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Review

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Recent advances in brown adipose tissue biology

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Abstract In mammals, white adipose tissue (WAT) store energy, whereas brown adipose tissue (BAT) burns energy. As a thermogenic organ, BAT can help maintain body temperature during cold exposure. Owing to its important roles in energy metabolism and regulating triacylglycerol levels, BAT has received great attention in treating obesity and its related diseases. Recent studies have suggested that BAT may secrete factor(s)—batokines—to regulate whole-body energy metabolism. In this review, we summarize the recent advances in the formation and function of BAT, as well as molecules that regulate the activity of BAT and beige fat.

Keywords Brown adipose tissue · Batokine · Energy regulation · Beige adipocyte · Anti-obesity

1 Introduction

The obesity epidemic is a major global health problem. Obesity can induce metabolic disorders, such as type 2 diabetes, hypertension, cardiovascular disease and other related diseases [1]. Obesity develops when energy intake exceeds energy consumption, and the excessive energy is stored in white adipose tissue (WAT) as triglycerides [2].

There are mainly two different types of fat in humans and small mammals: white adipose tissue (WAT) and brown adipose tissue (BAT) [3]. The main function of WAT is to store energy; however, as a thermogenic organ, BAT is characterized by multilocular lipid droplets and is enriched in mitochondria [4]. BAT plays important roles in maintaining the body temperature of human infants and small mammals [5]. When the brown adipocytes are activated, the respiratory chain is uncoupled in the mitochondria, and the chemical energy that would be stored as ATP is converted to heat [6].

1.1 White adipose tissue

WAT is mainly divided into 2 types according to the fat distribution: visceral (VIS) fat and subcutaneous (SC) fat. Individuals with increased visceral fat show apple-shaped obesity and have a high risk of metabolic syndrome. In contrast, pear-shaped obesity, characterized by excessive subcutaneous fat, shows a low risk of metabolic syndrome [7]. Accumulating evidence has indicated that increased VIS fat has detrimental effects on metabolism. First, Mice that received SC fat tissue transplantation in the SC or VIS fat region showed beneficial effects on energy metabolism [8]. Second, aging is associated with many metabolic syndrome disorders characterized by increased VIS fat and reduced SC fat [9]. Third, obese individuals with metabolic

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disease have less SC fat than those without metabolic disease [10]. Fourth, SC fat can secrete factors such as leptin and adiponectin, which have beneficial effects on metabolism [11, 12], whereas inflammatory factors such as retinol binding protein (RBP4), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein 1 (MCP1), interleukin 8 (IL8), and interleukin 6 (IL6) are highly expressed in VIS fat [13–16]. Fifth, M1 macrophages, which are pro-inflammatory cells, are more abundant in VIS fat than in SC fat [17].

1.2 Brown adipose tissue

BAT is a thermogenic organ. It is estimated that, when BAT is maximally stimulated, 50 g of BAT on average in adult humans could contribute to 20 % of the total resting energy expenditure [18]. Cold exposure drastically activates BAT and clears excessive triglycerides in the plasma by increasing lipid uptake into BAT, resulting in increased energy metabolism and weight loss [19–21]. Because of the important role of BAT in energy expenditure, an increase in the amount and/or activity of BAT is a promising avenue to treat obesity. Studies by our group and others have shown that BAT transplantation can reverse metabolic disorders such as high fat diet (HFD)-induced obesity and type 2 diabetes [22, 23]. These lines of evidence clearly indicate that BAT would be a great target organ to treat obesity and its related diseases.

