LncRNA SNHG8 Promotes Liver Cancer Proliferation and Metastasis by Sponging miR-542-3p and miR-4701-5p

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Research

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Abstract

Growing evidence suggests that long non-coding RNAs (lncRNAs) are associated with carcinogenesis and could function as competing endogenous RNAs (ceRNAs) to regulate microRNAs (miRNAs). LncRNA small nucleolar RNA host gene 8 (LncRNA SNHG8) is up-regulated in various cancers and positively associated with poor prognosis of these cancers. However, the molecular mechanisms by which lncRNA SNHG8 contributes to hepatocellular carcinoma (HCC) still remains unclear. In the present study, we reported that lncRNA SNHG8 was abnormally up-regulated in liver cancer tissues and HCC cell lines. Moreover, knockdown of lncRNA SNHG8 significantly attenuated the proliferation, migration, invasion process of HCC cell line HepG2 in vitro. Mechanistically, we first reported that suppression of lncRNA SNHG8 evidently enhanced miR-542-3p and miR-4701-5p expression and decreased TET3 expression at posttranscriptional level. Furthermore, lncRNA SNHG8 upregulated TET3 expression by sponging miR-542-3p and miR-4701-5p by competing binding. Taken together, our results confirmed the oncogene role of lncRNA SNHG8 and discovered the underlying mechanism that lncRNA SNHG8 upregulated TET3 through the sponging or decaying of miR-542-3p and miR-4701-5p in human hepatocellular carcinoma, suggesting that lncRNA SNHG8 may serve as a potential diagnostic marker and therapeutic target for patients with hepatocellular carcinoma.

1 Introduction

Hepatocellular carcinoma (HCC), one of the most common malignancies worldwide, has a high incidence and high mortality rate and is especially difficult to discover during the early stages. With the characteristics of invasion, metastasis, and frequent recurrence, HCC accounts for 70% – 90% of primary liver cancer and has been a major public health problem in a global context(Forner et al., 2012; Kuo, 2009). Therefore, exploring the detailed molecular mechanism for HCC and identifying novel therapeutic targets is necessary for prevention and treatment.

To date, a large set of non-coding RNAs (ncRNAs) have been recognized as controlling almost every level of gene expression and pathway activation, including the activation and repression of the EMT process(Beermann et al., 2016; Z. Zhao et al., 2017). Among them, lncRNA is defined as transcripts containing secondary structures larger than 200 bp. The sequence of lncRNA is long, so it has the potential to form a variety of complex conformations. Structural studies have found that the following three mechanisms in the lncRNA system may exist(Harrow et al., 2012): One is that RNA silencing sequences and interference can specifically regulate gene expression; ribose Somatic switch RNA regulates gene expression through secondary structure; ribosome synthesizes protein through complex tertiary structure. lncRNA may regulate gene expression in any one or all of the following three ways. Similarly, lncRNA RACGAP1P promotes breast cancer invasion and metastasis via miR-345-5p/RACGAP1-mediated mitochondrial fission(Zhou et al., 2021). LncRNA 00665 promotes melanoma cell growth and migration via regulating the miR-224-5p/VMA21 axis(X. Wang et al., 2020).

LncRNA not only participates in the normal biological functions of the body, including aging, cardiovascular tissue homeostasis and oxidative stress, etc(Kopp & Mendell, 2018; Lim et al., 2019; Y. Zhao et al., 2016), but also participates in or affects many disease processes including neurodegenerative and inflammation(Abdelmohsen et al., 2014). A large number of studies have focused on the role and mechanism of lncRNA in cancer. Some lncRNA acts in the nucleus and affects ANRIL (CDKN2B-as1) and other proximal localization gene expression, mediate the epigenetic silencing of two genes CDKN2A and CDKN2B at the same site to induce cell proliferation(Michalik et al., 2014); HOTAIR by targeting away from transcription sites such as the genes in the HOXD cluster promote the metastasis of breast cancer through the epigenetic silencing of the PRC2 complex(Loewer et al., 2010); Other nuclear lncRNAs play a role in post-transcription, such as ZEB2 NAT blocks ZEB2 mRNA splicing and promotes internal ribosome entry The translation of the corresponding site is initiated, and a high level of ZEB2 protein is transmitted, thereby inducing EMT(Feng et al., 2018); Conversely, some cytoplasmic lncRNA can be used as a molecular sponge or miRNA of miRNA. For example, PTENP1 binds to microRNA, and miRNA binds to the 3'UTR of PTEN mRNA, thereby reducing its expression and cancer suppressing activity (Spurlock et al., 2014). It is also found in breast cancer that StarD13 3'UTR exerts the ceRNA function of TP53INP1 by regulating the activity of miR-125b, preventing the migration and invasion of breast cancer cells(Kotake et al., 2011). Accumulating evidence has shown that lncRNAs play a critical role in tumor occurrence, invasion, metastasis and drug resistance by acting as a competitive RNA (ceRNA) for microRNAs (miRNAs)(Tan et al., 2018; Xie et al., 2021).

Dysfunction and deregulation of miRNAs exert a critical role in the initiation and progression of human cancers. miR-542-3p is previously found to be a cancer-related miRNA in tumor. MiR-542-3p inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting UBE3C(Tao et al., 2017). MiR-542-3p inhibits colorectal cancer cell proliferation, migration and invasion by targeting OTUB1(Yuan et al., 2017). Downregulation of miR-542-3p promotes cancer metastasis through activating TGF-β/Smad signaling in hepatocellular carcinoma(T. Zhang et al., 2018). SLCO4A1-AS1 promotes cell growth and induces resistance in lung adenocarcinoma by modulating miR-4701-5p/NFE2L1 axis to activate WNT pathway(Wei et al., 2020). Alpha-2, 3-sialyltransferases regulate the multidrug resistance of chronic myeloid leukemia through miR-4701-5p targeting ST3GAL1(Li et al., 2016).

Members of the ten-eleven translocation (TET) gene family, including TET3, play a role in the DNA methylation process(Langemeijer et al., 2009). By using ICGC database(Klonowska et al., 2016), we performed GO analysis, and showed that TET3 is involved in 5-MC catabolic process, DNA demethylation and epigenetic regulation of gene expression. Increased expression of TET3 predicts unfavorable prognosis in patients with ovarian cancer-a bioinformatics integrative analysis(Cao et al., 2019). TET3 expression was elevated with lipopolysaccharide stimulation via p38/ERK-MAPK pathway in ESCC and negatively correlated with patients' survival. TET3 induced the stemness of ESCC cells(Xu et al., 2020). Our results indicated that TET3 declined in TGF-β1 stimulation and TET3 overexpression inhibited TGF-β1-induced EMT and EMT-mediated metastasis of SKOV3 and 3AO cells by demethylating miR-30d precursor gene, indicating a novel mechanism of epigenetic regulation in ovarian cancer. Targeting the

TGF-β1-TET3-miR-30d signaling axis might be a promising therapeutic strategy for ovarian cancer treatment(Ye et al., 2016). No studies have reported the role of miR-542-3p targeting TET3 in liver cancer.

In the present study, we found that lncRNA SNHG8 was up-regulated in human liver cancer cell lines (HepG2, SK-Hep-1 and MHCC97H) and the abnormal expression of SNHG8 was found to be closely related to the development and progression of HCC. Knocking down lncRNA SNHG8 reduced the migration and invasion ability of HepG2 cells. The restoration of lncRNA SNHG8 was suggested to promote cell metastasis and reverse the EMT phenotype. Additionally, the bioinformatics database revealed that lncRNA SNHG8 could bind to miR-542-3p and miR-4701-5p, which was confirmed by the dual luciferase reporter assay. We also provided evidence that lncRNA SNHG8 promoted growth and invasion of human hepatocellular carcinoma cells via sponging miR-542-3p and miR-4701-5p to regulate TET3 probably and it represents a potential target for the treatment of hepatocellular carcinoma.

2 Materials And Methods

2.1 Cell culture

The human hepatoma cell lines SK-hep1, HepG2, MHCC97H, MHCC97L were used in this study and routinely cultured in DMEM (HyClone, USA). The immortalized normal liver epithelial cell line HL7702 was cultured in RPMI-1640 medium (Biological Industries, USA). Cancer cells and normal cell were supplemented with 10% fetal bovine serum (FBS; Biological Industries, USA) and 1% penicillin/streptomycin (Sigma, USA). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 .

2.2 Transfection

Two short hairpin RNAs (shRNAs) targeting lncRNA SNHG8 (sh lncRNA SNHG8-1 and sh lncRNA SNHG8-2) and negative control (sh-Ctrl) and miR-542-3p/miR-4701-5p inhibitor/mimics and its negative control (miR-NC) were purchased from GenePharma (Suzhou, China). The sequences for sh lncRNA SNHG8 were as follows: sh lncRNA SNHG8-1: 5'-GGTAATGGGCGAAGTTTATTC-3'; sh lncRNA SNHG8-2: 5'-GGTGGTCCGTGATAATTAAAT – 3'. Cells were transfected using Lipofectamine 2000 Reagent (Invitrogen, USA).

