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Review

Transcriptional brakes on the road to adipocyte thermogenesis☆

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ABSTRACT

White adipocytes represent the principle site for energy storage whereas brown/beige adipocytes emerge from seemingly distinct cellular lineages and burn chemical energy to produce heat. Thermogenic adipocytes utilize cell-type selective master regulatory transcription factors to drive the expression of their adipocyte thermogenic gene program. White adipocytes harbor transcriptional mechanisms to suppress the thermogenic gene program and maintain an energy-storing function. Here, we summarize some of the key developmental and transcriptional mechanisms leading to the postnatal recruitment of thermogenic adipocytes under physiological conditions, with a particular emphasis on the transcriptional "brakes" on the thermogenic gene program. We highlight a number of recent studies, including our own work on the transcription factor, ZFP423, that illustrate the potential to engineer the subcutaneous and visceral white fat lineages to adopt a thermogenic fat cell fate by releasing the inhibition of the adipocyte thermogenic gene program. These transcriptional brakes on adipocyte thermogenesis may represent potential targets of therapeutic interventions designed to combat obesity and associated metabolic disorders.

1. Introduction

For several decades, adipocytes were broadly classified as energy-storing "white adipocytes" or energy-burning "brown adipocytes". Over the past decade it has become increasingly clear that this designation is too simplistic [1]. White adipocytes, characterized by their large unilocular lipid droplet appearance, reside throughout the body. White adipocytes accumulate within distinct white adipose tissue (WAT) depots, or "mini-organs," and also reside interspersed within the parenchyma of other tissues. Molecular and cellular analyses now suggest that anatomically distinct white adipocytes are functionally, molecularly, and developmentally distinct [2,3]. As such, it appears likely that anatomically distinct white adipocytes represent functionally distinct adipocyte subtypes altogether. These differences are potentially of great importance since those individuals who preferentially accumulate abdominal/visceral adipose tissues are at a higher risk for developing cardiometabolic disease than those who preferentially expand subcutaneous adipose tissue.

Likewise, there is growing evidence that multiple types of thermogenic (UCP1$^+$) adipocytes exist. Brown adipocytes are found within distinct brown adipose tissue (BAT) depots. These "classical" brown adipocytes are specified in rodents during gestation and arise from a *Myf5*-expressing progenitor originating from the dermomyotome [4,5]. The most prominent classical BAT depot in mice is found in the interscapular region; however, additional depots are present throughout the organism. Interscapular BAT is also present in human infants but largely involutes with age [6,7]. Initially, it was widely believed that adult BAT was limited to individuals with pheochromocytoma (an adrenal catecholamine-producing tumor) and in outdoor workers with chronic exposure to cold temperatures [8,9]. Nearly a decade ago, this view changed with the unexpected finding that distinct and active BAT depots exist in adult humans [10–12]. The amount of BAT correlates inversely with body mass index (BMI), raising the possibility that variations in the amount or activity of BAT may contribute to the propensity for weight gain [10]. Importantly, human supraclavicular BAT can be activated in response to chronic cold exposure and contribute to nutrient homeostasis [13,14].

It has long been recognized that WAT depots of cold exposed rodents can adopt a thermogenic phenotype, elicited by the emergence of UCP1$^+$ energy-burning adipocytes [15]. It has become apparent only over the past several years that this "browning" of WAT depots involves the emergence of thermogenic adipocytes that are developmentally, molecularly, and functionally, distinct from the classic brown adipocytes. Notably, lineage-tracing studies revealed that most UCP1$^+$ cells within WAT depots are not derived from a *Myf5*$^+$ lineage [3,5]; these data strongly suggested a developmental origin distinct from the brown

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adipocytes formed during the fetal period. These cells activate *Ucp1* expression upon cold exposure and exhibit a multilocular lipid droplet phenotype [16]; however, they appear to be molecularly and likely functionally distinct from brown adipocytes present in brown adipose depots. Pioneering studies from Wu et al. characterized UCP1$^+$ adipocytes differentiated from WAT-derived clonal precursor cell lines and revealed that these cells exhibit properties of both brown and white adipocytes [17]. The term "beige adipocytes" has thus been adopted to describe these thermogenic cells; however, others have described them in the literature as "BRITE" cells or "inducible brown adipocytes". Importantly, BAT depots in humans appear to have both classical brown fat cells and beige adipocytes; the precise identity of human thermogenic adipocytes may depend on the location of the BAT depots examined [12,18].

A preponderance of data now support the notion that the ability to induce "browning" of WAT is protective against obesity and can reverse insulin resistance in metabolic syndrome. Some of these effects may be mediated by their thermogenic capacity; others may be mediated by distinct endocrine roles of these cells. Nevertheless, tremendous effort is now placed on developing strategies to recruit thermogenic adipocytes from precursors or stimulate activity of existing brown and beige fat in humans. Critical to these efforts will be a better understanding of the cellular and molecular mechanisms controlling the establishment and maintenance of the adipocyte lineages. Below, we summarize some of the natural developmental and transcriptional mechanisms leading to the postnatal recruitment of thermogenic adipocytes under physiological conditions. Furthermore, we highlight recent studies of the transcription factor, ZFP423, that illustrate the potential to engineer adult white adipocytes and white adipocyte precursors to adopt a thermogenic fat cell fate and exert a beneficial impact on nutrient homeostasis in obesity.

