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ORIGINAL RESEARCH

The Long Non-Coding RNA SNHG16 Negatively Regulates Let-7 and Predicts Recurrence in Hepatocellular Carcinoma

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Purpose: Let-7, a family of microRNA (miRNA) that regulates the timing of cell division, is associated with tumorigenesis and tumor progression. The factors that regulate let-7 and affect the prognosis of hepatocellular carcinoma (HCC) warrant further investigation.
Patients and Methods: In this study, we first utilized the data from The Cancer Genome Atlas (TCGA) and employed bioinformatics methods to analyze the expression and function of let-7 in HCC. Subsequently, we retrieved the long non-coding RNAs (lncRNAs) that regulate let-7 from the StarBase database. Finally, through bioinformatics analysis of the TCGA-LIHC data and validation with clinical samples, we explored the relationship between let-7 and its related lncRNAs and clinical indicators such as HCC recurrence and survival.
Results: Let-7c was significantly downregulated in HCC, regulating tumor progression via pathways like PI3K-Akt and tumor-related miRNAs. LncRNAs SNHG16 negatively regulated let-7c expression in HCC ($r = -0.160$, $p = 0.002$). Both bioinformatics analysis and clinical sample validation revealed that high SNHG16 expression in HCC tissues was associated with shorter disease-free survival (HR = 1.711, 95% CI: 1.144–2.559, $p = 0.009$), higher recurrence rates ($p < 0.001$), and shorter overall survival (HR = 1.837, 95% CI: 1.283–2.629, $p = 0.001$).
Conclusion: SNHG16 negatively regulates let-7c and serves as a prognostic biomarker for HCC recurrence and survival.
Keywords: let-7, long noncoding RNAs, hepatocellular carcinoma, prognosis

Introduction

Hepatocellular carcinoma (HCC) is a common and highly fatal malignant liver tumor, with a high recurrence rate (up to 70% within 5 years after treatment) that severely affects patient prognosis. Factors associated with HCC recurrence include patient age, liver function, tumor size, tumor differentiation, and treatment plans, among others.[1] With continuous in-depth research into the molecular characteristics of liver cancer, it has been found that certain microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) influence the progression of HCC. Monitoring their expression changes can help predict the risk of recurrence and provide a basis for the diagnosis and treatment of HCC.[2,3]

The let-7 miRNA was first discovered in Caenorhabditis elegans, where it induces cell differentiation and acts as key regulatory factors in the development of the nematode.[4] In humans, there are 13 members of the let-7 family (let-7a-1, let-7a-2, let-7a-3, let-7b, let-7c, let-7d, let-7e, let-7f-1, let-7f-2, let-7g, let-7i, miR-98 and miR-202). Except for miR-202, the seed sequences of the other 12 let-7 family members are highly conserved. When targeting the 3′ untranslated region (3′UTR) of mRNA, they exhibit similar binding characteristics and functional potential. The expression of let-7 miRNAs

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increases gradually with embryonic development and remains at a high level in mature tissues and organs. Abnormal expression of let-7 miRNAs is associated with tumorigenesis, progression, and bad prognosis.[5–7] LncRNAs are a class of noncoding RNAs longer than 200 bp that function as miRNA sponges. They work by specifically adsorbing miRNAs and competitively interfering with the binding of miRNAs to their target mRNAs.[8] The regulatory role of lncRNAs on let-7 has been a research hot spot in recent years regarding the mechanisms by which let-7 influences diseases. However, the complexity of let-7 family functions and their diverse roles in the same disease warrant further exploration. For example, Allela et al found that let-7 has a dual role in tumor immune evasion, highlighting its potential as a therapeutic target and biomarker in cancer therapy.[9,10] This study utilized data from The Cancer Genome Atlas (TCGA) and clinical samples of HCC to analyze the role of 12 let-7 miRNAs with similar seed sequences, and the correlation between lncRNAs that regulate let-7 and clinical indicators. Unlike previous studies that focused on the role of a specific lncRNA and let-7 in cancer, this study screened 12 members of the let-7 family and their related lncRNAs. We then selected targets that have predictive value for the prognosis of HCC and conducted clinical validation. The aim was to identify effective biomarkers for evaluating HCC recurrence and prognosis.

Materials and Methods

Patients and Datasets

Expression RNA-seq and clinical information of HCC were obtained from TCGA dataset (https://portal.gdc.cancer.gov/repository), including 370 primary tumors and 50 normal samples. In the Starbase database (http://starbase.sysu.edu.cn), analyses were conducted to identify let-7 target mRNAs (Supplementary Tables S1–S9) and the lncRNAs that regulate let-7 (Supplementary Tables S10–S18).

Hepatic tumor tissues were collected from 68 hepatocellular carcinoma patients at the Department of Hepatobiliary Surgery, Central Hospital of Tianjin University, between 2015 and 2018. The clinical records of the patients were listed in Table 1 (For more detailed information, please refer to Table S19). The study was approved by the hospital's Medical Ethics Committees, with all patients providing informed consent. HCC diagnosis followed the criteria in the "Primary Liver Cancer Diagnosis and Treatment Guidelines (2022 Edition)" from National Health Commission of China. Inclusion

Table 1 The Summary Table of Patient Baseline Characteristics and Clinical Outcomes

Parameter		No-Recurrence	Recurrence	*p*
Sex	Male Female	16 7	36 9	0.253
Age (median, Quartiles)		56 (46, 60)	60 (56, 66)	0.005
TNM stage	I/II III/IV	17 6	21 24	0.029
Tumor number	Single Multiple	20 3	28 17	0.030
Tumor size (cm)	<10 ≥10	19 4	34 11	0.368
Differentiation	Well Moderate/poor	11 12	10 35	0.031
DFS (days) (median, quartiles)		2250 (1996, 2474)	348 (172, 796)	0.000
OS (days) (median, quartiles)		2345 (2192, 2587)	931 (337, 2128)	0.000
Status	Alive Dead	23 0	13 32	0.000

Abbreviations: DFS, disease-free survival; OS, overall survival.

criteria: (1) Primary malignant tumor originating from hepatocytes; (2) No extrahepatic metastasis; (3) Complete clinical and follow-up data. Exclusion criteria: Severe diseases in other organs such as heart, lung, kidney, and brain. Tumor tissues were collected within 30 minutes after surgery and stored at −80°C until use. All patients were followed until death or closure of data analysis on May 4, 2023.

Expression and Functional Analysis of Let-7 in HCC

To clarify let-7 expression in HCC, we analyzed the differences of 12 let-7 miRNAs between 370 primary tumor and 50 normal tissues in TCGA, and their expression in tumors at different pathological stages. For the functional analysis of let-7, we used the R package "DESeq2" to analyze the differentially expressed mRNAs between the tumor and normal tissues in TCGA, and then intersected them with let-7 target mRNAs. We performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses on the differentially expressed let-7 target mRNAs, and visualized the results with the "ggplot2" package.

Screening of Let-7-Related lncRNAs

LncRNAs that regulate let-7 (Supplementary Tables S10–S18) were intersected to identify the lncRNAs commonly associated with let-7 isoforms. The expression of these let-7-related lncRNAs in HCC was analyzed in the Starbase database (Supplementary Table S20). LncRNAs with FoldChange > 1, $p < 0.05$, and previously reported to be associated with tumor prognosis were selected for subsequent HCC prognosis analysis. The correlation between the selected lncRNAs and let-7, as well as the expression differences in tumor tissues at different pathological stages were analyzed.