2.3 quantitative real-time PCR

Total RNA was isolated from cultured cells to verity the mRNA expression level in HCC cell lines. The relative mRNA levels were determined using the comparative Ct method with β-actin or U6 as the reference gene, and the formula 2 -ΔΔCt.

2.4 Cell proliferation assay

The cell proliferation was determined using MTS. After incubation for 24 h, 48 h, 72 h, and 96 h, 20 uL of MTS reagent was added incubation for three hours and determined at a wavelength of 490 nm.

2.5 Cell cycle assay

The cell suspension was diluted to 5×10^6 cells/mL; the supernatant was removed and added 70% 500 μL of cold ethanol was placed in a refrigerator at 4°C for 2 h; the cell pellet was added with 100 μL RNaseA and placed in a 37°C water bath for 30 min; PI staining of buffer, avoid light for 30 min at 4°C; The flow cytometer detects and records the red fluorescence at 488 nm.

2.6 Wound healing assay

Transfected HepG2 cells were placed in 6-well plates. When cells grow to 90%-95% confluence, the cells monolayers were wounded by scratching with plastic micropipette tips and washed 2 times with PBS. Images of the different stages of wound healing were photographed via microscopy at 0 h, 24 h and 48 h. Relative cell motility was quantified using Image-Pro Plus.

2.7 Transwell migration and invasion assay

Cells were transfected as described above for 48h. For migration and invasion assays, cells were seeded in the upper chamber of transwell plates (Corning, USA) and serum-free medium with 10% FBS medium was added to the lower chamber of the transwell. After 24 h for the migration assay and 48 h for the invasion assay, cells that have migrated or invaded through the membrane were stained with crystal violet and counted.

2.8 Luciferase reporter assay

The vectors of lncRNA SNHG8 wild type (GP-miRGLO-lncRNA SNHG8-W) or mutant (GP-miRGLO-lncRNA SNHG8-M) were framed and co-transfected, respectively, with miR-542-3p/miR-4701-5p mimics or corresponding control (miR-NC). After 48 hours, we used Dual-Luciferase Reporter Assay System (Promega) to measure luciferase activity differences.

2.9 RNA-seq library preparation and sequencing

To further verify the function of lncRNA SNHG8 to promote the migration and invasion of cancer cells, we knocked down lncRNA SNHG8 in HepG2 cells and used it as a control group to create an RNA library for each phenotype and perform RNA-sEq. The differentially expressed lncRNAs that displayed a > 1.5-fold change in expression and $p < 0.01$ based on at least two pairwise comparisons with the same trend were selected for further examination in the test phase.

2.10 Statistical analysis

The statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 21.0. Difference between two groups were compared by independent-sample t-test. $P < 0.05$ was considered to be statistically significant.

3 Results

3.1 LncRNA SNHG8 expression was up-regulated in HCC tissues and cell lines

In order to identify the role of lncRNA SNHG8 in cancer, the expression level of lncRNA SNHG8 in the TCGA database were analyzed (Fig. 1A). The expression level of lncRNA SNHG8 was noticeably increased in hepatocellular carcinoma tissues as compared with the adjacent normal tissues as showed by The Cancer Genome Atlas (TCGA) database analysis (Fig. 1B). Notably, the expression level of lncRNA SNHG8 was significantly up-regulated in hepatocellular carcinoma cell lines HepG2, SK-Hep-1, Huh7, and MHCC97H compared with normal human liver cell line HL-7702 (Fig. 1C). To further explore the biological function of lncRNA SNHG8 in HCC, we performed GO enrichment analysis on the up-regulated genes affected by lncRNA SNHG8 and found that the genes upregulated by lncRNA SNHG8 plays a vital role in cell proliferation and migration (Fig. 1D). This indicates that lncRNA SNHG8 may function as an oncogene in HCC carcinogenesis.

3.2 LncRNA SNHG8 knockdown reduced cell proliferation,migration and invasion in HepG2 cells

To further investigate the biological function of lncRNA SNHG8 in regulating cancer cell phenotypes, gene knockdown study was performed in HepG2 cell line. We purchased shRNAs targeting lncRNA SNHG8 (sh lncRNA SNHG8-1 and shlncRNA SNHG8-2) was designed and transfected into the HepG2 cells. lncRNA SNHG8 was knocked down effectively confirmed by RT-PCR as showed in Fig. 2A(Fig. 2A). Depletion of lncRNA SNHG8 in HepG2 cells resulted in significant inhibition of cell proliferation as showed by MTS assays (Fig. 2B). Moreover, knockdown of lncRNA SNHG8 inhibited the cell cycle progression in HepG2 cells showed by cell cycle analysis (Fig. 2C). Compared with the control group, the proportion of G0/G1 cells in the sh lncRNA SNHG8 group was significantly increased, and the proportion of cells in the S phase was decreased (Fig. 2C). Furthmore, knockdown of lncRNA SNHG8 suppressed the migratory ability of HepG2 cells as shown in wound healing assay (Fig. 2D) and the invasive activity of HepG2 cells as showed in transwell assay (Fig. 2E,F). To conclude, knockdown of lncRNA SNHG8 functionally inhibited proliferation and migration of hepatocellular carcinoma cell line HepG2 cells, therefore, lncRNA SNHG8 must play an important role in promoting proliferation and the progression of malignant hepatocellular carcinoma.

3.3 Differentially expressed genes in lncRNA SHNG8 knockdown HepG2 cells

To illustrate the the gene profiles regulated by lncRNA SHNG8, we performed RNA-seq using HepG2 and lncRNA-SHNG8 knockdown HepG2 cells. There were 5142 differentially expressed genes between HepG2 and lncRNA-SHNG8 knockdown HepG2 cells analyzed by Cutdiff v2.1.1 software (Fig. 3A). In lncRNA-SHNG8 knockdown HepG2 cells group, there were 2791 up-regulated and 2351 down-regulated genes compared to HepG2 cells without knockdown by volcano plot analysis (Fig. 3B). To confirm the accuracy

of the RNA-seq sequencing data, 3 randomly selected mRNAs were validated by qPCR. The qPCR results were in consistent with those of the sequencing data (Fig. 3C). In order to further confirm the genes affected by lncRNA SNHG8 in HepG2, Gene cluster analysis of the DEGs were performed. P53 related genes were significantly up-regulated (Fig. 3D) and genes associated with proliferation, migration and invasion were significantly down-regulated in lncRNA-SHNG8 knockdown HepG2 cells group (Fig. 3E, F). To conclude, lncRNA SNHG8 may function as a potient oncology gene through inhibiting the tumor suppressor p53 related gene expression and promot the proliferation, migration and invasion of hepatocellular carcinoma.

3.4 LncRNA SNHG8 acted as a sponge of miR-542-3p/miR-4701-5p respectively in HepG2 cells

Mechanically, lncRNA SNHGs can act as miRNA sponges by binding to miRNAs using their complementary sequence to prevent the binding of miRNAs to their target mRNA therefore inhibit function of miRNA. For example, lncRNA SNHG1/5/6/12 promotes tumor progression by binding different miRNAs such as miR-101-3p, miR-32, miR-199a/b(Cui et al., 2017; Lan et al., 2017; J. Z. Wang et al., 2017; L. Zhao et al., 2017), and miR-101(Chang et al., 2016; Yan et al., 2017), to prevent binding of these miRNAs to target mRNA and inhibit function of miRNA. To clarify the molecular mechanism underlying the sponge role of lncRNA SNHG8 in hepatocellular carcinoma, we used Starbase 3.0 (http://starbase.sysu.edu.cn/ago) to identify 28 miRNAs that could bind to lncRNA SNHG8. According to the AGO-Clip-seq data, we found that miR-542-3p and miR-4701-5p had the highest binding affinity with lncRNA SNHG8 (Fig. 4A). To further confirm that lncRNA SNHG8 served as a sponge of miR-542-3p and miR-4701-5p, we co-transfected the wild type or mutated lncRNA SNHG8 reporter and the miR-542-3p and miR-4701-5p in the HepG2 cell lines. Luciferase gene reporter assays showed miR-542-3p and miR-4701-5p bind to the 3'UTR of lncRNA SNHG8 (Fig. 4B). Further testing found that miR-542-3p and miR-4701-5p daughter hepatocellular carcinoma cells were down-regulated (Fig. 4C). RT-PCR results showed that miR-542-3p and miR-4701-5p expression was up-regulated when lncRNA SNHG8 was knockdown (Fig. 4D). On the contrary, whether it was transfected with miR-542-3p and miR-4701-5p mimic or miR-542-3p and miR-4701-5p inhibitor, lncRNA SNHG8 remained unchanged (Fig. 4E). To conclude, lncRNA SNHG8 bound to miR-542-3p and miR-4701-5p as a sponge and decay factor to promote the degradation of miR-542-3p and miR-4701-5p.

