Epigenetic regulation of beige adipocyte fate by histone methylation

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Abstract. Adipose tissue harbors plasticity to adapt to environmental thermal changes. While brown adipocyte is a thermogenic cell which produces heat acutely in response to cold stimuli, beige (or brite) adipocyte is an inducible form of thermogenic adipocytes which emerges in the white adipose depots in response to chronic cold exposure. Such adaptability of adipocytes is regulated by epigenetic mechanisms. Among them, histone methylation is chemically stable and thus is an appropriate epigenetic mark for mediating cellular memory to induce and maintain the beige adipocyte characteristics. The enzymes that catalyze the methylation or demethylation of H3K27 and H3K9 regulate brown adipocyte biogenesis through their catalytic activity-dependent and -independent mechanisms. Resolving the bivalency of H3K4me3 and H3K27me3 as well as “opening” the chromatin structure by demethylation of H3K9 both mediate beige adipogenesis. In addition, it is recently reported that maintenance of beige adipocyte, beige-to-white transition, and cellular memory of prior cold exposure in beige adipocyte are also regulated by histone methylation. A further understanding of the epigenetic mechanism of beige adipocyte biogenesis would unravel the mechanism of the cellular memory of environmental stimuli and provide a novel therapeutics for the metabolic disorders such as obesity and diabetes that are influenced by environmental factors.

Key words: Beige adipocyte, Brown adipocyte, Histone methylation, Epigenetics, Adaptive thermogenesis

Introduction

Adipose tissues maintain systemic energy homeostasis by flexibly altering their characteristics in diverse ways to adapt to metabolic and thermal changes. White adipocyte stores excess energy as the form of triglycerides, while brown adipocyte and beige adipocyte (also known as brite adipocyte) dissipate energy by producing heat through non-shivering thermogenesis. Brown adipocyte locates in the dedicated brown adipose tissue (BAT) that is well recognized in the interscapular regions of rodents and human infants. BAT also resides in adult humans at several locations such as cervical, supraclavicular, axillary, abdominal subcutaneous, and paravertebral regions (reviewed in [1]). The beige adipocyte is an inducible form of thermogenic adipocytes which emerges mainly in white adipose tissue (WAT) and possibly in other organs. Beige adipocyte biogenesis, also called beige adipogenesis or beige-ing, is induced with chronic exposure to the external cues such as long-lasting coldness, continuous adrenergic stimulation, and long-term treatment with a ligand for peroxisome proliferator-activated receptor-γ (PPARγ). Even after the withdrawal of such environmental stimuli, beige adipocyte maintains their thermogenic characteristics for a while (~2 weeks in rodent) and then gradually loses the thermogenic function while directly acquiring white adipocyte-like characteristics without passing through the intermediate precursor stage, which is called beige-to-white transition or “whitening” [2, 3]. Thus, beige adipogenesis is a temporary adaptive response which lasts even after the dissipation of external environmental cues. Epigenetics is a mechanism to encode cellular memory without changing the sequence of DNA bases for maintaining homeostasis persistently with environmental changes. Among various epigenetic mechanisms, methylation of histone is chemically stable [4] and therefore is an appropriate mechanism for regulating beige adipogenesis. In this review, the recent advances in the molecular mechanisms of beige adipogenesis regulated by histone methylation are summarized.
White, Brown, and Beige Adipose Cell Fate

Adipocyte differentiation from a pluripotent stem cell undergoes the commitment phase to a lineage-specific progenitor cell and eventually differentiates to a mature adipocyte, which is regulated by a complex network of the transcriptional factors, epigenetic regulators and chromatin remodelers [5-7]. Pluripotent stem cells possess a globally “open” and dynamic chromatin state, which allows widespread expressions of both coding and non-coding elements [8]. Under the such situation, the lineage-specific genes are focally repressed in pluripotent stem cells by being associated with “bivalent domains” which contains both active H3K4me3 and repressive H3K27me3 in their promoter [9]. This bivalent H3K4/K27me3 domain is considered to maintain the lineage-specific genes silenced and keep their transcription in a “poised” state for a future rapid activation.

