Transcriptional Control of Brown and Beige Fat Development and Function

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Adipose tissue, once viewed as an inert organ of energy storage, is now appreciated to be a central node for the dynamic regulation of systemic metabolism. There are three general types of adipose tissue: white, brown, and brown-in-white or “beige” fat. All three types of adipose tissue communicate extensively with other organs in the body, including skin, liver, pancreas, muscle, and brain, to maintain energy homeostasis. When energy intake chronically exceeds energy expenditure, obesity and its comorbidities can develop. Thus, understanding the molecular mechanisms by which different types of adipose tissues develop and function could uncover new therapies for combating disorders of energy imbalance. In this review, the recent findings on the transcriptional and chromatin-mediated regulation of brown and beige adipose tissue activity are highlighted.

White, Brown, and Beige Adipose Tissue

Adipocytes, skeletal muscle cells, chondrocytes, and bone cells descend from mesenchymal progenitor cells. Adipogenesis is initiated when mesenchymal progenitors undergo progressive fate restrictions to become committed preadipocytes. This is followed by differentiation, as preadipocytes acquire the morphological and functional characteristics of mature adipocytes (Figure 1A). White adipose tissue (WAT) stores energy in the form of triglycerides, secretes hormones, provides cushioning to protect organs and bones, and insulates the body (1). The predominant function of WAT is to balance lipid storage (lipogenesis) and breakdown (lipolysis) in response to the nutritional state of the organism. The ability of white adipocytes to store energy in the form of lipids (triglycerides) protects other tissues such as muscle, pancreas, and liver against the harmful effects of lipid overload, called lipotoxicity. When energy demand increases, such as during fasting or exercise, triglycerides undergo lipolysis in adipocytes. This generates glycerol and free-fatty acids for use as an energy substrate in other tissues. Sympathetic nerves play a major role in promoting lipolysis via the secretion of the β-adrenergic agonist norepinephrine (NE) in adipose tissue. This triggers a cAMP/protein kinase A (PKA)-dependent signaling cascade leading to the breakdown of lipid droplets (2-5). Conversely, in response to feeding, insulin induces energy storage in adipocytes while simultaneously suppressing lipolysis (2).

WAT is remarkably flexible and can respond to energy surplus through adipocyte hypertrophy and hyperplasia. How are new adipocytes made during periods of energetic overload? Genetic tracing of adipocyte fate in mice demonstrated that, initially, both subcutaneous and visceral white adipose undergo hypertrophy to accommodate the increased demand for lipid storage. However, following prolonged high-fat diet and even before existing adipocytes reach capacity, visceral adipose tissues undergo a wave of new adipogenesis when adipocyte progenitors proliferate and differentiate, a phenomenon not observed in subcutaneous depots (6,7). Thus, different WAT depots adapt to changing systemic energy levels via distinct mechanisms.

The adipose tissue extracellular matrix also undergoes extensive remodeling in response to changing energy flux. Obesity induces the differentiation of fibrogenic precursor cells into myofibroblast-like cells, which deposit extracellular matrix and contribute to fibrosis (8). Activation of the protein myocardin-related transcription factor A (MRTFA) induces myofibroblast-like differentiation and blocks adipocyte development through stimulating the transcription of extracellular matrix and profibrogenic genes (9). Mice lacking MRTFA have reduced levels of adipose fibrosis and increased thermogenic fat development, leading to improved glucose tolerance and higher energy expenditure. Upon high-fat-diet feeding, MRTFA knockout mice are further protected from obesity-induced fibrosis and show increased adipose hyperplasia compared with control animals (10). Together, these results implicate MRTFA in a fibro-adipogenic branch point, and therefore targeting this pathway may hold promise for combating obesity-induced adipose tissue fibrosis.

WAT also secretes many endocrine factors (termed “adipokines”) that communicate the nutritional status of adipose tissue to the central nervous system and peripheral tissues. A significant number of adipokines have been identified, including leptin, adiponectin, resistin, chimerin, and inflammatory cytokines such as tumor necrosis factor alpha (TNFα) and IL-6. The
critical function of leptin is underscored by the finding that loss-of-function mutations in leptin or the leptin receptor lead to monogenic forms of obesity in mice and humans (11-13). Of note, adipocyte-derived lipid species with signaling activity (“lipokines”) such as C16:1n7-palmitoleate and fatty acid–hydroxy fatty acids have also been identified (14,15).