3.5 MiR-542-3p/miR-4701-5p promoted cell proliferation migration and invasion abilities in HepG2 cells

HepG2 cells were transfected of miR-542-3p and miR-4701-5p mimics and inhibitors. The successful transfection of miR-542-3p and miR-4701-5p mimics and inhibitors was verified by qRT-PCR (Figure 5A,F). miR-542-3p significantly inhibited the proliferation of HepG2 cells as showed by MTS assay Figure 5B. However, both miR-4701-5p mimics or inhibitors had no significant difference in proliferation of HepG2 cells Figure 5G. Furthermore, miR-542-3p and miR-4701-5p mimics significantly decreased migration

while miR-542-3p and miR-4701-5p inhibitors promoted migration in HepG2 cells as showed by wound healing assay (Fig. 5C, H). Transwell migration and invasion assay also revealed that miR-542-3p and miR-4701-5p mimics significantly decreased invasion while miR-542-3p and miR-4701-5p inhibitors promoted invasion in HepG2 cells as showed by wound healing assay (Fig. 5D, E,I,J). To conclude, miR-542-3p and miR-4701-5p inhibited HepG2 cell proliferation, migration and invasion, but its inhibitor reversed these effects.

3.6 Inhibition of miR-542-3p/miR-4701-5p reversed silencing lncRNA SNHG8 induced suppression of malignant phenotype in HepG2 cells

LncRNA SNHG8 is a potential oncogene indicated by our study so far. Knockdown of LncRNA SNHG8 reduces cell proliferation, migration and invasion in hepatocellular carcinoma cell line HepG2 cells as showed in Fig. 2. Actually, miR-542-3p and miR-4701-5p expression was up-regulated when lncRNA SNHG8 was knockdown (Fig. 4D) because LncRNA SNHG8 acted as a sponge/decay factor of miR-542-3p and miR-4701-5p in HepG2 cells as showed in Fig. 4. We speculated that it was the upregulated miR-542-3p and miR-4701-5p indeed carried out the inhibition of cell proliferation, migration and invasion in the LncRNA SNHG8 knockdown experiment. To confirm, we further knocked down of miR-542-3p and miR-4701-5p in the lncRNA SNHG8 knockdowned HepG2 cells by miR-542-3p and miR-4701-5p inhibitor. Further knockdown of upregulated miR-542-3p and miR-4701-5p dismissed the inhibition of the sh-lncRNA SNHG8 on the proliferation, migration, invasion of HepG2 cells (Fig. 6A,B,C,D, F,G,H,I). To conclude, potential oncogene LncRNA promoted the proliferation, migration, invasion of HepG2 cells through sponging and inhibiting the function of miR-542-3p and miR-4701-5p.

In addition, lncRNA SNHG8 knockdown increased the expression of E-cadherin and decreased the expression of N-cadherin and knockdown of miR-542-3p and miR-4701-5p attended this phenomena by reducithing the expression of E-cadherin and increasing the expression of N-cadherin (Fig. 6E, J).

3.7 LncRNA SNHG8 upregulated TET3 expression via sponging miR-542-3p and miR-4701-5p in HepG2 cells

According to the bioinformatics analysis (TargetScan 6.2 and miRanda), TET3 was confirmed as a potential target of miR-542-3p and miR-4701-5p. The 3'UTR of TET3 gene contained the complementary sequence of miR-542-3p and miR-4701-5p (Fig. 7A). In addition, we performed quantitative RT-PCR (qRT-PCR) to validate the expression levels of miR-542-3p and miR-4701-5p. Our data showed that, TET3/ E2F2 were negatively correlated with both miR-542-3p and miR-4701-5p (Fig. 7B). The expression of TET3/ E2F2 was significantly up-regulate in hepatocellular carcinoma tissue (Fig. 7C)and in HepG2 cells (Fig. 7D). RT-PCR assays were used to explore whether TET3 are regulated by lncRNA SNHG8. The results showed that the expression of lncRNA SNHG8 was positively correlated with expression of TET3/ E2F2 in HCC cell as showed by RT-PCR (Fig. 7E) and transcriptome data (Fig. 7F). lncRNA SNHG8 upregulated

TET3 expression by compete binding and inhibiting miR-542-3p and miR-4701-5p, hence, promoted malignancy of hepatocellular carcinoma cells in vitro as shown in Fig. 7G.

Discussion

Growing evidence has suggested that long non-coding RNAs (lncRNAs) play a key role in the morbidity and progression of cancer("Integrated Genomic and Molecular Characterization of Cervical Cancer.," 2017). LncRNA SNHG8 was identified as an oncogene in the development of various cancers, including osteosarcoma, cervical cancer and ovarian carcinoma(Miao et al., 2020; Qu et al., 2020; Zhong et al., 2020). In our study, we reported that lncRNA SNHG8 was significantly up-regulated in in hepatocellular carcinoma tissues (Figure 1B) and in hepatocellular carcinoma cell lines HepG2, SK-Hep-1, Huh7, and MHCC97H (Figure 1C). Then, we found that knocking down of lncRNA SNHG8 expression in HepG2 cells resulted in significant inhibition of cell proliferation (Figure 2B), cell cycle progression (Figure 2C), cell migratory (Figure 2D) and cell invasive activity in HepG2 cells. lncRNA SNHG8 plays an important role in promoting proliferation and the malignant hepatocellular carcinoma.

Notably, massive articles have pointed that lncRNAs regulate miRNAs through a 'miRNA sponge' mechanism as a competing endogenous RNA (ceRNA)(Fan et al., 2018; Huang, 2018). For instance, lncRNA DDX11-AS1 facilitates gastric cancer progression by regulating miR-873-5p and SPC18 axis(Ren et al., 2020). Mechanically, lncRNA SNHGs can act as miRNA sponges by binding to miRNAs using their complementary sequence to prevent the binding of miRNAs to their target mRNA therefore inhibit function of miRNA. We revealed that lncRNA SNHG8 acted as a microRNA sponge targeting miR-542-3p and miR-4701-5p (Figure 4A). (Figure 4B) (Figure 4C).

Actually, miR-542-3p has been pointed to act as a suppressor in several cancers such as prostate cancer, ovarian cancer(Sun et al., 2020; C. Zhang et al., 2020) and chronic myeloid leukemia(Williams et al., 2016), but its functions and mechanism in hepatocellular carcinoma is unclear. Here, we found that miR-542-3p and miR-4701-5p was down-regulated in HepG2 cells and sponged by lncRNA SNHG8. Actually, miR-542-3p and miR-4701-5p expression was up-regulated when lncRNA SNHG8 was knockdown (Figure 4D). Most important, miR-542-3p significantly inhibited the proliferation of HepG2 cells (Figure 5B and miR-542-3p and miR-4701-5p significantly decreased migration of HepG2 cells (Figure 5C, H (Figure 5D, E,I,J). Thus, in hepatocellular carcinoma, lncRNA SNHG8 bound to miR-542-3p and miR-4701-5p as a sponge and decay factor to promote the degradation of miR-542-3p and miR-4701-5p and promoted the malignecy of hepatocellular carcinoma through.

We further confirm that lncRNA SNHG8 promoted the malignecy of hepatocellular carcinoma through sponging and promoting the degradation of miR-542-3p and miR-4701-5p. We further knocked down upregulated miR-542-3p and miR-4701-5p in the lncRNA SNHG8 knockdowned HepG2 cells by miR-542-3p and miR-4701-5p inhibitor. Further knockdown of upregulated miR-542-3p and miR-4701-5p dismished the inhibition of the sh-lncRNA SNHG8 on the proliferation, migration, invasion of HepG2 cells (Figure 6A,B,C,D, F,G,H,I). All together, we further confimed that potential oncogene LncRNA promoted the

proliferation, migration, invasion of HepG2 cells through sponging and inhibiting the function of miR-542-3p and miR-4701-5p.

Subsequently, we revealed TET3 was the downstream targets of miR-542-3p and miR-4701-5p. Increased expression of TET3 can predict unfavorable prognosis in patients with ovarian cancer(Cao et al., 2019). Interestingly, the expression of TET3 was significantly up-regulate in hepatocellular carcinoma (Figure 7C) and in HepG2 cells (Figure 7D) and positively correlated with expression of lncRNA SNHG8 (Figure 7E). The expression of TET3 was inhibited by miR-542-3p and miR-4701-5p (Figure 7B). Furthermore, lncRNA SNHG8 upregulated TET3 expression via sponging miR-542-3p, thereby enhances the tumorigenesis in hepatocellular carcinoma (Figure 7A, B). Thus, lncRNA SNHG8 upregulated TET3 expression by compete binding and inhibiting miR-542-3p and miR-4701-5p, hence, promoted malignancy of hepatocellular carcinoma cells.

In conclusion, our data revealed lncRNA SNHG8 acted as competitive endogenous RNAs (ceRNAs) to bind miR-542-3p and miR-4701-5p and increase the expression of TET3, therefore, promoted the malignancy of hepatocellular carcinoma.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HS and TH performed the experiments and collected the data; TH wrote the manuscript; TH and LKX provided suggestions and comments on the project and manuscript preparation; WCS and LPX participated in the project design and provided funds. TH and WCS participated in the manuscript preparation and revision. All authors read and approved the final manuscript.