2 Functions of BAT

2.1 Thermogenesis and energy expenditure

BAT can produce heat through non-shivering thermogenesis to protect small mammals against cold environments [24]. Human infants cannot shiver to produce heat; therefore, BAT mediated heat production is important to maintain the body temperature [25]. Diet can also induce BAT thermogenesis. Scientists have found that when rats were fed a HFD, their weight gain is less than expected; therefore, they hypothesized that the excessive energy intake may have been consumed by activated brown fat [26, 27]. The activity of brown fat showed a negative correlation with body weight gain and body fat percentage. More than half of all adults may have sufficient brown fat to burn off white fat; however, brown fat is not fully activated in 97 % of adults on average [18, 28]. The naturalist Gessner [29, 30] first described BAT as being "neither fat nor flesh" in 1551. Thermogenic BAT was finally discovered in 1960s; in the 1970s, researchers began to believe that BAT is the tissue used for non-shivering thermogenesis. The function of BAT depends on the uncoupling protein (UCP1, thermogenin), which was first discovered in 1978 [31]. In 1985, the 32,000-Da protein UCP1 was cloned by Jacobsson et al. [32]. UCP1 dissipates the proton electrochemical gradient in mitochondria, and then ATP is catalyzed by ATP synthase to generate heat (Fig. 1) [2, 33]. Mice with complete loss of BAT achieved by overexpression of the diphtheria toxin fused *Ucp1* gene were extremely obese and severely insulin resistance [34]. These results further imply the important role of BAT in whole-body energy metabolism. In 1997, researchers found that *Ucp1* knockout (KO) mice are cold insensitive, but they cannot become obese when fed a HFD [35]. Conversely, Feldman et al. [36] showed that UCP1 ablation in mice leads to obesity and metabolic dysfunction when mice are housed under thermoneutral conditions (29 °C) where thermal stress was eliminated, and the mice were metabolically resting. Adipose tissue-specific overexpression of *Ucp1* in transgenic (TG) mice resulted in mice with resistance to HFD-induced obesity [37]. These results highlight that the increase in the thermogenic function of BAT may contribute to the treatment of obesity. Adaptive thermogenesis in BAT plays important roles in regulating energy balance and defending the organism from cold environments, and the loss of BAT thermogenesis would result in obesity [38]. During the past few years, scientists have found that adult humans also have functional BAT. Remarkably, after cold stimulation at 19 °C, the skin temperature of the supraclavicular region, which is close to the BAT region in the BAT-positive individuals, is higher than that in BAT-negative individuals [39]. These lines of evidence highlight that BAT is an important tissue in regulating energy expenditure.

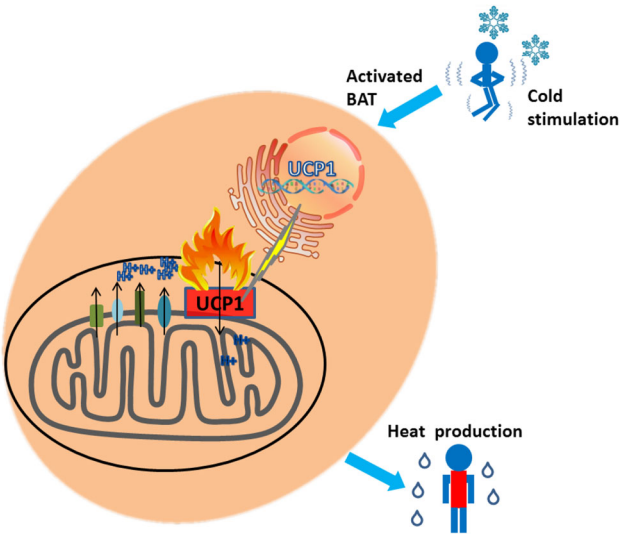

Fig. 1 (Color online) The mechanism of BAT thermogenesis

2.2 Blood glucose regulation

In addition to the thermogenic function of BAT, increasing evidence has shown that BAT participates in blood glucose regulation. Reports have demonstrated some *Ucp1*$^{+}$ brown fat-like cells emerging in SC fat tissue in *aP2-Prdm16* TG mice. In addition, glucose metabolism was improved in the *aP2-Prdm16* TG mice compared with that in the wild type (WT) mice after a HFD [40]. Ning et al. [41] found that BAT-specific marker genes are highly expressed in BAT of *Lgr4* KO mice, and some brown fat-like cells have also been observed in WAT. Importantly, *Lgr4* KO mice showed improved insulin sensitivity and glucose metabolism. Cyclooxygenase 2 (*Cox2*) TG mice showed improved energy metabolism after being anti-obesity induced by a HFD [42]. Nishio et al. [43] programmed brown adipocytes from human pluripotent stem cells (hESC-derived BAs, hESCdBAs), and they expressed brown adipocyte marker genes such as *Ucp1* and *Prdm16*. Notably, hESCdBA-transplanted mice showed improved lipid and glucose metabolism. Increasing glucose utilization has been noted in human BAT after cold exposure [21]. Interestingly, cold exposure strikingly increased the glucose uptake in BAT compared with that in muscle [44]. All together, these results demonstrated that BAT could consume a large portion of glucose.