In addition to WAT, placental mammals are equipped with brown adipose tissue (BAT), which serves as a critical site of nonshivering thermogenesis (16). Brown adipocytes are specialized to burn energy, including lipid and glucose, for the production of heat. Brown fat cells have a high density of mitochondria and display high rates of fatty acid oxidation. BAT-mediated thermogenesis is activated by cold and protects mammals against hypothermia. Cold is sensed through the central nervous system, which rapidly elicits sympathetic outflow to brown and white adipocytes. Mesenchymal precursors also give rise to alternative cell types, including muscle, bone, cartilage, and dermis. Mesenchymal precursors also give rise to alternative cell types, including muscle, bone, cartilage, and dermis.

Figure 1 Adipocyte lineage commitment and differentiation. (A) Mesenchymal precursors (gray) undergo progressive cell fate restrictions and commit to the adipocyte lineage. Brown adipocytes develop from a Myf5+Pdgfra+ cells, whereas white and beige preadipocytes arise from a Myf5-Pdgfra+ lineage (multiple steps indicated by dashed arrows). Upon terminal differentiation signals, preadipocytes accumulate lipid and become mature PPARα+ adipocytes. Cold exposure and rewarming can provoke chromatin state shifts between beige and white adipocytes. Mesenchymal precursors also give rise to alternative cell types, including muscle, bone, cartilage, and dermis. (B) Beige adipocytes have been proposed to develop from smooth muscle-like precursor cells (blue shaded box).

Sustained cold/NE also induces the differentiation of new brown adipocytes to increase BAT mass and augment thermogenic capacity (21,22). Furthermore, NE-mediated activation of the cAMP/PKA signaling pathway increases the expression of genes that support thermogenesis, including the hallmark brown fat-specific protein uncoupling protein 1 (Ucp1) (23,24). Brown fat mitochondria contain a high concentration of UCP1 within the inner mitochondrial membrane. When activated, UCP1 partially dissipates the proton gradient generated by oxidative phosphorylation (25,26). The UCP1-mediated decrease in membrane potential drives very high levels of substrate oxidation with the inherent inefficiency of all the metabolic reactions generating heat as a by-product. Accordingly, brown fat is highly vascularized, allowing for efficient delivery of oxygen and substrate to brown fat cells and the resulting heat to be distributed to the rest of the organism.
Recent work challenged the dogma that lipolysis in brown adipocytes is required for nonshivering thermogenesis. Brown adipocyte-specific deletion of ATGL or CGI-58, which control lipolysis, does not affect cold-stimulated brown fat activity (27,28). Thus, circulating nutrients obtained from diet or generated by lipolysis in WAT can fuel brown fat thermogenesis. Of note, long chain fatty acids released from WAT are converted to acylcarnitines by liver, released into circulation, and taken up by activated BAT to support thermogenesis (29). A recent study also demonstrated that BAT avidly takes up succinate; this stimulates ROS production to activate UCP1 and thermogenesis. Striking, feeding mice a succinate-supplemented diet increased energy expenditure and reversed diet-induced obesity in a UCP1-dependent manner (30).

Brown-like (“beige”) adipocytes that express UCP1 and other thermogenic components develop in WAT in response to cold and other stimuli. Despite their differences in developmental history and location, beige and brown adipocytes can achieve similar levels of uncoupling on a per-cell basis and express comparable levels of UCP1 protein when maximally stimulated (31,32). Ribosomal profiling studies have shown that cold-recruited beige adipocytes express smooth muscle-specific genes, such as actin, alpha 2, smooth muscle, aorta (Acta2) and myosin heavy chain 11 (Myh11). Lineage tracing studies with Myh11Cre and Acta2Cre drivers indicate that beige adipocytes develop from smooth muscle-like cells (33,34). These results suggest a developmental relationship between smooth muscle cells and beige adipocytes (Figure 1B).

