tumor gene expression profile and immunotherapy response [27]. A high TIDE score was associated with poor immune checkpoint blockade (ICB). Survival after ICB treatment is short.

2.5. The expression of five genes in mRNA and protein levels from different databases

We downloaded the RNA-seq of five genes in HCC and normal tissues from the TCGA database and ICGC database. The Wilcoxon

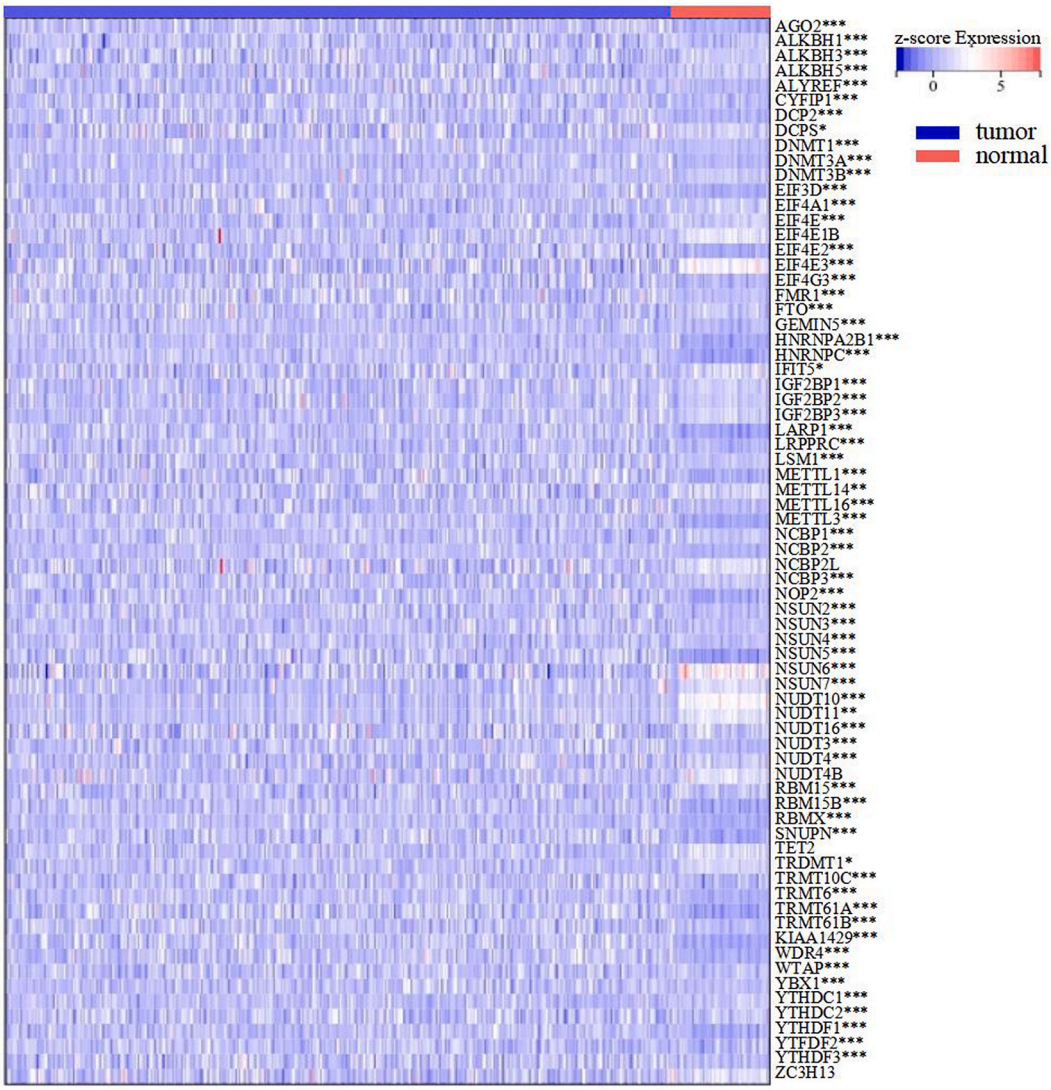

Fig. 1. Heapmap of m6A/m5C/m1A/m7G regulated genes in HCC patients and normal tissues (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

method was used to detect the differential expression of five genes in HCC and normal tissues. Then, we utilized the UALCAN database to study the protein expression of five genes in HCC and normal samples.

2.6. Analysis of drug sensitivity

Gene expression data and sensitivity of targeted therapeutics were obtained from the genetics of drug sensitivity in cancer (GDSC). We used the pRRophetic package in R to analyze differences in sensitivity to different drugs between the two risk groups. IC50 (semi-inhibitory concentration) is an important indicator for evaluating the efficacy of a drug or the response of a sample to treatment.

2.7. Gene functional enrichment analysis

GSEA (Gene Set Enrichment Analysis) is a powerful online tool for analyzing different physiological functions between high- and low-risk groups [28].

2.8. Statistical analysis

R version 4.2.3 and SPSS 17.0 were used for statistical analysis. Wilcoxon method was used to detect different analysis between groups. Chi-square tests or Fisher's exact tests were applied for the analysis of clinical characteristics. $P < 0.05$ was set as a significant difference.

3. Results

3.1. Screening differentially expressed genes, and enrichment analysis of differentially expressed genes

The mRNA expression of 71 m6A/m5C/m1A/m7G regulatory genes from HCC tissues and normal tissues were downloaded, and 66 differentially expressed genes (DEGs) were obtained through the Wilcoxon test. Heapmap was shown in Fig. 1. Moreover, we investigated the biologic function and related pathways of DEGs by GO and KEGG analysis. GO analysis showed that m6A/m5C/m1A/m7G regulatory genes were enriched in the regulation of translation, RNA modification, macromolecule methylation, and RNA methylation (Fig. 2A). KEGG analysis displayed that m6A/m5C/m1A/m7G regulatory genes participated in RNA transport, Spliceosome, mRNA surveillance pathway, RNA degradation, and cysteine and methionine metabolism (Fig. 2B).

3.2. Consensus clustering analysis

To reveal the role of prognostic signature in HCC, we conducted an unsupervised clustering algorithm based on the expression data for 71 m6A/m5C/m1A/m7G regulatory genes.

