REVIEW

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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Summary

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.

Key words

Brown adipose • Beige adipose • Browning • Hormones • Transcription regulators

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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 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.* (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). Wu *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 *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).

Guerra *et al.* (1998) 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. 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*. (2014) 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. 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.

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 and describe them in detail.

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 *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, 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.

Table 1. 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-1α and 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

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 coregulatory.

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).

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. Choi *et al.* (2016) recently reported that a *PPARγ* agonist increased browning of WAT and energy expenditure. In brief, three *PPARs* play different roles in differentiation of brown fat cells and WAT browning.

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 *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.

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 group studied *BMP7* systematically and comprehensively (Tseng *et al*. 2008). 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 *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 *et al.* (2014) found that *BMP4* and *BMP7* have the same effect on human preadipocytes such that both can promote white fat browning.

Recently, 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.

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*.

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 *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.

When subjected to cold stimulation, *miRNA133* (Yin *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.

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).

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).

RB family (retinoblastoma protein)

The *RB* family includes *pRB*, *P130* and *P107*.

pRB was the first gene discovered to regulate adipose differentiation. Lee discovered that *pRB* knockout mice died in the first 16 days of the embryonic period (Lee *et al.* 1992). 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.

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.* (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 *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.

RXR (retinoid X receptor)

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.

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 *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.* (2016) and 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.

Notch

Inhibition of *Notch* has been shown to promote white fat browning and to reduce obesity. 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.

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

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

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.

Catecholamines

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

Krief *et al.* (1993) found that adrenergic receptors were rich in BAT and that the main subtype was β3-adrenergic receptor. Giving rats the β3-adrenergic receptor stimulant CL-316243 (CL), 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. 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 *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. Barbatelli *et al.* (2010) found that when mice were exposed to cold stress for 6 days, some *UCP1* positive cells appeared in both in subcutaneous and visceral WAT. 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 β3AR.

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 β3AR is one of the most important methods of white fat browning.

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 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 h; 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 *et al.* (1993) 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, Martínez-Sánchez *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.

Irisin

In 2012, Spiegelman 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).

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. Liu *et al.* (2013) found that the glucocorticoid suppressed expression of *PRDM16* through 11β-HSD and *miRNA-27b*, thus finally producing an inhibitory effect on white fat browning.

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. Lockie *et al.* (2012) found that ICV administration of the preproglucagon-derived peptides (GLP-1) increased BAT thermogenesis by increasing SNS activity. 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, Xu *et al.* (2016) reported that GLP-1R agonist promotes browning of WAT in a *SIRT1*-dependent manner. These findings provide us with a promising therapeutic target to treat obesity and its associated metabolic disorders by GLP-1R agonist.

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 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 *et al.* (2016) 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. 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.

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.* (2012) 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. 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 (Fig. 1). 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.

