Original Article

Identification of N7-methylguanosine-related lncRNAs for the risk stratification of hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. The N7-methylguanosine (m7G) modification is related to the biological processes and regulation of various diseases. This study investigated the role and predictive value of m7G-related long non-coding RNAs (lncRNAs) in HCC.

Methods: HCC patients were clustered by consensus clustering, and a prognostic signature was developed using Least Absolute Shrinkage and Selection Operator (LASSO)-Cox regression analysis. The immune landscape and clinicopathological features of the distinct clusters and subgroups were investigated.

Results: A total of 32 m7G-related lncRNAs were confirmed to be prognostic lncRNAs. Two molecular clusters showed significant differences in terms of their clinicopathological features, prognoses, and immune checkpoint gene (ICG) expression levels. Cluster II was associated with upregulated ICG expression and poor overall survival (OS). The Cancer Genome Atlas training cohort was then used to create an m7G-related lncRNA signature for predicting OS. The signature exhibited excellent predictive performance in the training, test, and all cohorts. The high-risk patients had worse clinical outcomes than the low-risk patients. Further study revealed that this signature was an independent prognostic indicator, and a predictive nomogram was developed based on the clinicopathological features and risk score. In addition, we discovered that this model was correlated with ICG expression and tumor immune cell infiltration.

Conclusions: Our findings demonstrated that m7G-related lncRNAs are associated with the tumor immune landscape and prognosis and can serve as independent prognostic markers for HCC. These findings provide new insights into the functions of m7G-related lncRNAs in HCC.

Keywords: Hepatocellular carcinoma (HCC); N7-methylguanosine (m7G); long non-coding RNAs (lncRNAs); prognostic signature; tumor landscape

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world with an increasing annual morbidity rate and a high mortality rate (1), and represents a significant risk to public health (2). Despite significant advances in the early detection and management of HCC, the rate of early detection is poor, as the majority of HCC cases are diagnosed at the late stage (3,4). Further, tumor recurrence occurs in up to 70% of HCC patients within 5 years of resection (5). As a result, the 5-year survival rate for HCC is still extremely low (1). Currently, commonly used staging approaches based on clinicopathological criteria, such as tumor-node-metastasis (TNM) criteria, provide rather unclear predictions in evaluating the prognostic outcomes and treatment options for HCC patients (6,7). Thus, it is critical to discover new and accurate biomarkers to improve the treatment specificity and prognosis of HCC patients.

N7-methylguanosine (m7G) is a methyl modification of the seventh nitrogen atom of RNA guanine (8,9). The m7G modification has been revealed to have significant effects on messenger RNA, ribosomal RNA, and transfer RNA (tRNA), and is important in various biological processes (10-12). Recent studies have investigated the function of the m7G modification in carcinogenesis and cancer development, notably in HCC (13). For example, Xia *et al.* revealed that the m7G methyltransferase, WD repeat domain 4 (WDR4), enhances HCC progression by increasing cyclin B1 mRNA stability and translation and is thus a potential therapeutic target for HCC (14). The abnormal tRNA m7G modification by methyltransferase-like 1 (METTL1)/WDR4 has been linked to the progression and incidence of various cancers, including lung cancer (15), intrahepatic cholangiocarcinoma (16), and head and neck squamous cell carcinoma (17). A study by Tian *et al.* showed that METTL1 is overexpressed in HCC and promotes HCC migration and proliferation via the phosphatase and tensin homolog (PTEN) and AKT signaling pathways (18). The oncogenic activity of METTL1 is mediated through inhibition of PTEN signaling, which suggests that the METTL1/PTEN axis is a potential target for HCC treatment (18,19). Altogether, these findings indicate that m7G modifications play an important role in HCC.

Long non-coding RNAs (lncRNAs) are non-protein-coding RNAs over 200 nucleotides in length (20). There is increasing evidence that lncRNAs play a role in a wide range of biological activities, including disease pathogenesis (21). Further, certain lncRNAs have been linked to the initiation and development of HCC. For example, lncRNA-ANRIL (antisense non coding RNA in the INK4 locus) has been shown to increase HCC proliferation (22), and lncRNA-HULC (highly upregulated in liver cancer) can act as a driver to promote HCC proliferation, migration, and invasion (23). Additionally, other lncRNAs have been shown to influence HCC prognosis. For example, lncRNA-MVIH (microvascular invasion in hepatocellular carcinoma) overexpression has been linked to poor overall survival (OS) in HCC patients (24). Meanwhile, lncRNA-PTTG3P (pituitary tumor-transforming 3, pseudogene) expression in HCC patients has been associated with poor survival and TNM stage (24). According to recent research, several lncRNAs may be useful predictive biomarkers for HCC (25-27). It was reported that m7G-related lncRNAs (LOC102555374 and LOC102554730) are markedly upregulated and exacerbate hypoxia-induced pulmonary hypertension in mice (28). Furthermore, several studies showed that m7G-related lncRNAs are associated with tumor treatment and prognosis (29-33). However, the prognostic value and precise role of m7G-related lncRNAs in HCC are still unclear.

This study used data from The Cancer Genome Atlas

Highlight box

Key findings

- This study successfully validated the significance and accuracy of a risk-score model in predicting hepatocellular carcinoma (HCC) prognosis.

What is known and what is new?

- We discovered 2 patient clusters using 32 N7-methylguanosine (m7G)-related long non-coding RNAs (lncRNAs) and created an HCC prognostic signature from 12 m7G-related lncRNAs, which showed considerable value in predicting the overall survival (OS), clinicopathological features, and immune landscape of HCC patients.
- We developed a novel m7G-related lncRNA-based risk signature that can be used to predict the immune landscape and prognosis of HCC patients.

What is the implication, and what should change now?

- The identified m7G-related lncRNAs were associated with various biological processes and pathways. Our findings extend understandings of the involvement of the m7G-related lncRNAs in the development and progression of HCC and may also contribute to to the development of predictive biomarkers for HCC.

(TCGA) data sets to examine the prognostic significance of m7G-related lncRNAs in HCC. Cluster subgroups were created according to the expression of the m7G-related lncRNAs to assess the associations between m7G-related lncRNAs and HCC immune checkpoints and prognosis. Further, we developed a novel m7G-related lncRNA-based risk signature that can be used to predict the immune landscape and prognosis of HCC patients. We present this article in accordance with the TRIPOD reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-227/rc).

Methods

Acquisition of data sets

Transcriptome profiling [RNA-sequencing (RNA-seq)] data and relevant clinical data of liver hepatocellular carcinoma (LIHC) were collected from TCGA. This study included the sequencing results of 374 HCC tissues and 50 normal liver tissues, and a total of 377 samples containing clinical data, including data on patients' survival status, time, age, gender, and TMN stage. Samples with a patient follow-up duration of <30 days (n=27) and without survival information (n=1) were removed to decrease errors due to confounding variables. After matching the 374 tumor samples with the clinical data, a total of 343 tumor samples with clinical data and RNA-seq data remained for further study (available online https://cdn.amegroups.cn/static/public/jgo-23-227-1.pdf). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All the data were obtained from an open-access database, and thus the approval of a medical ethics committee was not required.

Selection of m7G-related genes

The Molecular Signature Database (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb) was searched to retrieve M7G-related genes using the keyword "N7 methylguanosine". A total of 13 m7G-related genes (i.e., *EIF4E*, *NCBP2*, *CYFIP1*, *LARP1*, *EIF4E1B*, *GEMIN5*, *CYFIP2*, *AGO2*, *DCPS*, *EIF4E3*, *NCBP1*, *NCBP3*, and *EIF4E2*) were used in our study.

Bioinformatics analysis

The correlation analysis of HCC mainly focused on the m7G-related genes and all the lncRNAs. LncRNAs with P values <0.001 and correlation coefficients >0.4 were regarded as m7G-related lncRNAs. A univariate Cox (uniCox) regression analysis was used to identify the m7G-related lncRNAs that were significantly related to OS, and 2 distinct clusters (clusters I and II) were subsequently identified by "Consensus ClusterPlus" to determine the potential function of the m7G-related lncRNAs in HCC (34). The clustering outcomes were then analyzed by a principal component analysis (PCA) and the t-distributed stochastic neighbor embedding (t-SNE) algorithm (35-37). The clinicopathological features and OS were compared between the two clusters, and the expression of immune checkpoint genes (ICGs) was examined in different clusters.

