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Using circular RNA SMARCA5 as a potential novel biomarker for hepatocellular carcinoma

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the most common malignant tumor worldwide. Circular RNAs (circRNAs), a new class of endogenous non-coding RNAs, are widespread and abundant in mammalian cells. Cumulative evidence showed that circRNAs play significant roles in the process of cancer. However, the expression and function of circRNAs in HCC remain to be investigated.

Methods: The expression of circular RNA SMARCA5 (circSMARCA5) in tissues and plasma samples was detected by quantitative real-time polymerase chain reaction (qRT-PCR). The role of circSMARCA5 in HCC progression was assessed by in vitro experiments. A receiver operating characteristic (ROC) curve was established to evaluate the value of circSMARCA5 as a biomarker in HCC.

Results: The expression of circSMARCA5 was significantly downregulated in HCC tissues compared with paracarcinoma tissues. CircSMARCA5 levels were correlated with tumor differentiation ($p = 0.023$), Tumor-node-metastasis (TNM) stage ($p = 0.001$), cancer invasion ($p = 0.004$), as well as cancer diameter ($p = 0.018$). In vitro cell experiments revealed that overexpression of circSMARCA5 resulted in inhibited proliferation, increased apoptosis and suppressed invasion. Moreover, we found that circSMARCA5 expression was downregulated in plasma samples of patients with HCC. The ROC curve analyses revealed that plasma circSMARCA5 showed a high accuracy (AUC = 0.938, 0.853, 0.711) for diagnosing HCC from healthy controls, hepatitis and cirrhosis. The area under the ROC curve of plasma circSMARCA5 in combination with AFP in diagnosing HCC from hepatitis and cirrhosis was 0.903 and 0.858. Especially, plasma circSMARCA5 presented a high accuracy (AUC = 0.847, 0.706) for detecting HCC with serum AFP below 200 ng/ml from those hepatitis and cirrhosis with AFP below 200 ng/ml.

Conclusion: Our study revealed that circSMARCA5 may promote apoptosis, inhibit proliferation, invasion and metastasis of HCC cells. CircSMARCA5 may serve as a potential prediction and monitor biomarker for HCC, especially in HCC patients with AFP blow the cutoff value.

1. Introduction

HCC ranks as the sixth most common cancer and the third commonest cause of death due to cancers worldwide [1,2]. Hepatitis B and C virus infection, consumption of aflatoxins, nonalcoholic fatty liver disease, and cirrhosis related to excessive alcohol consumption and smoking, are the main risk factors of HCC [3–5]. Despite treatment of HCC in an early stage by surgical or liver transplantation [1], the 5-year survival rate remains poor [6]. Due to lack of early diagnosis biomarker, most patients with HCC continue to be diagnosed at their advanced stages and thus miss the best opportunity for therapy. Thus, an early diagnosis appears to be a promising measure for improving the prognoses of patients with HCC. Over the last few decades, numerous biomarkers were reported to diagnostic for HCC [7], but their sensitivity and specificity remain suboptimal. Therefore, it is urgent to search for new biomarkers for improving the early prediction of HCC.

Abbreviations: HCC, hepatocellular carcinoma; circRNAs, circular RNAs; circSMARCA5, circular RNA SMARCA5; TNM, Tumor-node-metastasis; ROC, receiver operating characteristic curve; EMT, epithelial mesenchymal transition; STR, short tandem repeat; OFR, open reading frame; ANOVA, one-way analysis of variance; MMPs, matrix metalloproteinases; AUC, area under the curve; CI, confidence interva

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CircRNAs, a new class of endogenous non-coding RNAs, are covalently form closed loops with no 5′ and 3′ ends [8]. CircRNAs often exhibit tissue-specific and developmental-stage-specific expression [9,10]. Cumulative evidence indicates that dysregulation of circRNAs is involved in many diseases, including cancer [2,11–13]. Notably, circRNAs played a crucial role in tumor growth, invasion, apoptosis and clinical outcome [14,15]. CircRNAs are highly conserved and stable, thus might be potential ideal biomarkers for cancer diagnosis [13,16].

CircSMARCA5 was derived from SMARCA5 gene and located at chr4: 144464662–144,465,125. CircSMARCA5 was reported to be induced during epithelial mesenchymal transition (EMT), which was widely accepted as an important mechanism of the metastatic process for HCC [17]. CircSMARCA5 expression was dysregulated and associated with cell proliferation in prostate cancer [18]. Moreover, recent research showed that the levels of circSMARCA5 were decreased in HCC tissues compared with paired para-carcinoma tissues [19]. We thought the diagnostic value of circSMARCA5 in HCC need to be further investigated and executed the present study.

2. Materials and methods

2.1. Sample collection

The 133 pairs of HCC tissues and para-carcinoma tissues, 33 control liver tissues (liver tissues from patients with liver hemangioma) and 31 cirrhosis tissues were collected from patients who underwent surgery from May 2013 to May 2017, in the Zhongnan Hospital of Wuhan University. Tissue specimens were immediately soaked in RNAlater@ RNA Stabilization Solution (Invitrogen, CA, USA) after surgery, then stored at −80 °C. All patients were confirmed by pathological diagnosis of liver biopsy. None of the patients had an extra history of solid organ tumors or preoperative treatment. Clinical features of patients with HCC are shown in Table 1.

