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Serum exosomal microRNA-370-3p and microRNA-196a-5p are potential biomarkers for the diagnosis and prognosis of hepatocellular carcinoma

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Abstract

Introduction. Evidence has shown that some microRNAs (miRNAs) play a role in tumorigenesis of hepatocellular carcinoma (HCC). Herein, we aimed to evaluate the diagnostic and prognostic values of serum exosomal miR-370-3p and miR-196a-5p in patients with HCC.

Material and methods. Serum exosomes in 90 HCC patients were extracted and identified. Serum exosomal miR-370-3p and miR-196a-5p expression in HCC patients were detected. The diagnostic value of miR-370-3p and miR-196a-5p, relationship between miR-370-3p and miR-196a-5p expression and clinicopathological features and prognosis of patients with HCC were analyzed. Relationship between miR-370-3p and miR-196a-5p expression and liver function indices such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TBIL) in HCC patients were analyzed. The effects of miR-370-3p and miR-196a-5p on Huh-7 HCC cells' proliferation, invasion and migration were determined.

Results. Lower expression of miR-370-3p and higher expression of miR-196a-5p were found in serum exosomes of HCC patients. Serum exosomal miR-370-3p and miR-196a-5p were associated with tumor size, tumor grade and TNM stage as well as prognosis and liver function indices of HCC patients. Overexpressed miR-370-3p or silenced miR-196a-5p suppressed proliferation, invasion and migration of Huh-7 HCC cells.

Conclusions. We suggest that miR-370-3p/miR-196a-5p in serum exosomes of HCC patients could be potential biomarkers for the diagnosis and prognosis of HCC. *(Folia Histochemica et Cytobiologica 2022, Vol. 60, No. 3, 215–225)*

Keywords: exosome; microRNA-370-3p; microRNA-196a-5p; liver function biochemical indexes; hepatocellular carcinoma; Huh-7 cell line

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Introduction

Hepatocellular carcinoma (HCC) is known as the most frequent primary liver cancer, and the 5th most common cancer around the world [1]. At present, surgical resection is the primary treatment modality for HCC at the early-stage, while the majority of patients cannot be diagnosed until the advanced stage [2]. Therefore, it is of great importance to seek both sensitive and specific markers for HCC's early diagnosis. To date, the most effective tools for the diagnosis of HCC are the hepatic ultrasonography and serum α-fetoprotein (AFP) level

[3]. Many efforts have been made toward the research of both genomics and molecular biology for the purpose of unraveling the potential mechanisms of liver carcinogenesis, thereby identifying new therapy targets and early diagnostic and prognostic markers in order to enhance the clinical management of patients with HCC [4]. Presently, cellular microRNAs (miRNAs) have been associated with HCC, and the availability of HCC cells makes them a target for early detection of tumors [5].

Exosomes, as carriers of material transport, play a role in the exchange of biological information and the regulation of the cellular microenvironment through the transfer of a variety of biomolecules, such as mRNAs, miRNAs, as well as proteins [6–8]. Altered miRNA expression could generally impair lipid metabolism and increase cell proliferation, as well as a miRNA-driven increase in HCC collagen synthesis [9]. Some miRNAs have been suggested to play a role in tumorigenesis of HCC, which could be considered prognostic markers of HCC, including miR-370 and miR-196a [10, 11]. Serum exosomal miRNAs may be of great utility as serological biomarkers for HCC [12] and could be effective, and noninvasive biological indicators for the diagnosis and prognosis of HCC [12, 13] . Also, serum exosomal miRNA can be internalized by neighboring or distant cells, and then regulate multiple target genes in recipient cells at the post-transcriptional level to affect cell functions [14]. Nevertheless, few studies concentrated on the value of exosomal miR-370-3p/miR-196a-5p in the diagnosis and prognosis of HCC. Therefore, we conducted this study to investigate the correlation between serum exosomal miR-370-3p/miR-196a-5p and the survival of patients with HCC.

Material and methods

Ethics statement. This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Hebei University, Hebei China. The patients and their families signed the relevant informed consent according to the voluntary principle.

Study subjects. A total of 90 patients with HCC diagnosed in the Affiliated Hospital of Hebei University from June 2014 to December 2015 were collected as the HCC group. The patients were enrolled in the study if they met the following criteria: patients were diagnosed with HCC combined with clinical symptoms, signs, laboratory tests as well as imaging, and pathological examination; patients did not receive any tumor-related surgery, radiotherapy, and chemotherapy and other tumor-related treatment before admission; patients had no other tumors; patients had no heart, lung, gastrointestinal and other endocrine diseases. The pathological data and postoperative follow-up of patients with HCC were collected. According to the patient's hospitalization number to review the patient's complete electronic case, mainly including age, gender, tumor size, blood test index, imaging examination, pathological diagnosis and other information records. The overall survival (OS) period is used as the final evaluation indicator. OS is calculated from the date of diagnosis to the date of the last follow-up or death (died of primary tumor or complications). The last follow-up date was December 31, 2018. Those who died of other diseases during the follow-up or were still alive at the date of the last follow-up were regarded as "sensor data". According to the basic data of patients in the HCC group, 90 healthy subjects who were of the same age (male : female = 5:4) were selected as the control group, including 50 males and 40 females.