UCP1 is the defining marker gene or protein for thermogenic brown and beige adipocytes. However, beige adipose tissue also employs UCP1-independent mechanisms that enable futile cycling and metabolic inefficiency. Even before the field had been reinvigorated by the discovery of adult human BAT depots, Kozak and colleagues identified UCP1-independent nonshivering mechanisms (35,36). When wild-type and Ucp1−/− animals were adapted to long-term cold exposure, Ucp1−/− animals responded by elevating fatty acid oxidation and enhancing AMP kinase activity in inguinal WAT. Interestingly, inguinal fat from Ucp1−/− animals had increased levels of phospholamban, a key regulatory component of calcium cycling (36). More recently, SERCA2b-mediated futile calcium signaling was further shown to mediate UCP1-independent thermogenesis in beige adipose depots (37). In this model, adrenergic signaling promotes calcium release from the sarco/endoplasmic reticulum that is taken back up by the SER Ca2+ ATPase 2b pump, thus consuming ATP and influencing whole-body energy expenditure.

Beige adipocytes can also expend energy through futile creatine cycling, enabled by coordinated upregulation of creatine metabolism genes and creatine kinase activity (38). Reducing creatine synthesis in adipocytes of mice decreases energy expenditure and sensitizes animals to high-fat-diet-induced obesity (39). These defects can be rescued by supplementation with dietary creatine, pointing to a key mechanism by which creatine regulates adaptive thermogenesis in vivo. The secreted enzyme PM20D1 also promotes UCP1-independent uncoupling by regulating the production of N-acyl amino acids such as N-oleoyl phenylalanine (40). These N-lipidated amino acids directly uncouple mitochondrial respiration and can increase energy expenditure in obese animals.

**Transcriptional Networks and Adipogenic Programming**

The capacity for adipose tissue to acquire new white or brown adipocytes improves metabolic disease by reducing lipotoxicity. Thus, understanding the transcriptional networks that control adipocyte differentiation is crucial for future translational efforts. The coordinated recruitment of transcription factors defines cell type-specific gene

**Figure 2** Chromatin state dynamics at brown fat-specific enhancers. A model brown fat specific enhancer (e.g., Ucp1, Cidea, etc.) has characteristic enhancer marks including H3K27ac (blue) and H3K4me1 (green). Enhancer RNAs are transcribed by RNA polymerase II at active enhancers. The transcription factor EBF2 interacts with the SWI/SNF chromatin remodeling complex and the long noncoding RNA Blnc1 to activate brown fat genes. PRDM16 interacts with PPARγ/RXR heterodimers and recruits the Mediator complex to promote enhancer looping and activation. The histone deacetylase HDAC3 deacetylates PGC1α to coactivate the nuclear receptor ERRα and promote transcription of Pgc1α as well as other brown fat-selective genes. NFIA binds to brown fat selective enhancers to facilitate chromatin accessibility early in differentiation.
expression during development. Transcription factors are broadly defined as DNA-binding proteins that activate or repress RNA polymerase II (Pol II)-mediated transcription. These proteins bind to cognate DNA sequences (motifs) in core promoter or regulatory sequences, namely enhancers, silencers, and insulators (Figure 2). These regulatory elements far outnumber coding genes in the human genome and are often mutated in the setting of disease (41,42). Over the past few decades, the field has gained a fundamental understanding of the transcriptional hierarchies that govern adipogenesis using both in vivo mouse models and in vitro systems. In particular, the terminal differentiation cascade has been studied in detail using preadipocyte cell lines that undergo differentiation into mature adipocytes upon treatment with a standard induction cocktail. Both white and brown adipogenesis can be modeled in cell culture using immortalized cell lines, such as 3T3-L1 (white) adipocytes or HIB-1B (brown) adipocytes (43,44). Furthermore, primary preadipocytes can be isolated from the stromal vascular fraction of WAT or BAT and then differentiated in cell culture (45).

