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ORIGINAL ARTICLE

LncRNA00978 contributes to cells growth and metastasis in hepatocellular carcinoma via mediating miR-125b-5p/SOX12 pathway

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Summary

Purpose: *Liver hepatocellular carcinoma (LIHC) is a malignant tumor with high recurrence and poor prognosis. However, the regulatory mechanism underlying tumorigenesis in LIHC remains largely unclear.*

Methods: *Different methods were used to predict the binding sites between LINC00978 and several miRs and SOX12.*

Results: *Herein, we found that long non-coding RNA00978 (LINC00978) was significantly upregulated in LIHC, and the high expression of LINC00978 was associated with poor LIHC prognosis. Furthermore, LINC00978 silence could suppress LIHC cells growth and metastasis. Mechanistically, we revealed that LINC00978 could sponge microRNA-125b-5p (miR-125b-5p) and identified SRY-Box Transcription Factor 12 (SOX12) as a direct target gene of miR-125b-5p. More importantly, the suppressed effect of silencing LINC00978 on LIHC cells growth and metastasis could be rescued by miR-125b-5p inhibition and SOX12 overexpression.*

Conclusion: *Our findings suggested that LINC00978/miR-125b-5p/SOX12 axis could serve as a novel promising therapeutic candidate for LIHC.*

Key words: *liver hepatocellular carcinoma, LINC00978, miR-125b-5p, SOX12, cells proliferation, migration, invasion*

Introduction

Liver hepatocellular carcinoma (LIHC) is a malignant tumor occurring in the liver, occupying approximately 90% of primary liver cancers [1,2]. It is currently believed that the occurrence of LIHC is highly correlated with certain chemical carcinogens such as viral hepatitis, aflatoxin, liver cirrhosis, and factors of soil and water [3,4]. Clinically, LIHC is characterized with liver pain, weight loss, fever, loss of appetite, and splenomegaly [5]. In terms of clinical treatment, liver transplantation, tumor ablation, transarterial treatment and systemic treatment are recently used to alleviate LIHC [6]. However, the high recurrence and metastasis rate of LIHC are responsible for its poor prognosis [7]. Thus, there is a urgent need to investigate the molecular mechanism underlying tumorigenesis more importantly, revealing that potential biomarkers predicting LIHC recurrence have far-reaching significance for ameliorating the disease.

Non-coding RNAs (ncRNAs), long non-coding RNAs (lncRNAs) and microRNAs (miRNAs/miRs) have been proved to significantly contribute to the pathogenesis and progression of LIHC. Specifically, Zhang et al [8] clarified that lncRNA-CCDC144NL-AS1 could sponge miR-940 to inhibit WDR5 expression, thereby inducing acceleration of the progression in hepatocellular carcinoma, and Wang et al [9] suggested that lncRNA XIST could facilitate the occurrence and progression of

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hepatocellular carcinoma via mediating miR-192/TRIM25 axis. Conversely, Chen B et al revealed that lncRNA TP53TG1 negatively regulated cells growth and metastasis in hepatocellular carcinoma in a PRDX4/β-catenin pathway-dependent manner [10]. In addition, the functional role of long non-coding RNA00978 (LINC00978) [11] and microRNA-125b-5p (miR-125b-5p) [12] in LIHC were respectively reported, nevertheless, the mechanism though which LINC00978 mediates miR-125b-5p to affect the development of LIHC remains elusive.

SRY-Box Transcription Factor 12 (SOX12) belongs to the sex determining region Y-box (SOX) family which is characterized with the highly conserved high mobility group (HMG) sequences [13]. According to the HMG sequences and other structural motifs, SOX family is classified into subgroups ranging from A to H [14]. Previous studies have reported that SOX gene including SOX11 and SOX12 are involved in the development of neuronal and epithelial-mesenchymal transition (EMT) [15,16]. Recently, accumulating researches have proved that SOX12 made significant contribution to the progression of multiple cancers, like myeloma [17], breast cancer [18] and glioma [19]. In addition, Wang et al [20] suggested that miRNA-370 suppressed the tumor growth and EMT in bladder cancer via inhibiting SOX12 transcription. Interestingly, SOX12 has been recently proved to participate in the metastasis in LIHC [21]. However, whether and how LINC00978/miR-125b-5p mediates SOX12 to affect the progression of LIHC has not been elaborated.

Therefore, we examined the expression levels of LINC00978, miR-125b-5p and SOX12 in LIHC, and investigated the correlation between them. Moreover, we revealed the functional effect of LINC00978/miR-125b-5p/SOX12 axis on LIHC cells growth and metastasis. To summarize, our study reveals a novel mechanism of LINC00978/miR-125b-5p/SOX12 axis in LIHC cells growth and metastasis, which may be considered as novel biomarkers and potential therapeutic targets for LIHC.

Methods

Cell culture and transfection

Human liver cancer cell lines Hep3b, SNU423 and SNU449 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured in ATCC-formulated medium containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA). MHCC-97H cell line was purchased from the Liver Cancer Institute (Fudan University, Shanghai, China) and incubated in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA). The sh-LINC00978-1, sh-LINC00978-2, miR-125b-5p-inhibitor, miR-125b-5p-mimics, pcDNA-SOX12 were purchased and designed by GeneChem Corporation (Shanghai, China) (Table 1). SNU449 and SNU423 cells were transfected with indicated plasmids according to the instructions of lipofectamine (Vision 2000, 11668-019, Invitrogen, Carlsbad, CA, USA).

Fluorescence in situ hybridization (FISH)

For the measurement of LINC00978, fluorescein isothiocyanate (FITC)-UTP (Roche, Basel, Switzerland) was performed to label the fragment of LINC00978 by a mMESSAGE T7 Ultra In Vitro Transcription kit (Life Technologies, Gaithersburg, MD, USA) according to the instructions of manufacturer. In brief, the probes were hybridized with slides overnight and were washed using saline-sodium citrate. The images were captured using a fluorescence microscope (Olympus, Tokyo, Japan).