Serum sample collection and observation index. Blood samples were collected from all subjects (HCC patients and healthy controls) on an empty stomach. Peripheral venous blood (5 mL) was centrifuged at 300 *g* for 10 min at room temperature (RT). The serum was collected and centrifuged at 4°C, 17000 *g* for 10 min, and the upper part of the serum sample was carefully stored in a –80°C freezer for later use.

Alanine transaminase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were performed using the Hitachi 7600 Series automatic biochemical analyzer.

Isolation and identification of serum exosome. Serum samples were subjected to three-step gradient centrifugation to remove impurities such as cell debris [15], and then 250 μL serum was used to extract serum exosomes according to the ExoQuick Exosome Precipitation kit (SBI, Mountain View, CA, USA). A drop (50 μL) of serum sample was added to a copper grid, fixed with 5% fixed solution at 4°C for 2 h, and rinsed with 0.1 mol/L phosphate-buffered saline (PBS) 5 times, each time for 10 min. Thereafter, dehydration was carried out in acetone with an increasing concentration gradient, and the samples were dyed with 3% phosphotungstic acid. After natural evaporation, the extracted serum exosomes of 90 patients were observed by a transmission electron microscope (TEM-100CX II, JEOL, Tokyo, Japan) at 100 KV. Nanoparticle Tracking Analysis (NTA) (NS300, MIL, Malvern, UK) was employed to characterize exosomes.

Western blot analysis. Exosomes and Huh-7 cells were lysed with 50 μL of radio-immune precipitation assay lysis buffer and electrophoresed (10–15 mA) at 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis for 3–4 h. The proteins were transferred to the nitrocellulose membrane by wet transfer and then sealed with 5% skim milk powder and PBS containing Tween-20 (TBST) for more than 1 h. The membranes were supplemented with primary antibodies, CD81 (1:1000) and CD9 (1:2000; Abcam, UK), and incubated overnight at 4°C. The membranes were rinsed with PBS (3 × 5 min), and reacted with the corresponding secondary antibody for 2 h. The chemiluminescent substrate was added for color development.

Cell culture. As reported, Huh-7 cell line is one of commonly used human hepatoma cell lines [16]. Huh-7 cell line has the characteristics of easy access, high differentiation, rapid cell

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growth, and simple culture conditions, and, therefore, selected for the experiment. Huh-7 cells (Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China) were routinely cultured at 37°C with 5% CO_2 in DMEM (100 U/mL penicillin, 100 mg/mL streptomycin and 10% fetal bovine serum). The medium was changed every 3 days, and the cells reaching 80% confluence were detached with 0.25% trypsin for passage. The cells at passages 2–3 with good growth were selected for further experiments.

Cell transfection. miR-370-3p mimic (transfected with miR-370-3p mimic), mimic negative control (NC, transfected with miR-370-3p mimic irrelevant control nucleotide sequences), miR-196a-5p inhibitor (transfected with miR-196a-5p inhibitor), and inhibitor NC (transfected with miR-196a-5p inhibitor irrelevant control nucleotide sequences) were transfected into Huh7 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instruction. The above oligonucleotides and NCs were synthesized by GenePharma (Shanghai, China).

A total of 5 x 104 Huh-7 cells were seeded in 12-well plates and incubated overnight at 37°C, followed by transfected with the above oligonucleotides using Lipofectamine 2000 reagent for 48 h. The final concentrations of miR-370-3p mimic/mimic NC and miR-196a-5p inhibitor/inhibitor NC were 50 nM. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed 48 h post transfection to test the transfection efficiency.

RT-qPCR. Trizol (TaKaRa, Dalian, Liaoning, China) method was used to extract total RNA and RNA concentration was estimated using a Nanodrop spectrophotometer (Thermofisher, Shanghai, China) and RNA quality was examined using denaturing formaldehyde agarose gel electrophoresis. Reverse transcription was performed by using One Step Prime Script miRNA cDNA Synthesis Kit (TaKaRa) The primers were designed and synthesized by Invitrogen (Carlsbad, CA, USA). miR-370-3p forward primer; 5'-GCCTGCTGGGGTGGAACCTGGT-3'; miR-196a-5p forward primer: 5'-TAGGTAGTTTCATGTTGTTGGG-3'; U6 forward primer: 5'-CGCTTCGGCAGCACATATAC-3'. The reverse primers were provided by the One Step Prime Script miRNA cDNA Synthesis Kit (TaKaRa) [17]. Three replicate wells were set for each sample, and the reliability of the PCR results was evaluated by the dissolution curve. The relative expression of the target gene was calculated by $2^{-\Delta\Delta Ct}$ [18].

MTT assay. Huh-7 cell proliferation after co-culture with serum exosomes containing a high or low expression of miR-370-3p or miR-196a-5p was determined by an MTT kit (Sigma-Aldrich, St Louis, MO, USA). Cells (2×10^5) were cultured in DMEM (Invitrogen) containing 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen) for 24, 48 and 72 h, respectively. Then, cells were supplemented with MTT solution 20 μL MTT solution μ5 mg/mL) for for 4 h, and dimethyl sulfoxide (200 μL) was supplemented to each plate for the dissolution of the precipitate. The optical density ($OD_{570\,nm}$) value was detected by a microplate reader (Bio-Tek, Winooski, VT, USA).

