Heart Metab. (2016) 69:4-8

Brown adipose tissue and the genetics of obesity

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Abstract

Obesity is associated with a number of metabolic diseases and various cancers. At the biological level, obesity is caused by a shift in the body's energy balance toward energy abundance. The main function of one type of adipocyte called brown adipocytes, found in brown adipose tissue (BAT), is to dissipate energy as heat—generated through action of uncoupling protein 1 (UCP1)—in response to certain physiological stimuli, a process called adaptive thermogenesis. A second type of UCP1-positive thermogenic adipocyte appears in the subcutaneous white adipose tissue (WAT) in response to chronic exposure to cold or to agonists for β_3-adrenergic receptor (β_3-AR) and peroxisome proliferator–activated receptor γ (PPARγ); these are called beige/brite adipocytes. Brown and beige/brite cells originate from different developmental lineages and the energy expenditure ability of these cells gave us great hope that BAT can be manipulated to treat obesity. Here, I review the progress made thus far in understanding the developmental origins of brown and beige cells and the molecular regulation of BAT-mediated thermogenesis, studied in various knockout and transgenic mouse models. ■ *Heart Metab*. 2016;69:4-8

Keywords: beige cells; brown fat; genetic mouse models; oxidative metabolism; PGC1α; thermogenesis; UCP1

Obesity is a significant risk factor for a number of diseases, such as chronic heart disease, hypertension, type 2 diabetes, fatty liver disease, and various cancers.[1] For the past few decades, obesity has steadily increased, not only in the well-developed Western nations, but also throughout the world. According to the World Health Organization (WHO), around 2.1 billion people are obese or overweight. Some of the important contributing factors of obesity include cheap energy-rich diets, sugary drinks, technology-dependent lifestyles, and lack of physical activity. At the biological level, these factors shift the body's energy balance toward energy abundance, which ultimately causes obesity. If energy (food) consumption is chronically higher than expenditure, the extra energy is stockpiled in the white adipose (fat) tissue (WAT) as triglycerides. Conversely, if energy consumption is lower than expenditure, triglycerides in the WAT are broken down for use by other organs. Thus, WAT functions as the body's energy storage and supply center.

Brown adipose tissue

Another type of adipose tissue, called brown adipose tissue (BAT), also exists in rodents and in humans.

Abbreviations

β_3-AR: β_3-adrenergic receptor; **BAT:** brown adipose tissue; **cAMP:** cyclic adenosine monophosphate; **Cidea:** cell death–inducing DNA-fragmentation factor, α-subunit–like effector A; **Ebf2:** early B-cell factor 2; **FOXC2:** forkhead box C2; **LXRα:** liver-X-receptor α; **NRF1:** nuclear respiratory factor 1; **PGC1α:** peroxisome proliferator activated–receptor gamma coactivator 1 α; **PKA:** protein kinase A; **PPARγ:** peroxisome proliferator–activated receptor γ; **pRB:** retinoblastoma protein; **PRDM16:** PR domain containing 16; **RIP140:** receptor-interacting protein 140; **SRC1:** steroid receptor coactivator 1; **Twist1:** Twist family basic helix-loop-helix transcription factor 1; **UCP1:** uncoupling protein 1; **WAT:** white adipose tissue

The main function of BAT is to dissipate energy as heat in response to cold temperatures and probably to diet. This process is called adaptive thermogenesis; small mammals living in cold conditions produce heat by efficiently employing BAT.[2] BAT is rich in mitochondria and has a high cellular respiration, but a very low capacity for ATP synthesis because of low levels of ATP synthase and expression of a unique protein called uncoupling protein 1 (UCP1). BAT generates heat through UCP1, which is located in the inner mitochondrial membrane. UCP1 uncouples oxidative phosphorylation from ATP production and releases chemical energy as heat, thereby increasing the body's energy expenditure.[3] Recent studies demonstrated a second type of UCP1-positive thermogenic adipocyte in the subcutaneous WAT; adipocytes of this type are called beige/brite (brown in white) cells. These cells appear only in response to exposure to chronic cold and agonists for β_3-adrenergic receptor (β_3-AR) or peroxisome proliferator–activated receptor γ (PPARγ) and display several morphological and biochemical characteristics similar to brown adipocytes.[4] The energy dissipation ability of BAT and beige/brite cells gave us the idea of exploiting BAT to treat an energy-abundant obese condition. However, in order to execute this idea it is essential to understand the development of brown and beige adipocytes and the molecular regulation of BAT-mediated thermogenesis. In this regard, extensive research in various genetic mouse models has provided in-depth insight into genes that play critical roles in BAT origin and differentiation and in BAT-associated thermogenesis.