2. Cellular origins of thermogenic adipocytes in adult animals

The abundance and/or activity of brown and beige adipocytes appear heavily regulated in adult animals. An increasingly large number of pharmacological and genetic manipulations can lead to alterations in the number or activity of these cells. Moreover, numerous circulating hormones, including thyroid hormone, FGF21, BMPs, IRISIN, and cardiac natriuretic peptides (ANP and BNP), have been shown to induce or enhance "beiging" and increase the thermogenic capacity of WAT [19–24]. The most potent and physiological driver of thermogenic adipocyte recruitment and activation remains to be the sympathetic nervous system, via β-adrenergic receptor signaling. Prolonged cold exposure leads to an expansion in mass of classical BAT depots, mediated by both brown adipocyte hyperplasia and hypertrophy [25]. As described above, cold exposure leads to a dramatic remodeling of white adipose tissue, with the emergence of beige adipocytes. Beige adipocytes have also been linked to a number of physiologic and pathological states characterized by a systemically increased metabolic rate. Most notably, exercise, also associated with increased β-adrenergic signaling, drives the activation of BAT [26]. The recruitment of subcutaneous beige adipocytes has been observed in the setting of cancer-associated cachexia, and may arise in response to parathyroid hormone-related protein/parathyroid hormone signaling [27–30]. Increased beige adipocyte accumulation in the gonadal WAT of mice has been observed after gastric bypass surgery, potentially contributing to the observed metabolic enhancements [31]. Browning of subcutaneous WAT can occur in humans suffering from burn trauma [32]. This condition is associated with adrenergic stress and hypermetabolic status; destruction of the skin barrier compromises heat retention and thus may require increased thermogenesis to maintain body temperature.

Great progress has been made in elucidating the transcriptional machinery and signaling apparatus that drives beige adipocyte accumulation in WAT depots; however, the actual cellular origin of emerging UCP1$^+$ adipocytes under various physiological conditions or in most genetic models has remained unclear. Multiple lineage tracing studies now indicate that beige adipocytes naturally arise predominantly, but not exclusively, by de novo beige adipogenesis in response to cold exposure [33–36]. Importantly, beige adipocytes have the capacity to revert to a white adipocyte phenotype. When animals are returned to thermoneutral conditions following cold exposure, beige adipocytes lose UCP1 expression and become unilocular [37]. These same adipocytes can then revert back to UCP1$^+$ cells upon re-exposure to cold. This inter-conversion of phenotypes is often referred to as "transdifferentiation" [38]. In the strictest sense, transdifferentiation most often refers to a direct conversion of one differentiated cell type into another in the absence of de-differentiation. In most instances, this type of event is difficult to prove experimentally. Alternatively, the white-to-beige phenotype switching may simply reflect beige adipocytes in active and inactive ("dormant") thermogenic states [39]. The adipocyte lineage-tracing approaches currently employed in the field do not easily distinguish between these two possibilities. The *adiponectin* promoter used for adipocyte targeting is expressed in all types of adipocytes; molecular markers that will discriminate between unilocular white and unilocular dormant-beige adipocytes are still needed. As such, current data supports at least two general mechanisms that lead to the natural formation of beige adipocytes during cold exposure: 1) de novo differentiation from precursors, and 2) conversion of unilocular adipocytes into multilocular UCP1$^+$ adipocytes. The latter event may represent an activation of "dormant" unilocular beige adipocytes and/or a true transdifferentiation event.

It is notable that studies from Lee et al. suggested that beige adipocytes arise predominantly from pre-existing mature adipocytes, supporting the initial hypothesis of transdifferentiation [25]. The existence of dormant beige adipocytes may in fact explain discrepancies in lineage tracing studies. Animals with a prior history of cold exposure, even mild cold exposure, may harbor numerous inactive beige adipocytes that quickly revert to a multilocular UCP1$^+$ phenotype upon re-exposure to cold temperatures. Under such conditions, de novo beige adipocyte differentiation may not be the major mechanism leading to functional WAT browning since pre-existing beige cells can be readily reactivated. Moreover, most recent work from Graff and colleagues highlight the potential for distinct modes of beige cell recruitment upon pharmacological vs. physiological stimulation of WAT browning [40]. It is clear that additional lineage tracing studies are needed, including those that take into account additional environmental and/or physiological factors.

A number of lineage tracing studies have begun to shed insight into the origin of beige adipocytes, and have converged on the hypothesis that beige adipocytes arise, at least in part, from perivascular cells within adipose tissue. Expression profiling revealed that beige adipocytes express a smooth muscle-like gene program, much like how classical brown adipocytes exhibit a skeletal muscle-like gene signature [16,41]. Indeed, at least a subpopulation of beige adipocytes formed in cold-exposed mice originates from *Myh11*-expressing cells [16]. Additional lineage tracing studies suggest that the pool of beige adipocyte precursors may be heterogeneous, and multiple distinct populations of beige preadipocytes may even exist. Our group employed pulse-chase lineage-tracing to follow the fate of adipose *Pdgfrb*-expressing cells during cold exposure [35]. After 7 days of cold exposure, we were unable to find beige adipocytes which differentiated from labeled *Pdgfrb*-expressing precursors. De novo beige cell differentiation indeed occurs during this period [36]; however, these data suggest that beige adipogenesis is initiated from cells not actively expressing *Pdgfrb* at the time of labeling. After two weeks of cold exposure, labeled beige adipocytes are readily apparent, although present at a relatively low frequency. These data suggest that multiple waves of beige adipogenesis may occur, each drawing upon somewhat distinct precursor populations. Similar results were observed in studies following the fate of *Myh11*-expressing precursor cells [33]. Interestingly, pulse-chase lineage tracing using a α-smooth muscle actin (*Acta2*)-driven reporter system

demonstrated that most beige adipocytes formed following acute cold exposure (within 7 days) originated from αSMA$^+$ precursors [33]. All of these data are consistent with the hypothesis that beige adipocyte precursors share characteristics of vascular smooth muscle cells; however, the beige precursor pool may be heterogeneous. Importantly, recent studies from Corvera and colleagues provide compelling evidence that beige adipocyte precursors reside within the vasculature of human subcutaneous WAT [42]. Capillary sprouts emerging from explanted human WAT contain precursors that can differentiate into beige adipocytes. Upon transplantation into mice, these human beige adipocytes elicit improvements in glucose homeostasis. The exact identity of the adipocyte precursor in these cultures remains unclear; however, future studies employing single-cell sequencing technology may help clarify the heterogeneity of the adipose stroma and perivascular compartment.