Transwell assay. The basement membrane on the upper Transwell chamber was pre-coated with 20 μg Matrigel and incubated overnight in a 24-well plate. Cell (5×10^4 cells/mL) was added to the upper chamber, and a culture medium was added to the lower chamber. After culturing for 12 h, cells were fixed with 90% formaldehyde, stained with crystal violet for 15 min and examined with an inverted microscope. For cell migration test, the upper Transwell chamber had no matrigel coating [19].

Statistical analysis. All statistical analyses were performed using the SPSS 19.0 software (IBM, Armonk, NY, USA). The measurement data were expressed as mean ± standard deviation. The *t*-test was used for comparison between two groups, and one-way analysis of variance (ANOVA) for comparison among multiple groups. Enumeration data were expressed as a ratio or percentage and analyzed using χ^2 test. The receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of miR-370-3p and miR-196a-5p in patients with HCC. The survival curve was obtained by the Kaplan-Meier method, and the survival difference of patients with different expression levels was obtained by log-rank method. COX regression model was used to predict the prognostic significance of each clinical indicator. P values ≤ 0.05 were considered statistically significant.

Results

Isolation and identification of exosomes

Western blot found that CD81 and CD9 were expressed in serum exosomes of HCC patients, however, not in Huh-7 cells, indicating that serum exosomes were successfully extracted (Fig. 1A). The morphology of exosomes was identified by transmission electron microscopy, showing a typical circular or elliptical cup-like structure and uneven size. Vesicles were surrounded by phospholipid bilayers and contained low electron density substances (Fig. 1B). Nanoparticle tracking analysis proved that the size of exosomes was approximately 100 nm (Fig. 1C).

miR-370-3p is down-regulated and miR-196a-5p is up-regulated in serum exosomes of HCC patients

miR-370-3p and miR-196a-5p levels in serum exosomes were detected by RT-qPCR. The results showed that miR-370-3p expression was 0.94 ± 0.23 in healthy controls and 0.50 ± 0.21 in HCC patients ($P < 0.05$; Fig. 2A). MiR-196a-5p expression in serum exosomes of healthy controls was 1.00 ± 0.25 and 2.53 ± 1.03 in HCC patients ($P < 0.05$) (Fig. 2B). Pearson correlation analysis showed that miR-370-3p and miR-196a-5p levels were negatively correlated in serum exosomes of HCC patients (Fig. 2C).

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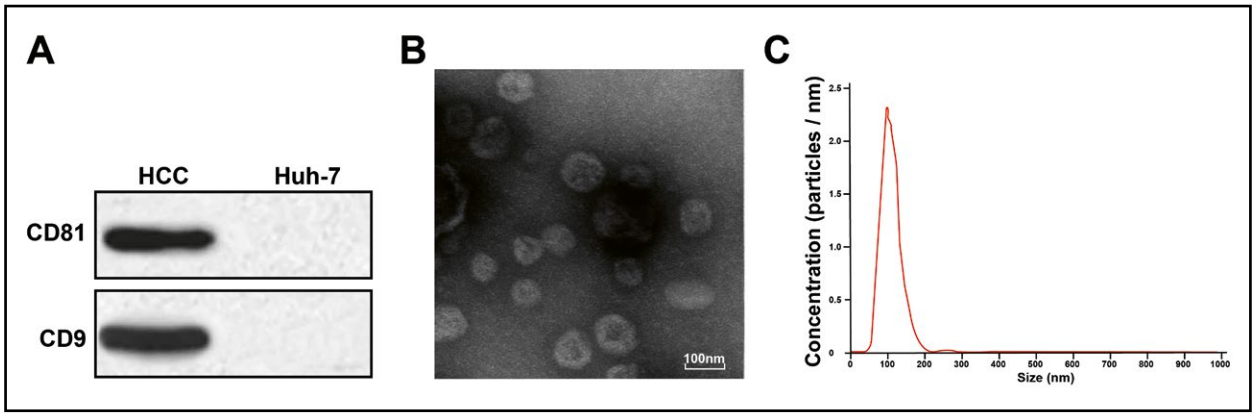

Figure 1. Isolation and identification of exosomes.**A.** Western blot analysis of exosomal markers CD81 and CD9. **B.** The morphology of exosomes observed under a transmission electron microscope. **C.** Nanoparticle tracking analysis of exosome size was performed as described in Material and methods.