A total of 498 plasma samples was collected in ethylenediaminetetraacetic acid (EDTA) tubes at Zhongnan Hospital of Wuhan University from August 2016 to September 2017, including 103 age- and sex-matched healthy controls, 117 hepatitis patients (hepatitis B and C), 143 cirrhosis and 135 patients with HCC. All the healthy controls were with no hepatitis, hepatic diseases and abnormal biochemical results of liver function tests. Patients who had an additional history of solid organ tumors, underwent radiotherapy, chemotherapy or targeted therapy were excluded. Clinical characteristics of plasma samples from HCC patients are summarized in Supplementary Table 2.

Written informed consent was obtained from all participants before the study began. All the investigation got the approval by Medical Institutional Review Board of Zhongnan Hospital of Wuhan University.

2.2. RNA extraction

Total RNA from tissues was isolated using TRIzol Reagent (Invitrogen, CA, USA) while plasma total RNA was extracted using Total RNA Separate Extraction Kit (Bioteke, Beijing, China), according to the manufacturer's instructions. Then, cDNA was synthesized using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO, Japan) according to the manufacturer's protocol.

2.3. Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed in triplicated with iTaq Universal SYBR Green supermix (Bio-Rad, Hercules, CA, USA) on a CFX96 system (Bio-Rad, CA, USA) following the manufacturer's instructions. Primers were listed in Supplementary Table 1. GAPDH served as the internal control. Relative mRNA expression was calculated by the $2^{-\Delta\Delta}$ Ct method. QRT-PCR data processing was shown in an additional file (Fig. S1).

Table 1
Associations between circSMARCA5 expression and clinical parameters in HCC.

Characteristics	Patient number	CircSMARCA5	*P*-value
		Mean ± SD	
Gender			
Male	124	0.508 ± 0.776	0.881
Female	10	0.667 ± 0.595	
Age			
< 55	64	0.403 ± 0.597	0.092
≥55	70	0.626 ± 0.881	
Alcoholism			
Negative	91	0.498 ± 0.776	0.504
Positive	40	0.526 ± 0.754	
Differentiation			
High/Moderate	107	0.595 ± 0.831	**0.023**
Low	27	0.222 ± 0.245	
TNM stage			
I + II	95	0.658 ± 0.865	**0.001**
III + IV	39	0.179 ± 0.161	
Invasion			
T0-T1	80	0.662 ± 0.817	**0.004**
T2-T4	54	0.309 ± 0.628	
Tumor Size			
< 5.0	42	0.86 ± 1.117	**0.018**
≥5.0	92	0.360 ± 0.458	
Tumor number			
1	99	0.563 ± 0.764	0.504
> 1	31	0.402 ± 0.806	
AFP			
Negative (<200)	48	0.532 ± 0.719	0.473
Positive (≥200)	47	0.383 ± 0.684	
ALT(U/L)			
< 46	79	0.519 ± 0.785	0.835
≥46	55	0.519 ± 0.739	
AST(U/L)			
< 46	79	0.531 ± 0.782	0.731
≥46	55	0.502 ± 0.744	

$P < 0.05$ was considered statistically significant (in bold).
Abbreviations: TNM, tumor node metastasis; AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

2.4. Cell culture and transfection

Human HCC cell lines (Huh7, HCCLM9 and HepG2) were cultured in Dulbeco's modified Eagel's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA), 100 μg/ml streptomycin and 100 U/ml penicillin. All cell lines were maintained at 37 °C in a humidified incubator with 5% (*v*/v) CO_2. HCC cell lines were authenticated by short tandem repeat (STR) profiling with ABI 3130 sequencing system (ABI, USA). The open reading frame (OFR) of SMARCA5 was amplified from HepG2 cells and subcloned into pcDNA 3.1. Transfection was performed with FuGENE® HD Transfection Reagent (Promega, USA) according to the manufacture's protocol.

2.5. Cell proliferation assay

Cell proliferation assay was performed using Cell Counting Kit-8 (CCK8, Dojindo, Japan) and 5-Ethynyl-2′-deoxyuridine (EdU) assays (RiboBio, Nanjing, China). CCK8 was performed according to the manufacturer's instructions. Briefly, after transfection with circSMARCA5 in 6-well plates for 24 h, cells were seeded into 96-well plates with 1000 cells per well and cultured for 0, 24, 48, 72 or 96 h, respectively. About 10 μl CCK8 reagent was added to the wells and incubated for 2 h at 37 °C. The absorbance was measured at 450 nm. Each experiment was replicated three times. For the EDU assays, cells

were indicated with 50 μM EdU at 48 h after transfection. An Apollo staining and DAPI staining were used to detect the EdU positive cells with a fluorescence microscope.

2.6. Apoptosis detection

To measure whether circSMARCA5 was involved in cell apoptosis, we carried out Annexin V-FITC/PI apoptosis assay and Tunel assay (Roche, Cat. No: 11 684 817 910). At 24 h after transfection, the level of apoptosis was detected with Annexin V-FITC Apoptosis Detection Kit (Beyotime Biotechnology, Shanghai, China) on a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA). Similarly, the analysis of apoptosis using Tunel assay was performed after transfection of 24 h. Tunel-positive cells were counted for different microscopic fields under fluorescence microscope.

