Brown and beige fat: Physiological roles beyond heat-generation

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Abstract

Since brown adipose tissue (BAT) dissipates energy through UCP1, BAT has garnered attention as a therapeutic intervention for obesity and metabolic diseases including type2 diabetes. As we better understand the physiological roles of classical brown and beige adipocytes, it is becoming clear that BAT is not simply a heat-generating organ. Increased beige fat mass in response to a variety of external/internal cues is associated with significant improvements in glucose and lipid homeostasis that may not be entirely mediated by UCP1. We aim to discuss recent insights regarding the developmental lineages, molecular regulation, and new functions for brown and beige adipocytes.

Keywords

Brown adipose tissue; brown adipocytes; beige adipocytes; obesity; diabetes; insulin resistance

Introduction

Thermogenic adipocytes and Ucp1

Brown and beige adipose cells have the capacity to burn glucose and fat to produce heat. This thermogenic function is mediated, in large part, by the action of Uncoupling Protein-1 (UCP1), a polypeptide that resides in the inner mitochondrial membrane of brown and beige adipocytes. UCP1, when activated, dissipates the proton gradient generated by the electron transport chain. This futile cycle of proton pump and leak reduces mitochondrial membrane potential which, in turn, drives high levels of substrate oxidation and results in the generation of heat (Cannon and Nedergaard, 2004; Lowell and Spiegelman, 2000).

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Brown adipose cells develop in dedicated deposits of brown adipose tissue (BAT). In mice, the large BAT depots, including the interscapular, axillary and cervical BAT develop prenatally and provide a source of heat to protect newborns against cold-exposure. The sympathetic nervous system (SNS) is intimately involved in regulating both the growth of BAT and its thermogenic function. Brown adipocytes are innervated by sympathetic fibers, which upon cold-exposure, release norepinephrine (NE) to acutely activate thermogenesis (Cannon and Nedergaard, 2004). NE also elicits a signaling cascade via P38 MAPK and PGC-1α to increase the transcription of thermogenic genes in brown adipocytes; this allows for sustained thermogenesis during longer term cold exposure (Cao et al., 2004). Finally, cold exposure stimulates BAT expansion through activating the proliferation and differentiation of brown adipose precursor cells (Bronnikov et al., 1992; Geloen et al., 1988). Another notable feature of BAT is its dense capillary bed that supplies adipocytes with substrate and oxygen for oxidation and enables the efficient distribution of heat to the rest of the body.

Brown-like adipocytes that have a multilocular morphology and express UCP1 can also be found in white adipose tissue (WAT) depots (Cinti, 1999; Collins et al., 1997; Guerra et al., 1998; Himms-Hagen et al., 2000; Vitali et al., 2012; Young et al., 1984). These so-called "beige" (Ishibashi and Seale, 2010) or "brite" adipocytes (Petrovic et al., 2010) are only readily detected in the WAT of animals that have been exposed to cold or other inducers. This dependency on external stimuli for UCP1 induction is a distinctive feature of beige fat. By contrast, brown fat cells express relatively high amounts of UCP1 even under non-stimulated conditions. This difference is, at least, partly fat cell-autonomous since preadipocytes from BAT differentiate *ex vivo* into adipocytes that express UCP1 and have high levels of mitochondria; under the same conditions beige fat precursors undergo adipocyte conversion but do not activate the brown fat program unless they are treated with certain inducers, such as β-adrenergic agonists or PPARγ activators (Klaus et al., 1995; Ohno et al., 2012; Petrovic et al., 2010; Wu et al., 2012). Notably, fully stimulated beige adipocytes express similar UCP1 levels as brown adipocytes and undergo UCP1-mediated uncoupled respiration (Long et al., 2014; Okamatsu-Ogura et al., 2013; Shabalina et al., 2013; Wu et al., 2012).

The induction of beige adipocytes is highly adipose depot-dependent. In mice, the subcutaneous inguinal WAT undergoes the most profound induction of beige adipocytes, whereas the epididymal WAT of male mice is particularly resistant to "beige-ing" (Ohno et al., 2012; Vitali et al., 2012). There also exists a great deal of variability in the beige-ing response amongst inbred strains of mice as first reported by Collins *et al.* (Collins et al., 1997). Kozak and colleagues used classical genetic approaches to study this trait in great detail and identified several loci that affect UCP1 induction in white fat (Guerra et al., 1998; Koza et al., 2000; Xue et al., 2007). The genetic variability controlling UCP1 activation was only observed in white fat, providing some of the earliest evidence that beige and brown fat cells may belong to different lineages (see later). Importantly, the induction of UCP1 in white fat (*i.e.,* beige-ing of WAT) is associated with a reduction in obesity in animals treated with the β3-adrenergic agonist, CL 316,243 (Guerra et al., 1998). Thus, the capacity for

UCP1 induction in white fat is strongly correlated with obesity-reduction caused by β3-agonists.

Human BAT

Do humans have thermogenic brown and/or beige fat? And if so, do these tissues affect systemic metabolism in a meaningful way? Through the use of ^{18}F-fluoro-2-deoxy-d-glucose (^{18}F-FDG) positron emission tomography computed tomography (^{18}F-FDG-PET) imaging, it is now evident that humans have substantial depots of UCP1+ adipose cells and that these tissues are activated to take up glucose by cold or β-adrenergic agonist-treatment (Cypess et al., 2014; Cypess et al., 2009; Cypess et al., 2015; Nedergaard et al., 2007; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). In adult humans, the supraclavicular region appears to be the most enriched with UCP1+ adipocytes. While ^{18}F-FDG-PET imaging relies on glucose uptake capacity, and may not necessarily reflect BAT mass *per se*, there are higher amounts of detectable BAT in young and lean subjects. This suggests that variation in BAT activity could participate in the natural regulation of body weight. Reduced BAT activity could also be a consequence of obesity and/or insulin resistance.

Various marker genes/proteins that are selectively expressed in mouse brown versus beige fat cells have been identified. The expression levels of these genes have been used as a way to classify human UCP1+ adipose depots as brown or beige. Human infants have a thin layer of interscapular BAT that shares morphological and molecular features with rodent brown fat (Heaton, 1972; Lidell et al., 2013a). This infant interscapular BAT expresses genes that are characteristic of classical brown adipocytes (Lidell et al., 2013b). In adult humans, BAT depots are generally heterogeneous, containing multiple cell types including UCP1 positive and negative adipocytes (Cypess et al., 2009; Cypess et al., 2013; Virtanen et al., 2009). At the molecular level, supraclavicular BAT possesses a molecular signature that closely resembles mouse beige adipocytes (Lee et al., 2014c; Sharp et al., 2012; Shinoda et al., 2015; Wu et al., 2012). Notably, global expression analyses of clonally-derived brown adipocytes indicate that adult human BAT depots in the supraclavicular region are largely composed of beige-like adipocytes (Shinoda et al., 2015). However, other depots, including those from the cervical and perirenal regions contain adipocytes that express classical brown markers like *ZIC1* and *LHX8* (Cypess et al., 2013; Nagano et al., 2015; Xue et al., 2015). In summary, the FDG-PET+ depots are heterogeneous with some composed mostly of beige-like cells while others resembling classic brown fat. In this review, we refer to the FDG-PET+ and UCP1+ human adipose depots collectively as BAT.

