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Fibroblast growth factor-21 is expressed in neonatal and pheochromocytoma-induced adult human brown adipose tissue

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ARTICLE INFO

Article history:
Received 12 August 2013
Accepted 19 November 2013

Keywords:
Obesity
Diabetes
Beige adipocyte
Brite adipocyte

ABSTRACT

Objective. In rodents, brown (BAT) and white (WAT) adipose tissues are targets and expression sites for fibroblast growth factor-21 (FGF21). In contrast, human WAT expresses negligible levels of FGF21. We examined FGF21 expression in human BAT samples, including the induced BAT found in adult patients with pheochromocytoma, and interscapular and visceral BAT from newborns.

Methods. The expression of FGF21 and uncoupling protein-1 (UCP1, a brown adipocyte marker), was determined by quantitative real-time-PCR and immunoblotting. The transcript levels of marker genes for developmentally-programmed BAT (zinc-finger-protein of the cerebellum-1, ZIC1) and inducible-BAT (cluster of differentiation-137, CD137) were also determined.

Results. FGF21 and UCP1 are significantly expressed in visceral adipose tissue from pheochromocytoma patients, but not in visceral fat from healthy individuals. In neonates, FGF21 and UCP1 are both expressed in visceral and interscapular fat, and their expression levels show a significant positive correlation. Marker gene expression profiles suggest that inducible BAT is present in visceral fat from pheochromocytoma patients and neonates, whereas developmentally-programmed BAT is present in neonatal interscapular fat.

Conclusions. Human BAT, but not WAT, expresses FGF21. The expression of FGF21 is especially high in inducible, also called beige/brite, neonatal BAT, but it is also found in the interscapular, developmentally-programmed, BAT of neonates.

Abbreviations: BAT, brown adipose tissue; CD137, cluster of differentiation 137; FGF21, fibroblast growth factor-21; UCP1, uncoupling protein-1; WAT, white adipose tissue; ZIC1, zinc finger protein of the cerebellum-1.

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http://dx.doi.org/10.1016/j.metabol.2013.11.014

1. Introduction

Fibroblast growth factor-21 (FGF21) is a hormonal factor that has systemic effects in promoting glucose uptake and oxidation [1]. The liver is the main site of FGF21 expression and production in rodents, but FGF21 is also expressed in white adipose tissue (WAT), where it may play an autocrine role [2], and in brown adipose tissue (BAT). In rodents, FGF21 targets BAT, where it induces mitochondrial uncoupling protein-1 (UCP1) gene expression, and favors glucose oxidation and energy expenditure [3]. Moreover, FGF21 promotes the "browning" of WAT; i.e. the appearance of brown adipocytes (also called "beige" or "brite" cells) in WAT depots [4]. Under thermogenic activation, FGF21 is highly expressed in BAT, which has been proposed to be source of FGF21 in this situation [5].

The liver is considered the main site of FGF21 expression in humans, and blood FGF21 levels and liver FGF21 expression have been correlated in some hepatic pathologies [6]. Muscle is also a potential source of FGF21 in patients with mitochondrial pathologies [7]. Unlike rodents, there is little or no expression of FGF21 in human WAT [6,8].

The presence of BAT has been traditionally recognized in human neonates, wherein the UCP1 content increases at the final trimester of gestation [9]. However, active BAT has also recently been found in adult humans [10]. BAT activity is reduced in obese individuals, and this has suggested, like in rodents, that BAT-mediated thermogenic energy expenditure may protect against obesity [10]. Some authors have recently claimed that BAT in adult humans arises largely via the inducible "browning" of WAT [11,12]. In fact, the capacity of adult humans to develop brown adipocytes has long been recognized, as brown adipocytes arise in WAT from pheochromocytoma patients due to the tumor-mediated release of catecholamines, which are known BAT-inducting agents [13,14]. However, recent data also claim for the presence of "classical" BAT in children and adult humans [15,16].

