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Review

Role of PRDM16 in the activation of brown fat programming. Relevance to the development of obesity

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Summary. From a histological and functional point of view, two types of adipose tissue can be identified. As opposed to the mainly unilocular white adipocytes, brown adipocytes possess plenty of small multilocular lipid droplets and dissipate energy as heat. Moreover, two distinct types of brown adipose cells exist. *In vivo* fate mapping experiments of brown adipose tissue (BAT) precursors suggest that classical brown adipocytes and skeletal myoblasts originate from a common mesenchymal, myogenic factor 5 (Myf5)-positive precursor cell. In addition to the classical brown adipocytes, thermogenic brown-like adipocytes (brite/beige cells) may appear within white adipose tissue (WAT) depots, sharing many of the morphological and functional features of brown adipocytes, but arising from a *Myf5*-negative lineage. In humans, the conversion of white fat cells into brite adipocytes could be a strategy to increase energy expenditure. The zinc finger transcription factor Prdm16 controls the bidirectional fate decision between brown adipocytes and myoblasts. Prdm16 determines the brown fat-like programme and thermogenesis in both brown and white adipose tissues. Moreover, the expression of this transcriptional regulator is strongly correlated with beige cell-selective genes. From a therapeutical point of view, the potential of inducing BAT or the transdifferentiation of WAT into beige cells by enhancing Prdm16 expression, as well as the identification of mechanisms of Prdm16 function and regulation represent potentially exciting new approaches for treatment or prevention of obesity and related diseases.

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Introduction

Obesity is becoming a major global health problem often associated with substantial morbidity and mortality, highlighting the urgent need for new therapeutic approaches (James, 2008; Frühbeck, 2012). Prevention and treatment of obesity both rely on the principles of energy balance regulation. Obesity results from a chronic imbalance between energy intake and total energy expenditure (Galgani and Ravussin, 2008). Adipose tissue mass is controlled by interactions between the individual's genetic background, the environment and behavioural factors. Two types of adipose tissues have been found in mammals, white (WAT) and brown adipose tissue (BAT), which accomplish opposite functions. Both adipose tissues can be distinguished from each other based on their morphology, physiological functions and gene expression profile. WAT represents the major site of energy storage in the form of triglycerides (TG) acting as a specialised fuel storage depot. Although this organ was previously considered as a passive tissue with relatively simple functions such as energy storage, heat insulation and mechanical protection, recent studies have demonstrated that WAT constitutes an active endocrine source of key hormones involved in the regulation of a wide variety of physiological functions, including energy balance (Ahima and Flier, 2000; Frühbeck and Gómez-Ambrosi, 2001; Frühbeck et al., 2001; Muruzábal et al., 2002; Frühbeck, 2006; Gómez-Ambrosi et al., 2006, 2008; Lancha et al., 2012). In contrast to WAT, BAT is able to contribute positively to energy expenditure due to

its prominent role in non-shivering thermogenesis (Frühbeck et al., 2009a). The proton transporter uncoupling protein UCP-1 located in the inner mitochondrial membrane mediates BAT thermogenesis through uncoupling ATP synthesis from the mitochondrial respiration chain, releasing the chemical energy in the form of heat (Gómez-Ambrosi et al., 1999). Due to these functional differences, attention has been focused on the balance between brown and white fat, since it affects energy balance and may contribute to the development of obesity (Cannon and Nedergaard, 2004).

BAT was initially considered physiologically important only in small animals and neonates, allowing the adaptation to a cold environment. Recent studies with positron-emission tomography (PET) and computed tomography (CT) have provided evidence of the existence of several discrete areas of metabolically active BAT in adult humans upon cold exposure, particularly in the supraclavicular and neck region (Nedergaard et al., 2007; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). BAT activity is inversely related to body mass index (BMI) and body fat percentage suggesting a significant role in metabolism (van Marken Lichtenbelt et al., 2009). These observations have renewed the interest in BAT non-shivering thermogenesis as a possible therapeutic target for the treatment of obesity (Frühbeck et al., 2009a).

The skeletal muscle is another important organ for thermorregulation. Three types of thermogenesis take place in skeletal muscle: exercise-induced thermogenesis, non-exercise activity thermogenesis (NEAT), and cold-induced shivering thermogenesis (Frühbeck, 2005a; Tseng et al., 2010). Moreover, muscle acts as a secretory organ through the production of different myokines affecting different organs, including BAT (Lancha et al., 2012). Several studies demonstrate the implication of skeletal muscle in heat production without muscle contraction, although the molecular underpinning of its involvement has not been fully unveiled. The mechanism for muscle-based thermogenesis involves ATP turnover and the maintenance of the Ca^{2+} gradient mediated by the sarco(endo)plasmic reticulum Ca^{2+} ATPases (SERCA proteins). The 31-aa membrane protein sarcolipin interacts with these proteins inhibiting their activity, with this interaction being the basis for skeletal muscle thermogenesis as well as an important mediator of whole-body energy metabolism (Bal et al., 2012).

Origen of adipose tissue

Although it has generally been considered that adipose tissue, muscle as well as bone and cartilage arise from a common mesenchymal progenitor (Rosen and MacDougald, 2006) the precise mesenchymal stem cell lineage that produces white and brown adipocytes is not fully known. The commitment of mesenchymal stem cells to myogenic, adipogenic and fibrogenic lineages can be considered a competitive process controlled by different inductive regulators. Although adipocytes from WAT and BAT have been shown to derive from a separate and distinct population of progenitors, they share many common features. Gene expression profiling reveals an extensive array of markers in common related to adipogenesis, lipid mobilization (lipolysis) or fat storage (fatty acid transport, synthesis and esterification). In fact, adipogenesis is controlled by a cascade of transcription factors that is in large part shared by both white and brown adipocytes (Gesta et al., 2007; Timmons et al., 2007; Farmer, 2008b; Kajimura et al., 2010). An initial step is the upregulation of the CCAAT/enhancer binding proteins ß and δ (C/EBPß and δ), which induces the expression of C/EBPα and the transcription factor peroxisome proliferator-activated receptor γ, PPARγ. Inactivation of C/EBPß in mice permits the differentiation into white and brown adipocytes, although with low efficiency in the brown fat lineage, as reflected by a loss of *Ucp1* expression in this tissue (Tanaka et al., 1997).

BAT emerged in parallel with the evolution of nonshivering thermogenesis and endothermo-regulation in birds through to mammals, evolving significantly later than WAT (Gesta et al., 2007). BAT development starts around the second trimester of gestation in humans and about embryonic day 15.5 (E15.5) in rodents. BAT exhibits all the features of the mature tissue at birth, being the only important organ for heat production (Cannon and Nedergaard, 1986), whereas WAT development starts at midgestation (humans) or post-natally (rodents) with its mass gradually increasing during postnatal life (Langin et al., 2009). Contrary to what was initially thought, the evolutionary, developmental and functional features of both tissues suggest separate and distinct origins. By lineage tracing using the homeobox transcription factor *Engrailed-1 (En-1)*, Atit et al. (2006) reported that cells expressing this gene in the dermomyotome of the early embryo (E9.5) gave rise not only to skeletal muscle and dermis, but also to BAT in the interscapular dorsal cervical region. Skeletal muscle develops from myogenic precursors through a complex process requiring the involvement of the transcription factor *Myf5*, among others. Timmons et al. (2007) demonstrated that brown preadipocytes exhibit a myogenic signature, sharing an early transcriptional programme with skeletal muscle cells. Most recently, Seale et al. (2007) in lineage tracing studies *in vivo* showed that brown but not white fat cells arise from *Myf5*-expressing precursors, demonstrating a close relationship between BAT and skeletal muscle during development (Walden et al., 2009) (Fig. 1). The common developmental origin can be observed at the level of the mitochondrial proteome when comparing differentiated brown fat and skeletal muscle (Forner et al., 2009). The ontogenic relationship between BAT and skeletal muscle may explain why brown adipose cells are involved in lipid catabolism rather than storage, similar to oxidative skeletal muscle tissue. It evidences a

close relationship between them, as well as helping to explain the frequent neighbouring zones of myotubes and brown adipocytes during fetal development, as can be observed in Fig. 2. PR domain containing 16 (PRDM16), as described below, has been identified as the major molecular determinant of the brown adipose/skeletal muscle fate switch from a common progenitor that expresses the myoblast marker *Myf5*.

Characteristics of WAT, BAT and skeletal muscle

Despite sharing the functional feature of storing energy in the form of triacylgycerols (TG), both WAT and BAT differentiate in anatomical locations, macroscopic and microscopic characteristics or specific regulation. As described previously, WAT accumulates excess energy in the form of TG whereas BAT plays an important role in thermoregulatory heat production (nonshivering thermogenesis) and in diet-induced thermogenesis (Frühbeck et al., 2009a). Oxidative skeletal muscle shares many features with BAT (Table 1). Both of them are innervated by the sympathetic nervous system and expend energy by oxidative phosphorylation, being specialized in lipid catabolism rather than storage (Smorlesi et al., 2012). Skeletal muscle expends the stored energy when there is an