Of note, BAT activity is increased after prolonged cold exposure in the supraclavicular region of adult humans who had previously lacked detectable BAT depots before treatment (Lee et al., 2014b; van der Lans et al., 2013; Yoneshiro et al., 2013). Given the inducible nature of rodent beige adipocytes, it seems likely that cold can similarly promote beige fat biogenesis within these adult human depots. However, again, since these studies utilized FDG-PET, which measures glucose uptake, it will be important to determine the cellular and molecular changes in these tissues before and after chronic cold. Interestingly, it has also been shown that prevalence of FDG-PET+ human BAT is lower in elderly populations

(Yoneshiro et al., 2011). This may be analogous to the reduction in beige fat mass that occurs in aging mice (Rogers et al., 2012). It will now be important to determine how aging suppresses beige and/or brown fat recruitment.

1. Developmental Lineages of Brown and Beige Adipocytes

The major classical BAT depots in mice, including the interscapular, cervical and axillary depots, are interspersed in and around deep back (epaxial) muscles and develop before WAT during embryogenesis. Most of the adipocytes in these tissues originate from precursors in the somites that also give rise to skeletal myocytes, dorsal dermis as well as a subset of white adipocytes in certain depots. The somitic multipotent precursor population is marked by the expression of certain transcription factors, including *Pax7*, *Engrailed-1* (*En1*) and the myogenic factor, *Myf5* (Atit et al., 2006; Lepper and Fan, 2010; Sanchez-Gurmaches et al., 2012; Seale et al., 2008; Wang et al., 2014b) (Fig. 1). These genes are almost certainly expressed at the earliest stages of BAT development, likely in multipotent cells, before adipogenic commitment factors, such as PPARγ are detectable. Through prospective analyses of different *Myf5^Cre* lineage-traced precursor populations, *Early B-Cell Factor-2* (*Ebf2*) was identified as a specific marker gene of embryonic brown preadipocytes (Wang et al., 2014b). EBF2 marks cells in the somitic mesoderm that do not express markers of other developmentally-related lineages, including muscle or dermis. The activation of *Ebf2* is likely to be an early step in brown adipose lineage commitment (Fig. 1). Further studies are needed to determine which inductive cues initiate *Ebf2* expression and adipogenic commitment. An obvious candidate is BMP7, which has been shown to play a pivotal role in BAT development. Loss of BMP7 or BMP-receptor signaling results in a near-complete absence of BAT development (Schulz et al., 2013; Tseng et al., 2008).

The common somitic origin of brown adipocytes and muscle suggested that these lineages may be closely related in development. Consistent with this, brown preadipose cells express many muscle-specific genes and the mitochondrial proteome of brown fat and muscle are highly related to one-another (Forner et al., 2009; Timmons et al., 2007). Interestingly, several factors influence muscle versus brown fat cell fate including PRDM16, C/EBP-β, EHMT1, EWS and ZFP516 (Dempersmier et al., 2015; Kajimura et al., 2009; Ohno et al., 2013; Park et al., 2013; Seale et al., 2008) (Fig. 1). Notably, ectopic expression of PRDM16 and C/EBP-β is sufficient to convert non-adipogenic fibroblasts, such as skin fibroblasts, into functional brown adipocytes *in vivo* (Kajimura et al., 2009). EHMT1, an important co-regulator of PRDM16, is required to suppress the expression of muscle-specific genes in BAT. Similarly EWS was shown to be required for BAT development and its deletion causes the ectopic expression of muscle genes in BAT (Park et al., 2013). PRDM16, which can potently suppress muscle gene expression, is however not required for BAT development in mice, presumably because other related factors can compensate in its absence (Harms et al., 2014).

The embryonic origin(s) of beige fat cells (and white fat cells) is more complicated. Lineage tracing analysis showed that beige adipocytes in the inguinal WAT are not derived from *Myf5*-expressing cells (Sanchez-Gurmaches et al., 2012; Seale et al., 2008). However, there may be beige adipocytes traced by *Myf5*-activation in other WAT depots, such as the

retroperitoneal WAT (Sanchez-Gurmaches et al., 2012). Through clonal analyses of adipogenic cell lines from the inguinal WAT of mice, Wu *et al.* discovered that beige and white adipocytes are distinct cell types that have distinctive molecular profiles (Wu et al., 2012). Importantly, only the beige clones activate UCP1 expression in response to β-adrenergic stimulation. Consistent with this finding, *Ebf2*-expression marks a subpopulation of adipogenic cells in WAT that are competent for *Ucp1* induction, whereas *Ebf2*-negative adipogenic cells differentiate into UCP1-negative adipocytes (Wang et al., 2014b). Distinct molecular signatures at the precursor stage between brown/beige and white adipocytes are also found in adult humans (Shinoda et al., 2015; Xue et al., 2015).

Recent studies show that some beige adipocytes originate from smooth muscle or smooth muscle-like cells and express various smooth muscle markers (Long et al., 2014). Interestingly, the smooth muscle versus beige adipogenic fate of mesenchymal precursors is controlled by cytoskeletal tension and RhoA signaling (McDonald et al., 2015). TGFβ activates Rho-activated protein kinase (ROCK) to increase G-actin and favor smooth muscle-like differentiation whereas BMP7 inhibits ROCK activity to facilitate beige adipogenesis. Smooth muscle cells are heterogeneous and have multiple origins during development, so further studies will be needed to delineate whether a particular subset of smooth muscle or smooth muscle-like cells are beige adipogenic precursors.

Another related and debated topic is whether beige adipocytes induced by cold exposure or other stimuli arise from pre-existing mature adipocytes or via the *de novo* differentiation/ maturation of resident precursor cells. There are compelling data to support both modes of WAT beige-ing (Fig.2). Studies by Cinti and others demonstrated that β-adrenergically-induced beige-ing occurs without significant increases in adipocyte proliferation or DNA content (Barbatelli et al., 2010; Himms-Hagen et al., 2000). This pointed to mature adipocytes as the likely precursor for the UCP1+ cells. Indeed, recent fate-mapping studies from the Granneman lab using a tamoxifen-inducible system suggest that most UCP1+ adipocytes in inguinal WAT come from mature adipocytes (Lee et al., 2015b). However, in sharp contrast to these results, the Scherer lab, using a Doxycycline-inducible (*Adipochaser*) system to label mature adipocytes, found that a large proportion of cold or β-agonist-induced beige cells do not come from pre-existing adipocytes (Wang et al., 2013). This implies that beige adipocytes can develop *de novo* from other sources, likely from resident beige precursor cells. How then does one explain these apparently conflicting fate-mapping results? First, it is important to note that the Adipochaser studies of Wang *et al*. demonstrate that mature adipocytes as well as non-adipocytes contribute to beige adipocytes. The inability to detect any contribution of precursor cells in the Granneman study could be due to the environmental history of animals (see next paragraph) and/or because of caveats associated with use of the tamoxifen-labeling system. Tamoxifen, given its hydrophobic properties is difficult to "wash-out" of fat tissue (Philipp Scherer, in preparation). This might then have led to the unintended labeling of newly differentiating beige adipocytes during the "chase" period after tamoxifen withdrawal.