Analysis of Let-7 and Its Related lncRNAs in HCC Prognosis

Univariate Cox and LASSO regression analyses were employed to screen for the factors influencing the overall survival (OS) of TCGA HCC patients based on the expression of let-7 and its related lncRNAs. A multivariate Cox regression model was constructed to develop a prognostic signature for HCC. The prognostic risk score was calculated based on the gene expression levels and regression coefficients (λ) from the model. The calculation formula is as follows: Score = $\sum$ (gene expression level × λ). The cutoff value of the risk score was determined by ROC curve analysis. Patients were divided into high-risk and low-risk groups based on this value. Kaplan-Meier analysis was used to evaluate the prognosis of HCC patients in the two groups. Display survival time scatter plots, risk score scatter plots, and expression heatmaps of prognosis-related genes using the R packages "ggplot2" and "pheatmap."

RNA Extraction and Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

We extracted RNA from 68 HCC tumor tissues using Trizol (Invitrogen) to detect let-7c and its related lncRNAs. RNA concentration and quality were assessed using a NanoDrop ND-2000 spectrophotometer (Life Technologies). For miRNA detection, first-strand cDNA synthesis was performed using TaqMan miRNA reverse transcription kit (Thermo Fisher Scientific), and SYBR Green real-time PCR was conducted using TaqMan® Universal PCR master mix (Thermo Fisher Scientific). For lncRNA detection, first-strand cDNA synthesis was performed using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara), and SYBR Green real-time PCR was conducted using PCR TB Green® Premix Ex Taq™II (Takara). Follow reagent instructions for all operations. Primer information of let-7c and its related lncRNAs can be found in Supplementary Table S21. MiRNA and lncRNA detection used U6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, forward prime: 5′-TGTCAAGCTCATTTCCTGGTATG-3′, reverse primer: 5′-TCTCTCTTCCTCTTGTGCTCTTG-3′) as endogenous controls, respectively, with three replicates for each. PCR conditions were optimized for a single Tm peak and equal amplification efficiency of target and reference genes. Relative gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 26.0). Differences between two or multiple groups were assessed using the Mann–Whitney *U*-test or Kruskal–Wallis H (K) test. ROC curve analysis was employed to determine the optimal cut-off thresholds for our biomarkers. The area under the ROC curve (AUC) was calculated to quantify the overall performance of the biomarkers. The cut-off threshold was selected based on the point that maximizes the sum of sensitivity and specificity, ensuring the highest diagnostic accuracy. A p value less than 0.05 was considered statistically significant, indicated as * for $p < 0.05$ and ** for $p < 0.01$.

Results

Let-7 is Abnormally Expressed in HCC and Regulates Tumor Progression

Analysis of let-7 expression in TCGA-LIHC tissues revealed that let-7a-1/2/3, let-7b, let-7c, let-7g, and let-7i were significantly downregulated in primary tumor tissues (370 cases) compared with normal tissues (50 cases) (Figure 1A). In paired normal and tumor tissues from 50 patients, let-7b, let-7c, and let-7g were still downregulated in tumors, and mir-98 was slightly upregulated in tumors (Figure 1B). The expression of let-7b, let-7d, and let-7i was correlated with tumor pathological grade (Figure 1C). Analysis of let-7 functions revealed that among the 1602 common target genes of the 12 let-7 miRNAs, 167 were upregulated and 66 were downregulated in tumor tissues (Figure 1D). GO analysis of these 233

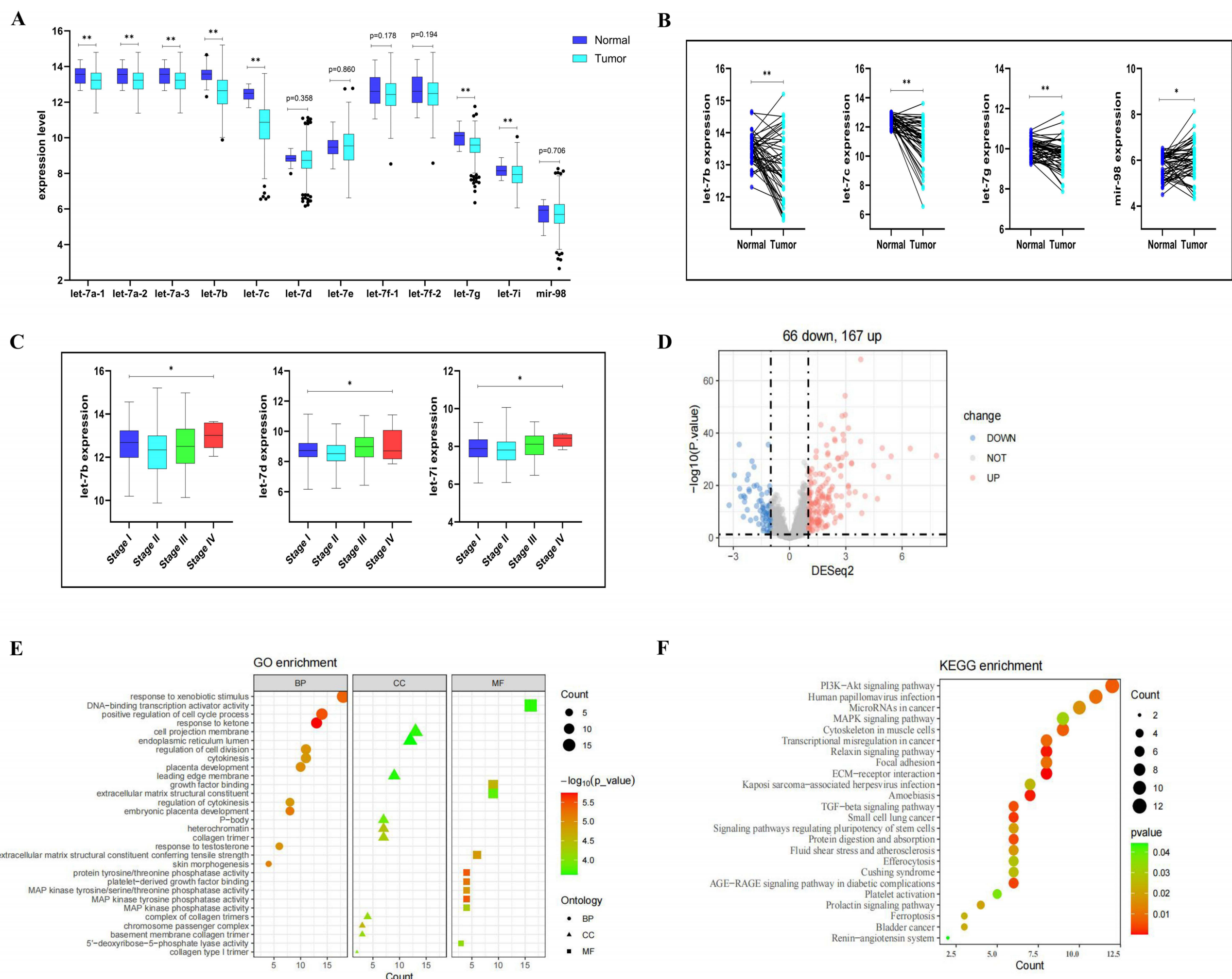


Figure 1 The expression and function of let-7 in TCGA-LIHC data. (**A**) The expression of let-7 in tumor tissues (370 cases) and normal tissues (50 cases). (**B**) The differentially expressed let-7 in 50 normal tissues and their paired tumor tissues. (**C**) The differentially expressed let-7 in TNM stages. (**D**) The volcano plot of let-7 target genes. (**E**) GO analysis of 233 DEGs in let-7 target genes. (**F**) KEGG analysis. * for $p < 0.05$ and ** for $p < 0.01$.

differentially expressed genes (DEGs) showed that let-7 target genes were enriched in biological processes (BP), cellular components (CC), and molecular functions (MF) related to cell development, such as positive regulation of the cell cycle process, cell membrane components, and regulation of DNA-binding transcription factor activity (Figure 1E). KEGG analysis also confirmed that the functions of let-7 target genes were enriched in the PI3K-Akt signaling pathway and tumor-related miRNAs (Figure 1F).