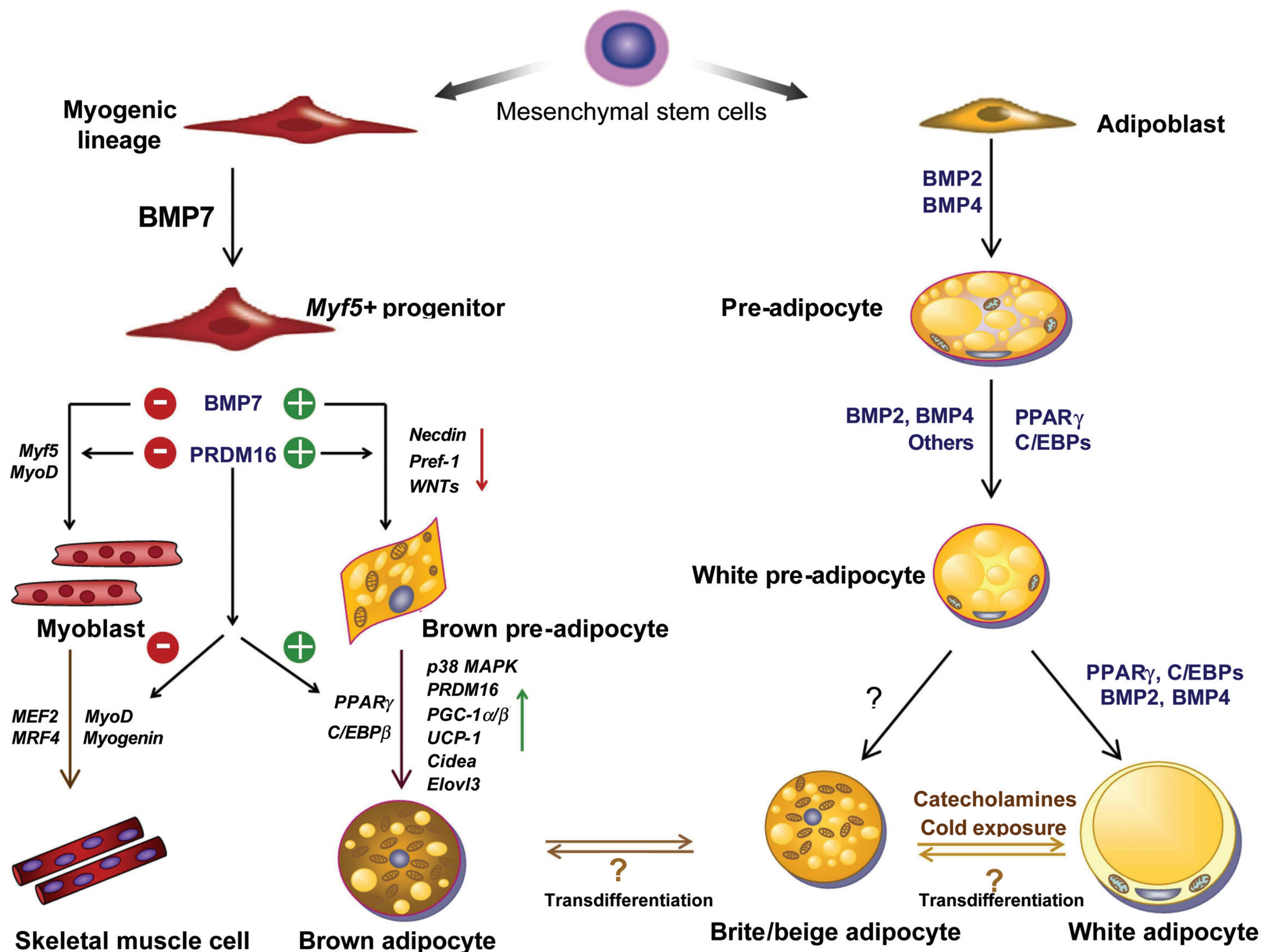

Fig. 1. Representation of the developmental pathways leading to the differentiation of fat cells. Adipocyte and myocyte differentiate from mesenchymal precursors. *Myf5*+ expressing precursors give rise to both brown adipocytes and myocytes, whereas *Myf5*- precursors differentiate into adipoblasts and then preadipocytes through the action of key regulators. BMP2 and BMP4 promote the differentiation of the white adipocyte lineage, whereas BMP7 specifically promotes brown-fat differentiation in both mesenchymal progenitor cells and committed brown preadipocytes. The brown fat differentiation program is stimulated by PRDM16, at least in part, via coactivating C/EBPß and inducing the expression of PPARγ, with the subsequent activation of key features that determine the brown-fat fate. At the same time, PRDM16 suppresses myogenesis, thus decreasing the expression of myogenic genes such as *MyoD*, *myogenin*, myocyte enhancer factor 2 (*MEF2*) and *myogenic related factor 4 (MRF4)*. Moreover, the adipocytes within the WAT depot may be induced to transdifferentiate into BAT cells under physiological conditions *in vivo*. Some conditions, such as cold exposure or chronic ß-adrenoceptor stimulation have been shown experimentally to drive the conversion from white to beige/brite adipocytes.

increase in energy demand, e.g. by exercise or shivering thermogenesis. However, despite containing abundant mitochondria, skeletal muscle does not express Ucp1 (Farmer, 2008a). The existence of a common progenitor cell confers the strongest link between brown fat and skeletal muscle. Not suprisingly, leptin and other more recently identified adipose-related factors such as aquaporins and caveolins are well known to exert important physiological effects in BAT and skeletal muscle (Frühbeck et al., 1995; Frühbeck, 2005b; Campo et al., 2007; Catalán et al., 2008; Sáinz et al., 2009). As previously described, skeletal muscle and brown adipocytes develop from progenitor cells expressing the transcription factor Myf5. The myogenic precursor cells arise from the dermomyotome expressing the paired-homeodomain transcription factors Pax3/7, important regulators of the myogenic cell fate. When myogenesis begins, Pax3 expression decreases and the expression of muscle regulatory factors such as Myf5, *MyoD, Myog and Myf4* significantly increases. Cells that continue to express myogenic determination factors develop into myocytes, whereas cells expressing the transcription factor Prdm16 become brown adipocytes (Buckingham et al., 2006; Yokoyama and Asahara, 2011).

Molecular switches in the differentiation of BAT

Despite the differences in functional and

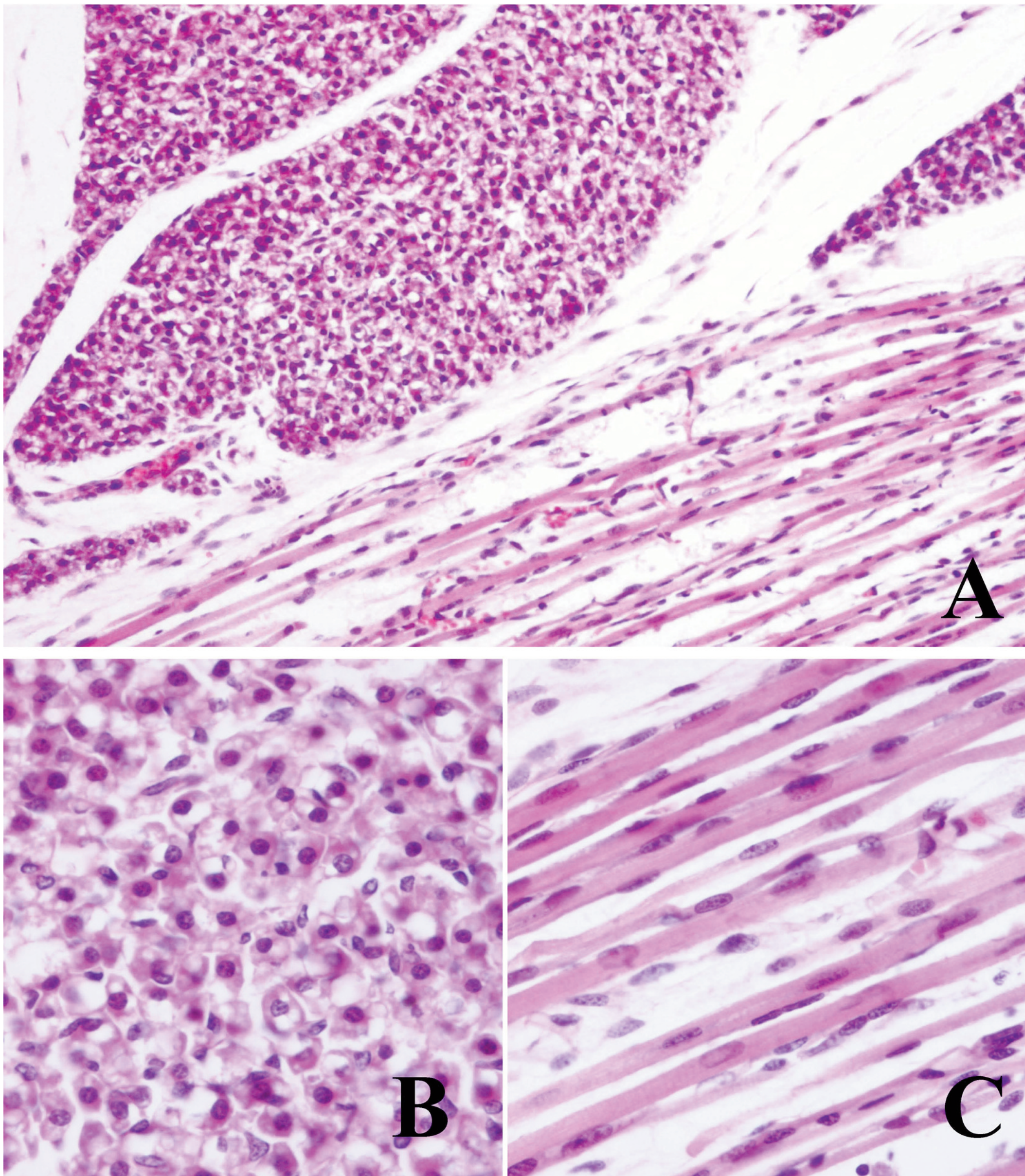

Fig. 2. A. Representative histological section of BAT and skeletal muscle myotubes in a paraffin section obtained from a 19 day-old mouse embryo. Developmental proximity between BAT and skeletal muscle reveals an evolutional and functional link between them. **B.** Detail of the developing BAT cells, showing round and often centrally located nuclei and lipid droplets in less numbers than adult BAT cells. **C.** Developing skeletal myotubes, which are formed by sequential fusion of myoblasts, are tubular and multinucleated. Haematoxylin-eosin stain.

developmental characteristics of white and brown adipocytes, both PPARγ and CCAAT/enhancer binding proteins (C/EBPs) transcription factors have been traditionally considered to participate in the terminal adipocyte differentiation process of brown and white adipocytes into mature fat cells (Rosen and MacDougald, 2006; Feige and Auwerx, 2007; Koppen and Kalkhoven, 2010). C/EBPα and PPARγ positively regulate each other's expression and they both coordinate the maintenance of the stable differentiated state of adipocytes. However, these molecules do not control the determination of the brown fat cell fate since their ectopic expression in mesenchymal cells induces a white, not brown, fat phenotype. Moreover, PPARγ is indispensable for both white and brown fat cell development, despite this factor alone not being sufficient to drive the mesenchymal cells into a brown fat programme (Seale et al., 2007). The browning activity of PPARγ is mediated through its Sirt-1-dependent deacetylation via regulation of the ligand-dependent coactivator/corepressor exchange at the PPARγ transcriptional complex (Qiang et al., 2012). Several studies have identified transcriptional regulators that control brown fat development that are briefly outlined below.

