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Effects of hsa_circ_0000711 expression level on proliferation and apoptosis of hepatoma cells

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Abstract. – OBJECTIVE: To investigate the role of human serum albumin (hsa)_circular (circ)_0000711 in hepatocellular carcinoma (HCC). Circular ribonucleic acids (circRNAs) are proven in numerous studies to play crucial role in tumor biology, but their roles in HCC remain unknown to a great extent.

PATIENTS AND METHODS: The circRNA expression profile microarray was employed to screen differentially expressed circRNAs in tumor tissues and adjacent tissues from HCC patients, and Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) assay was performed for further verification. Next, the target micro RNAs (miRNAs) and their messenger RNAs (mRNAs) of key circRNAs were predicted by bioinformatics software, and a circRNA-miRNA-mRNA regulatory network was constructed. Subsequently, KEGG and GO enrichment analyses were applied to predict the possible biological processes regulated by hsa_circ_0000711 and relevant signaling pathways. The miRNAs playing a key role in the circRNA-miRNA-mRNA regulatory network were then selected as the objects, and their direct binding to hsa_circ_0000711 was confirmed *via* luciferase reporter gene assay. Thereafter, hsa_circ_0000711 was overexpressed or knocked out, and the biological function of hsa_circ_0000711 was detected by cell counting kit-8 (CCK-8) assay, apoptosis detection, and 5-Ethynyl-2'-deoxyuridine (EdU) staining assay *in vitro*.

RESULTS: The results of expression profile screening revealed that there was a significant difference in the expression profile of circRNAs between tumor tissues and adjacent tissues in HCC patients. Based on the circRNA expression profile and RT-qPCR results, the expression level of hsa_circ_0000711 was overtly reduced in HCC tissues. In addition, miR-103a-3p had the highest eigenvector centrality in the circRNA-miRNA-mRNA regulatory network, suggesting that miR-103a-3p is a vital participant in the pathological mechanism of hsa_circ_0000711. The KEGG enrichment analysis results pointed out that the target genes regulated by hsa_circ_0000711 were clearly enriched in the tumor-associated signaling pathways. Besides, the results of GO enrichment analysis demonstrated that the biological processes regulated by hsa_circ_0000711 were mainly related to cell cycle regulation, so cell proliferation might be affected. The results of luciferase reporter gene and RT-qPCR assays showed that hsa_circ_0000711 directly bound to has-miR-103a-3p to serve as a molecular sponge. The results of CCK-8 and EdU staining assays revealed that the proliferation of hepatoma cells in hsa_circ_0000711 overexpression group was evidently enhanced. In addition, it was further found *via* flow cytometry that the apoptosis rate of cells was significantly raised in hsa_circ_0000711 low-expression group and dramatically declined in hsa_circ_0000711 overexpression group.

CONCLUSIONS: Overexpression of hsa_circ_0000711 promoted the proliferation and inhibited the apoptosis of hepatoma cells *via* targeting has-miR-103a-3p.

Key Words:
Hsa_circ_0000711, HCC, Molecular sponge.

Introduction

Hepatic carcinoma, a disease with a high mortality rate, ranks third in terms of the mortality rate among all tumors, and hepatocellular carcinoma (HCC) accounts for 80-90% of primary hepatic carcinoma[1]. As to the treatment of early HCC, surgery, liver transplantation, as well as percutaneous ablation are mainly adopted at present. With the advances in early detection techniques for patients with hepatic cirrhosis, the ac-

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curacy of early diagnosis of HCC is increasing[2], but most patients are still definitely diagnosed with advanced hepatic carcinoma. For patients with advanced hepatic carcinoma, such existing treatment methods as transarterial chemoembolization and molecularly targeted therapy are proven to be effective[3].

Currently, great progress has been made in the treatment approaches for HCC in clinical practice, but more new effective treatment methods are urgently needed. Many research results have pointed out that further analyzing the molecular mechanism of tumorigenesis is conductive to the discovery of new therapeutic targets[4]. Given this, finding out the core effect of circular ribonucleic acids (circRNAs) on tumorigenesis helps understand tumorigenesis at the molecular level and is of great significance for the development of new diagnostic and therapeutic methods[5].

For human cancers, the discovery of target genes is a critical step to assess the role of abnormally expressed genes in human cancers and a basis for the further development of targeted therapies[6]. CircRNAs are a class of RNAs with specific structures, and thousands of tissue-specific and pathomechanism-specific highly expressed circRNAs have been found in human tissues nowadays. CircRNAs are able to bind to micro RNAs (miRNAs) to function as a "miRNA sponge", modulating the expression level of target genes at the transcriptional and post-transcriptional levels and thereby playing a vital role in human diseases[7].

At present, circRNAs have become new hotspots in the field of cancer research, and the relationships of circRNAs with tumors, as well as their mechanisms affecting tumor progression are still elusive. This investigation aims to find out the abnormally expressed circRNAs in the tumor tissues of HCC patients through the high-throughput microarray technique, and to investigate their roles in the pathophysiological process of HCC *via* function research.

Patients and Methods

General Information

Tumor tissues and adjacent normal tissues surgically extracted from 27 HCC patients in our hospital from May 2018 to July 2018 were immediately placed in RNA-fixer reagent (Bioteke, Beijing, China) after removal and stored in a refrigerator at –80°C for use. To exclude the factors influencing the expression profile of circRNAs, the following exclusion criteria were used in this study: participants did not take drugs before surgery, including drugs for chemotherapy, radiation therapy, and targeted therapy. All tissue samples were definitely diagnosed as HCC tissues or adjacent normal tissues via histomorphology. This study was approved by the Ethics Committee of The People's Hospital of Guangxi Zhuang Autonomous Region. Signed written informed consents were obtained from all participants before the study.

