

CircASPH promotes hepatocellular carcinoma progression through methylation and expression of HAO2

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Abstract

Background

CircRNAs have been reported to be related to hepatocellular carcinoma (HCC) development. Limited studies have revealed the expression profile of circRNAs in tumor and paratumour normal samples in HCC patients.

Methods

The expression of circRNAs, mRNA and miRNA was detected by Real-time PCR. An immunofluorescence assay was performed to detect the level of 5mC and 5hmC. Dual luciferase assay was used to confirm the interaction between miRNA and circRNA. CCK8, wound healing and transwell assays were used to check the viability, migration and invasion of HCC cells. Migration and invasion assays was used to confirm the metastasis *in vivo*.

Results

We found that circASPH was significantly increased in HCC tumor samples and that the level of circASPH was closely related to the overall survival (OS) of HCC patients. Mechanistically, circASPH could regulate the methylation of promoter and gene expression of Hydrocyanic Oxidase 2 (HAO2) to promote HCC progression by acting as a sponge for miR-370, which targeted the DNMT3b and increased the 5mC level.

Conclusions

Our study determined that circASPH could promote the methylation and expression of HAO2 and could be considered an important epigenetic regulator in HCC progression.

Introduction

Hepatocellular carcinoma (HCC) is becoming one of the most common malignancies, with a high rate of cancer-related death worldwide, increasing the burden worldwide[1]. hepatitis C virus (HCV) and hepatitis B virus (HBV) infection and non-alcoholic fatty liver are risk factors to result in HCC[2–4], as well as excessive drinking, genetic factors, smoking, excess body weight, type 2 diabetes[5–7]. HCC possesses its unique characters because the prognosis depends on tumor stage and severity of liver disease. The effective intervention includes liver transplantation (LT) and surgical resection only in early stages[8]. Therefore, the manism of HCC should be uncovered to improving patient prognosis and treatment effects.

Circular RNAs (circRNAs) has been identified as a new type of non-coding RNA. Unlike, circRNA has a covalently closed loop structure, which is different from traditional linear RNA, as well as without a 5' cap and a 3' tail[9]. Because of its special structure, circRNAs could be resistant to ribonuclease cleavage, making itself more stable[10–12]. At the same time, circRNAs has developmental stage-related patterns, tissue specificity, and a high species conservation[13–15]. Thereby, circRNAs have been used as biomarkers in disease diagnosis, prognosis evaluation, progress monitoring. CircRNAs are divided into exon circular RNAs (EcircRNAs), exon-intron circular RNAs (EicRNAs) and intron circular RNAs (ciRNAs) depends on sequences spliced from mRNA[9]. More and more circRNAs were identified and verified to play a crucial role in cancers, especially in HCC. Hepatocyte nuclear factor 4 alpha (HNF4a) can activate circ_104075 transcription by binding to its promoter, sponging miR-582-3p to upregulate YAP to advance the process of HCC[16]. circTMEM45A could interact with miR-665 to inhibit the expression of insulin like growth factor 2 (IGF2) to promote HCC tumorigenesis[17]. However, the role of circASPH remains unclear in HCC.

Here, two circRNA-seq databases were used to identify that circASPH was significantly upregulated in tumor tissues in HCC patients. The expression of circASPH level in tumor tissues was closely related to the prognosis of HCC patients. Mechanistically, it indicated that circASPH could promote the methylation process of the HAO2 promoter and then further activated the transcription and increased the expression of HAO2 by acting as a sponge for miR-370, which targeted DNMT3b to increase the 5mC level. Therefore, circASPH could be considered an important epigenetic regulator in HCC progression.

Methods

Clinical data

In total, 181 samples were collected from consecutive patients with HCC who underwent curative resection in Hepatobiliary Center, The First Affiliated Hospital of Nanjing Medical University. Fresh human HCC and adjacent nontumor liver tissue samples were blindly collected from the cohort. Informed consent was obtained from each patient, and ethical approval was granted by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University. The follow-up procedures were described in detail in our previous studies. The information was summarized in Table 1.

Cell culture, RNA extraction and Transfection

The human HCC cell lines Huh-7, SMMC-7721, HepG2, MHCC97H, and HCCLM3 were routinely maintained in our laboratory. Transfection was carried out by using Lipofectamine 3000 (Invitrogen), and total RNA was obtained using the mirVana™ miRNA Isolation Kit (Life Technologies) according to the manufacturer's instructions.

