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MiR-205 modulates abnormal lipid metabolism of hepatoma cells *via* targeting acyl-CoA synthetase long-chain family member 1 (ACSL1) mRNA

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ABSTRACT

The abnormal lipid metabolism is considered as a hallmarker of tumorigenesis. Liver is the central organ for metabolic homeostasis. Hence, the development of hepatocellular carcinoma (HCC) always exhibits alterations of metabolism. MicroRNAs emerge as key post-transcriptional modulators of gene expression in physiologic and pathologic states. Here, we aim to explore the mechanism of abnormal lipid metabolism of hepatoma cells. Previously, our group reported that miR-205 as a tumor suppressor was down-regulated in HCC. Therefore, we supposed that miR-205 might be involved in the event. Interestingly, in this study we uncover that miR-205 deregulates lipid metabolism in HCC through targeting acyl-CoA synthetase long-chain family member 1 (ACSL1) mRNA, which is an important and abundant lipid metabolism enzyme in liver. We identified that miR-205 was able to down-regulate ACSL1 *via* targeting its 3′UTR in the cells. Oil red O staining showed that miR-205 disordered the lipogenesis in hepatoma cells and anti-miR-205 resulted in the accumulation of triglyceride in the cells depending on ACSL1. Moreover, we validated that the low levels of miR-205 were negatively related to high levels of ACSL1 in clinical HCC tissues. The expression levels of ACSL1 and its metabolite triglyceride levels were remarkably increased in hepatitis B virus X protein (HBx)-induced liver cancer tissues from the HBx transgenic mice model. Thus, we conclude that miR-205-targeted ACSL1 may contribute to the abnormal lipid metabolism of liver cancer. Our finding provides new insights into the dysregulation of lipid metabolism in HCC mediated by miR-205 targeting ACSL1 mRNA.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide [1]. Up to date, there is mounting evidence that cancer cells exhibit dramatically altered metabolic circuitry. These alterations can affect the availability of structural lipids for the synthesis of membranes, the synthesis and degradation of lipids that contribute to the energy homeostasis and the abundance of lipids with signaling functions [2]. As a central organ for metabolic homeostasis, liver is a major site for synthesis, metabolism, storage and redistribution of carbohydrates, proteins and lipids [3]. Hence, the advent of HCC is always accompanied by metabolism reprogramming [4]. However, the mechanism of metabolism reprogramming, such as abnormal lipid metabolism in liver cancer remains ill-identified. Acyl-CoA synthetase long-chain family member (ACSL) catalyzes the ATP-dependent acylation of fatty acids into long-chain acyl CoAs (LCA-CoAs), which is the first step in lipid metabolism after fatty acid entry into the cell [5]. ACSL1 is one of five isoforms, which is abundant in adipose tissue, liver, heart and important in activating fatty acid destined for triacylglycerol synthesis [6].

MicroRNAs (miRNAs) are small, non-coding RNAs which emerge as key post-transcriptional modulators of gene expression. Hundreds of miRNAs have been identified in vertebrates, with varying patterns of expression that range from ubiquitous to highly tissue- or developmental-stage-restricted. In addition, numerous miRNAs are involved in human cancer and undertake opposite roles of oncogenes or tumor suppressor genes depending on the different target genes [7,8]. As a miRNA, miR-205 is regarded as a

Abbreviations: HCC, hepatocellular carcinoma; miRNA, microRNA; ACSL1, acyl-CoA synthetase long-chain family member 1; 3′UTR, 3′untranslated region; qRT-PCR, quantitative real-time polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; HBx, hepatitis B virus X protein.

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tumor suppressor gene since its disease-specific expression pattern. It has been reported that down-regulation of miR-205 contributes to the tumorigenesis of melanoma and breast cancer [9,10]. Previously, our group found that miR-205 as a tumor suppressor was down-regulated in the tissues of liver cancer mediated by Hepatitis B virus X protein (HBx) [11]. However, whether miR-205 participates in abnormal lipid metabolism is poorly understood.

In this study, we aimed to illuminate the mechanism of abnormal lipid metabolism in HCC. Strikingly, our data demonstrate that low levels of miR-205 contribute to the event. MiR-205 targeted ACSL1 is involved in the abnormal lipid metabolism in HCC. Our finding provides fascinating insights into the mechanisms of abnormal lipid metabolism mediated by miR-205 in HCC.

2. Materials and methods

2.1. Patient samples

Twenty-five HCC tissues utilized in this study were obtained from Tianjin First Center Hospital (Tianjin, China) after surgical resection. Written consents approving the use of their tissues for research purposes were obtained from patients. The study protocol was approved by the Institute Research Ethics Committee at the Nankai University.