Altogether, the available data suggest that cold/β-adrenergic signaling promotes both *de novo* beige fat differentiation as well as induction of UCP1/thermogenic characteristics in mature adipocytes. The latter process may or may not reflect *bona fide* "transdifferentiation"

as it has been called in many papers. It seems more likely that there are latent or "masked" beige adipocytes that persist when the thermogenic stimulus subsides. These adipocytes may appear morphologically white but have a beige identity (lineage) and thus the capacity to quickly re-initiate the thermogenic program in response to cold (Fig. 2). Instead of "transdifferentiation" from a different cell type/lineage, cold/β-agonist treatment may simply "activate" (or unmask) the thermogenic properties of pre-existing beige cells. This concept is supported by a recent study showing that cold-induced UCP1+ cells acquire a white-like unilocular morphology after the animals are warmed and that these same cells re-activate UCP1 in response to another bout of cold (Rosenwald et al., 2013). Given this model, the extent to which beige fat cells arise by recruitment or activation may depend on the environmental history and age of the animal. For example, animals housed in warmer conditions may not acquire many beige adipocytes during normal tissue turnover and may therefore depend on higher levels of *de novo* beige adipogenesis when subjected to cold. This does not preclude the possibility that some white fat cells transdifferentiate to beige fat cells under unusual circumstances, perhaps very extended cold.

It is important to note that many of the studies described above have used the inguinal WAT of mice as a model of WAT beige-ing. The inguinal WAT is a major subcutaneous depot that expresses relatively high levels of PRDM16 and has an inherently high beige-ing response (Seale et al., 2011). Mechanisms of beige fat development (i.e., emergence of UCP1+ adipocytes) may differ greatly in other WAT depots. For example, the epididymal WAT of mice, a depot that is much less prone to beige-ing, contains bipotent adipogenic precursor cells that can give rise to both white and beige (UCP1+) adipocytes depending on environmental conditions (Lee et al., 2012). Ongoing and future studies will determine whether there are functionally distinct types of beige adipocytes in different WAT depots of mouse and man. In adult humans, UCP1-positive beige adipocytes emerge in subcutaneous WAT depots of severely burned subjects (Sidossis et al., 2015). The emergence of beige adipocytes appears to be highly location-dependent, such that future studies will need to establish the consensus regarding the locations of adipose tissue biopsies.

2. Molecular Regulation of Beige Adipocyte Development

Because beige adipocyte development is highly inducible, a special emphasis has been given to this cell type as an appealing cellular target of new obesity therapeutics. Given the recent reports that adult human BAT largely contains beige-like adipocytes (Lee et al., 2014c; Sharp et al., 2012; Shinoda et al., 2015; Wu et al., 2012), understanding the molecular circuits that promote WAT beige-ing may lead to new therapeutic opportunities in obese and/or elderly people who lack sufficient amounts of active BAT depots.

Over the past few years a number of studies have identified positive or negative regulators of brown and beige adipocyte development, as summarized by recent reviews (Harms and Seale, 2013; Kajimura and Saito, 2014; Wu et al., 2013). Another important advance is the development of new expression databases. As an example, independent groups have developed publically available expression databases of brown and beige adipocytes, including RNA-sequencing in mice and in humans (Alvarez-Dominguez et al., 2015; Ohno et al., 2013; Shinoda et al., 2015; Wang et al., 2014b), microarrays (Wu et al., 2012; Zhao et

al., 2014), translating ribosome affinity purification (TRAP) analysis (Long et al., 2014), mitochondrial proteome (Forner et al., 2009), and ChIP-sequencing (Loft et al., 2015; Rajakumari et al., 2013). These databases serve as important resources for the scientific community to search for novel regulators and cell-type selective markers. Here, we review recent progresses on molecular circuits that control beige adipocyte development in response to a variety of external and internal cues.

Transcriptional and epigenetic regulators—While many of the transcriptional regulators act as negative regulators of brown/beige adipocyte biogenesis, a few transcriptional regulators function as powerful activators for brown and beige adipocyte development. Such regulators include Forkhead box C2 (FoxC2) (Cederberg et al., 2001), and PRDM16 and its binding partners in the PRDM16 transcriptional complex, such as PPARγ coactivator 1α (PGC1α), C/EBP-β, and Euchromatic histone-lysine N-methyltransferase 1 (EHMT1) (Fig. 1). Intriguingly, PRDM16 expression in white adipocytes not only activate the brown fat-selective gene program but also represses the white or muslce-selective gene program (Kajimura et al., 2008; Seale et al., 2008; Seale et al., 2007). Genetic ablation of these regulators leads to a substantial impairment in the determination and/or maintenance of brown adipocytes (Harms et al., 2014; Harms et al., 2015; Kajimura et al., 2009; Ohno et al., 2013; Rajakumari et al., 2013; Seale et al., 2008). Furthermore, beige adipocyte development is significantly attenuated when PRDM16 or EHMT1 are deleted in an adipose-specific manner (Cohen et al., 2014; Ohno et al., 2013; Ohno et al., 2012). Recent studies identified several new transcriptional regulators that control brown and beige adipocyte differentiation through the PRDM16 pathway. For example, ZFP516 promotes beige adipocyte development by directly interacting with PRDM16 and activating its transcriptional activity (Dempersmier et al., 2015). PLAC8 acts as an upstream activator of C/EBP-β and induces brown fat differentiation (Jimenez-Preitner et al., 2011) (Fig. 1). On the other hand, TLE3 antagonizes the function of PRDM16 and suppresses brown fat differentiation and thermogenesis (Villanueva et al., 2013). Currently, all the identified transcriptional and epigenetic regulators appear to act both in classical brown adipocytes and beige adipcoytes.

Modulation of cellular fate is associated with dynamic chromatin remodeling. Recent ChIP-sequencing analyses of histone modifications identified distinct chromatin artetectures between BAT and WAT (Loft et al., 2015; Rajakumari et al., 2013). In addition, histone modifying enzymes, such as EHMT1 and JMJD1A (also known as KDM3A), are essential for the maintenance of chromatin artitechtures that favor brown/beige adipocytes through interacting with the key transcriptional complexes. EHMT1 function as an essential lysine methyltransferase in the PRDM16 transcriptional complex and required for brown/beige adipose cell fate (Ohno et al., 2013). We have previously shown that PRDM16 potently represses the muscle-selective or WAT-selective gene programs in a EHMT1-dependent fashion (Harms et al., 2014; Ohno et al., 2013). Consistent with the clinical observations that approximately 40-50% of the EHMT1 haploinsufficiency patients exhibit an obese phenotype (Cormier-Daire et al., 2003; Willemsen et al., 2012) and that EHMT1 expression correlate well with UCP1 in adult human BAT (Nagano et al., 2015), adipose-specific deletion of EHMT1 in mice reduces BAT-mediated thermogenesis and causes obesity and

insulin resistance (Ohno et al., 2013). JMJD1A forms a transcriptional complex with the SWI/SNF nucleosome remodeling complex and controls β1-adrenergic receptor (b1-AR) and it's downstream BAT-selecive genes in BAT (Abe et al., 2015). Whole body knockout mouse for *Jhjd1a* gene reduces β-AR-induced fatty oxidation and oxygen consumption in BAT and confers an obese phenotype (Inagaki et al., 2009; Tateishi et al., 2009).

