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Transcription Regulators and Hormones Involved in the Development of Brown Fat and White Fat Browning

----- Transcriptional and Hormonal control of brown/beige fat development

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Abstract

The high prevalence of obesity and related metabolic complications has inspired research on adipose tissues. Three kinds of adipose tissues are identified in mammals: brown adipose tissue (BAT), beige or brite adipose tissue and white adipose tissue (WAT). Beige adipocytes share some characteristics with brown adipocytes such as the expression of *UCP1*. Beige adipocytes can be activated by environmental stimuli or pharmacological treatment, and this change is accompanied by an increase in energy consumption. This process is called white browning, and it facilitates the maintenance of a lean and healthy phenotype. Thus, promoting beige adipocyte development in WAT shows promise as a new strategy in treating obesity and related metabolic consequences. In this review, we summarized the current understanding of the regulators and hormones that participate in the development of brown fat and white fat browning.

Keywords brown adipose, beige adipose, browning, hormones, transcription regulators.

Introduction

Historically, mammalian adipose tissue has been divided into two types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the most common adipose tissue, and it mainly distributes subcutaneously and viscerally; white adipocytes contain large fat droplets, in which excess energy is stored in the form of triglycerides. WAT is also an endocrine organ that secretes adipocytokines to regulate energy metabolism in the

whole body.

BAT is abundant in lower mammals and human neonates, and it distributes mainly in the neck, supraclavicular region, and around abdominal vasculature and the perirenal area. BAT appears brown or red brown, is rich in blood vessels and is tightly regulated by sympathetic nerves. Brown adipocytes contain plenty of small droplets, are rich in mitochondria and cytoplasm, and express uncoupling protein 1 (*UCP1*), which generates heat by respiratory uncoupling to maintain body temperature in cold environments or to consume excessive energy to maintain the balance of energy.

UCP1 is a biomarker of brown adipocytes (Kozak LP and Anunciado-Koza 2008). A number of *UCP1* homologues have been identified, with the main two being *UCP2* (Fleury *et al.* 1997) and *UCP3* (Vidal *et al.* 1997). *UCP2* is mainly expressed in WAT and other tissues, while *UCP3* is rich in BAT and skeletal muscle. *UCP3* was considered to be involved in mitochondrial fatty acid oxidation (Boss *et al.* 1998, Himms and Harper 2001), Hilse et al (Hilse *et al.* 2016) find that, similar to those of *UCP1, UCP3* expression levels are sensitive to body temperature, and knockout of *UCP1* can lead to a marked reduction in *UCP3* expression. However, current studies showed that *UCP3* is not involved in BAT thermogenesis in the absence of *UCP1* (Barger and Barnes 2006, Costford *et al.* 2006, Tsuboyama *et al.* 2001).

It is well accepted that some cells distributed in WAT can highly express *UCP1* upon prolonged cold stimulation, and further analysis showed that the origin of these cells is distinct from the classical brown adipocyte (Young and Arch *et al.* 1984, Cousin *et al.* 1992). In 2012, Wu J et al (Wu J *et al.* 2012) cloned these brown-like adipocytes and identified the specific gene expression profiles of these cells. These beige cells are distributed in WAT and express *UCP1* at very low levels; however, unlike classical white adipocytes, beige cells possess a remarkable ability to robustly activate *UCP1* expression. Therefore, beige adipocytes are identified as a distinct type of thermogenic adipocytes that can trigger a significant increase in respiration and energy expenditure that is equivalent to that of classical brown adipocytes.

Brown and white adipocytes originate from different progenitor cells. Brown adipocytes and skeletal muscle cells share the same progenitor lineage; brown

adipocyte progenitors are *Myf5+* and express *PRDM16* and *BMP7* during development, which drive progenitors to differentiate into mature brown adipocytes (Seale *et al.* 2008, Tseng *et al.* 2008). Although beige fat cells have some characteristics of brown adipocytes, their gene expression profiles are distinct from those of brown adipocytes (Wu J *et al.* 2012). However, the lineage of beige adipocytes is not yet fully elucidated. A previous study suggest that beige cells can either originate directly from mesodermal stem cells or trans-differentiation from mature white adipocytes (Harms and Seale 2013).

In early 1998, Guerra C et al found that white fat can change into brown fat when exposed to cold stimulation by a process termed white fat browning; however, this phenomenon is dependent on genetic background (Guerra *et al.* 1998). In addition, stimulation of BAT in rats can suppress the occurrence of obesity and type 2 diabetes mellitus (Kopecky *et al.* 1996). Recent studies (Chondronikola *et al.* 2014, Lee *et al.* 2014) showed that BAT activation facilitates an increase in glucose disposal and ameliorates insulin resistance. This improvement of glucose metabolism may reduce glucotoxicity and may also improve β-cell dysfunction.

In 2009, multiple studies reported that active BAT were detected in adult human by 18-FDG-PET-CT (Cypess *et al.* 2009, van Marken *et al.* 2009, Saito *et al.*2009). Petruzzelli et al found a phenotypic switch that prompted white fat to change to brown fat in the early stages of cachexia, suggesting that white adipocytes directly transform into brown adipocytes (Petruzzelli *et al.*2014). Moreover, animal studies confirmed that white fat cells can directly convert into brown fat cells (Himms *et al.*2000, Almind and Kahn *et al.* 2004, Groneman *et al.* 2005).