Screening of Differentially Expressed CircRNAs Through CircRNA Array Analysis

Total RNAs were extracted from tumor tissues and adjacent normal tissues and then quantified using a NanoDrop kit (Thermo Fisher Scientific, Waltham, MA, USA). Next, the integrity of RNAs was evaluated using a Bioanalyzer 2100 (Agilent, Santa Clara, CA, USA). In this study, complementary RNAs (cRNAs) were prepared using a 3' IVT Express kit (Affymetrix, Santa Clara, CA, USA) and 100 ng of total RNAs, and hybridized at 45°C for 16 h on a PrimeView Human array (Affymetrix, Santa Clara, CA, USA) according to the instructions of a GeneChip 3' Array (Affymetrix, Santa Clara, CA, USA). In addition, the array was processed on a FS-450 fluid station (Affymetrix, Santa Clara, CA, USA) for washing and staining and scanned using a GeneChip scanner (Affymetrix, Santa Clara, CA, USA) in accordance with the protocol of the manufacturer. The raw data of the CEL file were imported into the Partek Genomics Suite 6.6 software, and the probe set was normalized using the Robust Multiarray Average method. One-way analysis of variance (ANOVA) was adopted to determine the significance of the differentially expressed genes, and the p was corrected using FDR[8].

Cell Culture

The human hepatoma cell line Bel-7402 was purchased from the Institute of Biology, Chinese Academy of Sciences (Shanghai, China), phosphate-buffered saline (PBS), trypsin, fetal bovine serum and Roswell Park Memorial Institute-1640 (RPMI-1640) medium from Gibco (Rockville, MD, USA), and siRNAs from Servicebio (Wuhan, China). SW 480 cells were cultured in a 5% CO_2 cell incubator at 37°C. After the culture dish was covered with the cells, digestion and passage were performed with 0.25% trypsin-EDTA (ethylenediaminetetraacetic acid).

Table I. Primer sequences.

Primer name	Primer sequence (5'-3')
Human serum albumin (hsa)_circ_0000711	5'-CACTAGACTGGCCTTTACC-3' 5'-CACAATCATCTGGCTCAA-3'
Hsa_circ_0000520	5'-GTCTGAGACTAGGGCCAGAGG C-3' 5'-GACATGGGAGTGGAGTGAC AGG-3'
Hsa_circ_0061265	5'-TCAACCTTTTGCCCCACACT-3' 5'-AAGACACGTCTGTGTGTTGT-3'
MiR-103a-3p	5'-CAGCATTGTACAGGGCT-3' 5'-GAACATGTCTGCGTATCTC-3'
GAPDH	5'-TCGACAGTCAGCCGCATCTTCTTT-3' 5'-ACCAAATCCGTTGACTCCGACCTT-3'
U6	5'-CTCGCTTCGGCAGCACATAT-3' 5'-TTGCGTGTCATCCTTGCG-3'

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) Assay

RT and qPCR were carried out to detect the expressions. Tissue samples were taken out from the cryogenic vials, drained, ground in liquid nitrogen, and collected to a 5 mL tube, followed by thorough homogenization using a tissue homogenizer. Next, the liquid was transferred to a clean imported 1.5 mL Eppendorf (EP) tube, let stand at room temperature for 5-10 min for full lysis, and centrifuged at 1200 rpm/min for 5 min. Thereafter, the precipitate was discarded, and chloroform was added at 200 μL chloroform/1 mL TRIzol, shaken and mixed, followed by still standing at room temperature for 15 min and then centrifugation at 12000 rpm/min and 4°C for 15 min. After that, the supernatant aqueous phase was absorbed to another centrifuge tube, added with isopropanol 0.7-1 times the supernatant in volume, placed at room temperature for 10-30 min, and centrifuged at 12000 rpm/min for 10 min, followed by discarding of the supernatant. RNAs subsided at the bottom of the tube and were added with 75% ethanol at 1:1 (75% ethanol: TRIzol). Then, the tube was gently shaken to suspend the sediment, followed by centrifugation at 12000 rpm/min and 4°C for 5 min. Afterwards, the supernatant was discarded as far as possible, and the sediment was dried on a super clean bench for 10-20 min and dissolved with 10-50 μL of diethyl pyrocarbonate (DEPC)-treated H_2O solution. The concentration was determined using a microspectrophotometer (NanoDrop). RT reaction conditions: 4.5 μL of RNase free dH_2O, 2 μL of 5×RT reaction buffer, 0.5 μL of Random primer, 0.5 μL of Oligo dT, 0.5 μL of reverse transcriptase and 2 μL of RNA. The complementary deoxyribonucleic acid (cDNA) samples were divided into three portions, and each group of total cDNA samples was diluted 20 times. Then, 3 μL of cDNA was taken for PCR amplification. The amplification level of the target genes was verified using 5% agarose gel electrophoresis. The LabWorks 4.0 image acquisition and analysis software were applied for data quantitation and processing. For each group of samples, the assay was repeated for three times to obtain reliable data. In this study, the $2^{-\Delta\Delta Ct}$ method was used to analyze the changes in the relative expression level of the target genes. The primers used in this study were synthesized by Shanghai Generay Biotech Co., Ltd. (Shanghai, China). The primer sequences are shown in Table I.

Construction of CircRNA-miRNA-messenger RNA (mRNA) Network and GO and KEGG Enrichment Analyses

The circRNA-miRNA-mRNA network of candidate circRNAs was constructed based on the bioinformation database TargetScan (http://www.targetscan.org/) and miRanda (http://www.microrna.org/). Then, the GO and KEGG enrichment analyses were employed to annotate the function of the target genes.

Luciferase Reporter Gene Assay

In this assay, wild-type and mutant hsa_circ_0000711 were amplified and cloned into psiCHECK™-2 luciferase liposomes (Promega, Madison, WI, USA) to generate wild-type and mutant reporter genes. HEK293 cells were cultured in a 24-well plate and co-transfected with miR-103a mimic or miR-con and wild-type or mutant liposomes for 48 h. Thereafter, the activity of luciferase was detected using Dual-Lu-

ciferase reporter reagent (Promega, Madison, WI, USA).