2.3 Cytokine secretion

A large body of evidence has shown that WAT can secrete adipokines such as leptin and adiponectin to regulate energy metabolism. Most recently, BAT has also been shown to secrete molecules called batokines. Batokines regulate whole-body metabolism through the activation of BAT or other tissue-like muscle and WAT. Fibroblast growth factor 2 (FGF2), which is stimulated by norepinephrine (NE), can regulate cell proliferation and capillary growth [45]. Fibroblast growth factor 21 (FGF21) was reported to be secreted from BAT during cold stimulation and can regulate whole-body metabolism [46]. BAT-secreted vascular endothelial cell growth factor (VEGF) and angiotensinogen could regulate blood vessel growth in BAT in response to sympathetic stimulation [47]. Another potentially important batokine is IL6 [48, 49]. Mice transplanted with BAT from healthy donor mice were resistant to obesity; however, transplantation of BAT in *Il6*$^{-/-}$ mice resulted in obesity after a HFD [23]. Our group found that the transplanted BAT showed dramatically decreased BAT-specific gene expression such as decreased *Ucp1* and *Prdm16* expression. Therefore, we hypothesized that some batokines secreted by BAT may play roles in regulating energy metabolism [22].

3 Origin and development of BAT

Investigators have found that white adipocytes and brown adipocytes share similarities in gene expression, cell morphology and lipid metabolism; thus, it was assumed that WAT and BAT were derived from a common developmental origin [7, 50, 51]. However, many intriguing studies have recently demonstrated that classical BAT and muscle come from a common precursor. In 2008, using the DNA microarray technique, Timmons et al. [52] found that BAT and muscle possessed a common myogenic transcriptional signature. Accordingly, the Seale group [53] demonstrated that brown fat cells are differentiated from the precursors that expressed the myogenic lineage marker gene-*Myf5*. Interestingly, a *CD34*$^{+}$ cell population isolated from fetal skeletal muscle cells was shown to differentiate into functional brown adipocytes *in vitro* [54]. Another study showed that the dermis, muscle and BAT arose from cells expressing engrailed-1 (*En-1*) in the central dermomyotome [55]. It is clear that BAT and skeletal muscle come from *Myf5*-positive cells (Fig. 2). The development of BAT is regulated by transcription factors, many types of cytokines and hormones. Here, we describe several important systemic factors that affect brown fat development.

3.1 Thyroid hormone

Thyroid hormone (TH) participates in both the development and function of BAT. There are 2 thyroid hormone analogs: triiodothyronine (T3) and thyroxine (T4). T3 is the active form produced by the deiodination of T4 by deiodinase activation [56, 57]. Two types of deiodinases are responsible for this process: iodothyronine deiodinase type-1 (Dio1) and iodothyronine deiodinase type-2 (Dio2) [57]. T3 binds thyroid hormone nuclear receptors (TR) to mediate its biology activity, and 4 TR isoforms primarily bind to T3: TRα1, TRβ1, TRβ2, and TRβ3 [56]. Silva and Larsen [58] found that cold exposure or NE can induce T3 production in BAT by stimulating Dio2. The adaptive thermogenesis of BAT in hypothyroid animals is impaired [59, 60], highlighting the importance of TH in BAT function. In the presence of T3, NE promotes the synthesis of UCP1 in BAT by binding to the beta-3 noradrenergic receptor. However, the absence of T3 inhibits UCP1 synthesis, leading to hypothermia [61]. Under thermoneutral conditions, TH synergistically interacts with the sympatho-adrenal system to stimulate thermogenesis [62]. Euthyroid rats treated with NE exhibited a 2- to 3-fold increase in *Ucp1* mRNA expression in BAT. By contrast, the level of *Ucp1* did not increase when the same treatment was applied to hypothyroid rats [63]. Hypothermia was often observed in hypothyroid rodents after cold exposure. Interestingly, hypothermia can be promptly corrected within 24–48 hours when hypothyroid rodents were

