REVIEW

Promoting brown and beige adipocyte biogenesis through the PRDM16 pathway

S Kajimura

Obesity develops from a chronic energy imbalance in which energy intake exceeds energy expenditure. As brown adipose tissue (BAT) dissipates energy and produces heat, increasing energy expenditure via BAT thermogenesis may constitute a novel therapeutic intervention for the treatment of obesity and obesity-related diseases. Studies over the past few years have identified key regulatory molecules of brown and beige adipocyte biogenesis, including a dominant transcriptional co-regulator PRDM16 (PR domain containing 16) and its co-factors, which allows for engineering functional BAT by genetic approaches. A next step toward the goal of promoting BAT thermogenesis by pharmacological approaches necessitates a better understanding of the enzymatic components and signaling pathways for brown and beige adipocyte development. This review covers recent advances regarding this topic, with a special emphasis on the PRDM16 transcriptional pathway.

International Journal of Obesity Supplements (2015) 5, S11–S14; doi:10.1038/ijosup.2015.4

INTRODUCTION

In contrast to white adipose tissue (WAT) that stores excess energy, brown adipose tissue (BAT) dissipates energy and produces heat as a defense against hypothermia and obesity in mammals. BAT's function to generate heat is largely mediated by the BAT-specific mitochondrial inner membrane protein, uncoupling protein 1 (UCP1). Upon cold exposure or excess caloric intake, UCP1 reduces the proton gradient across the mitochondrial inner membrane, thereby uncoupling cellular respiration and mitochondrial ATP synthesis.

Over the past few years, greater attention has been paid to the field of BAT biology since the wide prevalence of active BAT depots has been appreciated in adult humans. Studies using ^{18}F -fluoro-2-deoxy-d-glucose positron emission tomography-computed tomography scans have demonstrated an inverse correlation between BAT prevalence and body mass index or adiposity, suggesting its physiological importance in whole-body energy homeostasis not only in rodents but also in adult humans.^{1–5} Coincident with this discovery in adult humans, a major advance has been made in the molecular regulation of brown adipocyte development and function. Several key transcriptional regulators, such as PRDM16 (PR domain containing 16) and its co-factors, have been identified, allowing researchers to generate functional BAT depots *in vivo* by genetic approaches.^{6–10}

As a next step toward novel anti-obesity strategies to pharmacologically promote BAT thermogenesis, we need to gain a better understanding of the enzymatic components and signaling pathways that control brown adipocyte development and thermogenesis. Here, I will overview recent advances in this topic with a special emphasis on the PRDM16 pathway.

DEVELOPMENTAL LINEAGES OF BROWN AND BEIGE ADIPOCYTES IN MICE AND HUMANS

From a developmental point of view, mammals possess two types of thermogenic adipocytes, referred to as classical brown adipocytes and beige adipocytes (also called brite adipocytes). Despite the fact that both cell types share a number of biochemical and morphological features including multilocular lipid droplets, large mitochondria with dense cristae, and expression of UCP1, brown and beige adipocytes are distinct cell types.^{8,11–13} Anatomically, classical brown adipocytes are localized in the major BAT depots of rodents and infants, including interscapular, perirenal and periaortic depots. The classical brown precursors diverge from a population of dermomyotome that can give rise to skeletal muscle during the prenatal stages between embryonic day (E) 9.5 and E11.5 in rodents.^{8,14} In contrast, beige adipocytes are sporadically localized in subcutaneous WAT and emerge under certain external cues, such as chronic cold exposure, exercise and long-term treatment with peroxisome proliferator-activated receptor gamma (PPAR γ) agonists (for example, thiazolidinediones, TZD). This phenomenon is often referred to as the 'browning' of WAT.^{11–13,15} Although cellular origins of beige adipocytes remain poorly understood, these cells in the epididymal WAT of rodents are derived through proliferation and differentiation of precursors that express PDGFR α (platelet-derived growth factor receptor α) and SCA1 (spinocerebellar ataxia type 1).¹⁶ A recent paper reported that ~10% of the UCP1-positive beige adipocytes in inguinal WAT arise from smooth muscle precursors that express *Myh11*.¹⁷ In addition, a subset of beige adipocytes in anterior subcutaneous and perigonadal WAT depots originates from *Myf5*-positive cells,¹⁸ whereas most of beige adipocytes in inguinal WAT derive from *Myf5*-negative precursors. These data suggest high cellular heterogeneity of beige adipocyte populations in multiple WAT depots.

Figure 1. (a) Function of the PRDM16 transcriptional complex in brown adipocyte differentiation. (b) Molecular mechanisms of TZD-induced beige adipocyte differentiation by PRDM16.

