Cellular heterogeneity in brown adipose tissue

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Brown adipose tissue (BAT) contains mitochondria-enriched thermogenic fat cells (brown adipocytes) that play a crucial role in the regulation of energy metabolism and systemic glucose homeostasis. It was presumed that brown adipocytes are composed of a homogeneous cell population. In this issue of the *JCI*, however, Song and colleagues report a previously uncharacterized subpopulation of brown adipocytes that display distinct characteristics from the conventional brown adipocytes in their molecular signature, regulation, and fuel utilization. The present study provides novel insight into our understanding of cellular heterogeneity in adipose tissues.

Roles of brown adipose tissue

Brown adipose tissue (BAT) contains numerous brown adipocytes with highly enriched mitochondria and multilocular lipid droplets, and possesses the remarkable capacity to dissipate energy in the form of heat. Thus, BAT has been viewed merely as a thermogenic organ for a long time; however, emerging evidence suggests that the role of BAT is far more than thermogenesis (1). As an example, BAT functions as a significant metabolic sink for glucose, fatty acids, and branched-chain amino acids (BCAAs), such that activation of BAT leads to a profound improvement in systemic glucose homeostasis, lipid homeostasis, and BCAA clearance in rodents and humans (2–4). Accordingly, BAT biology has become a significant area of research in the field of metabolic disorders beyond obesity because a better understanding of this organ may lead to new interventions to improve metabolic health, such as insulin resistance, dyslipidemia, and type 2 diabetes (5).

Adipose cell heterogeneity – more than brown, beige, and white

The adipose tissue was once thought to be a monolithic organ that was composed of homogeneous adipose cell populations. However, it is becoming clear that each adipose tissue depot contains a variety of fat cells, including brown adipocytes, beige adipocytes, and white adipocytes, that arise from distinct developmental origins (6, 7). Even within an adipose tissue depot, recent studies indicate the existence of heterogeneous cell populations. For instance, Chen et al. recently described a previously uncharacterized type of beige adipocyte whose biogenesis is regulated independently from the canonical β3-adrenergic receptor (β3-AR) signaling (8). This beige fat population stems from a distinct pool of progenitor cells and possesses a unique fuel utilization (e.g., glycolytic metabolism as opposed to fatty acid oxidation), and thus, these cells are termed glycolytic beige fat (or g-beige fat). Relative to beige adipocytes, brown adipocytes in the interscapular BAT region were thought to be homogeneous, although Cinti's group has previously reported that BAT contained two types of brown adipocytes that expressed high or low levels of uncoupling protein 1 (UCP1) following cold exposure or administration of a β3-AR agonist (CL316,243) (9). Owing to the recent advance in the single-cell RNA sequencing (scRNA-seq) technology and inducible lineage-tracing methods, the field has gained a momentum to better understand the cellular heterogeneity in adipose tissues.

In this issue of the *JCI*, Song et al. set out to characterize the cellular heterogeneity in the interscapular BAT (10). First, the authors employed the Adipo-Chaser system, an inducible animal model in which all the differentiated adiponectin-expressing (*Adipoq*-expressing) adipocytes were permanently labeled as LacZ$^+$ cells following doxycycline administration (10, 11). The authors found that LacZ$^+$ brown adipocytes (*Adipoq* high-expressing cells) constituted 38% of total brown adipocytes in the BAT at room temperature (24°C), whereas only 6% were LacZ$^+$ cells under a thermoneutral condition (30°C). Notably, cold exposure robustly increased the number of LacZ$^+$ cells (76% of total brown adipocytes at 6°C), although some cells remained LacZ$^-$ (*Adipoq* low-expressing brown adipocytes). Further analyses by transmission electron microscopy showed that these LacZ$^+$ and LacZ$^-$ brown adipocytes were morphologically distinct in their lipid droplet size and mitochondrial contents (Figure 1). The authors next investigated whether the differences in the percentage of LacZ$^+$ cells involve de novo adipogenesis or cell death. When mature adipocytes were derived from prelabeled mice that were maintained at 24°C then subsequently pulse-chased at 6°C or 30°C, the percentages of LacZ$^+$ brown adipocytes were comparable with mice that were consistently held at 24°C. Also, apoptosis signaling remained unchanged. These data suggest that there are two types of brown adipocytes, such that adiponectin-high and adiponectin-low cells in the BAT are interconvertible without cell death following acclimation to cold or thermoneutral conditions (10).