Western Blotting

An appropriate amount of radioimmunoprecipitation assay (RIPA; Beyotime, Shanghai, China) was taken, added with the protease inhibitor phenylmethylsulfonyl fluoride (PMSF) at a ratio of 100:1 and mixed to prepare cell lysis buffer. After trypsinization, the cells were collected and added with the lysis buffer, and the resulting product was collected, transferred to an EP tube, and centrifuged at 4°C and 14000 rpm for 30 min using a low temperature high-speed centrifuge, followed by collection of the protein supernatant. Thereafter, the proteins were denatured at 95°C for 10 min via hot bath. The prepared protein samples were placed in the refrigerator at –80°C for later use. A bicinchoninic acid (BCA) kit (Pierce, Rockford, IL, USA) was adopted to quantify proteins. After that, the gel for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared, and the protein samples were loaded into the well of the above gel for electrophoresis at constant pressure (80 V) for 2.5 h. Next, the protein samples were transferred onto polyvinylidene difluoride (PVDF) membranes using the semi-dry method. After that, the PVDF membranes were immersed in Tris-Buffered Saline with Tween®20 (TBST) buffer containing 5% skim milk powder, and blocked using a shaker for 1 h slowly. After that, antibodies were diluted with 5% skim milk powder, and the membrane was incubated with primary antibodies and rinsed with TBST solution for 3 times (10 min/time). Next, the membrane was incubated with secondary antibody at room temperature for 2 h and then rinsed twice with TBST and once with TBS (10 min/time). ECL reagents were used to detect proteins, with exposure in a dark room. Finally, the relative expression level of the proteins was analyzed using Image-Pro Plus v6 (Media Cybernetics, Silver Spring, MD, USA).

Detection of Effect of Hsa_circ_0000711 Expression Level on Proliferation of Hepatoma Cells Through Cell Counting Kit-8 (CCK-8) Assay

The Bel-7402 cells in the logarithmic growth phase were uniformly inoculated on a 96-well plate at 1×10^4 cells/well, added with different diluted concentrations of MTA, with 6 replicate wells set for each concentration, and cultured in an incubator for 72 h. After that, the original medium was discarded, and the cells were added with 20 μL of CCK-8 reaction solution (Dojindo Laboratories, Kumamoto, Japan) and 170 μL of cell medium, incubated at 37°C for 2 h in the dark and shaken on a micro-vibrator for 3 min. Then, the absorbance at a wavelength of 450 nm was measured using a microplate reader.

Determination of Effect of Hsa_circ_0000711 Expression Level on Apoptosis Via Annexin V/ Propidium Iodide (PI) Assay

An Annexin V/PI double staining kit was utilized to determine the changes in apoptosis after treatment. 5×10^5 cells were trypsinized, rinsed with PBS at 4°C twice, centrifuged, re-suspended in 500 μL of staining buffer, and stained with 5 μL of Annexin V-FITC staining solution and 5 μL of PI staining solution in the dark at 37°C for 15 min. A flow cytometer (Guava) was used for flow sample detection.

Statistical Analysis

All data were expressed as mean ± standard deviation ($\bar{\chi}\pm s$). Unpaired Student's *t*-test and one-way analysis of variance were used for data analysis in the two groups. In this study, Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA) was employed for statistical analysis. $p<0.05$ suggested a statistically significant difference.

Results

Differentially Expressed CircRNAs in HCC Patients Screened Via Expression Profile

The results of screening through expression profile (Figure 1) revealed that the expression profile of circRNAs in tumor tissues was significantly different from that in adjacent normal tissues of HCC patients, and there were 72 circRNAs with significant differences (fold change >2, $p<0.01$) in the tumor tissues of HCC patients, of which 31 were overtly up-regulated and 41 were evidently downregulated.

Differences in the Expression of Differentially Expressed Circular RNAs Verified Via RT-qPCR Assay

Considering the reliability of the high-throughput screening method, three significantly up-regulated circRNAs (hsa_circ_0000711, hsa_circ_0000520, and hsa_circ_0061265) were

