



Mini Review

MicroRNA Functions in Brite/Brown Fat – Novel Perspectives towards Anti-Obesity Strategies

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ABSTRACT

Current anti-obesity strategies are aiming at restricting energy uptake, but still, obesity treatment is far from being satisfactory. The discovery of active brown adipose tissue (BAT) in adult humans currently opens new avenues to combat obesity and follow-up complications as it tackles the other side of the energy balance: energy expenditure via non-shivering thermogenesis. This process of energy dissipation in the adipose tissue is tightly controlled, and the elucidation of its regulatory network is a key plank for therapeutic applications. MicroRNAs (miRNAs) belong to a novel class of regulatory determinants which are small non-coding RNAs with vital roles in regulating gene expression that also play a role in many human diseases. In this review we summarize miRNAs which have been shown to govern thermogenic, i.e. brite or brown, adipocyte recruitment and physiology. Notably, most miRNAs in this context have so far been characterized solely in mice, revealing a great demand for more human studies. As in the context of other diseases, RNA-based therapeutics have meanwhile entered clinical trials, further exploring the functions of miRNAs in brown and white adipose tissues could result in novel therapeutic approaches to treat obesity and its follow-up complications.

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1. Introduction

The whole body energy balance depends on two sides of the same coin: energy uptake and energy expenditure. As obesity is a consequence of energy uptake that exceeds energy expenditure, current

anti-obesity strategies are aiming at restricting energy uptake. Nevertheless, this treatment strategy acting on one side of the coin is far from being satisfactory so far, unfortunately [1]. Nowadays, there is emerging evidence that a lifestyle-modification program characterized by a balanced diet and combined with an increase in energy expenditure can reduce obesity and the risk of obesity-related comorbid conditions [2]. Increased energy expenditure can be achieved either by physical activity, mainly in the muscle [3], or by non-shivering thermogenesis in the adipose tissue [4], owing to the fact that active (i.e., thermogenic)

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brown adipose tissue (BAT) has recently been “re-discovered” in adult humans [5–9]. Indeed, human BAT recruitment and activation upon cold exposure have very recently been demonstrated to increase non-shivering thermogenesis, to elevate energy expenditure and finally to contribute to body fat reduction [10–12]. These findings underline the paradigm shift that recruitment and activation of thermogenic adipocytes, i.e. increasing energy combustion in the adipose organ by non-shivering thermogenesis, might contribute to anti-obesity strategies [13].

Thermogenic adipocytes are distinct from white adipocytes in that they have more mitochondria, and in that uncoupling protein 1 (UCP1) is highly enriched in these mitochondria. UCP1 uncouples substrate oxidation from ATP production so that heat is generated instead [14]. In addition to thermogenic brown adipocytes which are located in BAT, upon cold exposure, UCP1-expressing thermogenic adipocytes can also be recruited in white adipose tissue (WAT), so-called brite (brown-in-white) or beige adipocytes, resulting in WAT ‘browning’ [15–19]. Thermogenic adipocytes have a substantial impact on the energy balance, as UCP1-promoted heat production – non-shivering thermogenesis – is a highly energy-dissipating process [20,21].

This attracts constantly growing attention in the search for molecular pathways, pharmacological agents and endogenous signals that regulate the formation of thermogenic adipocytes [22–24]. As proof of concept, first promising targets in human have already been identified comprising endocrine and paracrine mediators such as FGF21 [25,26] and natriuretic peptides [27–29], as well as transcriptional regulators such as PPAR γ 2, C/EBP and PRDM16 [30].

However, the role of non-coding RNAs (ncRNAs), a novel class of regulatory determinants, is still mostly unknown in this context. The best explored subclass of ncRNAs comprises microRNAs (miRNAs), small RNAs of approximately 23 nucleotides, which play a central role in RNA interference (RNAi), a post-transcriptional gene silencing mechanism existing in many eukaryotes [31–34]. MiRNAs interact with partially complementary sites in the 3'UTR of mRNAs to diminish protein output, via both inhibition of translation and mRNA destabilization [35]. Despite their recent discovery, it is already well-known that miRNAs play pivotal roles in numerous biological processes. Importantly, fat-selective inactivation of Dicer, a necessary factor for miRNA biogenesis, has been shown to result in mice that were almost devoid of WAT [36], which implicates miRNAs in adipocyte development. Indeed, we [37–39] and others [40,41] have identified miRNAs that influence white adipocyte differentiation. In this review we summarize the currently known roles of miRNAs in the recruitment and function of thermogenic adipocytes.