In consensus clustering, the optimal clustering stability k = 2 was selected (Fig. 3A–C). Patients with cluster 2 had a higher

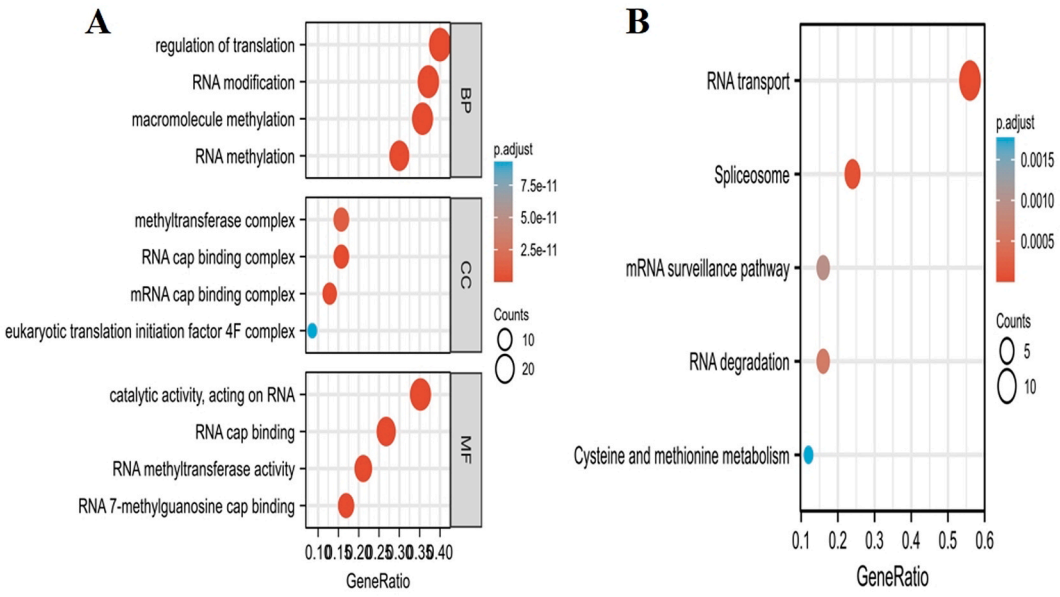

Fig. 2. GO and KEGG enrichment analysis of m6A/m5C/m1A/m7G regulated genes.

probability of survival than cluster 1 by Kaplan-Meier curve (Fig. 3D). The results showed that patients were classified into two clusters with good resolution in PCA, UMAP, and tSNE(Fig. 3*E*–G). SsGSEA analysis showed that in cluster 1 group, B cell memory, T cell regulatory (Treg), macrophage M0, and neutrophil had a higher infiltration degree, and in the cluster 2 group, T cell $CD8^+$ and T cell $CD4^+$ memory activated had a higher infiltration degree (Fig. 3H). In cluster 1, immune checkpoints (PD-1, PD-L1, CTLA4, HAVCR2, PDCD1LG2, and TIGIT) indicated higher expression compared to cluster 2 (Fig. 3I). The TIDE score in cluster 1 was higher than cluster 2, indicating that cluster1 was less responsive to immunotherapy (Fig. 3J).

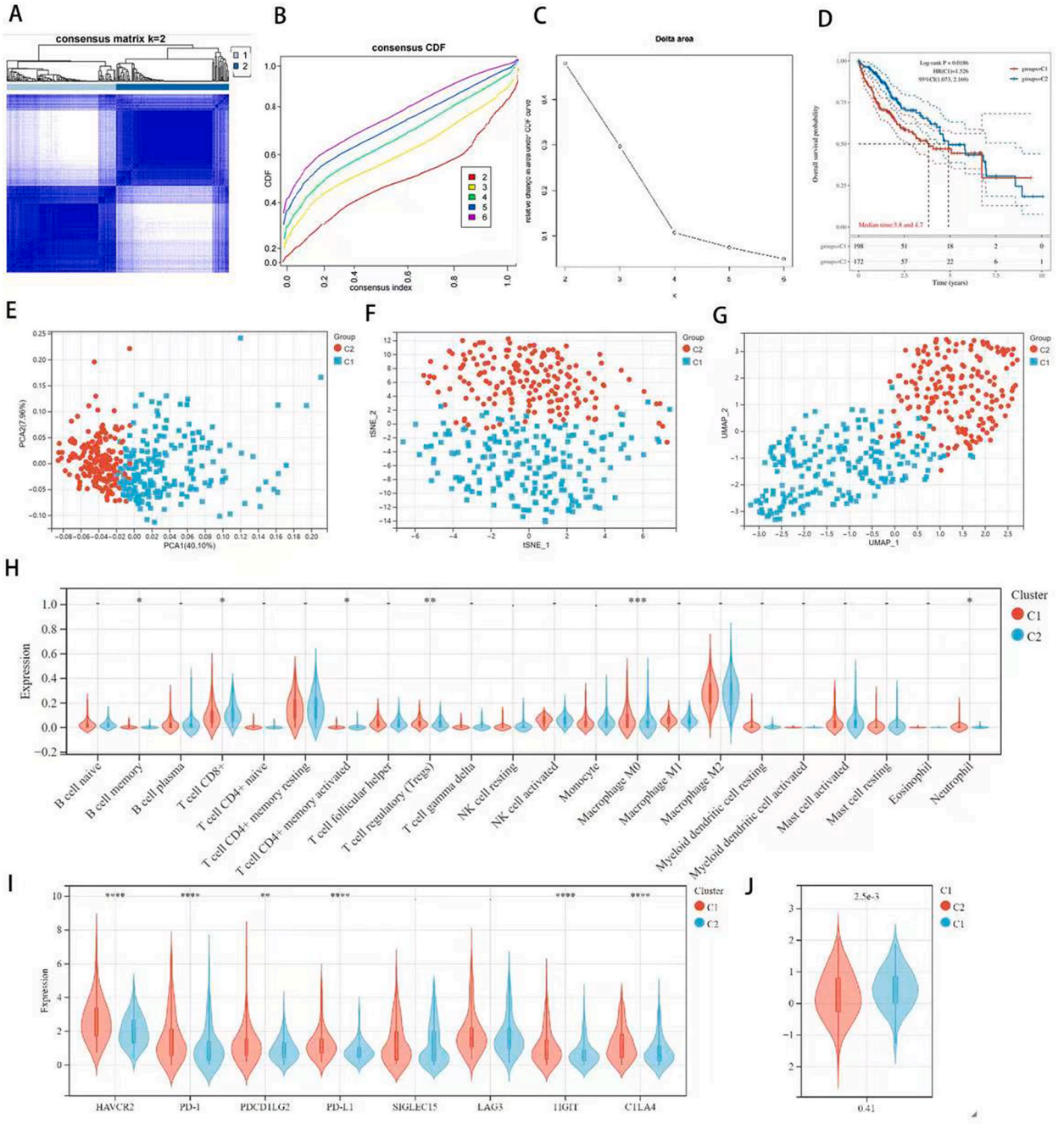

Fig. 3. The clinical values in m6A/m5C/m1A/m7G regulated gene subgroups in HCC patients based on consensus clustering. (A–C) The consensus clustering of m6A/m5C/m1A/m7G regulated genes. (D) Kaplan-Meier survival analysis in cluster1 and cluster2 groups. (*E*–G) The significant differences in the transcriptome in PCA, tSNE, and UMAP analysis. (H)The difference in expression of immune cell infiltration in both clusters. (I) The expression of immune checkpoints in both subgroups. (J) The difference of TIDE scores in both subgroups. (*$P < 0.05$, **$P < 0.01$,***$P < 0.001$).

