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Original Article

MicroRNA-708 is downregulated in hepatocellular carcinoma and suppresses tumor invasion and migration

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ABSTRACT

Background: MicroRNA-708 (miR-708) has been identified as one of down-regulated miRNAs in hepatocellular carcinoma (HCC) tissues by Taqman miRNAs array and confirmed quantitatively by reverse transcription polymerase chain reaction (qRT-PCR). However, its involvement in HCC remains unclear. The aim of this study was to investigate the roles of miR-708 in carcinogenesis and cancer progression of HCC.

Materials and methods: QRT-PCR was performed to detect the expression of miR-708 in 100 pairs of HCC and adjacent non cancerous tissues. Then, its associations with various clinicopathological features of HCC patients were statistically evaluated. After that, we also observed the effects of enforced miR-708 expression on migration and invasion of HCC cells in vitro.

Result: Our data confirmed that the expression level of miR-708 in HCC tissues was significantly lower than those in adjacent non-cancerous tissues ($P = 0.001$). In addition, low miR-708 expression was found to be closely correlated with high Edmondson-Steiner grading ($P = 0.02$) and advanced Tumor Node Metastasis (TNM) stage ($P = 0.01$). Furthermore, the enforced expression of miR-708 could suppress the migration and invasion of HCC cell lines in vitro.

Conclusion: Our findings support that miR-708, which is frequently down-regulated in HCC, may contribute to the aggressive progression of HCC and inhibit HCC cell mobility. Further studies on the identification of its target genes are required to be performed.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common malignant primary liver cancer and the third leading cause of cancer-related mortality worldwide with about 0.7 million deaths annually [1]. There are various factors involved in the development of HCC, including multiple viral infections, chronic inflammation, chronic alcohol abuse and obesity [2]. Surgical resection and liver transplantation are two promising treatment strategies for HCC, however, the clinical outcome of patients remains poor, with a median survival of 6–9 months following diagnosis [3]. Especially for advanced-stage HCC patients, the 5-year overall survival rate is less than 5% due to high recurrence and metastasis rates [4]. Growing evidence show that it is a complex and multi-step process of hepatocarcinogenesis which is associated with various genetic and epigenetic changes [5,6]. Therefore, it is of great significance to uncover the complicated molecular and cellular mechanisms of HCC development and progression in order to identify potential therapeutic targets for improving the prognosis of HCC patients.

MicroRNAs (miRNAs), an abundant group of small and endogenous non-coding single strand RNAs of 19–23 nucleotides, regulate gene expression negatively at the post-transcriptional level by binding to the untranslated regions of target mRNAs [7,8]. Through translational repression or degradation of target mRNA, miRNAs are implicated in various biological process including cell development, cell growth, cell cycle, apoptosis and stem cell renewal [9]. Growing evidence show that miRNAs play crucial roles in the initiation and progression of human cancers, including HCC. Aberrant expression of many miRNAs has been observed in different types of cancers and function as oncogenes or tumor suppressors in a tumor-type manner [10]. MiRNA expressing profiles which can differentiate cancer and normal tissues have been identified using microarray or real-time polymerase chain reaction (PCR), suggesting the importance of miRNAs in tumor pathogenesis and the implications of miRNAs

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in diagnosis, treatment, and prognosis of tumors. Especially in HCC, Wang et al. identified 10 up-regulated miRNAs (miR-217, miR-518b, miR-517c, miR-520 g, miR-519a, miR-522, miR-518e, miR-525-3p, miR-512-3p, and miR-518a-3p) and 11 down-regulated miRNAs (miR-138, miR-214, miR-214#, miR-199a-5p, miR-433, miR-511, miR-592, miR-483-3p, miR-483-5p, miR-708 and miR-1275) by Taqman miRNAs array combined with qRT-PCR in HCC and adjacent non-tumor tissues [11]. Among them, miR-708 has been reported to play different roles in several types of cancers [12–15]. However, little is known about the function of miR-708 in human HCC.

To address this problem, qRT-PCR was performed here to detect the expression of miR-708 in 100 pairs of HCC and adjacent noncancerous tissues. Then, its associations with various clinicopathological features of HCC patients were statistically evaluated. After that, we also observed the effects of enforced miR-708 expression on migration and invasion of HCC cells in vitro.

2. Materials and methods

2.1. Patients and tissue samples

This study was authorized by the Research Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan Province, China. All patients provided signed, informed consent. All specimens were handled and made anonymous according to the ethical and legal standards.

Our cohort contained 100 matched fresh HCC and adjacent non cancerous tissues which were obtained from 100 clinically confirmed HCC patients during hepatic resection at the First Affiliated Hospital of Xinxiang Medical University during December 2010 to December 2013. All patients enrolled in this study did not receive preoperative chemotherapy or radiotherapy. The clinicopathological data are summarized in Table 1. All samples were immediately snap-frozen in liquid nitrogen or stored at −80 °C before qRT-PCR.

