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N6-methyladenosine Modification-Related Long Non-Coding RNAs are Potential Biomarkers for Predicting the Prognosis of Patients With Osteosarcoma

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Abstract

Background: The role of N6-methyladenosine (m6A)-related long non-coding RNAs (lncRNAs) in osteosarcoma (OS) has not been fully studied yet. We aimed to identify m6A-related lncRNAs that could act as prognostic biomarkers for OS. **Methods:** Pearson correlation was performed to identify m6A-related lncRNAs. Univariate and multivariate Cox regression analyses were performed to construct the risk model and assess whether the risk score was an independent prognostic factor for patients with OS. Gene Set Enrichment Analysis (GSEA) was performed to analyze the functions of genes in high-risk and low-risk groups. StarBase and Cytoscape were used to construct a competing endogenous RNA (ceRNA) network based on m6A-related prognostic lncRNA signature. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to analyze the function of genes involved in the ceRNA network. **Results:** We extracted 122 common lncRNAs from TCGA and Gene Expression Omnibus (GEO) databases. Pearson correlation results revealed 59 significant m6A-related lncRNAs in The Cancer Genome Atlas (TCGA) database, from which 2 were screened to construct a risk signature in TCGA dataset, which was then validated in the GEO dataset. A corresponding risk score was calculated and shown to be an independent prognostic factor for patients with OS. Enrichment analysis indicated that cell proliferation-related biological processes were more common in the high-risk group, while immune-related biological processes were more common in the low-risk group. Moreover, we established a nomogram that had a good ability to predict the overall survival of patients with OS. Additionally, a ceRNA network based on small nucleolar RNA host gene 7 (*SNHG7*) and small nucleolar RNA host gene 12 (*SNHG12*) was constructed, with genes that were enriched in hepatocellular carcinoma, gastric cancer, and non-small-cell lung cancer pathways. **Conclusion:** Our study revealed the prognostic role of m6A-related lncRNAs in OS and identified *SNHG7* and *SNHG12* as potential biomarkers for predicting the prognosis of patients with OS. These findings have enriched our understanding of the role of m6A modification in the dysregulation of lncRNAs in OS.

Keywords

osteosarcoma, m6A modification, long non-coding RNA, competing endogenous RNA, prognosis

Abbreviations

AUC, area under the curve; aDCs, activated dendritic cells; ceRNA, competing endogenous RNA; DCs, dendritic cells; GEO, Gene Expression Omnibus; GO, gene ontology; GSEA, gene set enrichment analysis; HMBOX1, homeobox-containing 1;

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iDCs, immature DCs; IGF1R, insulin-like growth factor 1 receptor; KLF2, Kruppel-like factor 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA; m6A, N6-methyladenosine; miRNA, microRNA; NK cells, natural killer cells; OS, osteosarcoma; pDCs, plasmacytoid DCs; ROC, receiver operating characteristic; SNHG7, small nucleolar RNA host gene 7; SNHG12, small nucleolar RNA host gene 12; ssGSEA, single-sample GSEA; TCGA, The Cancer Genome Atlas; Tcms, central memory T Cells; Tems, effector memory T Cells; TFHs, follicular helper T Cells; Tgds, gamma delta T cells; Th1, T helper 1 cells; Th2, T helper 2 cells; Th17, T helper 17 cells; Tregs, regulatory T cells.

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Introduction

Osteosarcoma (OS) mainly occurs in the metaphysis of long bones and is one of the most malignant bone tumors in children and adolescents.[1] OS shows a worldwide incidence of 3.4 per million people per year.[2] Comprehensive therapies including surgical resection, neoadjuvant chemotherapy, and radiotherapy are currently used in the treatment of patients with OS.[3] The 5-year survival rate of patients with OS has increased from 20% to 68%.[4,5] However, the overall survival rate has remained markedly unchanged owing to its potent invasiveness and high rate of local recurrence and distant metastasis.[6] Furthermore, patients with OS and distant metastasis at the first clinical visit or chemoresistance fail to achieve a favorable 5-year survival rate.[7] Therefore, new biomarkers and novel therapeutic targets based on the molecular mechanisms of OS need to be developed for early diagnosis and treatment.

Long non-coding RNAs (lncRNAs) are defined as transcripts larger than 200 nucleotides with little or no protein-coding ability.[8] Accumulating evidence shows that lncRNAs function as pivotal regulators of gene expression at the level of chromatin modification, transcription, and posttranscriptional processing.[9,10] LncRNA alterations are associated with changes in biological processes and in the development of diseases.[11,12] Aberrant expression of lncRNAs contributes to carcinogenesis and progression of OS tumors.[12] Multiple studies have shown that lncRNAs are differentially expressed in OS tissues and are implicated in its initiation, invasion, and migration.[13–15] Moreover, abnormally expressed lncRNAs can serve as valuable biomarkers in predicting the prognosis of patients with OS.[16,17] Deeper insights into the functions of lncRNAs hold great promise for the identification of novel therapeutic targets and development of effective drugs.

N6-methyladenosine (m6A) modification occurs at the N6 position of adenosine and has been identified as the most prevalent and conserved posttranscriptional modification of messenger RNAs (mRNAs).[18] m6A modification is involved in regulating gene expression via modulating the processing, translation, splicing, and stability of mRNAs.[19–22] It is a dynamic and reversible process that is facilitated by writers (eg METTL3/14 and WTAP), reverted by erasers (eg ALKBH5 and FTO), and recognized by reader proteins (eg YTHDF1/2/3 and IGF2BP1).[23] Alteration of m6A regulators is implicated in the initiation, migration, and progression of various cancers.[24] Notably, recent studies have revealed that m6A modification is strongly associated with tumor metastasis and prognosis of patients with OS.[25–27] For instance, it was found that WTAP is highly expressed in OS tissues and is involved in the proliferation and metastasis of OS by inhibiting the expression of homeobox-containing 1 (HMBOX1) in an m6A-dependent manner.[25] Interestingly, a considerable amount of research has been carried out on m6A modification of lncRNAs.[28] The m6A-related lncRNAs may play critical roles in the pathogenesis of OS. However, few studies have focused on the prognostic value and potential mechanisms of m6A-related lncRNAs in OS.

In this study, we collected transcriptome data and clinicopathological features of patients with OS from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases and performed bioinformatics and statistical analyses to identify m6A-related lncRNAs and their prognostic value in OS. Furthermore, to enrich the regulatory network of prognostic m6A-related lncRNAs, a network of competing endogenous RNAs (ceRNAs) associated with the occurrence and progression of OS was created to show the microRNA (miRNAs) targets of m6A-related lncRNAs and the corresponding mRNA targets of these miRNAs. The biological functions of mRNAs in the ceRNA network were analyzed to better understand the underlying mechanisms.

Materials and Methods

Data Acquisition

We downloaded the level 3 RNA-seq expression data comprising lncRNAs and 21 m6 A regulators, namely METTL3, METTL14, LRPPRC, WTAP, KIAA1429, ZC3H13, CBLL1, RBM15, RBM15B, FTO, ALKBH5, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, IGF2BP1, FMR1, ELAVL1, HNRNPC, and HNRNPA2B1, of 88 patients with OS from TCGA database (https://portal.gdc.cancer.gov).[29] Subsequently, the latest clinical data of patients with OS were downloaded from the TARGET database (https://ocg.cancer.gov/programs/target). Finally, both RNA-seq expression data and valid clinical information of 85 patients were used as a training set for analysis in this study. The clinical information of patients with OS in TCGA is shown in Supplementary Table S1. For the validation set, the RNA-sequencing data and corresponding clinicopathological features of 37 patients with OS were downloaded from the GSE39055 database.[30]

Construction and Evaluation of Risk Score

Common lncRNAs were first extracted from TCGA and GSE39055 datasets. Pearson correlation analysis was then

performed to find m6A-related lncRNAs with cor > 0.3 and $P <$ .05, and univariate Cox regression analysis was performed to filter genes significantly related to survival. Consequently, five m6A-related lncRNAs were identified and an m6A-related lncRNA prognostic signature was established using the multivariate Cox regression analysis. Finally, the risk score was calculated using the following formula:

$$Exp\text{Gene1} \times Coef1 + Exp\text{Gene2} \times Coef2 + Exp\text{Gene3} \times Coef3 \ldots$$

where *Coef* is the coefficient and *Exp* is the normalized expression value of each signature gene. The risk score system of 2 m6A-related lncRNAs was built in the TCGA-TARGET-OS training set and evaluated in the GSE39055 validation set. The patients were divided into high-risk and low-risk groups based on the median risk score. Univariate and multivariate Cox regression analyses were then performed to assess whether the risk score was an independent prognostic factor for the survival of patients with OS.