Based on the recent findings, some researchers proposed the theory of conversion between different types of adipocytes under special conditions, such as cold temperature or tumor burden; for example, while energy is sufficient, white fat cells could convert to brown or beige cells to produce heat, and to increase energy storage, beige or brown adipocytes could convert to white fat cells. We can suppress over-development of white fat cells by increasing the number of brown or beige fat cells to consume excessive calories. This method provides a new therapeutic strategy to

combat obesity. However, mechanisms of white fat browning are not well known. This review summarizes our current understanding of the transcription factors and hormones that are involved in the development of brown/beige fat and white fat browning.

1. Important regulators of brown or beige fat cells development

A large number of transcription factors are involved in the development and differentiation of brown and beige fat, some of which play a crucial role. We summarize the important transcription factors in table 1(Table1) and describe them in detail.

1.1 *PRDM16* (PR domain containing 16) *PRDM16* was first discovered and reported by Spiegelman Laboratory as a zinc finger protein that was especially expressed in BAT (Seale *et al.* 2008). The study revealed that inhibition of *PRDM16* expression resulted in an almost complete loss in BAT integrity and that overexpression of *PRDM16* in mesenchymal cells strongly activated the expression of *PGC-1α* and *UCP1*, thus revealing a brown fat cell phenotype in these cells. The authors also found that inhibiting *PRDM16* promoted the differentiation of *Myf5* positive precursor cells into muscle cells and that ectopic expression of *PRDM16* induced pre-muscle cells to differentiate into brown fat cells such that *PRDM16* was the key regulator that determined the direction of *Myf5* positive cell differentiation. Kajimura S et al (Kajimura *et al*. 2009) found that *PRDM16* interacts with *CtBP1/2* and forms a complex with a specific gene promotor in white adipocytes to inhibit the expression of white fat cell-related genes and that *CtBP1/2* can be replaced by *PGC1a*, which can effectively activate brown fat cell-related gene expression. These results indicate that *PRDM16* is a key regulator of brown adipocyte differentiation and may be a crucial factor of the adipocyte phenotypic switch.

However, adipocyte-specific *PRDM16* knockout mice did not exhibit decreased interscapular BAT mass and function (Cohen *et al.* 2014). Another study reported that *PRDM16* was dispensable for embryonic BAT development because cell lineage ablation of *PRDM16* disrupted BAT integrity; however, ablation of *PRDM16* caused upregulation of white fat cell-selective genes in adult mice (Harms *et al.* 2014),

suggesting that *PRDM16* plays a critical role in orchestrating BAT development rather than executing BAT functionality.

More recently, Qiyuan Yang et al（Yang *et al.* 2016）found that *AMPKα* mediated DNA demethylation in the *PRDM16* promoter and regulated brown adipogenesis. Indeed, *PRDM16* was found to be essential for beige cell induction. Therefore, it is necessary to study other transcription factors in addition to *PRDM16* during classic BAT development.

1.2 *PPARs* (peroxisome proliferator-activated receptors). *PPARs* are nuclear receptors and fatty acids are their ligands. *PPARs* consist of *PPARα, PPARγ,* and *PPARβ. PPARγ* is expressed in both BAT and WAT, and *PPARγ* activation is more conducive to BAT features. *PPARγ* and *PRDM16* synergistically induce white fat browning (Ohno *et al*. 2012). *PPARγ* agonist TZDs was found to promote the interaction between *PRDM16* and *PGC1β* and to induce the expression of *PGC1α* (do Nascimento *et al.* 2004). *PPARγ* agonists also induced the interaction between *CtBPs* and *PRDM16*, which then inhibited expression of *RIP140* and thus maintained the white adipocyte phenotype (Castriota *et al.* 2007).

Barbea MJ et al (Barbera *et al.* 2001) found that the expression of *PPARα* in BAT was higher than that in WAT and that using a stimulus such as the *PPARα* ligand WY-14643 could induce *UCP1* expression in primary brown fat cells. Sunsil Choia et al recently reported that a *PPARγ* agonist increased browning of WAT and energy expenditure (Choi *et al.* 2016). In brief, three *PPARs* play different roles in differentiation of brown fat cells and WAT browning.

1.3 *PGC1α* (peroxisome proliferator-activated receptor γ coactivator 1α) *PGC1α* is highly expressed in BAT and regulates *UCP1* expression in brown fat cells (Liang and Ward 2006). *PGC1α* is widely regarded as the pivotal regulator of adaptive thermogenesis in brown adipocytes. Inguinal subcutaneous white fat overexpression of *PGC1α* appears multilocular, similar to brown fat cells, and is accompanied by the upregulation of *UCP1* and other thermogenesis-related genes. In *PGC1α* gene knockout mice, BAT pads were not obviously changed; however, cAMP-induced thermogenesis was significantly inhibited, mitochondrial protein synthesis was

dysfunctional, and the brown adipocyte phenotype was disrupted (Uldry *et al.* 2006). However, Gantner ML et al (Gantner *et al.* 2014) reported that adipose tissue-specific deletion of *PGC1α* had only minor effects on heat production, suggesting that other regulating factors exist. This study further found that *GAAD45γ* was a coactivator of *PGC1α* that could induce expression of *UCP1* and BAT oxidation capacity.