During the commitment to a lineage-specific preadipocyte from ES cells, a recent study showed that H3K4/K27me3 bivalent domains in master regulatory genes for terminal differentiation (Pparg and Cebpa) are replaced by other novel bivalent domains comprised of active H3K4me3 and repressive H3K9me3 [6]. In 3T3-L1 preadipocyte, which is one of the well-characterized cell models of adipogenesis, the repressive H3K27me3 mark constituting the bivalent domain is lost in most of the lineage-specific genes compared to ES cell [6]. Thus, while the resolution of the H3K4/K27me3 bivalent domains contributes to preadipocyte lineage commitment, it is insufficient for the terminal differentiation to adipocytes. The novel H3K4/K9me3 bivalent histone mark is essential for keeping the expression of master regulatory genes “poised” in the preadipocyte state and would work as a preparation mechanism for the prompt biogenesis of adipocyte in the future requirements [6, 10].

While the methylation of H3K27 and H3K9 are both implicated in condensed heterochromatin, they show distinct localization patterns. The bivalent domain of H3K4me3 and H3K27me3 locates focally on the separate H3 tails in the same nucleosome near the transcription start site (TSS) of target genes [11], while the bivalent domain of H3K4me3 and H3K9me3 locates in tandem across TSS and widely spreads through the gene body [6, 12]. Recent studies revealed that both of the repressive histone marks are involved in beige adipocyte biogenesis as discussed below, although it is not yet completely elucidated how these repressive marks cooperatively produce characteristics of beige adipocyte.

In addition to the above roles of epigenetic regulation, nuclear transcription factors are also critical for adipocyte differentiation. For example, the chemically-induced terminal differentiation of 3T3-L1 preadipocytes is mediated by rapid induction of C/EBPβ and C/EBPγ within 4 hours after the induction and the following expressions of PPARγ and C/EBPα, each of which subsequently activates the other’s expression after 24 hours of induction [7, 13]. PPARγ and C/EBPβ are also the key transcription factors that regulate both brown and beige adipocyte development by forming a co-regulatory complex with PPARγ co-activator-1α (PGC1α) and PRDM16 [5]. A number of other transcription factors involved in the differentiation of brown and beige adipocytes are considered to function through those four factors: PPARγ, C/EBPβ, PGC1α, and PRDM16 (reviewed in [5]). Although both brown adipocyte and beige adipocyte are thermogenic and their biogenesis requires common factors, they arise from distinct precursors and harbor discrete characteristics [1]. Brown adipocyte originates from sub-population of dermomyotome precursors expressing Myf5, Pax7, and engrailed 1 (En1). This dermomyotome precursor cell differentiates into either brown preadipocyte which expresses early B cell factor (EBF2) or MyoD-expressing myoblast [1, 5, 14]. The nature of precursor cells of beige adipocyte is more complicated as is reported that the concept of beige adipocyte does not include only one identical type of thermogenic cells, but encompasses diverse types of thermogenic cells which emerge in white adipose tissue and possibly in other organs in response to the external stimuli [1, 15]. Beige adipocyte thus originates from heterogeneous precursors including progenitor cells expressing Sma, Myh11, Pdgfra, or Pdgfrb [15-18] (reviewed in [1, 5]). Such a diversity of progenitor cells makes the situation more complicated to elucidate how methylation of histones regulates the biogenesis of beige adipocyte from multiple precursors although there are several lines of evidence for the regulation of beige adipogenesis by histone methylation as summarized below (Table 1).