The identity and in vivo profile of adipose precursor cells is not well defined because of a paucity of specific marker genes. Two recent studies have employed single-cell RNA sequencing (scRNA-seq) to provide unbiased insight into the cellular populations that are capable of undergoing adipocyte differentiation. scRNA-seq analysis of the subcutaneous adipose tissue revealed three population clusters stratified by gene expression profiles. Interestingly, these populations showed different tendencies toward adipocyte differentiation, including a novel “Areg” population shown to inhibit adipocyte differentiation in a paracrine manner in both mice and humans (46). An independent experiment utilized the β3-adrenergic agonist CL316, 243 (CL) to stimulate de novo adipogenesis in adipose tissues. scRNA-seq analysis resolved distinct adipose precursor populations along the differentiation trajectory and further identified tissue-resident immune cells in the adipogenic niche (47). Together, these studies provide a valuable transcriptomic resource and lay the groundwork for future studies aimed at elucidating the mechanisms controlling adipocyte stem or precursor cell activity in different depots.

The nuclear receptor peroxisome proliferator activated receptor gamma (PPARγ) is the master regulator of adipocyte differentiation and function. Ectopic expression of peroxisome proliferator activated receptor gamma (PPARγ) drives adipose differentiation in fibroblasts and muscle cells. Conversely, PPARγ is required for adipocyte differentiation in vivo and in vitro (48,49). At a mechanistic level, PPARγ cooperates extensively with the basic leucine zipper factor C/EBPα to regulate adipocyte-specific genes (50). Genome-wide chromatin immunoprecipitation (ChIP) studies have shown that PPARγ binds to most, if not all, adipocyte-selective genes and that C/EBPα is bound to the majority of these sites in adipocytes (51,52). PPARγ and C/EBPα function predominantly in the second of two defined “waves” of adipogenesis. In the first wave, expression of the proadipogenic factors C/EBPβ and C/EBPδ is induced. Interestingly, C/EBPβ/δ can bind to DNA or chromatin within just a few hours of adipogenic stimulus, and profiling of C/EBPβ in preadipocytes demonstrates some level of chromatin occupancy even before hormonal cocktail induction (53). C/EBPβ both facilitates the binding of and cooperates with the adipogenic transcription factor glucocorticoid receptor (GR) early in 3T3-L1 adipocyte differentiation (54). GR also interacts with phosphorylated STAT5A, which is required for efficient adipogenic differentiation. Indeed, short hairpin RNA-mediated depletion of C/EBPβ, GR, or STAT5A reduces the recruitment of other early-acting adipogenic factors, implying that these early transcription factors facilitate cooperative binding. ChIP-sequencing studies have revealed that C/EBPβ/δ, GR, RXR, and STAT5A bind transiently at transcriptional “hot spots” (4 hours post induction) during differentiation and cooperate to regulate cell cycle and cell growth genes (55).

The transcriptional cascade set in motion in the first wave of adipogenesis activates the second wave, which centers on PPARγ and C/EBPα. Indeed, many of the early transcriptional hot spots occupied by C/EBPβ/δ, GR, RXR, and STAT5A become bound by PPARγ during the second wave (“inherited” hot spots) (56). PPARγ and C/EBPα sustain each other’s expression through a positive feedback loop. These factors also cooperate to establish late transcriptional hot spots at genes linked to the adipocyte phenotype, including lipid and glucose metabolism genes.

Transcription Factors in Brown Adipogenesis

In addition to activating the general adipocyte gene program, brown adipocytes express a broad set of so-called “thermogenic genes” that support uncoupled respiration and high levels of fatty acid and/or glucose oxidation. Transcriptomic studies have identified a core brown versus white fat-selective gene signature. These genes include Ucp1, Ppara (fatty acid metabolism), Cidea (lipid droplet remodeling), Dio2 (thyroid hormone metabolism), Elovl3 (fatty acid elongase), Pgc1α (mitochondrial biogenesis), Cpt1b (fatty acid transport), and genes encoding respiratory chain components (both nuclear and mitochondrial encoded).