Forkhead Box C2 (FOXC2)

Forkhead box C2 belongs to the forkhead/winged helix transcription factor family and constitutes an important regulator of adipocyte metabolism. Intra-abdominal WAT of mice overexpressing *FoxC2* acquired a brown-fat phenotype with increased number of mitochondria, elongated mitochondrial morphology and elevated expression of thermogenic genes including *Ucp1* and *Pparg1a*. Moreover, transcription factors

Table 1. Main differential characteristics between white and brown adipocyes, as well as myocytes.

Characteristic	White adipocyte	Brown adipocyte	Skeletal muscle cell
Differentiation	Adipoblast (*Myf5*-lineage)	*Myf5+* progenitor	*Myf5+* progenitor
Funtional properties	Energy storage (TG)	Dissipation of energy (thermogenesis)	Contraction - Oxidative phosphorylation
Macroscopic properties			
Main depot localization	Abdominal, inguinal, perirenal, retroperitoneal, gonadal	Interscapular, paravertebral, axillary, perineal	Throughout the body attached to bones
Vascularization	Good vascularization/capillaries	Rich vascularization/capillaries	High vascularization/capillaries
Innervation	Mainly sympathetic; also parasympathetic	Sympathetic	Sympathetic
Tissue organization	Densely packed cells	Lobular arrangement with gland-like structure	Multinucleated fibers grouped in bundles
Colour	White, ivory, yellow	Brown (variable from dark red to tan)	Red (from pale to dark red) depending on myoglobin
Microscopic properties			
Shape	Spherical, round	Polygonal, polyhedral	Elongated, cylindrical, threadlike cells
Size	25-200 μm	15-60 μm	10-100 μm diameter, length variable
Lipid droplets	Unilocular	Multilocular	Unilocular when present in certain conditions (athletes and metabolic diseases)
Nucleus	Peripheral semilunar, flattened	Centrally located, round or oval shape	Multinucleated, peripheral, oval
Mitochondria	Few, small, elongated	Abundant, large, round	Abundant, round elongated
Proteins	Metabolism of fatty acids/detoxification	Pyruvate and fatty acid oxidation activities	Pyruvate and fatty acid oxidation activities
Function	Lipogenic function	Metabolic function	Metabolic function
Fatty acid composition	Monoeic acids	Cholesterol and phospholipids	-
Gene expression profile			
UCPS	*Ucp2*, no *Ucp1*	*Ucp1*, *Ucp2*, *Ucp3*	*Ucp3*, no *Ucp1*
Adrenoreceptors	Mainly β_1-AR (β_3-AR, $\alpha_{1/2}$-AR)	Mainly β_3-AR (β_3-AR, $\alpha_{1/2}$-AR)	β_2, β_2-AR
PGC1	Downregulated	Upregulated	Upregulated
PRDM16	Downregulated	Upregulated	Downregulated
Leptin	Upregulated	Present at birth, not in adulthood	Upregulated
Other genes	*Dio2*, *Elovl3*, *Cox8b*, *Tr3*, *Ppara* are downregulated	*Dio2*, *Elovl3*, *Cox8b*, *Tr3*, *Ppara*, *Pparg*, *C/ebpb* are upregulated	*MyoD*, *Myf5*, *Myf6*, *Miogenin* are upregulated

involved in adipocyte differentiation such as C/EBPα, PPARγ or SREBP1 are upregulated (Cederberg et al., 2001; Lidell et al., 2011). The browning effect of FOXC2 is achieved by increasing the sensitivity of the ß-adrenergic cAMP protein kinase A (PKA) signalling pathway. Furthermore, FOXC2 stimulates angiogenesis to support elevated metabolism in active BAT by activating the expression of angiopoietin-2 (Xue et al., 2008).

PGC-1α and its transcriptional regulators

The PPARγ coactivator-1 α (PGC-1α) is involved in the regulation of crucial aspects of energy metabolism, such as mitochondrial biogenesis or oxidative metabolic pathways (Handschin and Spiegelman, 2006; Scarpulla et al., 2012). It was first discovered as a cold-inducible transcription coactivator of adaptive thermogenesis driving the activation of the thermogenic gene program of brown fat.

The regulation of the transcriptional activity or gene expression of *Ppargc1a* influences brown fat development and function. Studies have suggested that PGC-1α activity is positively regulated by several transcriptional regulators; these include the steroid receptor coactivator-1 (SRC-1) (Picard et al., 2002), SIRT-1 (Lagouge et al., 2006), leucine-rich protein 130 (LRP130) (Cooper et al., 2006) and PRDM16 (Seale et al., 2007), among others. PRDM16 directly binds to PGC-1α, allowing the activation of *Ucp-1* and other brown fat-specific genes (Seale et al., 2007; Frühbeck et al., 2009b). Conversely, *Ppargc1a* mRNA expression in the brown fat is inhibited by the orphan nuclear receptor SHP, the retinoblastoma protein (RB), as well as by p107, another member of the RB pocket protein family (Scime et al., 2005; Wang et al., 2005). Two factors, GCN5 and $p160^{MBP}$, have been shown to suppress PGC-1α activity (Fan et al., 2004; Lerin et al., 2006). The corepressor RIP140 also affects PGC-1α, antagonising its transcriptional function through a physical interaction (Hallberg et al., 2008). Furthermore, the SRC (steroid receptor coactivator) family member SRC-2/TIF2/GRIPI represses PGC-1α transcriptional activity, inhibiting its interaction with PPARγ (Picard et al., 2002). Twist1 also participates in the maintenance of energy homeostasis, inhibiting PGC-1α function (Pan et al., 2009).

BMPs

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor ß (TGF-ß) superfamily (Wozney et al., 1988) and exert pleiotropic effects in embryonic development and differentiation including adipogenesis (Chen et al., 2004). It has been described that BMPs 2 and 4 are able to increase the commitment of mesenchymal cells to the adipocyte lineage (Tang et al., 2004). Both proteins also participate in the achievement of the characteristics of mature adipocytes by the acquisition of all the machinery needed for lipid transport, synthesis of TG or secretion of proteins (Bowers and Lane, 2007; Frühbeck et al., 2009b).

BMP7

Contrary to BMP2 and BMP4, which promote the differentiation of the white adipocyte lineage, BMP7 drives brown fat cell fate in mesenchymal precursor cells. It selectively induces brown preadipocyte differentiation, activating a full program of brown adipogenesis, including induction of the regulator PGC-1α, as well as the key molecular determinant PRDM16, at the same time as suppressing early adipogenic inhibitors, such as necdin, Pref-1 and WNTs (Seale et al., 2007; Tseng et al., 2008). Moreover, BMP7 upregulates the expression of the biomarker specific of brown adipocytes *Ucp1*, the adipogenic transcription factors PPARγ and C/EBPs, as well as increasing mitochondrial biogenesis. BMPs may act as a controller of brown adipogenic instead of skeletal muscle cell fate since these proteins negatively regulate myogenesis in certain contexts (Murray et al., 1993; Katagiri et al., 1997; Reshef et al., 1998) (Fig. 1).

BMP8B

Whittle et al. (2012) have recently described the induction of BMP8B in response to nutritional and thermogenic factors in mature BAT, acting as a component of the thermogenic machinery through enhanced p38MAPK/CREB signalling. In fact, $Bmp8b^{-/-}$ mice have impaired thermogenesis and are susceptible to diet-induced obesity. Moreover, BMP8B is expressed in key hypothalamic nuclei controlling energy balance acting together with AMPK. This group demonstrated that BMP8B increases the peripheral response of BAT to adrenergic stimulation while acting centrally to increase sympathetic output to BAT.

Myostatin

Myostatin, another member of the TGF-ß superfamily, acts as a potent negative regulator of brown adipogenic differentiation suppressing the expression of brown -adipocyte-specific genes, including *PRDM16, Ppargc1a* and *Ucp1* (Kim et al., 2012).

PRDM16

PRDM16 (PRD1-BF-1-RIZ1 homologous domain-containing protein 16) is a 140 kDa zinc finger transcription factor identified as the key molecular switch determining brown fat development from a myoblastic progenitor. Seale et al. (2007) identified this transcription factor together with two others (*Zic1* and *Lhx8*) greatly enriched in brown as compared to white fat (expression of *Prdm16* mRNA showed a 15-fold enrichment in BAT cells). PRDM16 controls brown adipocyte formation by inducing expression of BAT-

specific genes while suppressing expression of WAT-specific genes.

Transdifferentiation of BAT

Of special interest is the capability of WAT to transdifferentiate into BAT. Transdifferentiation consists of a process whereby a mature differentiated cell transforms into a new phenotype with a different morphology and physiology without going through dedifferentiation (Eberhard and Tosh, 2008). Adipocytes possess this physiological property. Two diverse types of brown or brown-like adipose cells, with different developmental origins can be identified. Classical brown fat cells, as described previously, are derived from a *Myf5*-positive lineage through the action of the transcriptional regulators PRDM16 and C/EBP (Fig.1). In addition, under certain conditions such as cold exposure, prolonged ß-adrenergic receptor activation or PPARγ agonist treatment UCP-1 positive brown fat-like cells that emerge within white fat pads are designated brite (corresponding to the contraction of "brown in white") or beige cells (Seale et al., 2008, 2010; Petrovic et al., 2010; Wu et al., 2012). These beige cells posses many of the biochemical and morphological characteristics of classical brown adipocytes, including multilocular appearance. Lipid deposition in a discrete subpopulation of multilocular cells morphologically similar to BAT can be observed in Figure 3. The multilocular adipocytes stained positive for UCP-1, demonstrating that brown fat-like adipocytes emerging in WAT are metabolically active (Fig. 4). Although beige cells present low basal levels of *Ucp1* gene expression, they respond to cyclic AMP stimulation with high levels of UCP-1, activating a robust program of respiration and energy expenditure as do classical brown adipocytes (Wu et al., 2012).