Non-coding RNAs—Non-coding RNAs provide another layer of regulation in the differentiation of brown and beige adipocytes. Several microRNAs (miR), including miR-133, miR-193b, and miR-365, target PRDM16 and negatively regulate brown and beige adipocyte development in mice (Liu et al., 2013; Sun et al., 2011; Trajkovski et al., 2012; Yin et al., 2013). miR-196a activates C/EBP-β expression and induces beige adipocyte differentiation through repression of HoxC8, a negative regulator of C/EBP-β (Mori et al., 2012), whereas miR-155 represses CEBP-β expression and impairs brown adipocyte differentiation (Chen et al., 2013). Additionally, miR-378 and miR-30 activate brown or beige adipocyte differentiation by targeting repressors of BAT thermogenesis, such as phosphodiesterase1b (PDE1b) and receptor-interacting protein 140 (RIP140), respectively (Hu et al., 2015; Pan et al., 2014). Similarly, long non-coding RNAs, such as Inc-BATE1 and Blinc1, are required for brown and beige adipocyte differentiation by forming a nuclear ribonucleoprotein complex to control the thermogenic gene program (Alvarez-Dominguez et al., 2015; Zhao et al., 2014). Conversely, miR-34 acts as a repressor of beige and brown adipocyte differentiation by repressing FGF21 and SIRT1 expression in mice (Fu et al., 2014). In cultured human adipocytes, miR-26 has been identified as activator of beige adipocyte differentiation and cellular respiration (Karbiener et al., 2014). While such non-coding RNAs are appealing tools to modulate BAT-thermogenesis, cell/tissue-type selectivity of the actions need be carefully examined.

3. Environmental Cues that Control Beige Adipocyte Recruitment

Table 1 summarizes recent findings regarding the molecular mechanisms of environmental cue-induced beige adipocyte biogenesis. Chronic cold exposure is a well-known stimulus that powerfully promotes brown and beige adipocyte development. Human studies using[18]F-FDG-PET/CT scans found that adult human BAT activity increases after chronic cold exposure even in subjects who do not possess appreciable amounts of BAT before cold exposure (Lee et al., 2014b; van der Lans et al., 2013; Yoneshiro et al., 2013). Upon cold exposure, NE may be released from the sympathetic nerve or M2 macrophages to activate the BAT/beige fat thermogenic program (Nguyen et al., 2011; Young et al., 1982). These pathways also stimulate *Ucp1* transcription through enhancing phosphorylation of transcriptional regulators including PGC1α, CREB, and ATF2 (Collins, 2011). While the β-adrenergic signaling pathway is clearly a dominant circuit, recent observations indicate that alternative pathways play roles in regulating beige adipocyte biogenesis. In many cases, it remains unclear whether these non-canonical stimuli that affect beige fat development also affect the recruitment and/or thermogenic activity of brown fat. Given the inducible nature of beige adipocytes as compared to classical brown adipocytes, it has been easier to identify factors that regulate UCP1 levels in the beige compartment. A key question for future studies will be to determine if increased beige fat development and UCP1-induction in white fat in response to various stimuli is associated with increases in local thermogenesis.

Recent studies, as summarized below, report new regulatory circuits of beige adipocyte development, some of which do not necessarily demand thermogenesis *per se* (*e.g.,* exercise and bariatric surgery). These studies imply that biological significance of beige adipocytes may go beyond simply generating heat in response to sympathetic nerve stimulation.

Exercise—Irisin and Meteorin-like (METRNL) are recently identified exercise-induced myokines that are induced in muscle through the PGC1α pathway. Administration of Irisin and METRNL via adenoviral vectors powerfully activates beige adipocyte development in mice (Bostrom et al., 2012; Rao et al., 2014). In adult humans, circulating irisin is also increased during cold exposure (Lee et al., 2014a). Mechanistically, irisin induces the beige adipocyte-gene program in a cell-autonomous manner, presumably through a selective receptor. Human irisin has recently been shown to employ a non-canonical initiator codon (ATA) for its translation, suggesting that it might be subject to complex regulation in muscle and/or other tissues (Jedrychowski et al., 2015). On the other hand, METRNL promotes an eosinophil-dependent activation of M2 macrophages to elicit beige adipocyte biogenesis (Rao et al., 2014). IL6 is a well-known exercise-induced myokine that has been shown to activate beige adipocyte development and also be required for exercise-induced WAT beige-ing in mice (Knudsen et al., 2014). In addition, metabolites derived from skeletal muscle after exercise, such as lactate and ß-aminoisobutyric acid (BAIBA), promote beige adipocyte biogenesis in the subcutaneous WAT depots in mice (Carriere et al., 2014; Roberts et al., 2014). Intriguingly, a recent study also showed that transplantation of the subcutaneous WAT from exercised-trained mice into the visceral cavity of sedentary mice significantly improves systemic glucose homeostasis. This improvement is associated with an enhanced insulin-stimulated glucose uptake in the skeletal muscle and BAT, suggesting a role for secreted molecules/metabolites from the subcutaneous WAT (Stanford et al., 2015).

Cancer Cachexia—Cancer cachexia, characterized by weight loss, chronic inflammation and muscle/adipose atrophy, is often associated with higher resting energy expenditure. This increased energy expenditure may be partly due to enhanced WAT beige-ing as has been seen in mouse models of K-ras induced pancreatic and lung cancer (Petruzzelli et al., 2014). For instance, blockade of IL6 or BAT denervation significantly impaired cachexia-associated beige adipocyte biogenesis (Petruzzelli et al., 2014). In addition, Parathyroid hormone related protein (PTHrP) derived from the Lewis lung carcinoma potently promotes beige adipocyte biogenesis (Kir et al., 2014). High level of serum PTHrP is associated with lean body mass in cachectic mice and humans. Treatment of cachectic mice with neutralizing antibody for PTHrP blocked WAT beige-ing and the loss of muscle mass and strength, indicating that PTHrP is *the* major lung-tumour-derived factor stimulating beige adipocyte biogenesis and hypermetabolism, at least in this experimental model of cachexia.

Burn-induced cachexia—In addition to cancer cachexia, a recent study by Sidossis's group reported that beige adipocyte development is highly induced in the human subcutaneous WAT of severely burned subjects. In the burn patients, significantly higher levels of urinary epinephrine and norepinephrine excretion are observed. In addition, several inflammatory cytokines, including IL-6, IL-8, and IL-10, are high. While the underlying

mechanisms here require further study, these circulating factors may contribute to the burn-induced WAT beige-ing in humans (Sidossis et al., 2015).