3. Transcriptional control of adipocyte thermogenesis

3.1. Specification of the brown/beige adipocytes lineages

The establishment and maintenance of functional adipocytes is dependent on a number of critical events, including *preadipocyte determination* (commitment of multipotent progenitors to the adipocyte lineage), *adipocyte differentiation,* (morphological and biochemical transition of preadipocytes into mature adipocytes in response to appropriate cues), and *maintenance of the adipocyte phenotype* (maintaining cellular identity and functional properties of the terminally differentiated cells). The nuclear hormone receptor, PPARγ, is undoubtedly the "master regulator" of adipocyte differentiation and plays a critical role in maintaining the mature adipocyte phenotype [43,44]. The initial discoveries of PPARγ, C/EBP family members, and other transcription factors, identified mechanisms critical for the development and maintenance of both white and brown adipocytes; the question of how the fates of specific subtypes of adipocytes were determined was left unresolved. Now it is clear that the coordinated recruitment of transcription factors, transcriptional co-regulatory proteins, and chromatin-modifying proteins, to brown adipocyte-specific regulatory elements guide brown/beige lineage specification during cellular differentiation (Fig. 1A). Excellent in-depth reviews on the chromatin remodeling factors/events leading to the activation of brown/beige adipogenesis have recently become available [45,46]. Among the first of transcriptional regulators described to drive the thermogenic gene program was PGC1α, a cold-induced co-activator of PPARγ, and FOXC2, which drives the expression of the RIα subunit of PKA [47,48]. Over the past few years alone, many other transcriptional regulators of the thermogenic gene program of adipocytes have been identified, and the list continues to expand [45,46,49,50]. Of particular interest are the transcription factors that function to specify/determine the brown/beige adipocyte lineage. Most notably, PRDM16, its co-regulator EHMT1, and the transcription factors, EBF2 and ZFP516, all specify the brown/beige lineage from mesenchymal precursors [51–54]. EBF2 expression defines brown/beige adipocyte precursors and coordinates with PPARγ and chromatin remodelers to directly activate the expression of *Prdm16* and other genes characteristic of the brown/beige adipocyte gene program [55]. More recently, Hiraike et al. identified nuclear factor I-A (NFIA) as a transcriptional regulator of brown adipocyte determination [56]. NFIA and PPARγ co-occupy brown adipocyte-specific enhancers. Importantly, the binding of NFIA precedes and facilitates the binding of PPARγ, leading to increased chromatin accessibility and active transcription. In this context, NFIA serves a "pioneering factor" and introduction of NFIA into myoblasts results in brown adipocyte differentiation. Many of these factors also cooperate with transcriptional co-repressors to simultaneously suppress the gene program of alternative cellular fates/states. EHMT1 activates PRDM16 and the brown adipocyte gene program while suppressing key skeletal muscle determining factors [53]. Likewise, PRMD16 interacts with CtBP1/2 to suppress the gene program characteristic of white adipocytes [57].

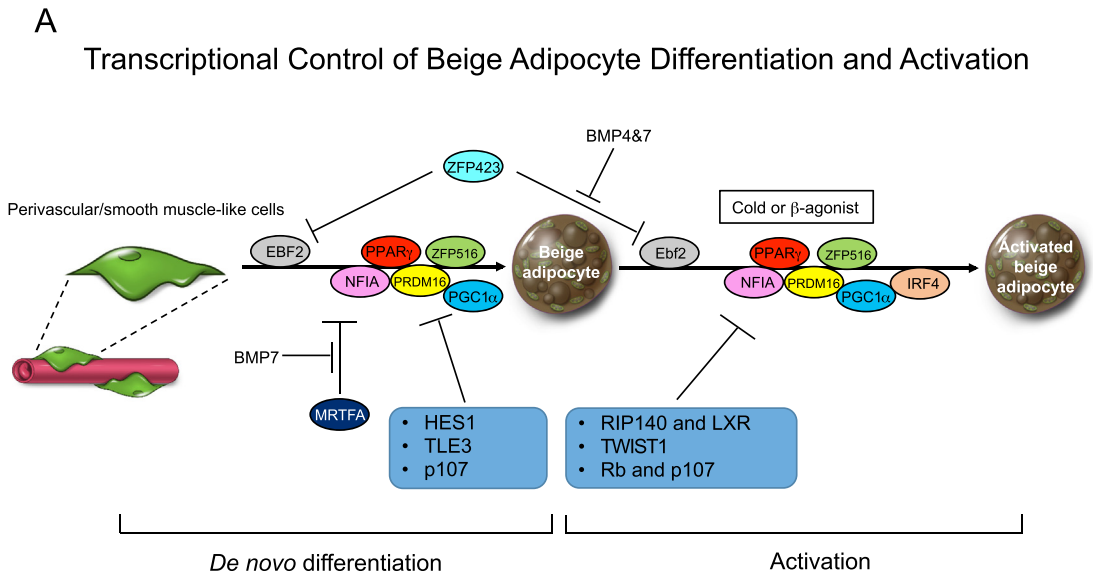
3.2. Transcriptional suppressors of the thermogenic gene program

A key feature of thermogenic adipocytes is their ability to appropriately activate and inactivate the thermogenic gene program in response to changes in environmental/physiological conditions. It is increasingly clear that integral to proper regulation of thermogenic gene is the presence of transcriptional inhibitors of the thermogenic gene program. Such "molecular brakes" may be turned on when the thermogenic gene program requires suppression, or these inhibitors may themselves be silenced in order to facilitate transcriptional activation of *Ucp1* and other thermogenic genes.