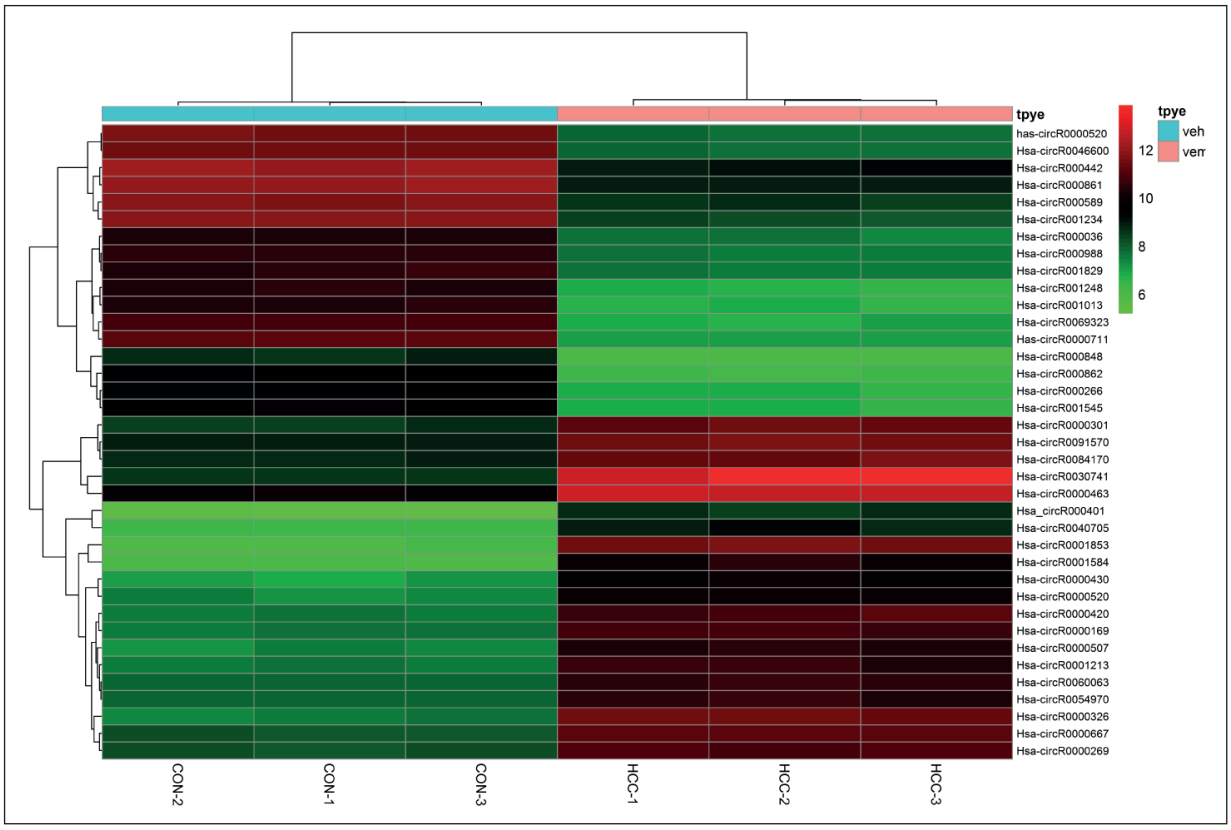

Figure 1. Differentially expressed circRNAs in tumor and adjacent tissues of HCC patients. Unsupervised cluster analysis heat map (HCC: tumor tissues, and Con: normal tissues, each row represents a circRNA, with red for a high expression level and green for a low expression level). In HCC patients, the expression profile of circRNAs is different between tumor tissues and adjacent normal tissues.

subsequently selected in this study for further verification through the more reliable RT-qPCR assay. The results of RT-qPCR showed that only hsa_circ_0000711 displayed a clearly higher expression in 27 cases of HCC tumor tissues than that in adjacent normal tissues, and the difference was statistically significant ($p<0.01$), while there were no significant differences in the expression levels of hsa_circ_0000520 and hsa_circ_0061265 between HCC tumor tissues and adjacent normal tissues ($p>0.05$), suggesting that the hsa_circ_0000711 expression level is notably reduced in HCC tumor tissues (Figure 2). Based on this, it was hypothesized in this study that hsa_circ_0000711 plays an important role in the pathological process of hepatic carcinoma, and it was selected as subject for further research.

CircRNA-miRNA-mRNA Regulatory Network

In this study, it was presumed that hsa_circ_0000711 regulates the circRNA-miRNA-mRNA network through the "miRNA sponge" mechanism, thus influencing the pathological process of HCC. Therefore, mirSVR was used for scoring, and the first seven target miRNAs of hsa_circ_0000711 were found and ranked. Next, the TargetScan and miRanda were applied to predict the mRNAs affected by these 7 target miRNAs.

Subsequently, the circRNA-miRNA-mRNA regulatory network of hsa_circ_0000711 was visualized using the Cytoscape software. The analysis results showed that miR-103a-3p had the highest eigenvector centrality in the circRNA-miRNA-mRNA regulatory network, suggesting that it plays a vital role in the pathological mechanism of hsa_circ_0000711 (Figure 3).

Results of KEGG and GO Pathway Enrichment Analyses

To further understand the potential roles of hsa_circ_0000711, GO and KEGG enrichment analyses were performed to evaluate the biological processes of the remarkable enrichment of all mR-

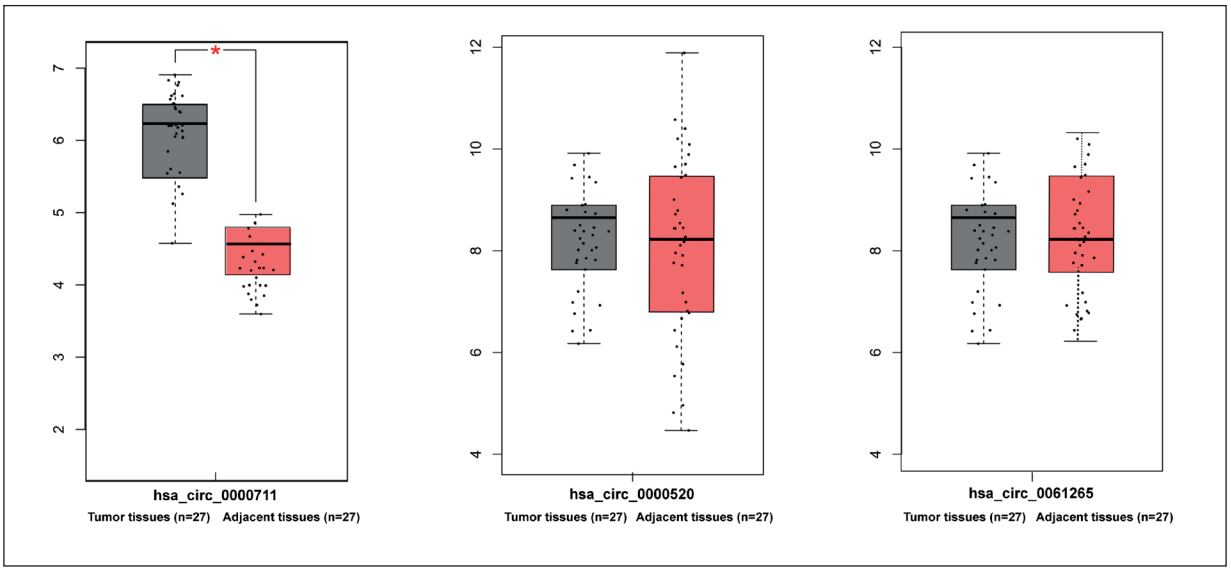

Figure 2. Differences in the expression levels of hsa_circ_0000711, hsa_circ_0000520 and hsa_circ_0061265 between tumor tissues and adjacent normal tissues detected via RT-qPCR assay. **$p<0.01$ *vs.* control group.