Environmental enrichment—Mice in an enriched environment with complex physical and social stimuli exhibit higher whole body energy expenditure and are more resistant to diet-induced obesity. Hypothalamic brain-derived neurotrophic factor (BDNF) mediates the environment-associated activation of the sympathetic nerve system (SNS) and promotes beige adipocyte differentiation. Notably, hypothalamic overexpression of BDNF is sufficient to promote beige adipocyte biogenesis and increased energy expenditure (Cao et al., 2011).

Innate immunity—Innate immune pathways are known to control adipose tissue function and systemic glucose homeostasis (Molofsky et al., 2013; Wu et al., 2011). Interestingly, decreased group 2 innate lymphoid cells (ILC2) in WAT are associated with obesity in humans and mice (Brestoff and Artis, 2015). IL-33 is required for the maintenance of ILC2s in WAT and also for beige adipocyte development in mice (Brestoff et al., 2015; Lee et al., 2015a). Conversely, IL-33 treatment is sufficient to promote beige adipocyte biogenesis and increased whole body energy expenditure. IL33-action in WAT involves multiple mechanisms including: (1) increasing the production of Met-enkephalin peptides by ILC2s which acts on adipocytes (Brestoff et al., 2015); and (2) stimulating IL4 secretion by eosinophils which acts to promote the proliferation and beige differentiation of adipogenic precursor cells (Lee et al., 2015a). In addition, alternatively activated (type2/M2) macrophages are recruited to the subcutaneous WAT and secrete catecholamines to activate BAT and induce beige adipocyte development (Nguyen et al., 2011). Mice that lacked IL4 signaling or the capacity to produce catecholamines selectively in macrophages appeared to have an impaired beige adipocyte development under cold (Qiu et al., 2014). However, relative contribution of M2 macrophage versus sympathetic nerve-mediated beige adipocyte development and whole body energy expenditure awaits further investigation.

Bariatric surgery—Bariatric surgery is an effective approach to reduce body weight and ameliorate type 2 diabetes. In a human study using FDG-PET scans, higher BAT activity (*i.e.,* glucose uptake) was observed in morbidly obese subjects one year after laparoscopic adjustable gastric banding surgery (Vijgen et al., 2012). Similarly, body weight reduction by the Roux-en-Y gastric bypass (RYGB) was associated with an increase in BAT activity under cold. This increase in BAT activity levels appears to be independent of hypothalamic activity (Rachid et al., 2015). In mice, higher expression of UCP1 was observed in the gonadal WAT but not in the inguinal WAT after RYGB surgery. This increase in UCP1 expression is associated with an increase in the gene expression of b3-adrenoceptor, natriuretic peptides, ANP, and BNP (Neinast et al., 2015). The underlying mechanisms by which bariatric surgery promotes beige adipocyte biogenesis remain elusive.

Extrinsic regulators—Studies over the past few years have identified a variety of secreted factors and hormones that control beige adipocyte development. Such factors include Bone Morphogenetic Protein (BMP)4 (Qian et al., 2013), BMP7 (Schulz et al., 2011; Tseng et al., 2008), BMP8b (Whittle et al., 2012), Fibroblast Growth Factor (FGF)21 (Emanuelli et al., 2014; Fisher et al., 2012), Growth Differentiation Factor (GDF)5 (Hinoi et

al., 2014), natriuretic peptides (Bordicchia et al., 2012), prostaglandins (Madsen et al., 2010; Vegiopoulos et al., 2010), and Vascular Endothelial Growth Factor (VEGF) (During et al., 2015; Sun et al., 2012). When treated with each factor, mice exhibit increase whole body energy expenditure and are protected from diet-induced body weight gain and insulin resistance. Conversely, Transforming Growth Factor (TGF)-β and Notch are up-regulated under obesity and blocks beige adipocyte differentiation. Blockade of TGF-β signaling by genetic ablation of Smad3 or by neutralizing antibody for TGF-β promote beige adipocyte development and protects animals from diet-induced obesity and insulin resistance (Koncarevic et al., 2012; Yadav et al., 2011). Similarly, genetic or pharmacological inactivation of Notch signaling induces beige adipocyte development (Bi et al., 2014). Therapeutic application of these endocrine regulators in obese humans would be an important topic in the near future.

Neuronal circuits—BAT thermogenesis is highly regulated by the core thermoregulatory neural network in the central nerve system (CNS). Series of neuroanatomical studies developed a neuroanatomical network model for thermoregulation in the interscapular BAT in response to cold and warm temperature (Morrison et al., 2014). Recent studies further identified new neuronal circuits that control brown and beige adipocyte thermogenesis. For example, activation of hepatic glucokinase leads to impaired thermogenesis in BAT through the BAT-liver connection between the afferent vagues from the liver and sympathetic efferents from the medulla to BAT (Tsukita et al., 2012). More recently, Elmquist's group reported that deletion of PPARγ in the *Phox2b*-positive vagal sensory neurons promotes beige adipocyte biogenesis (Liu et al., 2014).

Activation of hypothalamic POMC neurons by leptin and insulin treatment promotes beige adipocyte biogenesis (Dodd et al., 2015), whereas inhibition of AgRP neurons by genetic deletion of O-linked β-N-acetylglucosamine promotes beige adipocyte biogenesis (Ruan et al., 2014). On the other hand, depletion of serotonergic neurons in the CNS attenuates the thermogenesis in the interscapular BAT as well as WAT beige-ing (McGlashon et al., 2015). These studies indicate new neuronal circuits that control brown and/or beige adipocyte thermogenesis in rodents. It would be important to address if such neuronal regulatory circuits are conserved in adult humans.

4. Therapeutic Opportunities in Adult Humans

A major goal in this field is to determine if activation of brown/beige fat thermogenesis can prevent body weight gain and reverse metabolic abnormalities in adult humans. Several studies clearly demonstrate that chronic cold acclimation is able to recruit higher levels of activated BAT depots in the supraclavicular region of adult humans (Lee et al., 2014b; van der Lans et al., 2013; Yoneshiro et al., 2013). Importantly, this chronic cold-stimulated BAT recruitment is associated with an improvement in post-prandial insulin sensitivity (Lee et al., 2014b) or with an increase in cold induced energy expenditure (van der Lans et al., 2013; Yoneshiro et al., 2013). Hence, promoting BAT recruitment is an important aspect to be considered for those who do not possess sufficient amounts of active BAT, such as obese and elderly population.

The β3-AR pathway is a dominant signaling pathway to activate BAT thermogenesis in rodents and in humans, however, adverse effects on the cardiovascular system hampered the use of β-AR agonists in the clinic (Villarroya and Vidal-Puig, 2013). Recently, Cypess and colleagues reported that oral administration of a selective β3-AR agonist Mirabegron robustly stimulated glucose uptake in the BAT depots in healthy adult humans, in parallel with an increase in resting metabolic rate (Cypess et al., 2015). On the other hand, minimal efficacy on BAT activity was observed in obese subjects after administration of a pan-adrenergic agonist Ephedrine (Carey et al., 2013). The lack of efficacy of β-AR agonists in obese subjects appears to be due to an impaired β-AR signaling and/or development of resistance to the β-AR agonists in adipose tissues. The underlying mechanisms for the β-AR resistance remain poorly understood and should be further investigated.

