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miR-199a-3p affects adipocytes differentiation and fatty acid composition through targeting SCD

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ABSTRACT

Body fat mass is closely associated to diseases related to obesity. MicroRNAs (miRNAs, miR) are important regulatory molecules that function as post-transcriptional gene regulators of adipocyte development. In the current study, we revealed that reduced expression of miR-199a-3p in adipose tissue resulting from high fat diet (HFD)-induced obesity in mice. Overexpression of miR-199a-3p promoted adipocyte proliferation by regulating the expression of regulating factors of the cell cycle. Furthermore, miR-199a-3p blunted lipid accumulation in 3T3-L1 adipocytes. This was accompanied by a marked decrease in the expression of adipocyte-specific genes involved in lipogenic transcription, fatty acid synthesis, and fatty acid transportation. Furthermore, the fatty acid oxidation process was enhanced. Luciferase activity assays confirmed that miR-199a-3p regulates adipocyte differentiation by directly targeting the 3′-untranslated region (3′-UTR) of stearoyl-CoA desaturase *(SCD)*. Moreover, miR-199a-3p regulates fatty acid composition by decreasing the ratio of unsaturated fatty acids (UFAs) in adipocytes transfected with miR-199a-3p mimics. These results suggest that miR-199a-3p may promote adipocyte proliferation, while also repressing adipocyte differentiation by down-regulating *SCD* and changing fatty acid composition during adipogenesis.

1. Introduction

Currently, there is an obesity epidemic that threatens human health. The increase in body weight is accompanied by an increase in the development of type II diabetes, hypertension, and cardiovascular disease in both adults and children [1]. In mammals, calorie-dense meals stimulate triglyceride accumulation, and it is primarily stored as white adipose tissue (WAT) in the subcutaneous region and around the viscera. WAT is comprised predominantly of adipocytes. In obese individuals, the hypertrophy and increased number of adipocytes alters metabolic homeostasis and promote obesity-related diseases [2]. All of these abnormalities progressively cause damage to tissue and create an inflammatory response [3]. Inflammatory cytokines produced by adipose tissue include interleukin-6 (IL-6), tumour necrosis factor-α (TNFα), and plasminogen activator inhibitor 1 (PAI1), all of which may be involved in various types of cancer [4,5]. Fatty acids released from adipose tissue are oxidized by both muscle and the liver, and this generates ketones that serve as important fuel for the peripheral organs. Once the oxidation of fatty acids is below the intake, obesity will

Abbreviations: PPAR-γ, peroxisome proliferator activated receptor-g coactivator; *C/EBP-α*, CCAAT/enhancer-binding proteins; *FAS*, fatty acid synthase; *ACC*, acetyl-CoA carboxylase; *GSK*, glycogen synthase kinase; *SREBP*, sterol regulatory element-Binding protein; *ELOVL*, elongation-of-very-long-chain-fatty acids; *LPL*, lipoprotein lipase; *DGAT*, diacylglycerol acyltransferase; *VLDL*, very-low density lipoprotein; *FATP4*, fatty acid transport protein 4; *FABP4*, fatty acid binding protein 4; *ACADL*, acyl-CoA dehydrogenase, long chain; *ACOX2*, acyl-CoA oxidase 2; *ACADSB*, acyl-CoA dehydrogenase, short/branched chain; *HSD11B1*, hydroxysteroid 11-beta dehydrogenase; *ACOT1*, acyl-CoA thioesterase 1; *ACSS1*, acyl-CoA synthetase short-chain family member 1; *ACS*, Acyl-CoA synthetase; *aP2*, adipocyte fatty-acid binding protein 2; *SCD*, stearoyl-CoA desaturase; *P53*, p53; *P21*, calcium binding protein 21; *CDK4*, cyclin dependent kinase 4.

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gradually occur. Research suggests that impaired and ineffective activity of fatty acid metabolism in skeletal muscle results in triglyceride accumulation [6]. Moreover, genetic factors, such as *PPAR-γ* and *C/EBP-α,* are also thought to be the main factors contributing to the propensity of obesity [7,8]. Currently, physical activity is employed to increase energy expenditure, but a treatment through medication could increase the treatment and prevention of obesity.

During the last decade, a large number of transcription factors have been shown to control hundreds of genes related to adipocyte maturation [9]. Evidence suggests that miRNA is involved in adipocyte fat metabolism during differentiation. For instance, miR-375 promotes adipocyte differentiation via modulation of ERK signaling [10], while miR-344, miR-27, and miR-181a impair this process [11–13]. T. Shatseva et al. suggested that miR-199a-3p promotes proliferation of endothelial cells, as well as breast cancer cells, by inhibiting caveolin-2 activity [14]. Recently, W. Jin et al. established that reduced expression of miR-199a-3p adipose tissues of obese subjects, which implies that miR-199a-3p may participate in regulating of lipometabolism [15]. Remarkably, the other product of the miR-199a precursor, miR-199a-5p, has been reported to promote the proliferation of adipocytes and attenuate lipid accumulation in porcine adipocytes [16]. Nevertheless, the investigation of miR-199a-3p on adipogenesis is insufficient because it is not known if miR-199a-3p affects lipid deposition in adipocytes. In the present study, our data demonstrated that the overexpression of miR-199a-3p promoted proliferation and inhibited the differentiation of adipocytes through regulating adipogenic genes. Additionally, we found that miR-199a-3p suppresses the accumulation of lipid droplets by directly targeting *SCD,* which is a key gene of adipocyte differentiation. Furthermore, our results indicate that the elevation of miR-199a-3p levels altered the fatty acid composition.