2. MicroRNAs in brite/brown adipocyte differentiation and function

2.1. MiR-193b-365 involvement in brown adipocyte differentiation

Although white and brown adipocytes share morphological similarities (most importantly, lipid droplets), some recent studies have accumulated evidence for a close developmental relationship between brown and myogenic lineages, at least in mouse [42–44]. In extension of these findings, a study by Lodish and co-workers demonstrated that miRNAs can act as gatekeepers between brown adipogenesis and myogenesis [45]. First, the authors found miR-193b and miR-365, which are expressed from the same locus, to be enriched in mouse BAT versus WAT and other tissues. Second, inhibition of both miRNAs (by locked nucleic acid modified antisense oligonucleotides (LNA-ASOs)) suppressed adipocyte differentiation of precursor cells isolated from either BAT or WAT. As in brown precursors, this inhibition elevated the mRNAs of myogenic factor 6 (Myf6) and myogenic differentiation 1 (Myod1), a possible involvement of the miRNAs in myogenesis could be anticipated, and indeed, overexpression of miR-193b and/or miR-365 in C2C12 myoblasts impaired myogenic differentiation while promoting adipogenesis. However, the relevance of these in vitro results

has recently been challenged by the investigation of a mouse model in which the miR-193b gene as well as upstream regulatory sequences were deleted, thereby preventing the expression of both miR-193b and miR-365 [46]. Interestingly, the morphology of BAT and the expression of brown markers (Ucp1, PR domain containing 16 (Prdm16)) were not different between miR-193b^{-/-} and control (miR-193b^{+/+}) mice. Likewise, miR-193^{-/-} mice exhibited normal cold tolerance and energy expenditure upon β 3-adrenergic stimulation. Further, Feuermann et al. did not observe defects in adipocyte differentiation of precursor cells isolated from miR-193^{-/-} BAT, which is in direct contrast to the previous study by Sun et al. Future studies would therefore be in demand to further investigate the knockout mouse model, or cells obtained thereof.

2.2. MiR-196a is a positive regulator of brite adipocyte differentiation

MiR-196a was recently introduced as the first miRNA to regulate brite adipogenesis in vitro and in vivo [47]. Indicating a function in the “browning process”, miR-196a was found to be induced by cold and β 3-adrenergic stimulation in mouse inguinal WAT (iWAT, i.e. posterior subcutaneous WAT). Furthermore, inhibition of miR-196a during adipocyte differentiation of precursor cells isolated from iWAT largely prevented the induction of thermogenic markers. Mechanistically, the effect of miR-196a was delineated to its direct target homeobox C8 (Hoxc8), a transcription factor that is highly enriched in WAT versus BAT and that represses CCAAT/enhancer binding protein β (C/EBP β), an important regulator of brown adipogenesis. Indeed, HOXC8 overexpression during adipogenesis of human WAT-derived precursors suppressed brown marker gene expression, yet only if the miR-196a binding site within the HOXC8 3'UTR was deleted. The importance of miR-196a for adipose tissue metabolism was further corroborated by a transgenic mouse model overexpressing the miRNA under the control of the α 2 promoter. These mice exhibited a substantial induction of Ucp1 and other brown markers in iWAT, displayed increased energy expenditure and hence were resistant to diet-induced obesity as well as more sensitive to insulin. In sum, miR-196a and Hoxc8 were identified as a novel regulatory axis in adipocyte development. However, it should be pointed out that miR-196a is also associated with an oncogenic phenotype in various malignancies, e.g. colorectal cancer [48], non-small cell lung cancer [49], glioblastoma [50], and cervical cancer [51], which might limit its potential therapeutic value.