Therefore, *PGC1α* is a key molecule that regulates adaptive thermogenesis in brown fat cells but is not the decisive molecule of brown fat cell development.

1.4 *BMPs* (bone morphogenic proteins). *BMPs* belong to the transforming growth factor beta (*TGF-β*) superfamily. Certain *BMPs,* such as *BMP2, BMP4, BMP5, BMP6, BMP7, BMP8B* and *BMP9*, are now known to be associated with adipocyte differentiation.

The most important of which is *BMP7*. The Tseng (Tseng *et al*.2008) group studied *BMP7* systematically and comprehensively. In an in vitro study, C3H10T1/2 cells were pretreated for 72 h with *BMP7*, and the lipid deposition of the mature cells was found to be significantly reduced; however, *UCP1, PPARγ, C/EBP, PGC-1α, NRF-1* (nuclear respiratory factor-1) and cytochrome C expression levels were significantly increased, and thus, C3H10T1/2 cells showed the characteristics of brown fat cells. Next, C3H10T1/2 cells were subcutaneously transplanted into nude mice for 6 weeks, and these cells eventually developed into adipose tissue. Histological examination confirmed that this adipose tissue was mainly composed of brown fat cells. Knockout of *BMP7 r*esulted in a substantial reduction in BAT fat pads and almost no expression of *UCP1*. In contrast, overexpression of *BMP7* significantly increased BAT content and *UCP1* expression, and the content of WAT was not changed (Tseng *et al.*2008). Further study showed that *BMP7* could promote the differentiation of brown adipose precursor cells by activating *PGC1α*.

Sharma A et al (Sharma *et al.*2014) found that overexpression of *BMP6* induced skeletal muscle precursor cells to differentiate into brown fat cells; *COX2* and *IGF-1R* (Insulin-Like Growth Factor-1 Receptor) may be key molecules involved in *BMP6* targeting.

BMP9 (Kuo *et al.*2014) is mostly expressed in hepatocytes; *MB109* is derived from

BMP9 and has been shown to promote the development of human brown fat cells; and increased *BMP9* expression induced the browning of subcutaneous fat.

BMP4(Elsen *et al.*2014) has been considered to be a factor that can induce stem cell differentiation into white adipocytes in a rodent study. However, Elsen M found that *BMP4* and *BMP7* have the same effect on human preadipocytes such that both can promote white fat browning.

Recently, Martins et al (Martins *et al.*2016) found that *BMP8b* increases brown fat thermogenesis through central and peripheral actions. Using *BMP8b* knockout mice, the authors demonstrated that *BMP8b* increases BAT thermogenesis and suggested that *BMP8b* has an effect not only on immature cells but also on fully differentiated mature adipocytes.

BMPs play an important role in regulating the development and function of brown fat.

1.5 *CEBPβ* (CCAAT/enhancer binding protein). *CEBPβ* was found to bind to *PRDM16* and form a transcription complex that determines myoblast precursor cell differentiation into brown adipocytes. Overexpression of *PRDM16* and *CEBPβ* in fibroblasts was found to induce the conversion of fibroblasts into functional brown fat cells, and deletion of *CEBPβ* and *PRDM16* was found to substantially hinder the differentiation of brown adipocytes; however, a reduction in *UCP1* expression enhanced the expression of skeletal muscle-specific genes (Kajimura *et al.*2009). This study showed that *CEBPβ* is the key cofactor of *PRDM16*.

1.6 *miRNAs* *miRNAs* are some small molecules that consist of 18-25 nucleotides. *miRNAs* regulate gene expression by repressing translation and degrading messenger RNAs that contain complementary target sequences. *miRNAs* are tissue specific, and therefore, the function of *miRNAs* is more specific to cell differentiation. The present study confirmed that *miRNAs* are involved in many diseases, such as cancer, heart disease, and diabetes. Recent studies showed that certain *miRNAs* were necessary for brown fat cell differentiation (Sun *et al*.2011).

SUN L (Sun *et al.*2011) found that expression of *miRNA193b-365* is abundant in BAT and that blocking the expression of *miRNA193b* or *miRNA365* in primary brown

adipocytes leads to a significant reduction in brown fat pads; moreover, enhanced expression of *miRNA193b* in muscle cells blocked the development of the cell line into muscle cells, ultimately inducing these cells to develop into brown fat cells. Further study showed that expression of *miRNA193b-365* was regulated by *PRDM16* and *PPARα* and that *miRNA193b-365* played a key role in the development of brown fat cells (Sun *et al.*2011).

When subjected to cold stimulation, *miRNA133* (Yin H *et al.*2013) expression decreased, which negatively regulated *PRDM16* and inhibited preadipocyte differentiation into brown fat cells.

Downregulating *miR34-a* white adipocytes increased the expression of the beige fat cell marker *CD137*, and these white adipocytes acquired the features of brown fat cells; the mechanism may involve *FGF21/SIRT1/PGC1α* (Fu *et al.*2014).