NAs in the circRNA-miRNA-mRNA network and related signaling pathways. KEGG enrichment analysis results demonstrated that the target genes regulated by hsa_circ_0000711 were markedly enriched in tumor (such as HCC, prostate cancer, breast cancer, and colon cancer)-related signaling pathways and cell cycle signaling pathways, indicating that hsa_circ_0000711 may exert a crucial effect in cell cycle regulation. Moreover, it was revealed that hsa_circ_0000711 might participate in modulating such signaling pathways as hippo signaling pathway, MAPK signaling pathway, mTOR signaling pathway, and TGF-β signaling pathway. The results of GO enrichment analysis manifested that the biological processes regulated by hsa_circ_0000711 included regulation of cell cycle process, cell cycle arrest, and cell cycle, implying that the biological processes regulated by hsa_circ_0000711 are mainly correlated with cell cycle regulation (Figure 4).

Target Genes of Hsa_circ_0000711 Predicted and Verified

The most typical mechanism of action of circRNAs is to act as a molecular sponge of miRNAs. According to the above results, a total of five potential miRNAs of hsa_circ_0000711 were obtained (Figure 5A). The mirSVR score indicated that has-miR-103a-3p had the highest bond strength to hsa_circ_0000711, so miR-124 was selected for further verification. The predicted binding sites of has-miR-103a-3p to hsa_circ_0000711 are shown in Figure 5B. Next, the direct binding relationship between has-miR-103a-3p and hsa_circ_0000711 was verified using the luciferase reporter assay. In HEK-293 cells transfected with wild-type hsa_circ_0000711, the fluorescence density of cells in has-miR-103a-3p mimic treatment group was clearly lower than that in miR-con treatment group, showing a statistically significant difference ($p<0.01$) (Figure 5C). Besides, RT-qPCR results confirmed that the transfection of si-hsa_circ_0000711 in hepatoma cells overtly decreased the expression level of has-miR-103a-3p, and the difference was statistically significant ($p<0.01$). It can be concluded that hsa_circ_0000711 is capable of directly binding to has-miR-103a-3p to exert the effect of molecular sponges.

Effect of Hsa_circ_0000711 Expression Level on Proliferation of HCC Cells

In accordance with the results of CCK-8 assay, the proliferation of hepatoma cells in hsa_circ_0000711 overexpression group was evidently enhanced at 12 h, 24 h, 36 h, 48 h, and 72 h, indicating that hsa_circ_0000711 can increase and promote the proliferation of hepatoma cells ($p<0.05$) (Figure 6A). In addition, the results of 5-Ethynyl-2'-deoxyuridine (EdU) staining assay manifested that the number of red fluorescence rose significantly after 72 h of hsa_circ_0000711 overexpression, implying that hsa_circ_0000711 expression facilitates the proliferation of hepatoma cells ($p<0.05$) (Figure 6B).