2.3. MiR-133 is a negative regulator of brite and brown adipocyte differentiation

The miR-133 family has an established role in the physiology of cardiac and skeletal muscle [52–54]. In addition, Trajkovski et al. have described an involvement of miR-133a and -133b in brown and brite adipogenesis [55]. Cold exposure led to a pronounced decrease of both miRNAs in mouse BAT and subcutaneous WAT (scWAT). Inhibition of miR-133a/b during differentiation of immortalized brown preadipocytes strongly increased the expression of Ucp1 and its upstream regulators Prdm16 and peroxisome proliferator activated receptor α (Ppar α), resulting in increased cellular respiration. Of note, these browning effects were accompanied by a general promotion of adipogenesis (assessed by expression of the adipogenic master regulator peroxisome proliferator activated receptor γ (Ppar γ), and the portion of lipid-filled cells). Conversely, transfection of miR-133 mimics blunted the expression of brown and general adipocyte markers. Similar effects of the miR-133 family were also observed with preadipocytes isolated from scWAT. Further in vitro experiments demonstrated a novel regulatory cascade in which β -adrenergic stimulation leads to suppression of the myocyte enhancer factor 2 (Mef2) transcription factor family, thereby reducing miR-133a/b levels, leading to increases of the direct miR-133 target Prdm16 and subsequent browning. A more recent study recapitulated the negative effect of miR-133a on brown and brite adipocyte differentiation [56]. The authors used a knockout mouse model in which 3 of the 4

alleles that normally generate mature miR-133a were deleted. While this strong reduction of miR-133a did not affect brown marker gene expression in BAT, pronounced “browning” of iWAT was observed. Further, glucose tolerance and insulin sensitivity were markedly improved compared to wild type mice of matched age, gender and genetic background. Interestingly, the miR-133a/b effects on brown/brite adipocyte development have recently been demonstrated to extend to the myogenic lineage. Inhibition of the miR-133 family in satellite cells (i.e., adult skeletal muscle stem cells) promoted the development of Ucp1⁺ and Prdm16⁺ adipocytes in vitro and in vivo [57]. Collectively, the studies by Liu et al. and Yin et al. suggest a dual action of miR-133a/b, governing non-shivering thermogenesis in genuine adipose tissues as well as in skeletal muscle.

2.4. MiR-155 hampers differentiation of brite and brown adipocytes

MiR-155 was identified by Chen et al. as another negative regulator of BAT development and thermogenesis [58]. Using murine brown preadipocytes, the authors found that miR-155 prevents adipocyte differentiation via direct targeting of *C/ebpβ*. In turn, *C/ebpβ* was demonstrated to suppress transcription of miR-155, thereby establishing a double-negative feedback loop in brown precursor cells. Strikingly, transgenic mice that overexpressed miR-155 (globally or specifically in BAT) exhibited smaller BAT depots, as well as reduced Ucp1 in the tissue, which was corroborated by decreased BAT-derived thermogenesis. The in vivo relevance of this miRNA was further supported by a mouse knockout model: miR-155^{-/-} mice were more resistant to cold stress than wild type littermates, presumably due to more metabolically active BAT and browning of iWAT. However, possible consequences of miR-155 overexpression/deletion on diet-induced weight gain have not been investigated so far. Further, it should be noted that miR-155 has profound functions in the hematopoietic system [59]. For instance, inactivation of the miR-155 gene in mice evoked severe defects in T-cell differentiation and B-cell differentiation [60]; thus, possible beneficial metabolic effects of miR-155 antagonism might be paralleled by detrimental effects on immune responses.

2.5. MiR-27 represses differentiation of brite and brown adipocytes

The miR-27 family was one of the first that was functionally linked to white adipogenesis [37,61,62], exhibiting a strong anti-adipogenic function due to direct targeting of the Pparγ 3'UTR [37]. A possible role in differentiation of brown precursor cells could thus be anticipated and was recently confirmed, as inhibition of miR-27a/b led to marked increases of Prdm16, Pparα, and peroxisome proliferator activated receptor, γ coactivator 1 α (*Pgc1α*) mRNAs, Ucp1 protein, as well as respiratory capacity [63]. Similar positive effects on brown markers were observed when miR-27a/b were inhibited during differentiation of stromal vascular cells from scWAT and visceral WAT. Conversely, these marker genes were repressed if miR-27a levels were elevated during differentiation via transfection of miRNA mimics. Of interest, four important transcriptional regulators of the brown gene expression program – Prdm16, Pparα, cAMP responsive element binding protein 1 (*Creb1*), and *Pgc1β* – were validated as direct targets of the miR-27 family, which revealed a direct molecular link of these miRNAs to the core protein network governing the development of thermogenic adipocytes.