Considering the role of FGF21 in the thermogenic activation of BAT, the potential of BAT as a source of FGF21 in rodents, and the absence of FGF21 expression in human WAT, we herein investigated the expression of FGF21 in human BAT. We employed a unique collection of neonatal human BAT samples, as well as BAT samples from adult pheochromocytoma patients.

2. Methods

Samples of visceral (perirenal, omental) adipose tissue were obtained during surgical removal of the pheochromocytoma tumors from Caucasian adult patients (Azienda Ospedaliero-Universitaria, Ancona, Italy, 6 patients; Hospital del Mar, Barcelona, Spain, 3 patients; clinical details provided in Supplemental Table 1). Visceral (omental) WAT samples from 10 Caucasian healthy control individuals were obtained during cholecystectomies. Mean age (51.3 ± 12.2 year in pheochromocytoma patients versus 57.5 ± 5.0 year in healthy controls, P = 0.17) and body mass index (24.2 ± 5.4 in pheochromocytoma patients versus 24.9 ± 2.1 in healthy controls, P = 0.75) were not significantly different in patient's population relative to controls. Samples were frozen in liquid nitrogen until use for RNA and protein preparation. When possible, tissue sections were fixed and further processed for light and electron (JEOL-1010, Japan) microscopy.

Samples of adipose tissue from the interscapular and visceral (perirenal/retrorenal) areas were obtained from human newborns (mostly premature neonates) who died during 1995–2006 in the Czech Republic. Autopsies were performed 2–3 h after the death. Some of these patients were previously reported in studies on developmentally-regulated gene expression in other tissues [17,18]. Clinical details are provided in Supplemental Table 2.

After RNA isolation, 1 μg RNA was retro-transcribed and gene transcript levels were quantified by quantitative RT-PCR, using TaqMan reagents as described by the supplier (Applied Biosystems) along with specific TaqMan probes (Supplemental Table 3). Expression levels of gene transcripts were considered negligible when, under the above standard RT-PCR conditions, cycle threshold was ≥ 40. Data of specific mRNA abundance were expressed relative to 18S rRNA. The protein levels of UCP1 and FGF21 were assessed using 35 μg protein/lane, standard immunoblotting procedures, and antibodies against FGF21 (sc-16842, Santa Cruz, USA) and UCP1 (a specific anti-serum kindly provided by E.Rial, CSIC, Madrid). Where appropriate, statistical analyses were performed using the Mann-Whitney test or Kruskal-Wallis test with pairwise post-hoc analysis (Tukey adjustment). Spearman's coefficients of correlation were determined. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and it was approved by the committees of medical ethics at all of the collaborating institutions. Informed consent was obtained from all neonate parents and adult patients.

3. Results

3.1. *FGF21 is expressed in BAT of adult human pheochromocytoma patients*

We analyzed visceral adipose tissues from the perirenal and omental regions of pheochromocytoma patients. These samples, particularly those from the perirenal region, contained adipocytes with multilocular lipid droplets and enhanced mitochondrial abundance, which are characteristic of the brown adipocyte morphology (Fig. 1A). All samples from patients expressed detectable levels of UCP1 mRNA (median 9.3×10^{-06}, interquartile range (IR): 3.3×10^{-05}) the marker of the brown adipocyte cell identity relative to white adipocytes. In contrast, visceral adipose tissues from healthy adult controls showed little UCP1 (median 6.4×10^{-08}, IR: 1.2×10^{-07}. P = 0.0002 relative to pheochromocytoma patients), indicative of a main WAT phenotype. FGF21 mRNA was expressed at substantial levels in adipose samples from the pheochromocytoma patients (median 3.3 $x10^{-08}$, IR: 3.2×10^{-08}), whereas it was not detected in omental WAT from healthy controls (Fig. 1B, left). Subcutaneous abdominal or dorsal fat from healthy individuals did not show