Histone Demethylases as Regulators of Beige Adipogenesis

Epigenetics is a mechanism of gene expression modulation without alterations of genomic sequence. Epigenetic mechanisms include methylation of DNA and RNA, post-translational modifications of histones, and non-coding RNAs. Somatic cell DNA is coiled around histone octamers comprised of two copies each of four types of core histones (H3, H4, H2A, H2B). Histones are subject to various post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitination and thus contribute to the regulation of chromatin states and transcriptional activities. Although most (if not all) of covalent histone modifications are
reversible, methylation of histone had been considered to be irreversible until the discovery of a histone lysine demethylase 1 (LSD1) [19] and subsequent finding of a series of JmjC domain-containing histone demethylases [4, 20, 21]. The chemically stable characteristics of methylated histone would contribute to the cellular memory of external stimuli by maintaining the transcription levels of adaptive genes even after the dissipation of environmental cues. Considering above, it is speculated that histone methylation would be involved in the regulation of beige adipogenesis, which is one of the mechanisms for adaptation to the external environment (e.g. coldness). Indeed, recent studies have revealed that the cell fate of beige adipocyte is regulated by diverse histone methyl-modifying enzymes such as JMJD3 (also known as KDM6B) [22], euchromatic histone-lysine N-methyltransferase 1 (EHMT1) (also known as GLP) [23], Jumonji domain containing 1A (JMJD1A) (also known as JHDMA2 or KDM3A) [24], and LSD1 (also known as KDM1A or AOF2) [25, 26] (reviewed in [5, 14]) (Table 1).

| Histone Methylation | Histone methyl-modifiers | Roles in beige and brown adipose cell fate | References |
|---------------------|--------------------------|--------------------------------------------|------------|
| Methylated H3K27    | JMJD3, a histone H3K27me2/me3 demethylase, regulates beige adipogenesis by enzymatically resolving bivalency of an H3K4/K27me3 during brown adipocyte differentiation and beige adipocyte biogenesis. | [22] |
|                     | UTX, a demethylase of histone H3K27me2/3, promotes the expression of thermogenic genes in brown adipocytes and is predicted to mediate beige adipocyte function. | [27] |
|                     | EZH2, GSK126-dependent inhibition of an enzyme subunit of polycomb repressive complex 2 (PRC2), which catalyzes trimethylation of histone H3K27me3, results in promoting beige adipocyte biogenesis in diet-induced obese mice. | [28] |
| Methylated H3K9     | EHMT1 induces brown adipogenesis by repressing muscle-selective genes through its methyltransferase activity of H3K9 and activating BAT-selective genes through stabilizing the PRDM16 protein. EHMT1 is also required for beige adipogenesis. | [23] |
|                     | JMJD1A, a histone H3K9me1/me2 demethylase, induces beige adipogenesis through its demethylase activity. In response to coldness, JMJD1A is phosphorylated at S265 and induces its recruitment to the regions of beige-selective genes to produce chronic beige adipogenesis by demethylating H3K9me2. In brown adipocyte, phosphorylated JMJD1A acutely remodels chromatin conformation and induces thermogenic gene expression for producing heat through an enzyme-independent mechanism. | [24, 30] |
|                     | LSD1, a H3K4me1/me2 and H3K9me1/me2 demethylase, maintains BAT properties. LSD1 also regulates beige adipogenesis and rescues age-related beige-to-white transition in mouse. It regulates OXPHOS-related genes and beige-selective genes in white adipocyte cell lines. It is not elucidated if these functions are through its enzyme-dependent mechanism. | [25, 26, 32-35] |
| H3K4me1 in the absence of H3K27Ac | The "poised" enhancer regions, which is characterized by global enhancer mark H3K9me1 in the absence of active enhancer mark H3K27Ac, is well associated with the genomic regions of thermogenic genes and genes involved in fatty acid metabolism specifically in cold-experienced warm beige adipocyte for their future activation. | [3] |