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References

Abdelmohsen, K., Panda, A. C., Kang, M.-J., Guo, R., Kim, J., Grammatikakis, I., Yoon, J.-H., Dudekula, D. B., Noh, J. H., Yang, X., Martindale, J. L., & Gorospe, M. (2014). 7SL RNA represses p53 translation by competing with HuR. *Nucleic Acids Research*, *42*(15), 10099–10111. https://doi.org/10.1093/nar/gku686

Beermann, J., Piccoli, M.-T., Viereck, J., & Thum, T. (2016). Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. *Physiological Reviews*, *96*(4), 1297–1325. https://doi.org/10.1152/physrev.00041.2015

Cao, T., Pan, W., Sun, X., & Shen, H. (2019). Increased expression of TET3 predicts unfavorable prognosis in patients with ovarian cancer-a bioinformatics integrative analysis. *Journal of Ovarian Research*, *12*(1), 101. https://doi.org/10.1186/s13048-019-0575-4

Chang, L., Yuan, Y., Li, C., Guo, T., Qi, H., Xiao, Y., Dong, X., Liu, Z., & Liu, Q. (2016). Upregulation of SNHG6 regulates ZEB1 expression by competitively binding miR-101-3p and interacting with UPF1 in hepatocellular carcinoma. *Cancer Letters*, *383*(2), 183–194. https://doi.org/10.1016/j.canlet.2016.09.034

Cui, Y., Zhang, F., Zhu, C., Geng, L., Tian, T., & Liu, H. (2017). Upregulated lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of Wnt/β-

catenin signaling pathway. *Oncotarget*, *8*(11), 17785–17794. https://doi.org/10.18632/oncotarget.14854

Fan, C.-N., Ma, L., & Liu, N. (2018). Systematic analysis of lncRNA-miRNA-mRNA competing endogenous RNA network identifies four-lncRNA signature as a prognostic biomarker for breast cancer. *Journal of Translational Medicine*, *16*(1), 264. https://doi.org/10.1186/s12967-018-1640-2

Feng, L., Liao, Y.-T., He, J.-C., Xie, C.-L., Chen, S.-Y., Fan, H.-H., Su, Z.-P., & Wang, Z. (2018). Plasma long non-coding RNA BACE1 as a novel biomarker for diagnosis of Alzheimer disease. *BMC Neurology*, *18*(1), 4. https://doi.org/10.1186/s12883-017-1008-x

Forner, A., Llovet, J. M., & Bruix, J. (2012). Hepatocellular carcinoma. *Lancet (London, England)*, *379*(9822), 1245–1255. https://doi.org/10.1016/S0140-6736(11)61347-0

Harrow, J., Frankish, A., Gonzalez, J. M., Tapanari, E., Diekhans, M., Kokocinski, F., Aken, B. L., Barrell, D., Zadissa, A., Searle, S., Barnes, I., Bignell, A., Boychenko, V., Hunt, T., Kay, M., Mukherjee, G., Rajan, J., Despacio-Reyes, G., Saunders, G., … Hubbard, T. J. (2012). GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Research*, *22*(9), 1760–1774. https://doi.org/10.1101/gr.135350.111

Huang, Y. (2018). The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. *Journal of Cellular and Molecular Medicine*, *22*(12), 5768–5775. https://doi.org/10.1111/jcmm.13866

Integrated genomic and molecular characterization of cervical cancer. (2017). *Nature*, *543*(7645), 378–384. https://doi.org/10.1038/nature21386

Klonowska, K., Czubak, K., Wojciechowska, M., Handschuh, L., Zmienko, A., Figlerowicz, M., Dams-Kozlowska, H., & Kozlowski, P. (2016). Oncogenomic portals for the visualization and analysis of genome-wide cancer data. *Oncotarget*, *7*(1), 176–192. https://doi.org/10.18632/oncotarget.6128

Kopp, F., & Mendell, J. T. (2018). Functional Classification and Experimental Dissection of Long Noncoding RNAs. *Cell*, *172*(3), 393–407. https://doi.org/10.1016/j.cell.2018.01.011

Kotake, Y., Nakagawa, T., Kitagawa, K., Suzuki, S., Liu, N., Kitagawa, M., & Xiong, Y. (2011). Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. *Oncogene*, *30*(16), 1956–1962. https://doi.org/10.1038/onc.2010.568

Kuo, M. T. (2009). Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities. *Antioxidants & Redox Signaling*, *11*(1), 99–133. https://doi.org/10.1089/ars.2008.2095

Lan, T., Ma, W., Hong, Z., Wu, L., Chen, X., & Yuan, Y. (2017). Long non-coding RNA small nucleolar RNA host gene 12 (SNHG12) promotes tumorigenesis and metastasis by targeting miR-199a/b-5p in hepatocellular carcinoma. *Journal of Experimental & Clinical Cancer Research : CR*, *36*(1), 11. https://doi.org/10.1186/s13046-016-0486-9

Langemeijer, S. M. C., Aslanyan, M. G., & Jansen, J. H. (2009). TET proteins in malignant hematopoiesis. *Cell Cycle (Georgetown, Tex.)*, *8*(24), 4044–4048. https://doi.org/10.4161/cc.8.24.10239

Li, Y., Luo, S., Dong, W., Song, X., Zhou, H., Zhao, L., & Jia, L. (2016). Alpha-2, 3-sialyltransferases regulate the multidrug resistance of chronic myeloid leukemia through miR-4701-5p targeting ST3GAL1. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *96*(7), 731–740. https://doi.org/10.1038/labinvest.2016.50

Lim, L. J., Wong, S. Y. S., Huang, F., Lim, S., Chong, S. S., Ooi, L. L., Kon, O. L., & Lee, C. G. (2019). Roles and Regulation of Long Noncoding RNAs in Hepatocellular Carcinoma. *Cancer Research*, *79*(20), 5131–5139. https://doi.org/10.1158/0008-5472.CAN-19-0255

Loewer, S., Cabili, M. N., Guttman, M., Loh, Y.-H., Thomas, K., Park, I. H., Garber, M., Curran, M., Onder, T., Agarwal, S., Manos, P. D., Datta, S., Lander, E. S., Schlaeger, T. M., Daley, G. Q., & Rinn, J. L. (2010). Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nature Genetics*, *42*(12), 1113–1117. https://doi.org/10.1038/ng.710

Miao, W., Lu, T., Liu, X., Yin, W., & Zhang, H. (2020). LncRNA SNHG8 induces ovarian carcinoma cells cellular process and stemness through Wnt/β-catenin pathway. *Cancer Biomarkers : Section A of Disease Markers*, *28*(4), 459–471. https://doi.org/10.3233/CBM-190640

Michalik, K. M., You, X., Manavski, Y., Doddaballapur, A., Zörnig, M., Braun, T., John, D., Ponomareva, Y., Chen, W., Uchida, S., Boon, R. A., & Dimmeler, S. (2014). Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. *Circulation Research*, *114*(9), 1389–1397. https://doi.org/10.1161/CIRCRESAHA.114.303265

Qu, X., Li, Y., Wang, L., Yuan, N., Ma, M., & Chen, Y. (2020). LncRNA SNHG8 accelerates proliferation and inhibits apoptosis in HPV-induced cervical cancer through recruiting EZH2 to epigenetically silence RECK expression. *Journal of Cellular Biochemistry*, *121*(10), 4120–4129. https://doi.org/10.1002/jcb.29646

Ren, Z., Liu, X., Si, Y., & Yang, D. (2020). Long non-coding RNA DDX11-AS1 facilitates gastric cancer progression by regulating miR-873-5p/SPC18 axis. *Artificial Cells, Nanomedicine, and Biotechnology*, *48*(1), 572–583. https://doi.org/10.1080/21691401.2020.1726937

Spurlock, C. F. 3rd, Tossberg, J. T., Matlock, B. K., Olsen, N. J., & Aune, T. M. (2014). Methotrexate inhibits NF-κB activity via long intergenic (noncoding) RNA-p21 induction. *Arthritis & Rheumatology (Hoboken, N.J.)*, *66*(11), 2947–2957. https://doi.org/10.1002/art.38805

Sun, F., Wu, K., Yao, Z., Mu, X., Zheng, Z., Sun, M., Wang, Y., Liu, Z., & Zhu, Y. (2020). Long noncoding RNA LINC00963 induces NOP2 expression by sponging tumor suppressor miR-542-3p to promote metastasis in prostate cancer. *Aging*, *12*(12), 11500–11516. https://doi.org/10.18632/aging.103236

Tan, X., Banerjee, P., Liu, X., Yu, J., Gibbons, D. L., Wu, P., Scott, K. L., Diao, L., Zheng, X., Wang, J., Jalali, A., Suraokar, M., Fujimoto, J., Behrens, C., Liu, X., Liu, C.-G., Creighton, C. J., Wistuba, I. I., & Kurie, J. M. (2018). The epithelial-to-mesenchymal transition activator ZEB1 initiates a prometastatic competing endogenous RNA network. *The Journal of Clinical Investigation*, *128*(7), 3198. https://doi.org/10.1172/JCI122740

Tao, J., Liu, Z., Wang, Y., Wang, L., Yao, B., Li, Q., Wang, C., Tu, K., & Liu, Q. (2017). MiR-542-3p inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting UBE3C. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *93*, 420–428. https://doi.org/10.1016/j.biopha.2017.06.070