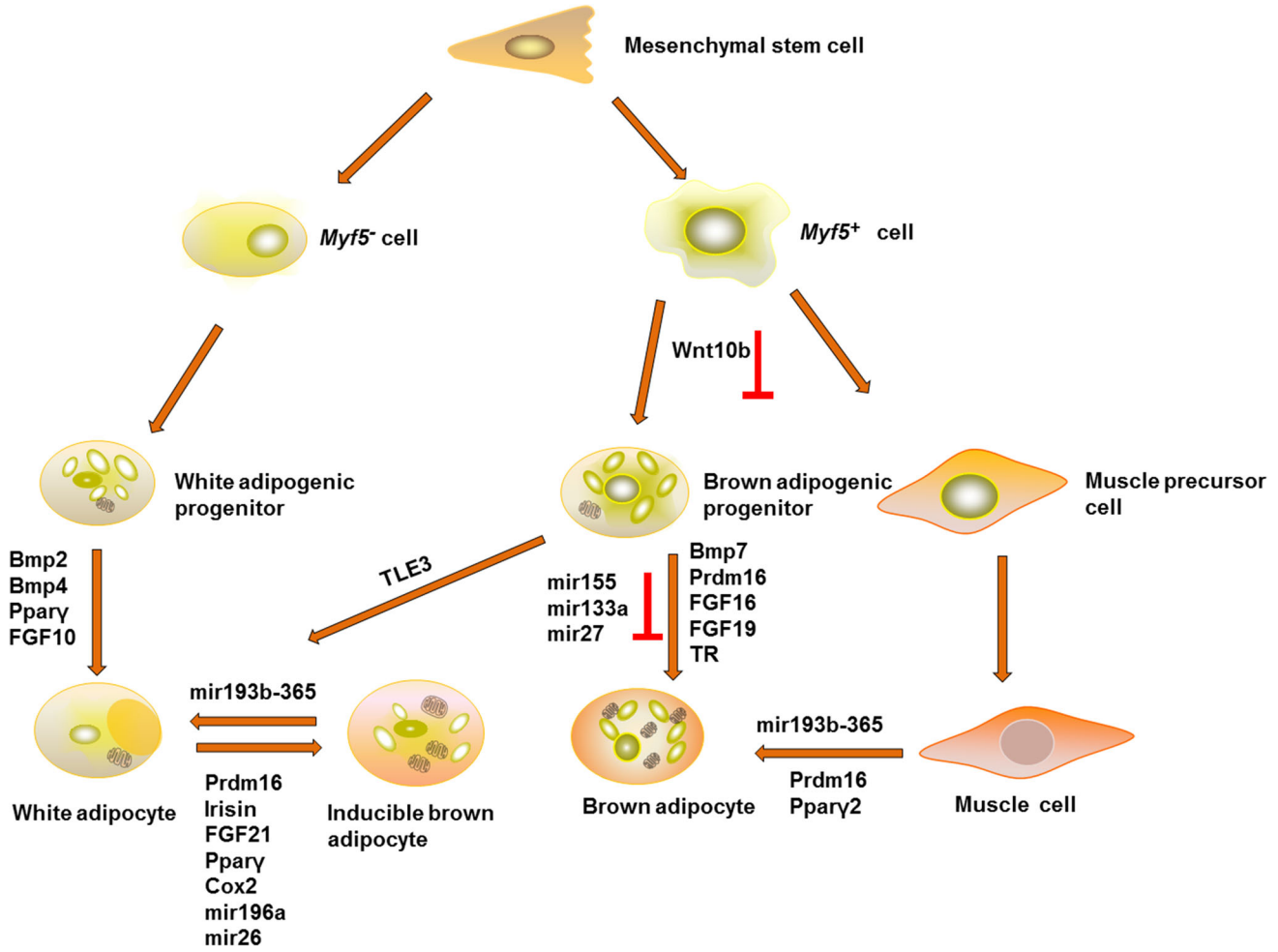

Fig. 2 (Color online) The model of BAT differentiation

given T4 to normalize the thyroid status [60]. Consistently, *TRα* KO mice are cold intolerant and exhibit impaired BAT thermogenesis when treated with NE. The latter finding suggests that TRα is important for the thermogenic response to NE stimulation [64]. Conversely, *Dio2* KO mice showed an impaired thermogenic response to cold exposure. More importantly, under thermoneutral conditions such as 30 °C, the *Dio2* KO mice became obese and glucose intolerant after 60 days of HFD treatment compared with the WT mice [65]. These results emphasize the important role of the TH axis in the regulation of BAT function.