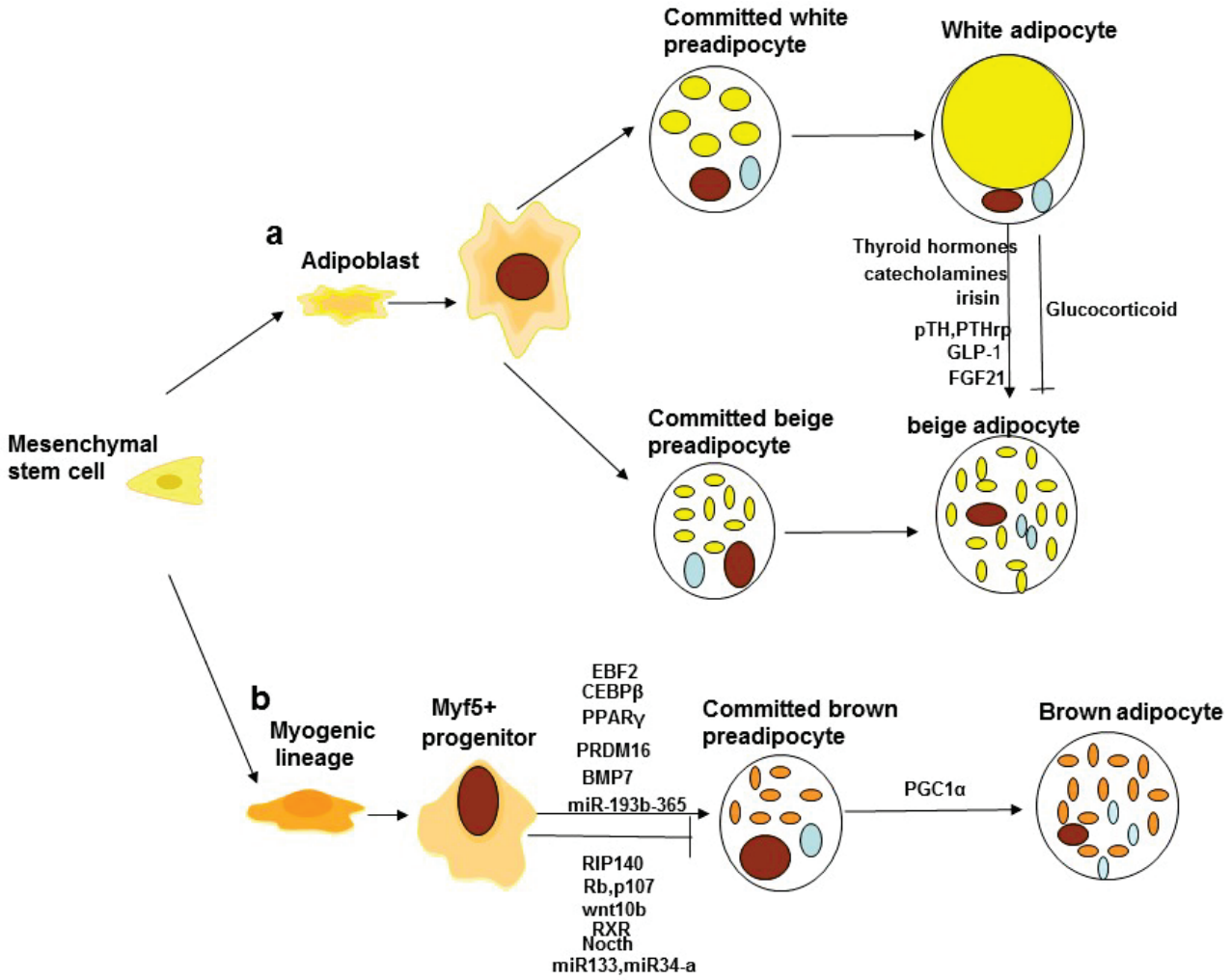

Fig. 1. Transcriptional regulation of brown adipogenesis and hormonal control of browning. (**a**) Hormonal control of browning. Thyroid hormones, catecholamines, irisin, PTH, PTH-rP, GLP-1 and FGF21 promote the browning of white adipose. On the contrary, glucocorticoid inhibits the browning. (**b**) 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.

Conflict of Interest

There is no conflict of interest.

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References

ALMIND K, KAHN CR: Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. *Diabetes* **53**: 3274-3285, 2004.

BARBATELLI G, MURANO I, MADSEN L, HAO Q, JIMENEZ M, KRISTIANSEN K, GIACOBINO JP, DE MATTEIS R, CINTI S: The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte trans-differentiation. *Am J Physiol Endocrinol Metab* **298**: E1244-E1253, 2010.

BARBERA MJ, SCHLUTER A, PEDRAZA N, IGLESIAS R, VILLARROYA F, GIRALT M: Peroxisome proliferator activated receptor alpha activate transcription of the brown fat uncoupling Protein-1 gene: A link between regulation of thermogenic and lipid oxidation pathways in the brown fat cell. *J Biol Chem* **276**: 1486-1493, 2001.

BARGER JL, BARNES BM: Regulation of UCP1 and UCP3 in arctic ground squirrels and relation with mitochondrial proton leak. *J Appl Physiol* **101**: 339-347, 2006.

BEIROA D, IMBERNON M, GALLEGO R, SENRA A, HERRANZ D, VILLARROYA F, SERRANO M, FERNØ J, SALVADOR J, ESCALADA J, DIEGUEZ C, LOPEZ M, FRÜHBECK G, NOGUEIRAS R: GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* **63**: 3346-3358, 2014.

BERGLUND ED, LI CY, BINA HA, LYNES SE, MICHAEL MD, SHANAFELT AB, KHARITONENKOV A, WASSERMAN DH: Fibroblast growth factor 21 controls glycemia via regulation of hepatic glucose flux and insulin sensitivity. *Endocrinology* **150**: 4084-4093, 2009.