2.2. Cell lines and cell culture

A human hepatoma cell line, HepG2 was maintained in Dulbecco's modified Eagle's medium (Gibco, CA, USA). The cells were supplemented with heat inactivated 10% fetal bovine serum (FBS, Gibco, CA, USA), 100 U/ml penicillin and 100 mg/ml streptomycin in 5% CO_2 at 37 °C.

2.3. Plasmid construction

One about 400 bp fragment of ACSL1 3′UTR was subcloned into pGL3-control vector (Promega Madison, WI, USA) immediately downstream of the stop codon of the luciferase gene to generate pGL3-ACSL1. Mutant construct of ACSL1 3′UTR (named as pGL3-PPARA-mut), carrying a substitution of 8 nucleotides within the core seed sequence of miR-205, was conducted by using overlapping extension PCR. The primers used in this study for construction were as follows: pGL3-ACSL1 forward, 5′-CTAGTCTAGATGGGA TTCACTTCTCCAGGGAT-3, reverse, 5′-GGGGGCCGGCCTCACCTGAAA TGCAGAAC-3′; pGL3 -ACSL1-mut forward, 5′-GGATTCACTTCTC-CAGGGATTTTTTAAAGTTAATTTGGGAA ATT-3, reverse, 5′-TCACCT-GAAATGCAGAAATATTTATTTTGGTTTGGAAGTAAGTT TT-3′.

2.4. Cell transfection

The cells were cultured in a 6-well or 24-well plate for 24 h and then were transfected with plasmids, miRNAs or siRNAs. All transfections were performed using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. ACSL1 siRNA oligonucleotides and a non-specific scrambled control (si-Ctrl), miR-205 (or anti-miR-205), miRNA control (miRNA Ctrl) and anit-miRNA control (anit-miRNA Ctrl) were synthesized by RiboBio (Guangzhou, China). The siRNA duplexes sequences used were as follows: ACSL1 siRNA, 5′-GGGCAGAUCCAACUCAGA AdTdT-3′.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR), reverse transcription-PCR (RT-PCR)

Total RNA was extracted from the cells (or tissues) using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. For mature miR-205 detection, total RNA was polyadenylated by poly (A) polymerase (Ambion, Austin, TX, USA) as described previously [12]. Reverse transcription was performed using poly (A)-tailed total RNA and reverse transcription primer with ImPro-II Reverse Transcriptase (Promega, Madison, WI, USA), according to the manufacturer's instructions. The qRT-PCR was performed as described in the method of Fast Start Universal SYBR Green Master (Rox) (Roche Diagnostics GmbH Mannheim, Germany). The primers used were as follows: ACSL1 forward, 5′-ATCTGCAAGCCAGGAAGAGTC-3′, reverse, 5′-CTTGCTTGATGCTT TGGTCTGT-3′; GAPDH forward, 5′-CATCACCATCTTCCAGGAGCG-3′, reverse, 5′-TGACCTTGCCCACAGCCTTG-3′; miR-205 forward, 5′-TCCTTCATTCCACCGGAGTCTG-3′, reverse, 5′-GCGAGCACAGAAT-TAATACGAC-3′; U6 forward, 5′-AGAGCCTGTGGTGTCCG-3′, reverse, 5′-CATCTTCAAAGCACTTCCCT-3′.

2.6. Luciferase reporter gene assays

Luciferase reporter gene assays were performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Cells were transferred into 24-well plates at 3×10^4 cells per well. After 24 h, the cells were transiently co-transfected with the pRL-TK plasmid (Promega, Madison, WI, USA) containing the Renilla luciferase gene, which was used for internal normalization, and with various constructs containing the seed sequence or mutant seed sequence of ACSL1 3′UTR, or pGL3-control. All experiments were performed at least three times.

2.7. Western blotting analysis

The Western blotting analysis protocol was described previously [1]. The primary antibodies used were mouse anti-β-actin (Sigma, St. Louis, MO, USA), rabbit anti-ACSL1 (Proteintech Group, USA). All experiments were repeated three times.

2.8. Oil red O staining

Cells were seeded in 6-well plates and incubated overnight. After cells were transfected with plasmid (siRNA or miRNA) for 48 h, cells were washed twice with phosphate saline and fixed with 10% formalin. The oil red O staining was performed according to the manufacturer's instructions.

2.9. Total triglyceride assay

The levels of triglyceride in cellular and tumorous (obtained from the transgenic mouse) were assayed using Tissue triglyceride assay kit (Applygen Technologies Inc., Beijing, China). All of the experiments were performed according to the manufacturer's recommended protocol.

2.10. Statistical analysis

Each experiment was repeated at least three times. Statistical significance was assessed by comparing mean values (6 standard deviation; SD), using a Student's t test for independent groups, and was assumed for $^{*}P < 0.05$, $^{**}P < 0.01$, No significant (NS). Pearson's correlation coefficient was used to determine the correlation between the expressions of each gene in HCC clinical tissues.