Developmental origins of brown and beige/brite cells

Brown adipocytes (interscapular and perirenal BAT) and beige/brite cells originate from different developmental lineages. The progenitor cells of the embryonic mesoderm that transiently express the myogenic factor 5/paired box 7 genes (*Myf5*$^+$*Pax7*$^+$) give rise to skeletal muscle cells or brown adipocytes. In these cells, early B-cell factor 2 (EBF2) and PPARγ cooperatively induce the expression of the gene named PR domain containing 16 (*Prdm16)*, which determines the brown-adipocyte-cell fate. The PRDM16 protein regulates the transcriptional activity of various thermogenic genes, such as *PPARα*, *PPARγ*, and PPARγ coactivator 1α (*PGC1α*), thus functioning as a central regulator of brown-adipocyte-cell fate. Genetic ablation of *Prdm16* disrupts cell fate determination between brown adipocytes and myocytes. *Prdm16*-deficient brown-adipocyte precursors display elevated expression of skeletal-muscle-cell genes such as *myogenin.*[5,6] *Myogenin*-knockout mice totally lack differentiated skeletal muscle, but have an enlarged interscapular BAT.[7] Conversely, forced expression of *Prdm16* stimulates beige-cell formation in subcutaneous WAT, and transgenic mice overexpressing PRDM16 display increased energy expenditure and reduced weight gain in response to a high-fat diet.[8] As an upstream regulator of PRDM16, EBF2 determines brown-adipocyte differentiation, and induced expression of *Ebf2* reprograms white preadipocytes into brown adipocytes. Consequently, brown adipose cells and tissues from *Ebf2*-knockout mice show a loss of brown-specific characteristics and thermogenic capacity.[9]

In response to various stimuli, such as cold and β_3-AR or PPARγ agonists, pools of UCP1-expressing beige/brite cells arise in WAT, mainly in inguinal WAT. Beige cells come from a *Myf5*-negative cell lineage, and recent studies indicate that they arise from platelet-derived growth-factor-receptor α (*Pdgfrα)*-positive progenitors in response to β_3-AR agonists.[10] Beige and brown cells share a number of thermogenic genes such as *Pgc1a*, *Ucp1*, *Cidea* (cell death–inducing DNA-fragmentation factor, α-subunit–like effector A), and *Prdm16;* however, beige cells also express several unique genes, such as *CD137*, *Tmem26* (transmembrane protein 26*),* and *Tbx1* (T-box tran-

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scription factor TBX1), that apparently reflect their distinct developmental origins.[11] Interestingly, human brown adipocytes isolated from different regions of the body express beige-cell markers, suggesting that these brown adipocytes have a molecular signature similar to that of beige cells.[12]

BAT-mediated thermogenesis and obesity

The relationship between BAT thermogenesis and obesity is evident in a transgenic mouse model in which *Ucp1* promoter–directed expression of diphtheria toxin caused obesity.[13] Furthermore, *Ucp1*-knockout mice exhibit an obese phenotype under thermoneutral conditions, and cold intolerance and impaired heat production under ambient temperatures, suggesting that activation of UCP1-mediated thermogenesis in BAT can have antiobesity effects.[14] Conversely, induced expression of *Ucp1* in vivo maintains mitochondria in an active uncoupled state in BAT; however, constitutively enhanced expression of *Ucp1* is cytotoxic and causes BAT atrophy.[15] The question arises as to what activates *Ucp1.* In response to certain physiological stimuli, such as cold or free fatty acids, norepinephrine released from sympathetic nerves act on β-adrenergic receptors of the brown adipocytes. Activation of β-adrenergic receptor/cyclic adenosine monophosphate (cAMP) signaling induces PGC1α via the protein kinase A (PKA)/cAMP-response-element–binding protein (CREB) pathway.[16] The β-adrenergic/cAMP pathway also increases PGC1α protein stability through p38 mitogen-activated protein kinase (MAPK). PGC1α regulates thermogenesis by directly inducing the expression of *Ucp1*. In addition, PGC1α also regulates a number of other factors, such as PPARα, PPARβ, PPARγ, PPARδ, nuclear respiratory factors 1 and 2 (NRF1 and NRF2), thyroid hormone receptor, liver X receptor (LXR), and forkhead box O1 (FOXO1), thus functioning as the central regulator of various pathways involved in mitochondrial biogenesis and thermogenesis. As a result, deletion of PGC1α in mice results in defective cold-induced thermogenesis due to impaired induction of thermogenesis genes.[17] Conversely, forced expression of PGC1α in white adipocytes stimulates various genes involved in mitochondrial biogenesis and thermogenesis, including *Ucp1*, suggesting a central role for PGC1α in mitochondrial thermogenesis.