Wang, J. Z., Xu, C. L., Wu, H., & Shen, S. J. (2017). LncRNA SNHG12 promotes cell growth and inhibits cell apoptosis in colorectal cancer cells. *Brazilian Journal of Medical and Biological Research = Revista Brasileira de Pesquisas Medicas e Biologicas*, *50*(3), e6079. https://doi.org/10.1590/1414-431X20176079

Wang, X., Wang, Y., Lin, F., Xu, M., & Zhao, X. (2020). Long non-coding RNA LINC00665 promotes melanoma cell growth and migration via regulating the miR-224-5p/VMA21 axis. *Experimental Dermatology*. https://doi.org/10.1111/exd.14246

Wei, Y., Wei, L., Li, J., Ma, Z., Zhang, Q., Han, Z., & Li, S. (2020). SLCO4A1-AS1 promotes cell growth and induces resistance in lung adenocarcinoma by modulating miR-4701-5p/NFE2L1 axis to activate WNT pathway. *Cancer Medicine*, *9*(19), 7205–7217. https://doi.org/10.1002/cam4.3270

Williams, A. E. G., Choi, K., Chan, A. L., Lee, Y. J., Reeves, W. H., Bubb, M. R., Stewart, C. M., & Cha, S. (2016). Sjögren's syndrome-associated microRNAs in CD14(+) monocytes unveils targeted TGFβ signaling. *Arthritis Research & Therapy*, *18*(1), 95. https://doi.org/10.1186/s13075-016-0987-0

Xie, D., Tong, M., Xia, B., Feng, G., Wang, L., Li, A., Luo, G., Wan, H., Zhang, Z., Zhang, H., Yang, Y.-G., Zhou, Q., Wang, M., & Wang, X.-J. (2021). Long noncoding RNA lnc-NAP sponges mmu-miR-139-5p to modulate Nanog functions in mouse ESCs and embryos. *RNA Biology*, *18*(6), 875–887. https://doi.org/10.1080/15476286.2020.1827591

Xu, F., Liu, Z., Liu, R., Lu, C., Wang, L., Mao, W., Zhu, Q., Shou, H., Zhang, K., Li, Y., Chu, Y., Gu, J., & Ge, D. (2020). Epigenetic induction of tumor stemness via the lipopolysaccharide-TET3-HOXB2 signaling axis in esophageal squamous cell carcinoma. *Cell Communication and Signaling : CCS*, *18*(1), 17. https://doi.org/10.1186/s12964-020-0510-8

Yan, K., Tian, J., Shi, W., Xia, H., & Zhu, Y. (2017). LncRNA SNHG6 is Associated with Poor Prognosis of Gastric Cancer and Promotes Cell Proliferation and EMT through Epigenetically Silencing p27 and Sponging miR-101-3p. *Cellular Physiology and Biochemistry : International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, *42*(3), 999–1012. https://doi.org/10.1159/000478682

Ye, Z., Li, J., Han, X., Hou, H., Chen, H., Zheng, X., Lu, J., Wang, L., Chen, W., Li, X., & Zhao, L. (2016). TET3 inhibits TGF-β1-induced epithelial-mesenchymal transition by demethylating miR-30d precursor gene in ovarian cancer cells. *Journal of Experimental & Clinical Cancer Research : CR*, *35*, 72. https://doi.org/10.1186/s13046-016-0350-y

Yuan, L., Yuan, P., Yuan, H., Wang, Z., Run, Z., Chen, G., Zhao, P., & Xu, B. (2017). miR-542-3p inhibits colorectal cancer cell proliferation, migration and invasion by targeting OTUB1. *American Journal of Cancer Research*, *7*(1), 159–172.

Zhang, C., Li, Y., Zhao, W., Liu, G., & Yang, Q. (2020). Circ-PGAM1 promotes malignant progression of epithelial ovarian cancer through regulation of the miR-542-3p/CDC5L/PEAK1 pathway. *Cancer Medicine*, *9*(10), 3500–3521. https://doi.org/10.1002/cam4.2929

Zhang, T., Liu, W., Meng, W., Zhao, H., Yang, Q., Gu, S.-J., Xiao, C.-C., Jia, C.-C., & Fu, B.-S. (2018). Downregulation of miR-542-3p promotes cancer metastasis through activating TGF-β/Smad signaling in hepatocellular carcinoma. *OncoTargets and Therapy*, *11*, 1929–1939. https://doi.org/10.2147/OTT.S154416

Zhao, L., Han, T., Li, Y., Sun, J., Zhang, S., Liu, Y., Shan, B., Zheng, D., & Shi, J. (2017). The lncRNA SNHG5/miR-32 axis regulates gastric cancer cell proliferation and migration by targeting KLF4. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *31*(3), 893–903. https://doi.org/10.1096/fj.201600994R

Zhao, Y., Li, H., Fang, S., Kang, Y., Wu, W., Hao, Y., Li, Z., Bu, D., Sun, N., Zhang, M. Q., & Chen, R. (2016). NONCODE 2016: an informative and valuable data source of long non-coding RNAs. *Nucleic Acids Research*, *44*(D1), D203-8. https://doi.org/10.1093/nar/gkv1252

Zhao, Z., Zhou, W., Han, Y., Peng, F., Wang, R., Yu, R., Wang, C., Liang, H., Guo, Z., & Gu, Y. (2017). EMT-Regulome: a database for EMT-related regulatory interactions, motifs and network. In *Cell death & disease* (Vol. 8, Issue 6, p. e2872). https://doi.org/10.1038/cddis.2017.267

Zhong, G. B., Jiang, C. Q., Yu, X. S., Liu, Z. D., Wang, W. L., & Xu, R. D. (2020). Long noncoding RNA SNHG8 promotes the proliferation of osteosarcoma cells by downregulating miR-542-3p. *Journal of Biological Regulators and Homeostatic Agents*, *34*(2), 517–524. https://doi.org/10.23812/20-97-61

Zhou, D., Ren, K., Wang, M., Wang, J., Li, E., Hou, C., Su, Y., Jin, Y., Zou, Q., Zhou, P., & Liu, X. (2021). Long non-coding RNA RACGAP1P promotes breast cancer invasion and metastasis via miR-345-5p/RACGAP1-mediated mitochondrial fission. *Molecular Oncology*, *15*(2), 543–559. https://doi.org/10.1002/1878-0261.12866