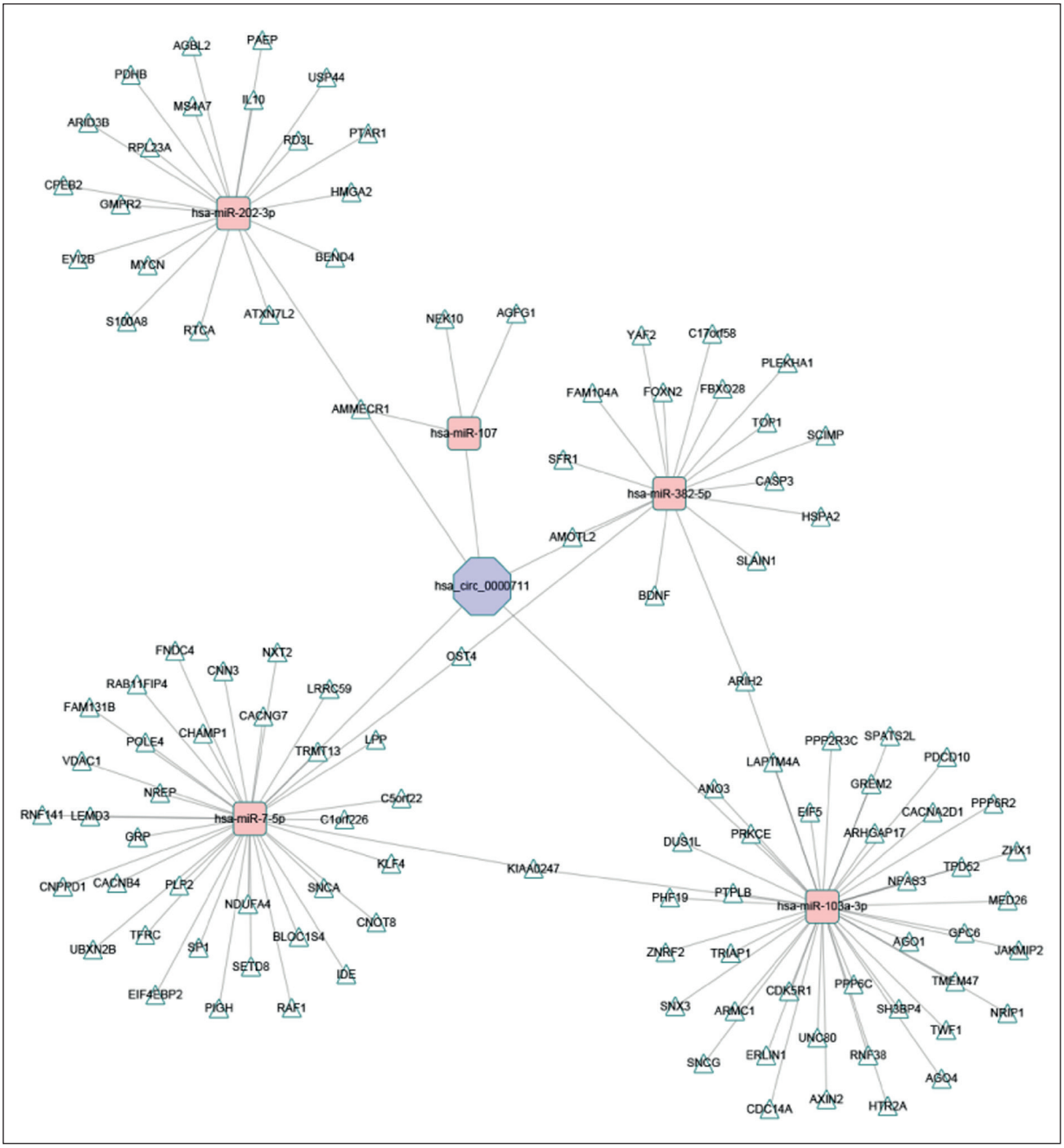

Figure 3. CircRNA-miRNA-mRNA regulatory network. Rectangle: lncRNA, triangle: miRNA, and arrow: mRNA. The darker graph and larger area represent that the eigenvector centrality of a node is higher, implying that the node is of great significance in the network. MiR-23b-5p exhibits the largest interaction network, followed by miR-93-3p, miR-581, and miR-23a-5p.

Influence of Hsa_circ_0000711 Expression Level on Apoptosis of HCC Cells

After that, flow cytometry was adopted in this study to detect the influence of hsa_circ_0000711 expression level on the number of apoptotic cells, and it was discovered that the number of apoptotic cells was notably greater in hsa_circ_0000711 low-expression group than that in control group, while it remarkably declined in hsa_circ_0000711 overexpression group, and the differences are of statistical significance ($p<0.05$), indicating that overexpressing hsa_circ_0000711 gene is able to repress the apoptosis of hepatoma cells (Figure 7).

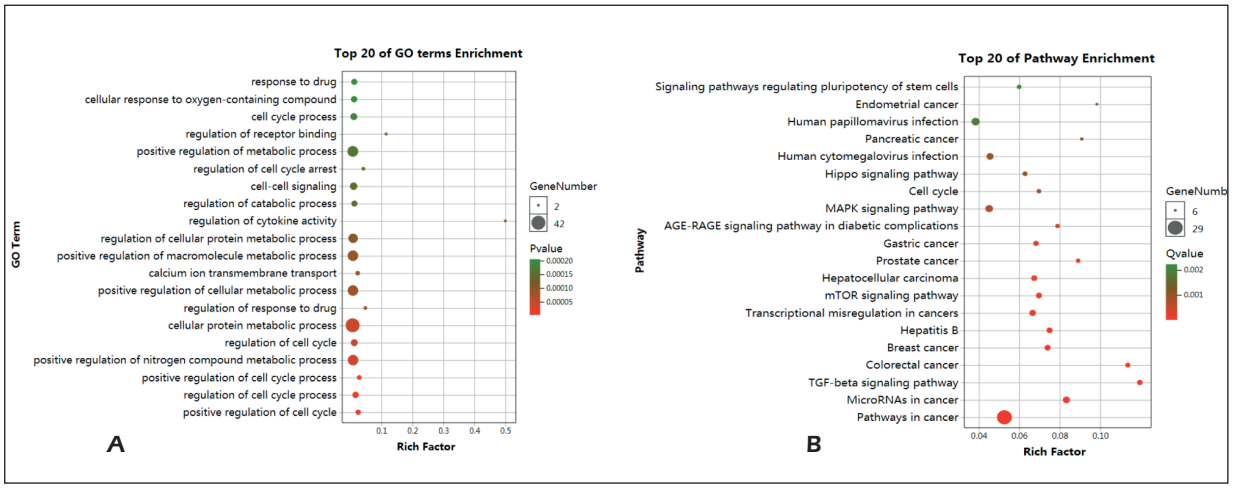

Figure 4. Results of KEGG and GO pathway enrichment analyses. **A**, Results of GO enrichment analysis. **B**, Results of KEGG enrichment analysis.

Discussion

According to the epidemiological studies, the incidence and mortality rates of HCC are increasing year by year all over the world. The leading factors resulting in HCC include hepatitis B virus infection, smoking, and drinking[9]. Many researchers are dedicated to finding out the pathogenic genes and diagnostic markers for liver cancer. Existing diagnosis and treatment strategies have been greatly improved, but the overall prognosis of hepatic carcinoma remains very