The Relationship Between Risk Score and Immune Cell Infiltration in OS

Single-sample gene set enrichment analysis (ssGSEA) was performed to analyze the enrichment score of 24 immune cell types in each sample: dendritic cells (DCs), activated DCs (aDCs), B cells, CD8 T cells, cytotoxic T cells, eosinophils, immature DCs (iDCs), macrophages, mast cells, neutrophils, natural killer (NK) cells, NK CD56 bright cells, NK CD56 dim cells, plasmacytoid DCs (pDCs), T cells, T helper cells, central memory T (Tcms) cells, effector memory T (Tems) cells, follicular helper T (TFH) cells, gamma delta T (Tgds) cells, T helper 1 (Th1) cells, T helper 2 (Th2) cells, T helper 17 (Th17) cells, and regulatory T (TRegs) cells. The relationship between risk score and immune cell infiltration was calculated by Pearson correlation.

Establishment of the Nomogram

A nomogram was built using multivariate Cox regression analysis, and calibration curves were created to show the concordance between the nomogram-predicted probability and observed overall survival of patients with OS. Decision curves were plotted to evaluate the clinical utility of the nomogram by estimating the net benefits at a range of threshold probabilities.

Construction of the ceRNA Network

First, the StarBase database was used to predict the target miRNAs of small nucleolar RNA host gene 7 (*SNHG7*) and small nucleolar RNA host gene 12 (*SNHG12*). Then, the corresponding target mRNAs of these miRNAs were selected from StarBase, and the lncRNA-miRNA and miRNA-mRNA regulatory relationships were integrated to construct the ceRNA network using Cytoscape.

Functional Analysis

Gene set enrichment analysis (GSEA) was performed to investigate the most common pathways in the high-risk or low-risk groups, and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the target mRNAs in the ceRNA network were performed using the clusterProfiler R package.

Statistical Analysis

The difference in overall survival between the high-risk and low-risk groups was assessed using the Kaplan–Meier method and log-rank test, and the predictive accuracy of the risk model was determined using the receiver operating characteristic (ROC) curves and area under the curve (AUC) values. Student's *t*-test was performed to compare the risk score values between subgroups according to the following clinicopathological features: age (<14 or >14 years), gender (male or female), and metastatic status (metastatic or non-metastatic). All data were analyzed using R software and results with a *P* value <.05 were considered as statistically significant.

Results

Identification of m6A-Related lncRNAs in Patients with OS

First, lncRNA data of patients with OS were downloaded from TCGA-TARGET-OS and GSE39055, and a total of 122 common lncRNAs were identified (Supplementary Table S2). Subsequently, data on the expression of 21 m6 A regulators were obtained from TCGA-TARGET-OS. Pearson correlation analysis was performed to identify m6A-related lncRNAs, the expression levels of which were correlated with one or more of the 21 m6 A regulators. Consequently, 59 m6 A-related lncRNAs were identified as significant in the TCGA dataset (Supplementary Table S3). LncRNAs with the highest correlation with each m6A regulator are shown in Figure 1. These significant m6A-related lncRNAs were further examined in the study.

Construction of Risk Score Signature Using m6A-Related lncRNAs with Prognostic Value

We further explored the role of these m6A-related lncRNAs in predicting the prognosis of patients with OS by performing univariate Cox regression analysis in TCGA training set. We found that five m6A-related lncRNAs were related to patient survival ($P <$ .05) and all were risk factors (HR > 1) for OS (Figure 2A). Furthermore, multivariate Cox regression analysis was performed to increase the predictive robustness of the five m6A-related lncRNAs in TCGA training set. Two m6A-related lncRNAs were screened to construct the risk model, in which the risk score was calculated as 0.062 × expression value of *SNHG7* + 0.115 × expression value of *SNHG12* (Figure 2B). Based on the median value of the risk score, we divided the patients from TCGA training set into high-risk and low-risk groups. The expression values of *SNHG7* and *SNHG12* are represented in the heat map (Figure 2C). The

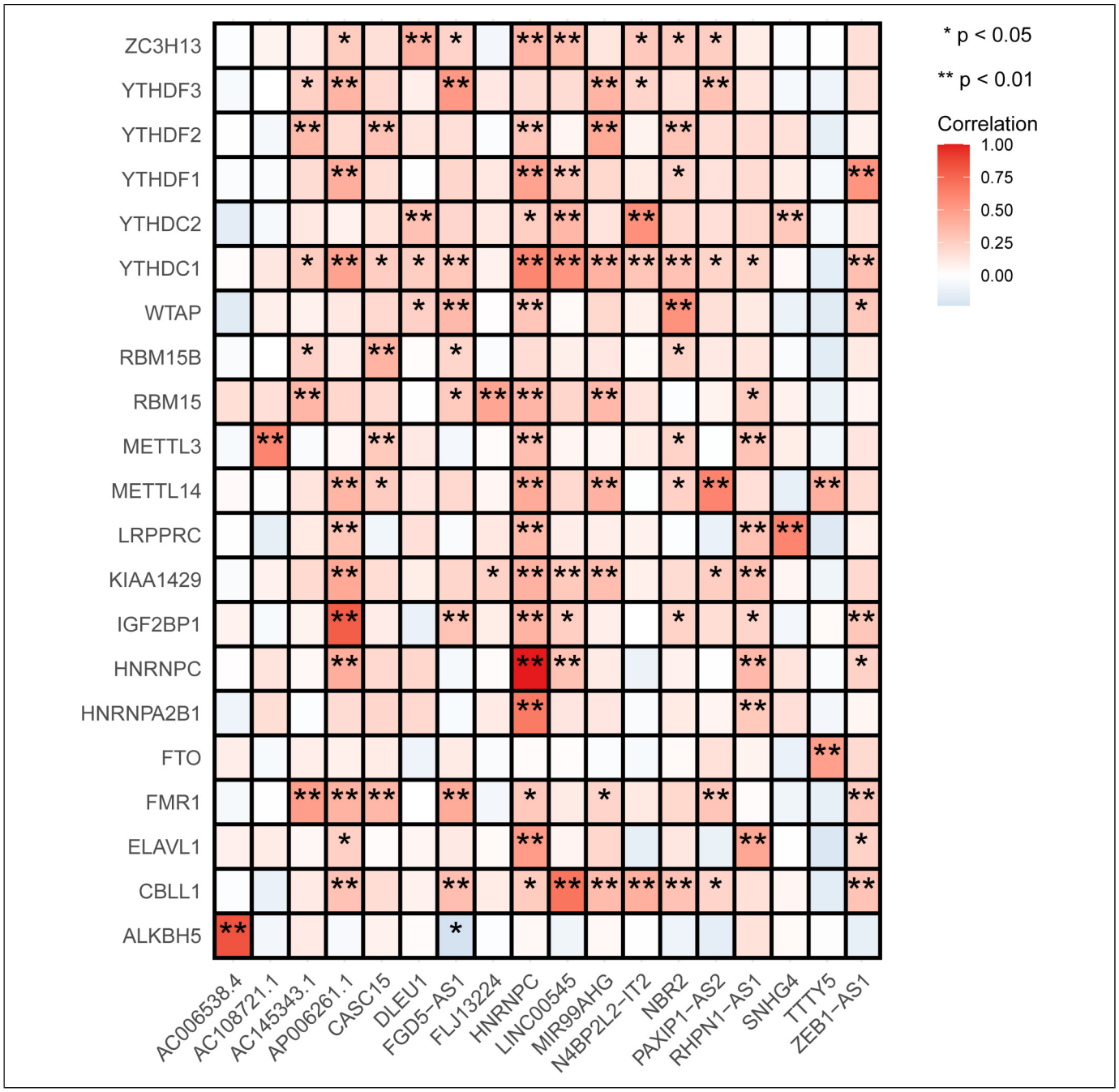

Figure 1. Heat map representing the correlation between lncRNAs and m6A-related genes.
Abbreviation: lncRNAs: long non-coding RNAs; m6A: N6-methyladenosine.

distributions of risk score and survival status of patients with OS in TCGA training set are represented in Figure 2D. The results of the Kaplan–Meier analysis showed that the high-risk group had a worse clinical outcome (Figure 2E), and the ROC curve indicated that the two m6A-related lncRNA signatures had high accuracy in predicting the overall survival in TCGA training set with an AUC value of > 0.65 (Figure 2F). Additionally, we investigated the relationship between the risk score value and clinicopathological features of patients with OS in TCGA training set. Although female patients aged >14 years and with metastatic disease had high-risk score values, the differences were not statistically significant (Figure 2G).