Figures

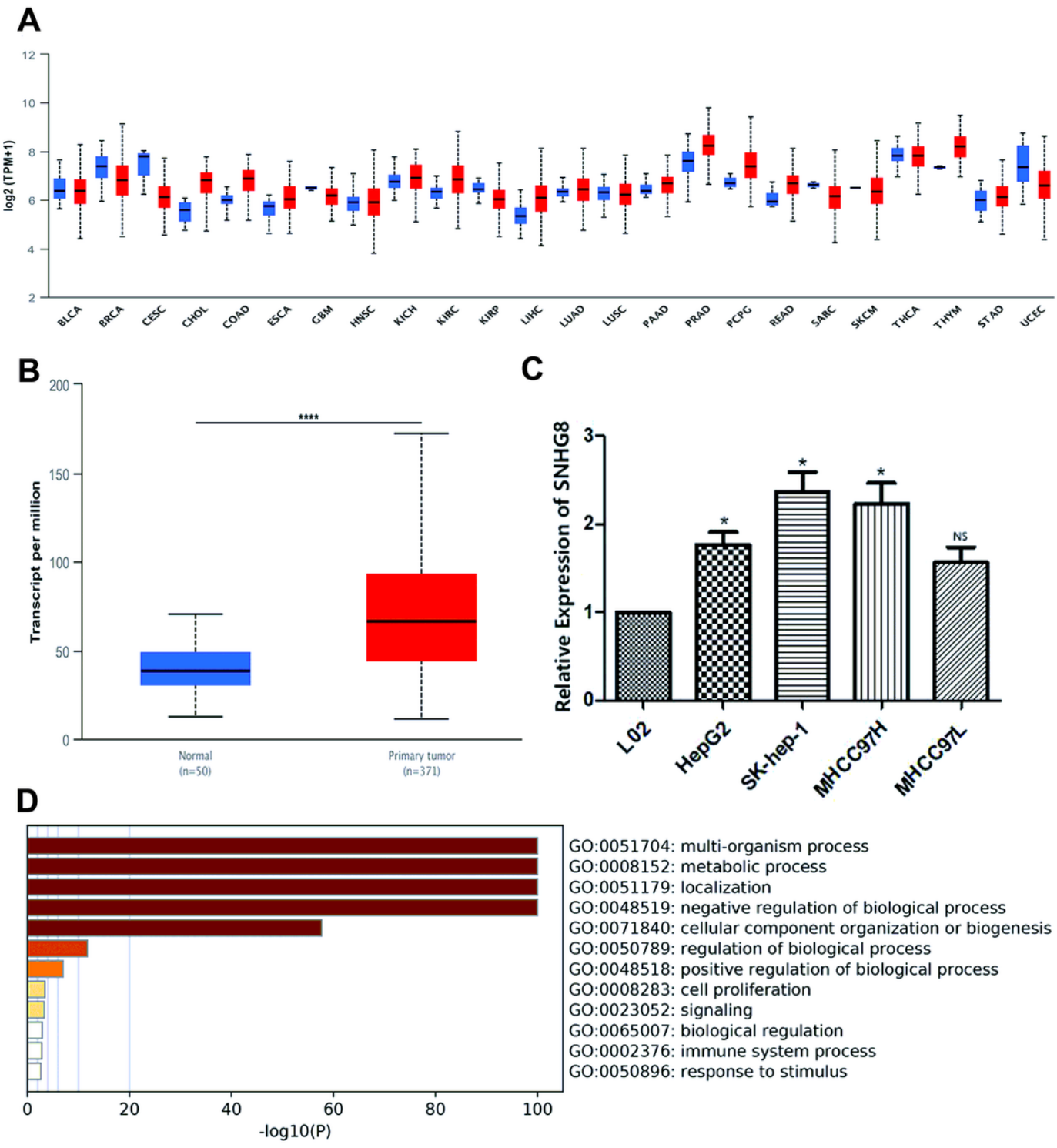

Figure 1

Expression of LncRNA SNHG8 increased in human hepatocellular carcinoma (A) TCGA dataset analysis of the expression of lncRNA SNHG8 in different cancers. (B) Expression level of lncRNA SNHG8 were up-regulated in liver cancer tissues compared with adjacent non-tumor tissues. (C) Relative expression of lncRNA SNHG8 in several human HCC cell lines compared that in normal epithelial cell line. (D) GO

analysis of lncRNA SNHG8 related up-regulated genes in TCGA dataset. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

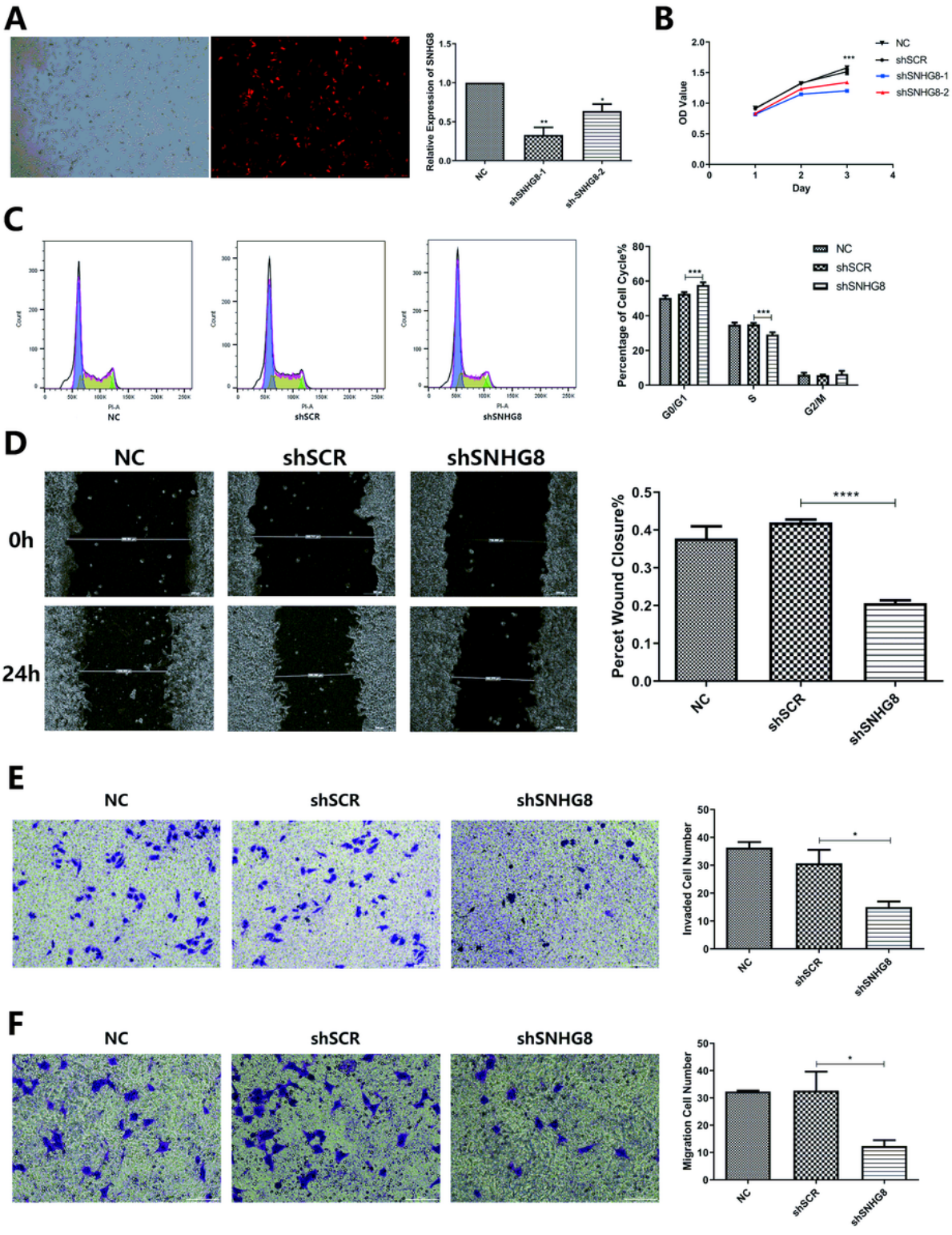

Figure 2

Knockdown of lncRNA SNHG8 reduced proliferation , migration and invasion of liver cancer cells in vitro. (A) The efficiency of shRNAs (sh lncRNA SNHG8-1 and sh lncRNA SNHG8-2) was confirmed by RT-PCR. (B, C) MTS assay and cell cycle assay showed that knockdown of lncRNA SNHG8 decreased the

proliferation of HepG2 cells. (D) Wound healing assay indicated that knockdown of lncRNA SNHG8 reduced the migratory ability of HepG2 cells. (E, F) Transwell migration and invasion assay indicated that knockdown of lncRNA SNHG8 reduced the invasive activity of HepG2 cells. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