3.3. Establishment and validation of the prognostic model

Through univariate regression analysis, 41 m6A/m5C/m1A/m7G regulatory genes were found to be associated with adverse outcomes (Fig. 4A). Subsequently, a prognostic model of 5 m6A/m5C/m1A/m7G regulatory genes was established by LASSO algorithm (Fig. 4B and C). Finally, multivariate Cox regression was used to determine the corresponding coefficients and calculate the risk score for each patient:The risk score is as followings: Riskscore=(0.0432 × YTHDF2+(0.2093)*YTHDF1+(0.5076)*

YBX1+(−0.222)*TRMT61A+(0.2913)*TRMT10C. The risk scores were separated into high-risk and low-risk groups based on the median scores (Fig. 5A). Kaplan-Meier analysis suggested that the patients with high-risk group had unfavorable outcome (Fig. 5B). PCA analysis demonstrated that two subgroups are separated after reducing characteristic dimension (Fig. 5C). AUCs for 5-gene signatures were 0.767, 0.69, and 0.693 at 1-year, 3-year, and 5-yearof overall survival(OS), respectively (Fig. 5D). To assess risk score as an independent predictor, univariate and multivariate regression analyses were conducted. Single-variable analysis demonstrated that tumor stage, T stage, and risk score were hazard predictors for HCC. For multiple variables analysis, only risk score was an independent risk predictor (Fig. 6). Therefore, the prognostic characteristics of five-gene are credible for HCC prognosis. Moreover, high-risk group had an unfavorable outcome in internal (randomly selected,n=186) and external (ICGC) testing cohorts (Fig. 7A and B). The AUCs of prognostic signature for 1-year, 3-year, and 5-year in internal testing cohort were 0.766, 0.740, and 0.674, respectively, and in external testing cohort, the AUCs of prognostic signature for 1-year, 3-year, 4-year were 0.706, 0.755, 0.733, respectively. (Figure 7C,D). Therefore, prognostic characteristics had a useful role in evaluating OS in HCC patients.

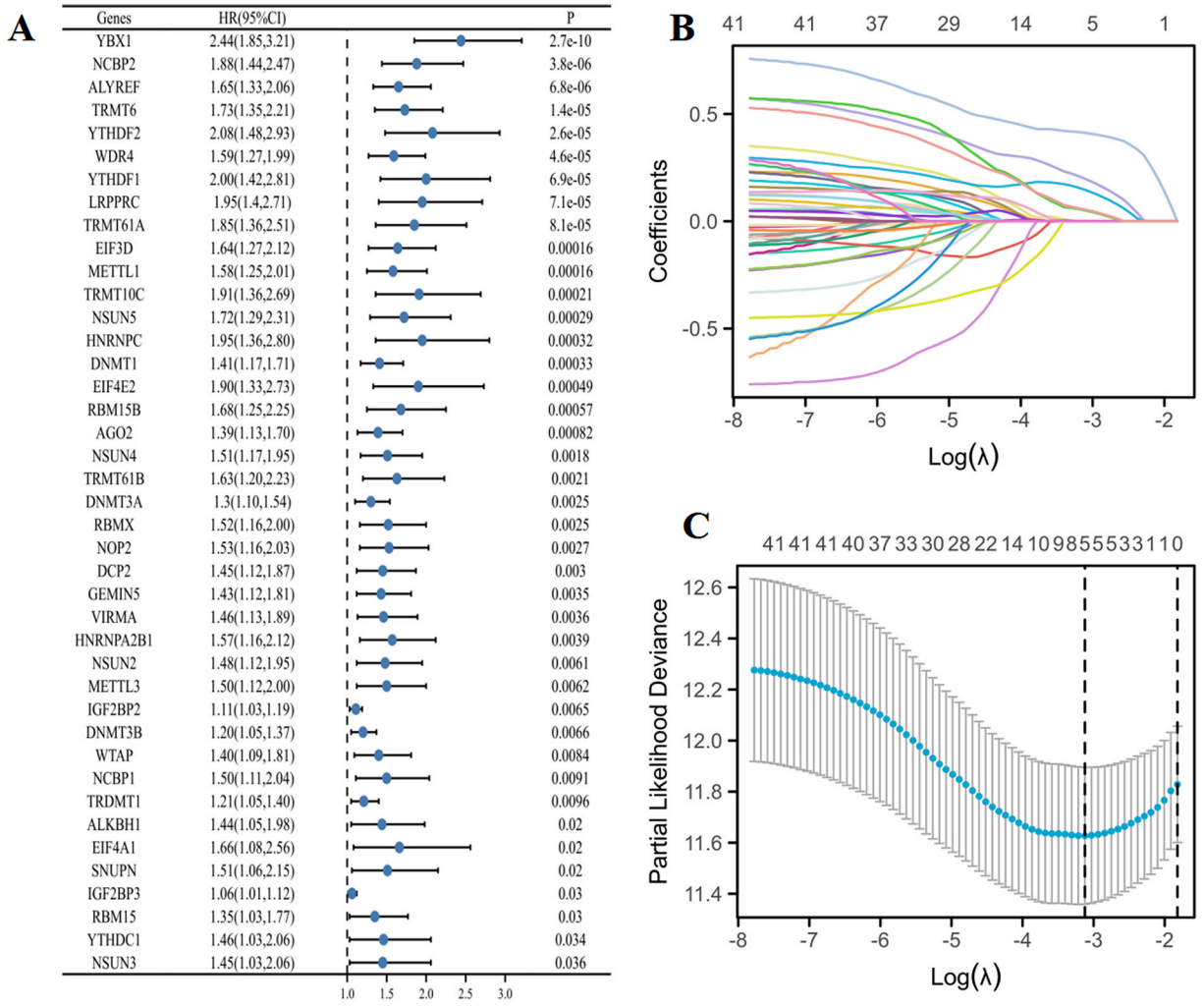

Fig. 4. Construction of prognostic model for m6A/m5C/m1A/m7G regulated genes. (A)Univariate Cox regression of differentially expressed m6A/m5C/m1A/m7G regulated genes. (B–C) The 5 m6A/m5C/m1A/m7G-regulated genes were identified using LASSO method.