### Methylation of H3K27

As described above, the bivalent domain of active H3K4me3 and repressive H3K27me3 contributes to maintaining the expression of lineage-specific genes in a poised state in ES cells [9]. In addition, a recent study revealed that the bivalent domain of H3K4/K27me3 is also involved in both brown adipocyte differentiation and beige adipocyte biogenesis. Adipocytes differentiated from either immortalized brown preadipocytes or primary subcutaneous inguinal white preadipocytes presented lower levels of H3K27me3 in the regions of thermogenic genes (e.g. Ucp1 and Cidea) compared with their respective preadipocytes. Such demethylation of H3K27me3, which resolves bivalency of an H3K4/K27me3 during brown adipocyte differentiation and beige adipocyte biogenesis, required JMJD3, a histone H3K27me2/me3 demethylase (Fig. 1, top). These findings in the cell-autonomous study were confirmed by using mouse models. The transgenic mice over-expressing Jmjd3 under the control of Fabp4 promoter
showed higher expressions of thermogenic genes (e.g. Ucp1 and Cidea) in BAT compared to their control litters. The similar change was observed in inguinal WAT when the mice were chronically treated (for 7 days) with an agonist for the β3-adrenergic receptor (β3-ADR) CL-316243. In addition, another line of transgenic mice which express an enzyme-defective point mutant (H1388A) of JMJD3 showed repressed beige adipogenesis. These data collectively demonstrated an enzyme activity-dependent regulation of beige adipogenesis by JMJD3. It is also shown that JMJD3 is recruited to the promoter regions of thermogenic genes (Ucp1 and Cidea) through a transcription factor, Rreb1, which binds to Rreb1-binding sites [22]. Thus, demethylation of H3K27 contributes to the induction of beige adipogenesis. While UTX, another demethylase of histone H3K27me2/me3, is reported to promote beige adipocyte biogenesis in diet-induced obese mice [28].

**Methylation of H3K9**

In a cold environment, homeotherms generate heat to maintain their body temperature through both shivering and non-shivering thermogenesis. While shivering thermogenesis takes place in skeletal muscle, non-shivering thermogenesis occurs in both brown and beige adipose tissues. Although brown adipose tissue as well as skeletal muscle generate heat in the acute phase of cold exposure (~ hours in mice), the emergence of beige adipocytes in subcutaneous white adipose tissues (scWAT) and the production of heat occurs only after long-term exposure to a cold environment (~ weeks in mice). The expression of thermogenic genes such as Ucp1 discriminates thermogenic BAT and non-thermogenic WAT, as is a constitutively higher expression in BAT while very low
expression of WAT. The constitutive expression of Ucp1 in BAT is associated with lower levels of H3K9me2 in the enhancer and promoter regions of Ucp1 compared with that in WAT. Notably, a recent report claimed that the induction of Ucp1 in BAT in response to acute cold exposure does not associated with a further reduction of H3K9me2, which is already at low levels in the enhancer and promoter regions of Ucp1 [24], while another study reported that the low levels of H3K9me2 is further reduced in the regulatory regions of Ucp1 in BAT in response to acute cold stimuli [29]. Such H3K9 demethylation-dependent induction of Ucp1 expression is more evident during beige adipocyte biogenesis. The induction of Ucp1 expression with beige adipocyte biogenesis in scWAT deposits in response to chronic cold exposure (4°C for 1 week) does associate with demethylation of highly methylated H3K9 in the enhancer and promoter regions of Ucp1 [24] (Fig. 1, middle). Collectively, the chromatin conformation in the regulatory regions of Ucp1 is in a relatively “open” state in BAT whereas it is “closed” in scWAT. Considering that focally distributed H3K27me3 represses lineage-specific genes in ES cells which also have a globally “open” chromatin state, the H3K4/K27me3 bivalent domain would be an appropriate mechanism for regulating Ucp1 expression in BAT. On the other hand, beige adipogenesis is regulated by the demethylation of both H3K9 and H3K27 repressive histone marks (Fig. 1, top and middle). As is discussed below, both histone H3K9 methyltransferase EHMT1 and H3K9 demethylase JMJD1A are reported to regulate beige adipocyte biogenesis and brown fat characteristics [23, 24, 30] (Fig. 2A-C). In addition, another histone H3K4/K9 demethylase LSD1 mediates beige adipose cell fate [25, 26] (Fig. 2B, C).