Validation of m6A-Related lncRNA Signature in the GEO Dataset

We further validated the risk model in the GSE39055 dataset. The patients were assigned into low- and high-risk groups

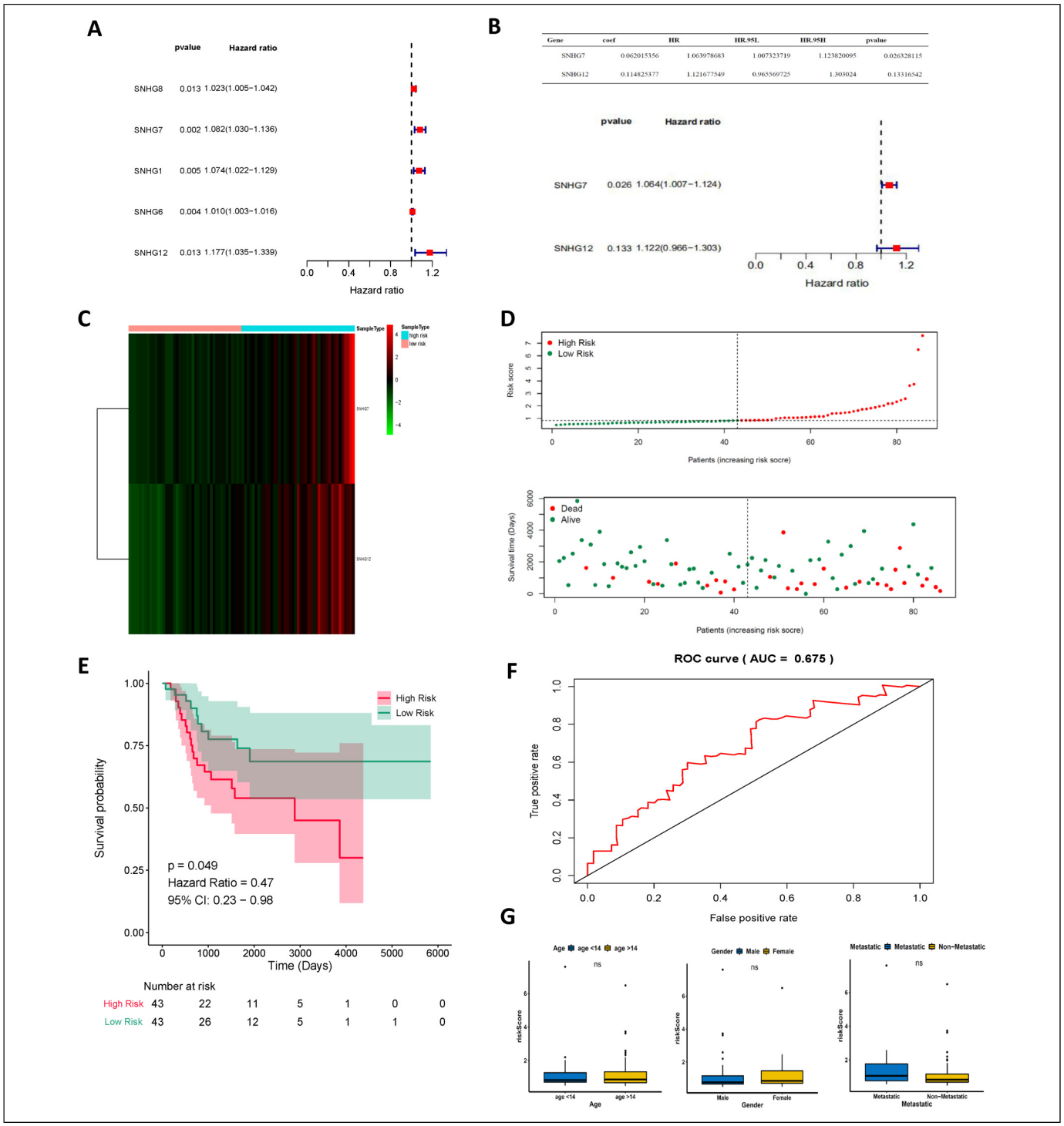

Figure 2. Screening for prognostic biomarkers and risk model construction. (A) Forest plot of univariate Cox regression analysis to screen for prognosis-related lncRNAs. (B) Forest plot of multivariate Cox regression analysis to screen for prognosis-related lncRNAs. (C) Heat map showing *SNHG7* and *SNHG12* expression levels in the training set. (D) Risk curve in the training set. (E) Kaplan–Meier curve in the training set. (F) ROC curve in TCGA training set. (G) Correlation between risk scores and clinical outcomes.
Abbreviations: lncRNAS: long non-coding RNAs; ROC: receiver operating curve; SNHG7: small nucleolar RNA host gene7; SNHG12: small nucleolar RNA host gene 12; TCGA: The Cancer Genome Atlas.

according to the median value of the risk score, which was calculated using the same formula as mentioned previously. A heat map was generated to show the expression values of *SNHG7* and *SNHG12* in the low- and high-risk groups (Figure 3A). Similar to the results in TCGA training set, we found that the mortality status was mainly distributed in patients with higher