Recent studies identified *miR378* (Pan *et al.*2014), *miRNA-26* (Karbiener *et al.*2014), *miRNA-30* (Hu *et al.*2015) as new positive regulators of the brown and beige fat development, whereas *miRNA-27*(Sun and Trajkovski 2014), *miRNA-106b-93*(Wu *et al.*2013), and *miRNA-155*(Chen *et al.*2013) were identified as negative regulators.

Although many *miRNAs* have been identified as central regulators of the brown/beige adipogenic program, the picture of the whole network of miRNAs is still incomplete. Further studies are required to fully understand the regulatory roles of miRNAs in brown/beige adipogenesis and to develop therapeutic approaches to combat obesity and related consequences.

1.7 *EBF2* (early B-cell factor 2). *EBF2* overexpression in cells isolated from embryos or white fat eventually differentiated into brown fat cells (or beige fat cells). Deletion of *EBF2* in brown preadipocytes resulted in reduced expression of brown fat cell markers, whereas ectopic expression of *EBF2* in myogenic cells induced the expression levels of brown adipocyte-specific genes. These results indicate that *EBF2* is a specific marker of brown fat and regulates the development of brown fat (Wang *et al.*2014).

1.8 *RIP140* (receptor-interacting protein 140) *RIP140* is a nuclear receptor corepressor and is highly expressed in adipose tissue. *RIP140* has an important role in regulating the development of brown or white fat cells. Inhibition of *RIP140 in* preadipocytes

led to substantially increased expression of brown adipocyte markers; in addition, overexpression of *RIP140* decreased the expression of the beige cell markers such as *TBX1, CD137, TMEM26 and PRDM16. RIP140* inhibits white adipose browning (Kiskinis *et al.*2014).

1.9 *RB* family (retinoblastoma protein) The *RB* family includes *pRB, P130* and *p107*.

pRB was the first gene discovered to regulate adipose differentiation. Lee EY discovered that *pRB* knockout mice died in the first 16 days of the embryonic period (Lee *et al.*1992). Hensen et al (Hansen *et al.*2004) found that *pRB* regulates white and brown fat cell differentiation. *pRB* knockout in stem cells resulted in cells differentiating into brown fat cells, which inhibited the expression of white adipose precursor cells, but these cells finally developed into brown fat cells. These results suggest that *pRB* plays a role in the differentiation switch of white and brown fat cells.

P107 plays a critical role in stem cell differentiation into adipocytes; inhibition of *P107* was essential for brown adipocyte differentiation. Brown fat was completely absent when *P107* was overexpressed. Additionally, the binding of *PRDM16* to the promoter of *P107* inhibited the expression of brown fat (De Sousa *et al.*2014).

Therefore, *P107* and *PRB* are key negative regulatory factors of brown or beige fat differentiation.

1.10 *WNTs* (wingless type MMTV integration site family members) *WNTs* are a family of 19 glycoproteins that regulate tissue homeostasis and remodeling by way of autocrine and paracrine signaling.

WNTs are key regulators of adipogenesis. *WNT10a* and *WNT10b* express in BAT, but with development and differentiation of brown fat, their expression levels gradually decline, suggesting that *WNTs* negatively regulate brown fat development (Christodoulides *et al.*2015). To study the effect of *WNT*10b on the differentiation of fat cells, Longo et al (Longo *et al.*2004) established transgenic mice that specifically expressed *WNT10b* on adipose tissue (*FABP4-WNT10b* mice) and found that brown fat development of the *FABP4-WNTl0b* mice was arrested, scapular brown adipose tissues appeared as white fat, and these tissues did not express the characteristic marker of BAT (*UCP1*) or molecular markers of WAT. Furthermore, under cold stress, the *FABP4-*

WNT10b mice were not able to maintain their core body temperature, which indicated that BAT was dysfunctional. Further studies found that *WNT10b* inhibited brown fat cell differentiation by inhibiting the expression of *PPARγ* and *CEBPα*. Overexpression of *WNT10b* on BAT led to a change in the appearance and structure of intracellular BAT to be similar to those of WAT, and the expression of *UCP1* and *PGC1α* was significantly decreased. Moreover, the synthesis of the mitochondria was inhibited, and these results confirmed that the overexpression of *WNT10b* could transform the mature brown fat cells into white fat cells and that *WNT10b* was an inhibitory factor of brown fat development (Kang *et al.*2005).

A recent study (Lo *et al.*2016) showed that using *WNT* inhibitors upregulated the expression levels of thermogenic genes in primary inguinal adipocytes but not epididymal adipocytes. This study also found that the induction and enhancement of browning are most prominent when *WNT* is blocked at the initial stages of differentiation, whereas later inhibition produces little or no effects.

In conclusion, the role of *WNTs* in the development and differentiation of brown adipocytes and the browning of white fat has not been fully elucidated; further research is needed.