Let-7-Related lncRNAs

Intersection of 9 let-7 miRNA-related lncRNAs identified 48 common lncRNAs. The expression of these 48 lncRNAs in TCGA-LIHC is shown in Supplementary Table S20. After excluding 3 lncRNAs without LIHC-related research data and 12 lncRNAs with no differential expression, 31 were upregulated and 2 downregulated in HCC. Based on literature review, we selected six lncRNAs that have been previously reported to be associated with HCC: NUT family member 2A antisense RNA 1 (NUTM2A-AS1), KCNQ1 opposite strand/antisense transcript 1 (KCNQ1OT1), nuclear paraspeckle assembly transcript 1 (NEAT1), small nucleolar RNA host gene 16 (SNHG16), long intergenic non-protein coding RNA 665 (LINC00665), and X–inactive specific transcript (XIST). We analyzed their expression in HCC and correlation with let-7. Results showed that NUTM2A-AS1, KCNQ1OT1, NEAT1, SNHG16, and LINC00665 were upregulated in tumor tissues (Figure 2A), with NUTM2A-AS1 and SNHG16 levels correlating with tumor pathological grade (Figure 2B). Correlation analysis showed that six lncRNAs were negatively correlated with let-7c expression, with significant negative correlation for NUTM2A-AS1 ($r = -0.195$, $p = 0.000$), KCNQ1OT1 ($r = -0.212$, $p = 0.000$), SNHG16 ($r = -0.160$, $p = 0.002$), LINC00665 ($r = -0.248$, $p = 0.002$) and XIST ($r = -0.168$, $p = 0.001$) (Figure 2C). Correlation with other let-7 members showed that only SNHG16 were negatively correlated with let-7b, while the six lncRNAs exhibited no or positive correlation with let-7 miRNAs (Supplementary Figures S1–S11).

The Prognostic Role of Let-7 and Its Related lncRNAs in HCC

Univariate Cox regression analysis was performed to assess the impact of let-7 and its related lncRNAs on overall survival in TCGA-LIHC patients. The results showed that let-7c (HR = 0.858, 95% CI: 0.752–0.979, $p = 0.023$), miR-98 (HR = 1.240, 95% CI: 1.005–1.529, $p = 0.045$), SNHG16 (HR = 1.446, 95% CI: 1.130–1.849, $p = 0.003$), and LINC00665 (HR = 1.249, 95% CI: 1.074–1.452, $p = 0.004$) had a hazard ratio (HR) with $P < 0.05$ (Figure 3A). Further screening using LASSO regression (Figure 3B) and cross-validation (Figure 3C) identified let-7i as another gene significantly associated with overall survival in HCC, in addition to the aforementioned four RNAs. To construct a stable prognostic gene model, we performed stepwise multivariate regression analysis on the five prognostic-related genes. Ultimately, let-7c (HR = 0.876, 95% CI: 0.766 −1.003, $p = 0.055$) expression was incorporated as a protective factor, while let-7i (HR = 1.278, 95% CI: 0.987–1.655, $p = 0.063$) and SNHG16 (HR=1.419, 95% CI: 1.106–1.821, $p = 0.006$) expression were included as risk factors in the model (Figure 3D). According to the multivariate Cox regression coefficients, the risk score of each sample in the TCGA-LIHC was calculated. The ROC curve determined the cut-off value for the risk score to be 0.40 (Figure 3E). Patients were divided into two groups based on this cut-off: the high-risk group (n=164) and the low-risk group (n=199). Comparison of the survival status and expression of the three RNAs between these two groups revealed that the high-risk group had a higher mortality rate ($p < 0.001$), lower expression of let-7c, and higher expression of let-7i and SNHG16 (Figure 3F–H).

Validation of the Expression of Let-7c and Its Related lncRNAs

We validated let-7c and six related lncRNAs in 68 HCC tissues and found that SNHG16 expression increased with TNM stage but decreased with lower cell differentiation. Let-7c and other lncRNAs showed minimal but detectable changes across different TNM stages and differentiation levels (Figure 4A and B). Besides tumor TNM stage and cellular differentiation affecting the expression, the level of NEAT1 is also associated with patient age, with lower expression in the tumor tissues of older patients; the level of XIST is associated with patient gender, with significantly higher expression in female patients (Figure 4C). We divided 68 HCC patients into recurrence and non-recurrence groups based on post-surgery follow-up. Let-7c and KCNQ1OT1 expression were lower, while SNHG16 expression was higher in the tumor tissues of the recurrence group (Figure 4D).

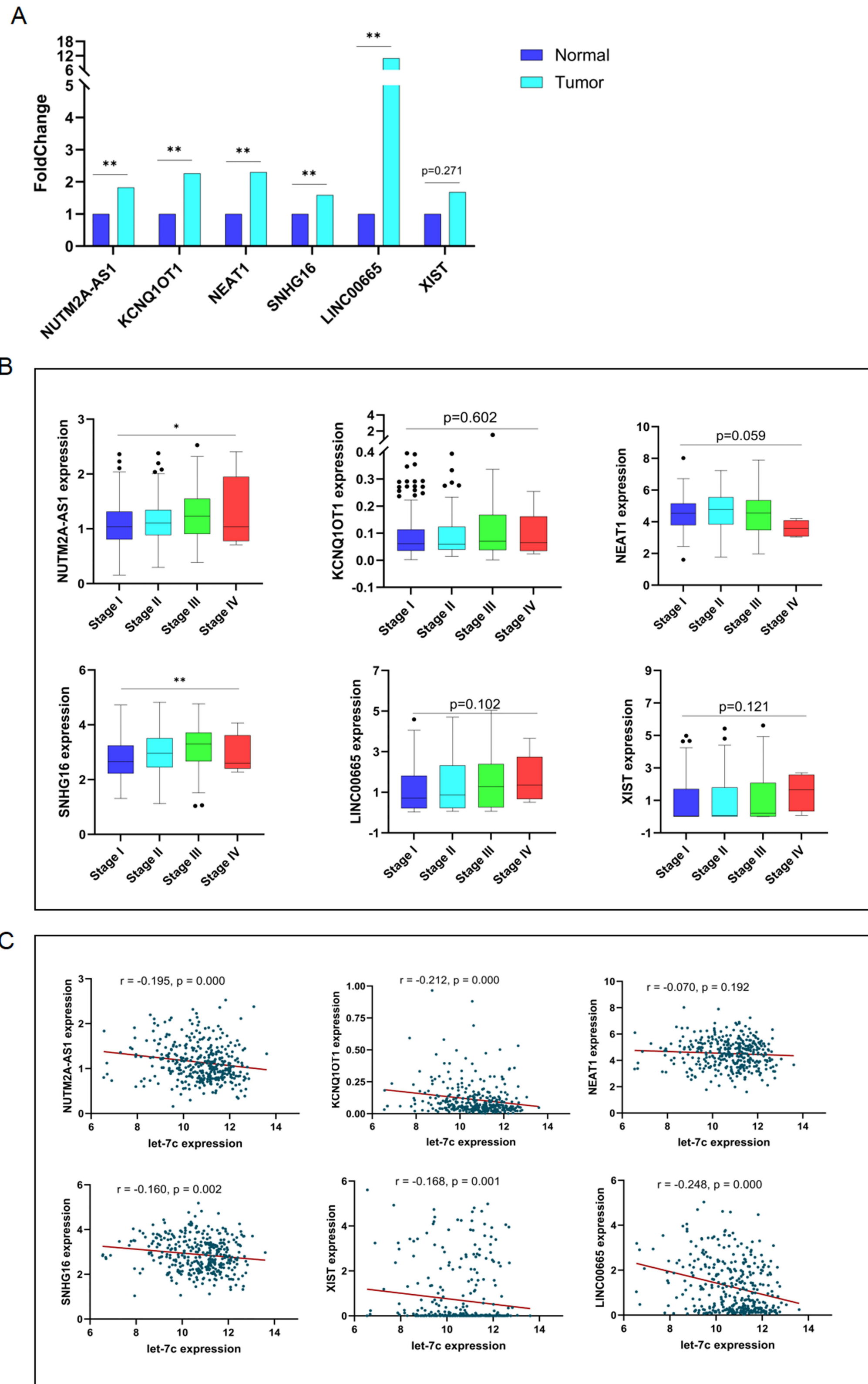


Figure 2 The six lncRNAs that regulate let-7. (**A**) Fold change in expression of six lncRNAs in tumors vs normal tissues. (**B**) Expression of six lncRNAs in tumor tissues by TNM stage. (**C**) Correlation of six lncRNAs with let-7c. * for $p < 0.05$ and ** for $p < 0.01$.