Positive and negative regulators of BAT thermogenesis

Because of their critical role in thermogenesis, PGC1α and UCP1 expression and activity are tightly controlled by inducers (positive regulators) or inhibitors (negative regulators). Some of the important factors that positively regulate PGC1α and UCP1 are forkhead box C2 (FOXC2), SRC1, CREB, sirtuin-3 (SIRT3), and p38 MAPK; however, receptor-interacting protein 140 (RIP140), LXRα, Cidea, retinoblastoma protein (pRB), SRC2, Twist1, transient receptor potential cation channel subfamily V member 4 (TRPV4), and inhibitor of differentiation 1 (Id1) negatively regulate PGC1α and/or UCP1 (*Figure 1*).

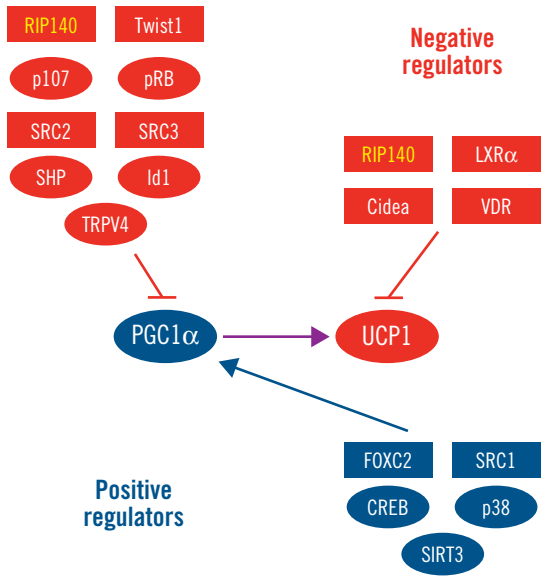

Fig. 1 *Regulators of BAT thermogenesis.*
Cartoon showing some of the important negative and positive regulators of PGC1α/UCP1-mediated thermogenesis. Detailed mechanisms are described in the text.
Abbreviations: *Cidea, cell-death–inducing DNA-fragmentation factor, α-subunit–like effector A; CREB, cAMP-response-element–binding protein; FOXC2, forkhead box C2; Id1, inhibitor of differentiation 1; LXRα, liver-X-receptor α; p38, p38 mitogen-activated protein kinase; p107, pocket protein 107; PGC1α, peroxisome proliferator–activated receptor γ coactivator 1α; pRB, retinoblastoma protein; RIP140, receptor-interacting protein 140; SHP, short heterodimer partner; SIRT3, sirtuin-3; SRC, steroid receptor coactivator; TRPV4, transient receptor potential cation channel subfamily V member 4; Twist1, Twist-family basic helix-loop-helix transcription factor 1; UCP1, uncoupling protein 1; VDR, vitamin D receptor.*

Positive regulators

FOXC2 belongs to the family of forkhead/winged-helix transcription factors. Transgenic expression of *FOXC2* in adipose tissues increases expression of *Ucp1* and other mitochondrial genes and induces