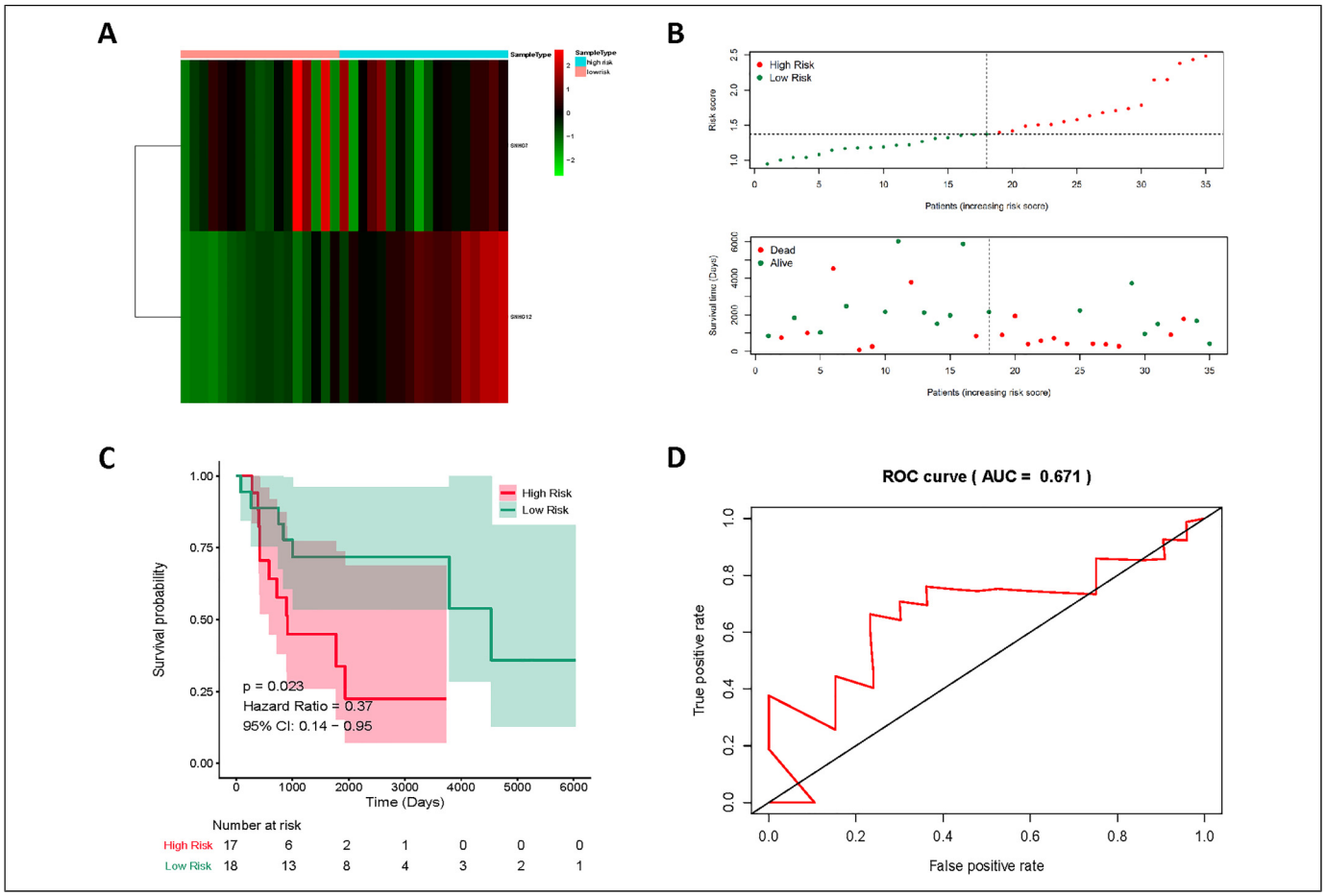

Figure 3. Validation of the risk model in the GEO dataset. (A) Heat map showing *SNHG7* and *SNHG12* expression levels in the validation set. (B) Risk curve in the validation set. (C) Kaplan–Meier curve in the validation set. (D) ROC curve in the GEO validation set.
Abbreviations: GEO: Gene Expression Omnibus; ROC: receiver operating curve; SNHG7: small nucleolar RNA host gene 7; SNHG12: small nucleolar RNA host gene 12.

risk score values (Figure 3B), and patients in the high-risk group had shorter survival time (Figure 3C). The ROC curve also demonstrated that the risk model had a strong efficiency for predicting the prognosis of patients with OS in the GSE39055 dataset (AUC = 0.671, Figure 3D).

Identification of Pathways Related to the Risk Model

To identify the pathways related to the risk model, we performed GSEA to investigate the potential biological processes and pathways in high- and low-risk groups. We found that the B-cell receptor signaling and NK cell pathways were enriched in patients in the low-risk group (Figure 4A), while RNA polymerase and cell cycle pathways were enriched in patients in the high-risk group (Figure 4B), suggesting that in OS, the m6A-related lncRNA risk signature may be related to immune system-related and cell proliferation. Thus, we calculated the infiltration levels of 24 immune cell types in each sample (Supplementary Table S4). The results of Pearson correlation showed that the risk score was significantly negatively correlated with iDCs (P value < .05, r = −0.43), macrophages (P value < .05, r = −0.4), neutrophils (P value < .05, r = −0.47), and NK cells (P value < .05, r = −0.41) (Figure 5).

Generation of Nomogram for Patients with OS

We then performed univariate and multivariate Cox regression analyses to explore whether the risk score and metastasis were independently associated with prognosis (Figure 6A). The univariate Cox regression analysis revealed that the risk score and metastasis were strongly associated with prognosis, and the multivariate Cox regression analysis further confirmed that the risk score was an independent prognostic factor (P < .001, Figure 6B). A nomogram for predicting the 1/3/5-year survival of patients with OS was created based on independent prognostic factors (risk score and metastasis) (Figure 6C). We found a good concordance between the observed and predicted 1- and 3-year overall survival rates, as shown in the calibration plot (Figure 6D). In addition, we plotted decision curves to assess the clinical utility of the nomogram, and found that it yielded more net benefit for predicting the 3- and 5-year survival

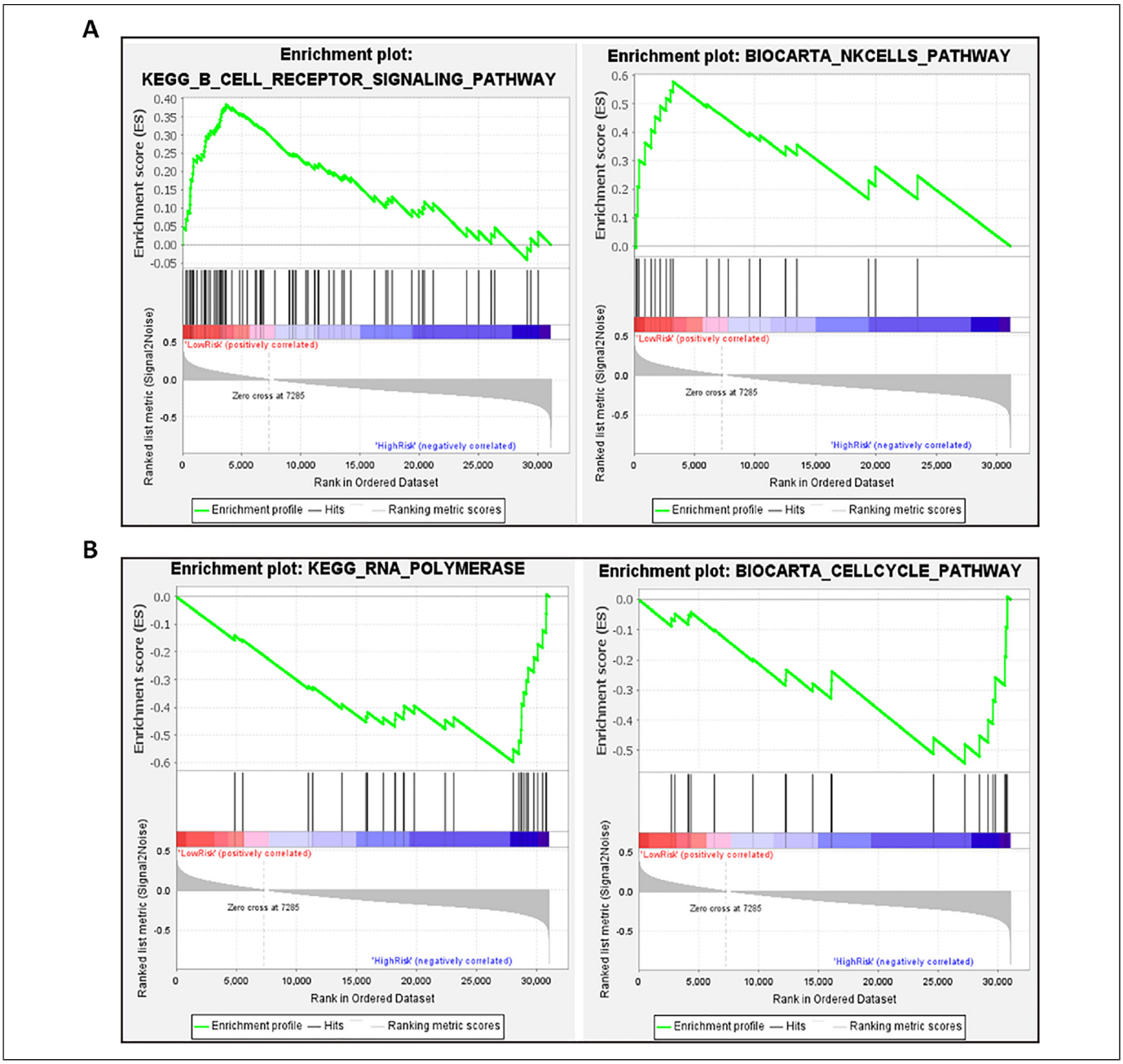

Figure 4. Identification of pathways related to the risk model. (A) GSEA analysis in low-risk group. (B) GSEA analysis in high-risk group.
Abbreviation: GSEA: gene set enrichment analysis.

rates (with the threshold probability > 0.2 and > 0.25, respectively) than the treat-all and treat-none strategies (Figure 6E, F).