1.11 *RXR* (retinoid X receptor) Nie et al (Nie *et al.*2017) identified bexarotene (Bex), a specific retinoid X receptor (*RXR*) agonist (Boehm *et al.*1995), as a potent molecule that induces brown adipocyte reprogramming. Their results showed that Bex selectively induced BAT features in multiple cell types while inhibiting WAT differentiation. Mice treated with Bex had a higher BAT mass, enhanced metabolic function, and constrained body weight. This study further confirmed that Bex treatment induced brown adipogenic reprogramming via the activation of *RXRα* and *RXRγ*. Bex/*RXR* was found to be a master regulator in controlling *PPARγ* and *PRDM16* expression and other downstream pathways such as *FGF21, PGC1α,* and *TBX15.* These studies established *RXRα* and *RXRγ* as new regulators of BAT development that control the expression levels of *PRDM16* and other browning-related molecules. However, many questions about how *RXRs* precisely control adipogenic subtype specification in development and tissue homeostasis remain unanswered.

1.12 mTORC1 (mechanistic target of rapamycin (mTOR) complex 1)

mTORC1 is a critical multiprotein hub that is nucleated around the protein raptor and that integrates intracellular and extracellular cues to regulate cellular growth and metabolism (Zoncu *et al.*2011, Dibble and Manning 2013, Goberdhan *et al.*2016).

The role of mTOR in adipocyte browning is not well understood. Loss of raptor in fat led to browning of WAT (Polak *et al.*2008). Xiang X et al (Xiang *et al.*2014) established a *FABP4-TSC1*$^{-/-}$ mouse model, of which white adipose tissues specifically lack the *TSC1* gene. Using this model, the authors found that the deposition of fat droplets in BAT of the *FABP4-TSC1*$^{-/-}$ mice was markedly increased, but the expression levels of brown adipocyte markers were significantly reduced; however, the expression levels of white adipocyte markers were elevated, and rapamycin treatment could reverse this change in *FABP4-TSC1*$^{-/-}$ mice.

Ablation of *TSC1* in brown preadipocytes substantially induced the differentiation of white adipocytes, and levels of *FoxC2* mRNA, which is a key transcription factor of brown fat development, were significantly reduced. However, *RIP140* and *P107* mRNA expression levels, which determines the phenotype of white fat cells, were increased. Liu et al (Liu *et al*.2016) and Tran et al (Tran *et al.*2016) found that loss of raptor in fat or pharmacological inhibition of *mTOR* blocked cold-induced browning of WAT.

A recent study revealed that *FLCN* (folliculin), *mTOR*, and *TFE3* (a member of the *MiTF* gene family) formed a key complex that integrated metabolic cues to coordinate mitochondrial biogenesis and browning of WAT (Wada *et al.*2016).

These results demonstrated that *mTOR* can promote white fat browning.

1.13 *Notch*

Inhibition of *Notch* has been shown to promote white fat browning and to reduce obesity. Bi P et al (Bi *et al.*2014) reported that specific inactivation of *Notch* or its signal medium RBPJ could cause the browning of white fat and increased *UCP1* expression. The results showed that the energy consumption of the *Notch* mutant mice increased, the mice had improved glucose tolerance and insulin sensitivity, and the mice had resistance to obesity induced by high fat.

Activation of the *Notch* pathway produced the opposite results. Sustained

activation of *Notch* reduced the expression levels of *PGC1α* and *PRDM16* in white adipose tissue, while inhibition of *Notch* could induce the expression of the above genes and thus could lead to WAT browning.

2. Hormone and peptides that regulate browning

Some hormones and peptides have been proven to promote the development of brown/beige fat and the browning of white fat as summarized in table 2(Table2).

2.1 Catecholamines

BAT is highly controlled by sympathetic nerves. Extensive studies recently confirmed that norepinephrine promotes brown fat thermogenesis and white fat browning.

In 1993, S Krief et al found that adrenergic receptors were rich in BAT and that the main subtype was β3-adrenergic receptor (Krief *et al*.1993). Giving rats the β3 adrenergic receptor stimulant CL-316243 (CL), Himms-Hagen et al (Himms *et al.*2000) found that the white adipocytes were multilocular and rich in mitochondrion. Histological examination showed that some of these cells were transdifferentiated from the original white adipocytes, and immunohistochemistry showed that approximately 8% of these cells were *UCP1* positive. In 2005, Granneman JG et al (Granneman *et al.*2005) came to the same conclusion that under stimulation of β3-adrenergic receptor agonist, mature white fat cells can be directly converted into brown fat cells.

Jimenez M et al (Jimenez *et al.*2003) found that after exposure to cold condition for 10 days, brown fat cells appeared in the WAT of wild-type mice, whereas they were not found in the WAT of β3-adrenergic receptor knockout mice. In 2010, Barbatelli found that when mice were exposed to cold stress for 6 days, some *UCP1* positive cells appeared in both in subcutaneous and visceral WAT (Barbatelli *et al.*2010). These cells were paucilocular and had more mitochondrial content. Further investigation revealed that cold stimulation had no impact on the expression of cell proliferation-related proteins, but expression of *CEBPα* was significantly increased; moreover, RT-qPCR confirmed that cold stimulation enhances the expression of brown adipose-specific genes in WAT, and β3-adrenergic receptor inhibitors could reduce the expression of these genes in WAT. Based on these results, the brown fat cells in WAT induced by

cold stress were confirmed to be from the direct transformation of mature white fat cells mediated by β3-AR.