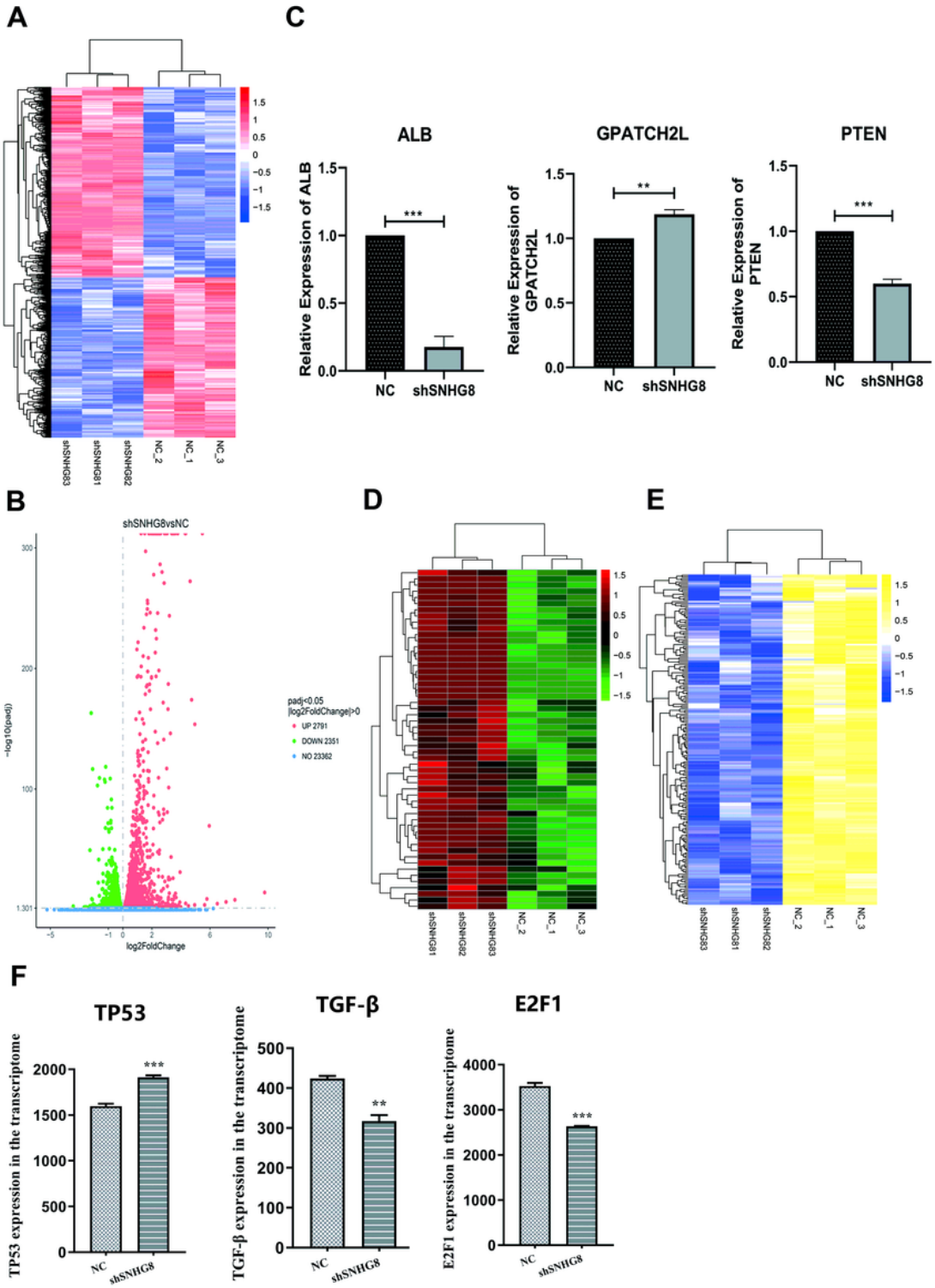

Figure 3

Differentially Expressed Genes in HepG2 cells (A) Volcano Plot of differentially expressed mRNAs. (B) Heat map of the differentially expressed mRNAs in HepG2 and HepG2(sh-SHNG8) (fold change ≥ 2 and q value ≤ 0.05). (C) The expression level of ALB、GPATCH2L、PTEN in HepG2 and HepG2(shSHNG8). (D) The heat-map of the P53 related genes in HepG2 and HepG2(sh-SHNG8) (fold change≥2; q-value≤0.05). (E) The heat-map of the proliferation, migration and invasion related genes in HepG2 and HepG2(sh-SHNG8) (fold change ≥ 2; q-value ≤ 0.05). (F)TP53、TGF-β、E2F1expression in the transcriptome. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

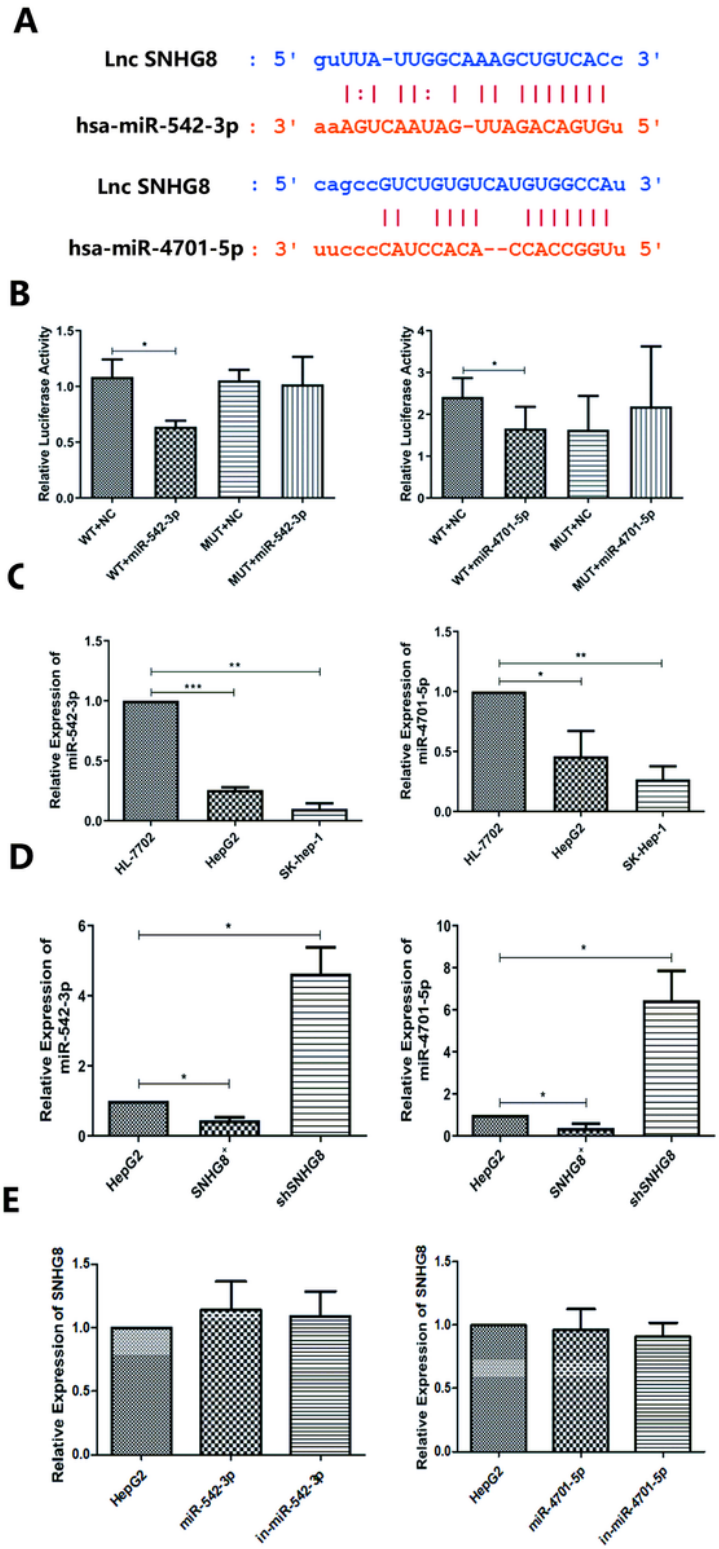

Figure 4

LncRNA SNHG8 functions as a ceRNA for miR-542-3p and miR-4701-5p in HCC cells. (A) Starbase3.0 predicted the binding site of lncRNA SNHG8 to miR-542-3p/miR-4701-5p. (B) Luciferase activity assay enzymatic activity decreases after co-transfection with the miR-542-3p/miR-4701-5p mimic and lncRNA SNHG8 WT vector. (C) The low expression of miR-542-3p/miR-4701-5p in HepG2 and SK-Hep cells compared with L02 cell by RT-PCR. (D) Relative expression level of miR-542-3p/miR-4701-5p in HepG2 cells after transfection were measured by RT-qPCR. (E) Regardless of the transfection of miR-542-3p/miR-4701-5p mimics or inhibitors, lncRNA SNHG8 has no significant changes. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

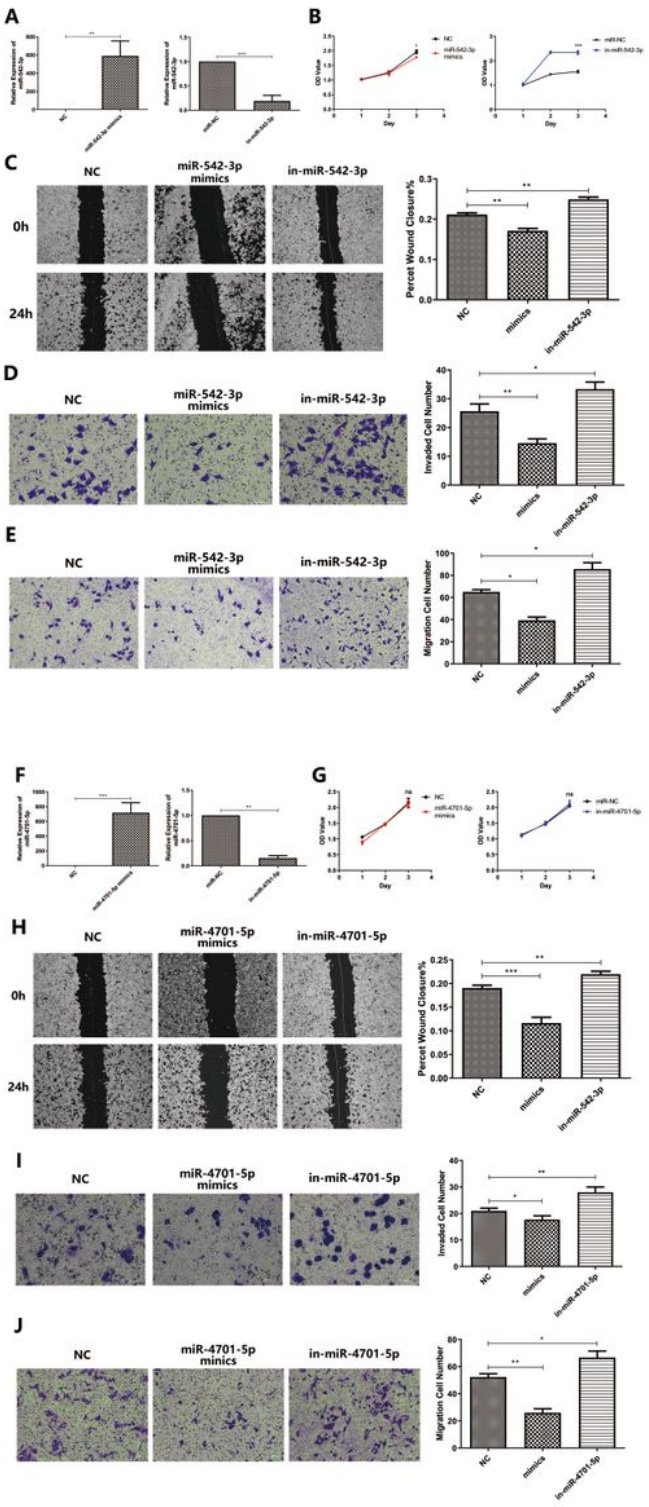

Figure 5

MiR-542-3p and miR-4701-5p are crucial for regulating the biological function of HepG2 cells. (A and F) Relative expression level of miR-542-3p and miR -4701-5p in HepG2 cells after transfection were measured by RT-PCR. (B and G) MTS assays were used to detect the viability of HepG2 cells after transfection. (C and H) Wound healing assays were used to detect cell migration capacities in HepG2 cells after transfection. (D, E, I, J) Transwell assays were used to detect cell invasion capacities in HepG2