2.7. Western blotting

Total protein was extracted at 24 h after transfection. Cell lysates were separated by 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred into polyvinylidene difluoride (PVDF) membranes. The following primary antibodies were used in this study: anti-MMP7 Antibody (Abcam, ab205525), anti-MMP9 Antibody (ABclonal, A0289), Anti-β-actin Antibody (ABclonal, AC026).

2.8. Statistical analysis

All experimental data were expressed as mean ± SD. Difference between two groups was analyzed by Student *t*-test. One-way analysis of variance (ANOVA) was adopted to analysis the difference in multiple groups. We combined the diagnostic value of circSMARCA5 with AFP based on multivariate ROC curve analysis. The steps were as follows: (1) Calculate the predicted probabilities. The Binary Logistic process of SPSS was used for logistic regression, then obtained the logistic regression equation, and a new variable containing the prediction probability of each individual was generated in the working data table of SPSS. (2) ROC curve analysis. The ROC curve was performed using GraphPad, the prediction probability was used as the test variable. The AUC, 95% CI, *P* value, sensitivity and specificity could obtain from the ROC curve. All statistical analysis was performed using SPSS 16.0 and GraphPad Prism 7.0. All statistical tests were two side, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. CircSMARCA5 was significantly down-regulated in HCC tissue specimens

To investigate the expression pattern of circSMARCA5, we first analyzed the specificity of the amplified circSMARCA5 product. The melting curve analysis showed that the amplified product yielded a single peak and the sequence was completely consistent with that from circBase (http://www.circbase.org; Fig. 1). These results showed that circSMARCA5 could specifically be amplified using the quantitative real-time PCR (qPCR) method we established.

The relative expression of circSMARCA5 in 133 HCC tissues and their matched para-carcinoma tissues was detected by qRT-PCR. As shown in Fig. 2A, its relative expression in tumor tissues was significantly lower than in matched para-carcinoma tissues ($p < 0.001$). In addition, the expression of circSMARCA5 in cirrhosis and control liver tissues was also investigated. Intriguingly, the results showed that circSMARCA5 expression was significantly lower in HCC tissues compared with cirrhosis and control liver tissues, and its levels in cirrhosis tissues were significantly lower than those in control liver tissues (Fig. 2B). CircSMARCA5 showed HCC-stage-specific expression features. This result indicated that circSMARCA5 might act as an anti-oncogene in HCC.

3.2. Correlations between circSMARCA5 expression and clinicopathological features

Correlation between circSMARCA5 and clinical characteristics are summarized in Table 1. Statistical analysis indicated that circSMARCA5 expression was significantly correlated with tumor differentiation, TNM stage, tumor invasion and tumor size (Fig. 2C–E). Nevertheless, we found no significant association between circSMARCA5 expression and other clinicopathological parameters, such as gender, age, alcoholism and tumor number.

3.3. The biological function of circSMARCA5 in HCC cell lines

The expression of circSMARCA5 was measured in HCC cell lines. As shown in Fig. 3A, endogenous circSMARCA5 expression was the lowest in Huh7, thus, we choose Huh7 for overexpression assays. The efficiency of transfection is examined by qRT-PCR. After transfection, the expression of circSMARCA5 was significantly overexpressed (Fig. 3B). CCK8 and EdU assays were used to test the effect of circSMARCA5 on proliferation of Huh7 cells. The CCK8 assay results showed that circSMARCA5 inhibited cell proliferation (Fig. 3C). Similar results were obtained with the EdU assay (Fig. 3D-E). Then, we investigated the role of circSMARCA5 in apoptosis in Huh7 cells. The Annexin V-FITC/PI apoptosis assay results indicated that circSMARCA5 overexpression promoted late apoptosis (Fig. 3F-H). Similarly, the Tunel assay results indicated that increased apoptosis was observed after circSMARCA5 overexpression (Fig. 3I–J).

Matrix metalloproteinases (MMPs) were reported to play a pivotal role in promoting tumor invasiveness and metastasis [20]. Down-regulated circSMARCA5 was associated with TNM stage and tumor invasion (Fig. 2D), so we carried on further research to investigate whether the expression of MMP7 and MMP9 was changed in circSMARCA5 overexpression cells. We found that overexpression of circSMARCA5 resulted in significantly decreased expression of MMP7 and MMP9 by qRT-PCR (Fig. 4A–B) and western blotting (Fig. 4C).

3.4. CircSMARCA5 expression in plasma among subgroups

In addition to the tissues, we also investigated the expression of circSMARCA5 in plasma. The detailed clinical data of plasma samples with HCC was showed in supplementary Table 2. The levels of plasma circSMARCA5 were investigated by qRT-PCR, in 135 HCC patients, 143 cirrhosis patients, 117 hepatitis patients and 103 healthy controls. The results showed that the expression of circSMARCA5 was lower in HCC samples than those in controls ($p < 0.001$), hepatitis ($p < 0.001$) and cirrhosis ($p < 0.001$) cases (Fig. 5A). Interesting, the levels of circSMARCA5 were decreased in cirrhosis cases compared to hepatitis patients ($p < 0.001$) and controls ($p < 0.001$), and its expression was lower in hepatitis samples compared with healthy controls ($p < 0.001$) (Fig. 5A).