The origin of these newly formed brown-like adipocytes is being discussed. Neither an increase in adipocyte number nor in DNA content in white fat depots is observed, suggesting that cell proliferation mechanisms are not implicated in this process (Cousin et al., 1996; Manieri et al., 2009; Barbatelli et al., 2010). Data obtained from independent research groups using different experimental methods have shown that the early marker of myogenesis Myf5 is not present in the lineage of beige cells, concluding that these brown-like adipocytes in WAT are ontogenically different from those of the classical interscapular BAT (Atit et al., 2006; Seale et al., 2008; Petrovic et al., 2010). Previously, it had been reported that the amounts of UCP-1 positive cells in the white and classical brown fat pads are controlled by different genetic loci, strongly suggesting a distinct regulation of these diverse types of thermogenic cells (Koza et al., 2000; Xue et al., 2005, 2007).

The brown-like transformation is more frequent in the inguinal subcutaneous fat pads, whereas the perigonadal depots are less susceptible to this change in the phenotype. Browning of WAT in rodents can be achieved by various factors (some of which have been already mentioned above); pharmacological activators of PPARγ (e.g. rosiglitazone), cytokines and hormones such as FGF21 (Fisher et al., 2012) and irisin (Böstrom et al., 2012) or by transcriptional modulation through PRDM16 (Seale et al., 2011), FoxC2 (Cederberg et al., 2001), Rip140 (Powelka et al., 2006) or 4E-BP1 (Tsukiyama-Kohara et al., 2001), among others. The

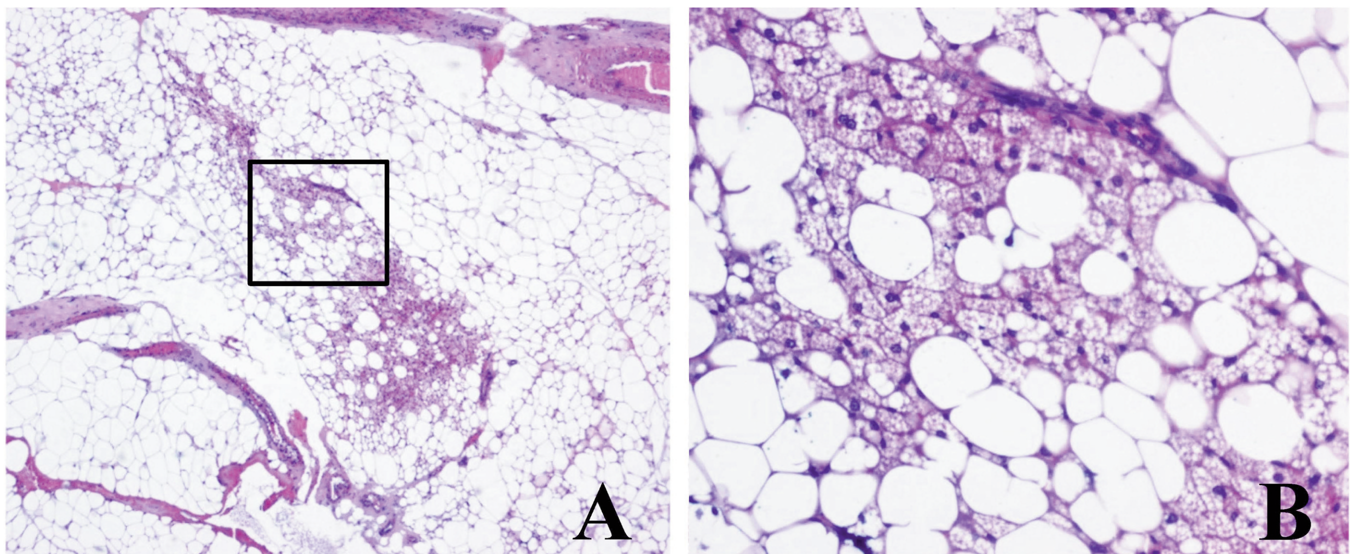

Fig. 3. A. Foci of multilocular adipose tissue, morphologically similar to BAT, can be found in white adipose tissue. **B.** Detail of the rectangle in **(A)** showing multilocular adipocytes, which are smaller and contain abundant lipid droplets, intermingled with bigger unilocular white adipocytes. Haematoxylin-eosin stain.

rate-limiting enzyme in prostaglandins synthesis cyclooxygenase-2 (COX-2) as well as the cardiac natriuretic peptides are also reportedly implicated in the *de novo* BAT recruitment in WAT depots (Vegiopoulos et al., 2010; Bordicchia et al., 2012). Nitric oxide is another important factor implicated in brown-like transformation, participating in the recovery of the BAT phenotype and the improvement of brown fat cell function, likely involving the transcriptional coactivator MED1 (Becerril et al., 2010, 2012).

The existence of beige fat cells may provide flexibility in adaptive thermogenesis, constituting an evolutionarily conserved cellular mechanism. The group of Spiegelman observed that the whole systemic improvement resulting from transgenic overexpression of muscle *Ppargc1a* was not only due to the local action of this transcriptional coactivator (Böstrom et al., 2012). They suggested the existence of a protein secreted by muscle that could mediate the beneficial effects on the whole-body metabolism. In this sense, exercise increased the expression levels of *Ppargc1a* in mouse skeletal muscle, leading to a "brown-like" adipose tissue programme through the induction of the type I protein FNCD5. This protein is cleaved and secreted as irisin, which reaches adipose tissue via the circulation and changes the genetic profile of WAT after binding to an unidentified receptor. Of particular interest, irisin induces the expression of *Ucp1* and other BAT-associated genes through the increase of *Ppara* expression, browning WAT and increasing energy expenditure. Nevertheless, studies in humans are not evident and FNCD5 has not been positively linked to an improvement in systemic metabolism, highlighting that the role of muscle FNCD5 in human metabolism needs to be further analysed (Pedersen and Febbraio, 2012; Timmons et al., 2012).

PRDM16

Structural features

PRDM16, also known as positive regulatory domain I-binding factor 1 and retinoblastoma-interacting zinc finger protein-1 [PRDIBF1-RIZ1] belongs to a 17 (human) or 16 (mouse) member family of PR (PRDI-BF1 and RIZ1homologous) domain containing proteins (PRDMs). PRDM16 was initially identified at a chromosomal breakpoint of t(1;3)(p36;q21)-positive human acute myeloid leukemia cells (Mochizuki et al., 2000).

As illustrated in Figure 5, PRDM16 is a 140 kDa zinc-finger protein that contains seven repeats of C2H2 zinc-finger DNA binding domain (ZF1 domain) after the N-terminus and another similar three zinc-finger domain (ZF2 domain) at its C-terminus. The first pair of zinc-coordinating residues are cysteines, and the second pair are histidines, conferring zinc-dependent DNA- or RNA-binding properties. The PR region also contains a SET domain, a conserved region with histone methyl-transferase activity and a PLDLS motif at position 804-808 (Nishikata et al., 2003; Kajimura et al., 2008, 2010).

Transcriptional control of brown fat determination by PRDM16

Molecular mechanisms involved in BAT differentiation

PRDM16 controls brown fat determination by stimulating brown fat selective gene expression while simultaneously suppressing the expression of white adipocyte- and myogenic-specific genes. This switching mechanism is due to the ability of PRDM16 to interact

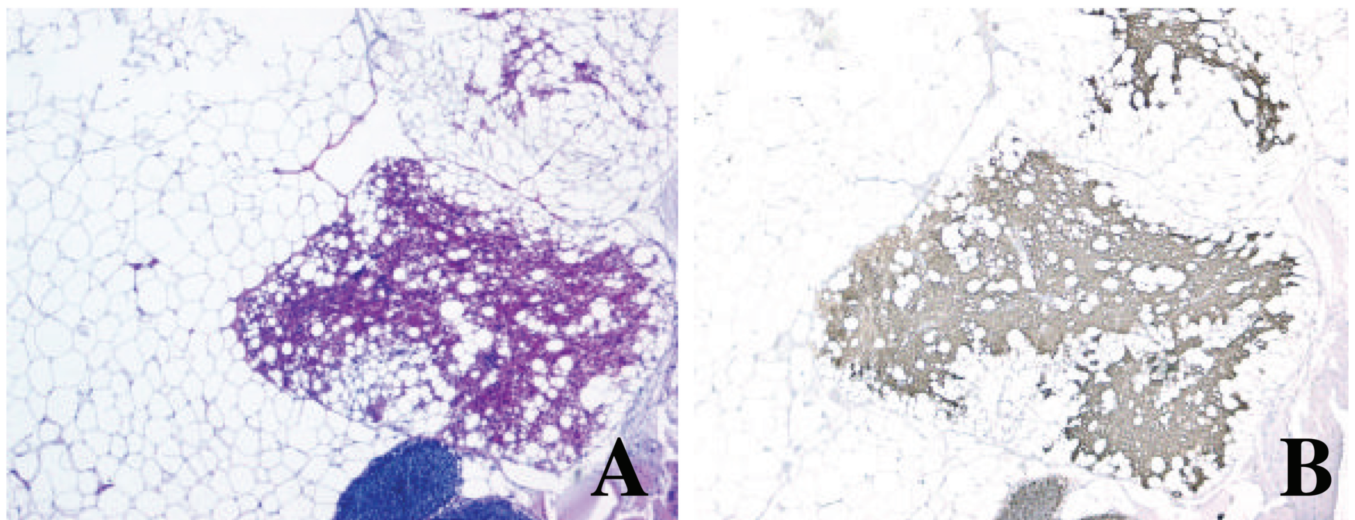

Fig. 4. A pair of paraffin serial sections showing that the area of multilocular adipose tissue, stained with haematoxylin-eosin in **(A)**, exhibits immunostaining with an antibody against uncoupling protein-1 (UCP-1) in **(B)**. **A.** Haematoxylin-eosin stain. **B.** EnVision method.

with specific transcription factors associated with the promoters and/or enhancers of the respective target genes (Frühbeck et al., 2009b). PRDM16 can bind through ZF1 and ZF2 and co-activate the transcriptional function of PPARγ, increasing the activity of PGC-1α y PGC-1ß. Indeed, PRDM16 activates the selective brown adipocyte gene programme (e.g. *Elovl3, Cidea* and *Ucp1*, among others) by binding to and stimulating PGC-1α and PGC-1ß (Seale et al., 2008). In this line, PRDM16 promotes adipogenesis by binding and activating the master regulator of adipogenic differentiation PPARγ. On the other hand, PRDM16 directly interacts with the corepressor proteins CtBP1 and CtBP2 through the PLDLS motif. This recruitment is supposed to specifically mediate the suppressive effects of PRDM16 on white fat- and myogenic-selective gene expression (e.g. resistin, angiotensinogen, myoD, myogenin, among others) (Kajimura et al., 2008) (Fig. 6). It has been recently discovered that the myogenic regulator *MyoD*, together with the growth factor *Igf2*, act simultaneously, but through independent pathways, to repress PRDM16 (Borensztein et al., 2012). The binding of PGC-1s or CtBPs to PRDM16 is mutually exclusive, and the involvement of other transcriptional factors cannot be completely ruled out. Although PRDM16, via the zinc-fingers, directly binds to a specific DNA sequence, abrogation of DNA binding by inserting a point mutation in ZF2 does not modify the capacity of PRDM16 to induce the brown fat phenotype. This observation suggests that PRDM16 action is principally mediated by protein-protein interactions, rather than by direct DNA binding (Seale et al., 2009).