Plasmid Construction

The lentiviral vectors pGMLV-SC5-shmiR-370 and negative sequences were purchased from GenePharm (China). The lentiviral cDNA templates of circASPH and DNMT3b were cloned into the pPB-CAG vector according to the manufacturer's instructions. Human HCC cells were then transduced with the appropriate lentivirus.

qRT-PCR

miRNAs were extracted with the mirVana™ miRNA Isolation Kit (Life Technologies). Real-time PCR was performed in triplicate with the SYBR Green PCR method. All miRNA levels were normalized to the U6 small nuclear RNA level; other RNA levels were adjusted using 18s as the reference. Relative expression was analysed by the comparative cycle threshold (Ct) method.

Immunofluorescence Assay

An immunofluorescence assay was performed as described previously. For 5mC and 5hmC staining, cells were fixed and then placed in 2 N HCl for 30 min. Then, it was placed in Tris-HCl (100 mM) and blocked with PBS containing 5% BSA.

Luciferase Assay

The 3'-untranslated region (UTR) of human DNMT3b was cloned into the pGL3 vector to generate pGL3-DNMT3b -3'UTR. Luciferase activity was measured after transfection. Alternatively, cells were cotransfected with plasmids and mimics.

Immunocytochemistry

Immunocytochemistry was performed as described previously. For 5-hmC staining, slides were fixed and then placed in 2 N HCl for 30 min. Then, it was placed in Tris-HCl (100 mM) and blocked with 5% normal goat serum at room temperature.

FISH

A FISH kit was used for the FISH assay. Specific probes for circMYH9 were synthesized by GenePharma (Shanghai, China). Briefly, cells were fixed with 4% paraformaldehyde, treated with 0.5% Triton X-100, and incubated with a circMYH9 probe overnight. Then, cell nuclei were stained with DAPI. Images were obtained with a fluorescence microscope.

CCK8 assay

Transfected BC cells at a density of 2,000 were seeded in 96-well plates. A CCK8 assay kit (Sigma, USA) was used to detect living cells. The absorbance was measured by a microplate reader at five time periods.

Wound-healing assay

Transfected BC cells were seeded in six-well plates. A 200 µl pipette tip was used to scratch to generate a linear gap. After 24h, we used the microscope to take pictures and measured the width (W) of the scratch wound. The rate of close distance of the wounds was calculated. All measurements were carried out at three times.

Transwell assay

Cell suspension was added into transwell chamber inserts (Millipore, USA), added with matrigel. 24h later, cells were stained and pictures taken were used to measure invasion assays in accordance with the manufacturer's instructions as described previously.

Migration and invasion assays, in vivo metastasis assays

Migration and invasion assays and in vivo metastasis assays were performed as described previously.

Statistical Analysis

Statistical analysis was performed with SPSS 19.0 software (SPSS). The sample number (n) indicates the number of independent biological samples in each experiment. Generally, all experiments were carried out with $n \geq 3$ biological replicates. T-tests were used to compare two groups and one-way ANOVA was used to compare multi groups. Correlations between groups were determined by Pearson's correlation test. Survival rates were analyzed by the Kaplan-Meier method., and $p < 0.05$ was considered statistically significant.

Results

CircASPH expression is significantly upregulated in HCC tissue and related to patient prognosis

In order to identify the differentially expressed circRNAs between normal tissues and HCC tumor tissues, we used two GEO databases (GSE28274 and GSE125469) (Figure 1A and 1B). The circRNAs with significant differential expression (fold change ≥ 2.0 and $P < 0.05$) between the groups were identified. From the results of two GEO databases, 1 circRNAs were preliminarily identified to be upregulated, while 2 circRNAs were downregulated in the HCC tissue samples compared to the paratumour normal samples (Figure 1C). Thereby, we chose the circASPH for further study.

To determine whether circASPH was associated with HCC, firstly, RT-qPCR indicated that the expression of circASPH was higher in HCC tissues (Figure 1D). Then, we used the FISH assay to identify the expression of circASPH in HCC tumor tissues. It showed that the level of circASPH expression was higher in tumor tissues (Figure 1E). We also investigated the level of circASPH in several HCC cell lines. The level of circASPH was higher in HCC cell lines (HepG2, SMMC-7721, Huh7 and HCCLM3) (Figure 1F). Then, we analyzed the relationship between the circASPH level and HCC patient prognosis. Multivariate Cox analysis revealed that the circASPH level in HCC tissue was an independent prognostic factor in HCC

patients (Figure 1G). These data suggest that the downregulated circASPH level plays important roles in HCC tumorigenesis and progression.