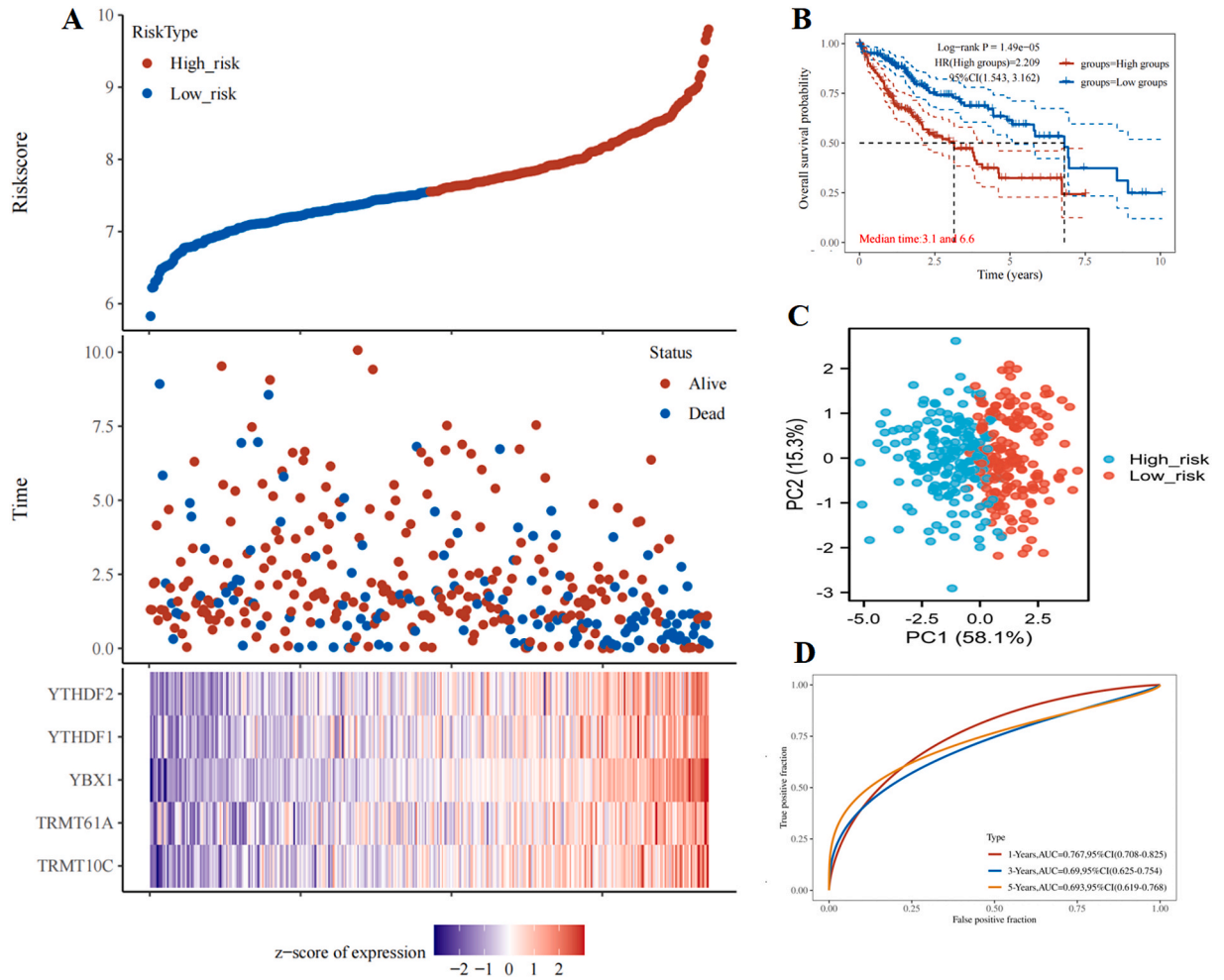

Fig. 5. Prognostic analysis of m6A/m5C/m1A/m7G regulated genes in the training cohort (whole TCGA). (A) The risk score was divided into high risk and low risk.(B)The low-risk groups had a better prognosis than high-risk groups. (C)Principal component analysis plot. (D)The AUC of 1-,3-, and 5-year was 0.767,0.69.0.693, respectively.

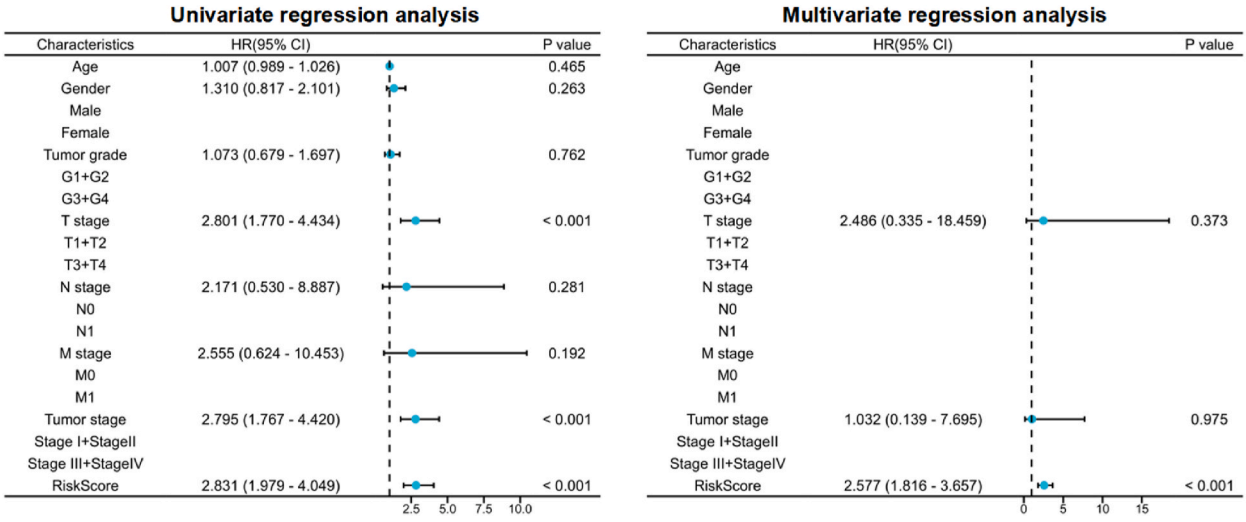

Fig. 6. Univariate and multivariate Cox regression of risk score and clinical features.