Recently, different studies have identified microRNAs (miRNAs) as being implicated in BAT determination and function. Sun et al. (2011) have identified a brown-fat-enriched miRNA cluster, miR-193b-365, as a key regulator of brown fat development. Ectopic expression of miR193b in C2C12 myoblasts represses the expression of the myogenic markers *Pax3* and *MyoD*, upregulating the expression of the brown-fat-selective markers *Ucp1, Cidea* or *Prdm16*. Moreover, it is also described that the miR-193b-365 cluster is upregulated by *Prdm16* partially through *Ppara*, creating a feedforward loop that ensures differentiation of brown adipocytes from bipotential brown adipocyte/myocyte progenitors. It has been reported as well that both miR193b and miR365 probably regulate other factors in addition to Prdm16 to control brown fat adipogenesis, acting as essential regulators of brown fat differentiation. Furthermore, the muscle-enriched miR-133 directly

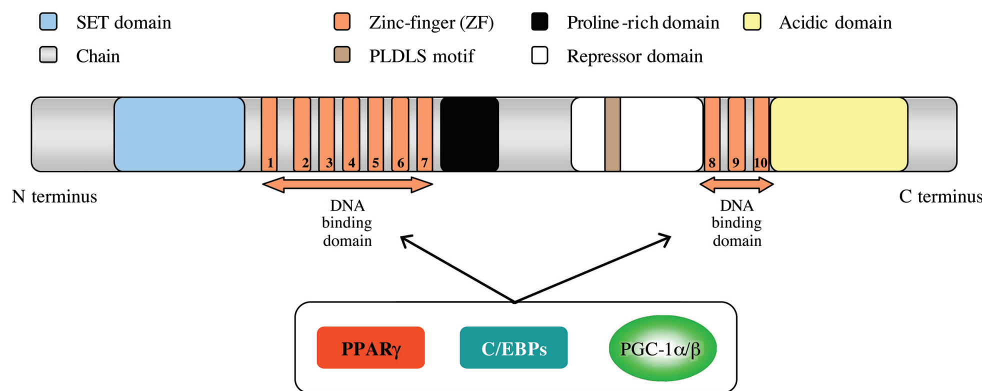

Fig. 5. Structure of PRDM16 and key functional domains. Following the N-terminal PR domain, PRDM16 has a zinc finger DNA-binding domain, a proline-rich domain, a repressor domain, a second zinc finger DNA-binding domain, and a C-terminal acidic domain. The two sets of zinc-finger DNA binding domains correspond to the classical C2H2-type. These two regions, called ZF1 and ZF2, have been identified to interact with canonical transcription factors such as PPARγ, whereas the correpresors CtBPs are associated to PRDM16 though the PLDLS motif.

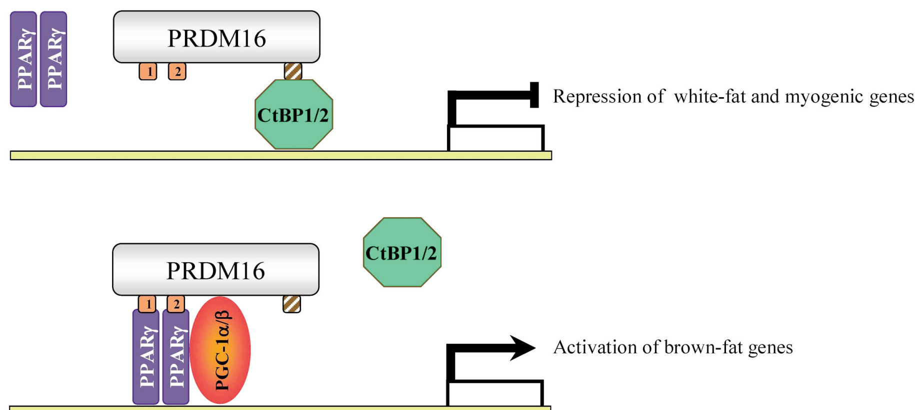

Fig. 6. Brown fat cell determination by PRDM16. The coregulatory protein PRDM16 functions as a bidirectional switch controlling brown fat development by inducing the expression of select brown adipocyte genes and simultaneously repressing the expression of white adipocyte- and myogenic-selective genes. The corepresor proteins CtBP1 and CtBP2 directly bind to PRDM16 through the PLDLS motif, suppressing white fat-selective gene expression. Moreover, PRDM16 directly binds to PPARγ through ZF1 and ZF2, thereby activating its transcriptional function, increasing the transcriptional activities of PGC-1α and PGC-1ß. The coactivation drives a brown fat differentiation program.

interacts with target sites in the *Prdm16* transcripts, decreasing its expression. Adrenergic stimulation leads to a marked downregulation of miR-133, resulting in a de-repression of *Prdm16* increasing brown adipocyte differentiation (Trajkovski et al., 2012).

PRDM16 expression induces mitochondrial biogenesis

BAT contains high amounts of densely packed mitochondria required to dissipate the energy as heat, so it is plausible that the BAT differentiation process is associated with mitochondrial biogenesis. As described previously, PRDM16 activates BAT-selective genes, including genes responsible for mitochondrial biogenesis and oxidative metabolism at least in part by increasing the transcriptional activity of PGC-1α and PGC-1ß. Seale et al. (2007) generated cells lacking the *Pparg* gene and induced them to differentiate into adipocytes by retroviral introduction of PPARγ in presence or absence of PRDM16. The studies revealed an intense elevation of mitochondrial density in differentiated adipocytes upon PRDM16 expression compared to cells expressing PPARγ only. Moreover, silencing of PRDM16 expression during brown fat cell differentiation blunts mitochondrial biogenesis and expression of BAT-selective genes. In summary, expression of PRDM16 affects mitochondrial DNA replication, gene expression and biogenesis during fat cell differentiation (Murholm et al., 2009).

Thermogenic activity

Initially, it was observed that when mice were exposed to cold temperatures (4°C for 4 h), there was no change in *Prdm16* mRNA expression. This suggested that *Prdm16* expression was connected to differentiation of preadipocytes into BAT cells, but not to adaptive thermogenesis (Seale et al., 2007).

The transcriptional coactivator PGC-1α, highly expressed in BAT, activates the thermogenic gene program through the control of the gene expression levels of *Ucp1* and *Ppargc1a* itself (Puigserver et al., 1998; Gómez-Ambrosi et al., 2001). PGC-1α has been recently identified as a direct target of PPARα transcriptional regulation in BAT. It has also been observed that PPARα directly interacts with PRDM16, suggesting that by interacting with PPARα, PRDM16 regulates the expression of thermogenic genes through induction of the *Ppargc1a* gene (Hondares et al., 2011).

Knockdown and transgenic expression of PRDM16

The determining role of PRDM16 in the brown fat phenotype has been investigated using short interfering RNA (siRNA) technology to specifically knock down PRDM16 in the brown fat cell lineage (Seale et al., 2007). PRDM16 knockdown of cultured brown fat preadipocytes resulted in a broad loss of brown fat gene expression, including *Ucp1, Ppargc1a, Cidea* or *Ppara*. The expression of a broad set of mitochondrial genes important to brown fat function was also significantly reduced. On the contrary, some white fat-selective genes such as resistin were increased. Despite siRNA-mediated depletion of PRDM16 causing a near total ablation in the differentiation into brown fat cells, chronic loss of PRDM16 in mice caused a significant but more modest reduction in brown fat cell characteristics and promoted overt skeletal muscle differentiation. It is suggested that one or more of the other 15 PR-domain containing family members could partially compensate for the loss of PRDM16 from BAT *in vivo* (Seale et al., 2007).

Transgenic overexpression of PRDM16, driven by the aP2 promoter in white fat cells, increased the expression of a broad set of BAT-selective genes, including activation of thermogenic and/or mitochondrial BAT-selective genes in white fat depots (Kajimura et al., 2008; Seale et al., 2011). Moreover, it resulted in the emergence of multilocular BAT-type cells that stained intensely for the UCP-1 protein within the WAT. In summary, PRDM16 activates a broad program of brown fat differentiation when expressed in cultured white fat preadipocytes or in white fat depots *in vivo*.

Transdifferentiation of WAT. Role of PRDM16

PRDM16, besides acting as the master regulator in the bifurcation between the myocyte and the brown adipocyte fates, has been recently considered to be involved in the development of brite cells in subcutaneous fat pads induced by PPARγ agonists (Walden et al., 2011). In fact, *Prdm16* is highly expressed in subcutaneous WAT (scWAT) relative to its levels in epididymal and retroperitoneal fat pads in mice. A full agonism of PPARγ is required to activate the thermogenic brown-fat gene program in scWAT. The activation requires the expression of *Prdm16*, since depletion of this transcriptional factor blunts the effects of the PPARγ agonist on the induced brown fat gene program. Furthermore, PPARγ agonists promote the development of beige adipocytes through the stabilization of the PRDM16 protein (Ohno et al., 2012).

