Gene 575 (2016) 761–764

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

MicroRNA-23a regulates 3T3-L1 adipocyte differentiation

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ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 4 September 2015
Accepted 22 September 2015
Available online 28 September 2015

Keywords:
3T3-L1 cell line
Adipocyte differentiation
Adipocyte lipid metabolism
MiRNA-23a

ABSTRACT

MicroRNAs (miRNAs) are small, non-coding RNAs, which are involved in regulation of a variety of biological processes. Since previous studies regarding the role of miRNAs in the regulation of adipogenic differentiation have shown that miRNA-27a, one member of miRNA-23a~27a~24 cluster, could suppress adipogenesis. We now investigated whether miRNA-23a regulates adipogenic differentiation. In the present study, we showed that the expression of miRNA-23a is decreased during the process of adipogenic differentiation. Over-expression of miRNA-23a decreased lipid accumulation and triglyceride content in 3T3-L1 adipocytes. Our results also demonstrated that miRNA-23a decreases mRNA levels of adipocyte-specific genes involved in lipogenic transcription, fatty acid synthesis and fatty acid transport. These findings suggested miRNA-23a to be a new type of adipogenic depressor and to play an important role in regulating adipocyte differentiation.

1. Introduction

MicroRNAs (miRNAs) are endogenous small, non-coding RNAs of about 22 nucleotide in length, and able to post-transcriptionally regulate gene expression by complementarily binding to the 3′-untranslated region (3′-UTR) of their target genes (Ambros, 2004). Among the large number of biological processes regulated by miRNAs, fat development and lipogenesis are particularly important because they are closely related to adipose tissue function. Adipose tissue is not only an energy storage organ, but also fulfills important immune functions (Tilg and Moschen, 2006). Regulatory mechanisms regarding adipose tissue gain more and more interest due to the increasing prevalence of obesity, diabetes and cardiovascular diseases in human being (Hajer et al., 2008). Exploring the role of miRNAs in the regulation of lipid metabolism may contribute to our understanding of these diseases.

The role of miRNAs in fat metabolism was first described in *Drosophila melanogaster*, where the loss of miRNA-14 resulted in an increased content of triacylglycerol and diacylglycerol (Xu et al., 2003). Furthermore, miRNA-375 was found to promote 3T3-L1 adipocyte differentiation (Ling et al., 2011). miRNA-143 may target mitogen-activated protein kinase, and thus stimulate adipocyte differentiation (Esau et al., 2004). Interestingly, miRNA-27a, miRNA-23a and miRNA-24–2 were determined to be a cluster, because they exhibited similar expression patterns and cooperative physiological effects in different disease condition (Chhabra et al., 2009; Chhabra et al., 2010; Hassan et al., 2010). A recent study demonstrated that miRNA-27a inhibited adipogenic differentiation by blockading the expression of *PPARγ* and *C/EBPα* gene (Lin et al., 2009). However, the underlying roles of miRNA-23a in adipogenic differentiation have not been clarified. The mouse 3T3-L1 pre-adipocyte cell line is widely used as an adipocyte differentiation model. In order to evaluate the effect of miRNA-23a regulating adipocyte differentiation, we treated 3T3-L1 cells with synthesized miRNA-23a mimic or inhibitor to augment or decrease its expression level, respectively in this study.

2. Material and methods

2.1. Cell culture

3T3-L1 pre-adipocytes were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone) containing 10% fetal bovine serum (FBS, Hyclone) at 5% CO_2 and 37 °C. To induce differentiation, post-confluent 3T3-L1 pre-adipocytes were incubated for 2 days in a differentiation media (DM) containing 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 5 μg of insulin/ml. The medium was replaced every second day with DMEM containing 10% FBS and 5 μM of insulin/ml, and the process was kept on till day 9.

Abbreviations: ACS, acyl-CoA synthetase; C/EBPα, CCAAT/enhancer-binding proteins; DM, differentiation media; DMEM, Dulbecco's modified Eagle's medium; DGAT, diacylglycerol acyltransferase; ELOVL, elongation-of-very-long-chain-fatty acids; FBS, fetal bovine serum; FAS, fatty acid synthase; GSK, glycogen synthase kinase; LPL, lipoprotein lipase; miRNAs, MicroRNAs; PPARγ, peroxisome proliferator activated receptor-γ coactivator; SREBP-1, sterol regulatory element-binding protein-1; SCD-1, stearoyl-CoA desaturase-1; UTR, untranslated region; VLDL, very-low density lipoprotein.

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http://dx.doi.org/10.1016/j.gene.2015.09.060
0378-1119/

2.2. Cell transfection

Mimic and inhibitor oligonucleotides of mmu-miRNA-23a were synthesized by Ribobio (Guangzhou, China). The transfection was carried out using the lipid carrier Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Briefly, cells grown in standard 24-well plates were covered with 500 μL of Opti-MEM, and a mixture of mimic or inhibitor (20 nM) with lipid carrier (2:1, *v*/*v*) was added. After 6 h of transfection, the medium was changed. Transfection was carried out every second day when replacing the medium.