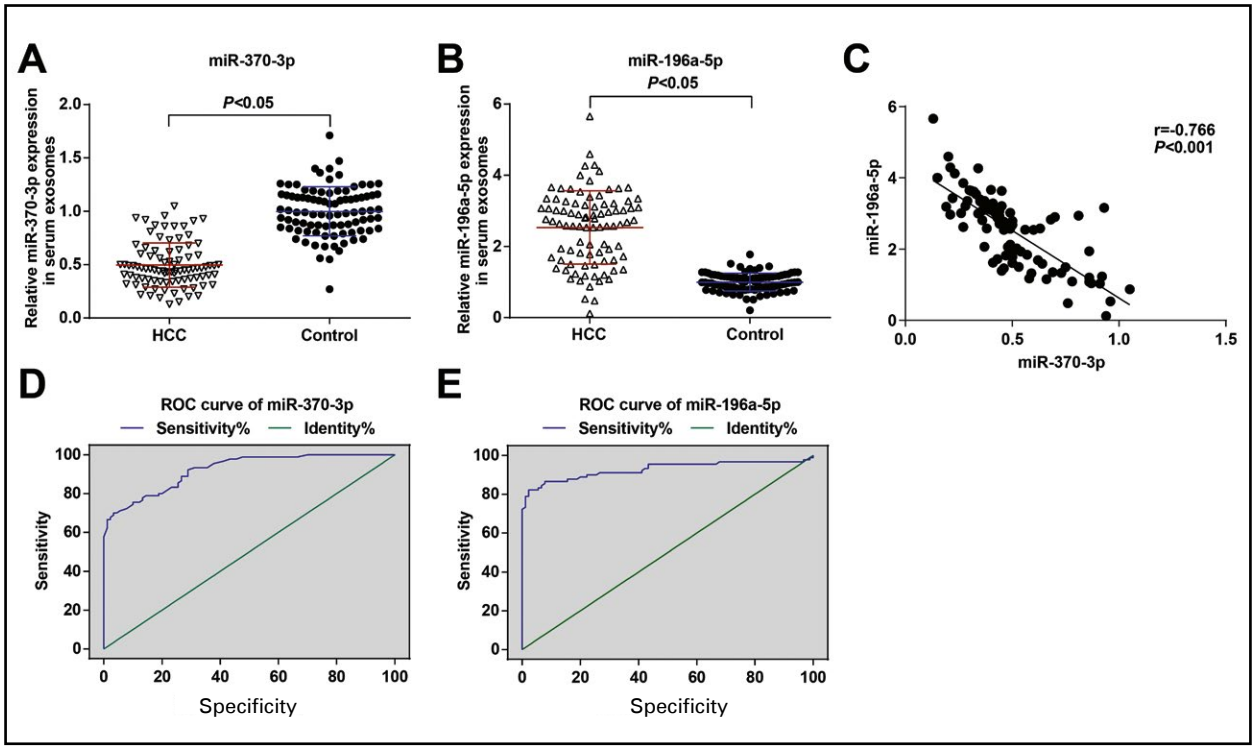

Figure 2. MiR-370-3p is down-regulated and miR-196a-5p is up-regulated in serum exosomes of HCC patients. **A–B.** MiR-370-3p and miR-196a-5p levels, respectively, in serum exosomes of patients with HCC detected by RT-qPCR. **C.** Correlation between miR-370-3p and miR-196a-5p levels in serum exosomes of patients with HCC. **D–E.** ROC curves of serum exosomal miR-370-3p and miR-196a-5p in the diagnosis of HCC.

To further evaluate the diagnostic value of miR--370-3p and miR-196a-5p in serum exosomes in HCC, ROC curve analysis was performed on 90 patients with HCC. The area under the curve (AUC) of miR--370-3p was 0.923 (95% CI: 0.887~0.959, P < 0.001) (Fig. 2D) and that of miR-196a-5p was 0.927 (95% CI: 0.883 ~ 0.971, P < 0.001) (Fig. 2E). Using maximum Youden's index (Youden index = sensitivity + specificity-1) [20], the diagnostic specificity and sensitivity of miR-370-3p and miR-196a-5p in serum exosomes were analyzed. The results indicated that the sensitivity and specificity of miR-370-3p were 0.967 and 0.700, respectively, whereas those of miR-196a-5p was 0.822 and 0.978, respectively.

Relationship between miR-370-3p and miR-196a--5p levels and the clinicopathological features and prognosis of patients with HCC

According to the average expression levels of miR-370-3p and miR-196a-5p in serum exosomes, 90

Table 1. The relationship between the expression of miR-370-3p and miR-196a-5p and the clinicopathological characteristics of patients with HCC

Variables	Serum exosomal miR-370-3p (n, %)		P value	Serum exosomal miR-196a-5p (n, %)		P value
	Low (n = 60)	High (n = 30)		Low (n = 34)	High (n = 56)	
Age (years)			0.881			0.522
< 65	33 (55.0%)	16 (53.3%)		17 (50.0%)	32 (57.1%)	
≥ 65	27 (45.0%)	14 (46.7%)		17 (50.0%)	24 (42.9%)	
Gender			0.764			0.827
Male	34 (56.7%)	16 (53.3%)		18 (52.9%)	32 (57.1%)	
Female	26 (43.3%)	14 (46.7)		16 (47.1%)	24 (42.9%)	
Tumor size (cm)			0.025			0.009
≤ 5	24 (40.0%)	20 (66.7%)		23 (67.6%)	21 (37.5%)	
> 5	36 (60.0%)	10 (33.3%)		11 (32.4%)	35 (62.5%)	
AFP (μg/L)			0.88			0.046
≤ 20	35 (58.3%)	18 (60.0%)		25 (73.5%)	28 (50.0%)	
> 20	25 (41.7%)	12 (40.0%)		9 (26.5%)	28 (50.0%)	
HBs Ag			0.551			0.666
+	30 (50.0%)	13 (43.3%)		15 (44.1%)	28 (50.0%)	
–	30 (50.0%)	17 (56.7%)		19 (55.9%)	28 (50.0%)	
Liver cirrhosis			0.878			0.128
+	23 (38.3%)	12 (40.0%)		13 (38.2%)	32 (57.1%)	
–	37 (61.7%)	18 (60.0)		21 (61.8%)	24 (42.9%)	
TNM stage			0.014			< 0.001
I + II	23 (38.3%)	20 (66.7)		28 (82.4%)	15 (26.8%)	
III + IV	37 (61.7%)	10 (33.3)		6 (17.6%)	41 (73.2%)	
Tumor grade			0.019			0.034
Undifferentiated	4 (6.7%)	0 (0.0%)		0 (0.0%)	4 (7.1%)	
Well	6 (10.0%)	10 (33.3%)		10 (29.4%)	6 (10.7%)	
Moderate	28 (46.7%)	14 (46.7%)		17 (50.0%)	25 (44.7%)	
Poor	22 (36.6%)	6 (20.0%)		7 (20.6%)	21 (37.5%)	