3.5. CircSMARCA5 was relatively stable in plasma

To investigate the potential application of circSMARCA5 in clinic, we test the stability of circSMARCA5 in blood samples. The plasma was incubated in different conditions (room temperature, 4 °C and − 80 °C). The expression of circSMARCA5 was assessed by qRT-PCR. The results showed that circSMARCA5 was relative stable when it was incubated at room temperature or 4 °C for 24 h. And its expression was relative stable when it was incubated at −80 °C for longer time (Fig. 5G).

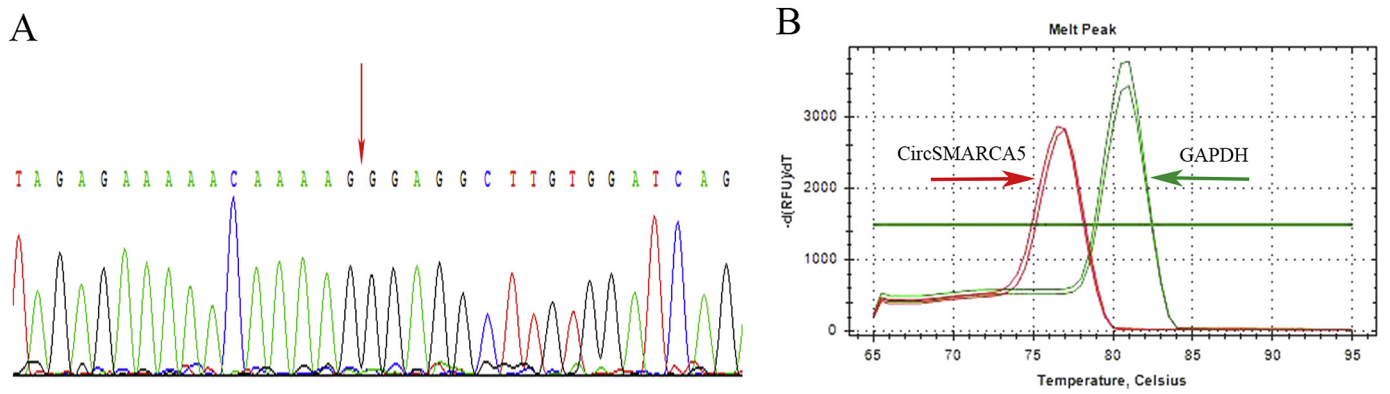

Fig. 1. CircSMARCA5 was specifically amplified by real-time PCR. A. The sequence of circSMARCA5. The splicing junction site was indicated by red arrow. B. The melt curve of circSMARCA5 and GAPDH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Potential values of CircSMARCA5 as a biomarker for HCC

To evaluate the prediction values of plasma circSMARCA5 in HCC, ROC curves by grouping controls, hepatitis, cirrhosis and HCC were generated (Table 2 and Fig. 5B–F). Plasma circSMARCA5 could be thought of as a good biomarker for discriminating HCC patients from healthy controls (AUC = 0.938, 95% CI: 0.910–0.966), hepatitis (AUC = 0.853, 95% CI: 0.806–0.900) and cirrhosis cases (AUC = 0.711, 95% CI: 0.650–0.772). When combined with AFP, the AUC was increased to 0.903 (95% CI: 0.866–0.940) and 0.858 (95% CI: 0.814–0.901) in detecting HCC from hepatitis and cirrhosis, respectively. More important, the result indicated that circSMARCA5 yielded an AUC of 0.847 (95% CI: 0.788–0.906) and 0.706 (95% CI: 0.634–0.778) in distinguishing HCC patients ($n = 68$) with AFP < 200 ng/ml from those hepatitis ($n = 110$) and cirrhosis patients ($n = 141$) with AFP < 200 ng/ml, respectively. So, it could be a good prediction biomarker for the HCC patients with low AFP levels.

4. Discussion

HCC is a common malignancy tumor worldwide, with extremely aggressive malignancy, high mortality and poor prognosis [21,22]. The serum α-fetoprotein (AFP) is commonly used as a prognostic marker for HCC. Unfortunately, even in 15–30% of patients of advanced HCC, the AFP levels remains normal [23,24]. Hence, seek a novel effective and reliable non-invasive biomarker for diagnosing HCC at early stage is urgent. Recently, the roles of circRNAs in HCC have attracted much attention. Previous study reported that circular RNAs might be used as new biomarkers for tumor diagnosis [25–27]. In our present study, we found that the expression of circSMARCA5 was decreased in HCC tissues to a greater extent than in para-carcinoma tissues. We also detected the levels of circSMARCA5 in plasma. Results indicated that plasma circSMARCA5 expression in HCC was lower than those in the controls, hepatitis and cirrhosis. These results were coincident with tissue circSMARCA5 profiles. In tissues and plasma, the levels of