The transcription factor T-box 15 (TBX15) is expressed predominantly in BAT, being capable of giving rise to brite adipocytes. *Tbx15* knockout mice reportedly reduce the expression of *Prdm16* and other phenotypic marker genes such as *Ppargc1a, Cox8b* or *Ucp1*. *Tbx15* knockdown also impairs adipogenesis in primary brown and brite adipocytes and mitochondrial biogenesis, concluding that this transcription factor seems to be essential for the enhancement of the brown phenotype in both brown and brite adipocytes (Gburcik et al., 2012).

Another factor involved in adipocyte transdifferentiation is the vascular endothelial growth factor VEGF-A. Repression of this growth factor

programmed brown-like adipocyte differentiation in WAT through stimulation of BAT-specific transcription factors, including PPARγ, C/EBPß or PRDM16 (Lu et al., 2012). The tumor suppressor PTEN also participates in brown adipocyte function due to its ability to improve the generation of subcutaneous BAT implants from PRDM16/C/EBPß-programmed fibroblasts (Ortega-Molina et al., 2012).

Transgenic expression of *Prdm16* in *scWAT* stimulates as well the development of brown-like adipocytes. Moreover, depletion of Prdm16 in isolated subcutaneous adipocytes provokes a decrease in the expression of thermogenic genes and a reduction of the brown-fat phenotype, concluding that PRDM16 determines the brown-fat-like and thermogenic gene program in scWAT of mice (Seale et al., 2011). In summary, PRDM16 can turn on a full set of BAT-selective genes when expressed in WAT precursors in culture or *in vivo*, while turning off the expression of several white fat-enriched genes.

The presence and relevance of beige fat cells in humans remains largely unknown. Sharp et al. (2012) studied the molecular signatures of human BAT isolated from different adipose depots and unexpectedly observed an undetectable expression of brown fat-selective genes but an abundance of beige selective ones. Moreover, PRDM16 expression correlated with beige fat-selective genes but not with that of brown fat, indicating that PRDM16 may be an important factor for the formation of human beige adipocytes. The relevance of PRDM16 and beige fat cells in the development of human obesity remains to be elucidated.

Role of PRDM16 in the potential therapeutic use of BAT for treating obesity

The differentiation program of both classical brown and beige adipocytes is complex and coordinated by multiple factors, representing potential sites for pharmacological manipulation. The potential role of PRDM16 in human energy balance has not been fully established. The master brown adipocyte differentiation factor PRDM16 acts as a dominant regulator of brown fat cell determination controlling the development of adipocytes, as well as the acquisition of the thermogenic function and phenotype of BAT. However, it is unknown whether this factor or related pathways can be exploited as new therapeutic alternatives for the treatment of obesity and linked metabolic diseases (Frühbeck et al., 2009a; Tseng et al., 2010; Whittle et al., 2011). Clarification of the functional relevance of brown adipocyte differentiation, the molecular determinants that enhance the brown fat cell commitment programme, as well as PRDM16 protein stability, might facilitate the design of PRDM16-modulating agents for effective therapeutic manipulation (Frühbeck et al., 2009b). Nevertheless, this strategy requires further characterization of the binding sites of PRDM16, the implication of the protein-protein interactions as well as the intrinsic activity of the PR-domain or its post-transcriptional regulation.

Concluding remarks and future perspectives

With the rising prevalence of obesity, attention has been focused on the control of the signalling underlying white and brown adipocyte differentiation. The confirmation of the existence and functionality of BAT in adult humans, and the evidence of its anti-obesity functions in rodents (Hansen and Kristiansen, 2006) has caused great interest in the development and function of brown adipocytes. The increase in BAT activity as well as the activation of conversion of white adipocytes into brown fat cells provides an attractive approach to the treatment of obesity and related metabolic complications. Changes in BAT activity affect not only body temperature, but also obesity and glucose homeostasis. In fact, subcutaneous transplants of embryonic BAT improve diabetes in mice with impaired glucose tolerance by inducing a significant loss of WAT, resulting in normalized glucose tolerance, reduced tissue inflammation and reversal of clinical diabetes markers such as polyphagia or polyuria (Gómez-Hernández et al., 2012). To unravel the therapeutic potential of activating brown fat-mediated thermogenesis in humans more studies are clearly needed.

Besides classical brown adipocytes, thermogenic brown-like adipocytes (beige adipocytes) appear within WAT depots, sharing many of the morphological and functional features of brown adipocytes while originating from a different source. The existence of beige fat cells may provide flexibility in adaptive thermogenesis constituting an evolutionarily conserved cellular mechanism. In humans, the conversion of white adipocytes into beige cells could be a strategy to increase energy consumption. From a therapeutical point of view, the potential of inducing BAT or transdifferentiation of WAT into beige cells in adults by enhancing PRDM16 expression would be an exciting new approach for the treatment or prevention of obesity. Gene silencing and microRNA tools will be further developed in the years to come and may provide new therapeutic avenues. In conclusion, defining the cues that promote brown fat induction, transdifferentiation and activation of the energy expenditure program will yield novel drug targets for the treatment or prevention of obesity and its comorbidities.

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References

Ahima R.S. and Flier J.S. (2000). Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327-332.

Atit R., Sgaier S.K., Mohamed O.A., Taketo M.M., Dufort D., Joyner A.L., Niswander L. and Conlon R.A. (2006). ß-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. Dev. Biol. 296, 164-176.

Bal N.C., Maurya S.K., Sopariwala D.H., Sahoo S.K., Gupta S.C., Shaikh S.A., Pant M., Rowland L.A., Goonasekera S.A., Molkentin J.D. and Periasamy M. (2012). Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. Nat. Med. 18, 1575-1579.

Barbatelli G., Murano I., Madsen L., Hao Q., Jimenez M., Kristiansen K., Giacobino J.P., De Matteis R. and Cinti S. (2010). The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am. J. Physiol. Endocrinol. Metab. 298, E1244-1253.

Becerril S., Rodríguez A., Catalán V., Sáinz N., Ramírez B., Collantes M., Peñuelas I., Gómez-Ambrosi J. and Frühbeck G. (2010). Deletion of inducible nitric-oxide synthase in leptin-deficient mice improves brown adipose tissue function. PLoS One 5, e10962.

Becerril S., Rodríguez A., Catalán V., Sainz N., Ramírez B., Gómez-Ambrosi J. and Frühbeck G. (2012). Transcriptional analysis of brown adipose tissue in leptin-deficient mice lacking inducible nitric oxide synthase: evidence of the role of Med1 in energy balance. Physiol. Genomics 44, 678-688.

Bordicchia M., Liu D., Amri E.Z., Ailhaud G., Dessi-Fulgheri P., Zhang C., Takahashi N., Sarzani R. and Collins S. (2012). Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J. Clin. Invest. 122, 1022-1036.

Borensztein M., Viengchareun S., Montarras D., Journot L., Binart N., Lombes M. and Dandolo L. (2012). Double Myod and Igf2 inactivation promotes brown adipose tissue development by increasing Prdm16 expression. FASEB J. 26, 4584-4591.

Böstrom P., Wu J., Jedrychowski M.P., Korde A., Ye L., Lo J.C., Rasbach K.A., Bostrom E.A., Choi J.H., Long J.Z., Kajimura S., Zingaretti M.C., Vind B.F., Tu H., Cinti S., Hojlund K., Gygi S.P. and Spiegelman B.M. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481, 463-8.

Bowers R.R. and Lane M.D. (2007). A role for bone morphogenetic protein-4 in adipocyte development. Cell Cycle 6, 385-389.

Buckingham M., Bajard L., Daubas P., Esner M., Lagha M., Relaix F. and Rocancourt D. (2006). Myogenic progenitor cells in the mouse embryo are marked by the expression of Pax3/7 genes that regulate their survival and myogenic potential. Anat. Embryol (Berl). 211 (Suppl) 1, 51-56.

Campo A., Frühbeck G., Zulueta J.J., Iriarte J., Seijo L.M., Alcaide A.B., Galdiz J.B. and Salvador J. (2007). Hyperleptinaemia, respiratory drive and hypercapnic response in obese patients. Eur. Respir. J. 30, 223-231.

Cannon B. and Nedergaard J. (1986). Brown adipose tissue thermogenesis in neonatal and cold-adapted animals. Biochem. Soc. Trans. 14, 233-236.

Cannon B. and Nedergaard J. (2004). Brown adipose tissue: function and physiological significance. Physiol. Rev. 84, 277-359.

Catalán V., Gómez-Ambrosi J., Rodríguez A., Silva C., Rotellar F., Gil M.J., Cienfuegos J.A., Salvador J. and Frühbeck G. (2008). Expression of caveolin-1 in human adipose tissue is upregulated in obesity and obesity-associated type 2 diabetes mellitus and related to inflammation. Clin. Endocrinol (Oxf). 68, 213-219.

Cederberg A., Gronning L.M., Ahren B., Tasken K., Carlsson P. and Enerback S. (2001). FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell 106, 563-573.

Chen D., Zhao M. and Mundy G.R. (2004). Bone morphogenetic proteins. Growth Factors 22, 233-241.

Cooper M.P., Qu L., Rohas L.M., Lin J., Yang W., Erdjument-Bromage H., Tempst P. and Spiegelman B.M. (2006). Defects in energy homeostasis in Leigh syndrome French Canadian variant through PGC-1α/LRP130 complex. Genes Dev. 20, 2996-3009.

Cousin B., Bascands-Viguerie N., Kassis N., Nibbelink M., Ambid L., Casteilla L. and Penicaud L. (1996). Cellular changes during cold acclimatation in adipose tissues. J. Cell. Physiol. 167, 285-289.

Eberhard D. and Tosh D. (2008). Transdifferentiation and metaplasia as a paradigm for understanding development and disease. Cell. Mol. Life Sci. 65, 33-40.

Fan M., Rhee J., St-Pierre J., Handschin C., Puigserver P., Lin J., Jaeger S., Erdjument-Bromage H., Tempst P. and Spiegelman B.M. (2004). Suppression of mitochondrial respiration through recruitment of p160 myb binding protein to PGC-1α: modulation by p38 MAPK. Genes Dev. 18, 278-289.