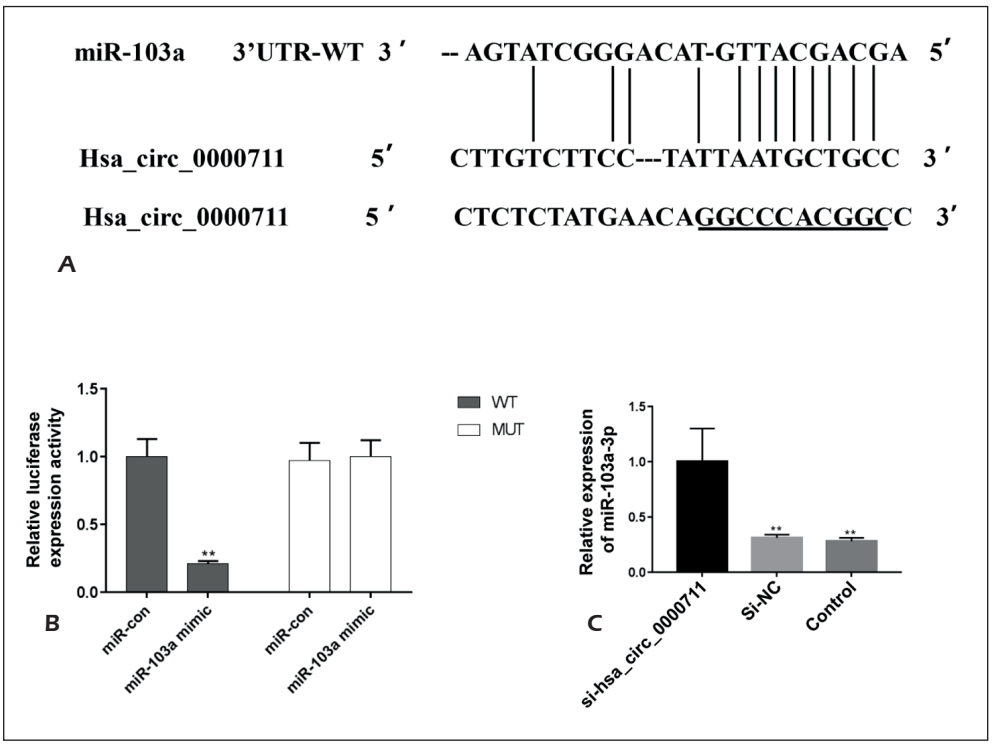

Figure 5. Hsa_circ_0000711 targets and acts on has-miR-103a-3p. **A**, Binding sites of hsa_circ_0000711 to has-miR-103a-3p predicted. **B**, Direct binding of hsa_circ_0000711 to has-miR-103a-3p verified via the luciferase reporter assay. WT: transfection with wild-type hsa_circ_0000711, MUT: transfection with mutant hsa_circ_0000711. **C**, Effect of transfection of si-hsa_circ_0000711 on the expression level of has-miR-103a-3p in HCC cells. $^{**}p<0.01$.

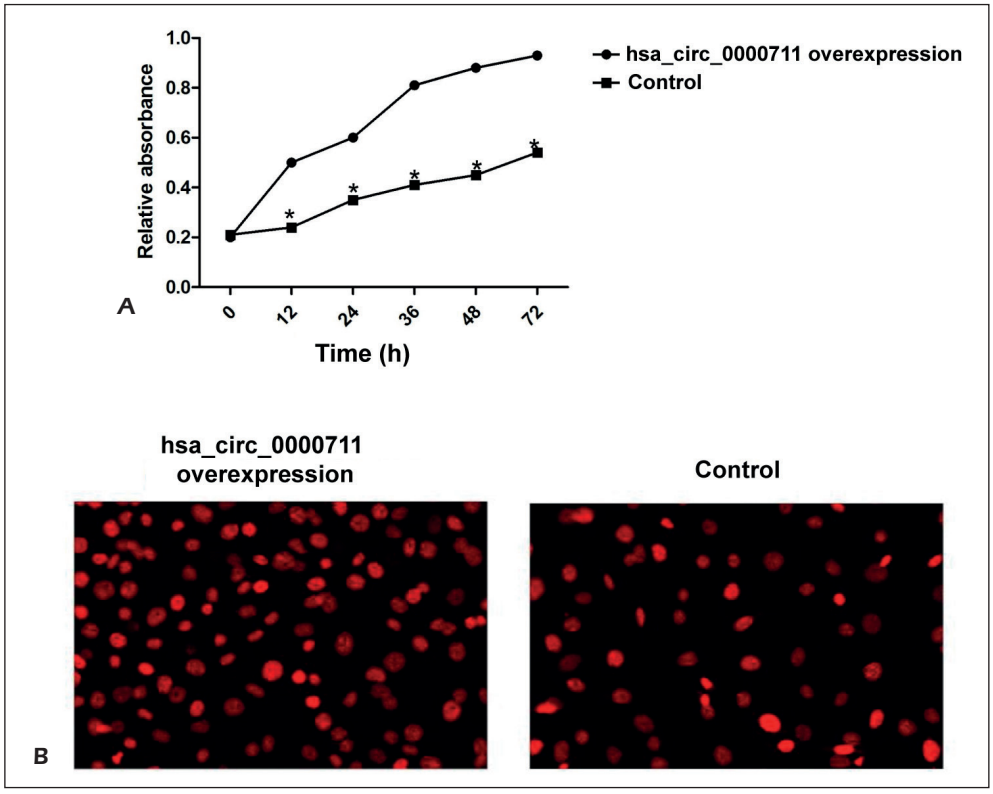

Figure 6. Effect of the hsa_circ_0000711 expression level on the proliferation of HCC cells. **A**, Effect of hsa_circ_0000711 expression level on the proliferation of HCC cells determined through CCK-8 assay, **B**, Effect of hsa_circ_0000711 expression level on the cycle of HCC cells determined through flow cytometry and PI staining assay (magnification: 40×).

poor. Hence, looking for key genes and proteins that lead to the development and progression of hepatic carcinoma is of great significance for the early diagnosis, precise treatment, and prognosis of hepatic carcinoma[10].

CircRNAs are highly expressed in human genomes, which are proven in a study to be widely expressed in human genomes in a complex tissue-, cell type- or developmental stage-specific manner[11]. Moreover, circRNAs have no terminals, so they are highly resistant to conventional RNA degradation pathways. As a result, massive researchers hold that circRNAs can serve as powerful and effective biomarkers for the diagnosis and monitoring of diseases[12,13]. In this study, the expression profile screening results showed that there was a significant difference in the expression profile of circRNAs between tumor tissues and adjacent normal tissues in HCC patients, and 72 circRNAs with significant differences (fold change >2, $p<0.01$) were detected in tumor tissues in HCC patients compared with adjacent normal tissues, including 31 circRNAs remarkably up-regulated and 41 circRNAs clearly down-regulated. Next, the expression profile of circRNA and RT-qPCR results manifested that the expression level of hsa_circ_0000711 was dramatically reduced in HCC tumor tissues.