browning in WAT. FOXC2 induces browning by sensitizing cells to the β-adrenergic PKA/cAMP pathway. As a result, *FOXC2*-transgenic mice are resistant to diet-induced obesity.[18] WAT browning due to loss of pRB is regulated through FOXC2, for which expression is elevated in *pRB*$^{-/-}$ cells. pRB regulates thermogenesis by directly binding to the PGC1α promoter and suppressing transcription. In addition, pRB also functions as a PPARγ corepressor, and loss of pRB results in increased PPARγ activity. Adipose-specific deletion of *pRB* in mice leads to browning of WAT, activation of BAT, and protection from diet-induced obesity.[19] Mice deficient for another pocket protein, p107, also display brown-like adipocytes within WAT. In p107-deficient adipocyte precursors, pRB levels are reduced, indicating that p107 function could be facilitated through regulation of pRB.[20] The members of the SRC family, SRC1 (nuclear receptor coactivator 1 [NcoA1]), SRC2 (transcriptional mediators/intermediary factor 2 [TIF2]), and SRC3 (p/CIP), have divergent functions in the regulation of thermogenesis. SRC1 positively regulates thermogenesis by mediating its effects via PGC1α. SRC1 augments coactivation of PPARγ by PGC1α, and genetic ablation of SRC1 leads to impaired thermogenesis due to reduced expression of UCP1. *SRC1*$^{-/-}$ mice are prone to obesity because of reduced energy expenditure.[21]

Negative regulators

In contrast to SRC1, SRC2 inhibits the interaction between PPARγ and PGC1α, leading to reduced activity of PGC1α. *SRC2*$^{-/-}$ mice are protected against obesity and show increased thermogenesis and improved energy expenditure.[21] RIP140 is a transcriptional corepressor that shares a number of downstream target gene promoters with PGC1α. RIP140 binds to PGC1α and inhibits its transcriptional activity on the target gene promoters. Genetic deletion of RIP140 leads to the formation of brown-like adipocytes within WAT, with increased expression of UCP1; *RIP140*$^{-/-}$ mice are lean and are resistant to diet-induced obesity. Conversely, induced expression of RIP140 in adipocytes impairs expression of mitochondrial and thermogenic genes.[22] Similarly, LXRα suppresses *Ucp1* gene expression by interfering with the transactivation of the *Ucp1* promoter by dismissing PPARγ from the *Ucp1* enhancer. LXRα achieves PPARγ discharge from the *Ucp1* enhancer by recruiting RIP140 as a corepressor to the LXRα binding site. In the absence of RIP140, LXRα fails to dismiss PPARγ from the *Ucp1* promoter. Mice lacking LXR have a lean phenotype, with increased expression of UCP1 in BAT and WAT.[23] Twist1 is a basic helix-loop-helix (bHLH) transcription factor that directly binds to PGC1α and suppresses its transcriptional activity. Adipocyte-specific overexpression of *Twist1* in a transgenic mouse model results in reduced mitochondrial density and suppression of UCP1 in BAT, leading to diet-induced obesity. Conversely, *Twist1*-knockout mice are resistant to diet-induced obesity owing to higher mitochondrial density and enhanced expression of UCP1.[24] Cidea, which is a member of the CIDE apoptotic family directly interacts with UCP1 and suppresses its uncoupling function, thus reducing energy expenditure. *Cidea*$^{-/-}$ mice are resistant to obesity and display increased energy expenditure.[25] Similarly, the TRPV4 receptor, which belongs to a family of ion channels, negatively regulates the expression of both PGC1α and UCP1. *TRPV4*$^{-/-}$ mice display higher energy expenditure and increased UCP1 expression in adipose tissues.[26] In addition, the translational inhibitor 4E-BP1 suppresses the translation of PGC1α mRNA. Mice deficient for 4E-BP1 (*Eif-4ebp1*$^{-/-}$) have increased translation of PGC1α, and enhanced expression of UCP1 results in an increased metabolic rate and a reduced WAT mass.[27]

Conclusions

The primary function of BAT is to generate heat in response to cold, thus protecting animals from hypothermia. The idea of utilizing BAT to increase energy expenditure and reduce body fat has generated tremendous interest in understanding BAT biology. As a result, much effort has been invested and significant progress has been made in understanding the developmental lineages of brown and beige/brite adipocytes, in cell-fate determination, and the molecular regulation of BAT thermogenesis. In this regard, genetic mouse models have played a pivotal role in identifying several critical transcription factors and their specific functions in these processes. On the basis of this knowledge, the next step toward increasing energy expenditure with an eye toward treating obesity should focus on expanding BAT mass and activating BAT thermogenesis in adult humans. ■

The author thanks Judith Chang for critically reviewing and editing the manuscript.

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