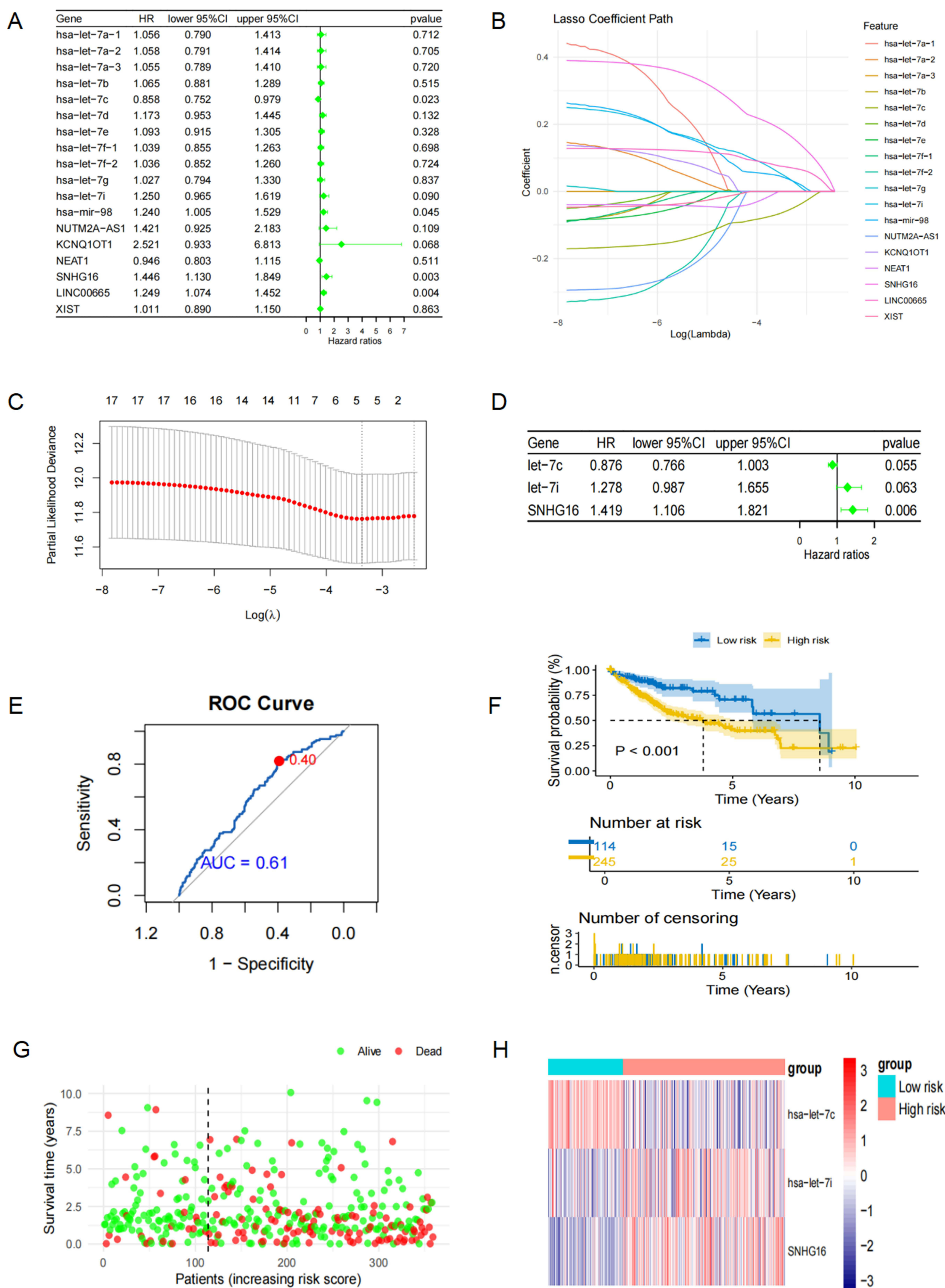
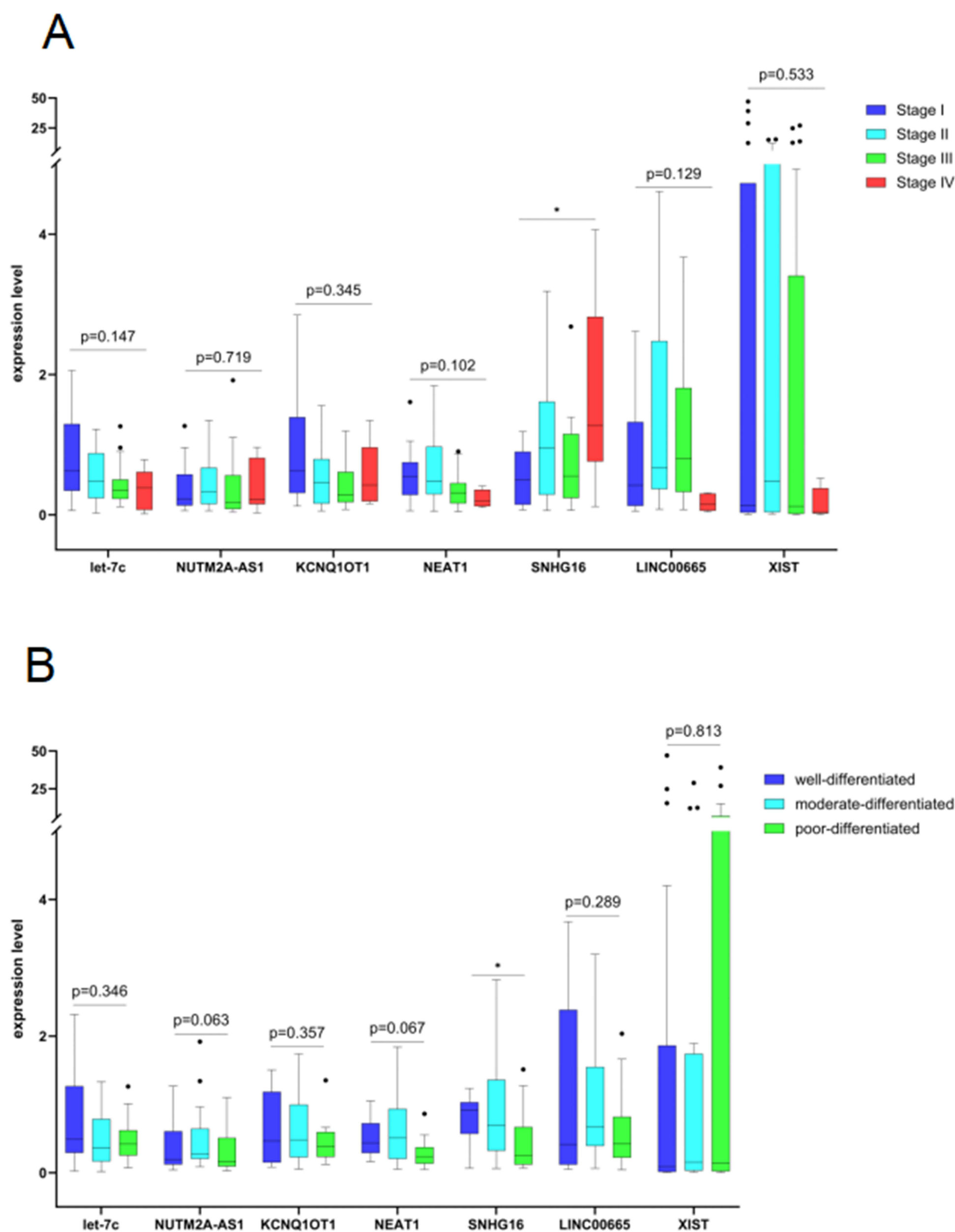