3.2 Prostaglandin (PG)

Prostaglandins are important hormones derived from arachidonic acid, which is a 20-carbon unsaturated fatty acid [66]. PG production depends on the activity of cyclooxygenases (COXs), which catalyze the important step in PG synthesis. There are 2 main COXs: COX1 and COX2—and both have peroxidase and cyclooxygenase activity [67]. The COX1 enzyme is thought to be produced constitutively because it is found to be expressed in nearly all tissues, whereas COX2 is an inducible enzyme [68]. Many studies have found that COX plays an important role in whole-body energy homeostasis. For example, inhibition of COX was shown to reverse weight loss and improve energy expenditure in both cancer patients and tumor-bearing mice model [69, 70], and $Cox2^{+/-}$ mice are obese compared with WT mice [71]. In addition, *Cox2* TG mice showed increased PG levels in WAT, showed no obesity, had lower levels of free fatty acids, and had brown-like adipocytes in the intra-abdominal WAT [42]. Madsen et al. [72] found that induction of *Ucp1* expression in WAT depends on COX activity.

3.3 Bone morphogenetic protein (BMP)

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-α (TGF-α) superfamily. BMPs

are required for the development and function of many other tissues such as bone, cartilage, kidney, skin, teeth, and lung [73]. In addition, BMPs can regulate adipogenesis. Tang et al. [74] found that BMP2 and BMP4 can induce C3H10T1/2 pluripotent stem cells to differentiate into adipocytes. There is report demonstrated a striking reduction of WAT mass in mice lacking Schnurri-2, which is an important mediator in the BMP2 signaling pathway [75]. Adipose tissue-specific *Bmp4* TG mice displayed an increased metabolic rate and improved insulin sensitivity and were resistant to HFD-induced obesity compared with WT mice [76]. The Tseng group [3] demonstrated that C3H10T1/2 cells could be differentiated into the brown adipocyte lineage by BMP7. Strikingly, adenovirus-mediated overexpression of *Bmp7* in the mice induced an increase in whole-body energy expenditure and reduced body weight gain. In contrast, the function and development of BAT is compromised in $Bmp7^{-/-}$ mice [77]. In 2012, Whittle et al. [78] found that BMP8b functioned in the regulation of BAT thermogenesis, and $Bmp8b^{-/-}$ mice displayed impaired thermogenesis, reduced energy expenditure and increased body weight. Additionally, mice treated with a BMP-9 derivative (MB109) were resistant to obesity [79]. Thus, BMP family members play important roles in the development and function of BAT.

3.4 Fibroblast growth factor (FGF)

The FGF family comprises approximately 23 members that play important roles in angiogenesis and wound repair [80, 81]. Sakaue et al. [82] found that the development of subcutaneous WAT was markedly impaired in $Fgf10^{-/-}$ neonate mice. In the rat, *Fgf16* was found to be highly expressed in BAT at embryonic days 17.5–19.5 [83], suggesting that FGF16 might play important roles in embryonic BAT development. Interestingly, *Fgf19* TG mice displayed higher energy expenditure and increased glucose tolerance due to a dramatic increase in the BAT mass [84]. The *Fgf21* mRNA levels in BAT increased significantly when the mice were treated with short-term cold exposure or β3-adrenergic stimulation [85]. In parallel, *Fgf21* TG mice were resistant to obesity and had a large amount of BAT and smaller subcutaneous adipocytes [86]. In line with this finding, Fisher et al. [87] found that FGF21 could induce the emergence of BAT-like cells in WAT. These studies show that FGF plays important roles in BAT development and function.

3.5 Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α)

Puigserver et al. [88] first cloned *Pgc1* from a brown fat cDNA library and found that, under cold stimulation, *Pgc1α* mRNA is highly increased in BAT and skeletal muscle of mice; *in vitro*, ectopic expression of *Pgc1α* in 3T3-F442A cells was shown to induce the expression of *Ucp1* and mitochondria-related genes. $Pgc1\alpha^{-/-}$ mice showed abnormal BAT and reduced adaptive thermogenic capacity in a cold environment [89]. Furthermore, Uldry et al. [90] found that PGC1α was not required for brown preadipocyte differentiation, but it was essential for the thermogenic activation of brown adipocytes. Importantly, skeletal muscle-specific *Pgc1α* TG (*MCK-Pgc1α*) mice showed improvement in insulin signaling in aged mice compared with WT mice [91].