For instance, a number of molecular inhibitors of PGC1α activity and thermogenic gene expression in adipocytes have now been described. Among the first to be identified are Retinoblastoma protein (Rb) and p107 [58–60]. These proteins negatively regulate *Ppargc1a* (PGC1α) promoter activity. In mice lacking p107, brown adipose tissue develops at the expense of white adipose tissue. Receptor-interacting protein 140 (RIP140) directly binds to PGC1α and suppresses its transcriptional activity. Liver X receptor (LXR) is a direct transcriptional inhibitor of cyclic AMP-dependent *Ucp1* gene expression [61]. LXR binds to the *Ucp1* promoter and recruits RIP140 to an LXRα binding site that overlaps with the PPARγ/PGC1α response element. This mechanism limits the recruitment of the PPARγ/PGC1α complex to the *Ucp1* promoter. Likewise, SRC2, a member of the steroid receptor co-activator family, and TWIST 1 (Twist-related protein 1) also bind to PGC1α and repress its transcriptional activity [62,63]. The activities of PRDM16 complexes are also highly regulated. TLE3 is a white fat-selective cofactor that physically interacts with PPARγ and disrupts the PPARγ-PRDM16 interaction [64]. Notably, TLE3 activates PPARγ at white-adipocyte gene loci while suppressing PRDM16 activity during beige adipogenesis. Genetic inactivation of these negative regulators in mice results in the induction of thermogenic gene expression and adipose tissue thermogenesis. Moreover, Lodhi et al. recently reported that PexRAP, a peroxisomal lipid synthetic enzyme, inhibits brown adipocyte gene expression by disrupting the PPARγ-PRDM16 interaction [65].

A number of important signaling cascades driving the activation of adipocyte thermogenesis are regulated themselves by specific transcriptional regulators [48,66]. These signaling pathways can also converge on transcriptional inhibitors of thermogenic gene expression and/or beige adipogenesis. Several lines of evidence support a role for BMP signaling in promoting brown/beige adipogenesis and thermogenesis [67,68]. Farmer and colleagues recently elucidated a signaling cascade mediating BMP7-induced beige adipogenesis [69]. A critical node in this pathway centers on the inhibition of myocardin-related transcription factor A (MRTFA), which is a globular actin-controlled transcriptional co-regulator of serum response factor (SRF). Upon treatment of adipocyte progenitors with BMP7, the SRF–MRTFA complex is repressed, thereby facilitating beige adipocyte differentiation at the expense of smooth muscle-like cells. Notably, overexpression of SRF or MRTFA inhibits beige adipogenesis in vitro and induces smooth muscle differentiation from mesenchymal cells. This observation provides functional data to support the aforementioned lineage tracing studies suggesting a smooth muscle-like origin of recruited beige adipocytes. Moreover, the impact of *Mrtfa* deficiency was selective to beige adipocytes; classic brown adipocytes were not overtly impacted in the *Mrtfa* knockout mice. These data further support the notion that classical brown and inguinal beige adipocytes are controlled by at least partially distinct molecular and developmental mechanisms. Likewise, the Notch signaling-activated transcriptional repressor, HES1, selectively represses *Prdm16* and *Ppargc1a* gene expression during beige, but not brown, adipogenesis [70].

Many of the aforementioned transcriptional inhibitors of adipocyte thermogenesis operate at the level of the brown or beige precursor to effect lineage commitment and/or differentiation. Recent studies from our group focusing on the transcription factor, ZFP423, have indicated