The HCC patients were randomly assigned to the training and test cohorts. The m7G-related lncRNAs with strong prognostic significance were determined by a Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression. A prognostic risk model was then established from the training cohort data using the prognostic m7G-related lncRNAs. The coefficients of the m7G-related lncRNAs were determined based on the best penalty parameter λ. Risk score (RS) was calculated using the following formula: RS = βlncRNA1 × lncRNA1 expression + βlncRNA2 × lncRNA2 expression + ... + βlncRNAn × lncRNAn expression. The HCC patients were categorized into the high-risk and low-risk groups using the median RS as the cut-off value. The predictive efficiency of prognostic risk model was verified by the receiver operating characteristic (ROC) curves and a Kaplan-Meier analysis (38). In addition, PCA and t-SNE analyses were conducted using the "Rtsne" and "ggplot2" packages, respectively, to determine the clustering capability of the risk signature. The test cohort and overall cohort were then used to confirm the accuracy of the model. Moreover, differences in immune function, clinicopathological features, immune cell infiltration, and ICG expression were compared between the high- and low-risk groups. The prognostic value of risk stratification (RS) was determined by uniCox and multivariate Cox (multiCox) regression analyses. The hazard ratios with 95% confidence intervals (CI) and the log-rank p values were determined by the "glmnet" and "survival" packages, respectively (39). A nomogram was established by combining the prognostic signatures for predicting OS in HCC patients.

To examine the biological functions correlated with the m7G-related lncRNAs, we performed a Gene Set Enrichment Analysis (GSEA). The GSEA was used to

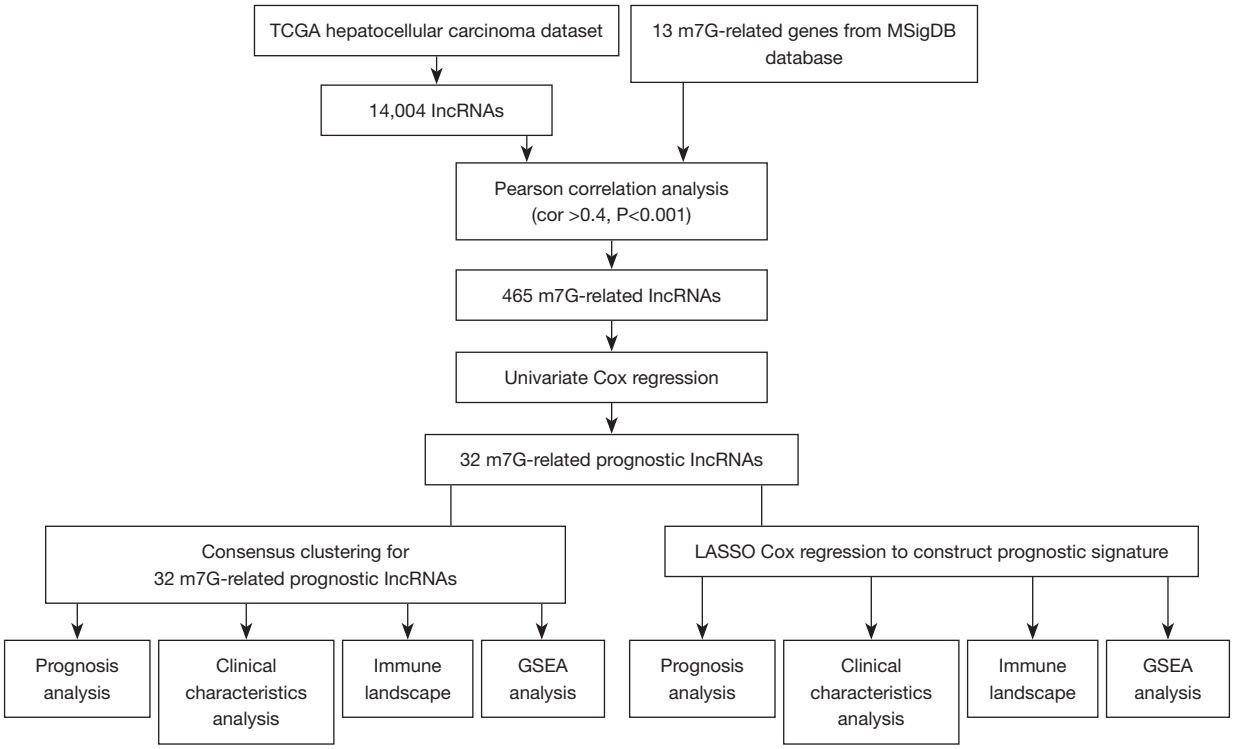


Figure 1 Flow chart of the study. TCGA, The Cancer Genome Atlas; lncRNAs, long non-coding RNAs; LASSO, Least Absolute Shrinkage and Selection Operator; GSEA, Gene Set Enrichment Analysis.

functionally annotate the genes in the different clusters and risk groups. A nominal P value <0.05, |NES| >1, and false discovery rate q<0.25 were considered statistically significant. *Figure 1* depicts a flow chart of the bioinformatics analysis.

Statistical analysis

A 2-group comparison was performed using the Wilcoxon signed-rank test. The OS curves were compared between the subgroups using the Kaplan-Meier estimator. The independent prognostic value of the risk model based on the m7G-related lncRNAs was assessed by uniCox and multiCox regression analyses. A P<0.05 was considered statistically significant. R version 4.1.1 was used for all the statistical analyses.

Results

Identification of the m7G-related lncRNAs in HCC patients

The expression data of 14,004 lncRNAs and 13 m7G genes were acquired from TCGA and analyzed. Ultimately, 9 m7G genes and 465 lncRNAs were found to be correlated (P<0.001), and their relationship was visualized in an interaction network diagram (*Figure 2A* and available online https://cdn.amegroups.cn/static/public/jgo-23-227-1.pdf). The uniCox regression analysis revealed that 32 of the 465 lncRNAs were linked to the OS of the HCC patients (*Figure 2B* and available online https://cdn.amegroups.cn/static/public/jgo-23-227-1.pdf). The expression levels of these 32 lncRNAs were significantly higher in the tumor tissues than in the normal tissues (*Figure 2C*,*2D*).

Correlation between consensus clustering of m7G-related lncRNAs and characteristics and survival of HCC patients

Consensus clustering analysis revealed that a k value of 2 was the best cluster parameter (*Figure 3A-3C*). We integrated the patients' survival time and the expression levels of the selected lncRNAs and removed any incomplete samples. Ultimately, 343 patients were identified and divided into cluster I (n=303) and cluster II (n=40) based on the m7G-related lncRNA expression (available online https://