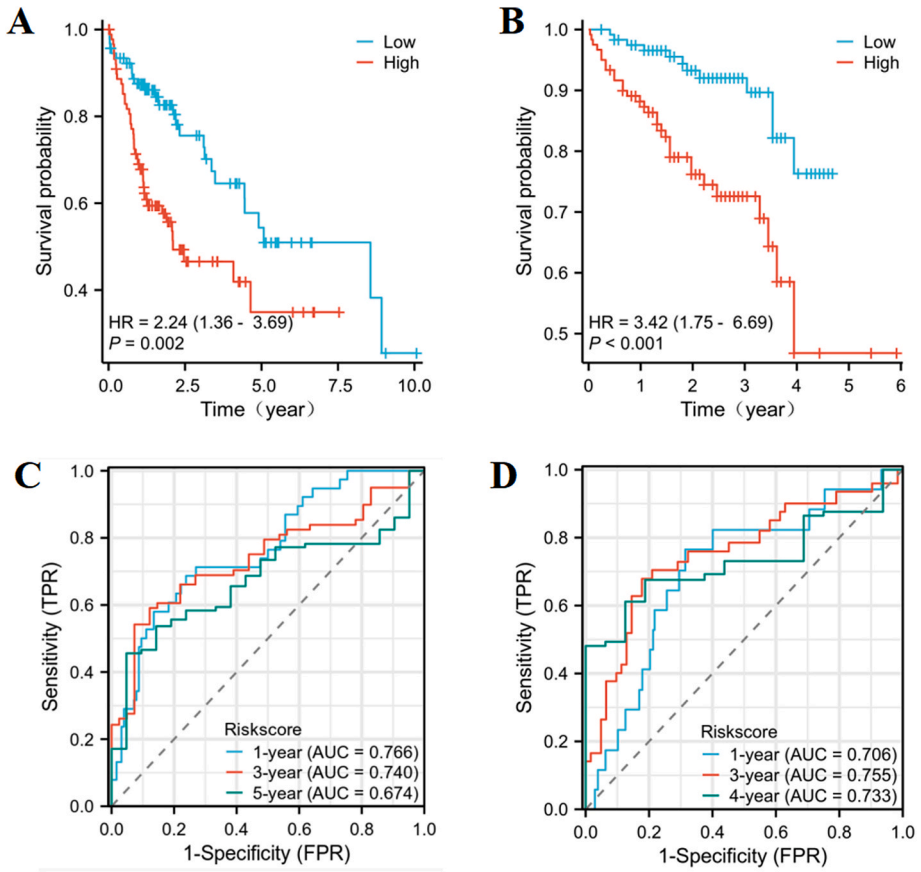

Fig. 7. Validation of the prognostic model of five-gene signature. (A, B)The low-risk groups had a better prognosis than high-risk groups in the internal testing cohort (randomly selected, n=186) and external validation cohort (ICGC). (C, D)Receiver operating characteristic (ROC) curves for the risk model in the internal and external testing cohort.

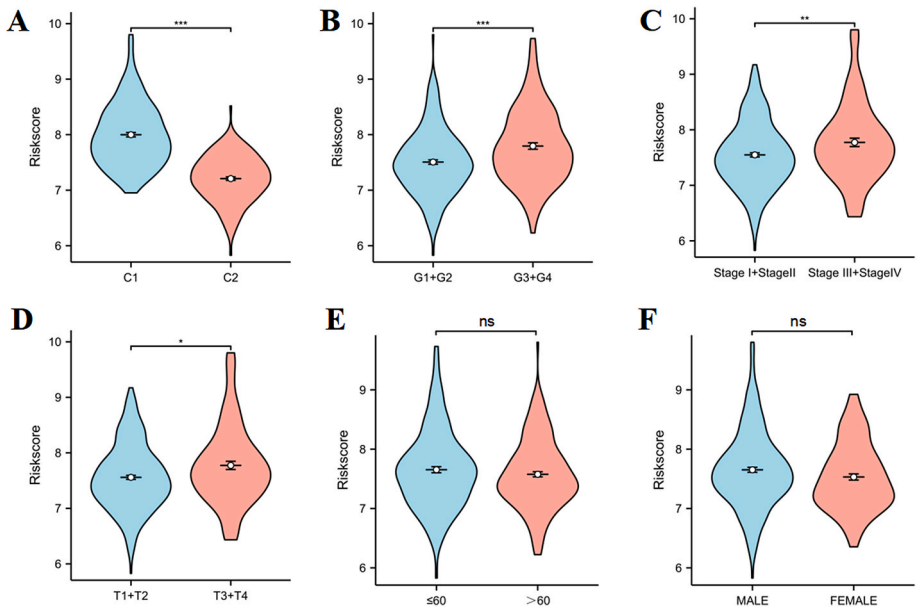

Fig. 8. The Correlation between risk score and clinicpathological features. (***$P < 0.001$,**$P < 0.01$,*$P < 0.05$, ns: no significance).