Table 1
Association of miR-708 expression with different clinicopathological features of hepatocellular carcinoma (HCC) patients.

Clinicopathological features	No. of cases (%)	miR-708 expression		*P*
		High (*n*, %)	Low (*n*, %)	
Age				
<50	40 (40.00)	20 (50.00)	20 (50.00)	NS
≥50	60 (60.00)	30 (50.00)	30 (50.00)	
Gender				
Male	80 (80.00)	40 (50.00)	40 (50.00)	NS
Female	20 (20.00)	10 (50.00)	10 (50.00)	
Serum AFP level (ng/mL)				
<400	30 (30.00)	20 (66.67)	10 (33.33)	NS
≥400	70 (70.00)	30 (42.86)	40 (57.14)	
HBV infection				
Negative	20 (20.00)	10 (50.00)	10 (50.00)	NS
Positive	80 (80.00)	40 (50.00)	40 (50.00)	
Cirrhosis				
Negative	35 (35.00)	20 (57.14)	15 (42.86)	NS
Positive	65 (65.00)	30 (46.15)	35 (53.85)	
Venous infiltration				
Negative	70 (70.00)	38 (54.29)	32 (45.71)	NS
Positive	30 (30.00)	12 (40.00)	18 (60.00)	
Edmondson-Steiner grading				
I∼II	75 (75.00)	45 (60.00)	30 (40.00)	0.02
III∼IV	25 (25.00)	5 (20.00)	20 (80.00)	
TNM stage				
I∼II	72 (72.00)	47 (65.28)	25 (34.72)	0.01
III∼IV	28 (28.00)	3 (10.71)	25 (89.29)	

"NS" refers to the difference without statistical significance.

2.2. RNA extraction and qRT-PCR

Total RNA, including miRNA, was extracted with the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. QRT-PCR was performed as previously described [16]. In brief, the PCR amplification for the quantification of the miR-708 and the small nucleolar RNA (RNU6B) was performed using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) and TaqMan Human MiRNA Assay Kit (Applied Biosystems, Foster City, CA, USA). RNU6B was used as an internal normalization control. The sequences of the primers used in this study were listed as follows: for miR-708, miR-708-RT 5′-GTT GGC TCT GGT GCA GGG TCC GAG GTA TTC GCA CCA GAG CCA ACC CCA GC-3′, miR-708 Forward Primer 5′-CGG CGG AAG GAG CTT ACA ATC TA- 3′; RNU6B-RT 5′-GTT GGC TCT GGT GCA GGG TCC GAG GTA TTC GCA CCA GAG CCA ACA AAA ATA T-3′, RNU6B-Forward Primer 5′-TTC CTC CGC AAG GAT GAC ACG C-3′; Universal Reverse Primer 5′-GTG CAG GGT CCG AGG T-3′. Detection of miRNA was performed with the miRNA assay system (Applied Biosystems, Foster City, CA, USA). The relative expression of miR-708 was shown as fold difference relative to RNU6B.

2.3. Cell culture and transfection

Two human HCC cell lines HepG2 and SMMC-7721 were both purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Science (Shanghai, China) and routinely cultured in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO BRL, Grand Island, NY) containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), 100 units/mL penicillin and 100 μg/mL streptomycin (Sigma, St-Louis, MO, USA) in a humidified containing of 5% CO_2 incubator at 37 °C.

The miR-708 mimics and the negative control (miR-NC) were purchased from Genepharma Co., Ltd. (Shanghai, China). Two human HCC cell lines HepG2 and SMMC-7721 were transfected using Lipofectamine-2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were verified and used for analysis.

2.4. Cell migration and invasion assay

The migration abilities of HepG2 and SMMC-7721 cells transfected with miR-708 mimic and NC were both tested by the wound-healing migration assay. In brief, the transfected cells were cultured in 24-well plates until confluent. Using a 200-μl pipette tip, a wound was created in the monolayer (at time 0). Then, the cells were washed with phosphate buffered saline (PBS) and incubated with fresh serum-free medium. After that, the distance between the two sides of the wound was measured using an Olympus CX71 microscope (Olympus). Serial images were obtained at 0, 24 and 48 h.

The invasion abilities of HepG2 and SMMC-7721 cells transfected with miR-708 mimic and NC were both tested in Matrigel coated cell culture chambers (8 μm pore size, Millipore, Billerica, MA, USA). In brief, the transfected cells were cultured in 24-well plates until confluent. Then, 48 h after transfection, infected cells were resuspended in 200 μl serum-free medium and were placed into the top chamber of the insert with Matrigel. Bottom chambers were filled with conditioned medium. After 48 h incubation periods, cells which remained on top of the filter were scrubbed off, and those that invaded the underside of the filter were fixed and stained with crystal violet. The number of invaded