BI P, SHAN T, LIU W, YUE F, YANG X, LIANG XR, WANG J, LI J, CARLESSO N, LIU X, KUANG S: Inhibition of Notch signaling promotes browning of white adipose tissue and ameliorates obesity. *Nat Med* **20**: 911-918, 2014.

BIANCO AC, SILVA JE: Nuclear 3,5,3'-triiodothyronine (T3) in brown adipose tissue: receptor occupancy and sources of T3 as determined by in vivo techniques. *Endocrinology* **120**: 55-62, 1987.

BOEHM MF, ZHANG L, ZHI L, MCCLURG MR, BERGER E, WAGONER M, MAIS DE, SUTO CM, DAVIES JA, HEYMAN RA: Design and synthesis of potent retinoid X receptor selective ligands that induce apoptosis in leukemia cells. *J Med Chem* **38**: 3146-3155, 1995.

BOSS O, SAMEC S, KUHNE F, BIJLENGA P, ASSIMACOPOULOS-JEANNET F, SEYDOUX J, GIACOBINO JP, MUZZIN P: Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. *J Biol Chem* **273**: 5-8, 1998.

BOSTRÖM P, WU J, JEDRYCHOWSKI MP, KORDE A, YE L, LO JC, RASBACH KA, BOSTRÖM EA, CHOI JH, LONG JZ, KAJIMURA S, ZINGARETTI MC, VIND BF, TU H, CINTI S, HØJLUND K, GYGI SP, SPIEGELMAN BM: A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* **481**: 463-468, 2012.

CASTRIOTA G, THOMPSON GM, LIN Y, SCHERER PE, MOLLER DE, BERGER JP: Peroxisome proliferator-activated receptor γ agonist inhibit adipocyte expression of α1-acid glycoprotein. *Cell Biol Int* **31**: 586-591, 2007.

CHEN Y, SIEGEL F, KIPSCHULL S, HAAS B, FRÖHLICH H, MEISTER G, PFEIFER A: miR-155 regulates differentiation of brown and beige adipocytes via a bistable circuit. *Nat Commun* **4**: 1769, 2013.

CHOI SS, KIM ES, JUNG JE, MARCIANO DP, JO A, KOO JY, CHOI SY, YANG YR, JANG HJ, KIM EK, PARK J, KWON HM, LEE IH, PARK SB, MYUNG KJ, SUH PG, GRIFFIN PR, CHOI JH: PPAR gamma antagonist gleevec improves insulin sensitivity and promotes the browning of white adipose tissue. *Diabetes* **65**: 829-839, 2016.

CHONDRONIKOLA M, VOLPI E, BØRSHEIM E, PORTER C, ANNAMALAI P, ENERBÄCK S, LIDELL ME, SARAF MK, LABBE SM, HURREN NM, YFANTI C, CHAO T, ANDERSEN CR, CESANI F, HAWKINS H, SIDOSSIS LS: Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes* **63**: 4089-4099, 2014.

CHRISTODOULIDES C, LAGATHU C, SETHI JK, VIDAL-PUIG A: Adipogenesis and WNT signalling. *Trends Endocrinol Metab* **20**: 16-24, 2015.

COHEN P, LEVY JD, ZHANG Y, FRONTINI A, KOLODIN DP, SVENSSON KJ, LO JC, ZENG X, YE L, KHANDEKAR MJ, WU J, GUNAWARDANA SC, BANKS AS, CAMPOREZ JP, JURCZAK MJ, KAJIMURA S, PISTON DW, MATHIS D, CINTI S, SHULMAN GI, SEALE P, SPIEGELMAN BM: Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* **156**: 304-316, 2014.

CONTRERAS GA, LEE YH, MOTTILLO EP, GRANNEMAN JG: Inducible brown adipocytes in subcutaneous inguinal white fat: the role of continuous sympathetic stimulation. *Am J Physiol Endocrinol Metab* **307**: E793-E799, 2014.

COSKUN T, BINA HA, SCHNEIDER MA, DUNBAR JD, HU CC, CHEN Y, MOLLER DE, KHARITONENKOV A: FGF21 corrects obesity in mice. *Endocrinology* **149**: 6018-6027, 2008.

COSTFORD SR, CHAUDHRY SN, SALKHORDEH M: Effects of the presence, absence, and overexpression of uncoupling protein-3 on adiposity and fuel metabolism in congenic mice. *Am J Physiol Endocrinol Metab* **290**: E1304-E1312, 2006.

