Original article

MOLECULAR METABOLISM

miR-125b affects mitochondrial biogenesis and impairs brite adipocyte formation and function

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ABSTRACT

Objective: In rodents and humans, besides brown adipose tissue (BAT), islands of thermogenic adipocytes, termed "brite" (brown-in-white) or beige adipocytes, emerge within white adipose tissue (WAT) after cold exposure or β3-adrenoceptor stimulation, which may protect from obesity and associated diseases. microRNAs are novel modulators of adipose tissue development and function. The purpose of this work was to characterize the role of microRNAs in the control of brite adipocyte formation.

Methods/Results: Using human multipotent adipose derived stem cells, we identified miR-125b-5p as downregulated upon brite adipocyte formation. In humans and rodents, miR-125b-5p expression was lower in BAT than in WAT. *In vitro*, overexpression and knockdown of miR-125b-5p decreased and increased mitochondrial biogenesis, respectively. *In vivo*, miR-125b-5p levels were downregulated in subcutaneous WAT and interscapular BAT upon β3-adrenergic receptor stimulation. Injections of an miR-125b-5p mimic and LNA inhibitor directly into WAT inhibited and increased β3-adrenoceptor-mediated induction of UCP1, respectively, and mitochondrial brite adipocyte marker expression and mitochondriogenesis.

Conclusion: Collectively, our results demonstrate that miR-125b-5p plays an important role in the repression of brite adipocyte function by modulating oxygen consumption and mitochondrial gene expression.

Keywords miR-125b-5p; White adipocyte; Brite adipocyte; Mitochondriogenesis

1. INTRODUCTION

A chronic imbalance between energy intake and energy expenditure leads to variation in body weight through modulation of adipose tissue mass. Today, such an imbalance is mostly manifested as obesity, which has become a worldwide socio-economic burden and constitutes a substantial risk factor for hypertension, type 2 diabetes (T2D), cardiovascular diseases, and certain cancers [1–3]. The adipose organ can be divided into two distinct types of adipose tissues, white and brown. White adipose tissue (WAT) is specialized in the storage and release of fatty acids while brown adipose tissue (BAT) dissipates energy in the form of heat by uncoupling mitochondrial respiratory chain from ATP synthesis [4,5]. BAT is predominantly composed of brown adipocytes that are characterized by a high mitochondrial content and the expression of uncoupling protein 1 (UCP1), resulting in an exceptionally high capacity of lipid and glucose utilization [6–9]. In contrast to earlier contention, it is now well accepted that brown adipocytes are present and active in human adults [10–13]. An inverse relationship between BAT activity and fat content and a decline with increasing age have been reported [10,14,15]. Interestingly, also WAT contains thermogenic (*i.e.*, UCP1-positive) fat cells, called "brown-in-white" ("brite"), "beige" or inducible brown adipocytes. These cells are formed upon chronic stimulation with cold, PPARγ ligands, β3-adrenergic agonists, or various other molecules [16–19]. The precise origin of brite adipocytes remains controversial, as it has been reported that brite adipocytes appearing in subcutaneous WAT (scWAT) upon cold exposure can originate either from trans-differentiation of white adipocytes [20–23] or from *de novo*-differentiation of precursors [24]. As BAT constitutes a target to combat obesity and associated diseases [25–28], identification of cellular and molecular mechanisms

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involved in the conversion and activation of white adipocytes into brite adipocytes may lead to the development of novel therapeutic tools. Several compounds have already been reported to play key roles [18]. Micro-RNAs (miRNAs) are small non-coding RNAs of approximately 23 nucleotides, which regulate gene expression through RNA interference [29–31]. miRNAs have recently been recognized as a novel class of modulators in adipose tissue development and function as tissue specific inactivation of Dicer resulted in a dramatic decrease of white fat mass as well as lipodystrophy [32–34]. Indeed, particular miRNAs have been shown to be involved in human white adipocyte differentiation, lipid metabolism, diabetes and obesity [35–40]. A few reports demonstrated that miRNAs might be involved in the switch between mature white and brite adipocytes [41,42]. The miR-193b-365 cluster was described as a novel regulator between closely related brown and myogenic lineages, and miR-196a has been introduced as the first non-coding RNA that acts as key regulator of brite adipocyte development in mouse [38,43–45]. Recently, it has been reported that miR-34a acts as an inhibitor of brite and brown adipocyte formation through modulation of FGF21 and SIRT1 function [46]. miR-26 has been reported to be the first in-depth characterized miRNA with an impact on human brite adipogenesis and the ability to improve insulin sensitivity [39,47]. However, the involvement of miRNAs in the process of conversion (trans-differentiation) of white into brite/brown adipocytes remains to be elucidated.

Herein, we have analyzed the role of miR-125b-5p in the browning/britening of white adipocytes in human and murine cell models and tissues. Altogether, our results show that miR-125b-5p plays an important role in the modulation of brite and brown adipocyte function by targeting mitochondriogenesis and oxygen consumption.

