Molecular and Cellular Endocrinology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

miR-199a-3p regulates brown adipocyte differentiation through mTOR signaling pathway

Yao Gao[1], Yan Cao[1], Xianwei Cui, Xingyun Wang, Yahui Zhou, Fangyan Huang, Xing Wang, Juan Wen, Kaipeng Xie, Pengfei Xu, Xirong Guo, Lianghui You**, Chenbo Ji*

Nanjing Maternity and Child Health Care Institute, The Affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, Nanjing, 210004, China

ARTICLE INFO

Keywords:
BAT
miR-199a-3p
Adipogenesis
Mitochondrial function
mTOR

ABSTRACT

Recent discoveries of functional brown adipocytes in mammals illuminates their therapeutic potential for combating obesity and its associated diseases. However, on account of the limited amount and activity in adult humans of brown adipocyte depots, identification of miRNAs and characterization their regulatory roles in human brown adipogenesis are urgently needed. This study focused on the role of microRNA (miR)-199a-3p in human brown adipocyte differentiation and thermogenic capacity. A decreased expression pattern of miR-199a-3p was consistently observed during the differentiation course of brown adipocytes in mice and humans. Conversely, its level was induced during the differentiation course of human white pre-adipocytes derived from visceral fat. miR-199a-3p expression was relatively abundant in interscapular BAT (iBAT) and differentially regulated in the activated and aging BAT in mice. Additionally, miR-199a-3p expression level in human brown adipocytes was observed decreased upon thermogenic activation and increased by aging-related stimuli. Using primary pre-adipocytes, miR-199a-3p over-expression was capable of attenuating lipid accumulation and adipogenic gene expression as well as impairing brown adipocytes' metabolic characteristics as revealed by decreased mitochondrial DNA content and respiration. Suppression of miR-199a-3p by a locked nucleic acid (LNA) modified-anti-miR led to increased differentiation and thermogenesis in human brown adipocytes. By combining target prediction and examination, we identified mechanistic target of rapamycin kinase (mTOR) as a direct target of miR-199a-3p that affected brown adipogenesis and thermogenesis. Our results point to a novel role for miR-199a-3p and its downstream effector mTOR in human brown adipocyte differentiation and maintenance of thermogenic characteristics, which can be manipulated as therapeutic targets against obesity and its related metabolic disorders.

1. Introduction

Adipose tissues are considered to be an important endocrine organ to regulate nutrient and energy homeostasis. At present, at least two distinctly different types of adipose tissues are recognized to exist in mammals: white and brown adipose tissues (WAT and BAT) (Rosen and Spiegelman, 2014). WAT mainly stores energy and plays a crucial role in endocrine signaling and crosstalk with the immune system. BAT, which is characterized by a high content of mitochondria, dissipates heat via specific expression of uncoupling protein 1 (UCP1), which leads to an exceptionally high utilization of lipid and glucose (Matthias et al., 2000; Cannon and Nedergaard, 2004). As revealed in rodent studies, manipulation of the content and/or activity of BAT plays a vital role in regulating whole-body energy, glucose homeostasis, and lipid metabolism (Bartelt et al., 2011; Liu et al., 2013, 2015; Stanford et al., 2013). Conventionally, in humans, BAT was believed to be solely apparent during the neonatal stage and then to disappear in adults. However, in 2009, functional BAT was identified in human adults by a combination of positron-emission tomography PET and computed tomography (CT) scanning with the glucose analog 18F-fluorodeoxyglucose (18F-FDG) (Cypess et al., 2009; van Marken et al., 2009; Virtanen et al., 2009). In line with previous findings from mice,

* Corresponding author. Nanjing Maternity and Child Health Care Institute, The affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, No 123 Tianfei Street, Nanjing, 210004, China. Tel.: +86 02552226162.

** Corresponding author. Nanjing Maternity and Child Health Care Institute, The affiliated Obstetrics and Gynecology Hospital of Nanjing Medical University, Nanjing Maternity and Child Health Care Hospital, No 123 Tianfei Street, Nanjing, 210004, China.