Figure 3 The prognostic role of let-7 and its related lncRNAs in TCGA-LIHC. (**A**) Forest plot showing the relationship of let-7 and lncRNAs with overall survival based on univariate Cox regression analysis. (**B**) LASSO coefficient profiles. (**C**) Cross-validation for the selection of tuning parameter in the LASSO regression. (**D**) Forest plot of stepwise multivariate Cox regression results. (**E**) ROC curves showed the predictive efficiency of the risk signature. (**F**) Kaplan-Meier survival curves of the risk signature. (**G**) Scatter plots displaying the survival status of each patient with HCC. (**H**) The expression pattern of the prognostic signature genes in the high- and low-risk groups.

Validation of the Prognostic Role of Let-7c and Its Related lncRNAs

Univariate Cox regression analysis in 68 HCC patients showed that age (HR = 1.046, 95% CI: 1.014–1.078, $p = 0.004$), TNM stage (HR = 1.615, 95% CI: 1.192–2.189, $p = 0.002$), tumor number (HR = 1.421, 95% CI: 0.999–2.020, $p = 0.050$), cellular differentiation (HR = 1.647, 95% CI: 1.131–2.398, $p = 0.009$), KCNQ1OT1 (HR = 0.484, 95% CI: 0.249–0.941, $p = 0.032$), and SNHG16 (HR = 1.691, 95% CI: 1.216–2.353, $p = 0.002$) had $P < 0.05$ for their HR, significantly affecting disease-free survival (DFS). KCNQ1OT1 was a protective factor, while the others were risk factors (Figure 5A). To establish a more stable recurrence prediction algorithm, we performed stepwise multivariate regression analysis on the six factors affecting DFS. Ultimately, we constructed a five-factor algorithm, which includes age (HR = 1.036, 95% CI: 1.001–1.072, $p = 0.045$), TNM stage (HR = 1.586, 95% CI: 1.142–2.203, $p = 0.006$), cellular differentiation (HR = 1.935, 95% CI: 1.250–2.995, $p = 0.003$), KCNQ1OT1 (HR = 0.572, 95% CI: 0.325–1.008, $p = 0.053$), and SNHG16 (HR = 1.711, 95% CI: 1.144–2.559, $p = 0.009$)



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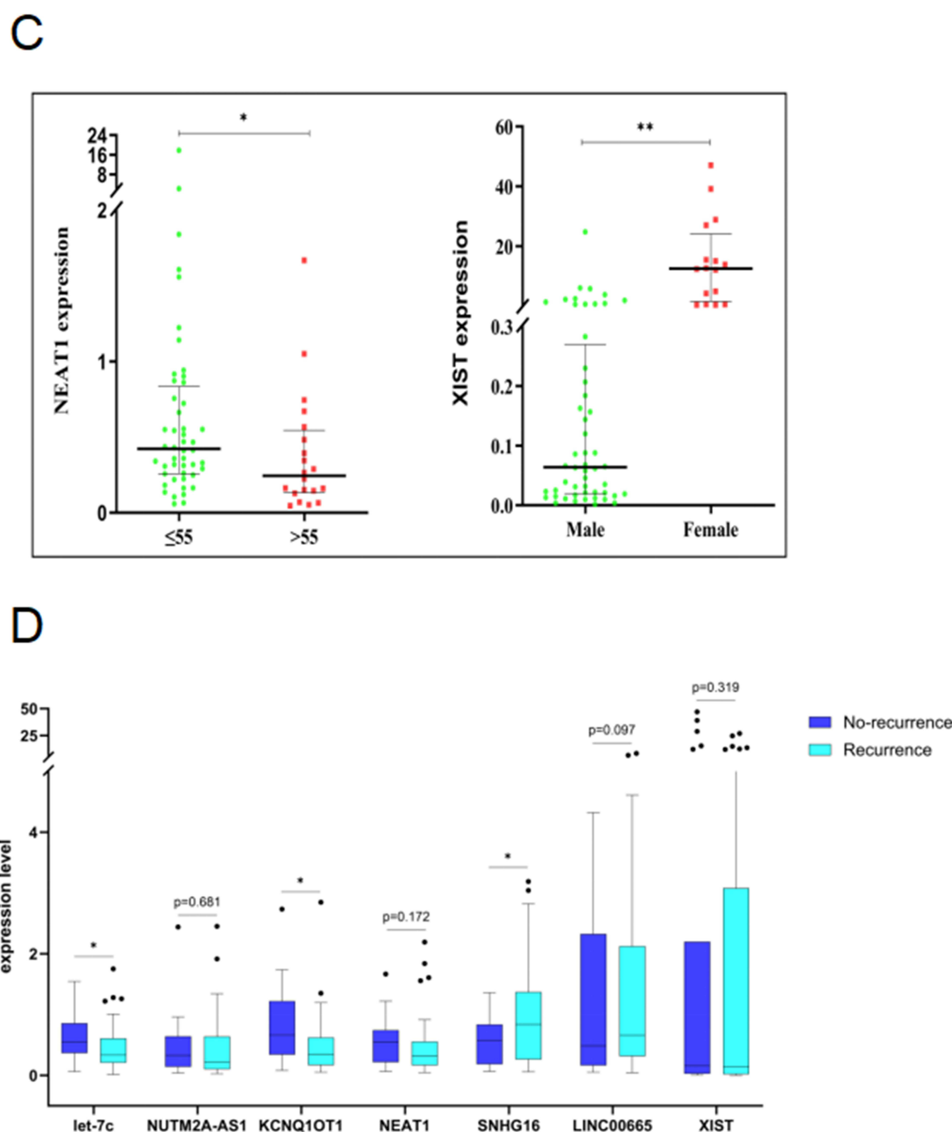

Figure 4 Expression of let-7c and six related lncRNAs in clinical HCC samples. (**A**) Expression across TNM stages. (**B**) Expression in different cell differentiation tissues. (**C**) NEAT expression across ages and XIST expression across genders. (**D**) Let-7c and 6 lncRNAs in recurrent vs non-recurrent HCC tissues. * for $p < 0.05$ and ** for $p < 0.01$.

(Figure 5B). Using Cox regression coefficients, we calculated risk scores for each tumor sample and set a cutoff value of 115.79 via ROC curve (Figure 5C). Patients were divided into two groups according to this cutoff value: the high-risk group (n=54) and the low-risk group (n=14). The high-risk group had significantly shorter DFS than the low-risk group ($p < 0.001$) (Figure 5D), with a higher probability of recurrence (Figure 5E). Using the same procedure, we also analyzed the impact of let-7c and its related lncRNAs on the overall survival (OS) and found that SNHG16 (HR = 1.837, 95% CI: 1.283–2.629, p = 0.001) is also a risk factor for OS (Figure 5F–J).