Construction and Analysis of the ceRNA Network

To further elucidate how the m6A-related lncRNAs (*SNHG7* and *SNHG12*) regulate mRNA expression via sponging miRNAs in OS, we searched for miRNAs interacting with *SNHG7* and *SNHG12,* and their target mRNAs in StarBase and constructed the ceRNA network (Figure 7A). Moreover, we investigated the biological functions of these target mRNAs and found that these genes were enriched in ficolin-1-rich granule, ficolin-1-rich granule lumen (GO cellular component), DNA polymerase binding, nuclear receptor binding (GO molecular function), estrogen signaling pathway, and hepatocellular carcinoma (KEGG pathway), as shown in Figures 7B and 7C.

Discussion

In recent years, m6A modification has been reported as a common internal modification of eukaryotic mRNAs and can

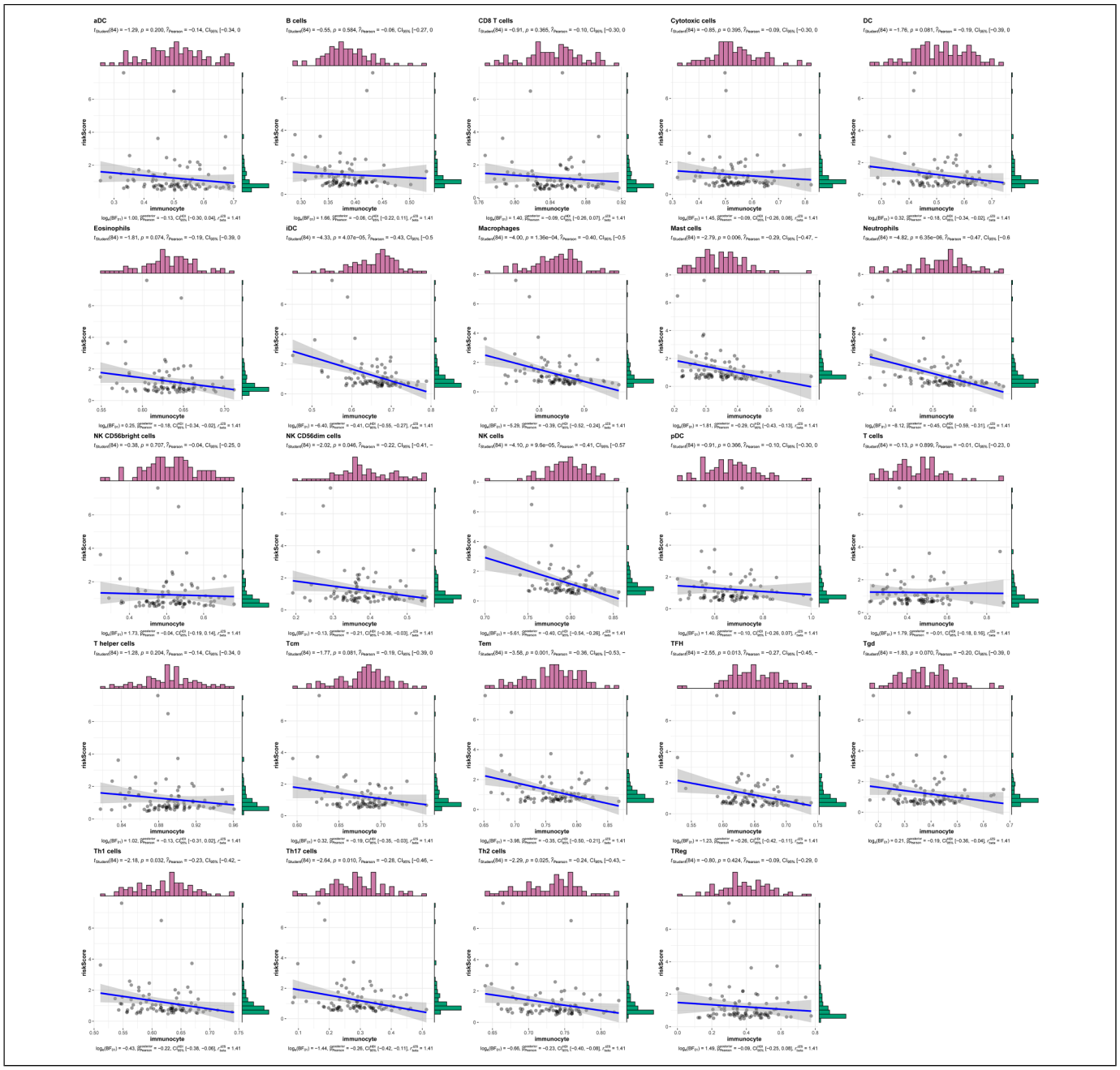

Figure 5. Relationship between risk score and immune cell infiltration in OS. ssGSEA was performed to analyze the enrichment score of 24 immune cell types in each sample. Pearson correlation between risk score and each immune cell type was calculated and visualized in a scatter plot.

Abbreviations: OS: osteosarcoma; ssGSEA: single-sample gene set enrichment analysis.

regulate cell proliferation, invasion and apoptosis, thereby modulating the progression of OS.[31] m6A-related methyltransferases, including METTL3, METTL14, and WTAP, have shown critical roles in OS cell growth and metastasis by modifying the expression of corresponding downstream genes.[25,32–35] YTHDF2, a key reader protein, was found to serve as the main factor involved in abnormal m6A modification of tripartite motif-containing 7, thus, promoting tumorigenesis and chemoresistance in OS.[35] Moreover, current evidence indicates that m6A modification of lncRNAs is involved in the tumorigenesis of OS.[36] However, it is still largely unknown how m6A modification acts in a lncRNA-dependent manner during OS development. Hence, a comprehensive bioinformatics analysis was performed to identify m6A-related lncRNAs and determine their prognostic value and potential underlying mechanisms in OS.

Several studies have suggested that dysregulated m6A-related lncRNAs are strongly associated with