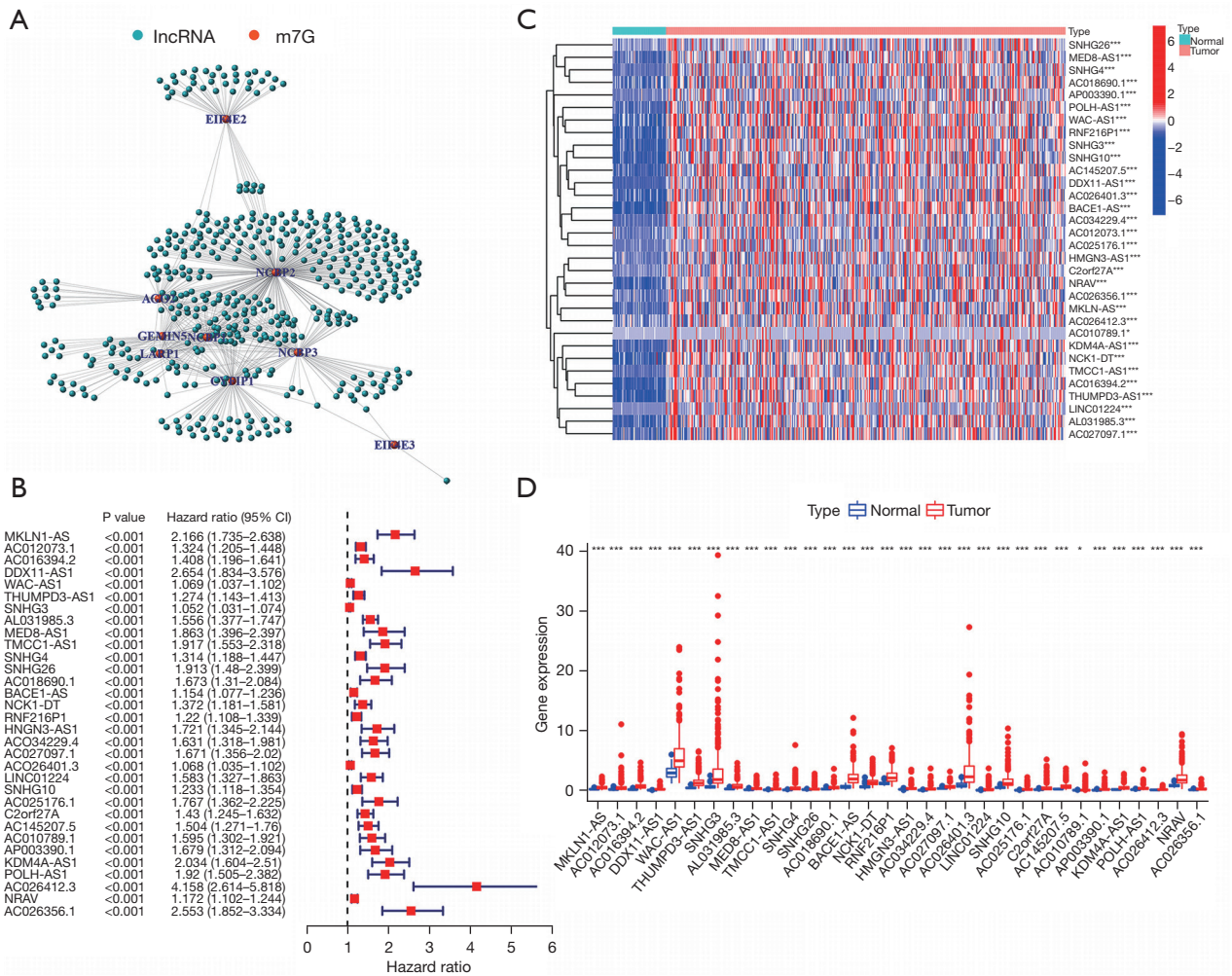


Figure 2 Identification of the m7G-related lncRNAs. (A) The coexpression network revealed m7G-related lncRNAs in HCC. (B) The forest plot of 32 prognostic m7G-related lncRNAs. (C,D) Expression of the 32 prognostic m7G-related lncRNAs. *, P<0.05; ***, P<0.001. lncRNAs, long non-coding RNAs; HCC, hepatocellular carcinoma.

cdn.amegroups.cn/static/public/jgo-23-227-1.pdf). The distribution patterns of the PCA and t-SNE analysis were generally consistent with the consensus clustering results (*Figure 3D*,*3E*), which suggests that the 2 clusters were successfully separated. The expression levels of the 32 lncRNAs were significantly higher in cluster II than in cluster I (*Figure 3F*). Moreover, a comparison of the clinicopathological characteristics between the 2 clusters revealed a significant association between cluster II and advanced T stage (T3-4) (P<0.05), grade (grade 3–4) (P<0.01), and stage (stage III–IV) (P<0.01). We performed a survival analysis to further analyze the differences in patient prognosis between the clusters and found that patient survival was significantly shorter in cluster II than in cluster I (P<0.001) (*Figure 3G*).

Relationship between immune checkpoint expression and the m7G-Related lncRNAs

According to previously published studies (40,41), the expression levels of key targets of the immune checkpoint blockade may be strongly correlated with the clinical outcomes of immune checkpoint inhibitors. Thus, we further evaluated the expression of immune checkpoints in different HCC clusters. We identified 6 key genes associated with the immune checkpoint blockade in HCC

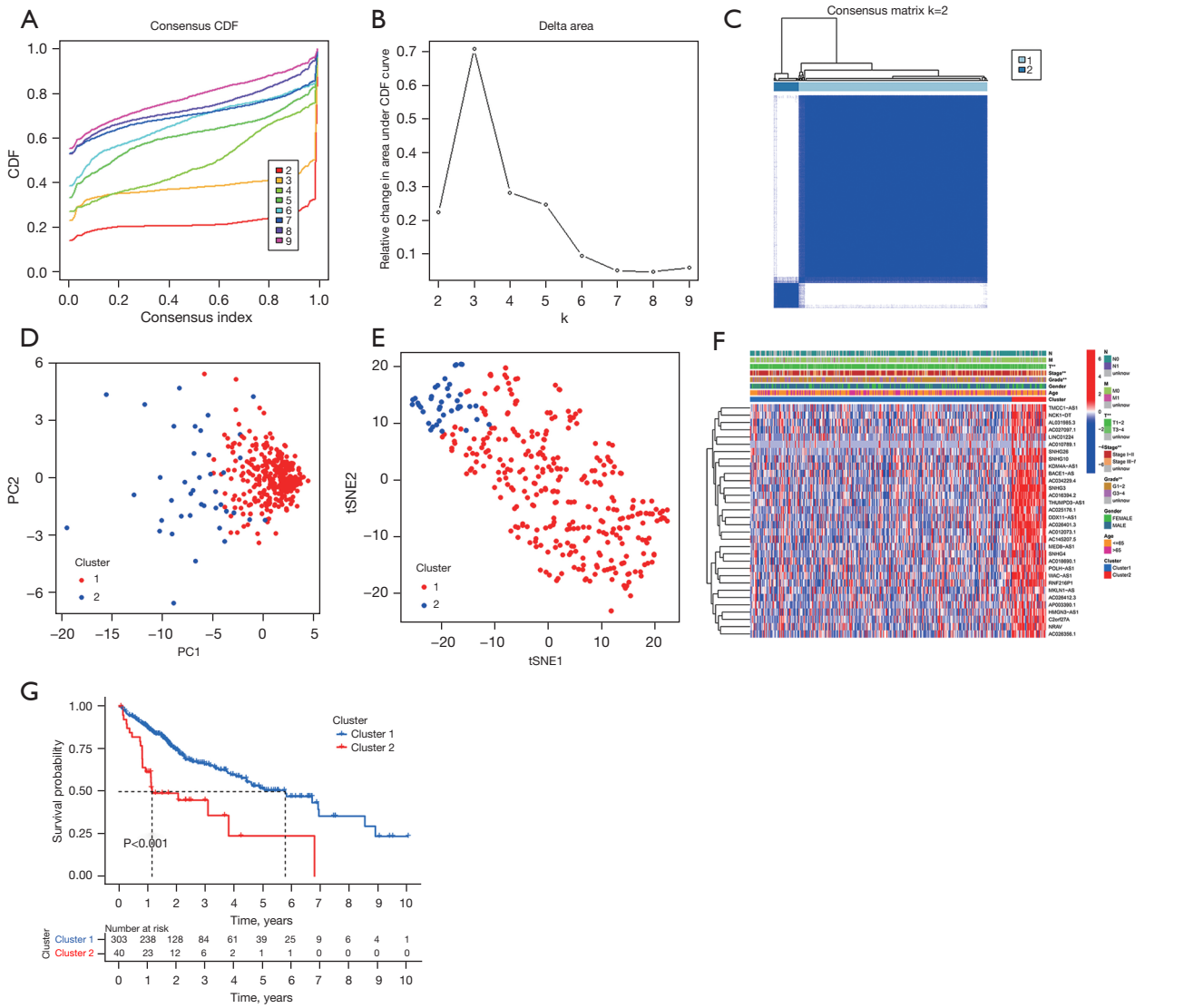


Figure 3 Consensus clustering analysis of the m7G-related lncRNAs in HCC patients. (A-C) Consensus clustering. (D) PCA and (E) t-SNE analysis. (F) The correlation of the two clusters with clinicopathologic features was visualized by heatmap. (G) Kaplan-Meier curves for the overall survival of HCC patients in the two clusters. **, P<0.01. CDF, Cumulative Distribution Function; lncRNAs, long non-coding RNAs; HCC, hepatocellular carcinoma; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding.

[i.e., *CTLA4*, *HAVCR2*, *TIGIT*, *CD276*, *PDCD1*, and *TNFRSF4* (41-44)], which were considerably upregulated in cluster II (*Figure 4A-4F*). The majority of the correlations between the 32 lncRNAs and 6 ICGs were significant (*Figure 4G-4L*). Based on these findings, we hypothesized that ICG overexpression was the contributing factor for a poor prognosis in cluster II.