**EHMT1**

The adipose tissue-specific Ehmt1-null mice produced by crossing an adiponectin-Cre line with an Ehmt1-floxed line present severely reduced expression levels of beige-selective genes such as Ucp1, Cidea, and Ppargc1a in inguinal WAT compared to their control mice with chronic administration of a β3-adrenergic receptor agonist [23]. EHMT1 is cell-autonomously required for beige-selective gene expression, which was demonstrated by using the stromal vascular fraction isolated from the inguinal WAT of Ehmt1-floxed mice subsequently infected with Cre-recombinase expressing adenovirus (Fig. 2B). Although the molecular mechanism of cell-autonomous regulation of beige adipocyte biogenesis by EHMT1 is not fully elucidated, the role of EHMT1 in the regulation of brown fat function is well determined. Adipose tissue-specific Ehmt1-null mice show impaired maintenance of body temperature in response to acute coldness and reduced fatty acid metabolism. In addition, mice with brown adipocyte precursor-specific knockout of Ehmt1 prepared by crossing the Myf-5-Cre line with an Ehmt1-floxed line show reduced BAT content at the embryonic stage, which revealed that EHMT1 regulates lineage specification between BAT and muscle. Cell-autonomous studies revealed that EHMT1 regulates brown adipose cell fate by both reducing expressions of muscle-selective genes and inducing expressions of BAT-selective genes [23]. While the repression of muscle-selective genes by EHMT1 is mediated by its H3K9 methyltransferase activity, the activation of BAT-selective genes is through stabilizing the PRDM16 protein (Fig. 2A). Because PRDM16 is a potential regulator of both brown and beige adipogenesis, stabilization of PRDM16 by EHMT1 could be applicable for the regulation of beige adipogenesis by EHMT1. However, considering that beige adipocytes are not derived from the sole type of precursor but from multiple cell types and at the same time they are distinct from the precursor of brown adipocytes, other mechanisms independent of PRDM16 would be applicable for the regulation of beige adipogenesis by EHMT1. Thus, further study would be required to elucidate how EHMT1 enzymatically contributes to the biogenesis of beige adipocyte.