Other studies (Contreras *et al.*2014) showed that brown-like fat cells were also rich in mammalian inguinal and subcutaneous white adipose tissue during preweaning, and the number of these cells was related to sympathetic nerve distribution density. Sustained sympathetic activity is essential for the expression of the phenotype of the brown fat cells in the white fat cells.

Activation of β3-AR is one of the most important methods of white fat browning.

2.2 Thyroid hormones

The effects of thyroid hormones on brown fat are complex and profound. In the present study, the interaction between thyroid hormones and the sympathetic nervous system plays an important role in the development and function of brown fat cells.

Early in the 1990s, Rubio A et al found that BAT responses to catecholamines were blocked in the condition of hypothyroidism and that the response rapidly recovered after sufficient thyroid hormone supplementation(Rubio *et al.*1995). Another study (Obregon 2008) found that the effects of thyroid hormone on BAT and WAT reaction to the β3 adrenergic receptor (β3AR) were different. In hypothyroidism, the content of β3AR protein and β3AR mRNA levels increased 4-6 times in BAT, but they were decreased in WAT. Furthermore, hypothyroid rats injected with triiodothyronine (T3) can reverse the above changes within 24 hours; however, while the excess of T3 caused a decrease in β3AR protein and mRNA levels by more than 90% in BAT, β3AR protein and mRNA levels were increased by 5 times in WAT. Hypothyroidism led to a significant reduction in cAMP in both BAT and WAT, but this decline was not recovered despite being given enough T3 for 2 days.

The development and differentiation of adipose tissue are also regulated by thyroid hormones. The development of adipose tissue is a complex process, including the proliferation and differentiation of preadipocytes into mature adipocytes; this process was activated by *CEBP*, *PPAR* and other brown adipocyte specific genes, and thyroid hormones participate in the regulation of these above genes. In 1987, T3 receptors

were found to be expressed on brown adipocytes, which suggests that BAT is one of the target organs of thyroid hormones （Bianco and Silva 1987). Tuca A et al found that the expression levels of the *CREB* gene were significantly increased during BAT differentiation, and the *CREB* expression peak appeared in 20 days of fetal development. Interestingly, nuclear T3 content and receptor binding rates also reached peak levels around embryonic day 20, suggesting that rat BAT won the mature thyroid function in 20 days of pregnancy. In addition, at 18-20 days of embryonic development, brown adipocyte marker *UCP1* expression was also induced, suggesting that thyroid hormones may be involved in brown adipocyte differentiation during the embryonic period (Tuca *et al.*1993). *UCP1* expression was also induced by T3 in the primary cultured brown fat cells of mouse embryos (Guerra *et al.*1994).

T3 in the tissues was obtained by deiodinase, and the differentiation of adipocytes was related to type 2 deiodinase (D2) (Bianco *et al.*1987). T4 was transformed into T3 by D2, which plays a key role in the action of T3 in tissues, and the expression of D2 in BAT was high but was severely diminished in WAT. Active D2 was found in the preadipocytes of human subcutaneous fat cells (Nomura *et al.*2011), and rats with adipose-specific knockout of D2 exhibited increased respiratory quotient, food intake and blood glucose. These rats then underwent an 8-week high-fat diet; compared to those of the control group, the body weight and fat mass of these rats was considerably higher, suggesting that BAT thermogenesis function is impaired and unable to oxidize excess fat (Fonseca *et al.*2014). More recently, Noelia Martínez-Sánchez et al (Martinez-Sanchez *et al.*2017) reported that the browning of WAT could be induced by central and specific administration of T3 in the ventromedial nucleus of hypothalamus (VMH) via a mechanism dependent of AMPK.

Therefore, thyroid hormones play a critical role in brown fat development and differentiation.

2.3 Irisin

In 2012, Professor Spiegelman BM Laboratory found that *PGC1α* increased *FNDC5* (a type of membrane protein) expression in rat muscle tissue, and after cleavage, *FNDC5* was converted into a new hormone, irisin (Boström *et al.*2012); expression of *UCP1*

was stimulated by irisin both in vitro and in vivo, which led to the browning of white fat cells. Exercise in both mice and humans can induce the generation of irisin. A moderate increase in irisin expression in mice could significantly increase energy consumption in the body. The mechanism of irisin-induced white fat browning is poorly understood. Some studies showed it may be achieved by *MAPK p38* and *ERK* (Zhang *et al.*2014).

2.4 Glucocorticoid hormone

It is well known that glucocorticoids (GC) have a very important influence on metabolism. As early as 2000, researchers found that GC inhibited the expression of *UCP1* and other brown fat-specific functional genes (Viengchareun *et al.*2001, Soumano *et al.*2000), and GC inhibitor RU486 (Rodriguez *et al.*2004) could increase *UCP1* expression in brown fat cells. Professor Ding guoxian et al found that the glucocorticoid suppressed expression of *PRDM16* through 11β-HSD and *miRNA-27b*, thus finally producing an inhibitory effect on white fat browning (Liu *et al.*2013).