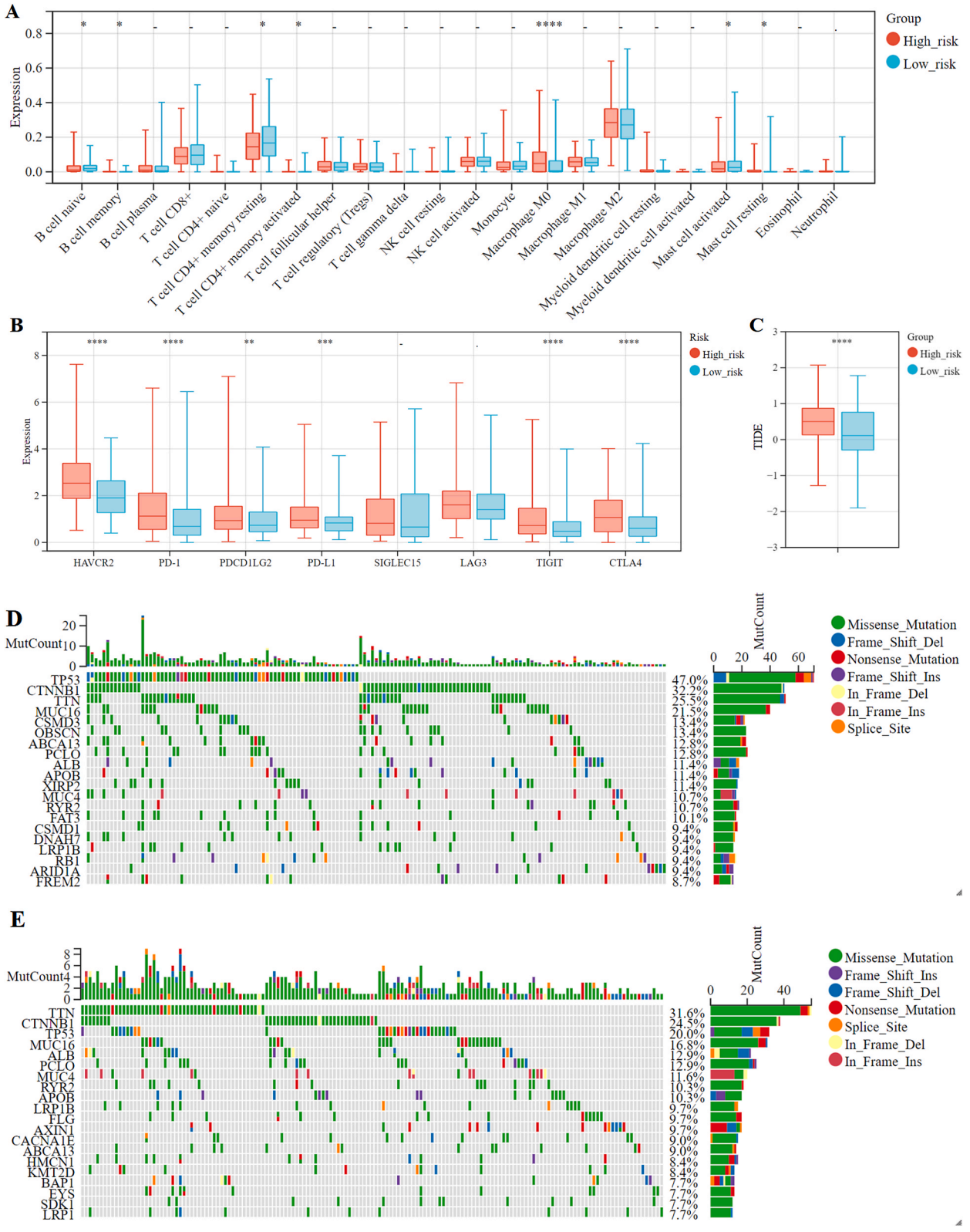

Fig. 9. The correlation between risk score and immunity and mutational analysis. (A)The correlation between risk score and immune cell infiltration. (B)The correlation between risk score and immune checkpoints. (C)The correlation between risk score and TIDE score. (D, E) The mutation alteration in high-risk group and low-risk group. (****$P < 0.0001$,***$P < 0.001$,**$P < 0.01$,*$P < 0.05$).

3.4. Correlation between prognostic signature and clinical features

We explored differences in risk scores for different pathological features. Risk scores were higher in cluster 1, G3+G4, T3+T4, and advanced pathological stages(stage III+IV) (Fig. 8A–D). However, risk scores were not significantly different with age and sex (Fig. 8E and F).

3.5. Correlation between risk signature and immune feature

To explore the correlation between m6A/m5C/m1A/m7G regulatory genes and tumor microenvironment (TME), firstly, using ssGSEA, we assessed differences in immunocyte infiltration for both risk groups. We found that risk scores had a positive association with B cell memory and macrophage M0, and negative association with B cell naive, T cell CD4$^+$ memory resting, T cell CD4$^+$ memory activated, mast cell activated, and mast cell resting (Fig. 9A). Secondly, we evaluated differences in immune checkpoint expression between the two risk subgroups. Immune checkpoints (PD-1, PD-L1, CTLA4, HAVCR2, PDCD1LG2, and TIGIT) had higher expression in the high-risk group. The expression of LAG3 and SIGLEC15 did not differ between the two risk groups (Fig. 9B). Thirdly, according to the TIDE score, we discussed the immunotherapeutic response of m6A/m5C/m1A/m7G regulatory genes. The TIDE score for the high-risk group was higher, indicating that the high-risk group had a poorer response to ICB and a shorter survival time after ICB (Fig. 9C). Finally, we studied the mutation status of two risk groups. The top three mutations between the two groups were TP53, TTN, and CTNNB1, which were dominated by missense mutations. The TP53 mutation rate was highest among the high-risk group (47 %) (Fig. 9D). TTN had the highest mutation rate (31.6 %) in the low-risk group (Fig. 9E). Established evidence suggests that TP53 mutations are participated in HCC development and linked with poor outcomes in HCC patients [29]. Besides, TTN and CTNNB1