cells after transfection. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

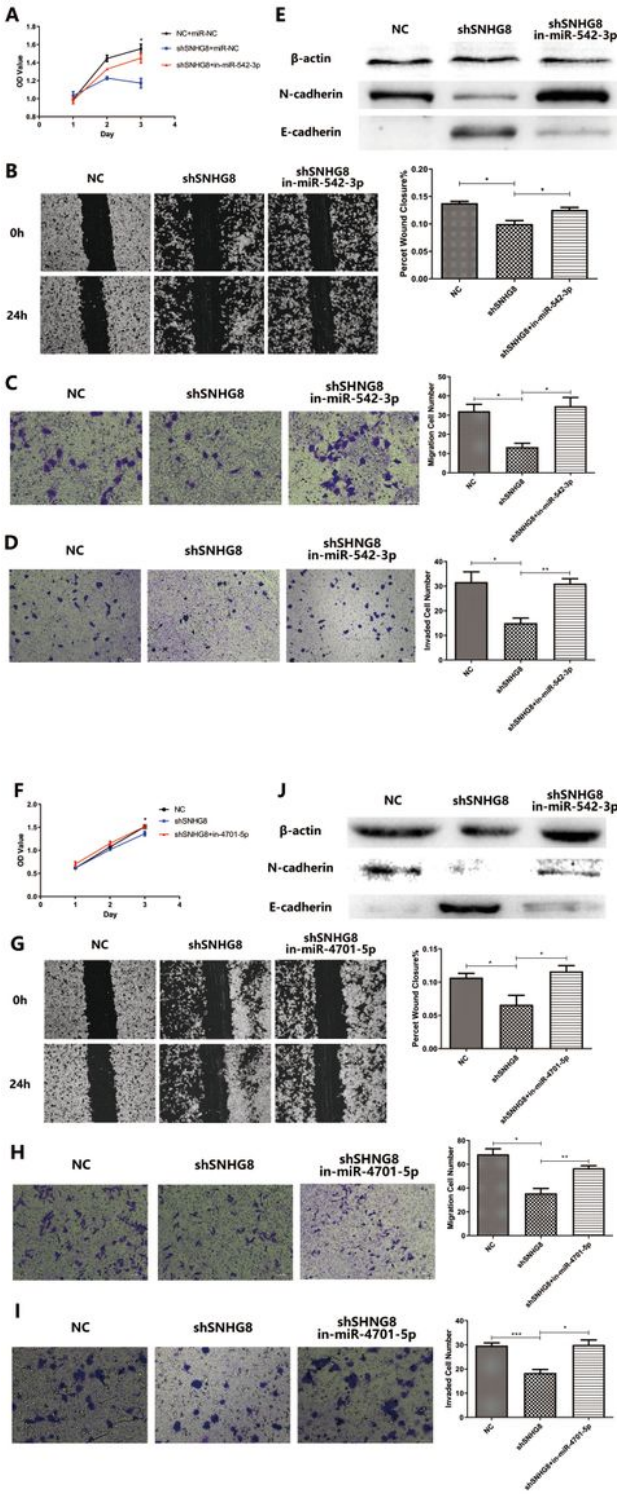

Figure 6

Silencing LncRNA SNHG8 reversed the tumor promoting effects of the miR-542-3p and miR -4701-5p inhibitor in HepG2 cells. (A and F) MTS assays were performed to investigate the effects of co-transfection with sh-SHNG8 and the miR-542-3p inhibitor on cell proliferation in HepG2 cells. (B and G)

Effects of co-transfection with sh-lncRNA SNHG8 and the miR-542-3p and miR-4701-5p inhibitor on cell migration were evaluated by wound healing assay in HepG2 cells. (C, D, I, H) Transwell assays of the effects of co-transfection on cell migration and invasion in HepG2 cell lines. (E and J) Expression levels of E-cadherin, N-cadherin were detected by western blot analysis after co-transfection. The data are presented as mean ± SD from three independent experiments. *P < 0.05, **P < 0.01.

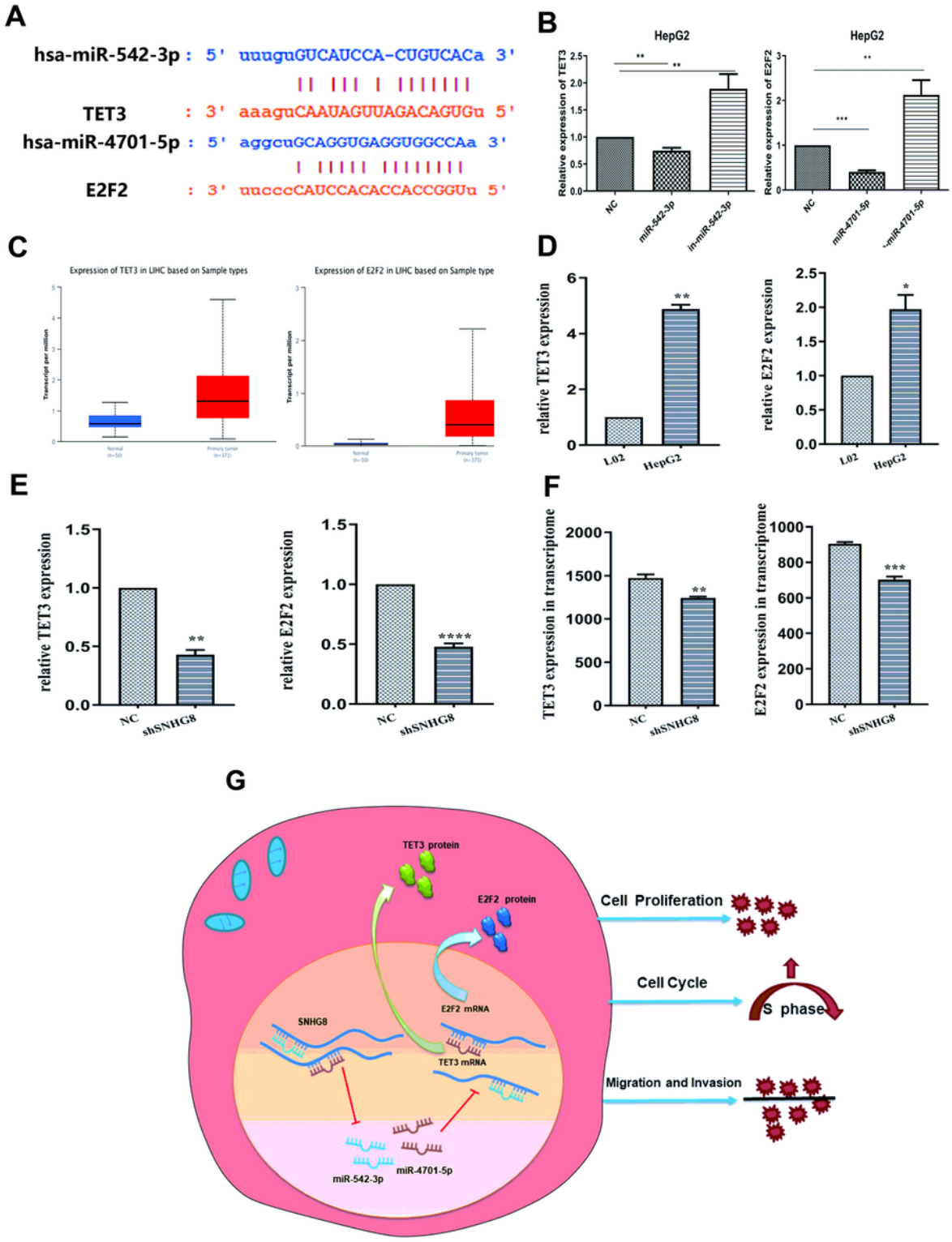

Figure 7

LncRNA SNHG8 regulates the expression of TET3 by targeting miR-542-3p and miR-4701-5p in HepG2 cells. (A) Predicted binding sites of miR-542-3p and miR-4701-5p in the TET3 3′UTR by TargetScan. (B) RT-qPCR was used to detect the relative expression of TET3 in miR-542-3p and miR-4701-5p transfected mimic and inhibitor HepG2 cells. (C) The expression level of TET in HepG2 cells detected by q-PCR was up-regulated compared with L02. (D) RT-qPCR was used to measure the expression level of TET3 in HepG2 cells. (E) Knock down SNHG8, the expression of TET3 was down-regulated. (F) Transcriptome data indicated that knocking down SNHG8 will down-regulate the expression of TET3. (G) Schematic illustration of lncRNA SNHG8/ miR-542-3p and miR-4701-5p/TET3 axis.