Saito's group demonstrated that six weeks treatment of with capsinoids, non-pungent capsaicin analogs, led to an increase in cold-induced thermogenesis that may have been associated with the recruitment of new BAT in subjects with undetectable BAT before the treatment (Yoneshiro et al., 2013). More recently, Saito's group found several dietary substances, such as catechins are able to increase BAT recruitment (In Preparation). These data indicate that new BAT can be recruited (and/or activated) by dietary supplements as well as cold-exposure in humans.

In addition to the above compounds that have been tested in adult humans, new candidates are identified through a variety of approaches and shown to be effective at least in rodent models. For example, we have recently developed a mouse model, termed *ThermoMouse* in which luciferase expression is regulated by the *Ucp1* gene regulatory elements. A cell based high-throughput screen platform using brown adipocytes from *ThermoMouse* identified a small compound WWL113 that induces UCP1 expression and whole body energy expenditure *in vivo* (Galmozzi et al., 2014). This study provides an important proof of concept that targeting UCP1 using small molecules can be a plausible approach for enhancing whole body energy expenditure *in vivo*. Additionally, a number of synthetic agonists or inhibitors, such as the PPARγ agonists (Ohno et al., 2012; Petrovic et al., 2010; Qiang et al., 2012), FXR agonist fexaramine (Fang et al., 2015), soluble guanylyl cyclase (Hoffmann et al., 2015), GLP-1 agonist (Beiroa et al., 2014), Notch inhibitor (Bi et al., 2014) and amlexanox, an inhibitor of TBK1 and IKK-e (Reilly et al., 2013), potently increase whole body energy expenditure by activating thermogenesis in BAT and/or by promoting beige adipocyte biogenesis. Clinical applications of the above compounds await further investigation.

5. New Function of Beige Fat in Energy Homeostasis

The biomedical interest in brown and beige fat cells is centered on the capacity of these cells to counteract obesity and metabolic disease. Classical experiments by Rothwell and Stock first demonstrated that brown fat is activated by various high-calorie diets and that this provided a potential mechanism to limit weight/fat gain (Rothwell and Stock, 1979). In support to this, BAT and beige fat-deficient mice expressing a *Ucp1*-driven toxygene, develop obesity and insulin resistance in the absence of hyperphagia (Lowell et al., 1993). Further evidence supporting a natural role for BAT in regulating body weight/composition

came from recent studies of UCP1-knockout mice. These animals display an increase in metabolic efficiency and gain more weight than wild-type controls when housed under thermoneutral conditions (Feldmann et al., 2009). Importantly, high fat diet increases the thermogenic capacity of BAT in wild-type but not in UCP1-knockout animals. Of note, UCP1-knockout mice are not obese when housed under standard room temperature conditions. This suggests that alternative thermogenic mechanisms, which are engaged in response to cold, also suppress weight gain and can conceal the role of UCP1 in body weight regulation.

Genetic or pharmacological elevation of brown and/or beige fat activity in mice provides further protection against metabolic disease and obesity. Many mouse models have increased brown/beige fat function and a correlated resistance to high fat diet-induced obesity and insulin resistance. Importantly, these results indicate that the ectopic increases in brown fat are not necessarily counteracted by increases in metabolic efficiency or higher food intake.

Currently there is no experimental system that allows for quantitative estimation of brown vs. beige in rodents and in humans. However, recent results, as discussed below, indicate that beige adipocytes contribute significantly to the regulation of whole-body energy expenditure and systemic glucose/lipid homeostasis.

Anti-diabetic role of beige fat—Perhaps the most obvious area of interest is in type 2 diabetes and insulin resistance. In fact, improvement in systemic glucose homeostasis and insulin sensitivity is associated with glucose uptake activity in adult human BAT (Chondronikola et al., 2014; Lee et al., 2014b). Loss of fat mass would be expected to be accompanied by improvements in insulin resistance and indeed that is usually observed. However, the improvements in glucose tolerance and/or insulin resistance often seem well out of proportion to the anti-obesity effects. For example, fat selective expression of PRDM16 in obese transgenic mice causes a modest but significant decrease in total adipose mass, accompanied by a small improvement in insulin resistance (Seale et al., 2011). On the other hand, glucose tolerance was vastly improved. This data strongly suggested that expansion and activation of (in this case) beige adipocytes improved glucose tolerance by mechanisms that may not be limited to insulin sensitivity. Perhaps the beige adipocytes clear glucose from the blood through mechanisms that are not totally insulin-dependent or perhaps systemic glucose tolerance is improved via some as yet unknown mechanisms. Conversely, adipose-specific deletion of PRDM16 (Cohen et al., 2014) or its co-activator EHMT1 (Ohno et al., 2013) significantly impaired adipocyte development and caused insulin resistance before the development of obesity. While this stimulates a mild obesity, with increased fat accumulation in the subcutaneous depot, a striking metabolic phenotype in the adipose-specific knockout mice of PRDM16 or EHMT1 is hepatic steatosis. Taken together, these data strongly suggest (but do not prove) that brown and beige fat play some important function in glucose homeostasis that goes beyond the dissipation of calories stored as fat. A specific role for beige fat in directly lowering circulating glucose and blood fatty acids via oxidation could explain a "kick-on" effect on other aspects of glucose homeostasis if the liver is forced to absorb this energy and become steatotic when beige fat is lost. In fact, BAT functions as a major "metabolic sink" not only for glucose but also for

lipoproteins and fatty acids (Bartelt et al., 2011), and presumably for other metabolites. Hence, therapeutic potential of brown and beige fat may not simply be limited to anti-obesity therapy, but can also be explored in the treatment of fatty liver diseases.

Endocrine Function—Since white fat cells secrete many adipokines, it is not far fetched to think that brown and beige fat cells might secrete "batokines" with various functions. Along the same lines, endurance exercise has been shown to stimulate beige-ing of the subcutaneous adipose tissue in rodents (Bostrom et al., 2012). Transplantation of the inguinal WAT depots from exercised mice improves various aspects of glucose homeostasis much more than equivalent fat transplantations from sedentary mice (Stanford et al., 2015). Hence, the notion that classical brown and/or beige fat may secrete other molecules that improve glucose homeostasis must be considered and studied. Regarding this hypothesis, two polypeptides secreted by muscle with exercise, irisin and METRNL, have also shown to be secreted by beige fat when challenged with cold. FGF21 and BMP8b have also been shown to be secreted by adipose tissues and induced in the cold, suggesting a paracrine or autocrine "batokine" function. More recently, it has been shown that neuregulin 4 (NRG4), a member of the epidermal growth factor (EGF) family of extracellular ligands, is secreted from BAT and regulates hepatic lipid metabolism by inhibiting lipogenesis in the liver (Wang et al., 2014a). The substrate depletion and "batokine" models are of course not mutually exclusive.