COUSIN B, CINTI S, MORRONI M, RAIMBAULT S, RICQUIER D, PÉNICAUD L, CASTEILLA L: Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J Cell Sci* **103**: 931-942, 1992.

CYPESS AM, LEHMAN S, WILLIAMS G, TAL I, RODMAN D, GOLDFINE AB, KUO FC, PALMER EL, TSENG YH, DORIA A, KOLODNY GM, KAHN CR: Identification and importance of brown adipose tissue in adult humans. *N Engl J Med* **360**: 1509-1517, 2009.

DE SOUSA M, PORRAS DP, PERRY CG, SEALE P, SCIMÈ A: p107 is a crucial regulator for determining the adipocyte lineage fate choices of stem cells. *Stem Cells* **32**: 1323-1336, 2014.

DIBBLE CC, MANNING BD: Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. *Nat Cell Biol* **15**: 555-564, 2013.

DO NASCIMENTO CO, HUNTER L, TRAYHURN P: Regulation of haptoglobin gene expression in 3T3-L1 adipocytes by cytokines, catecholamines, and PPARγ. *Biochem Biophys Res Commun* **313**: 702-708, 2004.

ELSEN M, RASCHKE S, TENNAGELS N, SCHWAHN U, JELENIK T, RODEN M, ROMACHO T, ECKEL J: BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. *Am J Physiol Cell Physiol* **306**: C431-C440, 2014.

FISHER FM, KLEINER S, DOURIS N, FOX EC, MEPANI RJ, VERDEGUER F, WU J, KHARITONENKOV A, FLIER JS, MARATOS-FLIER E, SPIEGELMAN BM: FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev* **26**: 271-281, 2012.

FISHER FM, MARATOS-FLIER E: Understanding the physiology of FGF21. *Annu Rev Physiol* **78**: 223-241, 2016.

FLEURY C, NEVEROVA M, COLLINS S, RAIMBAULT S, CHAMPIGNY O, LEVI-MEYRUEIS C, BOUILLAUD F, SELDIN MF, SURWIT RS, RICQUIER D, WARDEN CH: Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* **15**: 269-272, 1997.

FONSECA TL, WERNECK-DE-CASTRO JP, CASTILLO M, BOCCO BM, FERNANDES GW, MCANINCH EA, IGNACIO DL, MOISES CC, FERREIRA AR, GEREBEN B, BIANCO AC: Tissue-specific inactivation of type 2 deiodinase reveals multilevel control of fatty acid oxidation by thyroid hormone in the mouse. *Diabetes* **63**: 1594-1604, 2014.

FU T, SEOK S, CHOI S, HUANG Z, SUINO-POWELL K, XU HE, KEMPER B, KEMPER JK: MiR-34a inhibits beige and brown fat formation in obesity in part by suppressing adipocyte FGF21 signaling and SIRT1 function. *Mol Cell Biol* **34**: 4130-4142, 2014.

GANTNER ML, HAZEN BC, CONKRIGHT J, KRALLI A: GADD45γ regulates the thermogenic capacity of brown adipose tissue. *Proc Natl Acad Sci U S A* **111**: 11870-11875, 2014.

GOBERDHAN DC, WILSON C, HARRIS AL: Amino acid sensing by mTORC1: intracellular transporters mark the spot. *Cell Metab* **23**: 580-589, 2016.

GÖKE R, FEHMANN HC, GÖKE B: Glucagon-like peptide-1(7-36) amide is a new incretin/enterogastrone candidate. *Eur J Clin Invest* **21**: 135-144, 1991.

GRANNEMAN JG, LI P, ZHU Z, LU Y: Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. *Am J Physiol Endocrinol Metab* **289**: E608-E616, 2005.

GUERRA C, KOZA RA, YAMASHITA H, WALSH K, KOZAK LP: Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest* **102**: 412-420, 1998.

GUERRA C, PORRAS A, RONCERO C, BENITO M, FERNANDEZ M: Triiodothyronine induces the expression of the uncoupling protein in long term fetal rat brown adipocyte primary cultures: role of nuclear thyroid hormone receptor expression. *Endocrinology* **134**: 1067-1074, 1994.

HANSEN JB, JØRGENSEN C, PETERSEN RK, HALLENBORG P, DE MATTEIS R, BØYE HA, PETROVIC N, ENERBÄCK S, NEDERGAARD J, CINTI S, TE RIELE H, KRISTIANSEN K: Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc Natl Acad Sci U S A* **101**: 4112-4117, 2004.