Differentiation of brown fat cells as well as the acute response to stimulation is regulated via different pathways. The transcription factor PR domain zinc finger 16 (PRDM16) is selectively expressed in brown or beige versus white fat cells and plays an important role in controlling the differentiation-linked brown fat gene program (57). Ectopic PRDM16 expression is able to activate the expression of thermogenic genes in several cell systems, including white preadipocytes, muscle precursors, and NIH-3T3 fibroblasts. Mechanistically, PRDM16 binds to brown fat gene enhancers and cooperates with the mediator complex to help establish enhancer-promoter contacts at genes such as Ppara and Pgc1α (58,59). PRDM16 also represses white fat and muscle gene expression through interactions with corepressor complexes (60,61). Loss of PRDM16 in cultured brown adipocytes abrogates brown fat gene expression and increases the expression of muscle genes under certain conditions (62). PRDM16 protein itself is posttranslationally modified by the polycomb group protein CBX4, which sumoylates PRDM16 at lysine 917, thus blocking ubiquitination-mediated degradation of PRDM16 (63).

Genetic experiments in mice show that PRDM16 is required for the maintenance of brown adipocyte identity, though the related factor PRDM3 can compensate for loss of PRDM16 in young animals (58). PRDM16 also plays a critical role in regulating the browning of WAT. Transgenic mice overexpressing PRDM16 in adipose tissue develop beige adipocytes in WAT, display increased energy expenditure, and resist high-fat-diet-induced obesity (64). PRDM16 expression in vascular smooth muscle cells induces their conversion to beige adipocytes (33). Conversely, Prdm16 deficiency in adipocytes inhibits beige adipocyte development (65). These animals develop obesity accompanied by insulin resistance and hepatic steatosis. PRDM16 also represses type 1 interferon response genes in brown and beige adipocytes through its DNA-binding domain. Ectopic interferon signaling causes
mitochondrial dysfunction in adipocytes and decreases Ucp1 expression (66). Thus, PRDM16 regulates brown and beige fat fate through multiple mechanisms.

Members of the “early B-cell factor” (EBF) protein family have also emerged as key regulators of general adipogenesis and thermogenic programming. EBF1 is expressed in 3T3-L1 adipocytes early in differentiation, and ectopic EBF1 expression is sufficient to stimulate adipocyte differentiation in embryonic fibroblasts. Conversely, fusing EBF1 to the engrafted repressor domain blocks adipocyte differentiation (67), and whole body Ebf1−/− mice are lipodystrophic (68). Ebf2 and Ebf3 are also expressed at lower levels in differentiated 3T3-L1 adipocytes, and all EBF isoforms (1, 2, and 3) are able to induce adipogenesis in NIH-3T3 fibroblasts (69). Short hairpin RNA-mediated depletion of Ebf1 or Ebf2 (but not Ebf3) blocks adipogenesis (69).

Genome-wide analysis of the PPARγ cisactome in WAT and BAT identified high enrichment of the EBF motif at brown adipocyte-selective PPARγ binding sites (70). Among the EBF isoforms, Ebf2 was the most highly enriched in BAT relative to WAT. Exogenous EBF2 expression in myoblasts or white preadipocytes induces the brown fat differentiation program and uncoupled respiration. Mechanistically, EBF2 forms a ribonucleoprotein complex with the brown fat-selective long noncoding RNA Blnc1 and cooperates with PPARγ at brown fat enhancers to activate transcription (70,71). Ebf2 deficiency ablates the brown fat-specific characteristics of BAT or isolated brown adipocytes (70). In a separate line of investigation, RNA-seq analysis identified Ebf2 as a marker of committed brown adipocyte precursors during mouse development. Ebf2 expression is activated in the dermomyotome of somites starting from E11, and only Ebf2+ cells isolated from embryos displayed brown adipigenic potential in vitro (72).

EBF2 is also required and sufficient for the beige of WAT. Overexpression of EBF2 in primary white adipocytes or WAT induces the expression of thermogenic genes, increases oxygen consumption, and suppresses high-fat-diet-induced weight gain (73). EBF2 does not induce the expression of identified “beige adipose specific genes” such as Cited1 or Tmem26. Thus, EBF2 may reprogram white adipocytes into bona fide brown adipocytes. The transcriptional activity of EBF2 (and possibly other EBFs) is normally inhibited in white adipocytes by the zinc finger protein ZFP423. In mature adipocytes, ZFP423 binds and represses EBF2 activity to maintain white fat cell identity. Deletion of ZFP423 in adipocytes leads to unrestrained EBF2 activity and induction of thermogenic genes (74).