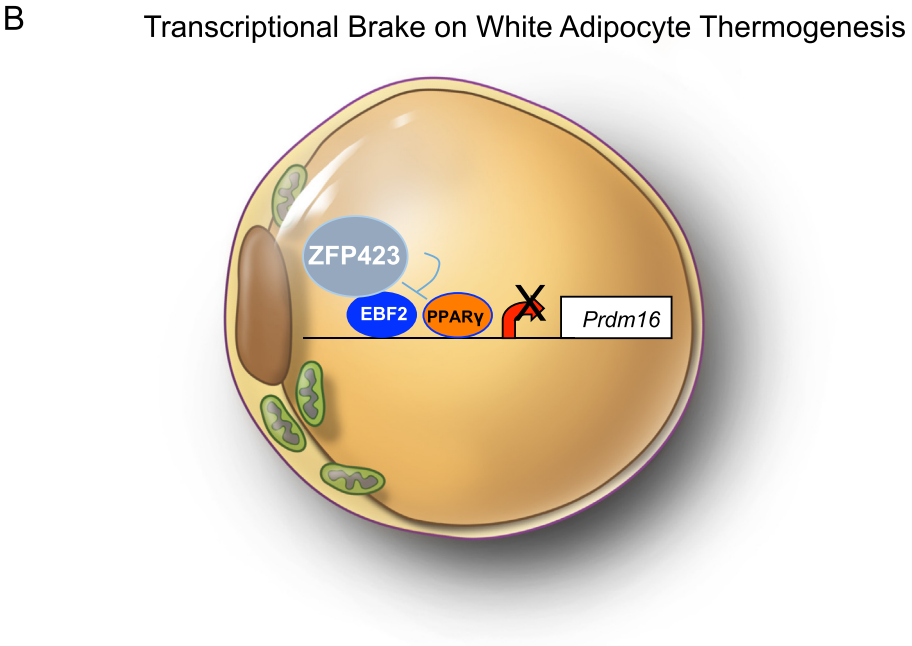


Fig. 1. Transcriptional regulation of adipocyte thermogenesis.
(A) In adult animals, beige adipocytes emerge, at least in part, from smooth muscle-like progenitors. Key transcriptional regulators driving the formation and activation of beige adipocytes in vivo are depicted (EBF2, PPARγ, ZFP516, PRDM16, NFIA, PGC1α, IRF4). A more complete list can be found in recent reviews [45,49]. Transcriptional suppressors of the thermogenic gene program are also depicted (MRTFA, ZFP423, HES1, TLE3, p107, RIP140, LXR, TWIST1, and Rb/p107). (B) Mature white adipocytes maintain their cellular identity through the actions of transcriptional brakes on beige determination factors. ZFP423 is a transcriptional co-repressor of EBF2, a brown/beige adipocyte determination factor.

that mature white adipocytes harbor mechanisms to actively suppress the global gene program characteristic of brown and beige fat cells (Fig. 1B). Importantly, the thermogenic potential of mature white adipocytes can be unveiled under physiological conditions by removing *Zfp423* from mature adipocytes [34].

ZFP423 (also referred to as OAZ, ROAZ, or EBFAZ) is a multi-C2H2 zinc-finger transcription factor that was originally identified by Massague and colleagues as a SMAD-interacting partner in BMP-signaling cascade [71]. Independently, Reed and colleagues identified ZFP423 as an interacting partner of EBF transcription factors in the olfactory epithelium [72]. ZFP423 plays a critical role in neuronal precursor differentiation, regulation of DNA damage response, and thus is linked genetically to abnormalities in neuronal development and cancer [73–77]. We previously identified *Zfp423* expression as a molecular marker and functional regulator of committed preadipocytes [78]. ZFP423 regulates preadipocyte levels of *Pparg*, and is required for

proper subcutaneous WAT development in vivo [79].

Zfp423 is also expressed in mature adipocytes, albeit at higher levels in white adipocytes than in brown adipocytes [34]. To explore the functional requirements of *Zfp423* in mature adipocytes, we employed an inducible system developed by Scherer and colleagues in which Cre expression is directed to *adiponectin*-expressing adipocytes in a doxycycline-inducible manner [36]. Remarkably, almost all inguinal adipocytes of the *Zfp423*-deficient mice appear as multilocular beige cells within four weeks after inducing *Zfp423* ablation in adult mice [34]