HARMS M, SEALE P: Brown and beige fat: development, function and therapeutic potential. *Nat Med* **19**: 1252-1263, 2013.

HARMS MJ, ISHIBASHI J, WANG W, LIM HW, GOYAMA S, SATO T, KUROKAWA M, WON KJ, SEALE P: Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell Metab* **19**: 593-604, 2014.

HILSE KE, KALINOVICH AV, RUPPRECHT A, SMORODCHENKO A, ZEITZ U, STANIEK K, ERBEN RG, POHL EE: The expression of UCP3 directly correlates to UCP1 abundance in brown adipose tissue. *Biochim Biophys Acta* **1857**: 72-78, 2016.

HIMMS-HAGEN J, HARPER ME: Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. *Exp Biol Med (Maywood)* **226**: 78-84, 2001.

HIMMS-HAGEN J, MELNYK A, ZINGARETTI MC, CERESI E, BARBATELLI G, CINTI S: Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am J Physiol Cell Physiol* **279**: C670-C681, 2000.

HOLST JJ, GRIBBLE F, HOROWITZ M, RAYNER CK: The role of gut hormones in glucose homeostasis. *J Clin Invest* **117**: 24-32, 2007.

HU F, WANG M, XIAO T, YIN B, HE L, MENG W, DONG M, LIU F: miR-30 promotes thermogenesis and the development of beige fat by targeting RIP140. *Diabetes* **64**: 2056-2068, 2015.

JIMENEZ M, BARBATELLI G, ALLEVI R, CINTI S, SEYDOUX J, GIACOBINO JP, MUZZIN P, PREITNER F: β3-adrenoceptor knockout in C57BL/6J mice depresses the occurrence of brown adipocytes in white fat. *Eur J Biochem* **270**: 699-705, 2003.

KAJIMURA S, SEALE P, KUBOTA K, LUNSFORD E, FRANGIONI JV, GYGI SP, SPIEGELMAN BM: Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature* **46**: 1154-1158, 2009.

KANG S, BAJNOK L, LONGO KA, PETERSEN RK, HANSEN JB, KRISTIANSEN K, MACDOUGALD OA: Effects of wnt signaling on brown adipocyte differentiation and metabolism mediated by pgc-1alpha. *Mol Cell Biol* **25**: 1272-1282, 2005.

KARBIENER M, PISANI DF, FRONTINI A, OBERREITER LM, LANG E, VEGIOPOULOS A, MÖSSENBÖCK K, BERNHARDT GA, MAYR T, HILDNER F, GRILLARI J, AILHAUD G, HERZIG S, CINTI S, AMRI EZ, SCHEIDELER M: MicroRNA-26 family is required for human adipogenesis and drives characteristics of brown adipocytes. *Stem Cells* **32**: 1578-1590, 2014.

KIR S, KOMABA H, GARCIA AP, ECONOMOPOULOS KP, LIU W, LANSKE B, HODIN RA, SPIEGELMAN BM: PTH/PTHrP receptor mediates cachexia in models of kidney failure and cancer. *Cell Metab* **23**: 315-323, 2016.

KISKINIS E, CHATZELI L, CURRY E, KAFOROU M, FRONTINI A, CINTI S, MONTANA G, PARKER MG, CHRISTIAN M: RIP140 represses the "brown-in-white" adipocyte program including a futile cycle of triacylglycerol breakdown and synthesis. *Mol Endocrinol* **28**: 344-353, 2014.

KOPECKÝ J, HODNÝ Z, ROSSMEISL M, SYROVÝ I, KOZAK LP: Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. *Am J Physiol* **270**: E768-E775, 1996.

KOZAK LP, ANUNCIADO-KOZA R: UCP1: its involvement and utility in obesity. *Int J Obes (Lond)* **32** (Suppl 7): S32-S38, 2008.

KRIEF S, LÖNNQVIST F, RAIMBAULT S, BAUDE B, VAN SPRONSEN A, ARNER P, STROSBERG AD, RICQUIER D, EMORINE LJ: Tissue distribution of β3-adrenergic receptor mRNA in man. *J Clin Invest* **91**: 344-349, 1993.