Genome-wide ChIP and RNA-seq analyses show that EBF2 binds directly to many brown fat-specific enhancer regions. The transcription factor NFIA also binds to brown fat-selective active enhancer marks along with PPARγ, C/EBPβ, and EBF2. Notably, NFIA is uniquely present at several brown fat enhancers prior to induction of differentiation and before PPARγ recruitment to these sites. This suggests that NFIA may play an early role in establishing a permissive chromatin landscape at lineage-specific cis-regulatory elements (75,76).

PPARγ coactivator 1A (PGC1α) was first discovered as an interacting partner of PPARγ in brown adipocytes (77). Pgc1α expression is highly induced by cold exposure and is further activated following phosphorylation by the cAMP-PKA-p38/MAPK signaling pathway. Upon interaction with its binding partners, PGC1α recruits histone acetyltransferases such as CBP/p300 and GCN5 to augment transcription (78). PGC1α binds to nuclear respiratory factors 1 and 2 to promote the activation of many mitochondrial genes. PGC1α also coactivates several nuclear hormone receptors, including PPARγ, PPARα, and ERRα/β/γ, all of which participate in the transcription of brown fat genes (79). Overexpression of PGC1α in adipocytes, myotubes, or cardiomyocytes promotes mitochondrial biogenesis and increases oxygen consumption (80,81). Pgc1α-deficient BAT displays mildly increased lipid droplet accumulation but expresses normal levels of Ucp1 and other brown fat-selective genes (82). Pgc1α-deficient brown fat cells in culture fail to efficiently activate the thermogenic machinery in response to adrenergic stimulation (83). These results demonstrate that PGC1α is required for the acute transcriptional activation of thermogenesis but not BAT development per se. Interestingly, deletion of Pgc1α in adipocytes severely impairs the development of beige adipocytes in WAT (84).

Peroxisome proliferator activated receptor alpha (PPARα) is a nuclear hormone receptor that regulates lipid metabolism, including mitochondrial and peroxisomal β-oxidation, fatty acid uptake, and lipoprotein transport. Ppara itself is a transcriptional target of EBF2, PRDM16, PPARγ, and C/EBPβ. It can also bind and directly activate Ucp1 expression (85). PPARα further cooperates with PRDM16 to activate Pgc1α expression, thus linking coordinated induction of fatty acid oxidation and mitochondrial metabolism (86). Surprisingly, mice lacking Ppara in brown adipocytes have normal Ucp1 expression and are able to defend their body temperature against cold stress (87). However, fasted Ppara−/− mice become hypothermic, likely because dampened hepatic fatty acid oxidation reduces the amount of fuel available for thermogenesis (88).

To date, many other transcriptional regulators, nuclear receptors, and long noncoding RNAs have been implicated in activating or repressing brown fat-specific gene expression. ZFP516, KLF11, IRF4, TAF7L, ZBTB16, EWS, PLAC8, the long noncoding RNA Blnc1, ERRα, ERRγ, and others have all been shown in different contexts to promote activation of brown fat genetic programming (89-91). Conversely, FOXO1, TWIST1, p107, LXRα, pRb, RIP140, TLE3, REV-ERBa, and ZFP423 repress brown fat gene expression either directly or by inhibiting activators such as PRDM16, EBF2, or PGC1α (89-91).

The growing number of whole-genome transcriptional and epigenomic studies continues to strengthen our understanding of how brown and beige adipogenesis is transcriptionally regulated (75,76,92,93). All of the transcription factors discussed in this review cooperate with epigenetic factors such as histone modification readers, writers, and erasers as well as chromatin remodeling enzymes (89) to establish a permissive chromatin environment for brown fat gene activation. Identification of brown fat gene enhancers has been enabled by several genome-wide interrogations of chromatin state. For example, lineage-specific enhancers are marked by high levels of activating histone marks such as H3K27ac and H3K4me1/2 in BAT but not WAT (93). Functional brown fat enhancers have been further defined by nascent enhancer RNA transcription (92).