**Chromatin Remodeling by Phosphorylated JMJD1A**

The BAT function and beige adipocyte biogenesis are also regulated by histone demethylase JMJD1A which catalyzes the removal of methyl-residue(s) from H3K9me1/me2 [20]. While JMJD1A regulates beige adipocyte biogenesis through its demethylase activity, it regulates BAT function by the mechanism independent of its enzyme activity [30]. Jmjdc1a-null mice present phenotypes similar to the human metabolic syndrome: an adult-onset obesity, a plasma hyperlipidemia, and a diabetic state characterized by insulin insensitivity [29, 31]. Metabolic cage analysis revealed that the fed Jmjd1a-null mice show a higher respiratory quotient compared to their control mice during the second half of dark phase, which indicates a time-dependent lower fat usage in Jmjdc1a-null mice [31]. In addition, Jmjdc1a-null mice show lower body temperature compared to their control mice under the conditions of fasting (for 12 hours during dark phase) as well as acute cold exposure (~6 hours) [29,31]. Thus, Jmjdc1a-null mice are deficient in thermogenesis due to their functional defects in BAT thermogenesis and/or energy utilization. Induction of both thermogenesis and lipolysis for supplying thermogenic fuel in brown adipocyte is regulated by the adrenergic
In response to coldness, the sympathetic nerve is stimulated and increases the secretion of catecholamine from its nerve-ending. The secreted catecholamine binds to the β-adrenergic receptor (β-ADR) localized in the cell membrane of brown adipocytes. Then, β-ADR activates adenylyl cyclase, which, in turn, increases cAMP, activates protein kinase A (PKA), and induces Ucp1 expression and activation of hormone-sensitive lipase which regulates lipolysis. JMJD1A is phosphorylated at S265 by PKA and represses muscle-selective genes through methylating H3K9 in their genomic regions. Notably, the phosphorylation of JMJD1A at S265 is essential for ISO-dependent induction of the genes encoding UCP1 and β-ADR in brown adipocytes. To prove above, immortalized mouse primary brown adipocytes, in which the expression of endogenous Jmd1a is knocked-down by stably expressing short hairpin RNA, were transduced with a wild-type (WT), a non-phosphorylated mutant (S265A), or an enzyme defect mutant (H1120Y or H1120F) of human JMJD1A. The induction of Ucp1 and Adrb1 expression by ISO was severely repressed in the cells transduced with non-phosphorylated JMJD1A, while it was intact in the cells transduced with enzyme defect mutants when compared to the cells transduced with wild-type JMJD1A. In addition, the flux analysis revealed that the functional induction of mitochondrial respiration and proton leak by β-ADR agonist dobutamine is also repressed only in the brown adipocytes transduced with a non-phosphorylated mutant of JMJD1A. However, the
enzyme activity of JMJD1A, determined by in vitro assays using either HTRF-FRET or MALDI-TOF/MS, is not altered by its phosphorylation at S265. Collectively, phosphorylation of JMJD1A at S265 is essential for thermogenesis in brown adipocytes without altering its enzyme activity. Although the phosphorylation of JMJD1A does not change its subcellular localization as well as its enzyme activity, it mediates the binding of JMJD1A with SWI/SNF chromatin-remodeling complex and PPARγ, which directly binds to enhancers and promoters of Ucp1 and Adb1. Thus, phosphorylated JMJD1A-dependent complex formation with SWI/SNF and PPARγ brings enhancers in close proximity to the promoter of thermogenic genes by forming long-range DNA loop and induces their transcription [30] (Fig. 2B). Notably, the process of JMJD1A phosphorylation and the following chromatin remodeling take place acutely in response to cold stimuli: the level of JMJD1A phosphorylation and the enhancer-promoter proximity increase toward the peak at 1 hour after the ISO administration and return to the basal level in several hours. This quick regulation of gene expression would be an appropriate mechanism for acute induction of thermogenic genes in brown adipocytes in response to coldness. In addition to the enzyme-independent function of JMJD1A, another histone demethylase FBXL10 regulates white adipocyte differentiation by forming a polycomb repressive complex-1 (PRC-1) independently of its enzyme activity [13]. These findings indicate that the concept of the enzyme-independent function of histone demethylases would be generally applicable for other histone demethylases.

**Enzyme-dependent Function of JMJD1A**

In addition to its regulation of BAT thermogenesis, JMJD1A also regulates beige adipocyte biogenesis [24, 30]. The physiological relevance of phosphorylation of JMJD1A to acute and cold thermogenesis was determined by generating knock-in mice which possess a serine 265-to-alanine (S265A) mutation in Jmd1a [24]. In response to acute cold exposure, Jmd1a-S265A knock-in mice showed a higher reduction of body temperature compared to the control mice in the similar extent with Jmd1a knockout mice. Notably, Jmd1a-S265A knock-in mice also presented severe reduction of beige adipogenesis in white adipose depots under chronic cold environment. This finding indicates the pre-requisition of JMJD1A phosphorylation for its enzyme-dependent regulation of beige adipocyte biogenesis. A cell-autonomous study confirmed that the phosphorylation of JMJD1A is required for its recruitment to the regulatory regions of beige-selective genes (i.e. Ucp1, Cidea, and Ppara) and the following demethylation of H3K9me2 in their genomic regions [24] (Fig. 2C). To prove above, immortalized beige adipocytes derived from subcutaneous WAT, in which endogenous expression of Jmd1a is suppressed by stably expressing shRNA, were transduced with a wild-type, a non-phosphorylated mutant (S265A), a phospho-mimetic mutant (S265D), or a double phospho-mimetic and catalytically-inactive mutant (S265D-H1120Y) of human JMJD1A.