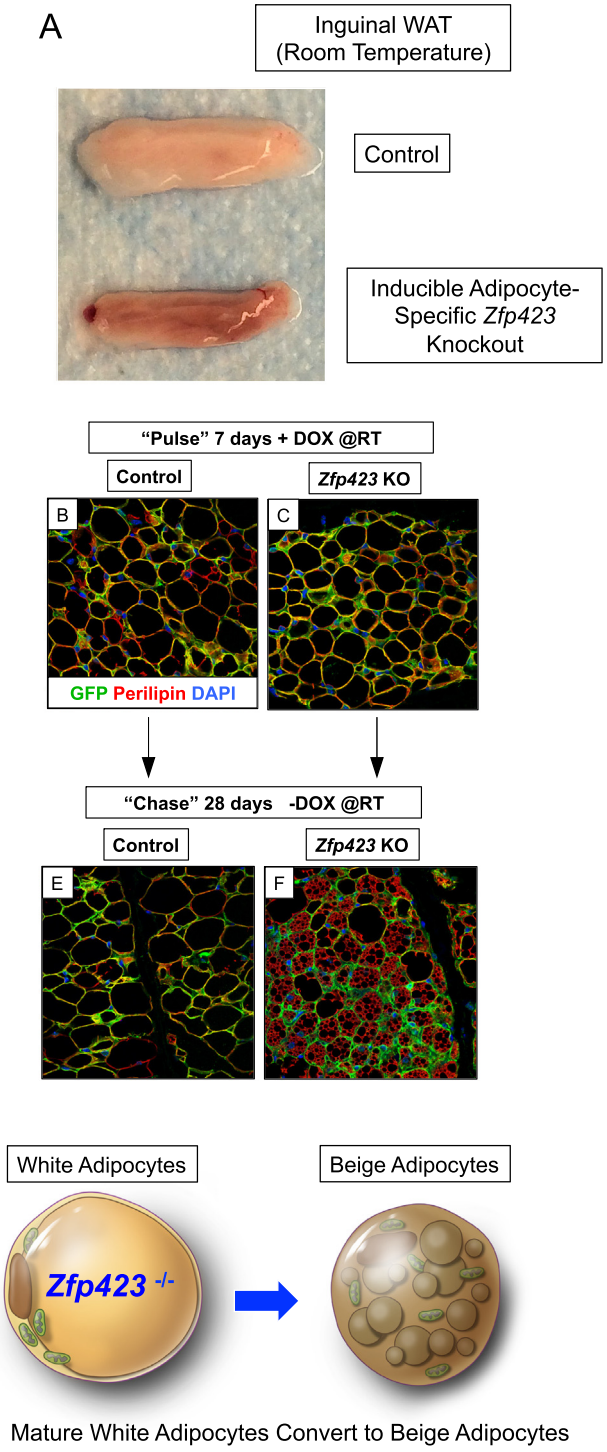


Fig. 2. *Zfp423* inactivation in mature white adipocytes triggers a white to beige adipocyte lineage conversion.
(A) Photograph of inguinal WAT depot isolated from control [*Adiponectin*^{rtTA}; *Zfp423*$^{loxP/loxP}$ mice (Cre negative)] and doxycycline-inducible adipocyte-specific *Zfp423* knockout mice [*Adiponectin*^{rtTA}; *TRE-Cre*; *Zfp423*$^{loxP/loxP}$ mice] 4 weeks after inducing *Zfp423* deletion in adult animals held at room temperature.
(B–E) The Cre-dependent *Rosa26R*$^{mT/mG}$ reporter allele was reconstituted to the control and adipocyte-specific *Zfp423* knockout background, allowing for indelible membrane GFP (mGFP) labeling of *Zfp423*-deficient adipocytes in the inguinal WAT depot. Mice were kept on normal chow until 8 weeks of age before switching to doxycycline (DOX)-containing chow for 7 days ("Pulse"). After the labeling period, mGFP is expressed in nearly all adipocytes (B, C) and *Zfp423* is inactivated. Mice were then switched back to a standard chow diet (devoid of DOX) for another 21 days ("Chase"). After the 21-day period, the presence of GFP+ multilocular adipocytes (perilipin+) indicates beige cells that arise directly from mature adipocytes targeted during the pulse-labeling period (D, E). The confocal images of Perilipin (red) and GFP (green) expression in iWAT sections obtained from these animals were previously published [34], and duplicated here with permission from Elsevier.

(Fig. 2A). Lineage tracing and molecular analyses indicated that *Zfp423*-deficient inguinal adipocytes undergo a lineage-conversion into beige adipocytes (Fig. 2B–E). Some of these emerging multilocular adipocytes may represent "dormant" beige adipocytes being activated by *Zfp423* deletion; however, the browning we observed in the animals is widespread and can be triggered in all major WAT depots, even those depots relatively resistant to cold-induced browning (e.g. gonadal WAT). Thus, a "reprogramming" of bona fide white adipocytes is likely occurring in this model. It is important to note that while *Zfp423* deficiency is sufficient to activate the thermogenic gene program, the accumulation of functional multilocular beige adipocytes in this model appears to be dependent on some level of active β adrenergic signaling. The white to beige lineage conversion does not occur in inducible adipocyte-specific *Zfp423* knockout mice housed under thermoneutral conditions. Physiological white adipose browning highlights the importance of de novo beige adipogenesis; however, our data highlight the potential for white adipocytes in obesity to activate a thermogenic program when the "brake" is removed and an "accelerator" of thermogenesis is applied.

The mechanism by which ZFP423 regulates adipocyte differentiation per se and thermogenic gene regulation are distinct. In response to BMP signals, ZFP423 co-activates SMAD1/4 to activate *Pparg* expression and adipogenesis [78]. ZFP423 functions to suppress the thermogenic gene program in adipocytes, at least in part, by antagonizing the actions of EBF2, an aforementioned brown fat determination factor [34] (Fig. 1B). Moreover, the interaction between ZFP423 and EBF2 is regulated by BMP signaling. BMPs trigger the ZFP423/SMAD interaction while disrupting the interaction between ZFP423 and EBF2. This ZFP423-mediated cross talk between BMP/SMAD signaling and EBF2 signaling may be one mechanism by which BMPs can promote the differentiation of thermogenic adipocytes.

Adipocyte *Zfp423* gene expression in vivo appears highly regulated. It is suppressed in mature white and brown adipocytes upon cold exposure or pharmacological activation of β3 adrenergic receptors. Moreover, we find that *Zfp423* expression in BAT increases in settings where morphological/molecular "whitening" of BAT occurs (aging, thermoneutral housing conditions, diet-induced obesity). Overexpression of *Zfp423* in brown adipocytes is sufficient to suppress *Ucp1* and other genes of the thermogenic program. Collectively, these data reveal that alterations in the expression of *Zfp423* are part of the normal physiological mechanisms leading to the activation/suppression of the adipocyte thermogenic gene program. Importantly, the loss of function data here shows that *Zfp423* is a major part of the cellular mechanism by which adipocytes stay or become "white".

4. Engineering cell fate to unlock the thermogenic potential of white adipose tissue

4.1. White to beige adipocyte lineage conversion in obese mice

Gain- and loss of function animal models corresponding to many of the transcriptional regulators of adipocyte thermogenesis described above have shed tremendous insight into the potential beneficial impact of brown/beige adipocytes on nutrient homeostasis [39,80,81]. Transgenic models overexpressing *Prdm16*, *Ebf2*, *Zfp516*, or other drivers of beige adipogenesis are protected against diet-induced obesity (DIO) due to increased energy expenditure [54,82,83]. Likewise, mice deficient in *Mrtfa* more readily accumulate beige adipocytes in WAT and are protected against DIO [69]. Beyond their impact on energy balance, activated beige adipocytes appear to drive improvements in systemic glucose and lipid metabolism. It is not yet entirely clear how beige adipocytes drive improvements in nutrient homeostasis independent of their impact on body weight. One strong possibility lies in the ability of brown/beige adipocytes to produce circulating hormones and cytokines (i.e. "BAToKines") [84–87].