Construction and verification of the m7G-related lncRNA prognostic signature

A m7G-related lncRNA signature was established from the training cohort by a LASSO analysis to assess the prognosis of the HCC patients based on the 32 candidate lncRNAs that were significantly correlated with OS. Ultimately, 12 lncRNAs were selected to generate a prognostic

signature and compute the RS (*Figure 5A*,*5B*). The patients in the training cohort (n=172) were separated into the high-risk and low-risk groups based on their median RS. The classification ability of the risk signature was validated by a PCA and t-SNE analysis (*Figure 5C*,*5D*). The following formula was used to calculate the RS based on the expression levels and coefficients of these 12 lncRNAs for each HCC patient (available online https://cdn.amegroups.cn/static/public/jgo-23-227-1.pdf):

$$\begin{aligned} RS = {} & 0.452253961 \times \text{MKLN1-AS expression level} \\ & + 0.156153421 \times \text{AL031985.3 expression level} \\ & + 0.274062674 \times \text{TMCC1-AS1 expression level} \\ & + 0.570698196 \times \text{SNHG26 expression level} \\ & + 0.034777968 \times \text{RNF216P1 expression level} \\ & + 0.105369596 \times \text{AC034229.4 expression level} \\ & + 0.545649233 \times \text{LINC01224 expression level} \\ & + 0.020778875 \times \text{SNHG10 expression level} \\ & + 0.000205483 \times \text{AC025176.1 expression level} \\ & + 0.465599554 \times \text{AP003390.1 expression level} \\ & + 0.239908465 \times \text{POLH-AS1 expression level} \\ & + 0.271425817 \times \text{AC026356.1 expression level} \end{aligned} \quad [1]$$

Survival and ROC curves were generated to evaluate the efficiency of the prognostic model. The Kaplan-Meier analysis showed that survival time was significantly shorter in the high-risk group than in the low-risk group (P<0.05) (*Figure 5E*). The areas under the curve (AUCs) of the 1-, 3-, and 5-year ROC curves were 0.878, 0.754, and 0.744, respectively (*Figure 5F*), which indicated that the survival model had a good predictive performance. A validation analysis was carried out in the test cohort (n=171) and overall cohort (n=343) to further confirm the validity of the 12 lncRNA-prognostic signature. Similar to the findings for the training cohort, the survival time was much shorter in the high-risk group than in the low-risk group (*Figure 5E*). The time-dependent ROC curves for the test and overall cohorts also demonstrated that the model had a good predictive performance, and the 1-, 3- and 5-year AUC values are shown in *Figure 5F*. The RS and survival distribution plot revealed that a higher RS was associated with more HCC deaths (*Figure 5G*,*5H*). A heatmap of the expression of the 12 prognostic m7G-related lncRNAs in the training, test, and overall cohorts indicated that the 12 selected lncRNAs were highly expressed in the high-risk group (*Figure 5I*).

Both uniCox and multiCox regression analyses were performed to determine whether the RS was an independent prognostic factor. Both analyses showed that the RS was closely related to OS in the training, test, and overall cohorts (P<0.001), indicating that the RS was an independent prognostic factor for HCC (*Figure 6A*,*6B*). To increase the clinical applicability of the m7G-related lncRNA signature model, we created a predictive nomogram model that included the RS and clinicopathological features. As *Figure 6C* shows, the RS was the main predictive index. A detrended correspondence analysis further showed that the RS was superior to other variables as a prognostic indicator in clinical decision-making (*Figure 6D*). The distribution of clinical features and patient clustering in the high- and low-risk groups are presented in *Figure 6E*.

Subgroup analysis of OS

We categorized the patients into subgroups based on age, gender, grade, and clinical stage to assess whether the m7G-related lncRNA prognostic model could predict OS among patients with different clinical characteristics. As *Figure* 7 shows, OS was considerably higher in low-risk patients than in high-risk patients based on age, gender, grade, clinical stage, stage T, stage M0, and stage N (P<0.05).

Tumor immune cell infiltration

A heatmap of immune cell infiltration was constructed based on the TIMER, CIBERSORT, QUANTISEQ, MCP-counter, XCELL, and EPIC algorithms (*Figure 8A* and available online https://cdn.amegroups.cn/static/public/jgo-23-227-1.pdf). A comparative analysis of the immune cell-related functions revealed differences in cytolytic activity, major histocompatibility complex class I (MHC class I) expression, and type I and II interferon (IFN) responses between the two risk groups (P<0.05, *Figure 8B*). Compared to the low-risk group, the high-risk group had lower cytolytic activity and type I and II IFN responses but higher MHC class I expression. Differences in immune checkpoint expression were also observed between the two risk groups (*Figure 8C*). Compared to the low-risk group, CD80, TNFRSF14, TNFSF18, CD48, CTLA4, CD200, TNFRSF18, CD276, CD28, ICOS, CD44, TNFRSF25, CD160, LAG3, HAVCR2, TNFRSF9, CD70, VTCN1, TNFRSF8, HHLA2, TNFSF4, TNFSF9, LAIR1, TIGIT, LGALS9, TNFRSF4, TNFSF15, CD200R1, CD27, CD86, PDCD1, and NRP1 have higher expression in the high-risk group.