2.6. MiR-106b-93 is a negative regulator of brown adipocyte differentiation

MiR-106b and miR-93 originate from a single (i.e., polycistronic) transcript and belong to the miR-17 family which comprises several other members that are partitioned between three distinct miRNA clusters. While the members of one of these clusters – miR-17-92 – were found to promote (white) adipocyte differentiation of 3T3-L1 preadipocytes [64], Wu et al. recently described a negative effect of

miR-106b and miR-93 on brown adipogenesis, as inhibition of either miRNA during differentiation of brown preadipocytes increased lipid accumulation and the levels of brown and general adipocyte markers [65]. These diverging results might be explained by the fact that the previous study overexpressed the entire miR-17~92 cluster which also comprises miRNAs not belonging to the miR-17 family (i.e. with another seed sequence). Alternatively, the different developmental origins of brown as opposed to white precursor cells might result in different repertoires of mRNAs that are targeted by members of the miR-17 family.

2.7. MiR-26 family induces and promotes human brite adipocyte differentiation

It should be noted that functional characterization of the above described miRNAs in brown and brite adipogenesis has been performed almost entirely in mouse. In contrast, a recent study has revealed the first “browning” effect of miRNAs in human adipogenesis [39]. MiR-26a and miR-26b levels were found to increase during adipocyte differentiation of human multipotent adipose-derived stem (hMADS) cells [66,67,18], and their inhibition strongly prevented lipid accumulation and expression of general adipocyte markers. In contrast, elevation of miR-26a/b by transfection of mimics modestly accelerated adipogenesis. Interestingly, global gene expression analysis of miR-26a-transfected cells revealed a significant induction of pathways related to energy dissipation. Brown marker genes – most importantly UCP1 – were indeed found to be strongly upregulated, leading to increased basal and uncoupled respiration upon treatment with miR-26. Mitochondrial morphology was likewise shifted towards a more brown phenotype. Mechanistically, the combination of transcriptomics, an RNAi screen, and reporter assays revealed that the effects of miR-26a/b are largely mediated via its direct target ADAM metalloproteinase 17 (*ADAM17/TACE*), a gene that was previously described to negatively regulate non-shivering thermogenesis [68,69]. Finally, miR-26a was found enriched in mouse BAT versus WAT, and to be induced in WAT upon cold exposure, which further supports a physiological relevance of the miR-26 family that could also be utilized for therapeutic purposes.

3. Summary and outlook

As overweight and obesity have reached pandemic dimensions, the demand for treatments which effectively promote weight loss is higher than ever. In this respect, approaches aimed to reduce energy intake (either intestinal absorption of nutrients, or central regulation of appetite) have been pursued for decades, yet have continuously failed to reduce body fat on a long-term perspective. Recently, the alternative concept of promoting energy expenditure via activation and recruitment of thermogenic adipocytes has been revitalized as several studies have demonstrated the presence of BAT in healthy adults. Indeed, the finding that BAT activity is inversely correlated with BMI in humans [7] suggests a physiological relevance for brown and brite adipocytes in the control of body weight, as has been demonstrated for mice [20]. The development of pharmacological compounds able to promote glucose and fatty acid oxidation, either by activation of pre-existing thermogenic adipocytes or their de novo recruitment, is therefore heavily pursued by both academia and the biotech industry, not least due to interesting therapeutic perspectives.

It is however evident that the molecular mechanisms governing white, brown and brite adipocyte development are still incompletely elucidated. This is especially true for human, as the vast majority of studies in adipocyte biology has been performed in mouse or cells derived thereof. Further, the more recently discovered classes of ncRNAs remain largely uncharacterized with respect to their impact on metabolism. MiRNAs are one such class, with only a few studies published to date that describe an impact on thermogenic adipocyte development. Due to the nature of miRNAs to simultaneously act on a large set of mRNA targets, they appear as powerful determinants in cell fate

decisions like stem cell self-renewal and differentiation [70–72]. Likewise, a strong involvement of particular miRNA families in complex diseases like obesity and diabetes can be anticipated. As for other diseases, it should be noted that therapeutic approaches aimed to antagonize or restore miRNA function have already made their way “from bench to bedside”: While an anti-miR-122 inhibitor compound has been shown to effectively reduce hepatitis C viral load in a phase 2a clinical trial [73], a miR-34 mimic with the perspective to treat liver cancer has recently entered phase 1 [74]. It is thus conceivable that future research further exploring the molecular actions of miRNAs in adipocytes and adipose tissue will reveal pharmacological ways to promote energy expenditure in fat cells that could eventually result in novel anti-obesity therapeutics.

Competing interests

The authors indicate no potential conflicts of interest.

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