AFP — alpha-fetoprotein; HBs Ag — Hepatitis B surface antigen.

patients with HCC were divided into two groups: the low expression group and the high expression group. As shown in Table 1, miR-370-3p expression in serum exosomes was closely related to tumor size (P = 0.025) TNM stage (P = 0.014) and tumor grade (P = 0.019). No significant difference was found in age, gender, AFP, Hepatitis B surface antigen (HBs Ag) and cirrhosis (all P > 0.05).

MiR-196a-5p expression in serum exosomes was closely related to tumor size (P = 0.009), AFP (P = 0.046), TNM stage (P < 0.001), and tumor grade (P = 0.034). Age, gender, HBs Ag and cirrhosis did not correlate with miR-196a-5p expression (all P > 0.05).

From the survival curves of 90 patients with HCC, the 3-year OS of patients with low miR-370-3p was significantly shorter than that of patients with high miR-370-3p (P = 0.035) (Fig. 3A) while the 3-year OS of patients with low miR-196a-5p was significantly longer than that of patients with high miR-196a-5p (P = 0.031) (Fig. 3B). Based on the survival curve of HCC patients, it could be seen that HCC patients with high expression of miR-370-3p in serum exosomes

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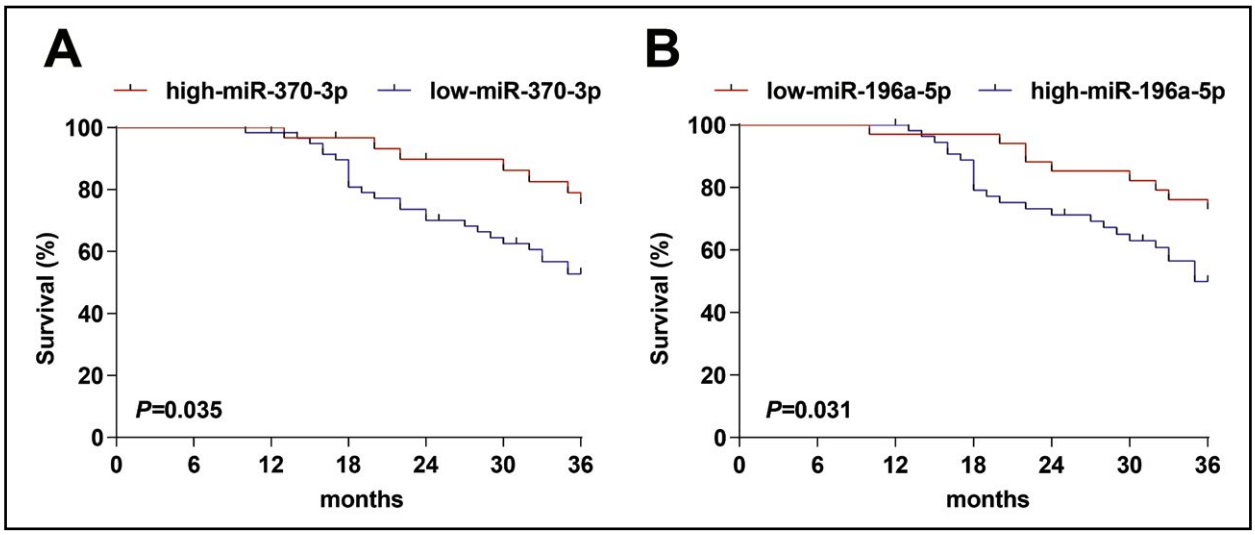

Figure 3. miR-370-3p and miR-196a-5p levels are associated with the prognosis of patients with HCC. **A.** Survival curves of HCC patients with low or high expression of miR-370-3p. **B.** Survival curve of HCC patients with low or high expression of miR-196a-5p.

were at a lower death risk than those with low expression of miR-370-3p; for HCC patients with high expression of miR-196a-5p in serum exosomes, the risk of death was higher than those with low expression of miR-196a-5p. These results further indicated the down-regulation of miR-370-3p and the up-regulation of miR-196a-5p in serum exosomes of HCC patients, and implying that their expression changes may be related to the development of HCC.

Tumor size, TNM stage, tumor grade, serum exosomal miR-370-3p, and miR-196a-5p are associated with prognosis in patients with HCC

To further analyze the effects of serum exosomal miR-370-3p and miR-196a-5p on the prognosis of patients with HCC, COX regression analysis was analyzed, finding that tumor size, TNM stage, tumor grade, serum exosomal miR-370-3p and miR-196a-5p were associated with prognosis in patients with HCC. The 3-year OS was worse for HCC patients with large tumor size (> 5 cm), advanced TNM stage (III + IV stage) and tumor grade (undifferentiated + poor), low expression of serum exosomal miR-370-3p, and high expression of serum exosomal miR-196a-5p. Age, gender, AFP, HBs Ag, and cirrhosis were not associated with the 3-year OS of patients with HCC (all $P > 0.05$) (Table 2).