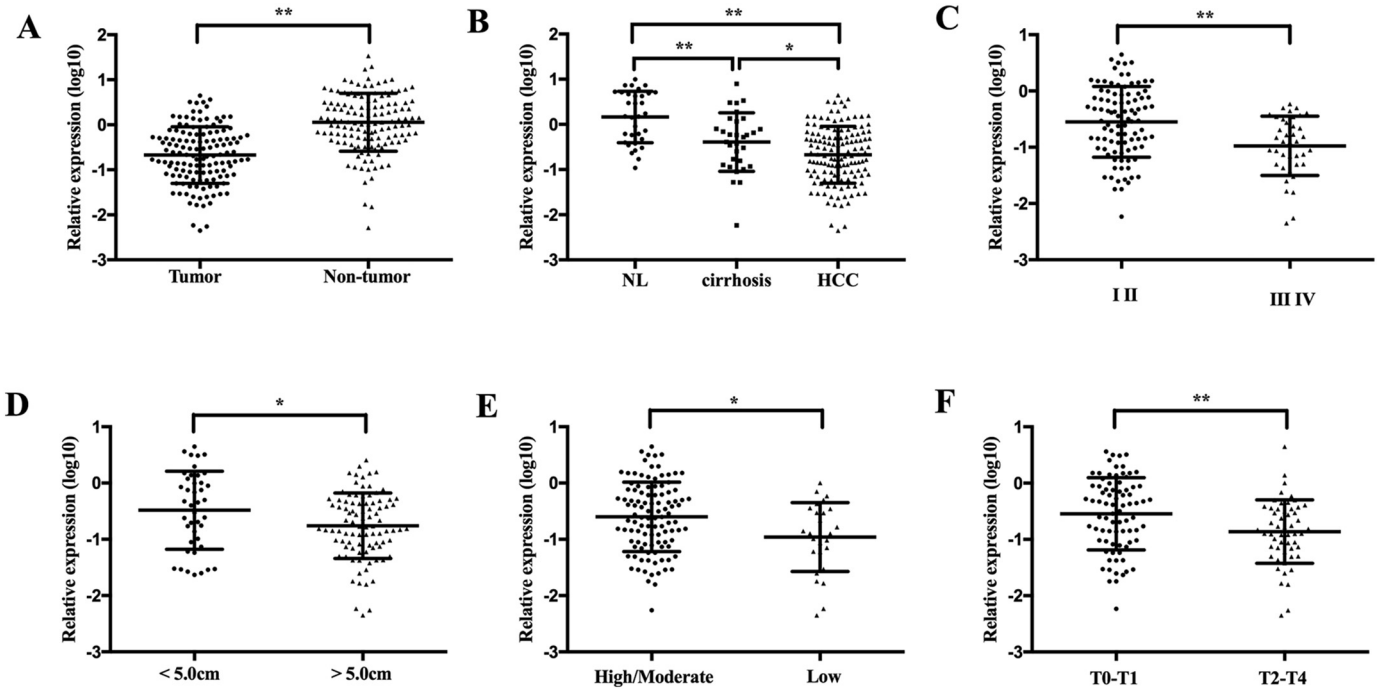

Fig. 2. CircSMARCA5 was downregulated in HCC tissues and was associated with TNM stage, tumor size, tumor differentiation and cancer invasion. A. The expression of circSMARCA5 was significantly downregulated in HCC tissues compared with para-carcinoma tissues ($p < 0.001$). B. CircSMARCA5 levels were lower in HCC tissues than that in cirrhosis tissues ($p = 0.024$) and control liver tissues ($p < 0.001$). C. Reduced circSMARCA5 was associated with TNM stage ($p = 0.001$). D. Decreased circSMARCA5 was corrected with tumor diameters ($p = 0.018$). E. Reduced circSMARCA5 was associated with cancer differentiations ($p = 0.023$). F. Decreased circSMARCA5 was related with cancer invasion ($p = 0.004$). **, $p < 0.01$, *, $p < 0.05$.