The induction of mRNA expression of beige-selective genes was associated with the recruitment of JMJD1A to their regulatory region and the reduction of H3K9me2 levels during differentiation of the beige adipocyte transduced with wild-type JMJD1A. However, such induction of the beige-selective genes and the reduction of H3K9me2 were not observed in the cells transduced with non-phosphorylated mutant JMJD1A, which indicates that the JMJD1A phosphorylation at S265 is pre-requisite for its recruitment and demethylation of H3K9me2 (Fig. 1, middle). Consistent with the suppression of beige-selective genes, the mitochondrial heat-producing function determined by flux analysis is severely repressed in the cells transduced with non-phosphorylated mutant JMJD1A compared to the cells with wild-type JMJD1A. Conversely, the expression of beige-selective genes and thermogenic function are highly induced in the cells transduced with phospho-mimetic mutant JMJD1A, which is totally suppressed in the cells with the double phospho-mimetic and catalytically-inactive mutant JMJD1A. Thus, phosphorylation at S265 of JMJD1A is required for its recruitment to the target genomic regions and the following demethylation of H3K9me2, which induces chromatin openness and subsequent induction of gene expression (Figs. 1, middle, and 2C). In contrast to the complex of JMJD1A with SWI/SNF and PPARγ in brown adipocyte, JMJD1A forms a complex in beige adipocytes with the factors previously reported critical for beige adipogenesis such as PRDM16, PGC1a, and PPARγ [24] (Fig. 2B, C). The cell type-specific factors in the complex are speculated to contribute to determining the target genes of JMJD1A and subsequent cell differentiation.

**LSD1**

LSD1 is an FAD-dependent amine oxidase homolog which demethylates histone H3K4me1/2 and H3K9me1/2. There are several lines of evidence that LSD1 regulates BAT function and beige adipogenesis [26, 32-35] (Fig. 2B, C). An adipose tissue-specific LSD1-deficient mice produced by crossing an adiponectin-Cre line and an LSD1-floxed line show impaired BAT thermogenesis by two mechanisms:
regulates OXPHOS-related genes and beige-selective controversy discussed below. Hino into both 3T3-L1 and C3H-10T1/2 [34]. Although the exact functions are still controversial whether beige adipocytes are differentiated only from the beige specific precursors or alternatively from the white adipocyte precursors [1]. Additional investigations would be required to elucidate the physiological role of LSD1 in beige adipocyte biogenesis. A recent study employing the mouse models of gain and loss of function of LSD1 also presented that LSD1 in adipocytes rescues the age-related transition from beige adipocyte to white adipocyte [25].

Epigenetic Memory in “Whitened” Beige Adipocyte

The beige adipocyte directly transits to white adipocyte in response to the released coldness [2]. Such “whitened” beige adipocyte is in preparation for future coldness and can return to the thermogenic state rapidly in response to subsequent cold exposure. It is expected that the cellular memory for the subsequent rapid induction of beige adipocyte in response to repeated coldness would be mediated by epigenetic mechanisms. It is recently reported that a “poised” enhancer region, which is characterized by global enhancer mark H3K4me1 in the absence of active enhancer mark H3K27Ac, is well associated with the genomic regions of thermogenic gene Ucp1 and genes involved in fatty acid metabolism specifically in cold-experienced warm beige adipocyte [3] (Fig. 1, bottom). This would be the first example showing that the cellular memory in whitened beige adipocyte is mediated by methylated histone marks. Further investigation would provide a more detailed insight into the epigenetic regulation mechanisms how the cold-experience is memorized (e.g. writing and erasing the poised enhancer marks) in warmed beige adipocytes. Regarding the enhancer regulation during thermogenic adipogenesis, it is also reported that histone H3K4 methyltransferases MLL3/4 are required for super-enhancer formation during brown adipogenesis [37, 38], while their roles during beige adipogenesis is still to be elucidated.