Serum exosomal miR-370-3p and miR-196a-5p are correlated to liver function indices of patients with HCC

We found that ALT and AST activities and TBIL levels were highly increased in serum of patients with HCC relative to healthy controls (all $P < 0.05$) (Fig. 4A–C). Pearson correlation analysis further discovered that serum exosomal miR-370-3p was negatively correlated with ALT ($r = –0.729$, $P < 0.001$), AST ($r = –0.613$, $P < 0.001$) and TBIL ($r = –0.618$, $P < 0.001$) (Fig. 4D–F); miR-196a-5p expression was positively correlated with ALT ($r = 0.724$, $P < 0.001$), AST ($r = 0.630$, $P < 0.001$) and TBIL ($r = 0.669$, $P < 0.001$) (Fig. 4G–I).

Restored miR-370-3p or depleted miR-196a-5p inhibits proliferation, invasion, and migration of HCC cells

To explore the effects of miR-370-3p and miR-196a-5p on the function of HCC cells, miR-370-3p mimic, miR-196a-5p inhibitor, mimic NC, and inhibitor NC were transfected into Huh-7 cells, respectively. RT-qPCR proved that the transfection was successful (Fig. 5A, B). MTT and Transwell assays found that Huh-7 cells with high expression of miR-370-3p or low expression of miR-196a-5p, their proliferation, invasion and migration were inhibited (Fig. 5C–F).

Discussion

The tumor microenvironment exerts function in tumor recurrence and drug resistance [21, 22], which is the main challenge for the treatment of advanced tumors. As a transport carrier of different biological molecules, exosomes along with exosomal active factors (*e.g.* mRNAs, proteins, miRNAs, and others) are implicated in the modulation of the tumor microenvironment [23, 24]. Therefore, the function of exosomes is of considerable interest [25]. In this present study, the obtained findings suggested that down-regulated miR-370-3p

Table 2. COX regression analysis

Variables	B	SE	Wald	df	P value	OR	95% CI for OR	
							Lower	Upper
Age	–0.381	0.303	1.579	1	0.209	0.683	0.377	1.238
Gender	–0.104	0.302	0.119	1	0.73	0.901	0.499	1.629
Tumor size	–0.813	0.325	6.273	1	0.012	2.256	1.194	4.264
AFP	–0.066	0.304	0.048	1	0.827	0.936	0.515	1.699
HBs Ag	–0.019	0.303	0.004	1	0.951	0.981	0.542	1.777
Liver cirrhosis	0.387	0.305	1.611	1	0.204	1.472	0.81	2.674
TNM	–0.885	0.332	7.108	1	0.008	2.422	1.264	4.642
Tumor grade	0.789	0.302	6.808	1	0.009	0.454	0.251	0.822
miR-370-3p	–0.824	0.361	5.224	1	0.022	0.439	0.216	0.889
miR-196a-5p	4.331	1.252	11.964	1	0.001	75.989	6.532	884.008

B — regression coefficient; CI — confidence interval; df — degree of freedom; OR — odds ratio; SE — standard error.