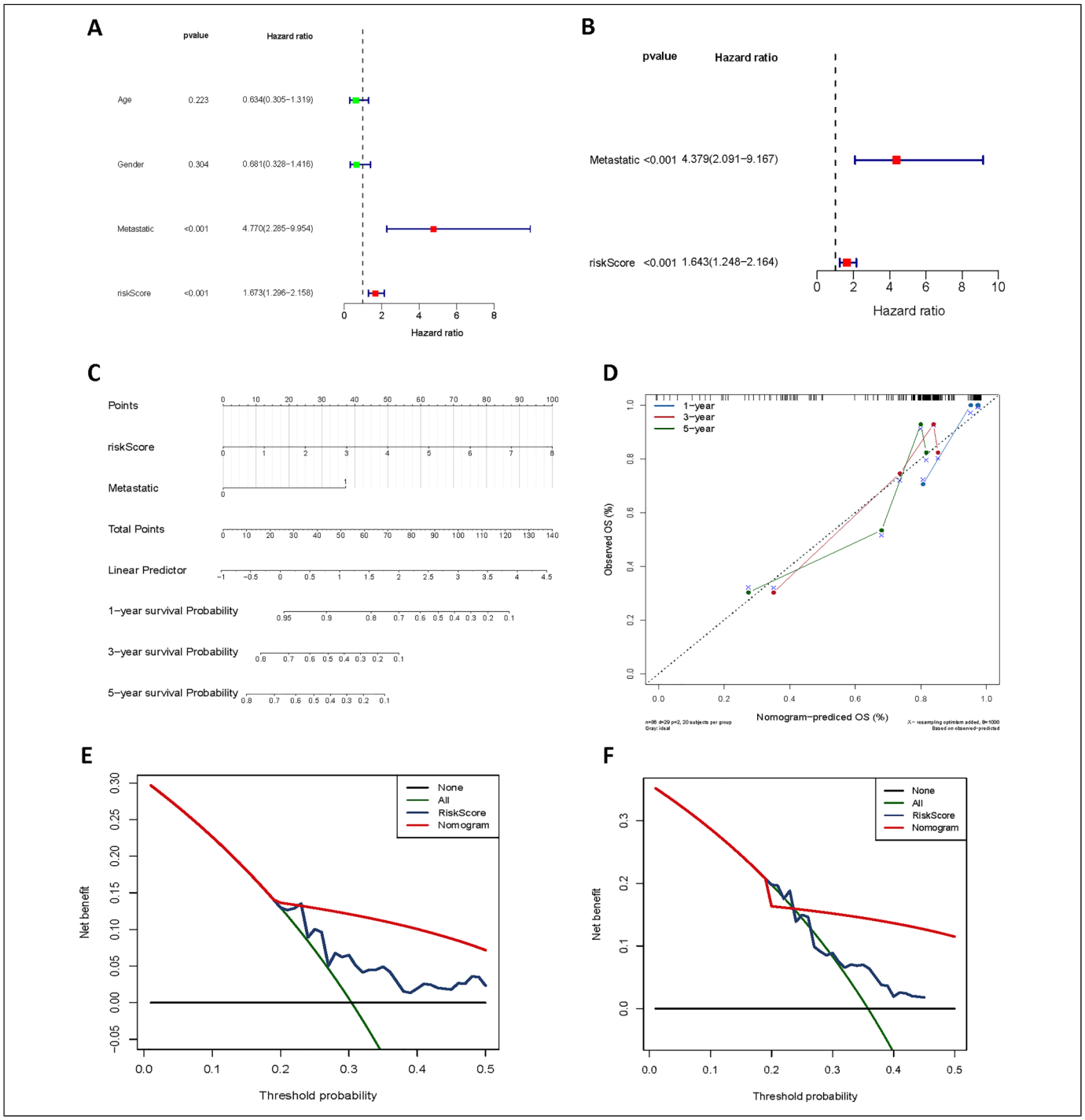

Figure 6. Construction of nomogram for patients with OS. (A) Forest plot of univariate Cox regression analysis to screen for factors associated with prognosis. (B) Forest plot of multivariate Cox regression analysis to screen for factors associated with prognosis (C) Nomogram constructed with risk scores and metastasis. (D) Calibration plot of nomogram. (E) Decision curves for predicting 3-year survival. (F) Decision curves for predicting 5-year survival.
Abbreviation: OS: osteosarcoma.

tumorigenesis and clinical outcomes of patients with tumors.[37] In the current study, data of 122 patients with OS from TCGA and GSE39055 datasets were analyzed to investigate the significance of m6A-related lncRNAs, and the pooled results showed that the expression of 59 lncRNAs was significantly correlated with the expression of one or more m6A-related regulators, suggesting that these lncRNAs may be modified in an m6A-dependent manner. Furthermore, we explored the role of these m6A-related lncRNAs in the prognosis of OS by performing univariate Cox regression analysis; five m6A-related

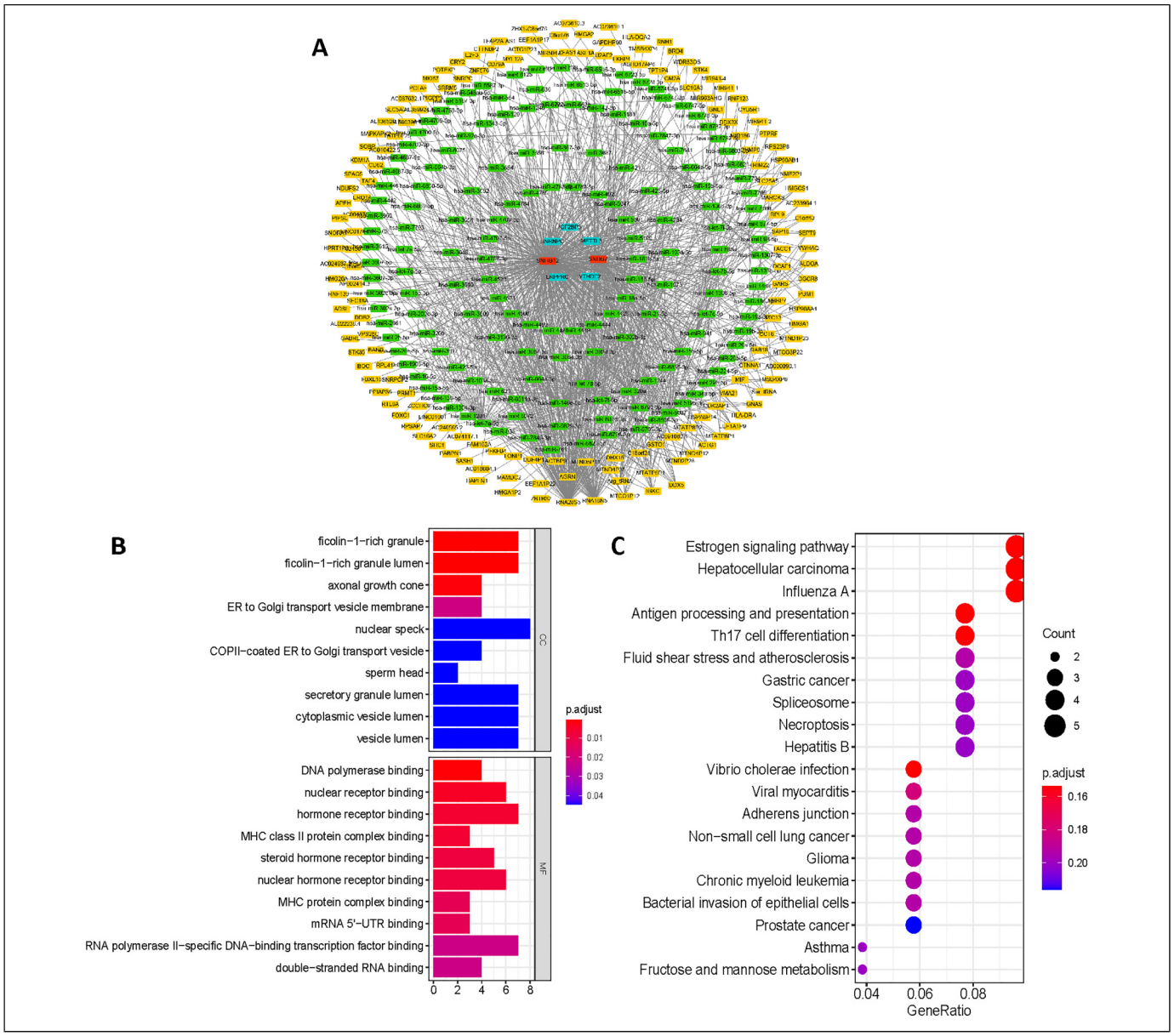

Figure 7. Establishment and analysis of the ceRNA network. (A) Construction of ceRNA network based on *SNHG7* and *SNHG12*. (B) GO analysis of mRNAs in the ceRNA network. (C) KEGG analysis of mRNAs in the ceRNA network.
Abbreviations: ceRNA: competing endogenous RNA; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; mRNAs: messenger RNAs; SNHG7: small nucleolar RNA host gene 7; SNHG12: small nucleolar RNA host gene 12.

lncRNAs, namely *SNHG1*, *SNHG6*, *SNHG7*, *SNHG8*, and *SNHG12*, were related to the overall survival of patients with OS.