Conclusions and Future Perspectives

The precise epigenetic mechanism for conferring functional heterogeneity on thermogenic adipocytes is not fully elucidated. Although brown adipocyte and beige adipocyte are both thermogenic, these two cell types harbor discrete characteristics. While classical brown adipo-
cyte stably retains thermogenic characteristics, beige adipocyte is inducible and reversible thermogenic cell in response to environmental changes. In addition, the thermogenic mechanisms are distinct between beige and brown adipocytes. Although Ucp1 plays a role in producing heat in both brown and beige adipocytes by uncoupling cellular respiration and mitochondrial ATP synthesis, recent studies revealed alternative Ucp1-independent thermogenic mechanisms: creatinine-driven substrate cycle [39-41] and ATP-dependent Ca^{2+} cycling by SERCA2b and RyR2 [42]. Notably, heat production through the Ca^{2+} cycling mechanism is indispensable for beige adipocyte thermogenesis, while it is not critical for brown adipocyte thermogenesis [42]. It is still an open question whether and how such a difference would be derived by the distinct epigenetic mechanisms. In addition, considering that beige adipocytes might originate from multiple precursors, it is also intriguing to know how the distinct epigenetic mechanisms regulate the differentiation of each precursor. Further investigation is required to get a clear answer for the above issues and to fully understand epigenetic mechanisms for beige adipocyte biogenesis.

As discussed above, recent studies revealed epigenetic regulation mechanisms of beige adipogenesis by histone methylation. However, there left a number of unsolved issues. While resolution of the bivalency of H3K4/K27 contributes to the induction of beige adipogenesis, an interactive action with another type of methylated histone marks such as the bivalency of H3K4/K9 is not elucidated. In addition, regulatory mechanisms of enzyme activities of histone modifiers during brown and beige adipogenesis are not well understood. Regarding the JMJD1A-mediated beige adipogenesis as an example, the mechanism for the time lag between the first phosphorylation-dependent recruitment step and the subsequent second demethylation step is to be elucidated. One possible factor is the level of intracellular metabolite that regulates the enzyme activity of histone modifier. Jumonji c (jmjc) domain-containing histone demethylases such as JMJD1A and JMJD3 require co-factors such as oxygen, ferrous iron (Fe(II)), and 2-oxoglutarate, which is a metabolite of tricarboxylic acid (TCA) cycle and amino acid metabolism, for thier enzyme activity [5, 20]. LSD1 is a flavin adenine dinucleotide (FAD)-dependent amine oxidase homolog [19]. In addition, S-adenosyl methionine (SAM) which derives from methionine is a methyl donor for histone methylation [5, 43]. Thus, under the cold environment, activated energy metabolism for heat production in adipocyte would change the levels of co-factors and co-substrates and consequently affect the activity of epigenetic enzymes. In this notion, it is conceivable that beige adipogenesis is mediated by sensing metabolic environment as well as thermal condition.

**Acknowledgments**

This work was supported in part by JSPS KAKENHI (grant numbers 18H04796, 17H03631, 25291002), Astellas Foundation for Research on Metabolic Disorders, the Novartis Foundation (Japan) for the Promotion of Science, the Tokyo Biochemical Research Foundation, the Naito Foundation, the Ichiro Kanehara Foundation, Japan Diabetes Foundation, Suzuken Memorial Foundation, and Kao Research Council for the Study of Healthcare Science (to T.I.).

**Conflict of Interest Statement**

The authors have no competing interests to declare.

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