In adult humans, it has been reported that supraclavicular BAT depots express several genes that are originally identified as selective markers for murine beige adipocytes.^{19–21} However, adult human BAT is a highly heterogeneous tissue as compared with murine BAT, such that molecular analyses of biopsied adult human BAT samples can be highly confounded by potential contamination of UCP1-negative cells, such as white adipocytes and myocytes. To understand the molecular nature of adult human brown adipocytes at a single-cell resolution, we have recently isolated clonally derived UCP1-positive adipocytes from adult human BAT. Global gene expression profiles based on RNA sequencing and unbiased clustering analyses indicate that molecular signatures of adult human brown adipocytes resemble those of murine beige adipocytes, rather than murine classical brown adipocytes.²² Intriguingly, recent reports found that chronic cold acclimation for 10 consecutive days up to 6 weeks recruited new active BAT in the supraclavicular region of adult humans who had previously lacked detectable BAT depots before treatment.^{23–25} These results indicate that adult human BAT is primarily, if not entirely, composed of beige-like adipocytes that are highly recruitable in response to appropriate external cues. Thus, decoding beige adipocyte biogenesis is critical for a better understanding of adult human BAT.

TRANSCRIPTIONAL REGULATION OF BROWN AND BEIGE ADIPOCYTE DEVELOPMENT BY THE PRDM16 PATHWAY

Recent studies identified a number of transcriptional regulators of brown and beige adipocyte development (reviewed in Kajimura *et al.*,⁷ Harms and Seale¹¹ and Kajimura and Saito¹³). PRDM16 is a 140-kDa zinc-finger nuclear protein and acts as a dominant activator of brown and beige adipogenesis. When expressed in myoblasts or preadipocytes, PRDM16 powerfully represses the pre-existing gene program (that is, myogenic genes or white adipocyte-selective genes) and activates the brown adipocyte-selective gene program, including *Ucp1*, *Pgc1a* and many mitochondrial genes.^{8–10} Conversely, depletion of PRDM16 in cultured brown adipocytes leads to a significant loss in brown

adipocyte identity and an ectopic activation of skeletal-muscle-selective genes, such as *Myf5*, *MyoD* and *Myogenin*.⁸

Although PRDM16 contains zinc-finger domains that can bind directly to a nucleotide consensus sequence,²⁶ the DNA-binding ability is dispensable for the function of PRDM16 to promote brown adipogenesis.⁹ Hence, it is considered that PRDM16 is a transcriptional co-regulator that functions through the canonical DNA-binding transcription factors (Figure 1a). Indeed, PRDM16 directly interacts and modulates the transcriptional activities of CCAAT/enhancer-binding protein-β, PPARγ and early B-cell factor 2 and PPARγ co-activator 1α (PGC1α).^{6,8,9,27} Consistent with these observations, genome-wide chromatin immunoprecipitation-sequencing analysis of PRDM16 revealed that PRDM16 binding in BAT is highly enriched at the binding sites for C/EBPs, PPARs and EBFs.²⁸ Importantly, ectopic expression of PRDM16 and CCAAT/enhancer-binding protein-β is sufficient to convert non-adipogenic fibroblasts, such as dermal fibroblasts from mice and humans, into brown adipocytes. When transplanted into nude mice, the engineered brown preadipocytes form UCP1-positive BAT depots that actively take up glucose, as assessed by ¹⁸F-fluoro-2-deoxy-d-glucose a positron emission tomography-computed tomography scan.⁶ As components that mediate the PRDM16's repression activity on WAT-selective genes and muscle-selective genes, C-terminal-binding proteins (CtBP1 and 2) and euchromatic histone-lysine N-methyltransferase 1 (EHMT1) have been identified.^{10,29} Notably, PRDM16 fails to repress the myogenic gene program in *Myf5*⁺ myoblasts in the absence of EHMT1.²⁹ Harms *et al.*³⁰ also reported that PRDM16 deletion in *Myf5*⁺ myoblast-derived BAT leads to an ectopic activation of WAT-selective genes through recruitment of EHMT1 in adult mice. Requirement of CtBPs for repressing the muscle gene program remains unknown. Intriguingly, embryonic BAT development is severely impaired in *Myf5*-specific EHMT1 null mice but not in *Myf5*-specific PRDM16 null mice, indicating that compensatory mechanisms may exist in the *Prdm16*-deficient BAT.

PRDM16 also has a major role in activating the thermogenic gene program in brown adipocytes. This is achieved in part by enhancing the transcriptional activity of PGC1α through direct interaction.⁹ Activation of β-adrenoceptor signaling by cold induces the expression of PGC1α and CCAAT/enhancer-binding protein-β. In addition, EHMT1 stabilizes PRDM16 protein through direct interaction. Importantly, deleting PRDM16 or EHMT1 in an adipose-selective manner significantly blunts activation of the BAT-specific thermogenic program in response to cold and β-adrenoceptor agonist (CL316243) *in vivo*.^{29,31} These results indicate that the PRDM16–EHMT1 transcriptional complex is required for the thermogenic function of brown adipocytes.