CircASPH promotes cell proliferation, migration and invasion in HCC cell lines

To investigate the biological function of circASPH in HCC progression, circASPH was stably overexpressed and knocked down in HCC cell lines respectively (Figure 2A). CCK8 assay and EDU staining assay were carried out to verify the proliferation of HCC cells. The results indicated that the cell proliferation was enhanced by circASPH overexpression, while reduced by circASPH knockdown (Figure 2B-2C). Meanwhile, wound healing assays were performed to evaluate the cell migration ability and transwell assays were used to indicate the cell invasion. The results showed that the migration and invasion capacities were significantly increased after circASPH was overexpressed. However, the ability of cell migration and invasion capacities were reduced when the circASPH level was altered *in vitro* (Figure 2D-2E). To further determine whether circASPH induce the tumor growth *in vivo*, we injected control or shRNA-circASPH transfected cells into nude mice. Our results revealed that 6 weeks after transplantation, the tumors composed of shRNA-circASPH transfected cells were significantly smaller than those in control cells (Fig. 2F-2H). Therefore, we verified that circASPH functioned as a tumor promoter to enhance the proliferation, invasion and metastasis of HCC *in vitro* and *in vivo*.

CircASPH regulates the level of the DNMT3b/5mC Axis by sponging miR-370 in HCC cells

CircRNAs have been reported to function as sponges for miRNAs. Then, we investigated the ability of circASPH to function as a sponge for miRNAs. Candidate targets were determined using the target prediction. According to the predicted results, the miR-370 gained the highest score (Figure 3A). Then, we used the molecular biological methods to investigate the direct interaction between circASPH and miR-370, the dual luciferase assay was performed and it indicated that circASPH could interact with miR-370 (Figure 3B-3C). We also designed a linear biotinylated circASPH probe. Our qRT-PCR results showed that miR-370 was abundantly pulled down by the circASPH probe in HCC cells. In addition, we designed biotinylated miR-370 to pull down circASPH, it showed the same result (Figure 3D). We also used FISH assay to detect the interaction between the circASPH and miR-370. The result showed that circASPH and miR-370 could be co-localized (Figure 3E). At the same time, overexpressed the circASPH could decrease the miR-370 expression level, while reduction of miR-370 was induced by circASPH knockdown (Figure 3F).

To further elucidate the molecular mechanism by which circASPH promotes HCC progression, we attempted to identify the target genes of miR-370. We used the bioinformatic analysis to indicate the function of target gene. It showed that the molecular function was enriched in chromatin binding (Figure 4A). And the biological process was enriched in the regulation of transcription, especially in apoptotic process (Figure 4B). KEEG pathway showed that target genes main functioned in pathways in cancer and transcription misregulation in cancer (Figure 4C). Previous studies revealed that DNMT3b played a key role in establishing de novo DNA methylation, and was upregulated in cancers. So, we used GEO databases (GSE124535) to analysis the expression of the family of DNMT. It showed that the family of

DNMT was enhanced in HCC tissues (Figure 4D). Based on online target prediction algorithms, we found that DNMT3b was a potential candidate target gene of miR-370 (Figure 4E). The dual luciferase assay was performed and it indicated that miR-370 could interact with DNMT3b (Figure 4F). Overexpressed the miR-370 could decrease the DNMT3b expression level, while reduction of DNMT3b was induced by miR-370 knockdown (Figure 4G). Then, we detected changes in 5hmC and 5-mC levels to assess whether miR-370 can remodel the epigenetic landscape by targeting DNMT3b genes and regulating 5-mC levels in the genome. As expected, miR-370 knockdown exhibited lower levels of 5hmC and enhanced the level of 5-mC (Figure 4G). Next, we verified the expression of DNMT3b and level of 5-mC under the circASPH changed condition. The result showed that circASPH knockdown could decrease the DNMT3b expression level and increase level of 5-mC (Figure 4H). These data indicated that circASPH could sponge miR-370 and regulate the DNMT3b/5mc axis in HCC cells.

