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White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma ☆

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ABSTRACT

In all mammals, white adipose tissue (WAT) and brown adipose tissue (BAT) are found together in several fat depots, forming a multi-depot organ. Adrenergic stimulation induces an increase in BAT usually referred to as "browning". This phenomenon is important because of its potential use in curbing obesity and related disorders; thus, understanding its cellular mechanisms in humans may be useful for the development of new therapeutic strategies. Data in rodents have supported the direct transformation of white into brown adipocytes. Biopsies of pure white omental fat were collected from 12 patients affected by the catecholamine-secreting tumor pheochromocytoma (pheo-patients) and compared with biopsies from controls. Half of the omental fat samples from pheo-patients contained uncoupling protein 1 (UCP1)-immunoreactive-(ir) multilocular cells that were often arranged in a BAT-like pattern endowed with noradrenergic fibers and dense capillary network. Many UCP1-ir adipocytes showed the characteristic morphology of paucilocular cells, which we have been described as cytological marker of transdifferentiation. Electron microscopy showed increased mitochondrial density in multi- and paucilocular cells and disclosed the presence of perivascular brown adipocyte precursors. Brown fat genes, such as UCP1, PR domain containing 16 (PRDM16) and β3-adrenoreceptor, were highly expressed in the omentum of pheo-patients and in those cases without visible morphologic re-arrangement. Of note, the brown determinant PRDM16 was detected by immunohistochemistry only in nuclei of multi- and paucilocular adipocytes. Quantitative electron microscopy and immunohistochemistry for Ki67 suggest an unlikely contribution of proliferative events to the phenomenon. The data support the idea that, in adult humans, white adipocytes of pure white fat that are subjected to adrenergic stimulation are able to undergo a process of direct transformation into brown adipocytes. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

1. Introduction

In small mammals and to some extent also in humans, white adipose tissue (WAT) and brown adipose tissue (BAT) are found together in several fat depots located in subcutaneous and visceral areas. These can be regarded as one multi-depot organ with distinct morphology and specific functions, and all of the depots exhibit extremely plastic properties [1].

The two main types of cell forming the adipose organ are white and brown adipocytes, which fulfill the opposing functions of storing lipids for survival and consuming lipids to produce heat, respectively. Thermogenesis in BAT is mediated by a brown fat-specific mitochondrial protein, uncoupling protein 1 (UCP1), which plays an important role in the control of energy homeostasis. Indeed, the loss of UCP1 causes cold-intolerance [2] and obesity at thermoneutrality in mice [3]. The transgenic expression of UCP1 in WAT has been shown to combat obesity [4], whereas BAT removal or inactivation has been shown to cause obesity and related disorders [5,6]. Cold- and diet-induced thermogenesis are dependent on the ß-adrenergically mediated activation of lipolysis and the subsequent degradation of fatty acids via UCP1 that dissipates large amounts of chemical energy [7].

Recent studies using non-invasive imaging technologies, such as 18fluorolabeled 2-deoxy-glucose positron emission tomography (18FDG-PET), have clearly demonstrated that adult humans have significant amounts of active BAT in the area at the base of the neck and cold exposure has revealed that this tissue can be activated [8–12]. In a case series of adult humans, a histological study demonstrated the presence of true brown adipocytes in the same anatomical area and

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identified brown fat precursors [13]. Furthermore, the glucose uptake capacity in BAT correlates inversely with adiposity, indicating that variation in the amount and/or thermogenic activity of BAT may contribute to the propensity for weight gain in humans [14].

The mechanisms by which WAT acquires BAT-like properties are generally referred as "browning" and have been proven to be of great importance in protecting against diet-induced obesity and metabolic diseases [15,16]. Browning occurs especially in the subcutaneous depots of mice that over-express PR domain containing 16 (PRDM16) [17] which is the key transcriptional regulator of the cellular lineage that gives rise to the classic brown adipose depots in mice [18].

In addition, UCP1-immunoreactive (ir), brown fat-like cells can emerge in predominantly "white" depots upon prolonged cold exposure or β3-adrenoreceptor (ADRB3) activation [19–22]. These brown-like fat cells, which are not derived from a Myf5$^+$ lineage, are designated as brown-in-white (brite) cells [23], beige cells [24,25] or simply brown adipocytes, mainly because they have anatomy and physiology corresponding to that of other brown adipocytes found in specific areas of the adipose organ considered as the "classic" brown depot [26,27].

The propensity to accumulate brown adipocytes differs in individual WAT depots of rodents and seems to be dependent on different innervations of the depot [27] or different cell subtype compositions [28]. A large accumulation of brown adipocytes in the adipose organ during cold exposure can be found most readily in the subcutaneous inguinal adipose tissue but is rarely observed in epididymal/perigonadal adipose tissue [17,27,29]. We have recently described UCP1-ir paucilocular as a cell marker of the intermediate steps in the process of white-to-brown adipocyte transdifferentiation in mice subjected to adrenergic stimulation [22].