Discussion

Initially, let-7 was found to regulate the development of Caenorhabditis elegans. Subsequently, it was discovered that let-7 is abnormally expressed in tumors, associated with altered transcriptional profiles and tumorigenicity of tumor cells, and is considered a target RNA for cancer therapy. At first, let-7 was found to be downregulated in tumors, where it

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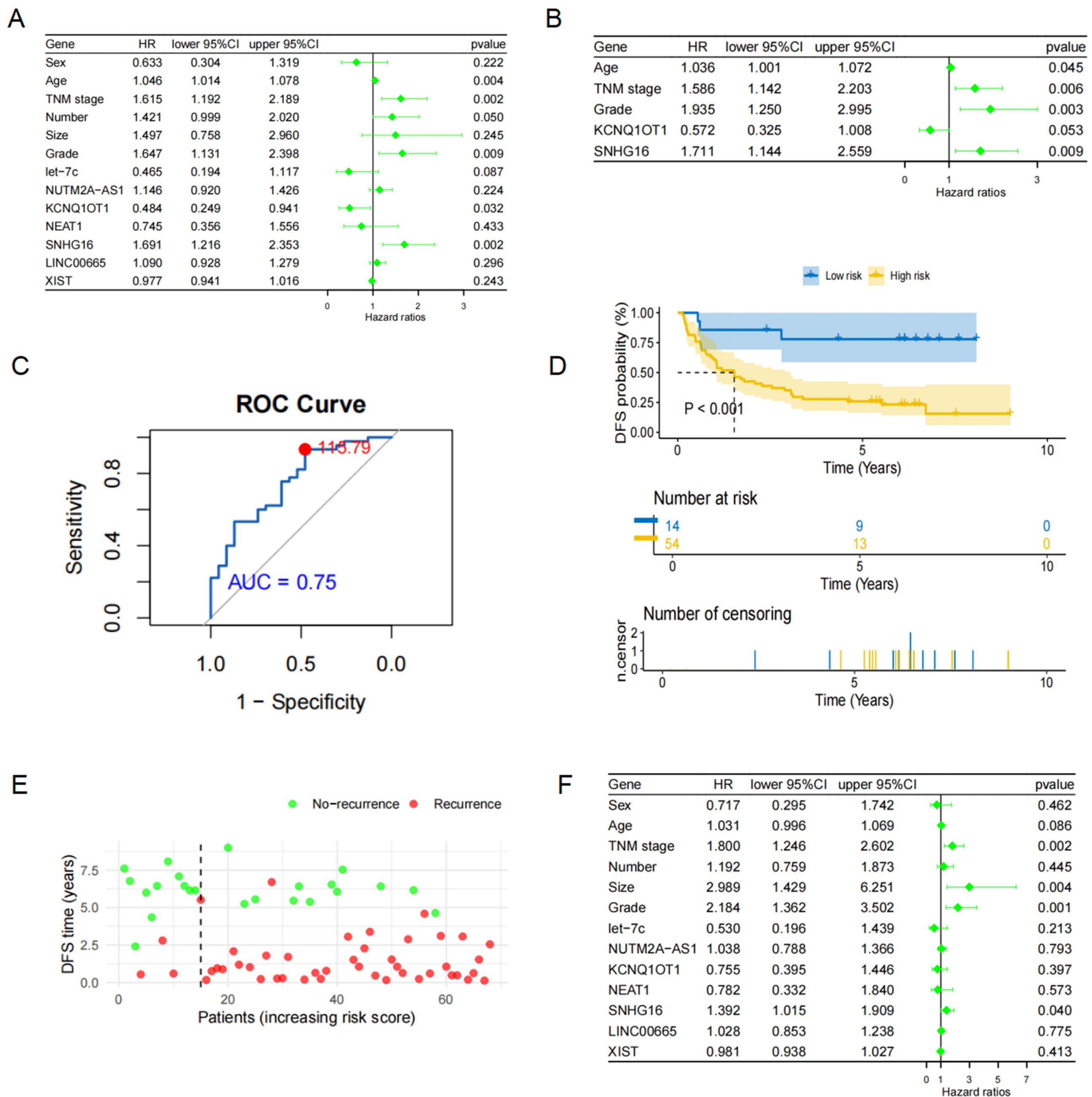
exerts tumor-suppressive effects by targeting and inhibiting the transcription of oncogenic mRNAs. Its expression is lower in poorly differentiated tumor cells than in well-differentiated tumor cells, making it a potential biomarker for tumor staging.[5,9–12] As research progressed, it was found that let-7 not only functions as a tumor suppressor in tumors, but its expression changes also have different impacts on tissue regeneration and tumor formation. For example, Wu[13] found that let-7g inhibits tumor formation in mouse hepatoblastoma, but also suppresses liver regeneration. Chronic over-expression of let-7g (12 times normal level for over 18 months) induces hepatocyte toxicity and hepatocellular carcinoma. Our prior study also found that let-7a/7b/7c/7e are highly expressed in hepatitis and cirrhosis liver tissues compared to normal ones.[14] Chronic hepatitis and cirrhosis are the risk factors for liver cancer. As observed in this study, let-7b, let-7c, and let-7g were downregulated in tumors, while other members showed no significant changes. Moreover, among the 12 let-7 miRNAs, only let-7c was confirmed to be significantly associated with HCC prognosis. This finding



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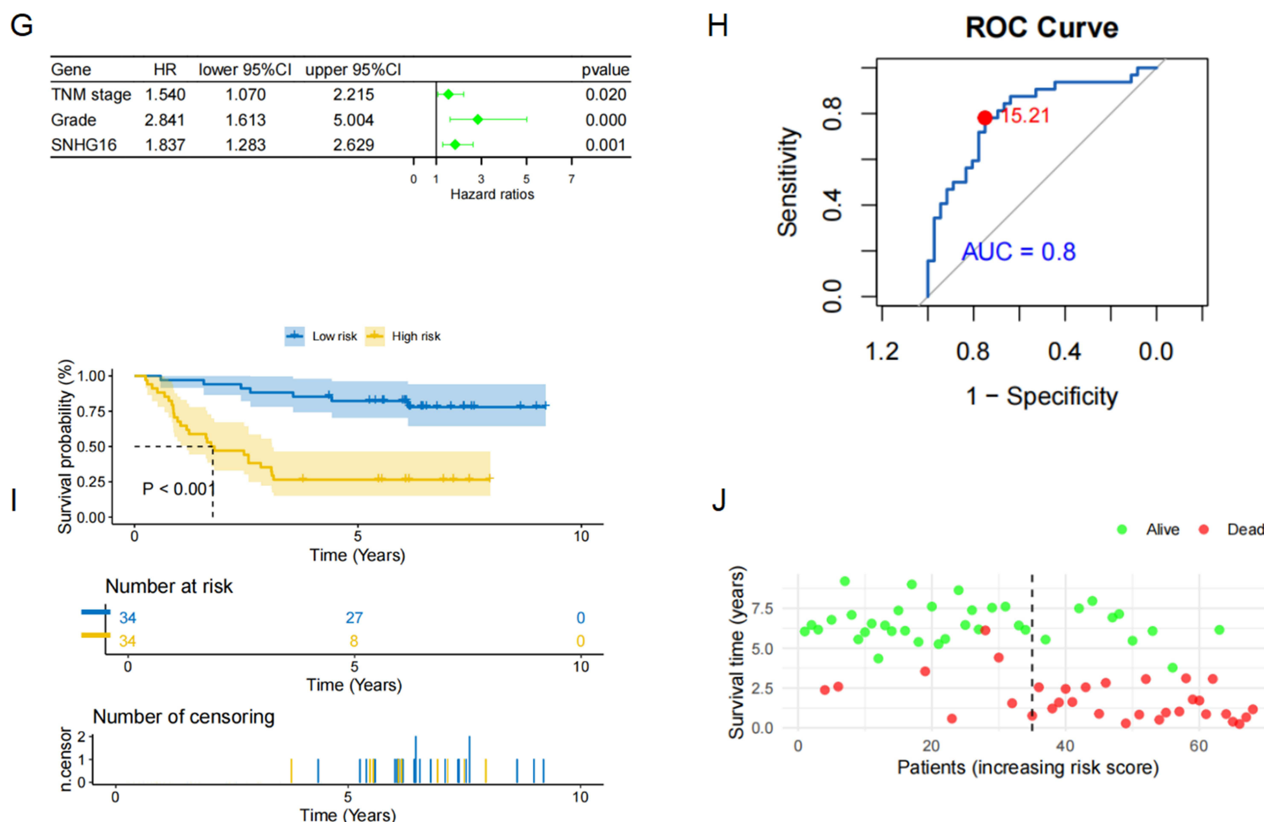