KUO MM, KIM S, TSENG CY, JEON YH, CHOE S, LEE DK: BMP-9 as a potent brown adipogenic inducer with anti-obesity capacity. *Biomaterial* **35**: 3172-3179, 2014.

LEE EY, CHANG CY, HU N, WANG YC, LAI CC, HERRUP K, LEE WH, BRADLEY A: Mice deficient for Rb are nonviable and show defects in neurogenesis and hematopoiesis. *Nature* **393**: 288-294, 1992.

LEE P, SMITH S, LINDERMAN J, COURVILLE AB, BRYCHTA RJ, DIECKMANN W, WERNER CD, CHEN KY, CELI FS: Temperature-acclimated brown adipose tissue modulates insulin sensitivity in humans. *Diabetes* **63**: 3686-3698, 2014.

LIANG H, WARD WF: PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ* **30**: 145-151, 2006.

LIU D, BORDICCHIA M, ZHANG C, FANG H, WEI W, LI JL, GUILHERME A, GUNTUR K, CZECH MP, COLLINS S: Activation of mTORC1 is essential for β-adrenergic stimulation of adipose browning. *J Clin Invest* **126**: 1704-1716, 2016.

LIU J, KONG X, WANG L, QI H, DI W, ZHANG X, WU L, CHEN X, YU J, ZHA J, LV S, ZHANG A, CHENG P, HU M, LI Y, BI J, LI Y, HU F, ZHONG Y, XU Y, DING G: Essential roles of 11β-HSD1 in regulating brown adipocyte function. *J Mol Endocrinol* **50**: 103-113, 2013.

LO KA, NG PY, KABIRI Z, VIRSHUP D, SUN L: Wnt inhibition enhances browning of mouse primary white adipocytes. *Adipocyte* **5**: 224-231, 2016.

LOCKIE SH, HEPPNER KM, CHAUDHARY N, CHABENNE JR, MORGAN DA, VEYRAT-DUREBEX C, ANANTHAKRISHNAN G, ROHNER-JEANRENAUD F, DRUCKER DJ, DIMARCHI R, RAHMOUNI K, OLDFIELD BJ, TSCHÖP MH, PEREZ-TILVE D: Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes* **61**: 2753-2762, 2012.

LONGO KA, WRIGHT WS, KANG S, GERIN I, CHIANG SH, LUCAS PC, OPP MR, MACDOUGALD OA: Wntl0b inhibits development of white and brown adipose tissues. *J Biol Chem* **279**: 35503-35509, 2004.

MARTÍNEZ-SÁNCHEZ N, MORENO-NAVARRETE JM, CONTRERAS C, RIAL-PENSADO E, FERNØ J, NOGUEIRAS R, DIÉGUEZ C, FERNÁNDEZ-REAL JM, LÓPEZ M: Thyroid hormones induce browning of white fat. *J Endocrinol* **232**: 351-362, 2017.

MARTINS L, SEOANE-COLLAZO P, CONTRERAS C, GONZÁLEZ-GARCÍA I, MARTÍNEZ-SÁNCHEZ N, GONZÁLEZ F, ZALVIDE J, GALLEGO R, DIÉGUEZ C, NOGUEIRAS R, TENA-SEMPERE M, LÓPEZ M: A functional link between AMPK and orexin mediates the effect of BMP8B on energy balance. *Cell Reports* **16**: 2231-2242, 2016.

NIE B, NIE T, HUI X, GU P, MAO L, LI K, YUAN R, ZHENG J, WANG H, LI K, TANG S, ZHANG Y, XU T, XU A, WU D, DING S: Brown adipogenic reprogramming induced by a small molecule. *Cell Reports* **18**: 624-635, 2017.

NOMURA E, TOYODA N, HARADA A, NISHIMURA K, UKITA C, MORIMOTO S, KOSAKI A, IWASAKA T, NISHIKAWA M: Type 2 iodothyronine deiodinase is expressed in human preadipocytes. *Thyroid* **21**: 305-310, 2011.

OBREGON MJ: Thyroid hormone and adipocyte differentiation. *Thyroid* **18**: 185-195, 2008.

OHNO H, SHINODA K, SPIEGELMAN BM, KAJIMURA S: PPARγ agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab* **15**: 395-404, 2012.

PAN D, MAO C, QUATTROCHI B, FRIEDLINE RH, ZHU LJ, JUNG DY, KIM JK, LEWIS B, WANG YX: MicroRNA-378 controls classical brown fat expansion to counteract obesity. *Nat Commun* **22**: 4725, 2014.