CircASPH promotes HCC process via the MiR-370/DNMT3b/5mc Axis

First, we used RT-qPCR to indicate that the level of DNMT3b was higher in HCC tumor tissues (Figure 5A). The Pearson analysis also showed the expression of DNMT3b level was positively related to the level of circASPH (Figure 5B). IHC assay also showed the 5mc level was enhanced in HCC tumor tissues (Figure 5C). These results prompted us to hypothesize that HCC tumor tissues may promote HCC progress via the MiR-370/DNMT3b/5mc axis. DNMT3b was knocked down in circASPH overexpression or miR-370 knockdown cells, and the proliferation ability of these cells was evaluated by CCK8 assays and EDU staining assays. We found that DNMT3b knockdown significantly reduced the cell proliferation, which was enhanced by circASPH overexpression or miR-370 knockdown (Figure 5D and 5E). In addition, the wound healing assays and transwell assays were also indicated DNMT3b knockdown significantly reduced the enhanced cell migration and invasion mediated by circASPH overexpression or miR-370 knockdown (Figure 5F and 5G). These results suggest that the DNMT3b/5mc axis plays an important role in the functions of miR-370 and circASPH in HCC cells.

CircASPH promotes HCC progression by regulating the DNA methylation and expression of HAO2

DNMT3b enzymes catalyzed the 5hmC to 5mC, leading to DNA methylation and gene expression regulation. Then, we used the methylation GEO database(GSE55752) in HCC tumor. It indicated the DNA methylation was enhanced in in HCC tumor and was enriched in promoter area (Figure 6A-6C). GSEA also showed the methylated genes also take part in negative regulation of growth. And HAO2 was one of high methylated genes in HCC tumor (Figure 6D). CHIP seq also showed that the methylation of HAO2 was occurred in promoter region (Figure 6F). Overexpressed the circASPH could increase the HAO2 expression level, while reduction of HAO2 was induced by circASPH knockdown (Figure 6G). Meanwhile, overexpressed the miR-370 could decrease the HAO2 expression level (Figure 6H). We also used CHIP assays to verified the 5mc enrichment on HAO2 promoter region (Figure 6I). It indicated that circASPH overexpression could enhance the enrichment and knockdown of circASPH reduce the binding.

Then, we detected the expression of HAO2 in HCC tumor. RT-qPCR indicated that the level of HAO2 was decreased in HCC tumor tissues (Figure 7A). The Pearson analysis also showed the expression of HAO2

level was negatively related to the level of circASPH (Figure 7B). HAO2 was overexpressed in circASPH overexpression or miR-370 knockdown cells, and the proliferation ability of these cells was evaluated by EDU staining assays. We found that HAO2 overexpression significantly reduced the cell proliferation, which was enhanced by circASPH overexpression or miR-370 knockdown (Figure 7C). In addition, the wound healing assays and transwell assays were also indicated HAO2 overexpression significantly reduced the enhanced cell migration and invasion mediated by circASPH overexpression or miR-370 knockdown (Figure 5D and 5E). These results demonstrated that circASPH promoted HCC progression by regulating the DNA promoter methylation and expression of HAO2.

Discussion

Here, we indicated that circASPH could regulate the promoter methylation and expression of HAO2 to promote HCC progression via the MiR-370/DNMT3b/5mC axis. First, two databases were used to identify that circASPH was only one upregulated circRNA in tumor tissues in HCC patients and that the expression of circASPH level was closely related to OS of HCC patients. Overexpression of circASPH could increase cell proliferation, migration and invasion in HCC cells. Mechanistically, it showed that circASPH could promote the methylation process of the HAO2 promoter, by acting as a sponge for miR-370, which targeted DNMT3b and increased the 5mC level. Therefore, circASPH could promote the methylation process and increase the expression of HAO2 in HCC process.

circRNAs are verified to play an important role in many diseases, especially in different types cancer. circASPH has been reported in other diseases. For instance, Upregulated circASPH contributed to glioma cell proliferation and aggressiveness by miR-599/AR/SOCS2-AS1 signaling pathway[18]. CircASPH also promoted KGN cells proliferation through miR-375/MAP2K6 axis in Polycystic Ovary Syndrome[19]. In lung adenocarcinoma, circASPH could be regulated by HMGA2 to promote tumor growth [20]. However, the function of circ-ASPH in HCC is still uncovered. Here, we indicated that circASPH led to increased cell proliferation, migration and invasion in HCC cells, which could be a diagnostic and treatment biomarker for HCC treatment.