Based on classic [30] and more recent studies [31], the presence of active BAT in patients affected by pheochromocytoma (pheo-patients), which is a neuroendocrine tumor that intermittently secretes excessive amounts of catecholamines, is known to be enhanced. It has also been documented by FDG-PET that patients with this type of tumor showed higher levels of active BAT in several locations of the body; this situation reverted to normal after tumor resection [32,33].

Because browning the human adipose organ may be a strategy to combat obesity and related disorders [34], it is important to investigate whether human white adipocytes can be converted into brown adipocytes and to determine the cell processes involved. Visceral fat deposits, especially mesenteric and omental fat, are generally considered "pure" white fat depots with little propensity to acquire a brown phenotype, even in rodents [27,29]. Thus, we selected these pure white depots in patients suffering from pheochromocytomas to investigate whether white-to-brown transdifferentiation can occur in this unique model of adrenergic hyper-stimulated human fat. In half of the samples analyzed, we observed all steps of the striking morphological changes in a typical transformation of WAT into BAT, including the presence of paucilocular adipocytes, an insignificant increase of adipocyte precursors and the re-organization of vascular and nerve supplies. These data, as well as data from gene expression analysis, strongly suggest that the transdifferentiation of white-to-brown adipocytes is the major phenomenon driving this transformation of omental fat in pheo-patients.

2. Material and methods

2.1. Subjects

Omental adipose tissue was removed from 20 control subjects undergoing surgery at the General Hospital Azienda Ospedaliero Universitaria (AOU; Ancona, Italy) for cholecystectomy. The subjects comprised 10 males and 10 females, aged from 32 to 65 years (average 46) with body mass index (BMI) from 18 to 29. The patients affected by adrenal pheochromocytoma included in this study totaled 12: 6 males and 6 females, ranging in age from 29 to 70 years (average 49) and in BMI, from 19 to 30.5. All of them underwent surgery for total resection of the tumor, and samples of omental fat were collected in comparable areas. The clinical details of the subjects are summarized in Table 1.

At the time of the surgery, all subjects had fasted for 15–18 h and had stopped all pharmacological treatment the day before. Informed consent was obtained from the subjects before the surgical procedure. The study protocol was approved by the Ethical Committee (Permission n. 209148) of the University of Ancona (Politecnica delle Marche).

2.2. Light microscopy (LM)

Immediately after removal, tissue pieces representing omental adipose tissue were fixed overnight by immersion at 4 °C in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The samples were then dehydrated, cleared, and embedded in paraffin. Sections from 3 different levels (100 μm apart) were hematoxylin and eosin-stained to assess their morphology, immunohistochemistry and morphometry. All observations were performed with a Nikon Eclipse 80i light microscope (Nikon, Tokyo, Japan) equipped with a CCD camera.

2.3. Immunohistochemistry (IHC)

UCP1, tyrosine hydroxylase (TH) and Ki67 (a cellular marker for proliferation) immunostaining were performed as follows. For each 3-μm-thick section level, dewaxed sections were incubated with anti-UCP1 (1:500; ab10983, Abcam, Cambridge UK), anti-TH (1:300; AB1542 Chemicon Millipore, Milan, Italy) or anti-Ki67 (1:3000; NCL-Ki67p, Novocastra Lab, Newcastle, UK), according to the avidin–biotin complex (ABC) method, briefly 1) endogenous peroxidase blocking with 3% hydrogen peroxide in methanol; 2) normal serum (1:75) for 20 min to reduce nonspecific background; 3) incubation with primary antibodies against UCP1, TH or Ki67 overnight at 4 °C; 4) secondary antibodies specific for each species in which the primary antibody was raised: IgG biotin conjugated (1:200; Vector Labs, Burlingame, CA, USA); 5) ABC kit (Vector Labs); and 6) enzymatic reaction to reveal peroxidase with Sigma Fast 3,3′-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) as the substrate. Finally, sections were counterstained with hematoxylin and mounted in Eukitt (Fluka, Deisenhofen, Germany).

IHC for PRDM16 (1:150; ab3789, Abcam, Cambridge UK) was performed with the same technique described above, and an antigen retrieval step using sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) was added before blocking with normal serum.

2.4. Morphometry

The 3-μm-thick sections stained were measured by live count command in Lucia Imaging for image analysis (version 4.82, Nikon Instruments, Florence, Italy).

The area of the TH-ir nerve fibers was measured in the 4 subjects showing at least 10–15% UCP1-ir adipocytes, based on images randomly captured with a Nikon DXM 1200 camera at ×100. Ten areas in the adipose lobules containing mixed brown/white adipocytes (pheo-patients) and 10 areas of pure white adipocytes (controls) were analyzed. The results are given as the total TH-ir area. The vascular area was measured in semi-thin sections from the same cases using images captured at 40× objective. The results are given as the total vascular area/mm^2.

Quantitative data on the density of precursor cells (preadipocytes) were produced as the number of cells per 100 capillaries.