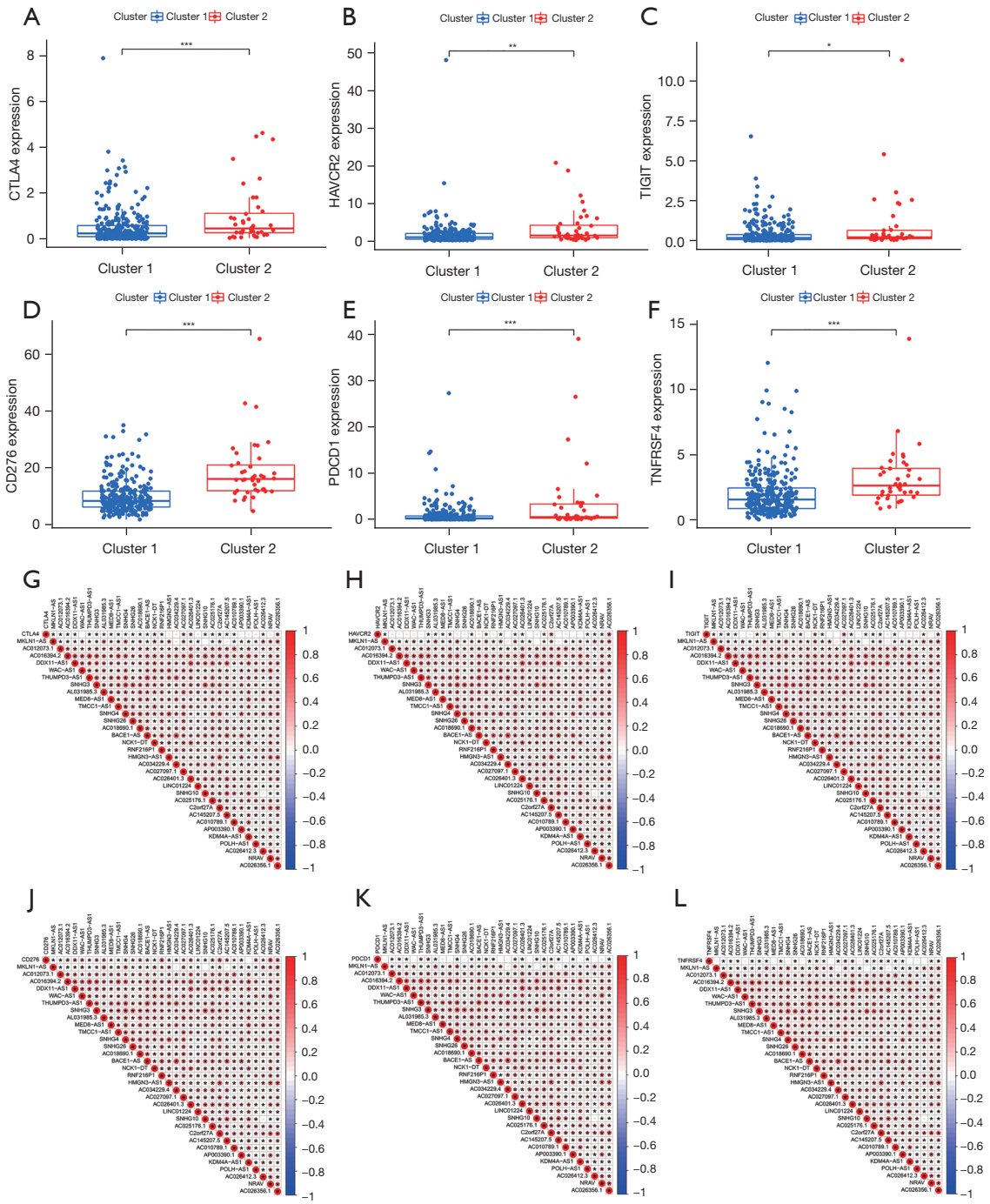
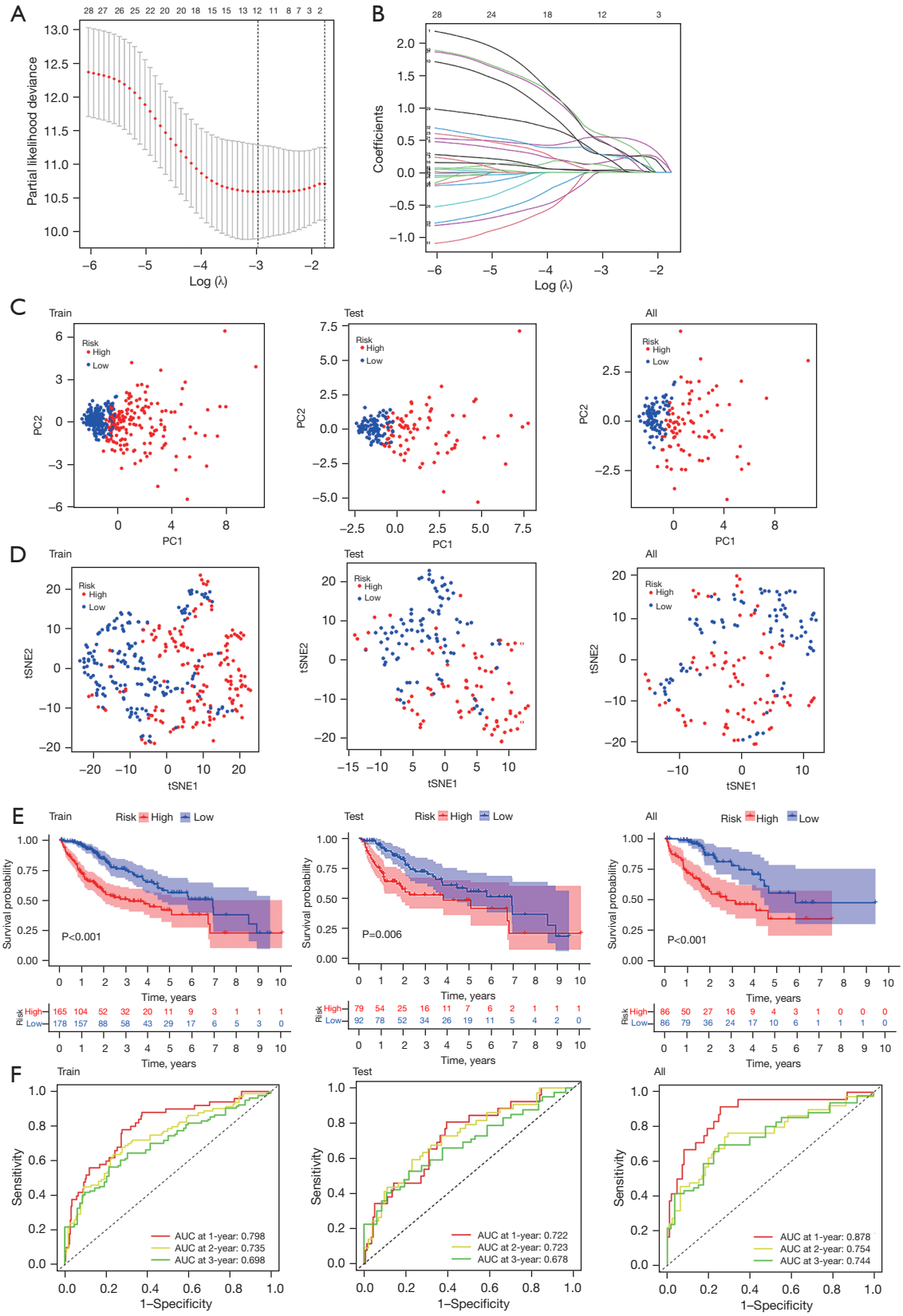


Figure 4 m7G-related lncRNAs are correlated with immune checkpoint expression. (A-F) The expression of immune checkpoints (A) CTLA4, (B) HAVCR2, (C) TIGIT, (D) CD276, (E) PDCD1, and (F) TNFRSF4 was significantly higher in cluster II than cluster I. (G-L) Co-expression analysis of the immune checkpoints and 32 prognostic m7G-related lncRNAs. *, P<0.05; **, P<0.01; ***, P<0.001. lncRNAs, long non-coding RNAs.



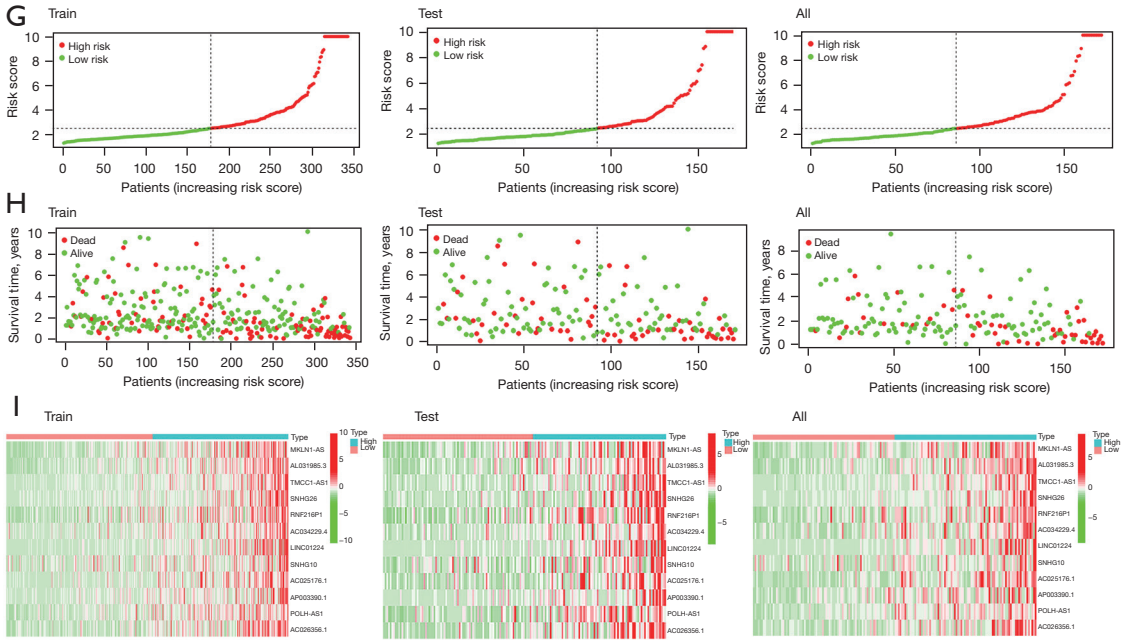


Figure 5 m7G-related lncRNA prognostic signature. (A,B) LASSO-Cox regression analysis. (C) PCA and (D) t-SNE analysis in the train group, the test group, and the all group. (E) Kaplan-Meier survival analysis in the train group, the test group, and the all group. (F) ROC analysis of 1, 3, and 5 years in the train group, the test group, and the all group. (G) Distribution of risk scores and (H) survival status of HCC patients. (I) Heatmap of the prognostic signature scores. AUC, area under the curve; lncRNAs, long non-coding RNAs; LASSO, Least Absolute Shrinkage and Selection Operator; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding; ROC, receiver operating characteristic; HCC, hepatocellular carcinoma.

Functional enrichment analysis

The functional differences between clusters I and II and between the high- and low-risk groups were examined by GSEA. Compared to cluster I, our findings revealed that cluster II had a lower 5-year survival rate. Cluster II was linked to cancer hallmarks such as "DNA repair", "unfolded protein response", "mitotic spindle", "G2M checkpoint", "E2F targets", and "Wnt/beta-catenin signaling". The cancer-related pathways were also enriched in the high-risk group, which were similar to those found in cluster II (*Figure 9*). Multiple signaling discrepancies between the two clusters and between the two risk groups suggested that the m7G-related lncRNAs may play a role in the development of HCC.

