Editorial

Beyond brown adipogenesis the inheritance of imprinted H19

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High-calorie food and sedentary living style give rise to the contemporary pandemics of overweight and obesity. Currently, nearly one-third of population in the world are either overweight or obese, and this number is expected to continuously increase in next decades. Obesity not only compromises glucose tolerance and insulin sensitivity, but also links to serious diseases, including type 2 diabetes, non-alcoholic fatty liver, cardiovascular diseases, and several types of cancers (1). Obesity stems from low energy expenditure by which the excess of energy is stored in white adipose tissue (WAT). However, excessive accumulation of WAT, especially in visceral fat, releases free fatty acids, inflammatory cytokines, and other mediators, which can interfere with the functions of other organs, such as brain, liver, and muscle. On the contrary, the expansion and activation of brown adipose tissue (BAT) improve energy homeostasis through non-shivering thermogenesis (NST). Recently, the discovery of substantial amount of BAT and beige adipocytes in human WAT provides a promising therapeutic target for combatting obesity and its induced complications (2).

To effectively manipulating BAT development and thermogenic activity, it is necessary to understand the molecular mechanism regulating brown adipogenesis. In the last decade, a group of principal factors have been identified, which regulate brown adipogenesis. Uncoupling protein-1 (UCP-1) is a predominant protein for NST in BAT and beige fat, though the endogenous creatine was recently identified to incur NST independent on UCP-1 (3). The presence of UCP-1 produces a futile proton cycle which dissipates proton gradient force as heat instead of synthesizing ATP. Due to the unique role of UCP-1, expression and activity of UCP-1 are considered as a hallmark for the acquisition of BAT identity. Available studies suggest that the regulation of *ucp-1* is mainly at the transcriptional level, although sulfenylation on Cys253 residue of UCP-1 is also required for its activity, which is up-regulated by reactive oxygen species (ROS) (4). In *ucp-1* gene, several nuclear regulatory elements have been identified. For example, its promoter region contains cAMP responsive element binding (CREB) and CCAAT/enhanced binding protein (C/EBP) sites, which can rapidly respond to cAMP, and transcription factors, C/EBPα and C/EBPβ; the enhancer region of *ucp-1* gene contains the binding elements responsive to nuclear receptors, harboring retinoid X receptor (RXR), peroxisome proliferator activated receptor (PPAR) γ, PPARα, retinoid acid receptor (RAR) or thyroid hormone receptor. In addition, several essential signaling mediators and transcriptional cofactors are also proven to regulate UCP-1 expression, such as protein kinase A (PKA), PRDM16 and PGC-1α. PRDM16 recruits PPARγ to activate *ucp-1* activity, indispensable for brown adipogenic commitment (5). Moreover, PGC-1α not only coordinates the dimerization of RXR/PPAR to bind and activate *ucp-1* transcription, but also initiates mitochondrial biogenesis (6). As a result, *ucp-1* transcription is inducible and can be enhanced by dietary bioactive compounds, nutrients, and drugs, including vitamin A, metformin, thiazolidinedione and fibrates, which is considered as alternative therapeutics for overweight, obesity and type 2 diabetes.

Although an extensive list of protein-coding genes have been identified, emerging evidences underscore the importance of non-coding RNAs (ncRNAs) in tuning brown adipogenesis. Long ncRNA (lncRNA) is

conceptually defined as over 200 nucleotides in length and no protein-coding potential. These lncRNAs were considered as "junk RNA" or "sequencing noise", but recently recognized as key players in fundamental cellular processes, such as chromatin remodeling, transcription, post-transcriptional regulations, and protein trafficking; as a result, the expression and function of lncRNA are tightly regulated, which are tissue and developmental stage dependent. Disturbance of lncRNA processing or binding targets is linked to numerous diseases, such as obesity, type 2 diabetes and cancer (7). Several lncRNAs in BAT have been identified to be essential for BAT differentiation and NST, including brown fat lncRNA 1 (Blnc1), brown adipose tissue enriched lncRNA 1 (lncBATE1), lncBATE10, and PR domain protein 16 lncRNA (lncdPRDM16).

H19 is a highly conserved lncRNA in mammalian animals, which is located on chromosome 11p15.5 in humans. It is also the first identified imprinted lncRNA and transcribes from the maternal allele. Interestingly, another imprinting gene, insulin-like growth factor 2 (*Ifg2*), is also located in the locus of *H19* gene, but is a paternal imprinted gene (PIG) (8). The *H19/IGF2* cluster is one of the well defined examples of gene imprinting.