DNA methylation is defined by the addition of a methyl group to the 5' position of a CpG dinucleotide cytosine pyrimidine ring. Active demethylation could be regulated by Ten-eleven translocation (TET) enzymes family, including Tet 1, Tet 2, and Tet 3. 5mC and 5hmC could be also changed to thymine or 5-hydroxymethyluracil (5-hmU) depends on an alternative pathway [21]. In HCC, TET1 and TET2 has been reported to be downregulated in tumor tissues and regulating DNA methylation[22–24]. DNA methylation is catalyzed by two types enzymes. In the first type, after DNA replication, DNA methyltransferase 1 (DNMT1) and its cofactor could unmethylated cytosine residues are methylated[25]. In the second type, DNMT3A and DNMT3B, together with their coactivator DNMT3L catalyze to new methyl groups to unmethylated cytosines[26]. DNMT1-mediated methylation of BEX1 regulates stemness and tumorigenicity in liver cancer[27]. However, the mechanism of DNMT3b/5mC activation and whether the DNMT3b/5mC axis is involved in the regulation of HCC remain unresolved. Here, we identified circASPH as an important noncoding RNA that regulated the expression of the DNMT3b and the 5mC level in HCC

progression. Mechanistically, circASPH acted as the sponge of miR-370, which binds to the 3'-UTR of DNMT3b mRNAs in HCC cells and upregulate 5mC levels. In HCC samples, there was a positive correlation between circASPH and DNMT3b expression. These data supported the conclusion that circASPH could regulate the level of the DNMT3B/5mC axis and act as key epigenetic modifiers in HCC.

Conclusions

We identified circASPH could act as a role of oncogene in HCC proliferation, migration and invasion that functions via the miR-370/DNMT3b/5mC /HAO2 axis. circASPH could be considered an important epigenetic regulator in HCC progression.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from each patient, and ethical approval was granted by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Chen Wu, Zhongming Tan designed the research; Han Zhuo, Jinguo Xia wrote the paper; Junwei Tang, Sheng Han, Qitong Zheng, Feihong Zhang performed the in vitro experiments; Han Zhuo, Jinguo Xia, Zhenggang Xu, Dongwei Sun analyzed data.

Acknowledgements

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Tables

Table 1: Clinicopathological features of HCC patients (n=20).

Patients	n (%)
Age(years)	
<60	12 (60.0)
≥60	8 (40.0)
Gender	
Male	14 (70.0)
Female	6 (30.0)
T stage	
T1-T2	9 (45.0)
T3-T4	11 (55.0)
Regional lymph node metastasis	
Yes	16 (80.0)
No	4 (20.0)
Distance metastasis	
Yes	3 (15.0)
No	17 (85.0)
Tumor size	
< 5cm	11 (55.0)
≥ 5cm	9 (45.0)

Figures

Figure 1

CircASPH Expression Was Identified to Be Significantly Downregulated in HCC Tissue and Related to Patient Prognosis. (A-B) Heat maps of differentially expressed circRNAs in BC tissues and non-tumor tissues obtained from GSE128274 and GSE125469. (C) The numbers of overlapping differentially expressed circRNAs from two GEO databases were shown in venn diagram. (D) The level of circASPH expression was evaluated by qRT-PCR in 20 paired BC tissues and non-tumor tissues. (E) FISH analysis of

expression of circASPH in HCC tissues and non-tumor tissues. (F) circASPH expression in HCC cell lines compared with THLE2 cells. (G) Kaplan-Meier analysis showed that the level of circASPH was predictive of overall survival. All experiments were carried out with $n \geq 3$ biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure2

