Review

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Formation and activation of thermogenic fat

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Thermogenic fat cells that convert chemical energy into heat are present in both mice and humans. Recent years have witnessed great advances in our understanding of the regulation of these adipocytes and an increased appreciation of the potential these cells have to counteract obesity. We summarize recent efforts to understand the formation of these fat cells and critically review genetic models and other experimental tools currently available to further investigate the development and activation of both classical brown and inducible beige fat cells. We also discuss recent discoveries about the epigenetic regulation of these adipocytes, and finally present emerging evidence revealing the metabolic impacts of thermogenic fat in humans.

Fighting fat with fat

Obesity affects one in three persons globally and constitutes an increasing burden on healthcare systems and an urgent challenge for the biomedical research community [1,2]. Effective prevention and treatment of obesity may drastically reduce the occurrence of comorbidities such as type 2 diabetes, cardiovascular disease, and other serious health problems, including many types of cancer. As a multifactorial disorder, obesity can be prevented through combinations of approaches that target different aspects of metabolism to decrease energy surplus. Much of energy homeostasis depends on the activity and function of adipose tissue. Two major types of adipocytes exist in mammals, white fat and thermogenic fat. The primary function of white adipose cells is to store energy and subsequently secrete hormones in response to nutritional signals. By contrast, thermogenic adipocytes defend against hypothermia and obesity through adaptive thermogenesis mediated by regulated expression and activity of mitochondrial uncoupling protein 1 (UCP1). Thermogenic adipocytes have also been classified into so-called 'classical' brown fat cells and newly identified beige adipocytes (discussed below).

It was long assumed that thermogenic fat was only present in humans at the infant stage. In 2009, however, thermogenic adipocytes were shown to exist in human adults [3–5], drawing intense interest as a potential target to increase energy expenditure and counteract obesity. These research efforts have led to significant advances in our knowledge of these cells [6–8]. It is becoming increasingly evident that thermogenic fat significantly influences whole-body metabolism in humans. To leverage the full potential of the metabolic benefits of these cells, it is essential to thoroughly understand the developmental history and distinct regulatory mechanisms of different types of thermogenic fat. In this review we discuss our current view of how these fat cells are regulated, particularly focusing on the distinguishing features of developmental formation and environmentally stimulated activation of thermogenic fat.

Thermogenic fat formation

The early developmental origins of fat remains an important question that is currently being intensively investigated [9,10]. It has been proposed that adipocytes arise from endothelial [11] or hematopoietic lineages [12,13]. Until recently, brown fat observed in the rodent interscapular depot and human infants was widely believed to share a common developmental origin with the rest of the fat cells throughout the body. These brown fat cells have high mitochondrial content, the iron in which gives them their eponymous color. A unique mitochondrial protein, UCP1, functions as a proton leak which effectively 'uncouples' ATP synthesis and oxidative phosphorylation through the electron transport chain (ETC). The inherent inefficiency of the biochemical reactions leads to the conversion of electrochemical energy into heat, such that mammals (small mammals in particular) can defend against hypothermia [14]. Despite these unique features, thermogenic fat cells share many characteristics with their white fat counterparts. They normally express most of the adipocyte-specific markers at comparable levels to white fat, including peroxisome proliferator-activated receptor γ (PPARG), adiponectin, and fatty acid binding protein 4 (FABP4, also referred as aP2). Both white and thermogenic fat cells contain intracellular lipid droplets, albeit unilocular morphology (one lipid droplet) is normally observed in white adipocytes and multilocular (many lipid droplets) in thermogenic fat.

Despite these common characteristics, however, the notion of a common developmental origin for all fat cells was disproved in 2008. After the discovery that PRD1-BF-RIZ1 homologous domain containing 16 (PRDM16) is a key regulator of the thermogenic program in brown fat [15], loss-of-function assays of PRDM16 were conducted in primary brown fat cells. These shPRDM16 brown preadipocytes differentiated into muscle-like cells instead of the expected white fat cells [16]. Cell fate-mapping

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experiments showed that some UCP1$^+$ fat cells from several depots, most notably the interscapular depot, arise from a *Myf5*$^+$ lineage which also gives rise to skeletal muscle [16]. This discovery, together with the rediscovery of thermogenic fat cells in human adults [3–5], which was reported one year later, significantly changed how we view thermogenic fat and its potential role in metabolism.

Not all UCP1$^+$ fat cells come from the *Myf5*$^+$ lineage, however [16]. Upon cold-exposure, UCP1$^+$ multilocular cells are detectable in many white adipose depots, most prominently in subcutaneous depots such as the inguinal depot in mice [14]. These 'inducible' thermogenic fat cells, together with white fat cells, come from one or more *Myf5*$^-$ lineage(s) [16]. To investigate the developmental origin and molecular identity of these *Myf5*$^-$ lineage-derived UCP1$^+$ adipocytes, clonal stable cell lines were generated from the subcutaneous depot, and unbiased analysis of transcriptional profiling revealed that a subset of these cell lines are functionally more similar to classical brown fat than to the rest of the lines from the subcutaneous depot. This provides direct evidence that these 'inducible' thermogenic fat cells (so-called beige fat cells) may be fundamentally dissimilar from the other fat cells of the inguinal depot, even at the precursor stage (Figure 1) [17]. The exact developmental lineage of this new type of fat cell is under intensive investigation. Using a ribosome-profiling approach, it was shown that an enriched expression of a smooth muscle gene signature is present in beige fat cells but not in brown fat cells. Cell fate-mapping experiments with a *Myh11*-driver (a smooth muscle marker) revealed that at least a subset of beige fat cells arise from a shared lineage with smooth muscle [18]. Other studies identified so-called 'brite' fat (brown in white), a distinguishable subpopulation of adipocytes from the visceral depot expressing UCP1 upon rosiglitazone treatment [19]. Rosiglitazone is a commonly used thiazolidinedione (TZD), a PPARγ agonist, which has been shown to induce activation of thermogenic gene expression in adipose tissue and cells [20–24].