The metabolic phenotypes of the animal models of increased WAT "browning" have generated considerable excitement in the prospect of increasing beige cell numbers and/or activity as a means to combat obesity and type 2 diabetes in humans. However, an important limitation to many of the models is that beige adipogenesis is triggered at the onset of WAT development. These models highlight the ability of beige adipocytes to protect against the development of DIO; however, genetic evidence that WAT browning can trigger weight loss in obese mice and/or improve nutrient homeostasis has been lacking. The inducible genetic models of *Zfp423* inactivation in adipocytes begin to shed light on this issue. Consistent with other models of increased beige cell accumulation, inactivation of adipocyte *Zfp423* in lean adult mice (6–8 weeks-old) and the subsequent browning phenotype confers resistance to DIO. When *Zfp423* is activated in mildly obese mice (i.e. following 8 weeks of high fat diet feeding), the thermogenic gene program is elevated in WAT; however, multilocular adipocytes do not emerge and animals continue to gain weight and remain insulin resistant [34]. As described above, thermogenic phenotype of *Zfp423*-deficient adipocytes is dependent on β-adrenergic signaling; it is well known that obesity is associated with augmented sympathetic activity [88,89]. We reasoned that the beige cells induced by *Zfp423*-deficiency in obese mice would require a stimulus to fully activate their thermogenic function in this setting. Indeed, obese knockout animals given the β3-agonist lost a significant amount of body weight and exhibited markedly improved glucose tolerance after four weeks of treatment. This was accompanied by reduced hepatic steatosis, significant accumulation of multilocular beige adipocytes in the inguinal WAT, and improved insulin sensitivity. Obese control animals were largely resistant to the effects of the β3-receptor agonist, consistent with the notion of catecholamine resistance in obesity. These data highlight the ability of mature white adipocytes to be reprogrammed into beige-like adipocytes in obese animals. Upon activation, these beige-like thermogenic adipocytes can reverse weight gain and metabolic dysfunction triggered by HFD feeding.

4.2. Directing visceral adipocyte precursors to adopt a thermogenic fat cell fate

A notable difference between the subcutaneous inguinal WAT and visceral WAT depots in rodents is the capacity to adopt a thermogenic phenotype. Unlike subcutaneous WAT, most visceral depots in mice, particularly the gonadal and mesenteric adipose tissues, are relatively resistant to browning in response to various physiological or pharmacological stimuli. With few exceptions [90], most engineered mouse models of white adipose tissue browning exhibit beige cell accumulation in subcutaneous WAT depots and not in visceral WAT [82,83]. The resistance to browning appears to be cell autonomous; visceral adipocyte cultures are relatively resistant to activating the thermogenic gene program in response to pharmacological stimuli [17,91]. Visceral adipocytes appear to harbor mechanisms to suppress thermogenesis in order to ensure its function as a white, energy-storing, cell type.

It is notable that visceral adipocytes lacking *Zfp423* were also capable of inducing their thermogenic gene program when animals or isolated cells are stimulated pharmacologically with a β3 adrenergic receptor agonist [34]. This observation afforded the possibility of examining whether the thermogenic capacity of visceral white adipose depots can be unlocked under physiological conditions, and whether thermogenic visceral WAT would be ultimately harmful or beneficial to systemic metabolic health. We recently described two mouse models of visceral adipose tissue browning derived through selective ablation of *Zfp423* in visceral adipose precursors [91]. The first model takes advantage of the *Wt1*-Cre line. Hastie and colleagues revealed that visceral white adipocytes, but not subcutaneous or classic brown adipocytes, descend from *Wt1*-expessing progenitors and are targeted by *Wt1-Cre* [92]. Specifically, *Wt1-Cre* targets the majority of gonadal adipocyte precursors and variable numbers of precursors in other visceral depots, including the mesenteric and retroperitoneal depots. Using this Cre-line, we derived animals in which *Zfp423* inactivation occurs in visceral, but not subcutaneous, WAT or classic BAT. By weaning age, visceral WAT *Zfp423* knockout mice housed at room temperature have smaller visceral WAT depots that contain large clusters of $UCP1^+$ multilocular adipocytes. Precursors isolated from these visceral depots differentiated into functional thermogenic adipocytes. Thus, visceral white preadipocytes can be redirected in a cell-autonomous manner to a beige-like adipocyte fate through the loss of *Zfp423*. The visceral browning in these animals is associated with improved cold tolerance and protection against the development of insulin resistance and hyperlipidemia in DIO. However, the degree of visceral browning that occurred in these animals did not drive any changes in overall body weight.

In a second model, we asked whether visceral mural preadipocytes in adult mice can be directed to a thermogenic cell fate, rather than a white adipocyte, in expanding visceral WAT depots of diet-induced obese animals. Visceral white adipocytes hyperplasia associated with DIO originates from cells expressing *Pdgfrb* [35,93]. Inducible deletion of *Zfp423* in *Pdgfrb*-expressing cells of adult mice led to the de novo differentiation of visceral beige-like adipocytes, rather than white adipocytes, following high-fat diet feeding [91]. This alone was not associated with improvements in glucose homeostasis; however, upon activation by β-3 adrenergic receptor agonism, these de novo differentiated beige-like adipocytes adopt a multilocular phenotype and animals exhibit improved insulin sensitivity. Together, these data provide proof of concept that the thermogenic capacity of visceral WAT can be induced under physiological conditions through removal of this molecular brake on the thermogenic gene program in adipose precursors. These data also highlight the potential of visceral WAT, much like subcutaneous WAT, to improve nutrient homeostasis in obesity when a thermogenic beige-like phenotype is induced.