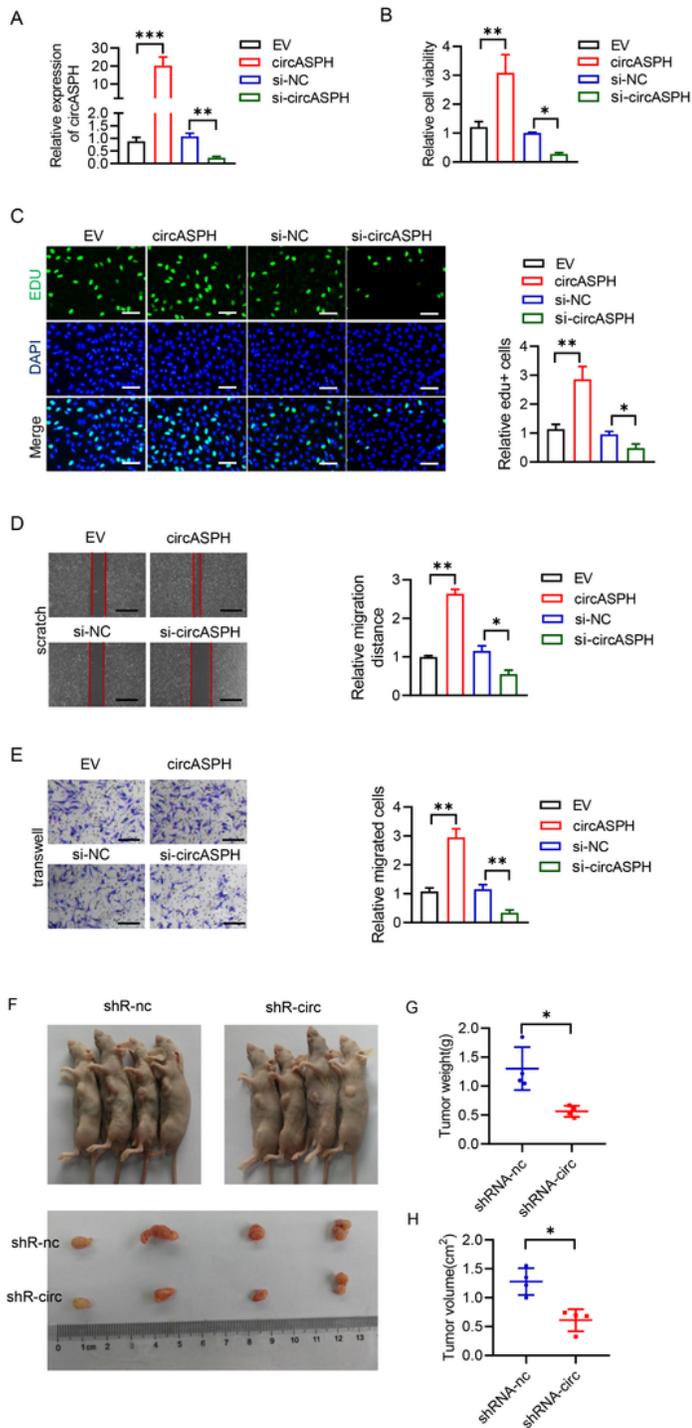


Figure 2

CircASPH Promoted HCC Cell Proliferation, Migration and Invasion In Vitro. (A) circASPH was overexpressed and knocked down in HCC cells. (B-C) Cell Counting Kit-8 (CCK-8) assays and EDU staining assays were used to indicate the function of circASPH in the proliferation of HCC cells. (D) Wound healing assays showed the role of circASPH in migration of HCC cells. (E) Transwell assays showed that function of circASPH in invasion of HCC cells. (F)-(H) Tumours composed of shRNA-circASPH transfected cells were significantly smaller than those composed of shRNA-nc cells. All experiments were carried out with $n = 3-4$ biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3

CircASPH sponged the miR-370. (A) Score list for circInteractome of predicting miRNAs sponged by circASPH. (B) Dual-luciferase reporter assay showed that co-transfection of WT and mimic miR-370 markedly decreased luciferase activity in 293T cells. (C) Predicted binding sites between circASPH and miR-370. (D) miR-370 was abundantly pulled down by a circASPH probe. (E) Images of circASPH and miR-370 co-localized in the cytoplasm; scale bar, 20 μm . (F) RT-qPCR indicated that the level of miR-370 was detected under circASPH overexpression or knockdown condition. All experiments were carried out with $n = 3$ biological replicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure4