Discussion

HCC is one of the most commonly diagnosed cancers, and its global mortality rate is on the rise (45). Due to its genetic and genomic variations, HCC is a highly heterogeneous solid tumor, both molecularly and clinically (46-48). The development of powerful techniques for predicting therapeutic outcomes and prognosis may help to better guide clinical intervention decisions in HCC. Previous studies have shown that lncRNAs play an important role in the development and prognosis of HCC and may serve as potential molecular therapeutic targets (25-27,49-51). m7G has been linked to the development and progression of HCC, and several studies have reported on the role of m7G-related lncRNAs in HCC (52-54). Previous studies have demonstrated that MKLN1-AS, AL031985.3, TMCC1-AS1, AC034229.4, LINC01224, POLH-AS1, and AC026356.1 were related to the development and progression of HCC, which is similar to our research (53,54). Our study showed that MKLN1-AS, AL031985.3, TMCC1-AS1, AC034229.4, LINC01224, POLH-AS1, and AC026356.1 were related to the development of HCC. Importantly, AP003390.1 and SNHG26, which were found to be closely associated with the development of HCC in this study, have not been reported in the above-mentioned

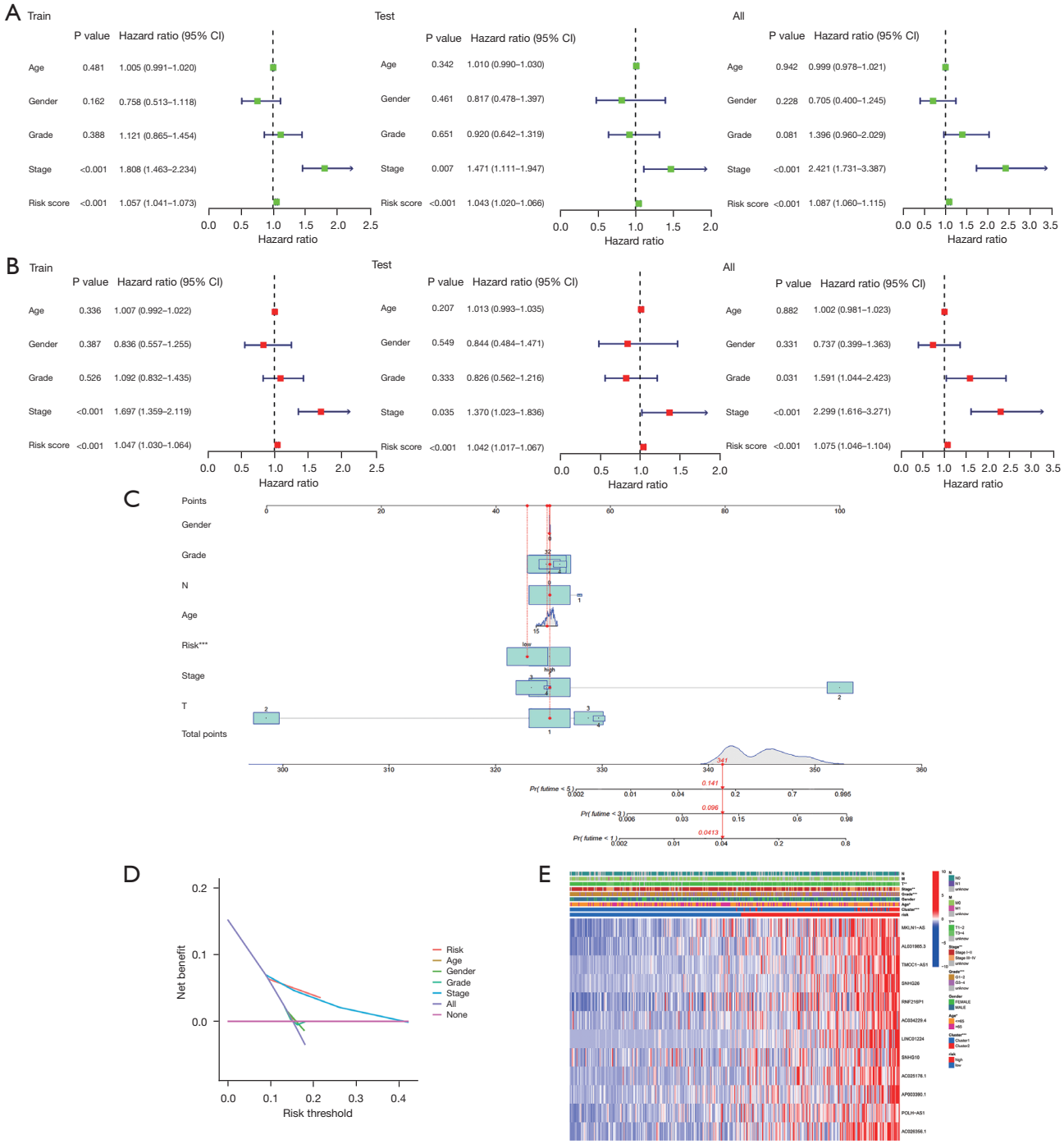


Figure 6 Relationship between the clinicopathological characteristics and risk score. (A) Univariate Cox regression analysis and (B) multivariate Cox regression analysis in the train group, the test group, and the all group. (C) Nomogram based on clinicopathological factors and risk score. (D) DCA analysis. (E) Heatmap showed the expression of 12 m7G-related prognostic lncRNAs, the distribution of clinicopathological characteristics and the clustering of patients in the high-risk and low-risk groups. *, P<0.05; **, P<0.01; ***, P<0.001. N, N stage in TMN lymph node dispersion; T, T stage in TMN staging tumor size; DCA, decision curve analysis; lncRNAs, long non-coding RNAs.

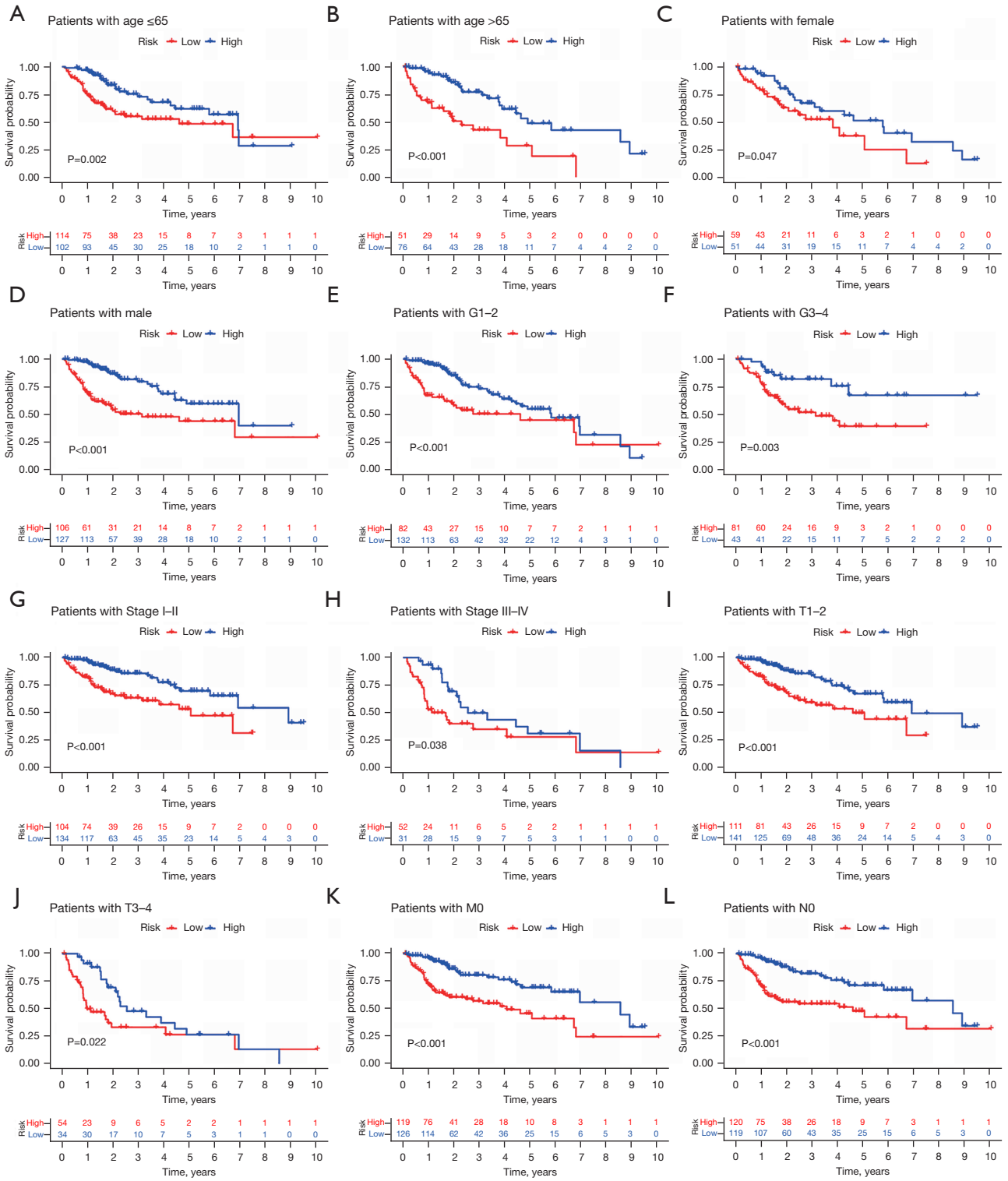


Figure 7 Subgroup analysis of overall survival based on the clinical features of HCC: (A) Age ≤65 years; (B) age >65 years; (C) female; (D) male; (E) G1–G2; (F) G3–G4; (G) stages I–II; (H) stage III–IV; (I) T1–T2; (J) T3–T4; (K) M0; and (L) N0. HCC, hepatocellular carcinoma.