E-mail addresses: y_lianghui@126.com (L. You), chenboji@njmu.edu.cn (C. Ji).

[1] Yao Gao and Yan Cao contributed equally to this work and should be regarded as joint first authors.

https://doi.org/10.1016/j.mce.2018.05.005
Received 27 January 2018; Received in revised form 23 April 2018; Accepted 7 May 2018

stimulating human BAT amount and/or activity also has the potential to substantially contribute to energy expenditure, decrease adiposity and restore glucose homeostasis (Lee et al., 2010; Jacene et al., 2011; Yoneshiro et al., 2013; Leitner et al., 2017).

As BAT has the potential of anti-diabetic and anti-obese action, identification of cellular and molecular mechanisms underlying brown adipocyte development and activation will result in the development of novel therapeutic methods. In addition to a full cascade of transcriptional factors and co-factors (Seale et al., 2007; Seale, 2015; Wang and Seale, 2016) as well as endocrine hormones (Tseng et al., 2008; Whittle et al., 2012; Hinoi et al., 2014) and metabolites (Fromme et al., 2018) implicated in the maintenance of BAT mass and function, microRNAs (miRNAs) have recently emerged as a novel kind of modulators in adipose tissue formation and function. Indeed, particular miRNAs have been shown to be involved in mouse brown adipocyte differentiation, lipid metabolism, and obesity. In particular, miR-365, miR-196a (Mori et al., 2012), miR-378 (Pan et al., 2014), and miR-32 (Ng et al., 2017) were discovered to promote mouse brown adipocyte development and function. In addition, miR-155 (Chen et al., 2013), miR-133a (Trajkovski et al., 2012; Yin et al., 2013), miR-106b-93 (Wu et al., 2013), miR-34a (Seok et al., 2014), miR-27 (Sun and Trajkovski, 2014) and miR-455 (Zhang et al., 2015) were found to be involved in the negative control of mouse brown adipocyte formation and function. Although some miRNAs, such as miR-26, miR-125b, and let-7i-5p, were found to function in the conversion from white to brite/brown adipocytes in humans (Karbiener et al., 2014; Giroud et al., 2016a, 2016b), the individual roles of miRNAs in human brown adipocyte differentiation and maintenance of thermogenic program are still not fully understood.

The miR-199 family has been shown to be widely expressed in multiple tissues and to play an important role in various biological events, including signal transduction, immune response, cancer progression, and muscle cell differentiation (Jia et al., 2013). Our previous studies identified that miR-199a-3p was up-regulated during human preadipocyte adipogenesis (visceral origin), and its expression was higher in visceral adipose tissues from obese subjects (Gu et al., 2016). Furthermore, the observation of a decreased expression of miR-199a-3p during the conversion of white to brite adipocytes indicated a potential role of miR-199a-3p in the thermogenic program of adipocytes (Giroud et al., 2016b). However, the specific action of miR-199a-3p and its possible targets in human brown adipocyte differentiation and the thermogenic program need to be further studied.

Herein, we have analyzed the role of miR-199a-3p in brown adipocyte differentiation and thermogenic function using human cell models. Our results showed miR-199a-3p presented a consistently decreased expression pattern during brown adipocyte differentiation in mice and humans, and its expression was differentially regulated in the activated and aging BAT in mice or human brown adipocytes. Additionally, functional studies *in vitro* validated the important role of miR-199a-3p in the modulation of human brown adipocyte lipid accumulation and mitochondrial function. By combining target prediction and examination, we identified the mechanistic target of rapamycin kinase (mTOR) as a direct target of miR-199a-3p that affected brown adipogenesis and thermogenic capacity, which can be manipulated as therapeutic targets against obesity and its related metabolic disorders.