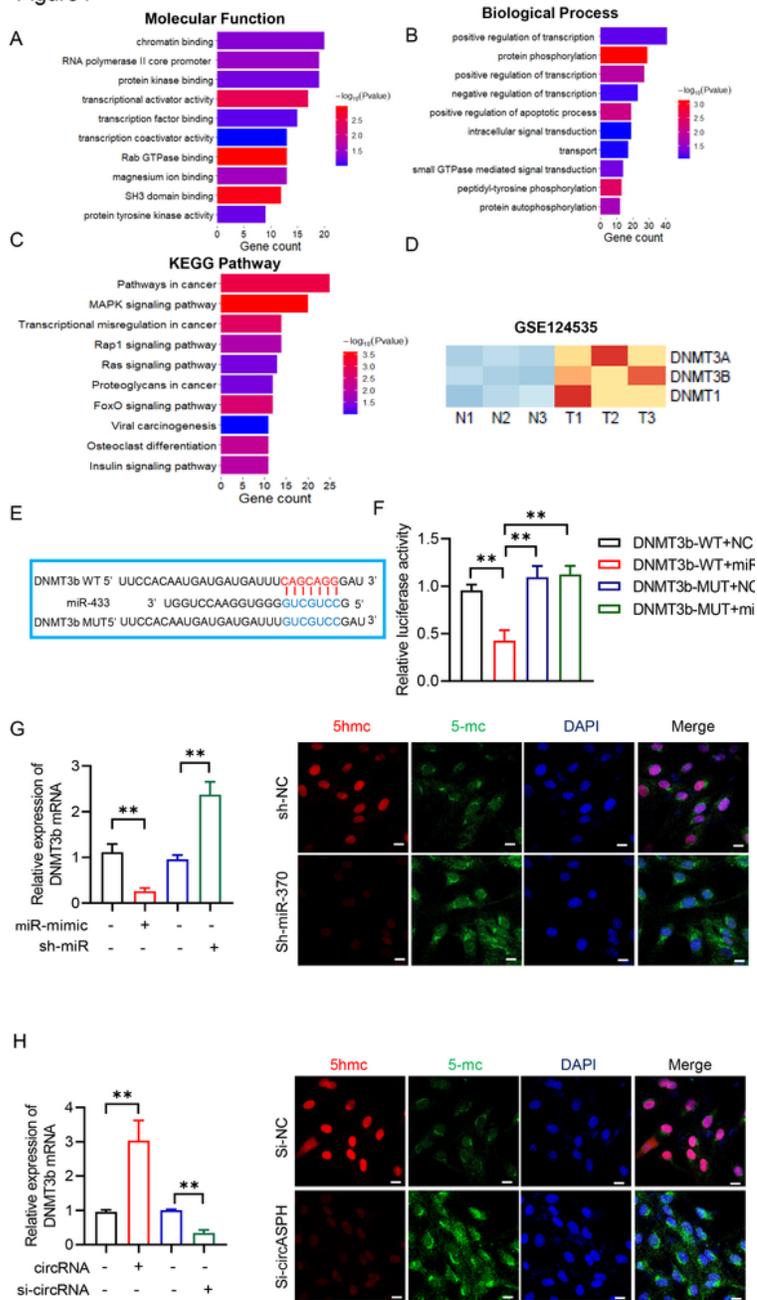


Figure 4

CircASPH regulates the level of the DNMT3b/5mC Axis by sponging miR-370 in HCC cells. (A-B) Molecular function and biological process of miR-370 target genes in Gene ontology analysis. (C) KEGG analysis of miR-370 target genes. (D) heatmap of expression of DNMT gene family level in GEO124535. (E) Predicted binding sites between DNMT3b and miR-370. (F) Dual-luciferase reporter assay showed that miR-370 could interact with the 3'UTR of DNMT3b mRNA. (G) Left, The expression of DNMT3b level by

miR-370 overexpression and knockdown. Right, images of 5hmC-, 5-mC- and DAPI-stained HCC cells with different level of miR-370; scar bar=50 μ m. (H) Left, The expression of DNMT3b level by circASPH overexpression and knockdown. Right, images of 5hmC-, 5-mC- and DAPI-stained HCC cells with different level of circASPH; scar bar=50 μ m. All experiments were carried out with n = 3 biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001.