Most available studies on genome regulation have focused on brown fat, with less known about chromatin regulation during beige fat induction. A recent study addressed this question using the NuTRAP mouse, which allows for isolation of nuclei from specific cell types within a heterogeneous tissue (94). Using this system, brown and beige adipocyte nuclei were purified and analyzed for changes in chromatin state in response to temperature shifts.
Histone acetylation is correlated with gene activation, as negatively charged acetyl groups neutralize the interactions between the DNA phosphate backbone and basic histone lysine residues. Several histone deacetylase enzymes (HDACs) have been implicated in control of brown adipose physiology. Pan-inhibition of class I HDACs (HDACs 1, 2, 3, and 8) promotes mitochondrial respiration and biogenesis in vitro through augmented PGC1α expression. Furthermore, administration of HDAC inhibitors in vivo promotes beige adipogenesis and augments energy expenditure in the db/db mouse model of obesity (103). In support of this result, HDAC1 silencing enhances brown fat-specific gene activation through coordinated removal of repressive H3K27me3 and increased H3K27ac deposition at promoters (104).

Histone acetyltransferases and deacetylases can also modify nonhistone substrates (105). In this regard, the histone deacetylase HDAC3, typically considered a transcriptional corepressor, was shown to coactivate ERRα in BAT via deacetylation of PGC1α (92). Mice lacking HDAC3 in brown fat do not express UCP1, fail to activate nonshivering thermogenesis in cold, and show markedly diminished mitochondrial respiration (92). Thus, HDACs likely have context-dependent roles in priming and activation of thermogenic gene expression.

Conclusion and Outlook for Human Health

A major goal in the adipose field is to establish methods for encouraging the differentiation of white and/or brown adipocytes to enhance insulin sensitivity and reduce diabetes in people. Thus, identifying the transcriptional and epigenetic factors that activate or repress adipose differentiation and function will provide new therapeutic targets. However, much of our current knowledge is based on rodent studies. It will therefore be important to assess the relevance of these identified pathways in human adipocytes.

While human BAT or beige adipose tissue is currently of limited availability, several labs have demonstrated that human mesenchymal stem cells or adipocyte-derived stromal vascular cells can be expanded ex vivo and are amenable to genetic and pharmacologic manipulations. For example, treatment of human mesenchymal adipose-derived stem cells with the PPARγ ligand rosiglitazone induces the thermogenic program (111,112). A key rosiglitazone-induced factor is KLF11, which is required downstream of PPARγ for activation of the thermogenic program in human adipocytes (113). Notably, human adipocyte progenitors can also be isolated and expanded ex vivo in three-dimensional Matrigel-based cultures (114). Following stimulation with proangiogenic and proadipogenic cocktail, this vascularized human adipose tissue can be implanted into mice, leading to metabolic benefits.
Embryonic stem or induced pluripotent cells hold great promise for human health, as they can be induced to differentiate into a variety of differentiated cell types. Human embryonic stem-derived mesenchymal progenitors can be differentiated into adipocytes via transduction with PPARγ virus and/or treatment with a PPARγ agonist. Addition of C/EBPβ and PRDM16 vectors, along with exogenous PPARγ2, further programs these mesenchymal progenitors into metabolically active UCP1+ brown adipocytes (115). These cells can engraft and uptake glucose when transplanted into immunocompromised mice, demonstrating that human stem cells can be programmed into functional brown adipocytes. A recent protocol established beige adipocytes from human induced pluripotent cells without gene transfer. Importantly, these cells can form differentiated and vascularized adipose tissue when injected subcutaneously (116).

Understanding the transcriptional hierarchies that regulate brown adipogenesis will advance these reprogramming-based strategies to augment thermogenic adipose tissue. For example, little is known about how Ebf2 is transcriptionally activated, with only one study in the literature demonstrating that the lateral plate mesoderm-derived BMP4 signaling induces Ebf2 and Ebf3 messenger RNA expression in chicken somites (117). Identifying the upstream signals that activate Ebf2 and/or other early browning factors in human cells could be used to induce the reprogramming of white adipocytes or ESCs into brown adipocytes. Future studies should prioritize understanding the upstream signals and transcriptional regulators that activate master lineage-determining factors during the commitment of brown precursors. Looking to the future, the field will continue to broaden and deepen our understanding of how signaling pathways, environmental factors, and/or metabolites regulate the activity of master transcriptional regulators, potentially leading to clinically useful methods for increasing brown and beige fat thermogenesis.

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