Adipose tissue remodeling—WAT beige-ing is associated with dynamic adipose remodeling in the subcutaneous WAT. For instance, increased microcapillary formation and tyrosine hydroxylase (TH)-positive nerve (*i.e.,* sympathetic nerve) innervation are tightly coupled with the clusters of beige adipocytes within WAT (Cinti, 1999). Xue et al., reported that chronic cold exposure activates angiogenesis in the inguinal WAT both in wild type and UCP1 null mice (Xue et al., 2009). Neutralizing antibody for VEGF-R2, but not VEGF-R1, significantly blocked the cold-induced angiogenesis in WAT. Conversely, adipose tissue-selective VEGF expression promotes beige adipocyte biogenesis in WAT (During et al., 2015; Sun et al., 2012), indicating that VEGF controls not only angiogenesis but also the beige adipocyte differentiation program. Nerve growth factor (NGF) is also abundantly expressed in BAT and regulated by cold and obesity (Nisoli et al., 1996). However, whether NGF is required for nerve innervation during WAT beige-ing is unknown.

In addition to angiogenesis and innervation, innate and adaptive immune cells, including eosinophil, macrophages, T-regs, and ILC2, are recruited within adipose tissues and controls not only inflammatory responses but also beige adipocyte biogenesis (Brestoff and Artis, 2015), providing an alternative pathway to regulate adipose tissue composition. Another intriguing aspect during the WAT beige-ing may be adipose fibrosis. TGF-β expression in the WAT is highly increased under obesity and powerfully drives adipose fibrosis, while blocking the TGF-β signaling pathway promotes beige adipocyte development (Koncarevic et al., 2012; Yadav et al., 2011). Given the recent studies showing that a population of beige adipocytes arises from a SMA+ smooth muscle lineage (Long et al., 2014) and that expression of SRF-target genes, such as SMA and Collagen1a1 and 3a1, are down-regulated

during beige adipocyte differentiation (McDonald et al., 2015), it is conceivable that beige adipocyte recruitment is coupled with inhibition of adipose fibrosis.

UCP1-independent thermogenesis—UCP1 dissipates the proton gradient in the form of heat by uncoupling cellular respiration from mitochondrial ATP synthesis. Because UCP1 null mice are cold sensitive and fail to maintain body temperature in response to prolong cold exposure (Enerback et al., 1997; Golozoubova et al., 2001), UCP1 is considered to be the major "thermogenin" that is responsible for adaptive non-shivering thermogenesis (Nedergaard et al., 2001). On the other hand, Granneman et al. reported that chronic stimulation of β3-AR significantly increased metabolic rate in UCP1 null mice, although the induction was lower than what was observed in wild type mice (Granneman et al., 2003). This UCP1-independent increase in metabolic rate is accompanied by an increase in mitochondrial biogenesis and lipid oxidation in the WAT of UCP1 null mice, indicating that beige adipocyte may possess UCP1-independent thermogenesis. Intriguingly, two recent papers showed that FGF21 treatment was able to reduce body weight gain and improved glucose and lipid homeostasis even in UCP1 null mice (Samms et al., 2015; Veniant et al., 2015). While the FGF21's effects appear to be partly due to reduced food intake (Samms et al., 2015), FGF21 also potently increases PGC1α expression in the inguinal WAT of UCP1 null mice (Veniant et al., 2015).

Recent data indicates that beige adipocytes contain a second thermogenic pathway that relies on futile cycling of creatine and creatine phosphate. It is noteworthy that this pathway is thermogenic but depends on coupled rather than uncoupled respiration. Interestingly, several components of this pathway are elevated in UCP1 KO mice, and chemical inhibition of this pathway suggests that it is indeed involved in thermal defense when the UCP1 pathway is ablated (Kazak et al., 2015). These data indicate the existence of UCP1-independent mechanisms that control thermogenesis and/or metabolism in adipose tissues and maybe in other organs.

6. Emerging Questions

A major function of BAT remains thermogenesis via UCP1. However, as we learn more about the two types of thermogenic adipocytes, *i.e.,* classical brown adipocytes and beige adipocytes, it is important to ask whether these cells have additional functions in regulating systemic metabolism that may go beyond UCP1-mediated thermogenesis. In this regard, it is likely that BAT is not simply a heat-generating organ: some of the metabolic improvements observed through increasing beige fat mass are not mediated by UCP1 (Fig.3). In particular, brown/beige fat secrete several "batokines" that may function in an endocrine, autocrine, and/or paracrine manner to control systemic glucose and lipid homeostasis. Furthermore, the metabolic significance of brown/beige fat acting as "metabolic sink" for toxic substances should be investigated. Finally, WAT beige-ing is associated with large-scale tissue remodeling, including increased micro-capillary formation, nerve innervation, and modulation of immune cell populations. These changes in other tissue components likely affect the local and systemic metabolism. Determining how the various cell types in adipose interact with one another is an important area for future study.

Another important goal is to establish reliable quantitative methods to measure human BAT mass regardless of environmental conditions. While ^{18}F-FDG-PET based measurement has recently been substantially improved, the assessment is highly variable depending on the conditions of measurement, such as outside temperature and length of cold exposure (Sidossis and Kajimura, 2015). Furthermore, FDG measurements are based on glucose uptake, which represents only one function of BAT. It will also be important to development new devices/techniques that have enough sensitivity and resolution to detect beige adipocytes that sporadically reside in subcutaneous WAT and other adipose depots. The identification of specific cell surface markers and circulating markers that reflect brown fat mass would be a huge advance.

Lastly, an improved understanding of the beige adipocyte-selective signaling pathways will allow us to selectively target WAT beige-ing for therapeutic effect. We also need to understand the mechanisms of beige adipocyte maintenance, as beige adipocytes gradually lose their thermogenic characteristics after removing the appropriate external cues. These studies will lead to a plausible approach to increase whole body energy expenditure and improve glucose/lipid homeostasis in human populations who have little or no existing active BAT, such as obese and/or elderly populations.