PETRUZZELLI M, SCHWEIGER M, SCHREIBER R, CAMPOS-OLIVAS R, TSOLI M, ALLEN J, SWARBRICK M, ROSE-JOHN S, RINCON M, ROBERTSON G, ZECHNER R, WAGNER EF: A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. *Cell Metab* **20**: 433-447, 2014.

POLAK P, CYBULSKI N, FEIGE JN, AUWERX J, RÜEGG MA, HALL MN: Adipose-specific knockout of raptor results in lean mice with enhanced mitochondrial respiration. *Cell Metab* **8**: 399-410, 2008.

RODRÍGUEZ AM, PALOU A: The steroid RU486 induces UCP1 expression in brown adipocytes. *Pflugers Arch* **449**: 170-174, 2004.

RUBIO A, RAASMAJA A, SILVA JE: Thyroid hormone and norepinephrine signaling in brown adipose tissue. II: Differential effects of thyroid hormone on beta 3-adrenergic receptors in brown and white adipose tissue. *Endocrinology* **136**: 3277-3284, 1995.

SAITO M, OKAMATSU-OGURA Y, MATSUSHITA M, WATANABE K, YONESHIRO T, NIO-KOBAYASHI J, IWANAGA T, MIYAGAWA M, KAMEYA T, NAKADA K, KAWAI Y, TSUJISAKI M: High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* **58**: 1526-1531, 2009.

SEALE P, BJORK B, YANG W, KAJIMURA S, CHIN S, KUANG S, SCIMÈ A, DEVARAKONDA S, CONROE HM, ERDJUMENT-BROMAGE H, TEMPST P, RUDNICKI MA, BEIER DR, SPIEGELMAN BM: PRDM16 controls a brown fat/skeletal muscle switch. *Nature* **454**: 961-967, 2008.

SHARMA A, HUARD C, VERNOCHET C, ZIEMEK D, KNOWLTON KM, TYMINSKI E, PARADIS T, ZHANG Y, JONES JE, VON SCHACK D, BROWN CT, MILOS PM, COYLE AJ, TREMBLAY F, MARTINEZ RV: Brown fat determination and development from muscle precursor cells by novel action of bone morphogenetic protein 6. *PLoS One* **21**: e92608, 2014.

SOUMANO K, DESBIENS S, RABELO R, BAKOPANOS E, CAMIRAND A, SILVA JE: Glucocorticoids inhibit the transcriptional response of the uncoupling protein-1 gene to adrenergic stimulation in a brown adipose cell line. *Mol Cell Endocrinol* **165**: 7-15, 2000.

SUN L, TRAJKOVSKI M: MiR-27 orchestrates the transcriptional regulation of brown adipogenesis. *Metabolism* **63**: 272-282, 2014.

SUN L, XIE H, MORI MA, ALEXANDER R, YUAN B, HATTANGADI SM, LIU Q, KAHN CR, LODISH HF: MiR193b-365 is essential for brown fat differentiation. *Nat Cell Biol* **13**: 958-965, 2011.

TRAN CM, MUKHERJEE S, YE L, FREDERICK DW, KISSIG M, DAVIS JG, LAMMING DW, SEALE P, BAUR JA: Rapamycin blocks induction of the thermogenic program in white adipose tissue. *Diabetes* **65**: 927-941, 2016.

TSENG YH, KOKKOTOU E, SCHULZ TJ, HUANG TL, WINNAY JN, TANIGUCHI CM, TRAN TT, SUZUKI R, ESPINOZA DO, YAMAMOTO Y, AHRENS MJ, DUDLEY AT, NORRIS AW, KULKARNI RN, KAHN CR: New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* **454**: 1000-1004, 2008.

TSUBOYAMA-KASAOKA N: Mitochondrial uncoupling protein 3 (UCP3) in skeletal muscle. *Front Biosci* **6**: D570-D574, 2001.

TUCA A, GIRALT M, VILLARROYA F, VIÑAS O, MAMPEL T, IGLESIAS R: Ontogeny of thyroid hormone receptors and CERBa expression during brown adipose tissue development: evidence of fetal acquisition of the mature thyroid status. *Endocrinology* **132**: 1913-1920, 1993.

ULDRY M, YANG W, ST-PIERRE J, LIN J, SEALE P, SPIEGELMAN BM: Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab* **3**: 333-341, 2006.