Similarly, previous studies have confirmed that these lncRNAs are involved in the oncogenesis and metastasis of OS. *SNHG1* was upregulated in OS tissues and promoted cell proliferation, invasion, and migration by serving as a ceRNA.[38–40] Highly expressed *SNHG6* was positively associated with poor overall survival of patients with OS and could enhance OS cell proliferation by regulating the miR-26a-5p/ULK1 axis and expression of p21 and Kruppel-like factor 2 (KLF2).[41,42] It has been reported that *SNHG7* was aberrantly expressed in OS tissues and elevated levels of *SNHG7* could facilitate tumor growth and epithelial-mesenchymal transition (EMT) by regulating p53 expression and miR-34a levels.[43,44] Recent studies have confirmed that *SNHG8* could stimulate the growth and migration of OS cells by acting as a ceRNA to sponge miR-876-5p and miR-542-3p, thereby suggesting that *SNHG8* may be a novel therapeutic target for the treatment of OS.[45,46] *SNHG12* was also found to be overexpressed in OS tissues and silencing *SNHG12* may attenuate Notch2- and insulin-like growth factor 1 receptor (IGF1R)-induced tumorigenesis and metastasis via regulating miR-195-5p.[47,48] Multiple studies have indicated that upregulation of these five lncRNAs are believed to promote the proliferation, migration,

and inhibit apoptosis of tumr cells, thereby enhancing chemoresistance.[49–53] Their dysregulation is positively correlated with poor clinical outcomes of patients with OS,[41,54] which is consistent with our findings. Furthermore, m6A-related lncRNAs may influence the expression of m6A regulators via cis-or trans-acting regulation, or by functioning as ceRNAs, thereby forming an integrated regulatory network.[55] Hence, additional studies are required to investigate the function of m6A-related lncRNAs in the initiation and development of OS, to identify novel prognostic biomarkers and therapeutic targets.

Furthermore, multivariate Cox regression analysis was performed to identify m6A-related lncRNAs that could predict the prognosis of OS more accurately and reliably. *SNHG7* and *SNHG12* were found to have such prognostic value and were used to create a risk model which was confirmed to be effective in predicting the clinical outcomes of patients with OS. Notably, patients enrolled from TCGA were divided into high-risk and low-risk groups based on the median value of the risk score. The expression levels of both *SNHG7* and *SNHG12* were elevated in the high-risk group, suggesting that these two m6A-related lncRNAs could be risk factors for OS. Moreover, the relationship between the risk score and the clinicopathological features of patients with OS was explored. The results showed that the risk score values were higher in female patients aged >14 years with metastatic disease when compared to each respective control group. However, no statistical significance was observed. These nonsignificant findings may be attributed to small sample sizes. Nevertheless, the univariate and multivariate regression analyses confirmed that the risk score based on *SNHG7* and *SNHG12* was an independent prognostic factor. Furthermore, a nomogram for predicting the 1/3/5-year survival of patients with OS was created based on independent prognostic factors. Both the calibration plot and decision curves showed that this nomogram indicated a good prognostic value.

To date, few studies have focused on the molecular mechanisms of these two lncRNAs in the progression of OS,[43,44,47,48,54,56] and further research regarding their role in the pathophysiological process of OS is needed. Nevertheless, there are no previous studies reporting the important roles that these lncRNAs might play in OS development via cross-talk with their downstream targets in an m6A-dependent manner. Hence, using bioinformatics analysis, a ceRNA network was created to search for potential miRNA and mRNA targets. Multiple OS-related m6A regulator-lncRNA-miRNA-mRNA networks were identified. Moreover, these genes were enriched in several cancer-related pathways including estrogen signaling pathway,[57] hepatocellular carcinoma, gastric cancer, and necroptosis.[58] Furthermore, the risk model based on *SNHG7* and *SNHG12* was enriched in immune system-related and cell proliferation pathways. The ssGSEA results further demonstrated the close relationship between risk score and immune cell infiltration. Considering these findings, these two m6A-related lncRNAs and their downstream target molecules may be positively associated with the tumorigenesis and prognosis of OS.

Nevertheless, there are some limitations in this study. First, transcriptome data and clinicopathological features were obtained from TCGA and GEO databases. However, there were only 85 patients in the training set and 37 in the validation set. The prognostic value of these m6A-related lncRNAs should be validated in more independent cohorts. Second, the underlying mechanisms of these m6A-related lncRNAs were evaluated only by bioinformatics-based prediction. These findings should be further corroborated through *in vitro* and *in vivo* experiments. Third, although higher risk score values were observed in female patients aged >14 years with metastatic disease, the differences were not significant. Studies with larger sample sizes are needed to further validate these results.

Conclusion

In summary, to our knowledge, this study is the first to comprehensively screen for m6A-related lncRNAs that influence the prognosis of patients with OS. Our results showed that *SNHG7* and *SNHG12* may be valuable prognostic biomarkers for OS, and a risk model that could accurately predict the survival of patients with OS was established based on these two m6A-related lncRNAs. A ceRNA network was created to gain further understanding of these two lncRNAs targeting downstream molecules in the pathogenesis of OS. These findings may provide novel insights into the molecular mechanisms of OS by giving a new perspective on m6A modification.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

Our study did not require an ethical board approval because no human subjects or animals were enrolled in current study.

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Supplemental Material

Supplemental material for this article is available online.

References

1. Jafari F, Javdansirat S, Sanaie S, et al. Osteosarcoma: a comprehensive review of management and treatment strategies. *Ann Diagn Pathol*. 2020;49:151654.
2. Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int J Cancer*. 2009;125(1):229–234.
3. Kager L, Tamamyan G, Bielack S. Novel insights and therapeutic interventions for pediatric osteosarcoma. *Future Oncol*. 2017;13(4):357–368.
4. Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res*. 2009;152:3–13.
5. Misaghi A, Goldin A, Awad M, et al. Osteosarcoma: a comprehensive review. *SICOT J*. 2018;4:12.
6. Anderson ME. Update on survival in osteosarcoma. *Orthop Clin North Am*. 2016;47(1):283–292.
7. Yang Y, Han L, He Z, et al. Advances in limb salvage treatment of osteosarcoma. *J Bone Oncol*. 2018;10:36–40.
8. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009;136(4):629–641.
9. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009;10(3):155–159.
10. Zampetaki A, Albrecht A, Steinhofel K. Long non-coding RNA structure and function: is there a link? *Front Physiol*. 2018;9:1201.
11. Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet*. 2016;17(1):47–62.
12. Goodall GJ, Wickramasinghe VO. RNA In cancer. *Nat Rev Cancer*. 2021;21(1):22–36.
13. Li JP, Liu LH, Li J, et al. Microarray expression profile of long noncoding RNAs in human osteosarcoma. *Biochem Biophys Res Commun*. 2013;433(2):200–206.
14. Xie L, Yao Z, Zhang Y, et al. Deep RNA sequencing reveals the dynamic regulation of miRNA, lncRNAs, and mRNAs in osteosarcoma tumorigenesis and pulmonary metastasis. *Cell Death Dis*. 2018;9(7):772.
15. Ren K, Ni Y, Li X, et al. Expression profiling of long noncoding RNAs associated with vasculogenic mimicry in osteosarcoma. *J Cell Biochem*. 2019;120(8):12473–12488.
16. Liu M, Yang P, Mao G, et al. Long non-coding RNA MALAT1 as a valuable biomarker for prognosis in osteosarcoma: a systematic review and meta-analysis. *Int J Surg*. 2019;72:206–213.
17. Xu S, Gong Y, Yin Y, et al. The multiple function of long noncoding RNAs in osteosarcoma progression, drug resistance and prognosis. *Biomed Pharmacother*. 2020;127:110141.
18. Wu Y, Zhou C, Yuan Q. Role of DNA and RNA N6-adenine methylation in regulating stem cell fate. *Curr Stem Cell Res Ther*. 2018;13(1):31–38.
19. Bartosovic M, Molares HC, Gregorova P, et al. N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3′-end processing. *Nucleic Acids Res*. 2017;45(19):11356–11370.
20. Barbieri I, Tzelepis K, Pandolfini L, et al. Promoter-bound METTL3 maintains myeloid leukaemia by m(6)A-dependent translation control. *Nature*. 2017;552(7683):126–131.
21. Shi H, Wang X, Lu Z, et al. YTHDF3 Facilitates translation and decay of N(6)-methyladenosine-modified RNA. *Cell Res*. 2017;27(3):315–328.
22. Wang X, Zhao BS, Roundtree IA, et al. N(6)-methyladenosine modulates messenger RNA translation efficiency. *Cell*. 2015;161(6):1388–1399.
23. Zhu ZM, Huo FC, Pei DS. Function and evolution of RNA N6-methyladenosine modification. *Int J Biol Sci*. 2020;16(11):1929–1940.
24. He L, Li H, Wu A, et al. Functions of N6-methyladenosine and its role in cancer. *Mol Cancer*. 2019;18(1):176.
25. Chen S, Li Y, Zhi S, et al. WTAP Promotes osteosarcoma tumorigenesis by repressing HMBOX1 expression in an m(6)A-dependent manner. *Cell Death Dis*. 2020;11(8):659.
26. Li J, Rao B, Yang J, et al. Dysregulated m6A-related regulators are associated with tumor metastasis and poor prognosis in osteosarcoma. *Front Oncol*. 2020;10:769.
27. Wang Y, Zeng L, Liang C, et al. Integrated analysis of transcriptome-wide m(6)A methylome of osteosarcoma stem cells enriched by chemotherapy. *Epigenomics*. 2019;11(15):1693–1715.
28. He RZ, Jiang J, Luo DX. The functions of N6-methyladenosine modification in lncRNAs. *Genes Dis*. 2020;7(4):598–605.
29. Wu G, Zhang M. A novel risk score model based on eight genes and a nomogram for predicting overall survival of patients with osteosarcoma. *BMC Cancer*. 2020;20(1):456.
30. Kelly AD, Haibe-Kains B, Janeway KA, et al. MicroRNA paraffin-based studies in osteosarcoma reveal reproducible independent prognostic profiles at 14q32. *Genome Med*. 2013;5(1):2.
31. Chen J, Tian Y, Zhang Q, et al. Novel insights into the role of N6-methyladenosine RNA modification in bone pathophysiology. *Stem Cells Dev*. 2021;30(1):17–28.
32. Ling Z, Chen L, Zhao J. m6A-dependent up-regulation of DRG1 by METTL3 and ELAVL1 promotes growth, migration, and colony formation in osteosarcoma. *Biosci Rep*. 2020;40(4):BSR20200282.
33. Liu Z, Liu N, Huang Z, et al. METTL14 overexpression promotes osteosarcoma cell apoptosis and slows tumor progression via caspase 3 activation. *Cancer Manag Res*. 2020;12:12759–12767.
34. Miao W, Chen J, Jia L, et al. The m6A methyltransferase METTL3 promotes osteosarcoma progression by regulating the m6A level of LEF1. *Biochem Biophys Res Commun*. 2019;516(4):719–725.
35. Zhou C, Zhang Z, Zhu X, et al. N6-Methyladenosine modification of the TRIM7 positively regulates tumorigenesis and chemoresistance in osteosarcoma through ubiquitination of BRMS1. *EBioMedicine*. 2020;59:102955.
36. Chen S, Zhou L, Wang Y. ALKBH5-mediated M(6)A demethylation of lncRNA PVT1 plays an oncogenic role in osteosarcoma. *Cancer Cell Int*. 2020;20:34.
37. Chen Y, Lin Y, Shu Y, et al. Interaction between N(6)-methyladenosine (m[6]A) modification and noncoding RNAs in cancer. *Mol Cancer*. 2020;19(1):94.
38. Deng R, Zhang J, Chen J. lncRNA SNHG1 negatively regulates miRNA-101-3p to enhance the expression of ROCK1 and