Farmer S.R. (2008a). Brown fat and skeletal muscle: unlikely cousins? Cell 134, 726-727.

Farmer S.R. (2008b). Molecular determinants of brown adipocyte formation and function. Genes Dev. 22, 1269-1275.

Feige J.N. and Auwerx J. (2007). Transcriptional coregulators in the control of energy homeostasis. Trends Cell Biol. 17, 292-301.

Fisher F.M., Kleiner S., Douris N., Fox E.C., Mepani R.J., Verdeguer F., Wu J., Kharitonenkov A., Flier J.S., Maratos-Flier E. and Spiegelman B.M. (2012). FGF21 regulates PGC-1α and browning of white adipose tissues in adaptive thermogenesis. Genes Dev. 26, 271-281.

Forner F., Kumar C., Luber C.A., Fromme T., Klingenspor M. and Mann M. (2009). Proteome differences between brown and white fat mitochondria reveal specialized metabolic functions. Cell Metab. 10, 324-335.

Frühbeck G. (2005a). Does a NEAT difference in energy expenditure lead to obesity? Lancet 366, 615-616.

Frühbeck G. (2005b). Obesity: aquaporin enters the picture. Nature 438, 436-437.

Frühbeck G. (2006). Intracellular signalling pathways activated by leptin. Biochem. J. 393, 7-20.

Frühbeck G. (2012). Obesity: Screening for the evident in obesity. Nat. Rev. Endocrinol. 8, 570-572.

Frühbeck G. and Gómez-Ambrosi J. (2001). Rationale for the existence of additional adipostatic hormones. FASEB J. 15, 1996-2006.

Frühbeck G., Alonso R., Marzo F. and Santidrián S. (1995). A modified method for the indirect quantitative analysis of phytate in foodstuffs. Anal. Biochem. 225, 206-212.

Frühbeck G., Gómez-Ambrosi J. and Salvador J. (2001). Leptin-induced lipolysis opposes the tonic inhibition of endogenous adenosine in white adipocytes. FASEB J. 15, 333-340.

Frühbeck G., Becerril S., Sáinz N., Garrastachu P. and Garcia-Velloso M.J. (2009a). BAT: a new target for human obesity? Trends

Pharmacol. Sci. 30, 387-396.
Frühbeck G., Sesma P. and Burrell M.A. (2009b). PRDM16: the interconvertible adipo-myocyte switch. Trends Cell Biol. 19, 141-146.
Galgani J. and Ravussin E. (2008). Energy metabolism, fuel selection and body weight regulation. Int. J. Obes (Lond). 32 (Suppl 7), S109-119.
Gburcik V., Cawthorn W.P., Nedergaard J., Timmons J.A. and Cannon B. (2012). An essential role for Tbx15 in the differentiation of brown and "brite" but not white adipocytes. Am. J. Physiol. Endocrinol. Metab. 303, E1053-1060.
Gesta S., Tseng Y.H. and Kahn C.R. (2007). Developmental origin of fat: tracking obesity to its source. Cell 131, 242-256.
Gómez-Ambrosi J., Frühbeck G. and Martinez J.A. (1999). Leptin, but not a β_3-adrenergic agonist, upregulates muscle uncoupling protein-3 messenger RNA expression: short-term thermogenic interactions. Cell. Mol. Life Sci. 55, 992-997.
Gómez-Ambrosi J., Frühbeck G. and Martínez J.A. (2001). Rapid in vivo PGC-1 mRNA upregulation in brown adipose tissue of Wistar rats by a β_3-adrenergic agonist and lack of effect of leptin. Mol. Cell. Endocrinol. 176, 85-90.
Gómez-Ambrosi J., Salvador J., Rotellar F., Silva C., Catalán V., Rodríguez A., Jesus Gil M. and Frühbeck G. (2006). Increased serum amyloid A concentrations in morbid obesity decrease after gastric bypass. Obes. Surg. 16, 262-269.
Gómez-Ambrosi J., Rodríguez A., Catalán V. and Frühbeck G. (2008). The bone-adipose axis in obesity and weight loss. Obes. Surg. 18, 1134-1143.
Gómez-Hernández A., Otero Y.F., de las Heras N., Escribano O., Cachofeiro V., Lahera V. and Benito M. (2012). Brown fat lipoatrophy and increased visceral adiposity through a concerted adipocytokines overexpression induces vascular insulin resistance and dysfunction. Endocrinology 153, 1242-1255.
Hallberg M., Morganstein D.L., Kiskinis E., Shah K., Kralli A., Dilworth S.M., White R., Parker M.G. and Christian M. (2008). A functional interaction between RIP140 and PGC-1α regulates the expression of the lipid droplet protein CIDEA. Mol. Cell. Biol. 28, 6785-6795.
Handschin C. and Spiegelman B.M. (2006). Peroxisome proliferator-activated receptor Á coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr. Rev. 27, 728-735.
Hansen J.B. and Kristiansen K. (2006). Regulatory circuits controlling white versus brown adipocyte differentiation. Biochem. J. 398, 153-168.
Hondares E., Rosell M., Díaz-Delfín J., Olmos Y., Monsalve M., Iglesias R., Villarroya F. and Giralt M. (2011). Peroxisome proliferator-activated receptor α (PPARα) induces PPARγ coactivator 1alpha (PGC-1α) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. J. Biol. Chem. 286, 43112-43122.
James W.P. (2008). The epidemiology of obesity: the size of the problem. J. Intern. Med. 263, 336-352.
Kajimura S., Seale P., Tomaru T., Erdjument-Bromage H., Cooper M.P., Ruas J.L., Chin S., Tempst P., Lazar M.A. and Spiegelman B.M. (2008). Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. Genes Dev. 22, 1397-1409.
Kajimura S., Seale P. and Spiegelman B.M. (2010). Transcriptional control of brown fat development. Cell Metab. 11, 257-262.
Katagiri T., Akiyama S., Namiki M., Komaki M., Yamaguchi A., Rosen V., Wozney J.M., Fujisawa-Sehara A. and Suda T. (1997). Bone morphogenetic protein-2 inhibits terminal differentiation of myogenic cells by suppressing the transcriptional activity of MyoD and myogenin. Exp. Cell Res. 230, 342-351.
Kim W.K., Choi H.R., Park S.G., Ko Y., Bae K.H. and Lee S.C. (2012). Myostatin inhibits brown adipocyte differentiation via regulation of Smad3-mediated ,-catenin stabilization. Int. J. Biochem. Cell. Biol. 44, 327-334.
Koppen A. and Kalkhoven E. (2010). Brown vs white adipocytes: the PPARγ coregulator story. FEBS Lett. 584, 3250-3259.
Koza R.A., Hohmann S.M., Guerra C., Rossmeisl M. and Kozak L.P. (2000). Synergistic gene interactions control the induction of the mitochondrial uncoupling protein (Ucp1) gene in white fat tissue. J. Biol. Chem. 275, 34486-34492.
Lagouge M., Argmann C., Gerhart-Hines Z., Meziane H., Lerin C., Daussin F., Messadeq N., Milne J., Lambert P., Elliott P., Geny B., Laakso M., Puigserver P. and Auwerx J. (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 127, 1109-1122.
Lancha A., Frühbeck G. and Gómez-Ambrosi J. (2012). Peripheral signalling involved in energy homeostasis control. Nutr. Res. Rev. 25, 223-248.
Langin D., Frübeck G., Frayn K.N. and Lafontan M. (2009). Adipose tissue: Development, anatomy and functions. In: Obesity science to practice. 1st. edition. Williams G. and Frühbeck G. (eds). Wiley-Blackwell. West Sussex. pp 79-103.
Lerin C., Rodgers J.T., Kalume D.E., Kim S.H., Pandey A. and Puigserver P. (2006). GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1α. Cell Metab. 3, 429-438.
Lidell M.E., Seifert E.L., Westergren R., Heglind M., Gowing A., Sukonina V., Arani Z., Itkonen P., Wallin S., Westberg F., Fernandez-Rodriguez J., Laakso M., Nilsson T., Peng X.R., Harper M.E. and Enerback S. (2011). The adipocyte-expressed forkhead transcription factor Foxc2 regulates metabolism through altered mitochondrial function. Diabetes 60, 427-435.
Lu X., Ji Y., Zhang L., Zhang Y., Zhang S., An Y., Liu P. and Zheng Y. (2012). Resistance to obesity by repression of VEGF gene expression through induction of brown-like adipocyte differentiation. Endocrinology 153, 3123-3132.
Manieri M., Murano I., Fianchini A., Brunelli A. and Cinti S. (2009). Morphological and immunohistochemical features of brown adipocytes and preadipocytes in a case of human hibernoma. Nutr. Metab. Cardiovasc. Dis. 20, 567-574.
Mochizuki N., Shimizu S., Nagasawa T., Tanaka H., Taniwaki M., Yokota J. and Morishita K. (2000). A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. Blood 96, 3209-3214.
Murholm M., Dixen K., Qvortrup K., Hansen L.H., Amri E.Z., Madsen L., Barbatelli G., Quistorff B. and Hansen J.B. (2009). Dynamic regulation of genes involved in mitochondrial DNA replication and transcription during mouse brown fat cell differentiation and recruitment. PLoS One 4, e8458.
Murray S.S., Murray E.J., Glackin C.A. and Urist M.R. (1993). Bone morphogenetic protein inhibits differentiation and affects expression of helix-loop-helix regulatory molecules in myoblastic cells. J. Cell. Biochem. 53, 51-60.
Muruzábal F.J., Frühbeck G., Gómez-Ambrosi J., Archanco M. and Burrell M.A. (2002). Immunocytochemical detection of leptin in non-

mammalian vertebrate stomach. Gen. Comp. Endocrinol. 128, 149-152.

Nedergaard J., Bengtsson T. and Cannon B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. Am. J. Physiol. Endocrinol. Metab. 293, E444-452.