3.6 PRDM16

Seale et al. [1] screened the transcribed murine transcriptional components in BAT and WAT cells using global expression analysis and found that the mRNA expression of *Prdm16* is approximately 15-fold enriched in BAT compared with that in WAT. PRDM16 is a PR (PRD1-BF1-RIZ1 homologous) domain-containing protein that belongs to the *Evi1* gene family [92]. Loss of PRDM16 in BAT preadipocytes induced the cells to differentiate into skeletal myoblasts. Conversely, ectopic expression of *Prdm16* in skeletal myoblasts promoted conversion of muscle cells into brown fat cells [53]. Mechanistically, PRDM16 regulates the switch of myoblasts to brown fat cells by forming a complex with C/EBPβ; formation of this complex leads to the expression of *Pparγ* and *Pgc1α*, which are key regulators of brown fat programming [93]. Brown-like adipocytes can be found in SC fat tissue of *aP2-Prdm16* TG mice, and the TG mice showed improved glucose tolerance, increased energy expenditure and reduced body weight gain upon HFD feeding [40]. Finally, PRDM16 can interact with PGC1α/β to activate brown fat-specific genes, and it can form a complex with CtBPs to suppress white fat gene expression [94].

3.7 MicroRNA

MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression, and they have been shown to participate in the regulation of various biological processes via destabilization of mRNA and inhibition of target mRNA translation [95]. MiRNAs were reported to regulate brown fat cell development. Sun et al. [96] showed that the miRNA cluster miR193b–365, which is abundant in BAT, is important for brown fat differentiation. Brown preadipocyte differentiation is impaired when miR193b and/or miR365 are blocked, and myogenic-specific gene expression is induced; in contrast, under adipogenic conditions, ectopic expression of *miR193b* can differentiate C2C12 myoblasts into brown adipocytes. MiR133a regulates

adipocyte browning by targeting the 3′ UTR of *Prdm16*, and BAT adipogenesis is inhibited by overexpression of *miR133a*. More importantly, *miR133a*$^{-/-}$ mice displayed elevated brown and thermogenic gene expression in SC adipose tissue [97]. Additionally, miR27 was found to be down-regulated in BAT and SC adipose tissue after cold exposure, and it can inhibit brown adipogenesis in BAT and SC preadipocytes. Mechanistically, miR27 may regulate BAT development by targeting some BAT-specific genes such as *Prdm16*, *Pparα* [98]. Further study has shown that *miR196a* is up-regulated in WAT when mice are treated with cold exposure or β-adrenergic stimulation. An abundance of brown adipocyte-like cells can be found in WAT in *miR196a* TG mice, which showed increased energy expenditure and resistant to obesity. MiR196a can induce brown adipogenesis by indirectly increasing C/EBPβ, which is essential for brown fat development [99]. MiR155 was also enriched in BAT, and blockade of *miR155* induced brown adipocyte differentiation and promoted browning in white adipocytes. Thus, *miR155*$^{-/-}$ mice exhibited increased BAT function. By contrast, BAT function is impaired in *miR155* TG mice. MiR155 regulates brown adipogenesis by forming a bistable feedback loop with C/EBPβ [100]. Karbiener et al. [101] also found that the miR26 family is involved in human white and brown adipocyte differentiation: miR26 was increased in WAT upon cold exposure, and miR26a significantly up-regulated *Ucp1* expression in WAT and promoted energy dissipation. Thus, accumulating evidence has indicated that various miRNAs are involved in brown adipocyte development and function, and microRNA-mediated gene therapy might be a great option for treating obesity and its related diseases.