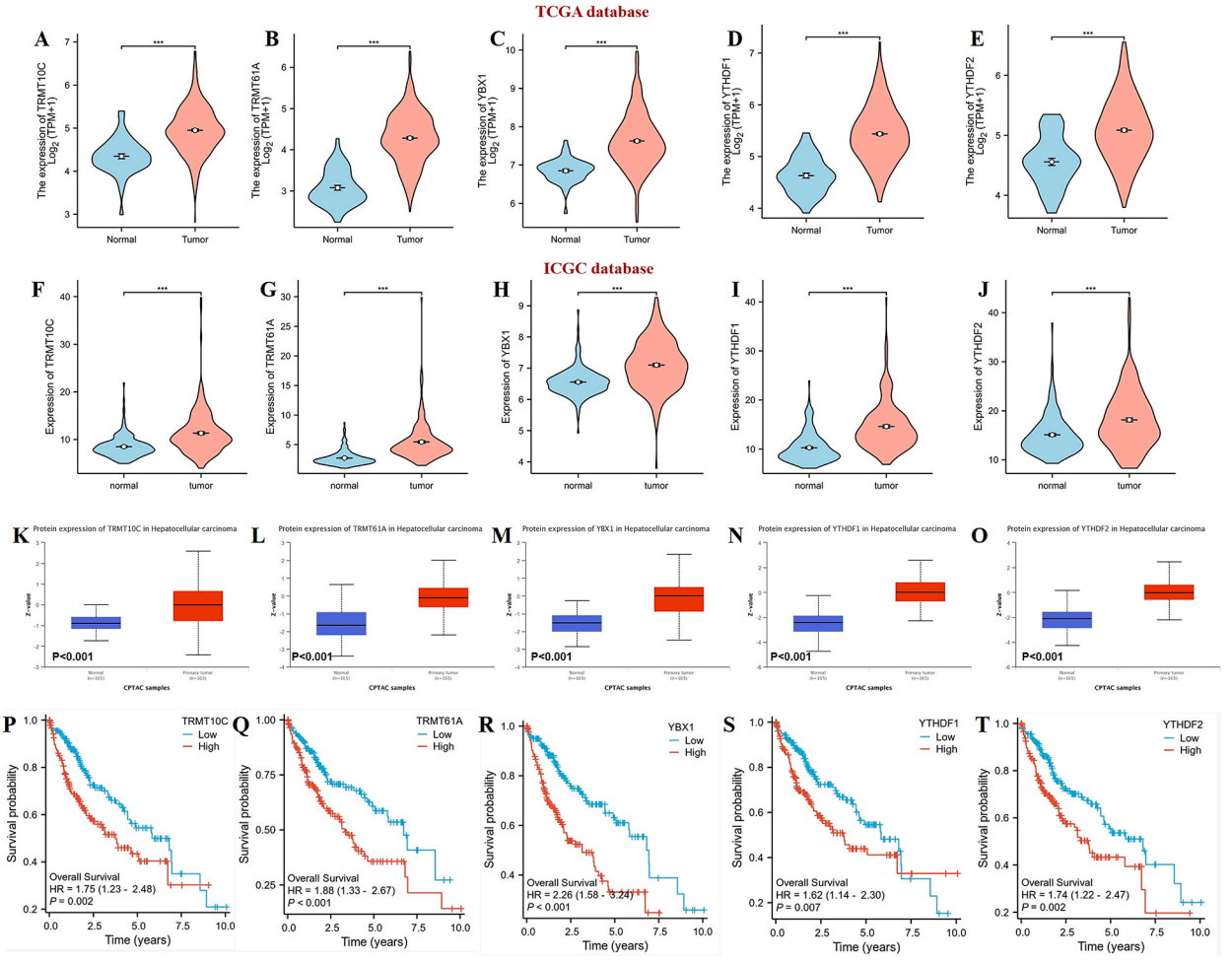

Fig. 10. The expression of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2. (A-E)The mRNA expression of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 in TCGA database. (F-J)The mRNA expression of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 in ICGC database. (K–O) The protein expression of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 in UALCAN database. (*P*–T) Kaplan-Meier survival analysis of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 in high-expression and low-expression groups. (***$P < 0.001$,**$P < 0.01$,*$P < 0.05$).

mutations have been identified as hazard factors for HCC [30].

3.6. The expression of five genes in HCC and normal tissues

TCGA database and ICGC analysis showed that the mRNA expression for TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 was higher in HCC than normal samples (Fig. 10A–J). UALCAN analysis suggested that the protein expression of TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 was higher in HCC compared to normal tissues (Fig. 10K-O). The expression of TRMT10C, TRMT61A, YBX1, YTHDF1 and YTHDF2 in high-expression groups had poor prognosis (Fig. 10P–T).

3.7. Analysis of drug sensitivity

We analyzed drug sensitivity of common chemotherapy drugs for high and low-risk patients. 5-Fluorouracil, docetaxel, doxorubicin, etoposide, gemcitabine, paclitaxel, sorafenib, and vinblastine were more suitable for high-risk patients, while sunitinib and gefitinib may be more suitable for low-risk patients (Fig. 11).

3.8. Functional enrichment analysis of prognostic signature

To clarify the biological process of the two groups, we used the KEGG set in GSEA for analysis. ECM receptor interaction, cell cycle, and Leishmania infection were significantly enriched in high-risk groups (Fig. 12A), while in low-risk groups, it was mainly involved in linoleic acid metabolism, histidine metabolism, and fatty acid metabolism(Fig. 12B).

4. Discussion

Liver cancer has become a common digestive disease, and accounts for the second-leading cause of cancer death [31]. Although surgical treatment, interventional therapy, and immunotherapy have achieved certain effects, the prognosis is still very poor. Therefore, we hope to provide a more reliable method for evaluating prognosis risk and improving the overall survival rate of HCC.

In recent years, more and more attention has been paid to epigenetic modification, especially methylation modification. Research suggests that epigenetic modifications are closely related to tumors, mainly through RNA methylation, DNA methylation, histone modification, non-coding RNA modification, and chromatin rearrangement to regulate gene function and expression level, thus affecting tumor progression [32]. RNA modification has the function of regulating RNA processing and metabolism, and its abnormal regulation is closely related to cancer progression [33,34]. RNA modification mainly includes m6A, m5C, m1A, and m7G. Some progress has been made in the study of RNA methylation in HCC. For example, METTL3 can accelerate the degradation of SOCS2 mRNA by modifying m6A at the 3′ end of SOCS2 mRNA, thus promoting the proliferation and metastasis of hepatoma cells [35]. WTAP acts as an oncogene in HCC and its up-regulated expression can cause bad prognosis. It accelerates the G2/M phase transition through the EST1-p21/p27 signaling pathway to regulate the cell cycle of HCC, thereby promoting the proliferation of liver cancer cells [36]. WDR4 is up-regulated in HCC, which can increase m7G methylation level for HCC. WDR4 not only enhances metastasis and sorafenib resistance through epithelial-mesenchymal transition (EMT) but also promotes HCC cell proliferation by inducing G2/M cell cycle transition and inhibiting apoptosis [37]. These findings suggested that m6A/m5C/m1A/m7G regulatory genes play an essential part in the progress of HCC. In our research, we integrated the expression data of m6A/m5C/m1A/m7G regulatory genes and established five-gene prognostic signature to predict HCC patients' prognosis.

We acquired the mRNA expression of 71 m6A/m5C/m1A/m7G regulatory genes from 374 HCC patients and 50 normal liver tissues by TCGA database and screened out 66 DEGs. We classified HCC patients into cluster 1 and cluster 2 according to the expression of 71 m6A/m5C/m1A/m7G regulator genes. Patients in cluster 1 had significantly poor OS. PCA, tSNE, and UMAP analysis suggested good resolution with two clusters. We further investigated the TME based on the two subtypes and found that there was significantly different immune cell infiltration in cluster 1 and cluster 2, revealing the characteristics of distinct tumor microenvironments in both clusters. Most immune checkpoints in cluster 1 had higher expression than cluster 2. A higher TIDE score displays a higher immune escape probability, indicating that patients are unlikely to gain from ICI treatment. The TIDE score of cluster 1 was higher than cluster 2, which indicated that cluster 1 patients benefited less from ICB, and cluster 2 patients benefited more from ICI therapy. Furthermore, univariate, LASSO and multivariable analysis confirmed that the prognostic signature of 5 genes (YTHDF2, YTHDF1, YBX1, TRMT61A, TRMT10C) was an independent factor for adverse prognosis of HCC. And high-risk group had an unfavorable outcome. Risk scores were positively related to cluster, T stage, histologic grade, and pathological stage. All these proved that m6A/m5C/m1A/m7G regulatory genes played a fundamental biological role in HCC. Past studies have demonstrated that YTHDF2 is overexpressed in glioblastoma, prostate cancer, endometrial cancer, and liver cancer [38–40]. YTHDF1 is an important tumorigenic factor that plays a significant process in various tumor growth [41–45] and is a hazard factor for an unfavorable prognosis in HCC [46]. YBX1 expression level is significantly increased in breast cancer and is linked with low survival, drug resistance, and high recurrence rates in all subtypes of breast cancer [47]. TRMT10C expression is elevated in ovarian cancer and cervical cancer, with worse prognosis and it participates in regulating the proliferation, colony formation, and migration of ovarian and cervical cancer cells [48]. In our study, TRMT10C, TRMT61A, YBX1, YTHDF1, and YTHDF2 had higher expression in HCC compared to normal samples. Overexpression of TRMT10C, TRMT61A,YBX1,YTHDF1 and YTHDF2 had unfavorable outcome. Our findings were consistent with previous studies.