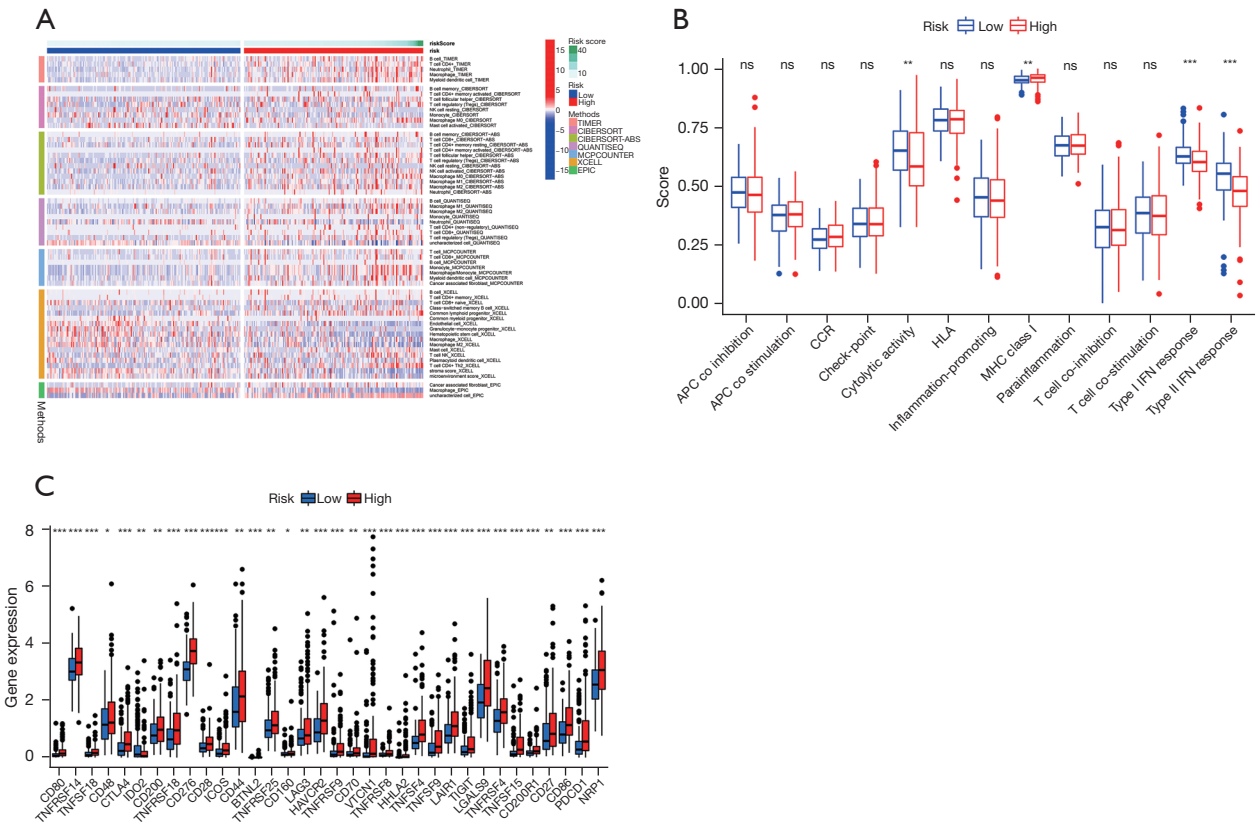


Figure 8 Immune infiltration analysis. (A) Heatmap of immune response based on TIMER, CIBERSORT, QUANTISEQ, MCP-counter, XCELL, and EPIC algorithms among high and low risk groups. (B) Box plot of immune function scores between high and low risk groups. (C) Immune checkpoint expression among high and low risk groups. *, P<0.05; **, P<0.01; ***, P<0.001. ns, not significant; APC, antigen presenting cell; CCR, chemotaxis cell ratio; HLA, human leukocyte antigen; MHC, major histocompatibility complex; IFN, interferon.

studies (52-54). The present study sought to generate a novel m7G-related prediction signature, elucidate its probable underlying molecular mechanisms, and assess its potential in predicting patient prognosis and determining the best therapy.

We first created a co-expression network and identified 465 m7G-related lncRNAs. We then performed uniCox regression analysis and found 32 prognostic m7G-related lncRNAs that were significantly associated with OS outcomes of HCC patients, and had higher expression levels in the HCC tissues than in normal tissues. Thus, these 32 m7G-related prognostic lncRNAs were selected for further investigation. We then performed consensus clustering to create 2 HCC patient groups based on the m7G-related prognostic lncRNAs to investigate if the m7G-related lncRNAs affected the prognosis and immune landscape of HCC. Patients in cluster II had a worse prognosis than those in cluster I, which suggested that the m7G-related lncRNAs were associated with HCC prognosis and could be used as independent prognostic biomarkers for HCC. Moreover, patients in cluster II expressed higher levels of ICGs, including CTLA4, HAVCR2, TIGIT, CD276, PDCD1, and TNFRSF4, than those in cluster I.

The activation of CTLA4 and TIGIT is associated with the progression of HCC (55,56). CTLA4 is known to induce immunosuppression (53). Immunotherapy with checkpoint inhibitors generate strong anti-tumor activity in HCC (57,58). Currently, immune checkpoint blockade for preventing immune evasion and reactivation of the functions of anti-tumor immunotherapies are strategies of great interest (59,60). Clinical studies with anti-CTLA-4 antibodies, such as ipilimumab and tremelimumab, have investigated their safety and efficacy and have yielded some promising results (61). Thus, immune checkpoint inhibitors against the above molecules may be novel therapeutic options for HCC. Several m7G-related

