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 (Table 1) 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 promoter in white adipocytes to inhibit the expression of white fat cell-related genes and that *CtBP1/2* can be replaced by *PGC1α*, 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 \textit{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 AMPK\(\alpha\) mediated DNA demethylation in the \textit{PRDM16} promoter and regulated brown adipogenesis. Indeed, \textit{PRDM16} was found to be essential for beige cell induction. Therefore, it is necessary to study other transcription factors in addition to \textit{PRDM16} during classic BAT development.

\textbf{1.2 PPARs (peroxisome proliferator-activated receptors).} PPARs are nuclear receptors and fatty acids are their ligands. PPARs consist of PPAR\(\alpha\), PPAR\(\gamma\), and PPAR\(\beta\). PPAR\(\gamma\) is expressed in both BAT and WAT, and PPAR\(\gamma\) activation is more conducive to BAT features. PPAR\(\gamma\) and \textit{PRDM16} synergistically induce white fat browning (Ohno et al. 2012). PPAR\(\gamma\) agonist TZDs was found to promote the interaction between \textit{PRDM16} and \textit{PGC1\(\beta\)} and to induce the expression of \textit{PGC1\(\alpha\)} (do Nascimento et al. 2004). PPAR\(\gamma\) agonists also induced the interaction between CtBPs and \textit{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 PPAR\(\alpha\) in BAT was higher than that in WAT and that using a stimulus such as the PPAR\(\alpha\) ligand WY-14643 could induce \textit{UCP1} expression in primary brown fat cells. Sunsil Choia et al recently reported that a PPAR\(\gamma\) 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.

\textbf{1.3 PGC1\(\alpha\) (peroxisome proliferator-activated receptor \(\gamma\) coactivator 1\(\alpha\))} PGC1\(\alpha\) is highly expressed in BAT and regulates \textit{UCP1} expression in brown fat cells (Liang and Ward 2006). PGC1\(\alpha\) is widely regarded as the pivotal regulator of adaptive thermogenesis in brown adipocytes. Inguinal subcutaneous white fat overexpression of PGC1\(\alpha\) appears multilocular, similar to brown fat cells, and is accompanied by the upregulation of \textit{UCP1} and other thermogenesis-related genes. In PGC1\(\alpha\) 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 resulted 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 WNT10b 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-WNT10b 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 CEBPa. 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 RXRa 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 RXRa 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 (Table 2).

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 (PTThrP), 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
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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Table 1: Transcription regulators and miRNAs involved in the process of browning

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

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

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Type</th>
<th>Model system</th>
<th>Role(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catecholamines</td>
<td>+, hormone</td>
<td>β3-adrenergic receptor knockdown</td>
<td>Positive stimulate CEBPα and induce browning</td>
</tr>
<tr>
<td>Regulator</td>
<td>Type</td>
<td>Mouse model or Adipocytes</td>
<td>Effect on BAT</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>---------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>+, hormone</td>
<td>Mouse model, Primary adipocytes</td>
<td>Essential for BAT function and induce browning</td>
</tr>
<tr>
<td>Irisin</td>
<td>+, hormone</td>
<td>Muscle-specific PGC-1α transgenic mice</td>
<td>Inducing browning in WAT, dependent on PPARα</td>
</tr>
<tr>
<td>Glucocorticoid hormone</td>
<td>-, hormone</td>
<td>Mouse model and Primary adipocytes</td>
<td>Suppress expression of PRDM16</td>
</tr>
<tr>
<td>GLP-1</td>
<td>+, hormone</td>
<td>Mouse model</td>
<td>Induce browning</td>
</tr>
<tr>
<td>PTH</td>
<td>+, hormone</td>
<td>Mouse model, human, WAT cell line</td>
<td>Induce browning</td>
</tr>
<tr>
<td>PTHrP</td>
<td>+, hormone</td>
<td>PTHrP knockout mice</td>
<td>Induce browning</td>
</tr>
<tr>
<td>FGF21</td>
<td>+, secreted protein</td>
<td>Primary adipocytes, FGF21 knockout mice</td>
<td>Inducing browning in WAT, dependent on PGC-1α</td>
</tr>
<tr>
<td>BMP7</td>
<td>+, secreted protein</td>
<td>Brown adipocyte cell line, C3H10T1/2 cell line, BMP7 null mouse</td>
<td>Essential for BAT development</td>
</tr>
</tbody>
</table>

Table 2: 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.

**Figure 1:** Transcriptional regulation of brown adipogenesis and hormonal control of browning.

(a) Hormonal control of browning. Thyroid hormones, catecholamines, irisin, PTH, PTHrP, 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.