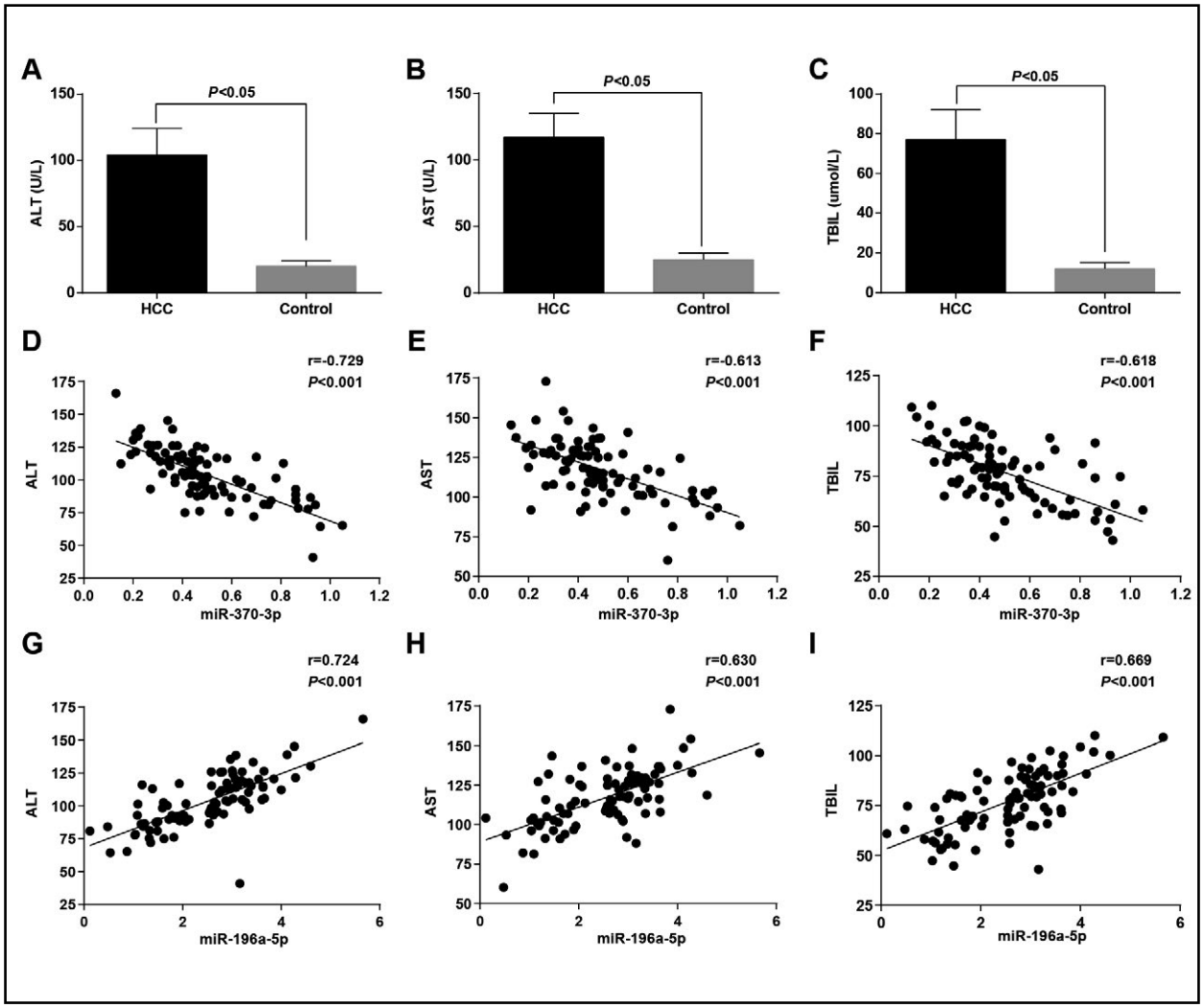

Figure 4. Serum exosomal miR-370-3p and miR-196a-5p are correlated with liver function indices of patients with HCC. **A–C.** ALT, AST and TBIL serum levels in HCC patients and healthy controls. **D–F.** Correlation analysis between miR-370-3p and ALT, AST and TBIL levels. **G–I.** Correlation analysis between miR-196a-5p expression and ALT, AST and TBIL levels. All measurement data were represented by mean ± SD.

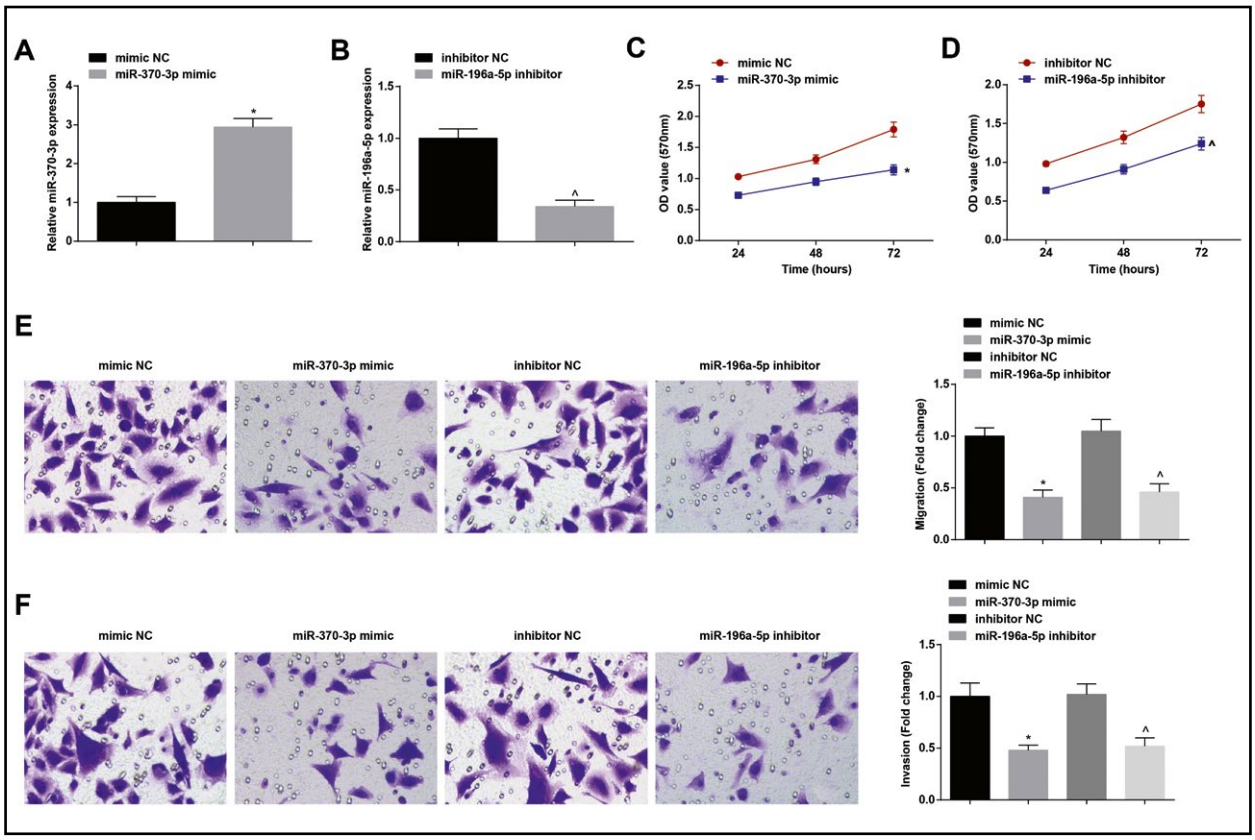

Figure 5. Restored miR-370-3p or depleted miR-196a-5p inhibits proliferation, invasion and migration of HCC cells. **A–B.** miR-370-3p and miR-196a-5p expression in HCC cells after co-culture with serum exosomes. **C–D.** Cell viability was tested by MTT assay. **E–F.** Cell invasion and migration were analyzed by Transwell assay. The cell experiments was repeated three times. * *vs.* the mimic NC group; ^ *vs.* the inhibitor NC group. Abbreviation: NC — negative control.