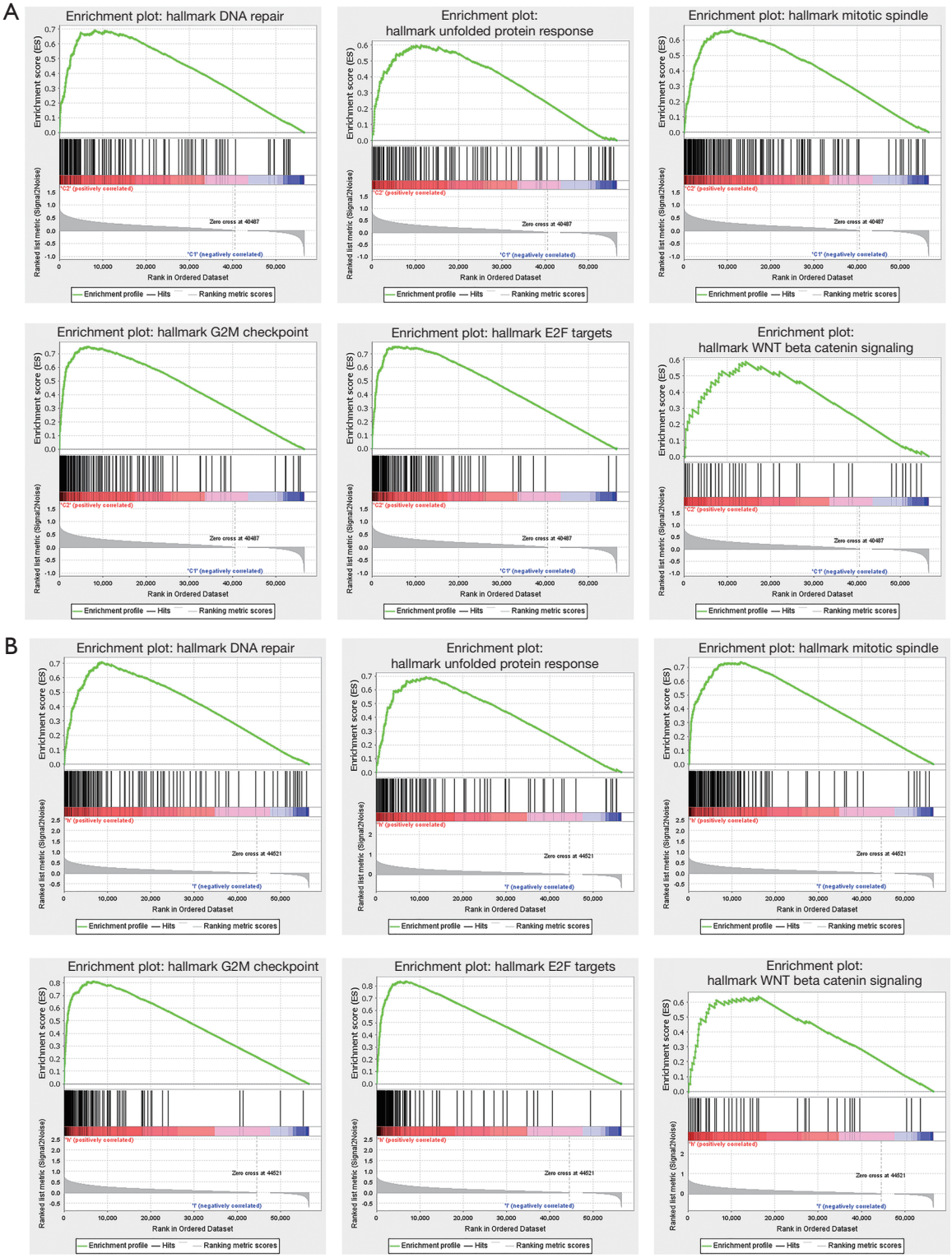


Figure 9 GSEA of m7G-related lncRNAs. (A) Enrichment of "DNA repair", "unfolded protein response", "mitotic spindle", "G2M checkpoint", "E2F targets" and "Wnt/beta-catenin signaling" in cluster II. (B) Enrichment of "DNA repair", "unfolded protein response", "mitotic spindle", "G2M checkpoint", "E2F targets" and "Wnt/beta-catenin signaling" in the high-risk group. GSEA, Gene Set Enrichment Analysis; lncRNAs, long non-coding RNAs.

lncRNAs, including SNHG3, AL031985.3, NCK1-DT, AC027097.1, AC026401.3, and AC025176.1, were shown to be highly correlated with the expression of these immune checkpoints, which indicates the potential function of m7G-related lncRNAs as immune checkpoint modulators.

Next, 12 m7G-related lncRNAs were selected to create the risk signature for predicting OS in HCC patients. The Kaplan-Meier analysis showed that high RS patients had shorter OS than low RS patients. Additionally, the ROC curve analysis suggested that the 12 m7G-related lncRNA signature may function as a highly sensitive and specific prognostic survival model in HCC. Compared to other clinicopathological features, this signature was found to be an independent predictor in both the uniCox and multiCox analyses. To offer clinicians a quantitative tool for predicting HCC prognosis, we combined the signature with the clinicopathological variables to create a nomogram with high reliability and reproducibility. In addition, the stratification analysis revealed that the signature maintained its predictive ability across all subgroups. The RS was more highly correlated with poor OC in cluster II than cluster I, which shows the consistency of the 2 methodologies in predicting HCC prognosis. Together, these findings suggest that the main prognostic m7G-related lncRNAs identified in this study may play a role in the initiation and development of HCC. This study successfully validated the significance and accuracy of the RS model in predicting HCC prognosis.

The proposed signature contained 12 m7G-related lncRNAs. High MKLN1-AS expression has been reported to exacerbate HCC progression and was associated with shorter OS and disease-free survival in HCC patients (62). MKLN1-AS affects HCC progression by regulating microRNAs (62). Several studies have also reported that AL031985.3, TMCC1-AS1, LINC01224, SNHG10, RNF216P1, and AC025176.1 are potential prognostic predictors in HCC (63-69). AL031985.3, an immune- and autophagy-related lncRNA, was shown to be a predictor for HCC prognosis (63,64). Chen *et al.* revealed that high expression of TMCC1-AS1 in HCC patients may lead to shorter survival (65). Gu *et al.* demonstrated that LINC01224 suppression not only inhibited colorectal cancer cell proliferation, migration, and invasion but also promoted apoptosis via the LINC01224/miR-485-5p axis (70). LINC01224 and SNHG26 may be potential biomarkers for predicting survival in tongue squamous cell carcinoma (71). The POLH-AS1 mutation is correlated with skin cancer development (72). Based on these findings, we speculate that the m7G-related lncRNAs are also associated with cancer progression. Importantly, AP003390.1 and SNHG26 have never been reported in HCC, and thus additional studies need to be conducted to assess their value in the early detection of HCC and the development of novel prognostic signatures.

Since gene alterations may contribute to an abnormal immune landscape in cancers, we examined the expression of m7G-related lncRNAs and immune cell infiltration in HCC. Immune infiltration is related to the carcinogenesis and prognosis of HCC (73,74). Using multiple algorithms, we created a heatmap of infiltrating immune cells in HCC patients to investigate the difference in immune landscapes between the two risk groups. T follicular helper cells, natural-killer cells, M0 macrophages, M2 macrophages, and neutrophils have been shown to highly infiltrate HCC and are associated with tumorigenesis, progression, and metastasis (75-78). As is well known, $CD8^+$ T cells have potent abilities to eradicate tumor cells through the Fas/FasL pathway (79). Macrophages can form an immunologic barrier to $CD8^+$ T cell-mediated anti-tumor immune responses (80).

In addition, the relationship between the RS and immune function scores was examined. The low-risk group had a considerably higher type II IFN response score than the high-risk group. IFN is an important part of the immune response to malignant tumors, and it is also an important promoter of the anti-tumor response (81). Our findings indicated that this m7G-related lncRNA signature may partially reflect tumor immune cell infiltration and may be useful for immunotherapies. Immunotherapies based on checkpoint inhibitors have increased the survival rate of metastatic cancer patients (82). The significant differences in ICG expression between the two risk groups suggested that the patients had different sensitivities to immunotherapy. Most ICGs were overexpressed in the high-risk group, which suggests that they may be potential therapeutic targets. Some of these ICGs, such as LAG3 (83), NRP1 (84), and LAIR1 (85), have been associated with a poor prognosis in HCC. In addition, LAG3 is a predictor of tumor response after HCC treatment (86). Further studies of targeting other immune checkpoints that are differentially expressed between the two risk groups may provide potential information for immunotherapy in HCC.

A GSEA was performed to annotate the function of the genes in different clusters or subgroups. The GSEA results revealed that "DNA repair", "unfold protein response", "mitotic spindle", "G2M checkpoint", "E2F targets",

and "Wnt/beta-catenin signaling" were all significantly correlated with cluster II and the high-risk group. E2F targets are genes that encode a family of transcription factors associated with tumor progression in various malignancies (87-89). Previous research has shown that "DNA repair", "mitotic spindle", "G2M checkpoint", and "Wnt/beta-catenin signaling" all play essential roles in the progression and development of HCC (90-94). LncRNA SNHG10 has been reported to enhance the proliferation and invasion of osteosarcoma through Wnt/beta-catenin signaling (95). Thus, we speculate that m7G-related lncRNAs may influence the progression of HCC via the aforementioned oncogenic pathways. These findings indicate that m7G-related lncRNAs play potential regulatory roles in the development of HCC.

Our findings provide an overall profile of the m7G-related lncRNAs in HCC. The limitations of our study should also be considered. First, the data were derived solely from a single TCGA data set, and a multi-data set study would have increased the credibility of the results. Second, we did not perform fundamental experiments to confirm the role of these lncRNAs.

Conclusions

We identified 2 clusters from 32 m7G-related lncRNAs and created an HCC prognostic signature using 12 m7G-related lncRNAs, which showed considerable value in predicting the OS, clinicopathological features, and immune landscape of the HCC patients. Furthermore, these m7G-related lncRNAs were associated with various biological processes and pathways. Our findings extend understanding of the involvement of m7G-related lncRNAs in the development and progression of HCC and may also contribute to the development of predictive biomarkers for HCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-23-227/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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