With the progress of tumor immunotherapy, the correlation between tumor and immunity has received more and more attention, and the degree of immunocyte infiltration in tumors is related to tumor growth, progression, and prognosis, which has become the

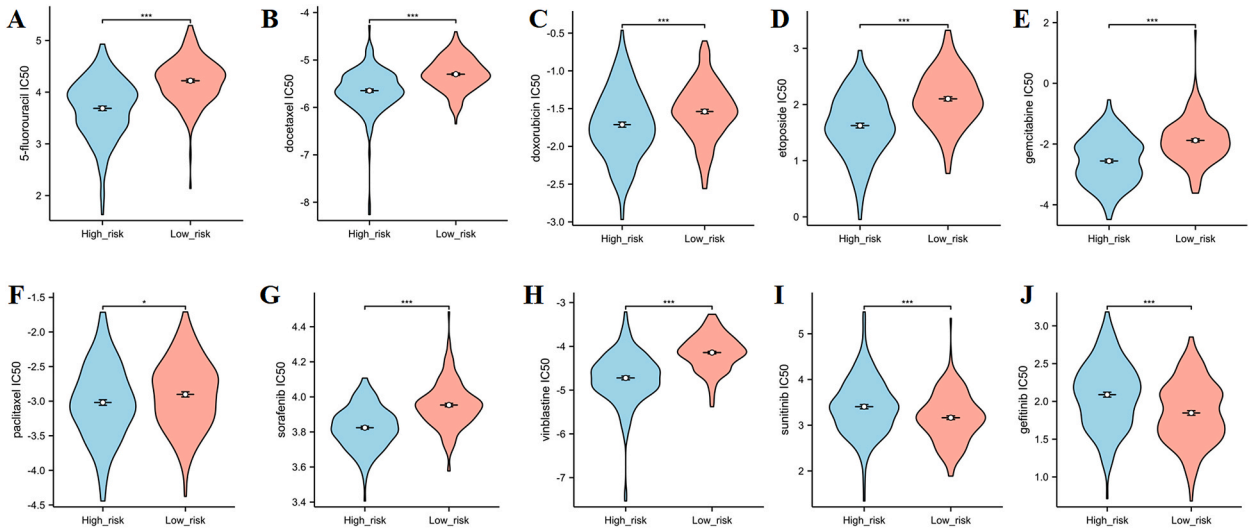

Fig. 11. The drug sensitivity analysis of risk score in HCC.

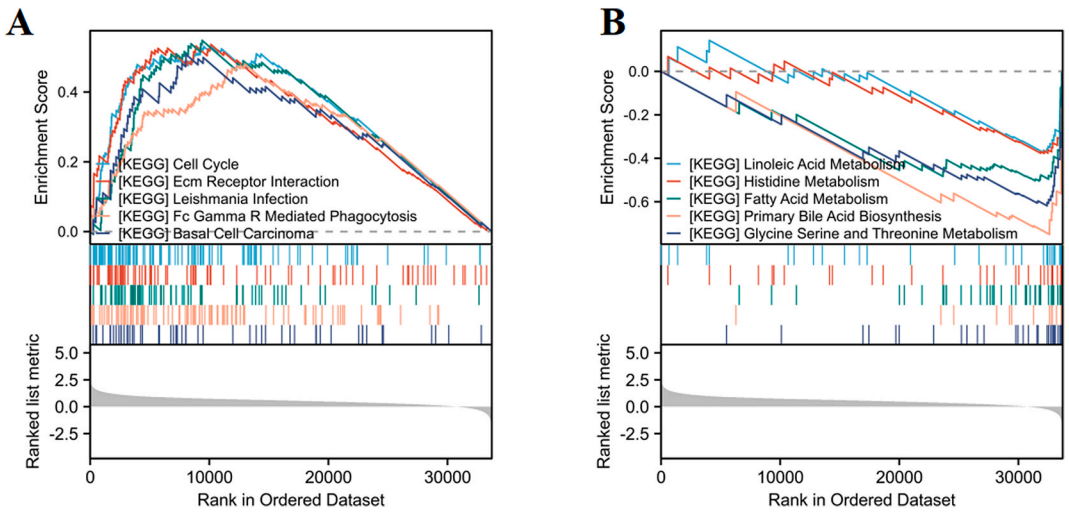

Fig. 12. The enrichment analysis in high-risk groups and low-risk groups. (A)High-risk groups. (B) Low-risk groups.

focus of attention in recent years [49,50]. In our research, we investigated immunocyte infiltration between different subgroups and it was found that expression of B cell memory and macrophage M0 were higher in the high-risk group, while B cell naive, T cell CD4$^+$ memory resting, T cell CD4$^+$ memory activated, mast cell activated, and mast cell resting were higher in the low-risk group. These results suggested that the prognosis model established by the m6A/m5C/m1A//m7G regulatory gene correlated with the immune cell infiltration of HCC. Several recent studies have found that tumor immunotherapy targeting immune checkpoint has made great progress and become an effective method for cancer therapy. For the high-risk group, most immune checkpoints had higher expression levels, and these results suggested that immune checkpoints can act as a treated target for HCC patients. After predicting the ICB of different subgroups, the TIDE algorithm simulates the tumor immune escape pathway to evaluate cancer treatment response [51]. It was discovered that the TIDE score in the low-risk group was lower, implying low-risk group of patients benefited more from ICI therapy. Finally, we compared the somatic mutagenesis of both subgroups and discovered that the mutation frequency of TP53 was significant higher in the high-risk groups. TP53 is considered to be a tumor suppression gene, with a lower frequency of mutation in the low-risk group, which may explain the favorable outcome of patients in the low-risk group. Finally, patients in the high-risk group may benefit from 5-fluorouracil, docetaxel, doxorubicin, etoposide, gemcitabine, paclitaxel, sorafenib, and vinblastine. Our work established a prognostic signature for m6A/m5C/m1A/m7G regulatory genes, which showed a good ability for predicting prognosis and could serve as a latent marker for HCC. However, this study still has some limitations, because it is based on bioinformatics analysis, so the prognostic functions of some related genes need to be further verified by related experiments.

5. Conclusion

We established two related subtypes of m6A/m5C/m1A/m7G regulatory genes to determine the potential effect of m6A/m5C/m1A/m7G regulatory genes on HCC. Furthermore, we also successfully developed a five-gene prognostic signature to predict characteristics, which can be considered as an independent prognostic indicator with HCC patients. Our study elucidates risk model associated with HCC prognosis, immune microenvironment, immunotherapy, and drug sensitivity. This prognostic model had certain clinical significance for drug selection and provided new insights for future immunotherapy of HCC.

Data availability

The data generated for this study can be found in the TCGA (https://portal.gdc.cancer.gov/)database and the ICGC (https://dcc.icgc.org/)datasets.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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