Figure 5 The prognostic role of let-7c and six lncRNAs in clinical HCC. (**A**) Forest plot showing the relationship of let-7 and lncRNAs with DFS based on univariate Cox regression analysis. (**B**) Forest plot of stepwise multivariate Cox regression results. (**C**) ROC curves showed the predictive efficiency of the risk signature. (**D**) Kaplan-Meier survival curves of the risk signature. (**E**) Scatter plots displaying the survival status of each patient with HCC. (**F–J**) Predictive value of let-7 and 6 lncRNAs for OS.

may explain the inconsistency in the tumor-suppressive or oncogenic roles of let-7 observed in previous studies, the complexity of let-7's roles in tumors may stem from its large family size and diverse functions. Different let-7 subtypes had varying correlations with their regulated lncRNAs. Thus, understanding the factors regulating let-7 expression is crucial for HCC diagnosis, treatment, and prognosis.

In mouse pre-osteoblasts (MC3T3-E1), KCNQ1OT1 targets and inhibits the expression of miR-98-5p.[15] In addition to being regulated by lncRNAs, let-7 can also form feedback loops with lncRNAs, affecting their expression. For example, in porcine alveolar macrophages (Marc-145), You showed that NEAT1 and let-7e are negatively correlated, with each regulating the other's expression.[16] In osteosarcoma cells, Liao found that SNHG16 promotes cell proliferation and invasion by downregulating miR-98-5p, while overexpression of miR-98-5p can reduce SNHG16 expression and reverse its effects.[17] The regulatory roles of LINC00665 and XIST on let-7 have also been confirmed.[18–20] The above studies reveal that lncRNAs negatively regulate let-7, with different let-7 members being regulated in various tissues and cells.

In HCC studies, NUTM2A-AS1, KCNQ1OT1, and LINC00665 are highly expressed in tumor tissues, where they promote cancer progression and are associated with poor prognosis. For example, Long found that NUTM2A-AS1 is highly expressed in LIHC, and patients with high levels of NUTM2A-AS1 have worse conditions, with lower overall survival and disease-free survival rates.[21] Li found that KCNQ1OT1 is highly expressed in cancer tissues, with even higher expression in larger tumors (diameter ≥ 5 cm). High KCNQ1OT1 expression correlates with cirrhosis, larger tumors, higher TNM stages, and lower survival rates.[22] Liu[23] and Zhang[18] confirmed that NEAT1 and LINC00665 respectively downregulate let-7b and let-7i to promote proliferation and invasion in HCC cells.

The research regarding the expression and functions of SNHG16 and XIST in HCC are not entirely consistent. For instance, Xu found that SNHG16 is downregulated in HCC tissues and cell lines, and its upregulation inhibits tumor cell proliferation and growth.[24] However, later studies showed that SNHG16 is upregulated in HCC, correlating with tumor

malignancy and poor prognosis.[25–28] Ma,[29] Lin,[30] and Zhang[31] found that downregulation of XIST in cancer tissues promotes tumor growth, metastasis, and poor prognosis. Conversely, Mo,[32] Wang,[33] and Duan[34] reported that upregulation of XIST in hepatocellular carcinoma has oncogenic effects. These conflicting findings highlight the complexity of understanding the roles of SNHG16 and XIST in HCC. Possible reasons for these discrepancies include differences in study populations, detection methods, and tumor heterogeneity.

This study confirmed that NUTM2A-AS1, KCNQ1OT1, NEAT1, SNHG16, and LINC00665 was significantly overexpressed in HCC, and XIST expression was significantly higher in female HCC patients than in males. The expression of these six lncRNAs was associated with tumor TNM staging and cellular differentiation. In particular, SNHG16 was upregulated with higher TNM stage and downregulated with lower differentiation. NEAT1 levels were also age-related, with lower expression in tumors of older patients. The functions of let-7 were enriched in the PI3K-Akt signaling pathway and tumor-related miRNA. Further screening revealed that let-7c was a protective factor for HCC prognosis. And the these six lncRNAs were negatively correlated with let-7c, suggesting that they might primarily exert their effects by regulating let-7c in HCC. Let-7c was downregulated in HCC, and HCC patients with low let-7c expression were more likely to relapse and had shorter survival periods. Although the six lncRNAs all negatively regulate let-7c, only SNHG16 was confirmed as a risk factor for recurrence and survival in HCC patients in this study.

The high expression of SNHG16 is associated with recurrence and poor prognosis in HCC, suggesting that SNHG16 can serve as a potential biomarker for recurrence risk stratification and surveillance. In clinical practice, detecting the expression levels of SNHG16 may more accurately identify patients at high risk of recurrence, thereby providing these patients with more intensive monitoring and more aggressive treatment strategies. Additionally, the expression levels of SNHG16 may also serve as a reference for treatment decisions, helping doctors choose more appropriate treatment plans and improve treatment outcomes. Although this study has provided important insights into the role of SNHG16 in HCC, there are still some limitations. Notably, the results from TCGA data are not entirely consistent with those from clinical samples. For instance, the TCGA HCC data analysis indicates that let-7i is a prognostic risk factor, whereas the clinical sample data analysis does not include it as a risk factor. The clinical sample analysis identifies KCNQ1OT1 as a protective factor for HCC recurrence, which is not observed in the TCGA HCC data analysis. These inconsistencies may arise from the differences in the samples from the two data sources. The relatively small sample size of this study may influence the generalizability and accuracy of the results. Future research should validate these findings in larger, multicenter cohorts to ensure the reliability and reproducibility of the outcomes. This study primarily focused on the expression levels and clinical relevance of SNHG16, the exploration of its functions and mechanisms was relatively limited. Future studies need to further investigate the specific mechanisms of action of SNHG16 in HCC, including its interaction with let-7c and its specific functions in tumor initiation and progression.

Conclusions

This study found that SNHG16 is highly expressed in HCC and negatively regulates let-7c to predict HCC recurrence. This finding provides a new potential biomarker for the diagnosis and treatment of HCC but requires further validation and exploration in future studies.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Approval Statement and Informed Consent

This study was approved by the Ethics Committee of Tianjin Third central Hospital (Approval number: IRB2019-034-01). All procedures involving human participants were in accordance with the ethical standards of national committee and the Helsinki declaration.