Table 1. Sequences of shRNA

sh-LINC00978-1	5'-3'	CACCGCCCAGATTTAAGGGCTATTTCAAGAGAATAGCCCTTAAATCTGGGCCTTTTTTG
sh-LINC00978-2	5'-3'	CACCGCCCAGATTTAAGGGCTATTTCAAGAGAATAGCCCTTAAATCTGGGCCTTTTTTG

Table 2. Sequences of PCR primers used in this study

Gene		*Primer sequences*
LINC00978	Forward(5'-3')	AGGCCCCAGGGAATCTTTCA
	Reverse (5'-3')	GCCTCTCCCTGAATAACTGGG
miR-125b-5p	Forward (5'-3')	CAGTCCCTGAGACCCTAAC
	Reverse (5'-3')	GTCCAGTTTTTTTTTTTTTTTCACAAG
GAPDH	Forward (5'-3')	GGAGCGAGATCCCTCCAAAAT
	Reverse (5'-3')	GGCTGTTGTCATACTTCTCATGG
SOX12	Forward (5'-3')	AAGAGGCCGATGAACGCATT
	Reverse (5'-3')	TAGTCCGGGTAATCCGCCAT

Luciferase reporter assay

Bioinformatics ENCORI and TargetScan database were respectively performed for the predictions of the binding site between LINC00978 and miR-125b-5p, as well as miR-125b-5p and SOX12. To detect the luciferase activity, miR-125b-5p mimics or NC mimics were co-transfected with LINC00978-MUT or LINC00978-WT, and SOX12-WT or SOX12-MUT into SNU423 and SNU449 cells in consistence with the manufacturer's protocols. The luciferase activity was detected using Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

RNA immunoprecipitation (RIP)

RIP was employed to analyze the correlation between LINC00978 and miR-125b-5p, as well as miR-125b-5p and SOX12. Briefly, using Anti-AGO2 (ab186733, 1:50, Abcam, Cambridge, MA, USA), the Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) was utilized, and then RNA bound complexes were measured by qRT-PCR; meanwhile, Anti-IgG was adopted as isotype control.

Cell counting kit-8 (CCK-8)

10% CCK-8 solution (cell counting-kit) (Sigma, St. Louis, MO, USA) was used to incubate SNU423 and SNU449 cells in 96-well plates. Cell counting kit reagent (Beyotime Biotechnology, Shanghai, China) was utilized for the measurement of absorbance at 450 nm at incubation time of 24, 48, 72 and 96 h, respectively.

Colony formation assay

For the analysis of cells proliferation rate, SNU423 and SNU449 cells at a density of 1×10^3 were cultured in 6-well plates for 14 days at 37°C. Then, cells were fixed by 4% paraformaldehyde and stained by 1% crystal violet. The visible colonies were counted and the images were recorded under a microscope.

Transwell assay

For the assessment of cells' migratory and invasive ability, SNU423 and SNU449 cells were plated into the upper chambers, and the DMEM with 10% FBS was maintained in the lower chambers. Then, the cells were removed and fixed by 4% paraformaldehyde while 0.1% crystal violet was performed for stain. The migratory and invasive cells were calculated and the images were captured under a microscope (Zeiss, Oberkochen, Germany).

Wound healing assay

SNU423 and SNU449 cells were maintained in 12-well plates for 1 day, followed by scratching using a sterile pipette tip and then cultured for another 2 days. Photographs were obtained and the migration rate was measured under a microscope.

Western blotting (WB)

The total protein of SOX12 from SNU423 and SNU449 cells was extracted by radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) and a bicinchoninic acid (BCA) protein Assay Kit (CWBIO) was adopted for the detection. Then, proteins were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and a Bio-Rad Mini PROTEAN 3 system (Bio-Rad, Hercules, CA, USA) was utilized to transfer onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), which were then mixed with phosphate buffered saline (PBS) containing 5% milk for 1 h, followed by incubation with the primary antibodies at 4°C overnight, and then were cultured with the anti-rabbit secondary antibodies conjugated with horseradish peroxidase. Amersham ECL Western Blotting Detection kit was performed for the visualization of blot bands in accordance with manufacturer's instructions, and GAPDH (ab8245, 1:1000, abcam, Cambridge, MA, USA) was adopted for the nor-

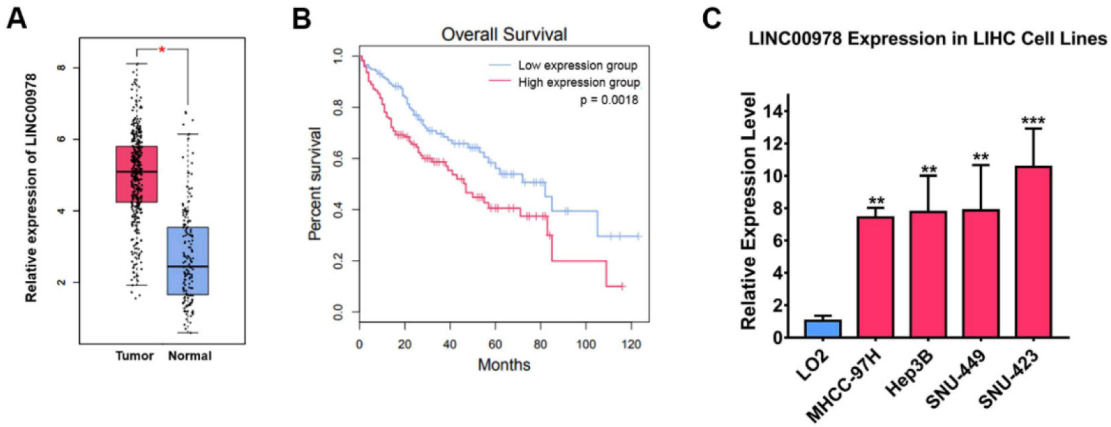

Figure 1. LINC00978 is upregulated in LIHC. **A:** TCGA database was performed to evaluate the different expression of LINC00978 in LIHC tissues and normal tissues. **B:** The overall survival analysis in the LIHC patients based on LINC00978 expression. **C:** qRT-PCR was used to detect the expression of LINC00978 in LIHC cell lines Hep3b, SNU423, SNU449 and MHCC-97H, and human normal liver cells LO2. * p<0.05, ** p<0.01, *** p<0.001.Data represent at least three independent sets of experiment.