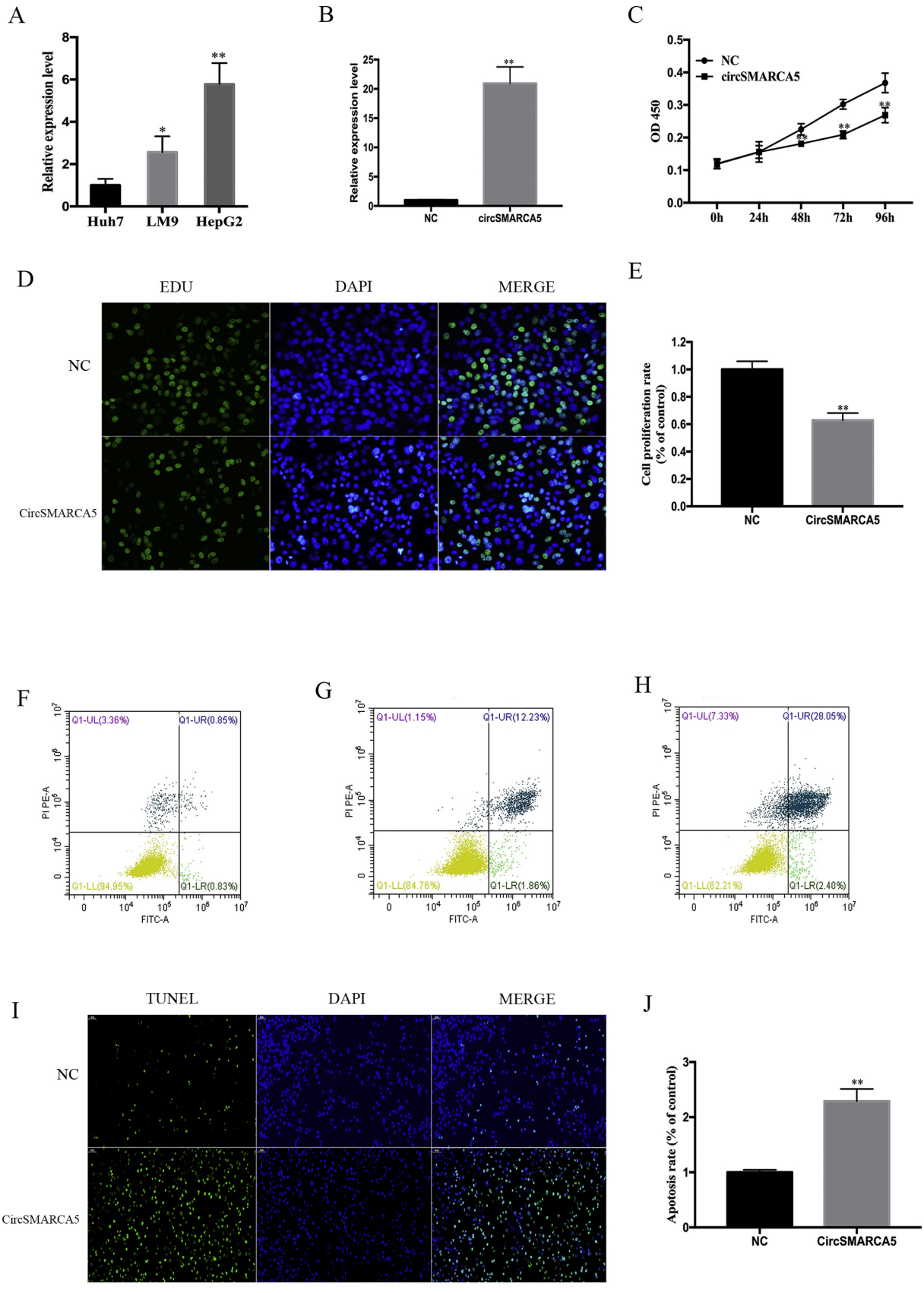

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Fig. 3. Overexpression of circSMARCA5 inhibited proliferation and promoted apoptosis in Huh7 cells. A. The expression patterns of circSMARCA5 in HCC cells. B. The expression of circSMARCA5 was significantly increased in Huh7 cells after transfection with circSMARCA5. C. Cell proliferation detected using the CCK8 assay. Overexpression of circSMARCA5 inhibited cell proliferation in Huh7 cells. D-E. Results of the Edu assay in Hhu7 cells. Edu staining appeared as a green fluorescence, whereas nuclei were stained with DAPI (blue). F–H. Overexpression of circSMARCA5 increased the late apoptotic cell fractions in Huh7 cells. F. Untransfected. G. Transfected with pcDNA 3.1. H. Transfected with circSMARCA5. Representative images (I) and quantification (J) of Tunel staining in Huh7 cells were presented. Tunel staining appeared as a green fluorescence, whereas nuclei were stained with DAPI (blue). NC: negative control. **, $p < 0.01$, *, $p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

circSMARCA5 were decreased from the healthy control group, to hepatitis, to cirrhosis, and then to HCC case groups gradually. This result indicated that circSMARCA5 could be used as an indicator to monitor the process of HCC. The ROC analysis indicated that plasma circSMARCA5 was of substantial value in distinguishing HCC patients from healthy controls, with AUC of 0.938 (86.67% sensitivity, 89.32% specificity). Hepatitis B and C infection are one of the most important risk factors of HCC. The ROC results elaborated that plasma circSMARCA5 was helpful for differentiating HCC patients from hepatitis patients, with AUC of 0.853. Importantly, cirrhosis is an important precancerous disease of HCC, circSMARCA5 yield an AUC of 0.711 for distinguishing HCC patients from cirrhosis patients. These data indicated that circSMARCA5 could be a good prediction marker in HCC. Combined circSMARCA5 with AFP would improve sensitivity and specificity significantly. After combination with AFP, the AUC increased from 0.938 to 0.992 for detecting HCC patients from controls, from 0.853 to 0.903 for diagnostic HCC patients from hepatitis patients, and from 0.711 to 0.858 for distinguishing HCC patients from cirrhosis patients. Moreover, circSMARCA5 have a good prediction value for detecting HCC patients with AFP below 200 ng/ml from hepatitis and cirrhosis patients with AFP below 200 ng/ml. This indicated that circSMARCA5 might be used for detecting HCC when the AFP level blows the cutoff value. Circular RNAs, unlike linear RNAs, form covalently loops through the jointing 5′- and 3′-ends. CircRNAs are more stable than linear RNA, presumably because of their resistance to RNA exonucleases and debranching enzymes [13]. Consistently, our study showed that circSMARCA5 was stable in plasma. On account of these data, we found that circSMARCA5 could be used as a potential biomarker for HCC prediction.

We found the expression of circSMARCA5 was decreased in HCC tissues compared with para-carcinoma tissues and was associated with TNM stage, tumor differentiation, tumor invasion and tumor size. This was agreeing with recent literature reported [19]. Yu et al. [19] further reported that circSMARCA5 was correlated with survival. However, as opposed to our present study that circSMARCA5 expression was unrelated with survival (data not shown). This could be probably due to the different sources of population.

The expression of circSMARCA5 was founded to be lowest in Huh7 compared with HepG2 and HCCLM9 in our study, but highest compared to HCCLM3, Hep3B, MHCC97H and SMMC-7721 in Yu et al. study [19]. CircSMARCA5 expression level may be moderately high in Huh7 cell lines compared to other kinds of HCC cell lines.