Acknowledgments

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Nevola R, Ruocco R, Criscuolo L, et al. Predictors of early and late hepatocellular carcinoma recurrence. *World J Gastroenterol*. 2023;29:1243–1260. doi:10.3748/wjg.v29.i8.1243
2. Badwei N. Molecular clues for prediction of hepatocellular carcinoma recurrence after liver transplantation. *J Clin Exp Hepatol*. 2023;13:804–812. doi:10.1016/j.jceh.2023.02.006
3. Niu ZS, Wang WH, Niu XJ. Recent progress in molecular mechanisms of postoperative recurrence and metastasis of hepatocellular carcinoma. *World J Gastroenterol*. 2022;28:6433–6477. doi:10.3748/wjg.v28.i46.6433
4. Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in caenorhabditis elegans. *Nature*. 2000;403:901–906. doi:10.1038/35002607
5. Boyerinas B, Park SM, Hau A, et al. The role of let-7 in cell differentiation and cancer. *Endocr Relat Cancer*. 2010;17:F19–36. doi:10.1677/ERC-09-0184
6. Allela OQB, Al-Hussainy AF, Sanghvi G, et al. Tumor immune evasion and the let-7 family: insights into mechanisms and therapies. *Naunyn Schmiedebergs Arch Pharmacol*. 2025. doi:10.1007/s00210-025-04283-9
7. Wei C, Chen J, Huang T, et al. The microRNA landscape and regulatory network in clonorchis sinensis-infected hepatocellular carcinoma: implications for tumor progression. *Parasit Vectors*. 2025;18:68. doi:10.1186/s13071-025-06689-z
8. Segal D, Dostie J. The talented LncRNAs: meshing into transcriptional regulatory networks in cancer. *Cancers*. 2023;15:3433. doi:10.3390/cancers15133433
9. Shell S, Park SM, Radjabi AR, et al. Let-7 expression defines two differentiation stages of cancer. *Proc Natl Acad Sci U S A*. 2007;104:11400–11405. doi:10.1073/pnas.0704372104
10. Schuh GM, Maschhoff KR, Minor A, et al. Repression of AGO1 by AGO2 via let-7 microRNAs facilitates embryonic stem cell differentiation. *RNA*. 2025;31:772–780. doi:10.1261/rna.080426.125
11. Chirshev E, Oberg KC, Ioffe YJ, et al. Let-7 as biomarker, prognostic indicator, and therapy for precision medicine in cancer. *Clin Transl Med*. 2019;8:24. doi:10.1186/s40169-019-0240-y
12. Ma Y, Shen N, Wicha MS, et al. The roles of the let-7 family of MicroRNAs in the regulation of cancer stemness. *Cells*. 2021;10:2415. doi:10.3390/cells10092415
13. Wu L, Nguyen LH, Zhou K, et al. Precise let-7 expression levels balance organ regeneration against tumor suppression. *Elife*. 2015;4:e09431. doi:10.7554/eLife.09431
14. Shi W, Zhang Z, Yang B, et al. Overexpression of microRNA let-7 correlates with disease progression and poor prognosis in hepatocellular carcinoma. *Medicine*. 2017;96:e7764. doi:10.1097/MD.0000000000007764
15. Wang F, Zhang F, Zheng F. lncRNA Kcnq1ot1 promotes bone formation by inhibiting miR-98-5p/Tbx5 axis in MC3T3-E1 cells. *Exp Ther Med*. 2022;23:194. doi:10.3892/etm.2022.11117
16. You X, Liu M, Liu Q, et al. miRNA let-7 family regulated by NEAT1 and ARID3A/NF-κB inhibits PRRSV-2 replication in vitro and in vivo. *PLoS Pathog*. 2022;18:e1010820. doi:10.1371/journal.ppat.1010820
17. Liao S, Xing S, Ma Y. LncRNA SNHG16 sponges miR-98-5p to regulate cellular processes in osteosarcoma. *Cancer Chemother Pharmacol*. 2019;83:1065–1074. doi:10.1007/s00280-019-03822-5
18. Zhang BC, Ma SY, Zhu P, et al. LINC00665 target let-7i/HMGA1 promotes the proliferation and invasion of hepatoma cells. *Mutat Res*. 2024;828:111852. doi:10.1016/j.mrfmmm.2024.111852
19. Liang D, Jin Y, Lin M, et al. Down-regulation of Xist and Mir-7a-5p improves LPS-induced myocardial injury. *Int J Med Sci*. 2020;17:2570–2577. doi:10.7150/ijms.45408
20. Yu CH, Hsieh PL, Chao SC, et al. XIST/let-7i/HMGA1 axis maintains myofibroblasts activities in oral submucous fibrosis. *Int J Biol Macromol*. 2023;232:123400. doi:10.1016/j.ijbiomac.2023.123400
21. Long J, Liu L, Yang X, et al. LncRNA NUTM2A-AS1 aggravates the progression of hepatocellular carcinoma by activating the miR-186-5p/KLF7-mediated wnt/beta-catenin pathway. *Hum Cell*. 2023;36:312–328. doi:10.1007/s13577-022-00802-5
22. Li C, Miao R, Zhang J, et al. Long non-coding RNA KCNQ1OT1 mediates the growth of hepatocellular carcinoma by functioning as a competing endogenous RNA of miR-504. *Int J Oncol*. 2018;52:1603–1612. doi:10.3892/ijo.2018.4313
23. Liu Q, Shi H, Yang J, et al. Long non-coding RNA NEAT1 promoted hepatocellular carcinoma cell proliferation and reduced apoptosis through the regulation of let-7b-IGF-1R axis. *Onco Targets Ther*. 2019;12:10401–10413. doi:10.2147/OTT.S217763

24. Xu F, Zha G, Wu Y, et al. Overexpressing lncRNA SNHG16 inhibited HCC proliferation and chemoresistance by functionally sponging hsa-miR-93. *Onco Targets Ther*. 2018;11:8855–8863. doi:10.2147/OTT.S182005
25. Li W, Xu W, Song JS, et al. LncRNA SNHG16 promotes cell proliferation through miR-302a-3p/FGF19 axis in hepatocellular carcinoma. *Neoplasma*. 2019;66:397–404. doi:10.4149/neo_2018_180720N504
26. Liu Q, Gao P, Li Q, et al. Long non-coding RNA SNHG16 as a potential biomarker in hepatocellular carcinoma: a meta-analysis. *Medicine*. 2021;100:e27178. doi:10.1097/MD.0000000000027178
27. Zhang QJ, Li DZ, Lin BY, et al. SNHG16 promotes hepatocellular carcinoma development via activating ECM receptor interaction pathway. *Hepatobiliary Pancreat Dis Int*. 2022;21:41–49. doi:10.1016/j.hbpd.2021.09.006
28. Xu Y, Luan G, Li Z, et al. Tumour-derived exosomal lncRNA SNHG16 induces telocytes to promote metastasis of hepatocellular carcinoma via the miR-942-3p/MMP9 axis. *Cell Oncol*. 2023;46:251–264. doi:10.1007/s13402-022-00746-w
29. Ma W, Wang H, Jing W, et al. Downregulation of long non-coding RNAs JPX and XIST is associated with the prognosis of hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol*. 2017;41:163–170. doi:10.1016/j.clinre.2016.09.002
30. Lin XQ, Huang ZM, Chen X, et al. XIST induced by JPX suppresses hepatocellular carcinoma by sponging miR-155-5p. *Yonsei Med J*. 2018;59:816–826. doi:10.3349/ymj.2018.59.7.816
31. Zhang Y, Zhu Z, Huang S, et al. lncRNA XIST regulates proliferation and migration of hepatocellular carcinoma cells by acting as miR-497-5p molecular sponge and targeting PDCD4. *Cancer Cell Int*. 2019;19:198. doi:10.1186/s12935-019-0909-8
32. Mo Y, Lu Y, Wang P, et al. Long non-coding RNA XIST promotes cell growth by regulating miR-139-5p/PDK1/AKT axis in hepatocellular carcinoma. *Tumour Biol*. 2017;39:1010428317690999. doi:10.1177/1010428317690999
33. Wang J, Yin G, Bian H, et al. LncRNA XIST upregulates TRIM25 via negatively regulating miR-192 in hepatitis B virus-related hepatocellular carcinoma. *Mol Med*. 2021;27:41. doi:10.1186/s10020-021-00278-3
34. Duan A, Li H, Yu W, et al. Long noncoding RNA XIST promotes resistance to lenvatinib in hepatocellular carcinoma cells via epigenetic inhibition of NOD2. *J Oncol*. 2022;2022:4537343. doi:10.1155/2022/4537343

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