2. MATERIALS AND METHODS

2.1. Reagents

Cell culture media and buffers were purchased from Lonza, foetal bovine serum, insulin and trypsin from Invitrogen, and other reagents from Sigma–Aldrich Chimie.

2.2. Animals

Experiments were conducted in accordance with the French regulation for the care and use of research animals and were approved by local experimentation committees (Nice University and Ciepal Azur: protocol NCE-2013-166). Procedures comply with the ARRIVE guidelines. Animals were maintained under constant temperature near thermoneutrality (28 ± 2 °C) and on 12:12-hour light–dark cycles, with *ad libitum* access to standard chow diet and water. 10 week-old male C57Bl/6J mice were from Janvier Labs. Thermogenesis activation consisted in an intra-peritoneal injection of the β-adrenergic receptor agonist, CL316,243 (1 mg/kg/day in saline solution, NaCl 0.9%) for 7 days. Control mice were injected with vehicle only.

For miRNA mimic injection, mice were anesthetized with a xylasin/ketamine mixture. miRNA and control mimics were delivered using *in vivo*-jetPEI® reagent (Ozyme) according to manufacturer's recommendation. *In vivo*-jetPEI® does not induce any significant inflammatory response. The intra-subcutaneous WAT administration was carried out after a longitudinal incision in the skin at the inguinal area or at the interscapular area for BAT injection as previously described [48]. Mice received five injections per inguinal fat pad and were sacrificed for analysis 2 weeks after injections. Each injection contained 40 ng of miR-125b-5p mimic in 10 μl saline solution. Mice were stitched and reanimated under warm light. Control mice were injected with vehicle (NaCl 0.9%) or mimic control. β-adrenergic receptor stimulation was carried out during the last week (CL316,243, 1 mg/kg/day in saline solution). Control mice were injected with vehicle only.

For Locked Nucleic Acid (LNA™) miR-inhibitor injection (miRCURY LNA™ microRNA Inhibitors, Exiqon), mice received five injections per inguinal fat pad and were sacrificed for analysis 2 weeks after injections. Each injection contained 200 ng LNA inhibitor miR-125b-5p in 10 μl saline solution. β-adrenergic receptor stimulation was carried out during the last week (CL316,243, 0.1 mg/kg/day in saline solution). Control mice groups were injected with vehicle or miRNA inhibitor control.

2.3. Subjects

2.3.1. Human BAT and WAT samples

The study protocol was reviewed and approved by the ethics committee of the Hospital District of Southwestern Finland, and subjects provided written, informed consent following the committee's instructions. The study was conducted according to the principles of the Declaration of Helsinki. All potential subjects were screened for metabolic status, and only those with normal glucose tolerance and normal cardiovascular status (as assessed on the basis of electrocardiograms and measured blood pressure) were included. The age range of the subjects was 23–49 years. We studied a group of 6 healthy volunteers (2 men and 4 women). BAT was sampled from positive FDG-PET scan sites in supraclavicular localization, and subcutaneous WAT was derived via the same incision.

2.3.2. Human adipose sample correlated to the BMI

Primary subcutaneous adipose tissue samples were obtained from patients (n = 35, 30 female, 5 male, age 18–54 years) with a wide range of body mass index (BMI 18.7–70.4 kg m^{-2}, mean 33.8+/−12.9 kg m^{-2}) who underwent abdominal surgery for nonmalignant diseases or plastic surgery for mammary reduction. The study was approved by the ethical committee of Ulm University (122/99), and all patients gave written, informed consent. Snap-frozen samples were homogenized in TriReagent (Zymo Research, Irvine, USA) and total RNA was subsequently isolated using the Direct-zol RNA MiniPrep kit (Zymo Research). miRNA was reverse transcribed and analyzed with the miScript miRNA PCR Assay System using specific primer assays (Qiagen, Hilden, Germany). Small nucleolar RNA 68 (sno68) was used as reference. cDNA was transcribed using SuperScript II (Thermo Fischer, Waltham, USA) and UCP1 expression was analyzed by qRT-PCR (Roche, Mannheim, Germany).

2.4. Cell culture and stromal vascular fraction preparation

2.4.1. hMADS cells culture

The establishment and characterization of hMADS cells have been described previously [49,50]. Cells were seeded at a density of 5000 cells/cm^2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS, 15 mM Hepes, 2.5 ng/ml hFGF2, 60 mg/ml penicillin, and 50 mg/ml streptomycin. hFGF2 was removed when cells reached confluence. Cells were induced to differentiate at day 2 post confluence (designated as day 0) in DMEM/Ham's F12 (1:1) media supplemented with 10 μg/ml transferrin, 10 nM insulin, 0.2 nM triiodothyronine, 1 μM dexamethasone and 500 μM isobutylmethylxanthine.

Media were changed every other day and cells used at day 18. miRNA mimic (miRIDIAN from Dharmacon), miRNA hairpin inhibitor (miRIDIAN microRNA Hairpin Inhibitors) or LNA inhibitor (miRCURY LNA™ microRNA Inhibitors Exiqon) transfections were performed at day 14 as described previously [39].