Overexpression of circSMARCA5 in Huh7 cells resulted in decreased proliferation and increased apoptosis. Moreover, the expression of MMP7 and MMP9 was downregulated when circSMARCA5 overexpression in Huh7 cells. In HCC tissues study, we have found that decreased circSMARCA5 was correlated with tumor invasion and TNM stage. CircSMARCA5 may exert its function by promoted proliferation, suppressed apoptosis, activated invasion and metastasis in the progress of HCC. The mechanism study on circular RNA is mainly focused on miRNA sponges and RNA binding protein sponges [28–30]. Previous study reported that circSMARCA5 might act as a sponge of miR-17-3p and miR-181b-5p [19]. CircSMARCA5 was reported that it would be induced during EMT, and RNA binding protein Quaking (QKI) was involved in circSMARCA5 formation [31]. While another study reported that DHX9 (DExH-Box Helicase 9), but not QKI, was involved in inhibiting circSMARCA5 expression [19]. Davide et al. showed that circSMARCA5 stimulated the expression of RNA binding protein serine and arginine rich splicing factor 1 (SRSF1) [32]. Detailed molecular mechanisms of circSMARCA5 functioning in HCC need further investigation.

Zhe et al. [18] reported that circSMARCA5 was upregulated in prostate cancer tissues compared with paired para-carcinoma tissues and circSMARCA5 knockdown inhibited cell proliferation and promoted cell apoptosis in prostate cancer cells. While another study reported that circSMARCA5 was downregulated in glioblastoma multiforme compared with normal brain tissues, and overexpression of circSMARCA5 in glioblastoma multiforme cells resulted in decreased migration, but had no effect on proliferation [32]. In this study, we found a decreased expression of circSMARCA5 in HCC tissues, and circSMARCA5 could inhibit proliferation, promote apoptosis and suppress tumor invasion and metastasis. Thus, most reports support that circSMARCA5 could be a tumor suppressor in different cancers, and the potential mechanism needs further study.

In conclusion, our study reveals that circSMARCA5 may promote apoptosis, inhibit proliferation, invasion and metastasis of HCC. CircSMARCA5 may serve as a potential prediction and monitor biomarker for HCC, especially in HCC patients with AFP blow cutoff values.

Conflict of interest

The authors declare no conflict of interest.

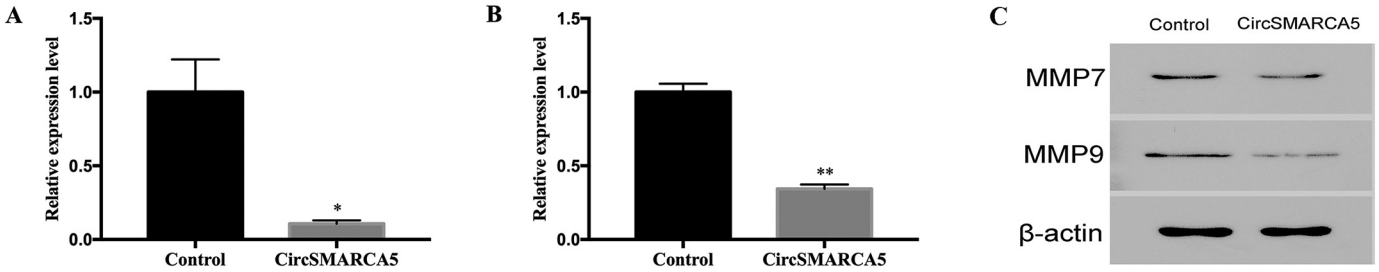

Fig. 4. Overexpression of circSMARCA5 showed reduced expression of MMP7 and MMP9 in Huh7 cells. A. The mRNA expression of MMP7 was significantly reduced in circSMARCA5 overexpression cells ($p = 0.002$). B. Overexpression of circSMARCA5 decreased the mRNA expression of MMP9 in Huh7 cells ($p < 0.001$). C. Western blot showed decreased protein expression of MMP7 and MMP9 after overexpression of circSMARCA5 for 24 h.