promote cell proliferation, migration and invasion in osteosarcoma. *Int J Mol Med*. 2019;43(3):1157–1166.
39. Li Z, Wang X, Liang S. Long non-coding RNA small nucleolar RNA host gene 1 knockdown suppresses the proliferation, migration and invasion of osteosarcoma cells by regulating microRNA-424-5p/FGF2 in vitro. *Exp Ther Med*. 2021;21(4):325.
40. Wang J, Cao L, Wu J, et al. Long non-coding RNA SNHG1 regulates NOB1 expression by sponging miR-326 and promotes tumorigenesis in osteosarcoma. *Int J Oncol*. 2018;52(1):77–88.
41. Ruan J, Zheng L, Hu N, et al. Long noncoding RNA SNHG6 promotes osteosarcoma cell proliferation through regulating p21 and KLF2. *Arch Biochem Biophys*. 2018;646:128–136.
42. Zhu X, Yang G, Xu J, et al. Silencing of SNHG6 induced cell autophagy by targeting miR-26a-5p/ULK1 signaling pathway in human osteosarcoma. *Cancer Cell Int*. 2019;19:82.
43. Deng Y, Zhao F, Zhang Z, et al. Long noncoding RNA SNHG7 promotes the tumor growth and epithelial-to-mesenchymal transition via regulation of miR-34a signals in osteosarcoma. *Cancer Biother Radiopharm*. 2018;33(9):365–372.
44. Zhang GD, Gai PZ, Liao GY, et al. LncRNA SNHG7 participates in osteosarcoma progression by down-regulating p53 via binding to DNMT1. *Eur Rev Med Pharmacol Sci*. 2019;23(9):3602–3610.
45. Hao H, Wang L, Liu Q, et al. LncRNA small nucleolar RNA host gene 8 promotes cell growth and migration of osteosarcoma in vitro and in vivo by functioning as a ceRNA of microRNA-876-5p. *Am J Transl Res*. 2020;12(7):3476–3488.
46. Zhong GB, Jiang CQ, Yu XS, et al. Long noncoding RNA SNHG8 promotes the proliferation of osteosarcoma cells by downregulating miR-542-3p. *J Biol Regul Homeost Agents*. 2020;34(2):517–524.
47. Xu N, Xu J, Zuo Z, et al. Downregulation of lncRNA SNHG12 reversed IGF1R-induced osteosarcoma metastasis and proliferation by targeting miR-195-5p. *Gene*. 2020;726:144145.
48. Zhou S, Yu L, Xiong M, et al. LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p. *Biochem Biophys Res Commun*. 2018;495(2):1822–1832.
49. Ge P, Cao L, Zheng M, et al. LncRNA SNHG1 contributes to the cisplatin resistance and progression of NSCLC via miR-330-5p/DCLK1 axis. *Exp Mol Pathol*. 2021;120:104633.
50. Sun T, Li K, Zhu K, et al. SNHG6 Interacted with miR-325-3p to regulate cisplatin resistance of gastric cancer by targeting GITR. *Onco Targets Ther*. 2020;13:12181–12193.
51. Zhang H, Zhang X Y, Kang X N, et al. LncRNA-SNHG7 enhances chemotherapy resistance and cell viability of breast cancer cells by regulating miR-186. *Cancer Manag Res*. 2020;12:10163–10172.
52. Song Y, Zou L, Li J, et al. LncRNA SNHG8 promotes the development and chemo-resistance of pancreatic adenocarcinoma. *Eur Rev Med Pharmacol Sci*. 2018;22(23):8161–8168.
53. Liu Y, Cheng G, Huang Z, et al. Long noncoding RNA SNHG12 promotes tumour progression and sunitinib resistance by upregulating CDCA3 in renal cell carcinoma. *Cell Death Dis*. 2020;11(7):515.
54. Zhou B, Li L, Li Y, et al. Long noncoding RNA SNHG12 mediates doxorubicin resistance of osteosarcoma via miR-320a/MCL1 axis. *Biomed Pharmacother*. 2018;106:850–857.
55. Dai F, Wu Y, Lu Y, et al. Crosstalk between RNA m(6)A modification and Non-coding RNA contributes to cancer growth and progression. *Mol Ther Nucleic Acids*. 2020;22:62–71.
56. Ruan W, Wang P, Feng S, et al. Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes cell proliferation and migration by upregulating angiomotin gene expression in human osteosarcoma cells. *Tumour Biol*. 2016;37(3):4065–4073.
57. Yang Z, Yu W, Liu B, et al. Estrogen receptor beta induces autophagy of osteosarcoma through the mTOR signaling pathway. *J Orthop Surg Res*. 2020;15(1):50.
58. Eskandari A, Flamme M, Xiao Z, et al. The bulk osteosarcoma and osteosarcoma stem cell activity of a necroptosis-inducing nickel(II)-phenanthroline complex. *Chembiochem*. 2020;21(19):2854–2860.