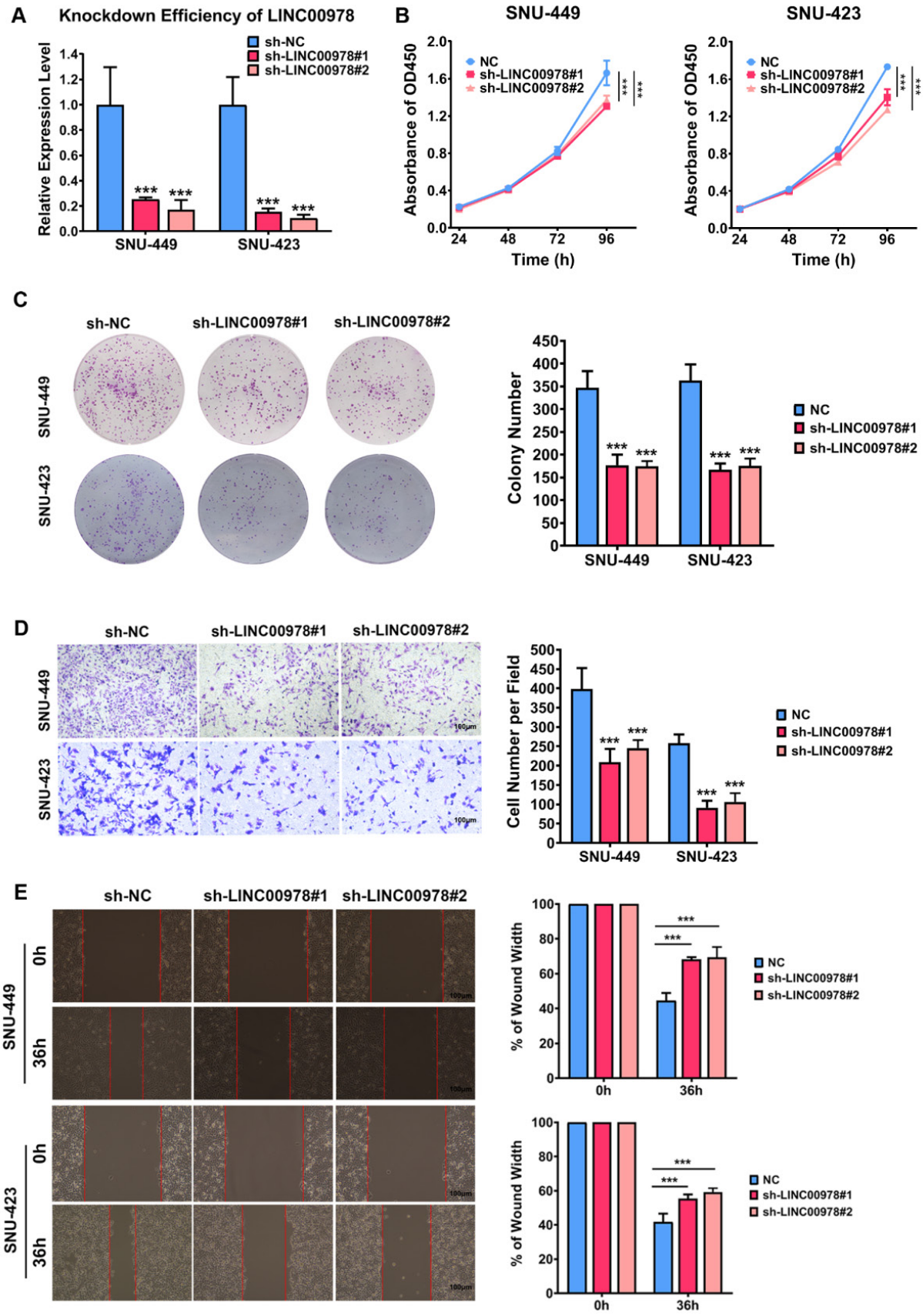

Figure 2. LINC00978 depletion suppresses LIHC cells growth and metastasis. For functional exploration, SNU423 and SNU449 cells were transiently infected with shRNA plasmid, and qRT-PCR was adopted to examine the transfected efficacy. **A:** CCK-8, **B:** colony formation, **C:** assays were used to evaluate the proliferation rate of SNU423 and SNU449 cells. Transwell **(D)** and wound healing **(E)** assays were performed to measure the migratory and invasive capacities. ***p<0.001. Data represent at least three independent sets of experiment.

malization. The primary antibodies SOX12 (PA5-103280, 1:1000) were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

qRT-PCR

TRIzol reagent (TaKaRa, Tokyo, Japan) was utilized for the extraction of total RNA from cells, and then PrimeScript RT Reagent kit (TaKaRa, Tokyo, Japan) was employed for the RNA reverse-transcription. The mRNA expression levels of LINC00978, miR-125b-5p and SOX12 were detected using qRT-PCR. GAPDH and U6 were respectively adopted to normalize the relative gene expression. The primer sequences are listed in Table 2.