Li et al[14] have indicated that hsa_circ_0000711 expression level is overtly decreased in colon cancer tissues, and clinical data show that hsa_circ_0000711 can be used as an independent predictor for the diagnosis and prognosis of colon cancer patients, which plays a crucial role in the development of colon cancer. Of note, the results of KEGG enrichment analysis of the target genes regulated by hsa_circ_0000711 displayed that the target genes regulated by hsa_circ_0000711 were significantly enriched in the signaling pathways relating to different types of tumors such as gastric cancer[15], colon cancer, prostate cancer and breast cancer, which is consistent with the above study results, demonstrating the reliability of the findings of this study. The results also indicated that hsa_circ_0000711 might be an important participant in the development of various different types of tumors, worthy of attention.

The function and mechanism of most circRNAs have not been clearly elucidated. Several

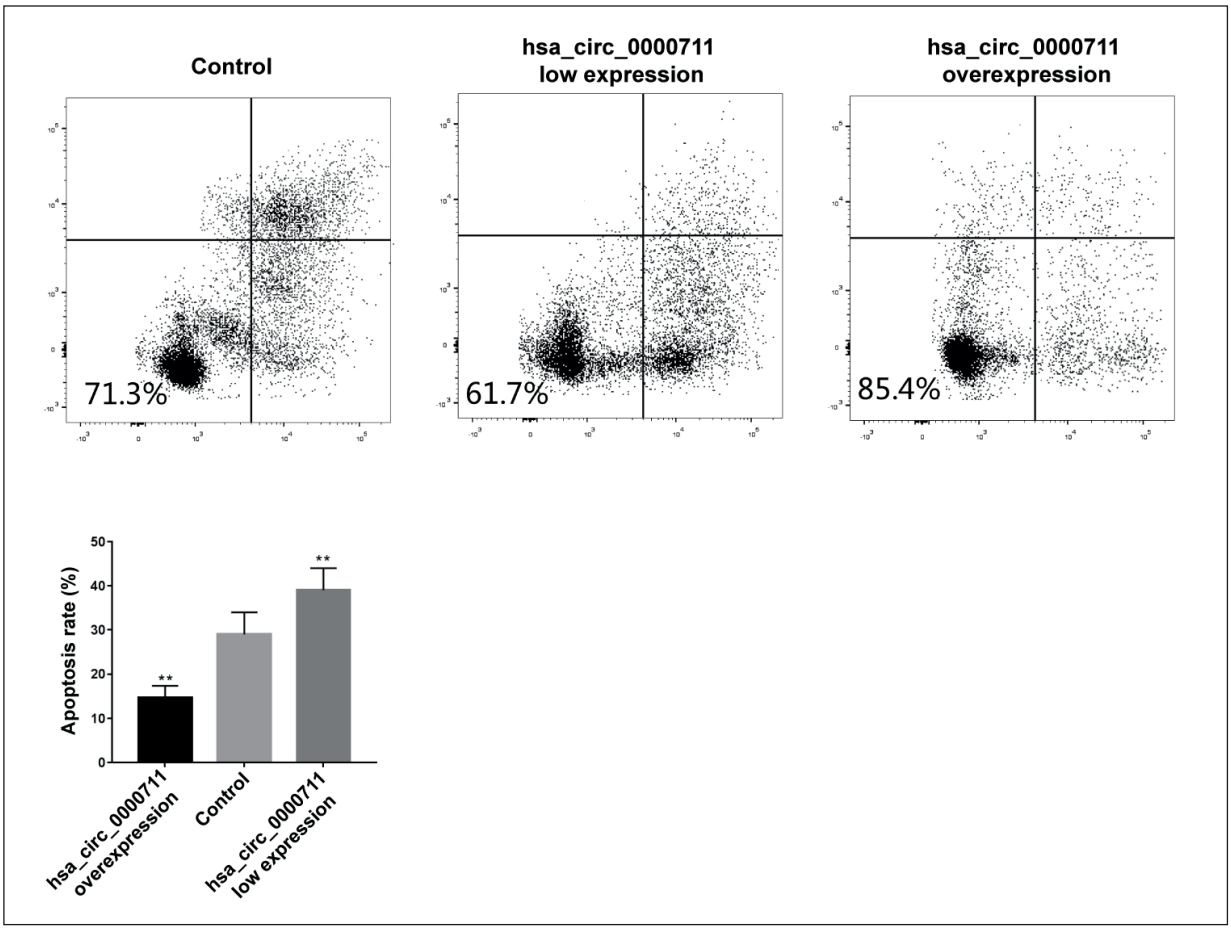

Figure 7. Influence of the hsa_circ_0000711 expression level on the apoptosis of HCC cells.

studies have demonstrated that circRNAs can bind to miRNAs to act as molecular sponges, thus regulating gene expression in cancers. Therefore, the circRNA-miRNA-mRNA regulatory network was constructed in this study, in which miR-103a-3p had the highest eigenvector centrality, implying that miR-103a-3p plays a vital role in the pathogenesis of hsa_circ_0000711. Xia et al[16] pointed out in their study that has-miR-103a-3p is capable of modulating the expression of AKAP12, thereby serving as a proto-oncogene in liver cancer. Furthermore, the results of GO enrichment analysis showed that the biological processes regulated by hsa_circ_0000711 were mainly related to cell cycle regulation, so cell proliferation might be affected, which was verified in this study. Moreover, the luciferase reporter gene and RT-qPCR results indicated that hsa_circ_0000711 directly bound to has-miR-103a-3p to act as a molecular sponge, and the subsequent *in vitro* CCK-8 and EdU staining assays uncovered that the proliferation of hepatoma cells in hsa_circ_0000711 overexpression group was distinctly enhanced. The flow cytometry further manifested that the apoptosis rate was significantly elevated in hsa_circ_0000711 low-expression group, and significantly decreased in hsa_circ_0000711 overexpression group.

Conclusions

In summary, the results indicated that hsa_circ_0000711 was markedly low-expressed in the tumor tissues of HCC patients. Hsa_circ_0000711 has an important role in the proliferation and apoptosis of hepatoma cells, while its mechanism may be associated with the binding to has-miR-103a-3p to serve as a molecular sponge. The underlying potential molecular mechanism should be further explored.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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