and up-regulated miR-196a-5p in serum exosomes of HCC patients may be potential tools for the diagnosis and prognosis of HCC.

First of all, our study examined the low expression of miR-370-3p in serum exosomes of patients with HCC which was related to tumor size, tumor grade and TNM stage. Meanwhile, we found that serum exosomal miR-370-3p was negatively associated with prognosis in patients with HCC. Similarly, Pan XP *et al.* [10] have found that low miR-370 is correlated with advanced TNM stage and shorter overall survival of patients with HCC. On the other hand, miR-370 low expression in patients with HCC is negatively correlated with poor survival and adverse clinicopathological features of HCC patients, which was in line with our findings [26]. In particular, Lin L *et al*. [27] found that upregulation of miR-370-3p could improve the survival rate of mice with ulcerative colitis-associated colorectal cancer. Also, Peng *et al*. [28] reported the down-regulation of miR-370-3p in glioma and further confirmed the correlation between miR-370-3p expression and tumor grade. From the functional effects on the activities of HCC cells, the present research uncovered that miR-370-3p overexpression suppressed the proliferation, invasion and migration of Huh-7 cells. As reported, miR-490-3p is downregulated in HCC cells, and restoration of miR-490-3p weakened the migration and invasion capabilities of Huh-7 cells [29]. Additionally, miR-296-3p was expressed at a low level in Huh-7 cells, and its overexpression diminished the capacities of HCC cells to migrate and invade [30]. Consistently, the functions of miR-370-3p in other types of human cancers have been investigated. For example, Lulli V *et al*. [31] have offered an explanation that miR-370-3p acts as a tumor-suppressor factor inhibiting proliferation, migration and invasion of glioblastoma cells. It was demonstrated in thyroid cancer cell lines that miR-370-3p overexpression induces apoptosis whereas suppresses viability and invasion of tumor cells [32]. Besides, miR-370-3p silencing is identified as an inducer for proliferation and survival of bladder cancer cells [33] while miR-370-3p restoration as a suppressor for migration and invasion of papillary thyroid carcinoma cells [34]. All these studies demonstrate that upregulation of miR-370-3p could retard the progression of human cancers.

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Additionally, our study also demonstrated that patients with HCC showed higher expression of miR-196a-5p, and serum exosomal miR-196a-5p was related to tumor size, tumor grade, TNM stage and prognosis of patients with HCC. Similar to our findings, Bao *et al*. [35] found that high miR-196a-5p expression in serum is positively correlated with advanced tumor stage of non-small cell lung cancer, and further determined that serum miR-196-5p has great diagnostic utility. It has been reported that miR-196-5p is overexpressed in gastric cancer, presenting a positive association with advanced lymph node metastasis and tumor stage [36]. In the context of renal cell carcinoma, high miR-196a-5p in serum has moderate diagnostic ability (AUC = 0.719) [37]. Concerning the role of miR-196a-5p in tumor malignancy, our research revealed that miR-196a-5p inhibition impeded the phenotype of HCC cells' proliferation, migration and invasion. As previously described, miR-224 was highly expressed in HCC cells, and up-/down-regulation of miR-224 accelerated/restrained Huh-7 cell proliferation and motility [38]. Consistently, the roles of miR-196a-5p in other types of human cancers have been explored. For instance, Xin *et al*. [39] showed that miR-196a-5p acts as a promoter for proliferation, migration and invasion of colorectal cancer cells. Mechanistically, it has been revealed that miR-196a-5p knockdown could limit the invasion of gastric cancer stem cells [40]. Evidence has shown the abnormal increase of miR-196a-5p expression in triple-negative breast cancer, and further verified that proliferation and invasion of tumor cells are limited when miR-196a-5p is silenced [41]. Zhao *et al*. [42] demonstrated that miR-196a-5p silencing impedes migration and invasion of human glioma stem cells. All these studies suggest that the downregulation of miR-196a-5p could suppress the progression of human cancers.

To sum up, our study showed that miR-370-3 and miR-196a-5p in serum exosomes of HCC patients could be potential biomarkers for the diagnosis and prognosis of HCC, implying that miR-370-3p and miR-196a-5p may be novel targets for HCC therapy. In addition, the relative efficacy of targeting miR-370-3p and miR-196a-5p in contrast to other miRNA delivery approaches, such as exosomes, may be important to select the optimal miRNA delivery ways for further clinical translation. Therefore, an in-depth recognition of the potential molecular mechanisms of tumor-derived exosomes in the progression of HCC has great significance for clarifying the function of exosomes in the initiation and development of HCC together with their potential therapeutic value.

Conflict of interests

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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