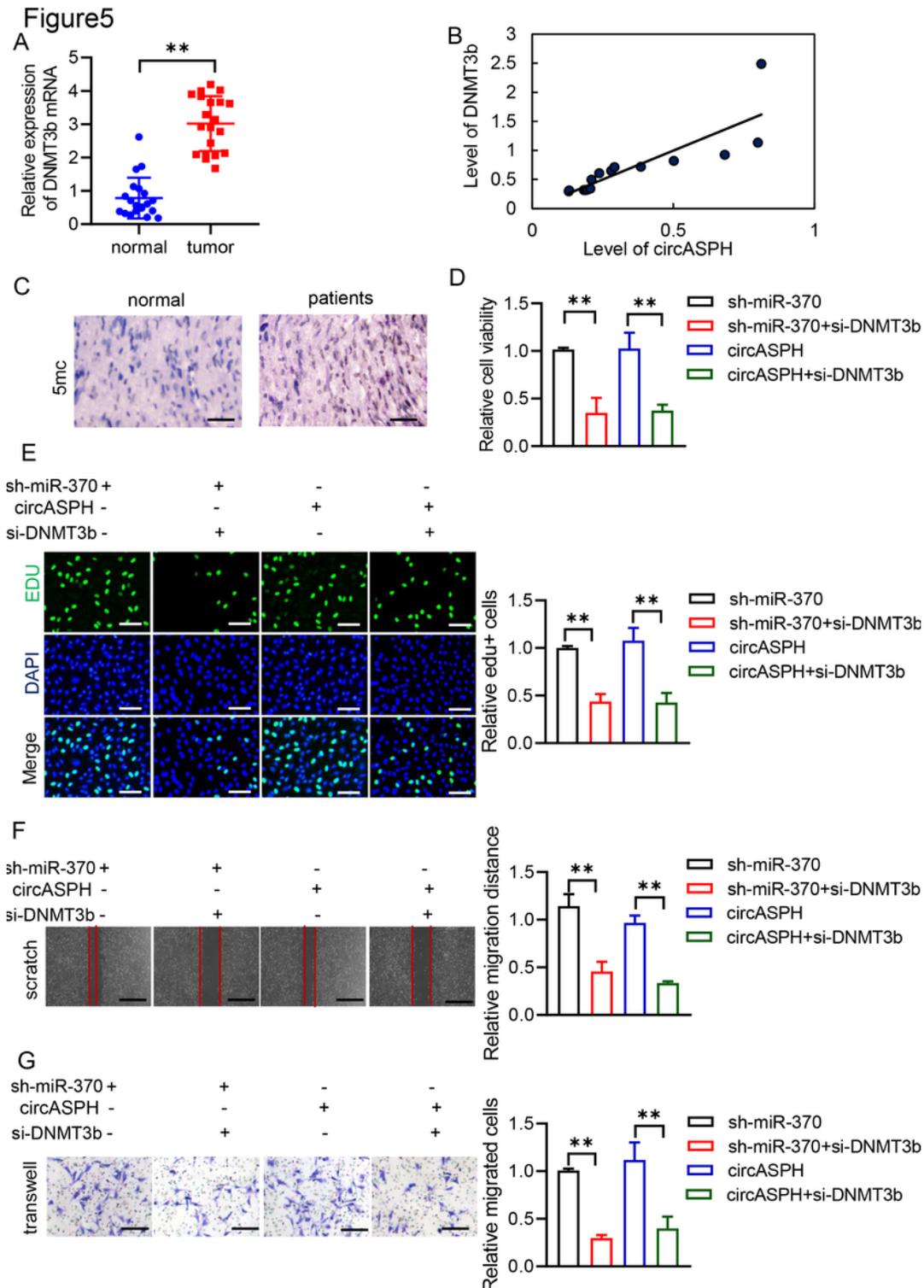


Figure 5

CircASPH promotes HCC process via the level of the miR-370/DNMT3b/5Mc. (A) The level of DNMT3b expression was evaluated by qRT-PCR in 20 paired BC tissues and non-tumor tissues. (B) Pearson analysis indicated that the relationship between circASPH and DNMT3b. (C) Immunohistochemical staining for 5mc in HCC tumor tissues; scar bar=100 μ m. (D-E) Cell Counting Kit-8 (CCK-8) assays and EDU staining assays indicated proliferation of cells expressing a combination of circASPH, sh-miR-370, and si-DNMT3b. (F-G) Wound healing assays and transwell assays indicated that the ability of migration and invasion of cells expressing a combination of circASPH, sh-miR-370, and si-DNMT3b. All experiments were carried out with n = 3 biological replicates. * p < 0.05, **p < 0.01, ***p < 0.001.

Figure 6

circASPH promotes HCC progression by Regulating the DNA methylation and expression of HAO2. (A) Volcano plot of methylation difference between HCC tumor tissues and normal tissues. (B) CpG island distribution of promoter differential methylated CpG sites. (C-D) Average profiles of 5mC and input DNA coverage across binding-site motif identified on 5mCp enhancers. (E) GSEA analysis of gene enriched in negative regulation of growth pathway. (F) IGV profile of 5mC-enriched regions and RNA-seq profiles in HCC tumor tissues. (G) The expression of HAO2 level by circASPH overexpression and knockdown. (H) The expression of HAO2 level by miR-370 overexpression and knockdown. (I) hMeDIP-qPCR assays showed that 5mC in HAO2 genes with different circASPH levels. All experiments were carried out with n = 3 biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 7

CircASPH promotes HCC process via the level of the miR-370/DNMT3b/5mC/HAO2 axis. (A) The level of HAO2 expression was evaluated by qRT-PCR in 20 paired BC tissues and non-tumor tissues. (B) Pearson analysis indicated that the relationship between circASPH and HAO2. (C) EDU staining assays indicated proliferation of cells expressing a combination of circASPH, sh-miR-370, and HAO2. (D-E) Wound healing assays and transwell assays indicated that the ability of migration and invasion of cells expressing a combination of circASPH, sh-miR-370, and HAO2. All experiments were carried out with n = 3 biological replicates. *p < 0.05, **p < 0.01, ***p < 0.001.