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ORIGINAL ARTICLE

Effects of miR-339-5p on invasion and prognosis of hepatocellular carcinoma

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Summary microRNAs (miRNAs) are short single-strand non-coding RNAs that regulate various cell processes at the post-transcriptional levels. Mounting evidences suggested that dysregulation of miRNA is associated with cancer progression and development. The aberrant expression of miR-339-5p has been found in some types of cancers, however, the association of miR-339-5p expression and hepatocellular carcinoma (HCC) is still unclear. Here, we measured the expression of miR-339-5p in HCC tissues and explored its clinicopathological and prognostic significance. The result showed that miR-339-5p expression level was significantly lower in HCC tissues compared with non-cancerous liver tissues. Moreover, patients with lower miR-339-5p expression level are associated with a poorer overall survival. Multivariate Cox regression analysis showed that the expression of miR-339-5p was an independent prognostic factor for HCC patients. In addition, we found that over-expression of miR-339-5p can inhibit HCC cell invasion. In conclusion, our results indicated that miR-339-5p may serve as a tumor suppressor and play important role in inhibiting tumor invasion. Our work implicates that miR-339-5p may serve as a prognostic marker and molecular therapeutic target in HCC.

Introduction

Hepatocellular carcinoma (HCC) is currently one of the most common malignant tumors worldwide, with an estimated more than 20,000 new cases diagnosed annually and accounting for about 700,000 deaths every year [1—3]. It has been reported that HCC patients have a relatively lower 5-year overall survival rate, and HCC has a poor prognosis because of the characteristic progressive overgrowth and diffuse invasion. Therefore, examining the pathogenesis and biological features of HCC is crucial to enhance early detection and treatment.

MicroRNAs (miRNAs) are a class of small, single-strand, non-coding RNAs with the size range of 22—25 nucleotides.

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They are key post-transcriptional regulators of gene expression and play important role at post-transcriptional regulation levels [4]. Through imperfect hybridization to target 3′-untranslated regions (UTR), microRNA can lead to mRNA degradation or inhibition of translation. Evidences have suggested that miRNAs play critical roles in many human biological processes, including cell growth, apoptosis, proliferation and differentiation [5,6]. In recent works, studies have demonstrated that aberrantly expressed miRNAs is involved in the tumorigenesis and progression of many cancer types, and a number of miRNAs have been reported to regulate tumor carcinogenesis and metastasis [7,8]. Some miRNAs may function as markers for cancer diagnosis and prognosis.

Dysregulation of miRNA expression have been documented to play crucial role in tumorigenesis and cancer progression [9]. Recently, the expression of miR-339-5p was shown to be dis-regulated in several human cancers, such as colorectal cancer and breast cancer [10,11]. However, the underlying molecular mechanism and clinical significance of miR-339-5p in HCC remains unclear. In this work, we examined the expression difference between HCC tissues and normal liver tissues, and evaluated the clinical significance of miR-339-5p in HCC patients. Moreover, we examined the effects of miR-339-5p on HCC cells invasion, which implicates the potential effects of miR-339-5p on HCC prognosis.

Materials and methods

Patients and tissue samples

Surgical specimens of HCC tissues and matched non-cancerous liver tissues were obtained from 100 patients with a diagnosis of HCC who underwent surgery at the Yi Jishan Hospital (Anhui Province, China) between April 2006 and June 2012. All patients had complete 5-year follow-up, and informed written consents were obtained from all cases. None of the patients recruited in this work had received radiotherapy before surgery excision. The HCC tissues and normal liver tissues were divided by tissue laser microdissection. All tissue samples were immediately frozen in liquid nitrogen after surgical removal and stored at $-80\,^{\circ}C$ until further use.

RNA extraction and RT-PCR

The total RNA from tissues or cell lines was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Quantitative RT-PCR was performed using All-in-OneTM miRNA quantitative RT-PCR Detection Kit (GeneCopoeia, Rockville, MD). The primer sequences of miR-339-5p and U6 were purchased from Applied Biosystems (ABI, Foster City, CA, USA). The expression of U6 was selected as an endogenous control. RT-PCR was performed using TaKaRa SYBR Green PCR Kit (TaKaRa), and measured in a LightCycler 480 system (Roche, Basel, Switzerland). Relative quantification of miR-339-5p expression was calculated by using the $2^{-\Delta\Delta CT}$ method.

Cell culture and transfection of miRNA

Four human HCC cell lines (HepG2, Hep3B, Huh7 and SMMC7721) and normal human liver cells (LO2) were obtained from the American Type Culture Collection and cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum, 100 μ/mL penicillin and 100 mg/mL streptomycin, at 37 °C in a humidified atmosphere containing 5% CO_2. The pre-miR miRNA-339-5p (Pre-miR-339-5p), pre-miR negative control (Pre-miR-nc) were purchased from Ambion (Austin, TX, USA). A final concentration of 2×10^5 cells were seeded into each well of a 6-well plate and transfected for 48 hours using Lipofectamine 2000 Reagent (Invitrogen) following the manufacturer's protocol.

In vitro invasion assays

The cell invasion abilities were evaluated using transwell inserts with 8 μm pores (BD Biosciences, San Jose, CA, USA). For invasion assay, a final concentration of 2×10^5 cells in serum free medium were placed to each upper compartment of the chamber pre-coated with matrigel matrix (BD Biosciences, San Jose, CA, USA). Medium containing 10% fetal bovine serum in the lower chamber was used as the chemoattractant. After the incubation for 48 hours and stained with hematoxylin for 20 minutes, we removed the non-invaded cells from the top chambers. The number of cells on the lower surface of the membrane were counted under a microscope at a magnification of X400 in five random fields.

Statistical analysis

All data in this work have been repeated for at least three independent experiments. The differences between different groups were assessed using the nonparametric test (Mann-Whitney U test) and categorical data were studied using Chi^2 test. Kaplan-Meier method was employed to examine the overall survival rate. A Cox proportional hazards model was used for the multivariate analysis. All statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). All of the *P*-values were two-sided, and the differences were considered to be statistically significant at *P*-value of < 0.05.

Results

The expression of miR-339-5p in HCC tissues and cell lines

At first, we examined miR-339-5p expression in 100 pairs of HCC tissues and adjacent non-tumor liver tissues using qRT-PCR method. The result indicated that miR-339-5p expression level was significantly lower in HCC tissues (2.1 ± 0.57) compared with adjacent non-tumor liver tissues (3.9 ± 1.2, $P < 0.0001$, Fig. 1A). Further analysis of miR-339-5p expression in liver cell lines showed that miR-339-5p expressions in HCC cell lines (HepG2, Hep3B, Huh7 and SMMC7721) were significantly lower compared with normal liver cell line (LO2) (Fig. 1B).