Brown adipocytes with high versus low thermogenic capacities

Is there any functional difference between the two forms of brown fat cells? To address

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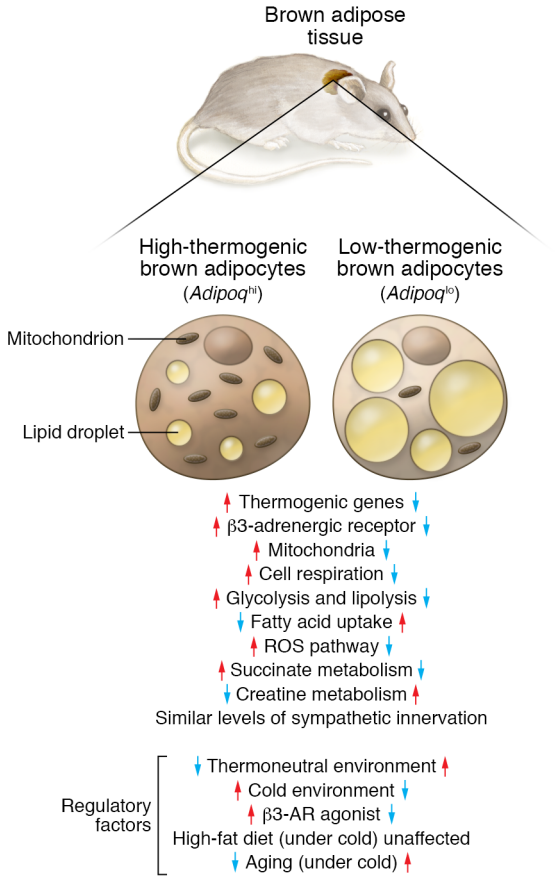

Figure 1. Two types of brown adipocytes in BAT. A population of brown adipocytes with low thermogenic activity and adiponectin expression coexists with brown adipocytes with high thermogenic activity and adiponectin expression.

this question, Song et al. performed scRNA-seq in primary brown adipocytes and identified 2352 *Adipoq* high-expressing cells and 1250 *Adipoq* low-expressing cells. In the *Adipoq* low-expressing cells, the authors found lower mRNA expression of genes related to oxidative phosphorylation, β3-adrenergic receptor, lipolysis, glycolysis, fatty acid oxidation, and the TCA cycle relative to those of their high-expressing counterparts. Similarly, the *Adipoq* low-expressing brown adipocytes expressed lower levels of genes involved in thermogenesis, reactive oxygen species (ROS), and succinate metabolism, as compared with the *Adipoq* high-expressing cells. On the other hand, the *Adipoq* low-expressing cells expressed higher levels of genes related to fatty acid uptake and UCP1-independent creatine futile cycle (12). Intriguingly, *Adipoq* high- and low-expressing cells disparately express two well-characterized master transcriptional regulators, *Cebpa* and *Pparg*, respectively, suggesting their roles in the transcriptional regulation of each cell type. Importantly, cell respiration was higher in *Adipoq* high-expressing cells relative to that in low-expressing cells. These data collectively suggest that the two populations of brown adipocytes have different cellular functions and metabolic profiles in that *Adipoq* high-expressing cells possess high thermogenic capacity while *Adipoq* low-expressing cells have low thermogenic capacity (10).

Aging affects the cellular composition in BAT

Given these distinct molecular features, the authors next aimed to address their regulatory pathways. Since BAT activity declines with obesity and aging (13, 14), the authors examined changes in the cellular composition of BAT by counting LacZ$^+$ cells (*Adipoq* high-expressing cells) under a high-fat diet condition and in aged mice. The authors found that high-fat diet feeding failed to impair the cold-induced recruitment of *Adipoq* high-expressing brown adipocytes. On the other hand, the number of *Adipoq* high-expressing brown adipocytes declined gradually in aged mice (below 40% in 30-week-old and 20% in 60-week-old mice) relative to young mice (approximately 70% in 10-week-old mice). These results indicate that the ability of BAT to recruit *Adipoq* high-expressing cells following cold stimuli declines with age (10).

In conclusion, the present study by Song et al. elegantly demonstrates the existence of two subpopulations of brown adipocytes that possess unique molecular and metabolic features (10). Do they arise from distinct progenitor pools? While embryonic brown adipocytes in the interscapular BAT arise from a *Myf5*$^+$ myogenic lineage (15), it remains unknown whether *Myf5*$^+$ cells persist as a progenitor source for *Adipoq* high-expressing and/or low-expressing brown adipocytes in adult BAT. Another key question is: what are the mechanisms by which aging alters the cellular composition of BAT? A recent study reported that mitochondrial lipoylation in the BAT declines with age-associated impairment in thermogenesis and fuel oxidation. Of note, supplementation of α-lipoic acids effectively restored BAT function, including thermogenesis and glucose oxidation, in aged mice (14). Thus, it would be interesting to test how α-lipoic acid supplementation affects the cellular composition of *Adipoq* high- and low-expressing cells in aged BAT. A better understanding of the molecular mechanisms that underlie age-associated changes in BAT may lead to effective prevention or therapeutic measures for age-associated metabolic disorders, including obesity, insulin resistance, and type 2 diabetes.

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