The discovery of a common lineage of skeletal muscle and brown fat has drawn much attention and has been confirmed by many groups in various cell fate-mapping models with different skeletal muscle markers. Experiments with an inducible *Pax7* tracing model revealed that the cell fate-diverging decision to become either brown fat or skeletal muscle takes place between embryonic (E) days E9.5 and E11.5 during gestation [25]. Detailed mapping analysis of multiple fat depots using *Myf5* and several other skeletal muscle-specific genes (*MyoD and Pax3)* confirmed the earlier discovery that UCP1$^+$ cells in the interscapular fat depot are from the *Myf5*$^+$ lineage, and that UCP1$^+$ cells in the perigonadal (visceral) and posterior-subcutaneous (inguinal) depots are from a *Myf5*$^-$ lineage [26]. Additional analysis in the same study revealed that certain depots (e.g., the cervical depot) contain UCP1$^+$ adipocytes of both *Myf5*$^+$ and *Myf5*$^-$ lineages [26]. It has

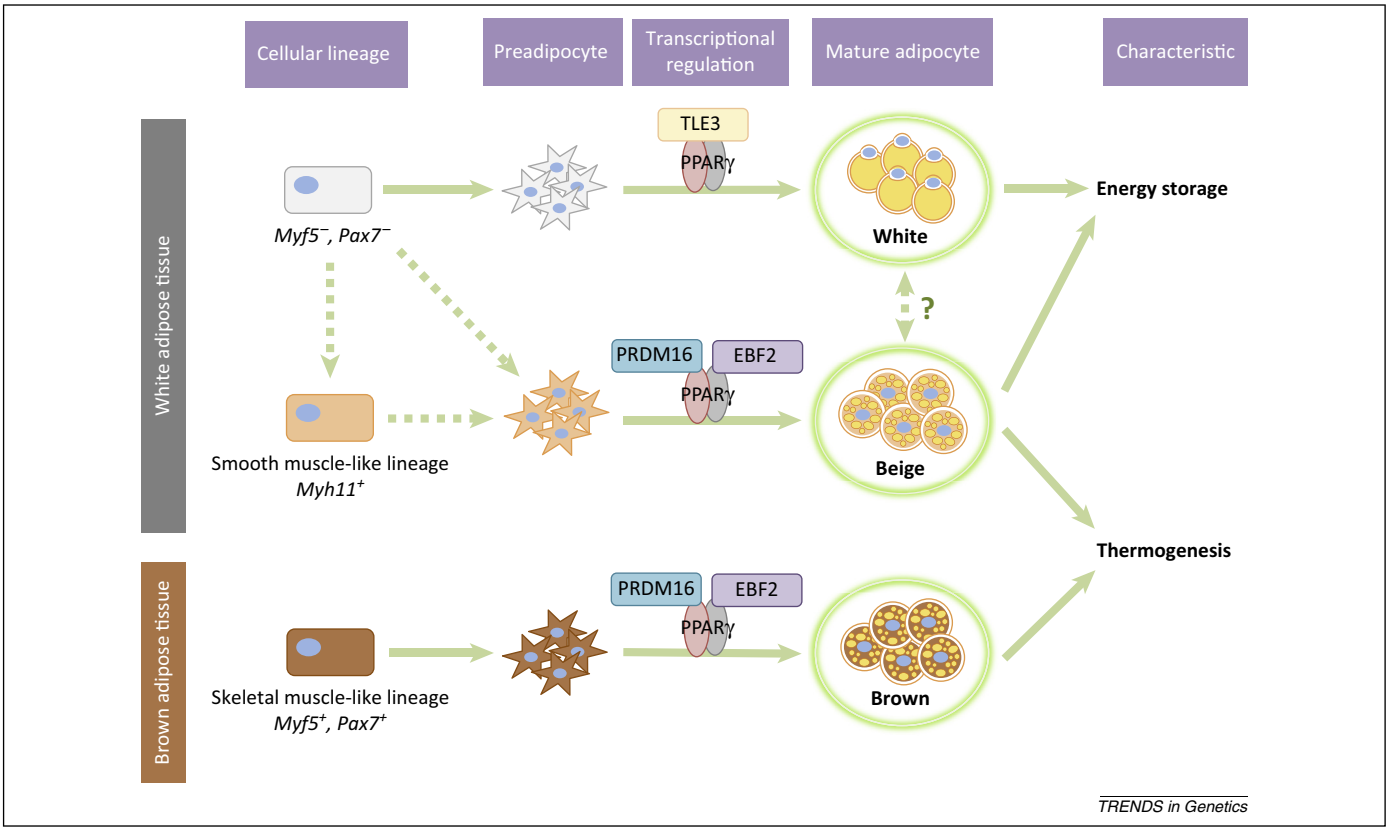

Figure 1. Development of three types of fat cells. White, beige, and brown adipocytes are derived from different lineages and subpopulations of precursors. Brown adipocytes come from *Myf5*$^+$/*Pax7*$^+$ lineages, whereas white and beige adipocytes arise from *Myf5*$^-$/*Pax7*$^-$ lineages. Recently, *Myh11*-expressing progenitors have been newly identified as a population giving rise to at least some of the 'inducible' thermogenic beige adipocytes. PPARγ coordinates the adipogenesis of three types of fat cells through interactions with different transcriptional coregulators. TLE3 functions as a coactivator of PPARγ during white adipogenesis [70]. Recruitment of PRDM16 and EBF2 to PPARγ leads cells to differentiate into thermogenic beige and brown adipocytes.