Acknowledgments

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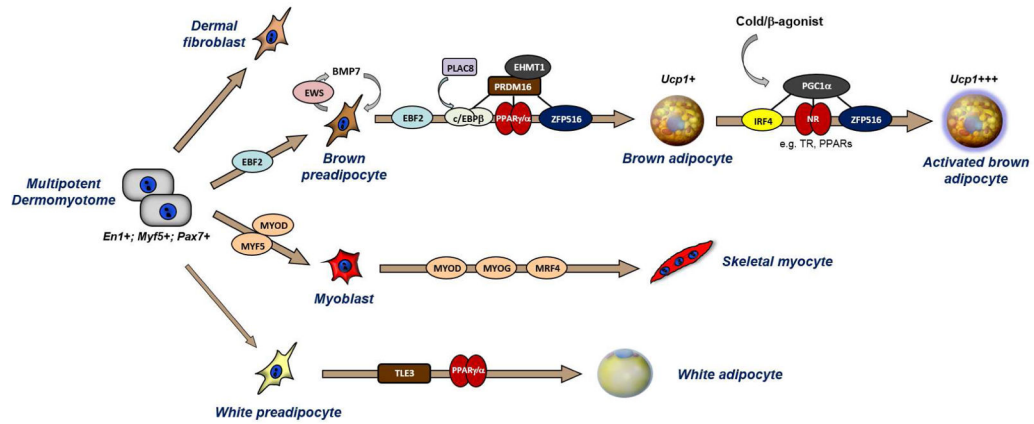
Figure 1. Brown adipocyte development
A multipotent cell population in the somitic mesoderm gives rise to: dorsal dermis, skeletal muscle, brown adipocytes and white adipocytes in certain depots. EBF2 marks committed brown preadipocytes and may also regulate the commitment process from upstream stem cells. EWS controls the expression of BMP7 by brown fat precursor cells, which in turn promotes brown adipocyte differentiation by acting in an autocrine manner. EBF2, PRDM16, C/EBP-β, and ZFP516 specifically regulate the induction of brown fat-specific genes during the differentiation process. PRDM16 binds and activates C/EBP-β, PPARγ, PPARα, Thyroid receptor (not shown) and ZFP516. Cold exposure/norepinephrine (NE) activates brown adipocytes to express high levels of thermogenic genes; this activation process is controlled, in large part, by PGC1α which interacts with IRF4 (Kong et al., 2014) and various Nuclear Receptors (NRs). MYF5 or MYOD control myoblast cell commitment, while MYOD, MYOG and MRF4 regulate myocyte differentiation and maturation. TLE3 plays an important role in driving a white fat-specific differentiation program.
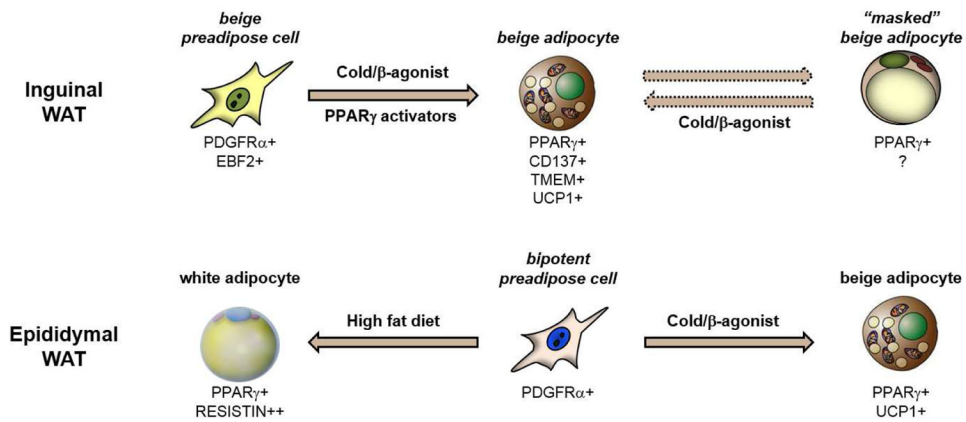
Figure 2. Beige fat biogenesis
(Top panel) In inguinal WAT, β-adrenergic stimulation stimulates the *de novo* differentiation of EBF2+ precursor cells into beige adipocytes. In the absence of stimuli, these beige adipocytes lose their expression of UCP1 and their multilocular morphology and could be considered as "masked". The thermogenic characteristics of these cells can be re-activated by repeated stimulation. **(Bottom panel)** In epididymal WAT, bipotent precursor cells can be induced to undergo beige adipocyte differentiation in response to cold/β-agonist. Under other conditions (such as high fat diet), these cells may differentiate into white adipocytes.
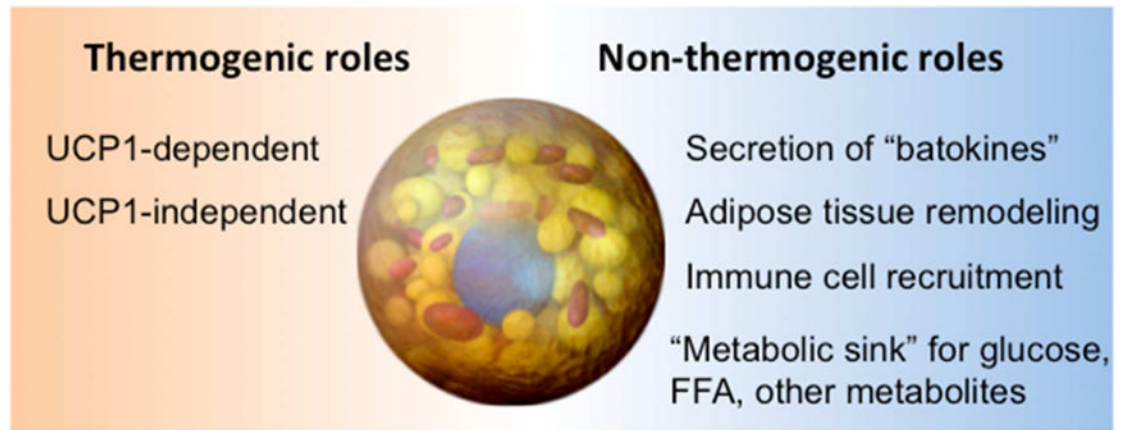
Figure 3. Physiological roles of brown and beige fat in energy metabolism
Thermogenic and non-thermogenic functions of brown and beige adipocytes are listed.

Table 1

Cues and mediators that promote beige adipocyte recruitment.

Stimuli	Mediators	References
Chronic cold exposure	beta-adrenergic receptors	Lee et al., 2014b; van der Lans et al., 2013; Yoneshiro et al., 2013 (Adult humans)
Exercise	Irisin Meteorin-like IL-6 BAIBA Lactate	Bostrom et al., 2012 Rao et al., 2014 Knudsen et al., 2014 Roberts et al., 2014 Carriere et al., 2014
Biatric surgery	ANP, BNP?	Rachid et al., 2015; Neinast et al., 2015
Cancer Cachexia	IL-6 PTHrP	Petruzzelli et al., 2014 Kir et al., 2014
Burn-induced cachexia	Epinephrine, norepinephrine, inflammatory cytokines?	Sidossis et al., 2015
Environmental enrichment	BDNF	Cao et al., 2011
Innate immunity	IL-33 IL-4	Brestoff et al., 2015 Qiu et al., 2014
Endocrine hormones	BMP4 BMP7 BMP8b FGF21 GDF5 Natriuretic peptides Prostaglandins VEGF	Qian et al., 2013 Schulz et al., 2011; Tseng et al., 2008 Whittle et al., 2012 Emanuelli et al., 2014; Fisher et al., 2012 Hinoi et al., 2014 Bordicchia et al., 2012 Madsen et al., 2010; Vegiopoulos et al., 2010 During et al., 2015; Sun et al., 2012 Ohno et al., 2012; Petrovic et al., 2010; Qiang et al., 2012
Synthetic compounds	PPARg agonists FXR agonist Notch inhibitor GLP1 agonist TBK1 and IKK-e inhibitor Soluble guanylyl cyclase TGF-beta antagonists	Fang et al., 2015 Bi et al., 2014 Beiroa et al., 2014 Reilly et al., 2013 Hoffmann et al., 2015 Koncarevic et al., 2012; Yadav et al., 2011