Statistics

In our study, the data were presented as the mean ± SD. Differences in internal groups were assessed by Student's t-test and one-way analysis of variance (ANOVA). In addition, the overall survival was evaluated using Kaplan-Meier method. P<0.05 was considered statistically significant.

Results

LINC00978 is highly expressed in LIHC

To explore the correlation between LINC00978 and LIHC, we first evaluated the expression of LINC00978 using TCGA database, and found that LINC00978 was differently expressed in LIHC tissues and normal tissues (Figure 1A). Moreover, the overall survival analysis showed that LIHC patients with high LINC00978 expression indicated poor overall survival (p<0.01, Figure 1B). In addition, for further confirmation, we detected the expression levels of LINC00978 in LIHC cell lines including Hep3b, SNU423, SNU449 and MHCC-97H, and human normal liver cells LO2 and we observed that LINC00978 was significantly upregulated in LIHC cell lines, compared with LO2 cells (Figure 1C). Collectively, these data suggested that LINC00978 was highly expressed in LIHC, and the high expression of LINC00978 was as-

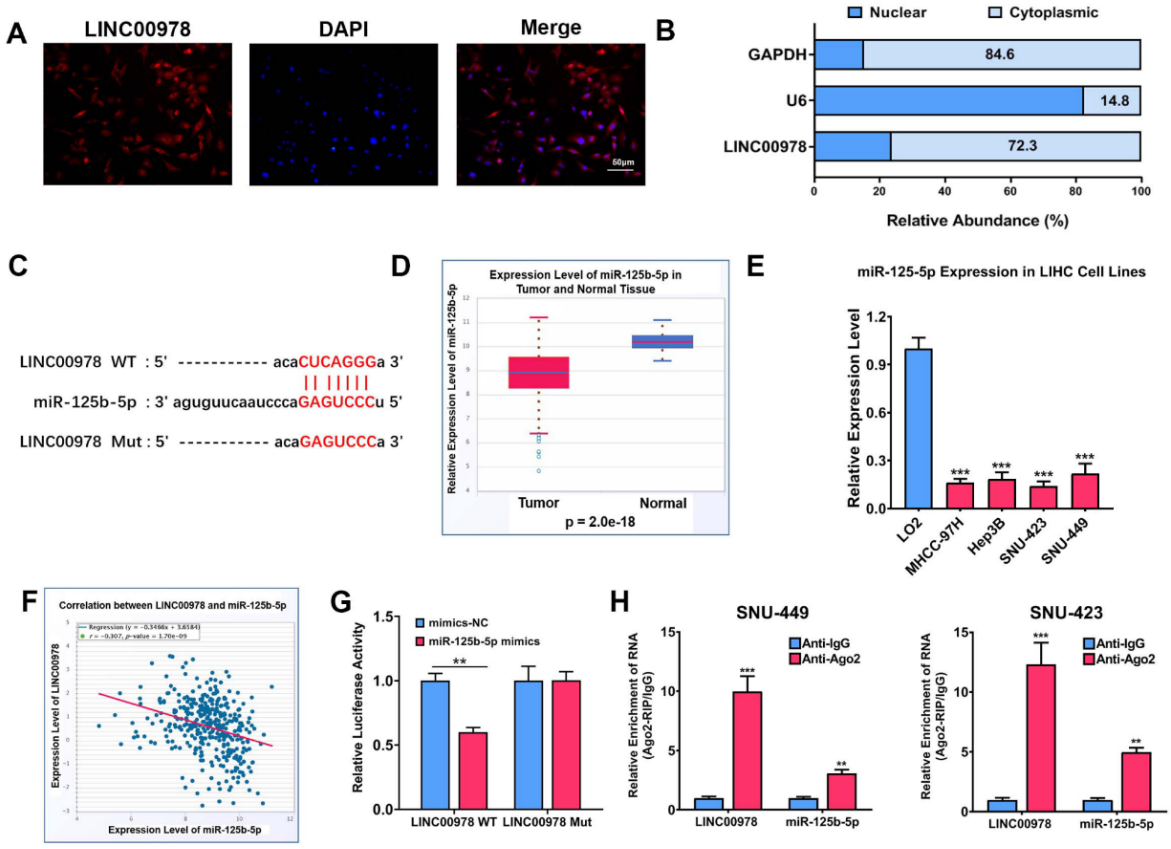

Figure 3. LINC00978 acts as a sponger of miR-125b-5p in LIHC. **A:** FISH was adopted to analyze the subcellular localization of LINC00978 in LIHC cells. **B:** qRT-PCR was used to measure the relative LINC00978 expression levels in the cytoplasmic and nuclear fractions of LIHC cells. **C:** ENCORI database was performed to predict the binding site between LINC00978 and miR-125b-5p. **D:** TCGA database was used to evaluate the different expression of miR-125b-5p in LIHC tissues and normal tissues. **E:** qRT-PCR was used to detect the expression of miR-125b-5p in LIHC cell lines Hep3b, SNU423, SNU449 and MHCC-97H, and human normal liver cells LO2. **F:** The correlation analysis between LINC00978 and miR-125b-5p. Luciferase activity **(G)** and RIP **(H)** assays were employed to evaluate the binding between LINC00978 and miR-125b-5p. **p<0.01, ***p<0.001. Data represent at least three independent sets of experiment.

sociated with poor LIHC prognosis, implying that LINC00978 may be involved in the progression of LIHC.

LINC00978 knockdown represses LIHC cells growth and metastasis

To estimate the functional effect of LINC00978 on LIHC cells, we generated stable LINC00978-silenced SNU423 and SNU449 cells (Figure 2A). CCK-8 (Figure 2B) and colony formation (Figure 2C) results exhibited the inhibited effect by silencing LINC00978 on LIHC cells proliferation. Similarly, LINC00978 depletion could efficiently suppress the migratory and invasive capacities of SNU423 and SNU449 cells (Figure 2D and 2E). Overall, these findings suggested that silencing LINC00978 could remarkably suppress LIHC cells proliferation, migration and invasion.

