Hot Cells, Raise Your Hands

Heejin Jun¹ and Jun Wu¹*

Obesity is a commonly used predictor for many metabolic diseases and is closely linked to type 2 diabetes and cardiovascular diseases. Essentially a disorder of energy balance, obesity occurs when energy intake and storage exceed expenditure. Much of the energy homeostasis depends on the activity and function of adipose tissue. White adipose tissue (WAT)² primarily functions to store energy and subsequently secrete hormones such as leptin in response to nutritional signals. In contrast, thermogenic adipose tissue defends against hyperthermia and obesity through adaptive thermogenesis mediated by the regulated expression and activity of mitochondrial uncoupling protein 1 (UCP1). There are 2 types of thermogenic adipocytes identified in mammals to date: brown fat and beige (brite) fat. Unlike brown fat cells that form distinct anatomical depots, newly identified and isolated beige fat cells, which are interspersed within white adipose tissues, arise from a different developmental lineage from the brown fat cells and have dual functions regulating both energy storage and energy dissipation (1).

Whereas the functional involvements of white adipocytes in human metabolic diseases have been studied for decades, how thermogenic fat cells may influence whole body metabolism in human remains elusive. Although it was widely accepted that thermogenic adipocytes are present in human infants, until 5 years ago few endocrinologists deemed healthy human adults to have these “energy-consuming” fat cells. The “rediscovery” of thermogenic fat cells in human adults ignited the enthusiasm of exploring the role of these cells in obesity and metabolism in general (2). However, concerns have been raised regarding whether the counteracting-obesity potential of these cells is restricted, because of the limited amount of these cells present in average individuals.

The current standard approach measuring thermogenic fat activity in humans is through positron emission tomography–computed tomography (PET/CT). Because this method scans for radioactively labeled glucose uptake by fat cells, PET/CT reveals only the activated but not the total deposit of thermogenic fat cells in the body. It is speculated that the amount of thermogenic fat cells in humans is considerably underestimated owing to the limitation of current measurement procedures.

In a recent study published in Science Translational Medicine, Kahn and colleagues reported cell surface markers that can distinguish both white adipocytes and the “metabolic-beneficial” brown or beige fat cells (3). The authors used computational approaches to identify molecules with enriched expression in white adipose tissues and brown adipose tissues using transcription-profiling data. Cell surface markers whose expression correlated with either adiponectin (as an adipocyte marker) or Ucp1 (as the thermogenic fat marker) were selected for further validations. The expression levels of these candidate markers were tested in multiple tissues, and the ones exclusively expressed in fat tissues were evaluated further. ASC-1 (neutral amino acid transporter, y+ system, also called solute carrier family 7 member 10, slc7a10) was identified as a white fat–specific cell surface marker. PAT-2 (proton/amino acid symporter member 2, also called solute carrier family 36 member 2, slc36a2), and P2RX5 (purinergic receptor P2X, ligand-gated ion channel 5) were specific for thermogenic adipocytes (Fig. 1).

These markers were expressed at increasing levels as the in vitro adipogenesis process proceeded and primary stromal vascular fractions differentiated into fat cells. The expression levels of these markers were also regulated in vivo. When mice were subjected to either cold exposure or sympathetic stimulation by a β-adrenergic agonist, mRNA levels of Slc36a2³ [solute carrier family 36 (proton/amino acid symporter), member 2; also known as PAT2] and P2rx5 (purinergic receptor P2X, ligand-gated ion channel, 5) were increased in both interscapular and subcutaneous fat depots, where thermogenic fat cells are most prominent. This observation was consistent with the notion that these markers correlate with the abundance of thermogenic fat cells. Conversely, these brown/beige fat markers were downregulated in the in-
interscapular depot of db/db mice, which harbor a spontaneous mutation of leptin receptor, and these mice become obese at 3 or 4 weeks of age. It is not yet fully understood how tissue remodeling is carried out in thermogenic fat depots as mice become obese. It is likely that the phenotypic switch is caused by several mechanisms: (a) inhibition of progenitor proliferation and differentiation into additional thermogenic fat cells; (b) regulated cell death of existing thermogenic fat cells; and (c) remaining brown and beige fat cells taking on a more “white fat–like” morphology, with reduced mitochondrial contents and enlarged lipid droplets. Further understanding of how PAT-2 and P2RX5 are involved in the fate commitment or function regulation of thermogenic fat cells will help to interpret the regulated expression pattern of these markers in the interscapular and subcutaneous fat depots.