REGULATION OF PRDM16 TRANSCRIPTIONAL ACTIVITIES IN WAT BROWNING

In addition to its critical role in the determination and maintenance of brown adipocyte fate, PRDM16 is required for the external cue-induced browning of WAT (that is, beige adipocyte development). For example, depletion of PRDM16 leads to a significant loss in the cold and TZD-induced browning of white adipocytes.^{31,32} We have previously shown that rosiglitazone robustly extends the half-life of PRDM16 protein from 5.9 to 17.5 h.³² The TZD-induced stabilization of PRDM16 protein is mediated through the ubiquitin–proteasome pathway, although specific E3 ubiquitin ligase(s) for PRDM16 has not been identified (Figure 1b). This finding explains well the slow kinetics of WAT browning in response to rosiglitazone treatment; TZD-mediated WAT browning requires at least 3 days or longer in cultured cells, whereas most of the direct target genes of PPARγ are activated by rosiglitazone within several hours. Theoretically, 3-day-treatment with rosiglitazone results in the accumulation of PRDM16 protein by ~250-fold, in accordance well with the slow kinetics of

browning effects by PPAR γ agonists.³² More recently, the Accili's group also proposed an additional mechanism in which Sirt1-dependent deacetylation of PPAR γ enhances the interaction between PRDM16 and PPAR γ .³³ These results imply a possibility that stabilization of PRDM16 protein may be a plausible strategy to induce WAT browning without directly agonizing the PPAR γ transcriptional activity.

GENETIC CONTRIBUTION OF EHMT1 AND ENERGY HOMEOSTASIS

As ectopic expression of PRDM16 by a recurrent reciprocal translocation is associated with human acute myeloid leukemia,²⁶ targeting PRDM16 gene transcription as an anti-obesity therapy may raise a safety concern. As an alternative approach, we aim to identify enzymatic components that control the activity of the PRDM16 transcriptional complex. We previously found that the PRDM16 transcriptional complex has a methyltransferase activity on histone3, which correlates well with the PRDM16 activity to activate brown adipogenesis. By biochemical purification followed by mass spectrometry analyses, we identified EHMT1 as a dominant methyltransferase that mediates the PRDM16 function to control brown adipose cell fate.²⁹

Notably, it has been reported that 40–50% of the human patients with EHMT1 haploinsufficiency exhibit an obese phenotype.^{34,35} We have shown that adipose-specific deletion of EHMT1 by an *Adiponectin-Cre* lowers whole-body energy expenditure and causes obesity, hepatic steatosis and insulin resistance under a high-fat diet. These results indicate that EHMT1 is a critical enzyme for whole-body energy homeostasis in rodents and in humans. Given the wide prevalence of human BAT and its importance in energy homeostasis, it is important to decipher genetic variances that affect human BAT prevalence and obese propensity.

SMALL-MOLECULE SCREENING TO IDENTIFY PHARMACOLOGICAL ACTIVATORS OF BAT THERMOGENESIS

Thermogenic capacity of BAT largely depends on UCP1, because other uncoupling proteins, such as UCP2 and UCP3 appear to have a minor role in adaptive thermogenesis in rodents.³⁶ UCP1 knockout mice are cold intolerant³⁷ and exhibit an obese phenotype under thermoneutrality.³⁸ Conversely, transgenic expression of UCP1 in an adipose-selective manner increases oxygen consumption in WAT and protects mice from obesity.³⁹ In contrast to general chemical uncouplers, such as 2,4-dinitrophenol, UCP1-mediated thermogenesis is a highly regulated process that requires physiological stimulation of the cAMP signaling pathway.⁴⁰ Hence, modulating UCP1 expression and activity in adipose tissue may constitute a safer avenue to increase energy expenditure.

To test this idea, we have recently generated a genetic mouse model, termed 'ThermoMouse' in which luciferase expression faithfully recapitulates the pattern of endogenous UCP1 expression. ThermoMouse allowed us to obtain real-time visualization and quantification of UCP1 expression *in vivo*. We further developed a cell-based high-throughput screen platform using brown adipocytes from ThermoMouse and identified a small compound that induces UCP1 expression *in vivo*. The compound WWL113 is annotated to be an inhibitor for serine hydrolases, carboxylesterase 3 (Ces3 or Ces1d) and Ces1f (CesML1), although the detailed mechanisms by which WWL113 activates UCP1 expression needs further study. Importantly, WWL113 treatment in mice was able to increase whole-body energy expenditure when co-treated with a β 3-adrenoceptor agonist (CL316243).⁴¹ This new screening platform offers an opportunity to identify novel regulatory pathways of BAT-mediated thermogenesis. This would also provide an important proof of concept that targeting UCP1

using small molecules is indeed a plausible approach for enhancing BAT thermogenesis.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

I thank Kathleen Jay for editorial assistance. The present study was supported by NIH grants DK087853 and DK97441 to SK. SK also acknowledges support from the DERC center grant (DK63720), UCSF PBBR program, the Pew Charitable Trust and PRESTO from Japan Science and Technology Agency. SK has received grant support from Novo Nordisk, and holds related patents No. WO/2010/080985 and No. WO/2011/091134.

DISCLAIMER

This article is published as part of a supplement sponsored by the Université Laval's Research Chair in Obesity, in an effort to inform the public on the causes, consequences, treatments and prevention of obesity.

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