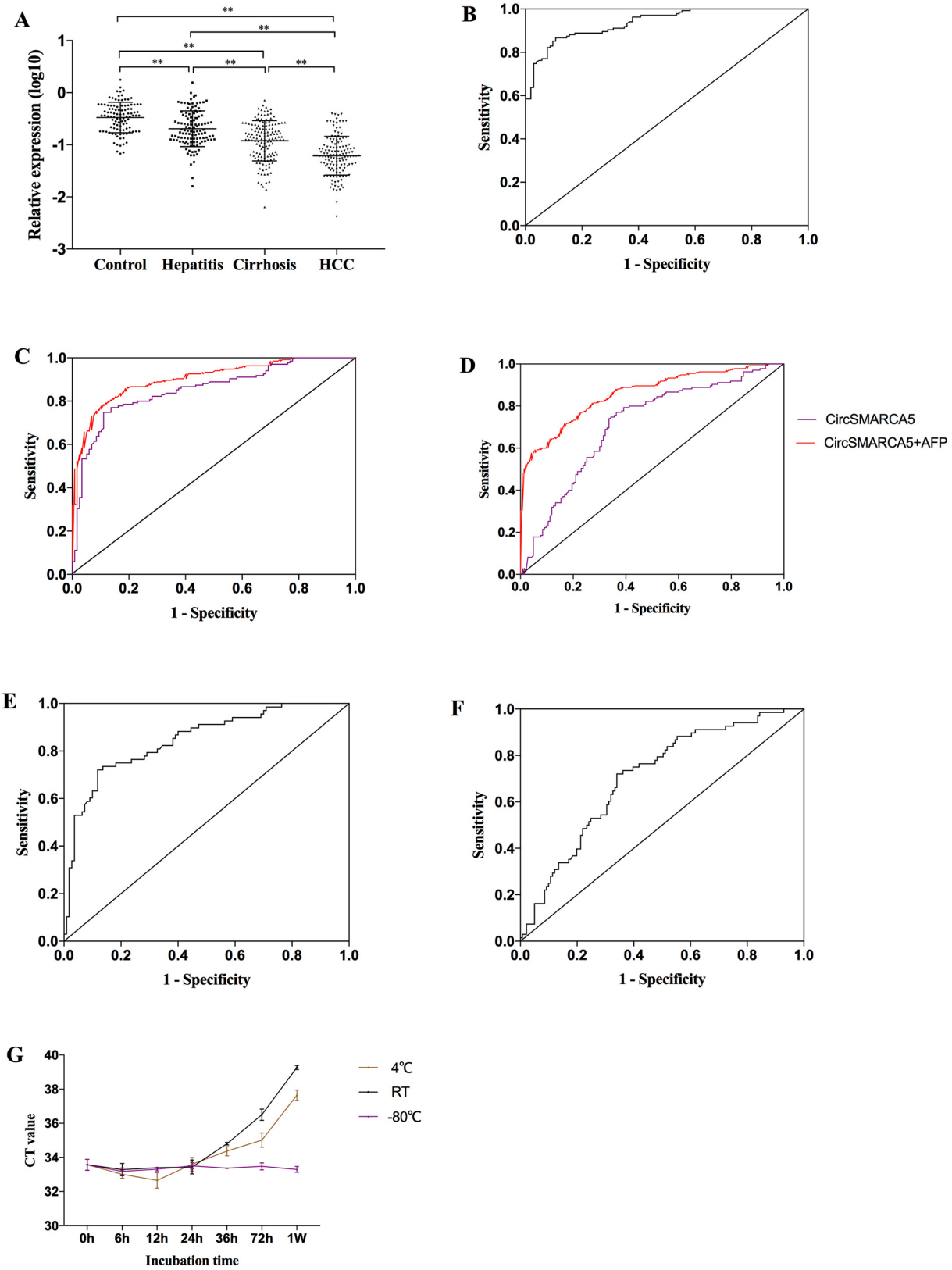

Fig. 5. Plasma circSMARCA5 was decreased in HCC samples and maybe a potential prediction biomarker for HCC. A. The expression of plasma circSMARCA5 in healthy controls, hepatitis samples, cirrhosis cases and HCC patients. B. ROC curve analyses of plasma circSMARCA5 for distinguishing HCC patients from healthy controls. ROC curve analyses of plasma circSMARCA5 or combined with serum AFP for detecting HCC patients from hepatitis (C) and cirrhosis (D). ROC curve analysis of plasma circSMARCA5 for discriminating HCC patients with AFP below 200 ng/ml from hepatitis (E) and cirrhosis (F) cases with AFP below 200 ng/ml. G. The plasma circSMARCA5 expression was relative stable when stored at room temperature and 4 °C for 24 h and was stable when incubated at −80 °C for longer time.

Table 2
Diagnostic abilities of plasma circSMARCA5 for the detection of HCC.

Biomarker	Group	Cutoff value	AUC	(95% CI)	*p* value	Sensitivity (%)	Specificity (%)
CircSMARCA5	HCC vs Control	15.28×10^{-2}	0.938	0.910–0.966	< 0.0001	86.67	89.32
CircSMARCA5 + AFP	HCC vs Control		0.992	0.983–1.002	< 0.0001	100	100
CircSMARCA5	HCC vs Hepatitis	9.385×10^{-2}	0.853	0.806–0.900	< 0.0001	74.81	88.89
CircSMARCA5 + AFP	HCC vs Hepatitis		0.903	0.866–0.940	< 0.0001	77.78	89.74
CircSMARCA5	HCC vs Cirrhosis	10.34×10^{-2}	0.711	0.650–0.772	< 0.0001	77.04	63.64
CircSMARCA5 + AFP	HCC vs Cirrhosis		0.858	0.814–0.901	< 0.0001	71.85	83.22
CircSMARCA5	HCC vs Hepatitis[a]	9.315×10^{-2}	0.847	0.788–0.906	< 0.0001	72.06	88.18
CircSMARCA5	HCC vs Cirrhosis[a]	9.19×10^{-2}	0.706	0.634–0.778	< 0.0001	72.06	65.96

[a] AFP levels below 200 ng/ml, AUC: area under curve, CI: confidence interval.

Author contributions

F.Z. conceived and designed the experiments. Z.H.L. Y.Z. and G.H.Y. performed the experiments. S.Y.H., X.P.Q. and Z.H.L. analyzed the data. Z.H.L., F.Z., Q.Y.D. and Y.Z. wrote the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.02.001.

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