4 Beige adipocytes

Studies have demonstrated that brown and white adipocytes are derived from different lineages: brown adipocytes come from a common *Myf5*$^{+}$ precursor of myogenic cells [53]. *Ucp1*-expressing cells are known to adopt a multilocular appearance in WAT of mice treated with cold exposure or β3-adrenoceptor agonists [102, 103]. The brown adipocytes appearing in WAT are often called "inducible, beige, or brite". Consequently, the Spiegelman group [104] found that the brown, white, and beige cells are derived from different precursors. Compared with visceral adipocytes, the gene expression profiles in subcutaneous adipocytes are similar to those in classical brown fat cells, and *Ucp1* gene expression profiles in the beige lines are similar to those observed in the interscapular BAT (iBAT) cell lines upon cAMP stimulation. Additionally, Vitali et al. [105] demonstrated that the beige adipocytes are enriched in inguinal WAT, and the white adipocytes can transform into beige adipocytes. Wang et al. [106] showed that the beige adipocytes in white adipose tissue mainly arise from *de novo* adipogenesis rather than transdifferentiation from mature white adipocytes. Recently, several landmark studies have discovered active BAT in adult humans. In those studies, the researchers proved the existence of brown fat in adults using positron-emission tomography and computed tomography (PET-CT), which uses ^{18}F-fluorodeoxyglucose (^{18}F-FDG) as a radioactive tracer. Under normal temperature conditions, the rate of functional brown fat in the human body is very low: 3.1 % in males and 7.5 % in females. However, under cold stimulation, functional brown fat can be detected in almost all individuals; brown fat activity was reduced gradually with aging, and brown fat activity decreased with the occurrence of obesity [107, 108]. Two exciting reports have confirmed that classical brown fat can be found in both adults and children. The Tseng group [109] accurately investigated that adipose tissues are different according to the depots (from the most superficial to the deepest) in the adult neck. The deeper fat displayed the classical BAT phenotype. Another study found that adipocytes in the perirenal and supraclavicular regions, in contrast to classical iBAT in infants, showed iBAT morphology and molecular characteristics [110]. These data suggest that there are two types of functional brown fat tissues in human body and that they may play important roles in the process of preventing obesity and its related metabolic diseases.

5 Activation of BAT and beige adipocytes

With the discovery of the existence of functional BAT in adults and the potential role of BAT in protecting against obesity and its related diseases, a major question arises: how can we activate BAT and beige fat? In this section, we will discuss some factors that can regulate both BAT and beige fat activation.

5.1 Cold exposure

Cold exposure is now believed to be the safest way to activate BAT, thereby significantly increasing thermogenesis-related protein expression in BAT [111]. The weight of iBAT and the energy expenditure capacity increased upon cold stimulation [112]. Accumulation of beige adipocytes occurs in WAT when mice are treated with cold exposure [105]. Using PET-CT analysis, Yoneshiro et al. [20] found that the energy expenditure is positively correlated with the activity of BAT: the activity of BAT was stimulated, as well as glucose utilization, in the participants under cold stimulation, and the participants showed a reduced weight with 6 weeks of cold exposure (17 °C/two

hours a day). Ouellet et al. [21] and Muzik et al. [113] used dynamic oxygen^{15} (^{15}O) PET imaging and ^{11}C-acetate imaging to study BAT thermogenesis in adults under cold stimulation. Acetate can be used to measure the oxidative metabolism of tissue and oxygen can reflect the oxygen intake in the tissue. Those studies confirmed that cold exposure could activate brown fat. Recently, Anouk et al. [114] showed that, after 10 days of cold stimulation, brown fat activity and non-shivering thermogenesis (NST) in the body increased. It is known that the sympathetic nervous system regulates the stimulation of BAT under a cold environment [115]. The sympathetic nervous system can activate BAT by releasing NE. The function of NE is mediated by the β-adrenergic receptor (β-AR) system [6]. Mice that lack the three receptors become obese [116], further implicating the importance of the sympathetic nervous system in BAT activation.

5.2 Small molecular compounds

Epinephrine and caffeine can synergistically stimulate sympathetic nerves to release noradrenaline, which can activate brown fat, leading to increased oxygen consumption in rats [117]. Both ephedrine and cold stimulation can increase whole-body metabolism; however, unlike cold stimulation, ephedrine can induce some side effects, such as an increased heart rate and increased blood pressure [118]. Capsaicin is an ingredient in red pepper, and 6.0 mg/kg of capsaicin can enhance the oxygen consumption in experiments with rats [119]. Yoshioka et al. [120] observed an immediate increase in energy expenditure in humans after consuming red pepper compared with control subjects. A rodent study found that the animals will not respond to capsaicin stimuli when they received β-AR inhibitor treatment in advance, indicating that capsaicin might enhance body metabolism through activation of β-AR directly or indirectly [121].