LINC00978 functions as a ceRNA to sponge miR-125b-5p in LIHC

To reveal the underlying mechanism of LINC00978 in mediating LIHC cells biological function, we first determined the subcellular localization of LINC00978 in LIHC cells using FISH, and observed that LINC00978 was mainly abundant

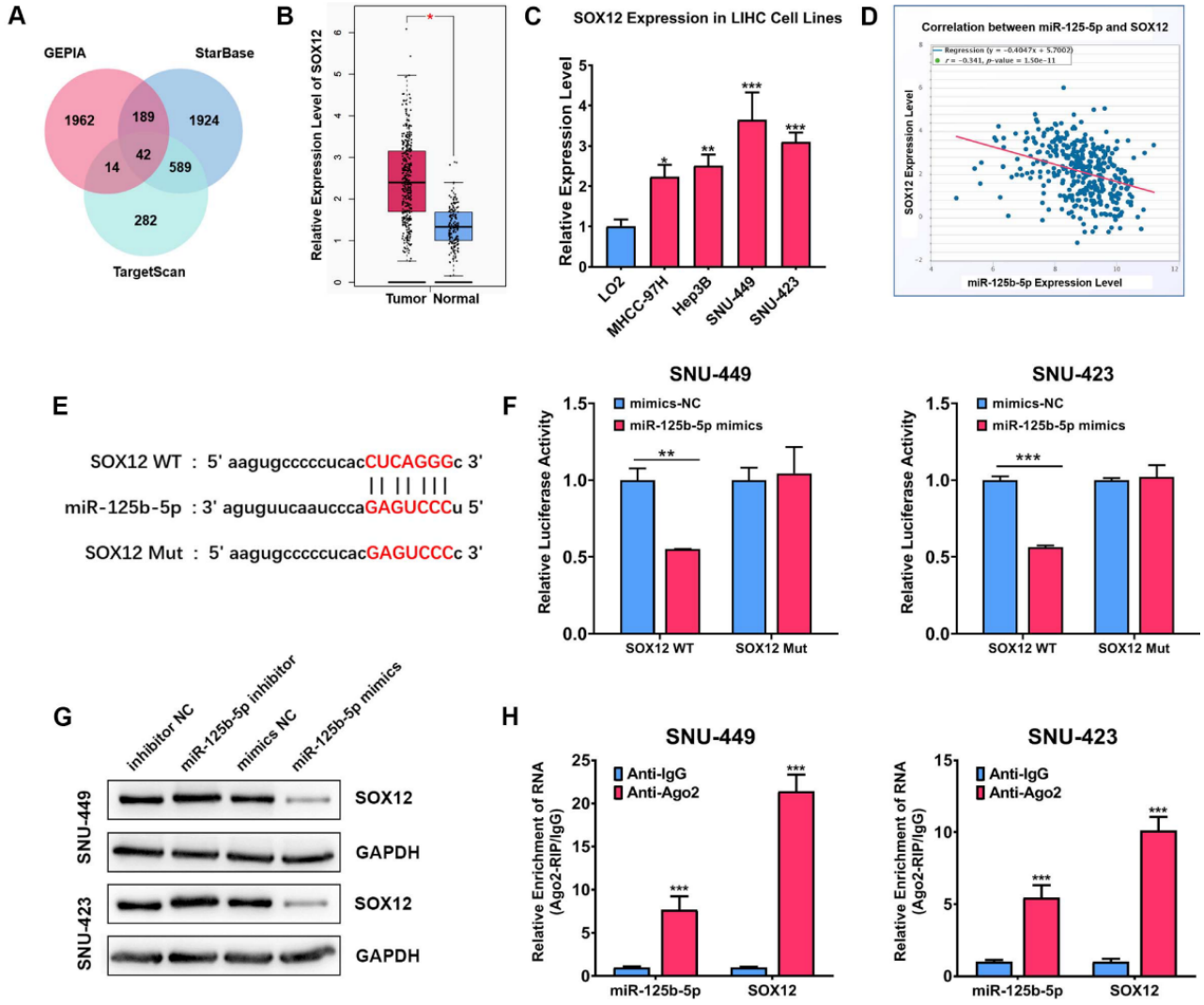

Figure 4. miR-125b-5p negatively mediates the expression of SOX12 in LIHC. **A:** To obtain the potential target gene of miR-125b-5p in LIHC, GEPIA, StarBase and TargetScan databases were performed. **B:** TCGA database was performed to evaluate the different expression of SOX12 in LIHC tissues and normal tissues. **C:** qRT-PCR was used to detect the expression of SOX12 in LIHC cell lines Hep3b, SNU423, SNU449 and MHCC-97H, and human normal liver cells LO2. **D:** The correlation analysis between miR-125b-5p and SOX12. **E:** StarBase database was performed to predict the binding site between miR-125b-5p and SOX12. **F:** Luciferase reporter assay was utilized to analyze the luciferase activity of SOX12. **G:** Western blotting was used to examine the expression of SOX12 in LIHC cells with overexpressing/inhibiting miR-125b-5p. **H:** RIP assay was used to evaluate the binding between LINC00978 and miR-125b-5p. *p<0.05, **p<0.01, ***p<0.001. Data represent at least three independent sets of experiment.