2. Materials and methods

2.1. Experimental animals

All experiments were performed in accordance with the guidelines of the U.K. Animals (Scientific Procedures) Act, 1986. In the current study, two groups of 7-week-old male Kunming mice were fed either a high-fat diet (HFD, 45% fat) or normal chow (NCW, 15% fat) for 3 months. Mice were given free access to food and water under controlled conditions of light and temperature during the experimental trial. For RNA samples and serum samples, mice were sacrificed by cervical dislocation and tissue samples were frozen immediately in liquid nitrogen and stored at −80 °C. Three samples of each were obtained and analyzed in triplicate.

2.2. Serum-sample analysis

The blood samples were allowed to naturally coagulate at room temperature to collect serum. Serum samples were stored at −20 °C until further analysis. Serum levels of cholesterol (TC) and triglycerides (TG) were determined (Chengdu Li Lai Biotechnology limited company, Chengdu, P. R. China).

2.3. Cell culture

Mouse 3T3-L1 cells (Stem Cell Bank, Chinese Academy of Science) were maintained in proliferation medium containing Dulbecco's modified Eagle's medium (DMEM, Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum at 37 °C with 5% CO_2, humidified atmosphere. After cells had grown to confluency, cells were stimulated in differentiation medium (DM) (10% FBS, 10 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM IBMX). Two days following DM stimulation, the media was changed to insulin media (10% FBS and 10 Lg/mL insulin) for 48 h. Cells were then maintained in DMEM containing 10% FBS, and media was changed every other day. 3T3-L1 cells were transfected with plasmid DNA, miRNA mimics, inhibitors, mimics negative control, or inhibitor negative control (all purchased from RIBOBIO, Guangzhou, China) using Lipofectamine 2000 (Invitrogen, Shanghai, China) according to the manufacturer's instructions. The sequence of synthesized RNA oligonucleotides is shown in supplement Table S1.

2.4. Edu proliferation and CCK-8 analysis

For the Edu assay, 3T3-L1 cells were seeded in 12-well plates. At 24 h post-transfection, 100 μl of 50 μM Edu reagent was added to each well (RiboBio, Guangzhou, China) and incubated for at least 24 h. Edu staining was done according to the manufacturer's protocol. Images were captured using a Nikon TE2000 microscope (Nikon, Tokyo, Japan). For CCK-8 analysis, 3T3-L1 cells were seeded in 96-well plates and maintained in growth medium. The cells were then transfected with miR-199a-3p mimics and inhibitors. Cell proliferation was assessed with a Cell Counting kit 8 (CCK-8, Beyotime, Shanghai, China) according to manufacturer's protocol.

2.5. Oil Red O staining

Cells were washed with PBS, fixed in 4% paraformaldehyde for 30 min, and then washed with PBS. Next, 0.5 g of oil Red O was dissolved in 60 ml isopropanol and 40 ml of water was added, and then it was filtrated. Cells were stained for 1 h, washed twice in water, and then photographed. For quantitative analysis, we used 100% isopropanol extracted Oil Red O, and absorbance was read at 510 nm wavelength.

2.6. qRT-PCR analysis

Tissue and cell total RNA was obtained using Trizol (Invitrogen) according to the manufacturer's instructions. Then total RNA was reverse-transcribed into cDNA using a commercial kit (TaKaRa, Dalian, China) and Mir-X™ miRNA First-Strand Synthesis (TaKaRa, Dalian, China) for mRNA and miRNA, respectively, according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) reactions were performed using a SYBR Premix Ex Taq kit (TaKaRa, China) run on a Bio-RAD IQTM5 system (Bio-Rad, Hercules, CA, USA). The relative expression ratio of mRNAs was calculated using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as an internal normalizing control for mRNA, and U6 was used for miRNA. The primer sequences used for qRT-PCR are listed in supplement Table S1.

2.7. Luciferase reporter assay

The 3′-UTR of *SCD* was amplified by RT-PCR (Table S1), then the product was inserted into the *Xho*I and *Not*I psiCHECK-2 dual-luciferase reporter plasmid (Promega, Madison, USA). Mutant-type *SCD* 3′-UTR was manufactured using commercial kits (TransGen Biotech, Beijin, China) according to the manufacturer's instructions. Hela229 cells were plated in 96-well plates at a density 60%. The, 0.25 μg psiCHECK-2 recombination vector and either 50 nM or 200 nM of miR-199a mimic or miRNA mimics Negative Control were co-transfection using Lipofectamine™ 3000 (Invitrogen, Shanghai, China). Three technical replicates were conducted for each combination. Forty-eight hours after transfection, cells were harvested and measured for *Renilla* and *firefly* luciferase activity using the Dual Luciferase Reporter Assay System (Promega).

2.8. Determination of fatty acid content

We collected 50 mg of adipocytes which transfected with mimic

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