H19 involves in embryogenesis, tumorigenesis and myogenesis through diverse functional patterns (9). During embryogenesis, methyl-CpG-binding domain protein 1 (MBD1) mediates the repressive effects of H19 on PIG, including *Igf2* (10). H19 guides MBD1 to DNA domain of PIG, subsequently recruiting histone methyltransferases (HMTs) to induce histone modifications, including H3K9me3 and H3K27me3. Given that miR-675 is transcribed from an exon of H19, H19 could exert its biological effects via miR-675. In placenta, H19 maintains the regular trophoblast cell proliferation through miR-675 mediated down-regulation of Nodal signaling (11). Moreover, H19 regulates myogenesis and tumorigenesis through miR-675 and K-homology splicing regulatory protein (KSRP). During myogenesis, H19 down-regulates SMAD signaling of the bone morphogenetic protein pathway by means of miR-675, which promotes skeletal muscle differentiation (12). Additionally, H19 dismisses RNA binding protein, KSRP, and stabilizes myogenin mRNA (13). Furthermore, H19 functions as an oncogene and inhibits the activity of tumor suppressor p53 under the help of miR-675 (14).

Up to now, only a handful of studies investigate the role of H19 in adipogenesis and lipid metabolism. A previous study suggested that H19 impedes white adipogenesis of bone marrow mesenchymal stem cells, which is mediated by miR675 (15). However, in macrophages treated by oxygenized low density lipoprotein, H19 enhances lipid accumulation and secretion of inflammatory cytokines (16). The discriminative responses of H19 to adipogenesis and lipid synthesis suggest that H19 interacts with different partners to exert context-dependent functions.

The recent study from Elena *et al.* [2018] reported that H19 activates BAT development and NST potentially through down-regulating the expression of PIG without influence on the expression of maternal imprinted gene (MIG) in BAT. Although the repressive response of H19 on differentiation of white adipocytes has been reported in the past, the regulation of H19 on BAT had not yet been explored (15). In this study, a positive link was discovered between H19 and BAT function, which was stimulated by cold stress but repressed by obesity. Gain and loss of function studies further confirmed that BAT development and thermogenic function were activated by H19, including enhanced fatty acid oxidization, mitochondrial biogenesis and NST. H19 interference in BAT discouraged BAT activity and drove mice prone to obesity, insulin resistance and glucose tolerance. Supportively, overexpression of H19 in BAT improved the functionality of BAT, rendering mice resistant to high fat diet-induced obesity. Therefore, H19 is a potential target to promote the thermogenic function of BAT, preventing obesity and metabolic disorders. Interestingly, in subcutaneous and visceral WAT, H19 expression was not altered by cold stress and obesity, indicating beige adipogenesis is potentially independent on H19. The difference between beige adipogenesis and classic BAT development could be due to different developmental origins and niche environment; brown adipocytes are derived from the muscle-like progenitor cells expressing Myf5 and Pax7, while beige and WAT adipocytes originate from non-myogenic potential cells (17,18). Consistent with the effects of H19 on BAT, H19 is abundantly expressed in skeletal muscle and also identified to be required for myogenesis (12,19).

Elena *et al.* [2018] also reported that interfering H19 expression increased PIG expression without affecting MIG expression. In addition, PIG expression was tended to be decreased by cold stress and increased by obesity. Taking these evidences together, authors provided an insightful explanation that PIG expression might inhibit BAT, but its expression was elaborately repressed by H19. Regulation of H19 on imprinted gene network (IGN) has been reported previously, but the specific repression of H19 on PIG is novel although it requires to be further studied (10,20).

This study delineates a link between the expression of monoallelically imprinted genes and brown adipogenesis. Our lab intensively explores the trans-generational effects of maternal obesity on energy homeostasis in mice (21-23). Strikingly, offspring from obese subjects are susceptible to obesity, even when young. Corresponding to obesity, the BAT development and functions are also impaired majorly due to impaired adipogenic commitment. As *H19* is MIG, an inappropriate epigenetic imprinting on *H19*, such as DNA hypermethylation, may explain the inheritance of maternal obesity to obesity and associated syndromes in offspring. Previous studies have demonstrated that dysregulation of *H19/IGF2* locus can also cause severe diseases, including Beckwith-Wiedemann's Syndrome and Silver-Russell's Syndrome (24). Authors also identified that MBD1 was responsible for mediating the repressive effect of H19 on PIG. MBD1 recruited HMTs to enhance H3K9me3 and H3K27me3 on PIG, causing gene expression repression. It has been reported that *igf2* is inhibited by H19 during embryogenesis via a similar mechanism (25). Although this study provided solid evidences to demonstrate that repression of PIG was related to MBD1, the mechanism by which H19 selectively targets PIG rather than MIG keeps elusive.

Altogether, Elena *et al.* [2018] provided insightful evidence on imprinted lncRNA H19 in regulating BAT development and function. The energy homeostasis improved by H19 via BAT activation suggests that H19 is a potential therapeutic target to prevent or reduce obesity. Meanwhile, since *H19* is a MIG, the dysregulated epigenetic modification on H19 in maternal subjects could pass to the next generation, rendering them prone to obesity and other diseases.

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Footnote

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