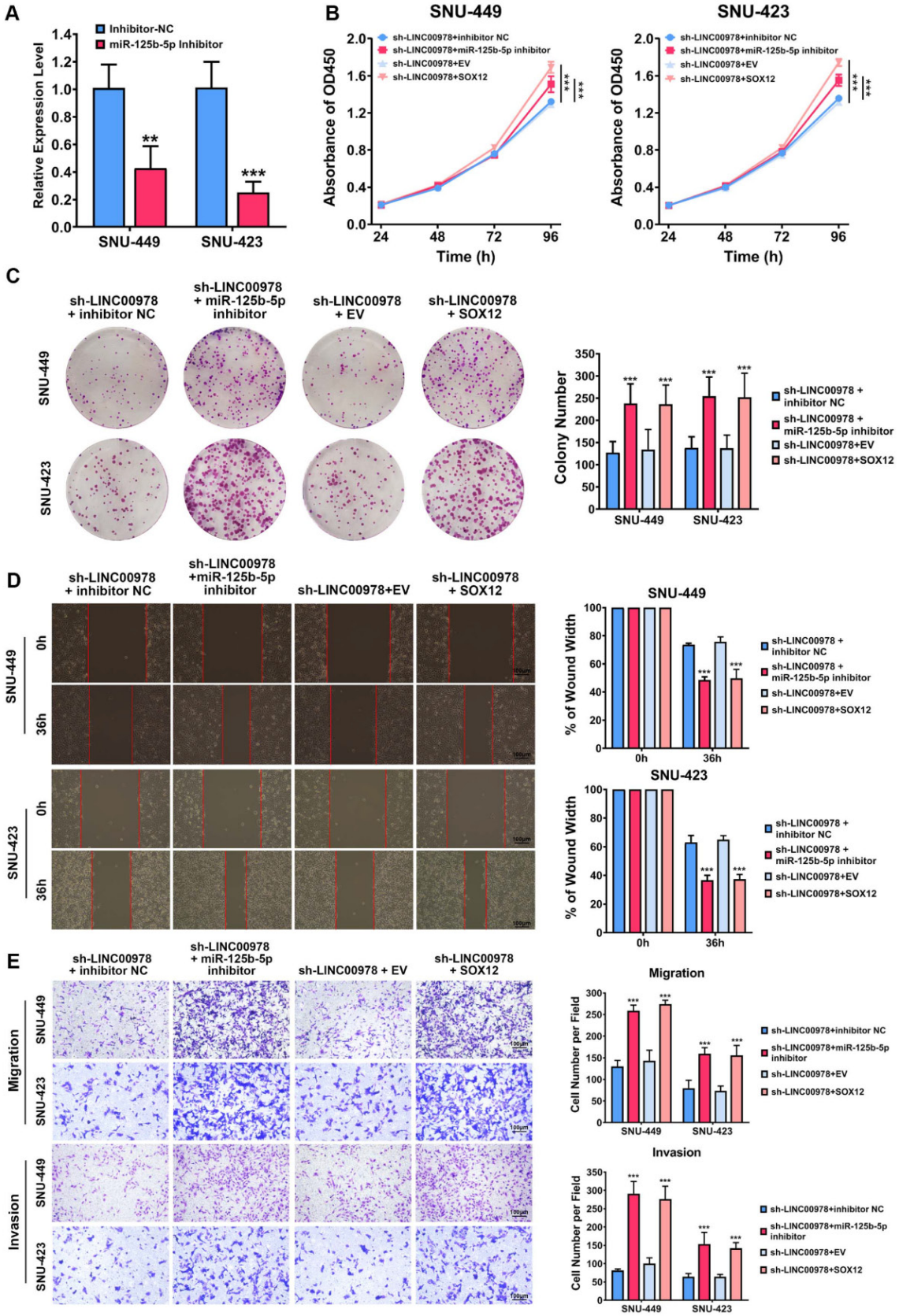

Figure 5. LINC00978 depletion repressed LIHC cells growth and metastasis via miR-125b-5p/SOX12 pathway. **A:** SNU449 and SNU423 cells were transfected with miR-125b-5p inhibitor, and the transfection efficacy was detected using qRT-PCR. Next, SNU449 and SNU423 cells were cotransfected with LINC00978 and miR-125b-5p depletion plasmid/LINC00978 depletion and SOX12 overexpression plasmid. CCK-8 **B:** and colony formation **C:** assays were used to evaluate the proliferation rate of SNU423 and SNU449 cells. Transwell **D:** and wound healing **E:** assays were performed to measure the migratory and invasive capacities. ***p<0.001. Data represent at least three independent sets of experiment.

in the cytoplasm (Figure 3A). Additionally, qRT-PCR assay demonstrated the relative LINC00978 expression levels in the cytoplasmic fractions of LIHC cells up to 72.3% (Figure 3B), implying cytoplasmic LINC00978 may function as a ceRNA via competitively binding target miRNAs.

Subsequently, ENCORI database exhibited a binding site between LINC00978 and miR-125b-5p (Figure 3C). In addition, we observed miR-125b-5p was differently expressed in LIHC tissues and normal tissues based on the TCGA database (Figure 3D), and miR-125b-5p was significantly downregulated in LIHC cell lines compared to LO2 cells (Figure 3E). To further reveal the association between LINC00978 and miR-125b-5p, we performed correlation analysis and found that there was a negative correlation between them (Figure 3F). Furthermore, miR-125b-5p mimic efficiently restricted the luciferase activity of a reporter gene with wild-type, but not mutant LINC00978 3'-UTR (Figure 3G). RIP assay further demonstrated that LINC00978 and miR-125b-5p were considerably enriched in anti-AGO2 microribonucleoprotein complexes in SNU423 and SNU449 cells, compared with that in anti-IgG group (Figure 3H). These results clarified that miR-125b-5p was lowly expressed in LIHC and LINC00978 could sponge miR-125b-5p in LIHC cells.