2.5 GLP-1 (glucagon-like peptide-1) GLP-1 is an incretin hormone released by L cells located in the ileum and colon (Göke *et al.*1991, Drucker *et al.*2007) and is currently one of the most widely and successfully used hormones in type 2 diabetes mellitus therapies. GLP-1 and its receptor (GLP-1R) are expressed in peripheral tissues and the central nervous system (CNS) and are involved in the control of energy balance. Sarah H. Lockie et al found that ICV administration of the preproglucagon-derived peptides (GLP-1) increased BAT thermogenesis by increasing SNS activity (Lockie *et al.* 2012). Another study showed that the central stimulation of GLP-1R induced not only BAT thermogenesis but also WAT browning and that this regulatory mechanism depends on *AMPK* (Beiroa *et al.*2014).

More recently, Fen Xu et al reported that GLP-1R agonist promotes browning of WAT in a *SIRT1*-dependent manner (Xu *et al.*2016). These findings provide us with a promising therapeutic target to treat obesity and its associated metabolic disorders by GLP-1R agonist.

2.6 PTHrP (parathyroid hormone-related protein) and PTH (parathyroid hormone)

Cancer-associated cachexia is a wasting syndrome, and its characteristic features are

systemic inflammation, weight loss, and atrophy of white adipose tissue and skeletal muscle, all of which ultimately results in severe weight loss and weakness in cancer patients. Approximately half of cancer patients suffering from cachexia that damaged to their quality of life and exhibited reduced survival rate of cancer. The characteristic feature of cachexia is higher energy consumption than normal individuals, which is considered to be related to the overproduction of heat.

Petruzzelli al found that expression of *UCP1* in WAT was increased in cachexia patients and that this overexpression led to enhanced cellular uncoupling respiration, increased heat production, and reduced ATP synthesis, suggesting that WAT browning occurred in cachexia patients (Petruzzelli *et al.*2014). Kir S et al identified parathyroid hormone-related protein (PTHrP), a tumor-derived small polypeptide, as an inducer of browning in the lung cancer cachexia rat model; the authors found that PTHrP promoted the body energy consumption by increasing the expression of the thermogenesis gene in white adipose tissue, and blocking PTHrP prevented the white fat browning and the reduction of muscle tissue weight and power in rats with tumors (Kir *et al.*2016). In addition, this study found that parathyroid hormone (PTH) was involved in stimulating thermogenic gene expression levels in mice suffering from cachexia and that adipose specific deletion of PTHR blocked adipose browning and wasting.

The above results confirmed that PTH/PTHrP mediates browning by a common mechanism that involves PTHR and that altering PTH/PTHrP function may be a selective therapeutic strategy to combat cachexia.

2.7 *FGF21* (fibroblast growth factor 21): *FGF21* is a type of peptide hormone that is synthesized in several organs, including liver, white adipose tissue and the pancreas. The function of *FGF21* is complicated owing to its extensive metabolic functions in multiple target organs and its ability to act as an autocrine, paracrine, and endocrine factor (Fisher *et al*.2016). The beneficial effects of *FGF21* on glucose metabolism and body weight were reported in 2009 by Coskun and Berglund (Coskun *et al.*2008, Berglund *et al.*2009) and results from this study have evoked a substantial interest in *FGF21* as a potential treatment for diseases such as obesity and diabetes (Xu *et*

al.2009).

Increased *FGF21* expression in BAT has been observed. Fisher et al found that *FGF21* can also increase thermogenic gene expression in specific WAT depots (IWAT and PRWAT), and *FGF21* appeared to induce the expression of many genes associated with the function of the brown/beige adipocytes (Fisher *et al.*2012). This study suggested that *FGF21* regulates white fat browning by mainly increasing *PGC1α* expression.

Due to the beneficial effects of *FGF21* on metabolism and bodyweight, *FGF21* has been proposed as a novel therapeutic for diabetes and fatty liver disease.

Conclusions

With the development of technology, multiple studies confirmed that functional BAT exists in adult humans and that it is inversely correlated with BMI, adiposity, glucose and lipid metabolism. As summarized above, several molecules and hormones are involved in the regulation of brown adipocyte differentiation and the browning of white fat (Figure1). However, a full understanding of the phenotype switching mechanism between white fat and brown fat is needed. We expect that regulating BAT and WAT browning will become new methods in the treatment of obesity and diabetes.

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Authors' contributions

J.Z. wrote the manuscript. H.W., F.J, S.M, L.G and J.Z revised the manuscript. All authors read and approved the final manuscript.

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Table1: Transcription regulators and miRNAs involved in the process of browning

Regulator	Type	Model system	Role(s)
PRDM16	+, core TF	Ap2-PRDM16 transgenic mouse, primary adipocytes	Needed for development of BAT and WAT browning
PPARγ	+, core TF	Primary adipocytes, mouse model	needed for browning and BAT differentiation
PGC-1α	+, coregulator	Human subcutaneous fat, PGC1-α knockout mouse myoblast precursor cells	Needed for induction of UCP1 and other specific BAT genes in WAT
CEBPβ	+, TF	Primary adipocytes, mouse model	CEBPβ is the key cofactor of PRDM16.
EBF2	+, TF	Mouse model and Preadipocytes	Needed for development of brown fat
RIP140	-, TF	Mouse model and Preadipocytes	Block development of brown fat
Rb and p107	-, TF	p107 knockout mice, primary adipocytes	Repressing expression of PGC-1α
Wnt10b	-, TF	wnt10b knockout mice and primary adipocytes	inhibited at the start stage of differentiation
RXR	-, coregulator	mouse model	inhibit RIP140 and P107 expression
mTORC1	+, coregulator	mTORC1 knockout mouse model, primary adipocytes	inhibit the transcription of PGC-1αand PRDM16
Nocth	-, coregulator	mouse model	Essential for brown-fat development
miRNA 193b−365	+, micro RNA	Primary adipocytes, mouse model	negatively regulated PRDM16
miRNA133	−, micro RNA	Myf5+ brown precursors, Myf5− preadipocytes from subcutaneous WAT	increased expression of FGF21 receptor and SIRT1