The most exciting part of the study comes from the analysis of these fat-type–specific markers in human adipose tissues. The first reports in 2009 and many follow-up studies have shown that metabolic active thermogenic fat cells are present in healthy human adults, particularly in the neck area (4). Molecular identity/identities of these human UCP1+ fat cells is of great interest and has been the focus of many reports and ongoing studies. Short of genetic lineage tracing evidences, these human studies have been dependent on use of molecular markers; some of them have been classified as cell-identity markers and others as functional markers. Beige fat cells have been identified and isolated in mice only very recently (1). Besides flexibility in “inducible” thermogenic capacities and a distinct developmental origin, how beige fat cells differ from brown fat cells remains to be answered. As more experimental evidence emerges and we better understand the unique functions of beige fat cells, more tools and assays will become available to definitively answer the question whether human thermogenic fat cells are brown or beige. Meanwhile, in this study (3) the markers identified from mice were confirmed in human fat cells. The brown/beige markers P2RX5 and PAT2 were detected in the carotid sheath and longus colli areas around the neck, where thermogenic fat cells are present, whereas the white fat cell marker ASC-1 was much more prominent in the subcutaneous and omental fat depots, both of which predominantly consist of white adipocytes.

These cell-type–specific, membrane-bound molecules provide a powerful tool for many future studies. Fluorescence-activated cell-sorting techniques have been optimized for sorting mature fat cells with satisfactory degrees of success in overcoming the fragility caused by intracellular lipid droplets in adipocytes. With these cell-surface markers (all have commercially available antibodies), it is conceivable that novel procedures can be designed to assay the total amount of thermogenic fat cells instead of only the highly activated ones that uptake huge amounts of oxygen.

**Fig. 1. Three types of fat cells and their therapeutic potential in humans.**

White fat cells are responsible for energy storage, whereas multilocular brown/beige cells dissipate energy in the form of heat using mitochondrial UCP1. They are distinguishable by recently identified cell surface markers, ASC-1, P2RX5, and PAT2. ASC-1 marks the surface of white fat cells, whereas P2RX5 and PAT2 are specific for brown/beige fat cells, of which P2RX5 is more specific for brown fat. Various adipokines and metabolites secreted by these adipocytes communicate with other metabolic organs and contribute to maintain metabolic homeostasis.
amounts of labeled glucose above the detection threshold of a PET/CT scan. More importantly, now that thermogenic fat cells from human fat biopsies can be separated and enriched, primary cultures of these cells along with white fat control cells from the same individual will help us uncover much-awaited information on human thermogenic fat cell regulation and function. Identification of unique thermogenic fat adipokines or secreted metabolites in humans will potentially lead to a much more convenient assay (molecules in the circulation) for accurate diagnosis. Many metabolic diseases are polygenic and multifactorial, demanding therapeutic approaches and treatment plans specifically tailored for each individual patient’s genetic makeup and lifestyle. With improved diagnostic tools, lack of thermogenic fat cells could be included as a potential risk factor for obesity and metabolic syndromes.

It has recently been reported that activated thermogenic fat cells can improve whole body glucose metabolism and insulin sensitivity in humans (5). These data attest to the physiological significance of thermogenic fat cells in metabolism, but it is not yet known how brown/beige fat regulated metabolic benefits are achieved. Chemical energy is converted into heat, and hence the reduced adiposity certainly could play a role in long-term metabolic maintenance. It remains to be determined whether different portfolios of secreted molecules from activated brown/beige fat cells directly change the information flow between various metabolic organs. Perhaps activated brown/beige fat cells communicate with skeletal muscle (where 70% glucose uptake happens in the body), liver, or brain, to acutely and intimately participate in metabolic control in real time. This study by Ussar et al. may very well provide a tool to isolate human primary thermogenic fat cells and help us to answer these interesting questions.

Many questions remain unanswered. Detailed understanding of developmental lineages of different types of fat cells will most likely be achieved through studies with model organisms. However, given it is evident that not all the regulation of thermogenic fat is conserved between rodents and humans, e.g., the interscapular brown fat depot is present and functional in adult rodents but only in human infants, it is essential for researchers in this field to investigate both systems.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: None declared.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health grant K01DK094824, National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health grant R03DK100698, and Young Investigator Grant RGY0082/2014 from the Human Frontier Science Program.

Expert Testimony: None declared.

Patents: None declared.

References