SOX12 is a target gene of miR-125b-5p

We next explored the potential target gene of miR-125b-5p in LIHC cells. We performed the prediction of intersection genes using GEPIA, StarBase and TargetScan databases (Figure 4A), and found that the intersection gene SOX12 was highly expressed in LIHC tissues (Figure 4B). Consistently, it was validated that SOX12 was upregulated in LIHC cell lines (Figure 4C). Next, we observed miR-125b-5p was negatively associated with the expression level of SOX12 (Figure 4D), and there was a binding site between them (Figure 4E). In addition, dual luciferase reporter assay exhibited that miR-125b-5p mimics obviously decreased the luciferase activity in SOX12-WT plasmid transfected cells ($p<0.01$), while there was no change in cells transfected with SOX12-MUT plasmid (Figure 4F). Furthermore, miR-125b-5p inhibition efficiently enhanced the expression levels of SOX12 in SNU423 and SNU449 cells, while upregulated miR-125b-5p exerted suppressed effect (Figure 4G). RIP assay further clarified that miR-125b-5p and SOX12 were remarkably enriched in anti-AGO2 complexes in SNU423 and SNU449 cells, in comparison with the anti-IgG group (Figure 4H). Taken together, these results implied that miR-125b-5p could negatively regulate SOX12 expression in LIHC.

LINC00978 depletion represses LIHC cells growth and metastasis via miR-125b-5p/SOX12 axis

To validate whether LINC00978 regulates LIHC progression in a miR-125b-5p/SOX12 axis-dependent manner, we respectively downregulated miR-125b-5p in SNU449 and SNU423 cells (Figure 5A), and performed cotransfection with LINC00978 and miR-125b-5p depletion plasmids, as well as LINC00978 depletion and SOX12 overexpression plasmids. CCK-8 (Figure 5B) and colony formation (Figure 5C) assays indicated that downregulated LINC00978 could inhibit the proliferation rate of SNU449 and SNU423 cells, which could be rescued by inhibiting miR-125b-5p and overexpressing SOX12. Similarly, the suppressed effect of silencing LINC00978 on LIHC cells migratory and invasive capacities could be retained by miR-125b-5p inhibition and SOX12 overexpression (Figure 5D and 5E). Taken together, our results demonstrated that the depleted LINC00978 could repress LIHC cells proliferation, migration and invasion though mediating miR-125b-5p/SOX12 axis.

Discussion

In this study, our results illustrated that LINC00978 was highly expressed in LIHC, and the high expression of LINC00978 was associated with poor LIHC prognosis. In addition, LINC00978 depletion repressed LIHC cells growth and metastasis. Moreover, LINC00978 sponged miR-125b-5p in LIHC cells and SOX12 was determined as a target gene of miR-125b-5p. Thus, we revealed the LINC00978/ miR-125b-5p/ SOX12 axis was involved in the regulation of the LIHC progression.

Previous studies have suggested that LINC00978 was an oncogenic LncRNA in diverse cancers, like breast cancer [22], melanoma [23] and bladder cancer [24]. Our data demonstrated that LINC00978 was highly expressed in LIHC, and, in addition, we also revealed that the high expression of LINC00978 was closely associated with poor LIHC prognosis, which was in consistence with previous studies. For example, Deng et al suggested that LINC00978 was highly expressed in breast cancer cell lines, and the high LINC00978 expression indicated poor overall survival [25]. In addition, accumulating studies have validated that LINC00978 significantly contributed to the regulation of cells proliferation, apoptosis, metastasis and inflammation. Specifically, Zhang et al suggested that depleted LINC00978 efficiently repressed the HCC cells proliferation rate, and meanwhile promoted the cells apoptosis and the arrest of cell cy-

cle, while Bu et al [26] reported that downregulating LINC00978 could suppress the cells proliferation and tumor progression in gastric cancer. Consistent with previous reports, we demonstrated that shRNA-mediated LINC00978 knockdown could repress the LIHC cells growth and metastasis.

Apart from mediating DNA, RNA, or proteins, LncRNAs also exerted functional effect though acting as a ceRNA of miRNAs [27]. For instance, LINC00978 could facilitated the progression in breast cancer though acting as a sponger of miR-4288 [22], and Li et al [28] revealed the regulatory mechanism that LINC00978 facilitated cell growth and metastasis in non-small cell lung cancer though sponging miR-6754-5p. In addition, Ma et al [23] reported that LINC00978 significantly promoted the progression of melanoma via recruiting miR-802 from FLOT2. Similarly, herein, we suggested that miR-125b-5p was lowly expressed in LIHC and exposed the underlying mechanism of LINC00978 in regulating the biological function of LIHC cells, founding that LINC00978 could inhibit the expression of miR-125b-5p via acting as its sponger.

As referred above, SOX12 belongs to the SOX family and has been proved to participate in the progression of multiple cancers. In our study, SOX12 was upregulated in LIHC tissues and cells lines, and could be negatively mediated by miR-125b-5p in LIHC cells. In addition, we found that the repressed effect acted by LINC00978 depletion on LIHC cells growth and metastasis could be rescued by inhibiting miR-125b-5p and overexpressing SOX12.

Conclusions

To summarize, our study demonstrated that LINC00978 was upregulated in LIHC and the high expression of LINC00978 indicated poor LIHC prognosis. In addition, depleted LINC00978 could inhibit LIHC cells proliferation, migration and invasion, and we suggested that LINC00978 could sponge miR-125b-5p in LIHC cells and identified SOX12 as the target gene of miR-125b-5p. Moreover, LINC00978 silence could repress LIHC cells growth and metastasis though mediating miR-125b-5p/SOX12 axis. These findings provided the rationale for considering LINC00978/miR-125b-5p/SOX12 axis as a novel therapeutic strategy against progression of LIHC.

Conflict of interests

The authors declare no conflict of interests.