Table1: Type indicates whether the regulator has a positive (+) or negative (−) effects on BAT differentiation or browning and whether the regulator is a transcription factor (TF) or coregulator

Table 2 Hormones, secreted proteins involved in the BAT development and process of browning

Regulator	Type	Model system	Role(s)
Catecholamines	+, hormone	β3-adrenergic receptor knockdown	Positive stimulate CEBPα and induce browning

		mouse	
Thyroid hormones	+, hormone	Mouse model, Primary adipocytes	Essential for BAT function and Induce browning
Irisin	+, hormone	Muscle-specific PGC-1α transgenic mice	Inducing browning in WAT, dependent on PPARα
Glucocorticoid hormone	-, hormone	Mouse model and Primary adipocytes	suppress expression of PRDM16
GLP-1	+, hormone	Mouse model	Induce browning
PTHrP	+, hormone	Mouse model, human, WAT cell line	Induce browing
PTH	+, hormone	PTHR knockout mice	Induce browning
FGF21	+, secreted protein	Primary adipocytes, FGF21 knockout mice	Inducing browning in WAT, dependent on PGC-1α
BMP7	+, secreted protein	Brown adipocyte cell line, C3H10T1/2 cell line, BMP7 null mouse	Essential for BAT development

Table2: Type indicates whether the regulator has a positive (+) or negative (−) effects on BAT differentiation or browning and whether the regulator is a transcription factor (TF) or coregulators.

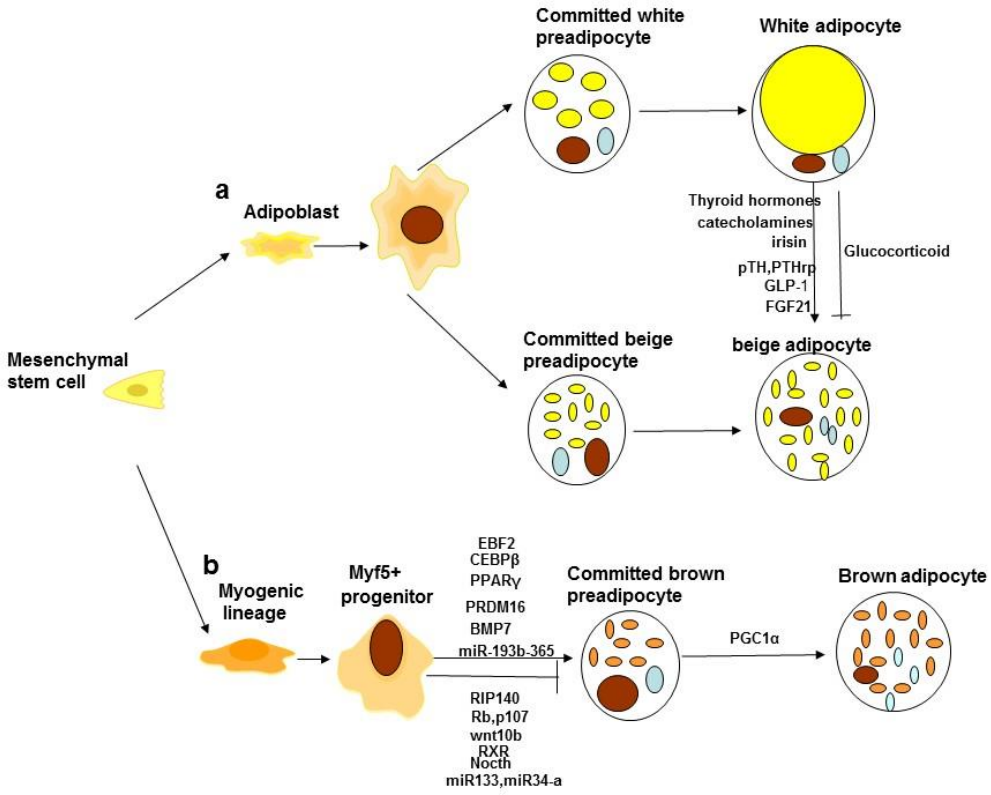

Figure1: Transcriptional regulation of brown adipogenesis and hormonal control of browning. (a) Hormonal control of browning. Thyroid hormones, catecholamines, irisin, PTH, PTH-rP, GLP-1 and FGF21 promote the browning of white adipose. On the contrary glucocorticoid inhibits the browning. (b) Transcription factors EBF2,CEBPβ，PPARγ，PRDM16，BMP7 and miR-193b-365 induce the development of brown adipose. Others such as RIP140, Rb, p107, WNT10b, RXR, Notch,miR133 and miR34-a inhibit the brown adipogenesis.