5.3 Secretory factors

The function of reducing body weight gain by brain-derived neurotrophic factor (BDNF) in the ventromedial nucleus of the hypothalamus (VMH) partially depends on BAT activation [122]. BMP7 and BMP8b are important for maintaining body energy balance; both proteins can increase brown fat thermogenesis [77, 78]. Orexin is a neuropeptide that can regulate appetite and arousal. *Orexin*$^{-/-}$ mice showed impaired BAT thermogenic function and became obese. Orexin is required for brown fat precursor cell differentiation because the developmental defects of brown fat precursor cells in *Orexin*$^{-/-}$ mice can be rescued when orexin injected into these mice [123]. Atrial natriuretic peptide (ANP) can stimulate the production of the second messenger guanosine 3′,5′-monophosphate by binding to its receptor [124]. A study found that ANP can activate lipolysis in mice and humans, and ANP strongly increases *Ucp1* and *Pgc1α* expression in WAT and BAT of mice [125]. FGF21 and irisin were recently identified as regulators of brown fat activity; FGF21 is mainly expressed in the liver, whereas irisin is expressed in skeletal muscle. Both proteins can increase brown fat-specific gene expression in white adipose tissue, further promoting body energy metabolism and weight loss [87, 126]. *Pgc1α* was shown to be induced by exercise and can stimulate mitochondria biogenesis in muscle. Muscle-specific *Pgc1α* TG mice showed that BAT-specific genes are dramatically induced in white adipose tissue. Using gene expression array and bioinformatics approaches, Boström et al. [126] demonstrated that PGC1α stimulates the expression of the muscle membrane protein FNDC5. Furthermore, the authors found that the *Fndc5* coding protein irisin is secreted into the blood, and then activates subcutaneous adipose tissue browning processes and *Ucp1* expression. Moreover, systemic irisin treatment increased whole-body energy metabolism. The identification of irisin as a new hormone that increases energy metabolism could open up a new avenue to treat obesity and related diseases such as type 2 diabetes. The BAT transplantation study found that IL6 secreted from brown fat promotes the activity of intrinsic brown fat in mice by increasing the levels of serum FGF21 [23]. Those studies illustrate that secretory factors can be therapeutic targets for the prevention of obesity by regulating the activity of brown fat and inducing white fat browning.

6 Prospects of future study

Many promising studies have shown BAT to play potential roles in body energy metabolism regulation [127, 128]. Thus, BAT may be a promising target to treat obesity and its related diseases. Because BAT is found in adults [18, 107, 129, 130], and BAT activity has a negative correlation with BMI and age [130, 131], researchers have been inspired to eliminate traditional therapeutic methods such as reducing energy intake and suppressing energy absorption and to focus more on increasing energy consumption. If BAT can be activated efficiently to burn redundant white fat *in vivo* or trans-differentiate a portion of redundant WAT to BAT, a significant advance would be made in the prevention and treatment of obesity. It has been demonstrated that WAT may partially convert to BAT under controlled conditions such as cold stimulation [132] or drug treatment such as rosiglitazone and β-adrenergic agonists [133–135]. However, thus far, such agents have demonstrated too many unwanted side effects, and further

investigations in clinical settings are needed [136, 137]. Most recently, C/EBPβ and PRDM16 were demonstrated to be important transcriptional factors that could convert murine fibroblasts to brown adipocytes [93]. In addition, some microRNAs (e.g., microRNA196a) could re-program fibroblasts into brown adipocytes [99]. Nevertheless, it remains unknown, which factors are involved in the activation of BAT, and the trans-differentiation techniques and efficiency of reprogramming into brown adipocytes are still under way. The long-term goal of our research is to identify proteins that can activate BAT, the results of which may lead to the development of a new class of anti-obesity/anti-diabetes drugs. Until now, many exciting results have come from animal experiments, and many differences have been found between human and rodents. Thus, it will be essential to study the BAT activation mechanism in humans in the future. Since Konrad Gesner [29] found brown fat in 1551, no direct study has shown that brown fat is involved in whole-body energy metabolism. Stanford et al. [23] and Liu et al. [22] proved almost at the same time that brown fat transplantation can improve original obesity and developing obesity. However, the underlying specific molecular mechanism has not been elucidated. Therefore, understanding the molecular mechanism of brown fat activation and finding an effective way to activate brown fat can provide a new method to prevent or treat obesity. Studying the activation mechanism of brown fat will be worthwhile for a period of time in the future.

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