VAN MARKEN LICHTENBELT WD, VANHOMMERIG JW, SMULDERS NM, DROSSAERTS JM, KEMERINK GJ, BOUVY ND, SCHRAUWEN P, TEULE GJ: Cold-activated brown adipose tissue in healthy men. *N Engl J Med* **360**: 1500-1508, 2009.

VIDAL-PUIG A, SOLANES G, GRUJIC D, FLIER JS, LOWELL B: UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and adipose tissue. *Biochem Biophys Res Commun* **235**: 79-82, 1997.

VIENGCHAREUN S, PENFORNIS P, ZENNARO MC, LOMBÈS M: Mineralocorticoid and glucocorticoid receptors inhibit UCP expression and function in brown adipocytes. *Am J Physiol Endocrinol Metab* **280**: E640-E649, 2001.

WADA S, NEINAST M, JANG C, IBRAHIM YH, LEE G, BABU A, LI J, HOSHINO A, ROWE GC, RHEE J, MARTINA JA, PUERTOLLANO R, BLENIS J, MORLEY M, BAUR JA, SEALE P, ARANY Z: The tumor suppressor FLCN mediates an alternate mTOR pathway to regulate browning of adipose tissue. *Genes Dev* **30**: 2551-2564, 2016.

WANG W, KISSIG M, RAJAKUMARI S, HUANG L, LIM HW, WON KJ, SEALE P: Ebf2 is a selective marker of brown and beige adipogenic precursor cells. *Proc Natl Acad Sci U S A* **111**: 14466-14471, 2014.

WU J, BOSTRÖM P, SPARKS LM, YE L, CHOI JH, GIANG AH, KHANDEKAR M, VIRTANEN KA, NUUTILA P, SCHAART G, HUANG K, TU H, VAN MARKEN LICHTENBELT WD, HOEKS J, ENERBÄCK S, SCHRAUWEN P, SPIEGELMAN BM: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150**: 366-376, 2012.

WU Y, ZUO J, ZHANG Y, XIE Y, HU F, CHEN L, LIU B, LIU F: Identification of miR-106b-93 as a negative regulator of brown adipocyte differentiation. *Biochem Biophys Res Commun* **438**: 575-580, 2013.

XIANG X, LAN H, TANG H, YUAN F, XU Y, ZHAO J, LI Y, ZHANG W: TSC1-mTORC1 signaling determines brown-to-white adipocyte phenotypic switch. *Diabetes* **64**: 519-528, 2014.

XU F, LIN B, ZHENG X, CHEN Z, CAO H, XU H, LIANG H, WENG J: GLP-1 receptor agonist promotes brown remodelling in mouse white adipose tissue through SIRT1. *Diabetologia* **59**: 1059-1069, 2016.

XU J, LLOYD DJ, HALE C, STANISLAUS S, CHEN M, SIVITS G, VONDERFECHT S, HECHT R, LI YS, LINDBERG RA, CHEN JL, JUNG DY, ZHANG Z, KO HJ, KIM JK, VÉNIANT MM: Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes* **58**: 250-259, 2009.

YANG Q, LIANG X, SUN X, ZHANG L, FU X, ROGERS CJ, BERIM A, ZHANG S, WANG S, WANG B, FORETZ M, VIOLLET B, GANG DR, RODGERS BD, ZHU MJ, DU M: AMPK/α-ketoglutarate axis dynamically mediates DNA demethylation in the PRDM16 promoter and brown adipogenesis. *Cell Metab* **24**: 542-554, 2016.

YIN H, PASUT A, SOLEIMANI VD, BENTZINGER CF, ANTOUN G, THORN S, SEALE P, FERNANDO P, VAN IJCKEN W, GROSVELD F, DEKEMP RA, BOUSHEL R, HARPER ME, RUDNICKI MA: MicroRNA-133 controls brown adipose determination in skeletal muscle satellite cells by targeting Prdm16. *Cell Metab* **17**: 210-224, 2013.

YOUNG P, ARCH JR: Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett* **167**: 10-14, 1984.

ZHANG Y, LI R, MENG Y, LI S, DONELAN W, ZHAO Y, QI L, ZHANG M, WANG X, CUI T, YANG LJ, TANG D: Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes* **63**: 514-525, 2014.

ZONCU R, EFEYAN A, SABATINI DM: mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* **12**: 21-35, 2011.