2. Materials and methods

2.1. Ethics statement

Our experiments involving mice were handled with the approved protocols by the Use of Experimental Animals published by the Ethics Committee at Nanjing Medical University. The human fetal adipose tissues were obtained from the interscapular position of aborted fetuses after the parental donors signed a written informed consent. This work was carried out with approval from the Human Research Ethics Committee of Nanjing Maternity and Child Health Care Institute (permit number [2015]110) and the methods were carried out in accordance with the approved guidelines.

2.2. Mice, cold exposure and CL-316, 243 administration in vivo

Male C57BL/6 J mice purchased from the Model Animal Research Center of Nanjing University were maintained with a 12-h light/dark cycle and allowed free access to food and water supplies. For the cold exposure experiment, mice at the age of six weeks were housed either at room temperature (RT) or 4 °C (cold) for 7 day as described in a previous study (You et al., 2018). For adrenergic receptor agonist treatment *in vivo*, mice were injected with saline or CL-316,243 (1 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally (i.p.) daily for 1 week as described in a previous study (You et al., 2018). At the indicated time points, interscapular BAT (iBAT) were collected for qPCR analysis (six mice for each group). Mice at specific ages (1 month, 2 months, 5 months, 8 months, and 10 months) were sacrificed, and their iBATs were harvested for qPCR detection (six mice for each time point).

2.3. Cell isolation, culture and induction of adipogenesis

Primary human pre-adipocytes were isolated from fetal interscapular depots and low passage number cells were maintained in Preadipocyte medium (PAM; Sciencell Research Laboratories, Carlsbad, CA, USA) supplemented with recombinant human FGF (20 ng/ml) (R& D, Minneapolis, USA) and ascorbic acid (16 nM) (Sigma-Aldrich). For brown human adipocyte differentiation, 2-day post-confluent cells were treated with adipogenic medium including 0.5 mM IBMX, 0.5 mM dexamethasone, 860 nM insulin, 1 nM T3, 1 μM rosiglitazone, 17 μM pantothenate, 33 μM biotin, and 10 μg/ml transferrin (all regents were purchased from Sigma-Aldrich). After 2–4 days induction, cells were changed into a maintenance medium containing 860 nM insulin and 1 nM T3 and sustained for 4–6 days until reaching a fully differentiated status according to our previous study (Wang et al., 2018). The mouse BAT progenitor cells derived from the stromal vascular fraction (SVF) were isolated from the iBAT of four-week-old C57BL/6 J mice, cultured and induced to mature adipocytes using a standard procedure described in our previous study (You et al., 2015, 2018). To induce thermogenesis, human brown adipocytes with fully differentiated status were treated with norepinephrine (NE), CL and forskolin (FSK) to a final concentration of 10 μM in DMEM/F12 basal medium for 4 h, following incubation in serum-free medium for 6 h. To induce ROS, the differentiated human brown adipocytes were treated with hydrogen peroxide (H_2O_2) at the concentration of 0, 10, 50 and 250 μM respectively according to previous studies (Liu et al., 2016). At the indicated time, cells were collected for the following experiments.

2.4. Transfection of miR-199a-3p mimic and inhibitor in human brown preadipocytes

The specific miR-199a-3p mimic and the negative control (NC) were purchased from Ribobio (Guangzhou, China) and used at the concentration of 75 nM. The miRCURY LNA™ inhibitor and negative control with full phosphorothioate (PS) backbones were obtained from Exiqon (Vedbaek, Denmark). LNA-miR-199a-3p-inhibitor (LNA-miR-199a-3p-I) was designed to work efficiently in the brown pre-adipocytes at the concentration of 75 nM and had the following sequence 5′-AACCAATGTGCAGACTACTG-3’. LNA-miRNA–Negative Control (LNA-NC) was used at the same concentration (75 nM) and as sequence follows 5′-TAACACGTCTATACGCCCA-3’. When cells reached the density of 60%–70%, transfection for over-expression or down-regulation of miR-199a-3p respectively was conducted using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Following transfection and 2 day post-confluency, the cells were stimulated to differentiation medium as described above.