References

1. Chung EM, Lattin GJ, Cube R et al. From the archives of the AFIP: Pediatric liver masses: radiologic-pathologic correlation. Part 2. Malignant tumors. Radiographics 2011;31:483-507.
2. Alzahrani B, Iseli TJ, Hebbard LW. Non-viral causes of liver cancer: does obesity led inflammation play a role? Cancer Lett 2014;345:223-9.
3. Yu SZ, Huang XE, Koide T et al. Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China. Jpn J Cancer Res 2002;93:1287-92.
4. Wild CP, Hall AJ. Primary prevention of hepatocellular carcinoma in developing countries. Mutat Res 2000;462:381-93.
5. Balogh J, Victor DR, Asham EH et al. Hepatocellular carcinoma: a review. J Hepatocell Carcinoma 2016;3:41-53.
6. Villanueva A. Hepatocellular Carcinoma. N Engl J Med 2019;380:1450-62.
7. Liu M, Hu Q, Tu M et al. MCM6 promotes metastasis of hepatocellular carcinoma via MEK/ERK pathway and serves as a novel serum biomarker for early recurrence. J Exp Clin Cancer Res 2018;37:10.
8. Zhang Y, Zhang H, Wu S. LncRNA-CCDC144NL-AS1 Promotes the Development of Hepatocellular Carcinoma by Inducing WDR5 Expression via Sponging miR-940. J Hepatocell Carcinoma 2021;8:333-48.
9. 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.
10. Chen B, Lan J, Xiao Y et al. Long noncoding RNA TP53TG1 suppresses the growth and metastasis of hepatocellular carcinoma by regulating the PRDX4/beta-catenin pathway. Cancer Lett 2021;513:75-89.
11. Xu X, Gu J, Ding X et al. LINC00978 promotes the progression of hepatocellular carcinoma by regulating EZH2-mediated silencing of p21 and E-cadherin expression. Cell Death Dis 2019;10:752.
12. Hua S, Quan Y, Zhan M, Liao H, Li Y, Lu L. miR-125b-5p inhibits cell proliferation, migration, and invasion in hepatocellular carcinoma via targeting TXNRD1. Cancer Cell Int 2019;19:203.
13. Sarkar A, Hochedlinger K. The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. Cell Stem Cell 2013;12:15-30.
14. Du F, Feng W, Chen S et al. Sex determining region Y-box 12 (SOX12) promotes gastric cancer metastasis by upregulating MMP7 and IGF1. Cancer Lett 2019;452:103-18.

15. Dy P, Penzo-Mendez A, Wang H, Pedraza CE, Macklin WB, Lefebvre V. The three SoxC proteins--Sox4, Sox11 and Sox12--exhibit overlapping expression patterns and molecular properties. Nucleic Acids Res 2008;36:3101-17.
16. Bhattaram P, Penzo-Mendez A, Sock E et al. Organogenesis relies on SoxC transcription factors for the survival of neural and mesenchymal progenitors. Nat Commun 2010;1:9.
17. Guo B, Xiao C, Liu Y et al. miR-744-5p Inhibits Multiple Myeloma Proliferation, Epithelial Mesenchymal Transformation and Glycolysis by Targeting SOX12/Wnt/beta-Catenin Signaling. Onco Targets Ther 2021;14:1161-72.
18. Xu J, Zhang J, Li L, Mao J, You T, Li Y. SOX12 expression is associated with progression and poor prognosis in human breast cancer. Am J Transl Res 2020;12:8162-74.
19. Wu B, Yang C, Fang Y, Ding W, Zhang Y. Long noncoding RNA DUXAP10 promotes the stemness of glioma cells by recruiting HuR to enhance Sox12 mRNA stability. Environ Toxicol 2021;36:840-9.
20. Wang Y, Ma DL, Yu CH, Sha KF, Zhao MJ, Liu TJ. MicroRNA-370 suppresses SOX12 transcription and acts as a tumor suppressor in bladder cancer. Eur Rev Med Pharmacol Sci 2020;24:2303-12.
21. Yuan P, Meng L, Wang N. SOX12 upregulation is associated with metastasis of hepatocellular carcinoma and increases CDK4 and IGF2BP1 expression. Eur Rev Med Pharmacol Sci 2017;21:3821-6.
22. Chen D, Tang P, Wang Y et al. Downregulation of long non-coding RNA MR4435-2HG suppresses breast cancer progression via the Wnt/beta-catenin signaling pathway. Oncol Lett 2021;21:373.
23. Ma DM, Sun D, Wang J, Jin DH, Li Y, Han YE. Long non-coding RNA MIR4435-2HG recruits miR-802 from FLOT2 to promote melanoma progression. Eur Rev Med Pharmacol Sci 2020;24:2616-24.
24. Wang W, Xu Z, Wang J, Chen R. LINC00978 promotes bladder cancer cell proliferation, migration and invasion by sponging miR4288. Mol Med Rep 2019;20:1866-72.
25. Deng LL, Chi YY, Liu L, Huang NS, Wang L, Wu J. LINC00978 predicts poor prognosis in breast cancer patients. Sci Rep 2016;6:37936.
26. Bu JY, Lv WZ, Liao YF, Xiao XY, Lv BJ. Long noncoding RNA LINC00978 promotes cell proliferation and tumorigenesis via regulating microRNA-497/NTRK3 axis in gastric cancer. Int J Biol Macromol 2019;123:1106-14.
27. Chen R, Xia W, Wang S et al. Long Noncoding RNA SBF2-AS1 Is Critical for Tumorigenesis of Early-Stage Lung Adenocarcinoma. Mol Ther Nucleic Acids 2019;16:543-53.
28. Li X, Ren Y, Zuo T. Long noncoding RNA LINC00978 promotes cell proliferation and invasion in nonsmall cell lung cancer by inhibiting miR67545p. Mol Med Rep 2018;18:4725-32.