A number of key questions about visceral WAT browning remain unresolved. *Zfp423*-deficient visceral WAT depots express many of the mitochondrial components and thermogenic genes found in subcutaneous inguinal beige adipocytes and classical brown adipocytes; however, it is still unclear whether visceral $UCP1^+$ cells in this model are functionally similar to the other well-studied thermogenic adipocytes. In fact, Kirichok and colleagues reported the existence of two distinct types of thermogenic beige adipocytes present in visceral depots of mice stimulated with the β3-adrenergic receptor agonist for 10 days [94]. Bertholet et al. revealed that most thermogenic adipocytes in visceral WAT are devoid of UCP1 protein and instead employ futile creatine cycling for thermogenesis. *Zfp423*-deficient visceral adipocytes express UCP1 protein; however, the precise contribution of UCP1-mediated uncoupling vs. UCP1-independent mechanisms in these

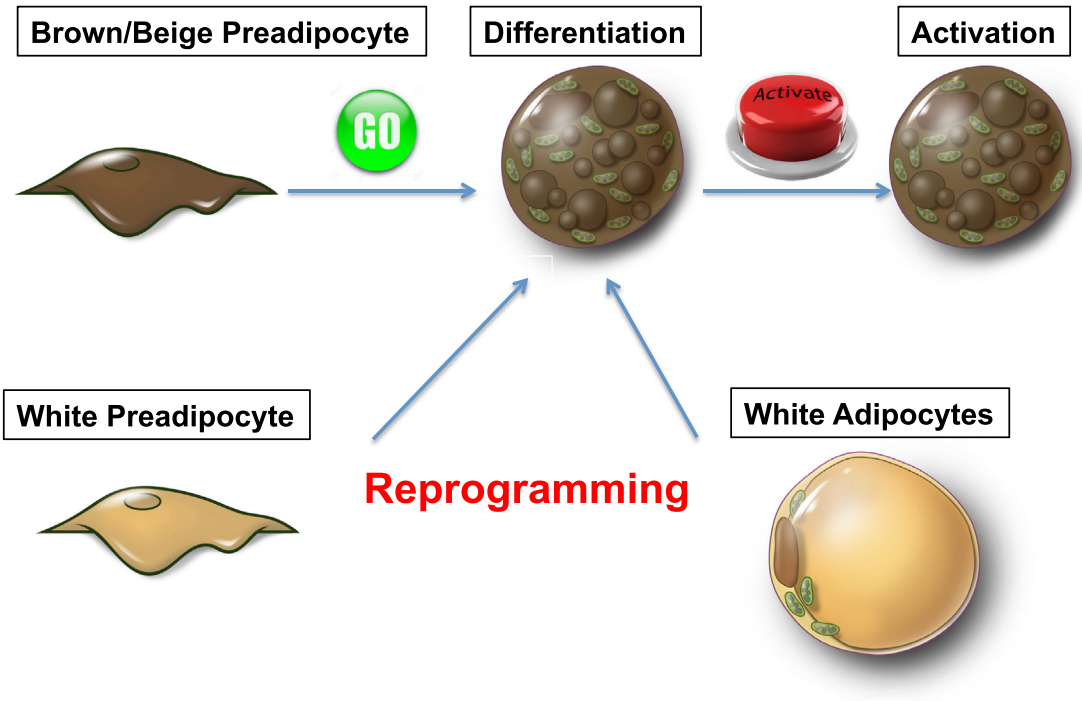


Fig. 3. Potential avenues to increase the abundance of thermogenic adipocytes in obesity.
Animal models highlight a number of potential strategies to increase the abundance of activated brown/beige thermogenic fat cells in obesity. This includes, activation of existing brown/beige adipocytes, driving de novo differentiation of brown/beige adipocytes from their respective precursors, re-directing white adipocyte precursors to adopt a thermogenic adipocyte fate, and direct conversion of existing white adipocytes into beige-like thermogenic fat cells.

cells remains unclear. Recently, Scherer and colleagues employed imaging techniques to identify previously unexplored beige-like WAT depots throughout the body [95]. As such, a number of important questions remain unanswered: Are anatomically distinct beige adipocytes functionally, molecularly, and developmentally distinct? It is notable that *Prdm16*, *Ebf2*, and other regulators of the thermogenic gene program are expressed at very low levels in visceral WAT depots. Do anatomically distinct beige adipocytes depend on the actions of distinct transcriptional regulators? Ultimately, more precise genetic tools allowing depot-specific gene targeting will be needed to unravel these questions. A deeper understanding of the heterogeneity, plasticity, and molecular control, of anatomically distinct white and brown adipose depots may lead to a better understanding of adipose tissue distribution in mammals, and suggest novel therapeutic strategies to combat visceral obesity.

5. Concluding remarks

It is now certain that adult humans have readily identifiable thermogenic adipose tissue consisting of brown and beige adipocytes that can influence glucose and lipid homeostasis [10–12,18] [13,14,96]. Nevertheless, it still remains unclear as to whether sufficient amounts of thermogenic adipose tissue are present in obese individuals to exert beneficial therapeutic effects, even when fully activated. There is tremendous interest in identifying strategies to increase the mass of functional thermogenic adipose tissue in obese patients with metabolic syndrome. The studies described here suggest, at least in principle, a number of possible strategies to increase the abundance and/or activity of thermogenic adipose tissue (Fig. 3). One possible approach to engineer thermogenic adipose tissue lies in stem cell biology; manipulation of renewable stem cell populations or patient-derived adipose stem cells combined with emerging advancements in transplantation approaches may be an option [97,98]. Moreover, a viable approach may be to target mature white adipocytes and induce a white to beige lineage conversion. Continuing efforts to identify activators of existing thermogenic adipose tissue are critical; however, efforts should also be placed on unveiling the mechanisms that function in white adipocytes to suppress the thermogenic program. Ultimately, a therapeutic approach may involve releasing the molecular brakes on thermogenesis while promoting the activity of the thermogenic machinery (e.g. β3 adrenergic receptor agonism). Identifying these transcriptional brakes on the adipocyte thermogenic gene program and their mechanisms of action may facilitate the development of novel strategies to increase energy expenditure and/or improve nutrient homeostasis in obesity.

Conflicting interests statement

The authors declare that they have no competing financial interests.

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