2.3. Quantitative PCR

Total RNA (including miRNA) was extracted with TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription of mRNA and miRNA was performed using a commercial kit (TaKaRa, China), following the manufacturer's recommendations. Quantitative PCR was performed using the SYBR Premix Ex Taq kit (TaKaRa) on the CFX96 system (Bio-Rad, USA). Relative expression levels of mRNAs and miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Sequences of all primers used for PCR are shown in Supplementary Table S1.

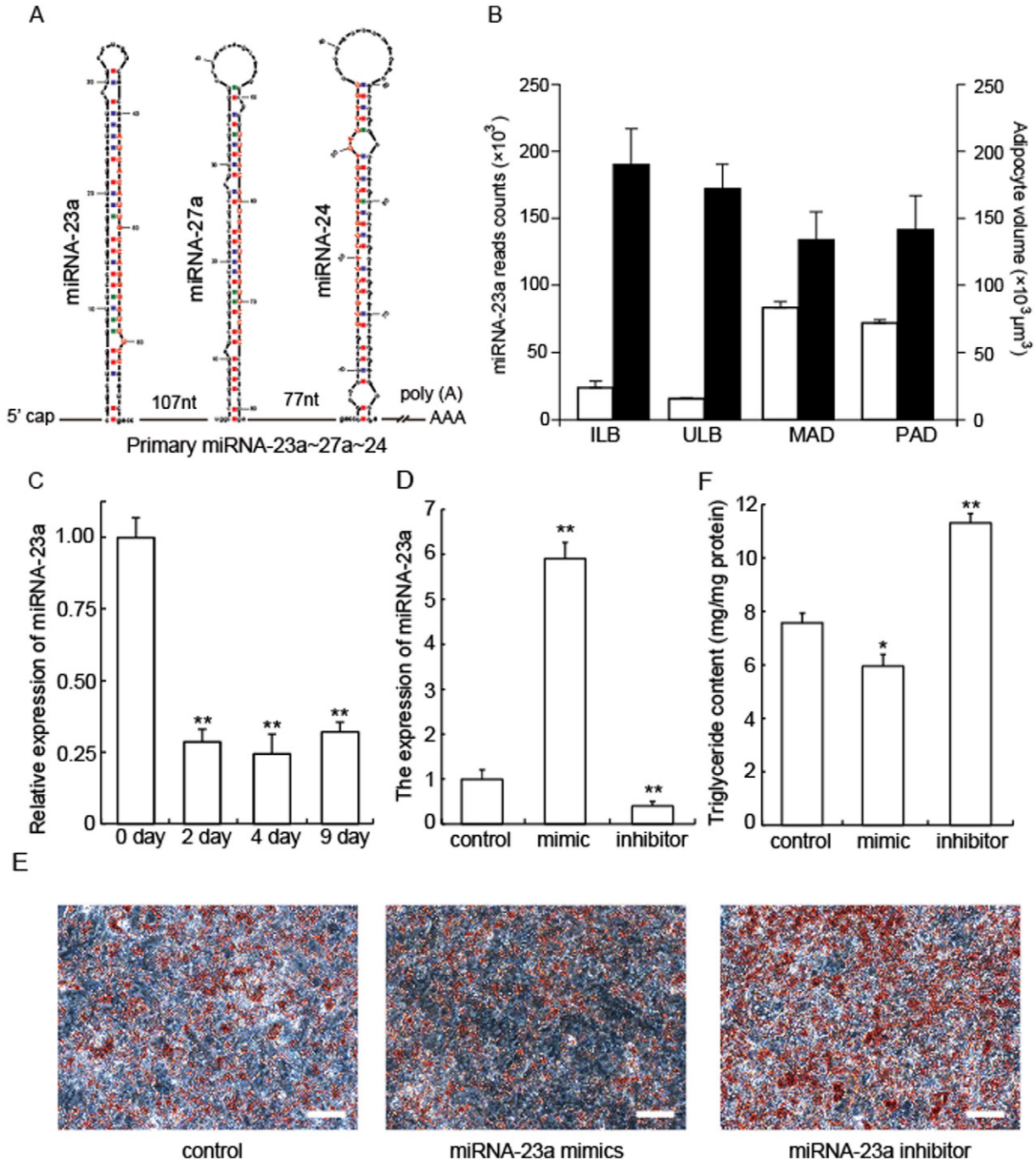

Fig. 1. The role of miRNA-23a in adipocyte differentiation. (A) The schematic structure of miRNA-23a ~ 27a ~ 24. (B) miRNA-23a expression levels and adipocyte volume in different adipose tissues. ILB: inner layer of backfat, ULB: upper layer of backfat, MAD: mesenteric adipose, PAD: pericardial adipose. (C) The relative expression of miRNA-23a during adipocyte differentiation. (D) The relative expression levels of miRNA-23a in the control group and 3T3-L1 pre-adipocytes transfected with miRNA-23a mimic and inhibitor. (E) Oil Red O staining of terminally differentiated adipocytes (day 9). Scale bar, 10 μm. (F) The content of triglycerides in terminally differentiated adipocytes. All data are expressed as means ± SE (n = 3). $^{*}P < 0.05$, $^{**}P < 0.01$.