Nishikata I., Sasaki H., Iga M., Tateno Y., Imayoshi S., Asou N., Nakamura T. and Morishita K. (2003). A novel EVI1 gene family, MEL1, lacking a PR domain (MEL1S) is expressed mainly in t(1;3)(p36;q21)-positive AML and blocks G-CSF-induced myeloid differentiation. Blood 102, 3323-3332.

Ohno H., Shinoda K., Spiegelman B.M. and Kajimura S. (2012). PPARγ agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. Cell Metab. 15, 395-404.

Ortega-Molina A., Efeyan A., López-Guadamillas E., Muñoz-Martín M., Gómez-López G., Canamero M., Mulero F., Pastor J., Martínez S., Romanos E., Mar González-Barroso M., Rial E., Valverde A.M., Bischoff J.R. and Serrano M. (2012). Pten positively regulates brown adipose function, energy expenditure, and longevity. Cell Metab. 15, 382-394.

Pan D., Fujimoto M., Lopes A. and Wang Y.X. (2009). Twist-1 is a PPARδ-inducible, negative-feedback regulator of PGC-1α in brown fat metabolism. Cell 137, 73-86.

Pedersen B.K. and Febbraio M.A. (2012). Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat. Rev. Endocrinol. 8, 457-465.

Petrovic N., Walden T.B., Shabalina I.G., Timmons J.A., Cannon B. and Nedergaard J. (2010). Chronic peroxisome proliferator-activated receptor γ (PPARγ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J. Biol. Chem. 285, 7153-7164.

Picard F., Gehin M., Annicotte J., Rocchi S., Champy M.F., O'Malley B.W., Chambon P. and Auwerx J. (2002). SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 111, 931-941.

Powelka A.M., Seth A., Virbasius J.V., Kiskinis E., Nicoloro S.M., Guilherme A., Tang X., Straubhaar J., Cherniack A.D., Parker M.G. and Czech M.P. (2006). Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. J. Clin. Invest. 116, 125-136.

Puigserver P., Wu Z., Park C.W., Graves R., Wright M. and Spiegelman B.M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829-839.

Qiang L., Wang L., Kon N., Zhao W., Lee S., Zhang Y., Rosenbaum M., Zhao Y., Gu W., Farmer S.R. and Accili D. (2012). Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Pparγ. Cell 150, 620-632.

Reshef R., Maroto M. and Lassar A.B. (1998). Regulation of dorsal somitic cell fates: BMPs and Noggin control the timing and pattern of myogenic regulator expression. Genes Dev. 12, 290-303.

Rosen E.D. and MacDougald O.A. (2006). Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7, 885-896.

Sáinz N., Rodríguez A., Catalán V., Becerril S., Ramírez B., Gómez-Ambrosi J. and Frühbeck G. (2009). Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1α in *ob/ob* mice. PLoS One 4, e6808.

Saito M., Okamatsu-Ogura Y., Matsushita M., Watanabe K., Yoneshiro T., Nio-Kobayashi J., Iwanaga T., Miyagawa M., Kameya T., Nakada K., Kawai Y. and Tsujisaki M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58, 1526-1531.

Scarpulla R.C., Vega R.B. and Kelly D.P. (2012). Transcriptional integration of mitochondrial biogenesis. Trends Endocrinol. Metab. 23, 459-466.

Scime A., Grenier G., Huh M.S., Gillespie M.A., Bevilacqua L., Harper M.E. and Rudnicki M.A. (2005). Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1α. Cell Metab. 2, 283-295.

Seale P., Kajimura S., Yang W., Chin S., Rohas L.M., Uldry M., Tavernier G., Langin D. and Spiegelman B.M. (2007). Transcriptional control of brown fat determination by PRDM16. Cell Metab. 6, 38-54.

Seale P., Bjork B., Yang W., Kajimura S., Chin S., Kuang S., Scime A., Devarakonda S., Conroe H.M., Erdjument-Bromage H., Tempst P., Rudnicki M.A., Beier D.R. and Spiegelman B.M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. Nature 454, 961-967.

Seale P., Kajimura S. and Spiegelman B.M. (2009). Transcriptional control of brown adipocyte development and physiological function--of mice and men. Genes Dev. 23, 788-797.

Seale P., Conroe H.M., Estall J., Kajimura S., Frontini A., Ishibashi J., Cohen P., Cinti S. and Spiegelman B.M. (2010). Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin. Invest. 121, 96-105.

Sharp L.Z., Shinoda K., Ohno H., Scheel D.W., Tomoda E., Ruiz L., Hu H., Wang L., Pavlova Z., Gilsanz V. and Kajimura S. (2012). Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS One 7, e49452.

Smorlesi A., Frontini A., Giordano A. and Cinti S. (2012). The adipose organ: white-brown adipocyte plasticity and metabolic inflammation. Obes. Rev. 13 (Suppl 2), 83-96.

Sun L., Xie H., Mori M.A., Alexander R., Yuan B., Hattangadi S.M., Liu Q., Kahn C.R. and Lodish H.F. (2011). Mir193b-365 is essential for brown fat differentiation. Nat. Cell. Biol. 13, 958-965.

Tanaka T., Yoshida N., Kishimoto T. and Akira S. (1997). Defective adipocyte differentiation in mice lacking the C/EBPß and/or C/EBPδ gene. EMBO J. 16, 7432-7443.

Tang Q.Q., Otto T.C. and Lane M.D. (2004). Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc. Natl. Acad. Sci. USA 101, 9607-9611.

Timmons J.A., Wennmalm K., Larsson O., Walden T.B., Lassmann T., Petrovic N., Hamilton D.L., Gimeno R.E., Wahlestedt C., Baar K., Nedergaard J. and Cannon B. (2007). Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. Proc. Natl. Acad. Sci. USA 104, 4401-4406.

Timmons J.A., Baar K., Davidsen P.K. and Atherton P.J. (2012). Is irisin a human exercise gene? Nature 488, E9-10.

Trajkovski M., Ahmed K., Esau C.C. and Stoffel M. (2012). MyomiR-133 regulates brown fat differentiation through Prdm16. Nat. Cell. Biol. 14, 1330-1335.

Tseng Y.H., Kokkotou E., Schulz T.J., Huang T.L., Winnay J.N., Taniguchi C.M., Tran T.T., Suzuki R., Espinoza D.O., Yamamoto Y., Ahrens M.J., Dudley A.T., Norris A.W., Kulkarni R.N. and Kahn C.R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454, 1000-1004.

Tseng Y.H., Cypess A.M. and Kahn C.R. (2010). Cellular bioenergetics as a target for obesity therapy. Nat. Rev. Drug. Discov. 9, 465-482.

Tsukiyama-Kohara K., Poulin F., Kohara M., DeMaria C.T., Cheng A., Wu Z., Gingras A.C., Katsume A., Elchebly M., Spiegelman B.M.,

Harper M.E., Tremblay M.L. and Sonenberg N. (2001). Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat. Med. 7, 1128-1132.

van Marken Lichtenbelt W.D., Vanhommerig J.W., Smulders N.M., Drossaerts J.M., Kemerink G.J., Bouvy N.D., Schrauwen P. and Teule G.J. (2009). Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. 360, 1500-1508.

Vegiopoulos A., Muller-Decker K., Strzoda D., Schmitt I., Chichelnitskiy E., Ostertag A., Berriel Diaz M., Rozman J., Hrabe de Angelis M., Nusing R.M., Meyer C.W., Wahli W., Klingenspor M. and Herzig S. (2010). Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. Science. 328, 1158-1161.

Virtanen K.A., Lidell M.E., Orava J., Heglind M., Westergren R., Niemi T., Taittonen M., Laine J., Savisto N.J., Enerback S. and Nuutila P. (2009). Functional brown adipose tissue in healthy adults. N. Engl. J. Med. 360, 1518-1525.

Walden T.B., Hansen I.R., Timmons J.A., Cannon B. and Nedergaard J. (2011). Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am. J. Physiol. Endocrinol. Metab. 302, E19-31.

Walden T.B., Timmons J.A., Keller P., Nedergaard J. and Cannon B. (2009). Distinct expression of muscle-specific microRNAs (myomirs) in brown adipocytes. J. Cell. Physiol. 218, 444-449.

Wang L., Liu J., Saha P., Huang J., Chan L., Spiegelman B. and Moore D.D. (2005). The orphan nuclear receptor SHP regulates PGC-1α expression and energy production in brown adipocytes. Cell Metab. 2, 227-238.

Whittle A.J., López M. and Vidal-Puig A. (2011). Using brown adipose tissue to treat obesity - the central issue. Trends Mol. Med. 17, 405-411.

Whittle A.J., Carobbio S., Martins L., Slawik M., Hondares E., Vázquez M.J., Morgan D., Csikasz R.I., Gallego R., Rodríguez-Cuenca S., Dale M., Virtue S., Villarroya F., Cannon B., Rahmouni K., López M. and Vidal-Puig A. (2012). BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 149, 871-885.

Wozney J.M., Rosen V., Celeste A.J., Mitsock L.M., Whitters M.J., Kriz R.W., Hewick R.M. and Wang E.A. (1988). Novel regulators of bone formation: molecular clones and activities. Science 242, 1528-1534.

Wu J., Bostrom P., Sparks L.M., Ye L., Choi J.H., Giang A.H., Khandekar M., Virtanen K.A., Nuutila P., Schaart G., Huang K., Tu H., van Marken Lichtenbelt W.D., Hoeks J., Enerback S., Schrauwen P. and Spiegelman B.M. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366-376.

Xue B., Coulter A., Rim J.S., Koza R.A. and Kozak L.P. (2005). Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots. Mol. Cell. Biol. 25, 8311-8322.

Xue B., Rim J.S., Hogan J.C., Coulter A.A., Koza R.A. and Kozak L.P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. J. Lipid Res. 48, 41-51.

Xue Y., Cao R., Nilsson D., Chen S., Westergren R., Hedlund E.M., Martijn C., Rondahl L., Krauli P., Walum E., Enerback S. and Cao Y. (2008). FOXC2 controls Ang-2 expression and modulates angiogenesis, vascular patterning, remodeling, and functions in adipose tissue. Proc. Natl. Acad. Sci. USA 105, 10167-10172.

Yokoyama S. and Asahara H. (2011). The myogenic transcriptional network. Cell. Mol. Life Sci. 68, 1843-1849.

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