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MicroRNAs in adipocyte formation and obesity

Marcel Scheideler, PhD and qualified as University Lecturer¹,²,³,⁴,*

¹ Institute for Diabetes and Cancer (IDC), Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

² Joint Heidelberg-IDC Translational Diabetes Program, Heidelberg University Hospital, Heidelberg, Germany

³ Molecular Metabolic Control, Medical Faculty, Technical University Munich, Germany

⁴ German Center for Diabetes Research (DZD), Neuherberg, Germany

* Phone: +49-89-3187-1047; E-mail address: marcel.scheideler@helmholtz-muenchen.de
Abstract

The worldwide epidemic of obesity demands novel and more effective therapeutic approaches. Fat cells are at the core of energy metabolism trying either to cope with a positive energy balance by hypertrophy and hyperplasia of energy storing white adipocytes or to counteract obesity by the induction of non-shivering thermogenesis in energy combusting brite/brown adipocytes. However, the comprehensive regulatory network of adipocyte formation remains to be elucidated. MicroRNAs are an emerging class of important regulatory determinants in many biological processes and diseases, including adipocyte formation and obesity. In this review, miRNAs governing the formation of white, brite and brown adipocytes as well as candidates with impact on obesity are overviewed, concluded with recommendations for further research that considers prerequisites for successful therapeutic applications.
Introduction

The worldwide prevalence of obesity has more than doubled since 1980 and is constantly rising, with recent WHO global estimates of more than 600 million adults being obese (body mass index (BMI: kg/m²) ≥ 30) and more than 1.9 billion adults being overweight (BMI ≥ 25). Overall, obesity currently applies to 13% of the world’s adult population (11% of men and 15% of women), while overweight concerns even 39% of adults (38% of men and 40% of women). Nowadays, there are more people obese than underweight, with overweight and obesity being linked to more deaths worldwide than underweight [1].

Obesity is a consequence of continuous energy uptake that exceeds energy expenditure leading to abnormal fat accumulation that may impair health. The organism tries to cope with that challenge which can be described as “allostatic adipose tissue expandability concept” [2]. Excessive energy is stored in adipocytes of the white adipose tissue (WAT) in the form of lipids leading to an increase in adipocyte volume and lipid content (hypertrophy). Adipocyte hypertrophy itself is associated with decreased insulin sensitivity, even in lean and apparently healthy subjects [3], and with increasing risk for developing type 2 diabetes [4,5]. Moreover, when maximal adipocytes’ storage capacity is reached once, then lipids start to be deposited ectopically in non-adipose organs which cause toxic effects e.g. in muscle, liver and the pancreas, also called “lipotoxicity” [6], finally leading to follow-up complications such as insulin resistance, cardiovascular diseases (mainly heart disease and stroke), musculoskeletal disorders (especially osteoarthritis), and various types of cancer (including endometrial, breast, ovarian, prostate, liver, gallbladder, kidney, and colon) [1].

The reaction to cope with this lipid spilling over and to buffer a continuous positive energy balance is the recruitment of more adipocytes (hyperplasia) which is correlated with obesity severity and is most marked in severely obese individuals [7,8]. In addition, adipocyte formation is not only an important response to obesity during adulthood, it also represents a pivotal factor in the growth of adipose tissue during childhood [9]. And, moreover, adipocyte formation is a vital process throughout life, with approximately 10% of fat cells being renewed every year at all adult ages which implies a tight regulatory control [8,10]. In this context, adipocyte formation emerges as a new therapeutic target for pharmacological intervention in obesity and other metabolic disorders.

The alternative to counteract lipotoxicity and obesity is to prevent a continuous positive energy balance by increasing energy expenditure. Increased energy expenditure can be achieved either via physical activity, mainly in the muscle [11], or by non-shivering thermogenesis in the adipose tissue [12], owing to the fact that active thermogenic brown adipose tissue (BAT) has recently been rediscovered in adult humans [13–16]. Thermogenic adipocytes differ from white adipocytes by having more mitochondria, in which also uncoupling protein 1 (UCP1) is highly enriched. UCP1 uncouples substrate oxidation from ATP synthesis so that heat is generated instead [17]. Indeed, recruitment of human thermogenic adipocytes and their cold-mediated activation have very recently been demonstrated to increase non-shivering thermogenesis, to elevate energy expenditure and finally to contribute to body fat reduction [18–20]. Moreover, thermogenic brown-like adipocytes can also be recruited in WAT, so-called brite (brown-in-white) or beige adipocytes, resulting in ‘browning’ of WAT [21–26].

These discoveries fuel the paradigm 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 [27]. Thus, a better understanding of the regulatory network in the formation of fat cells, in particular of white, brite and brown adipocytes and their impact on obesity is in demand.
In this context, microRNAs (miRNAs) have emerged as a novel class of regulatory determinants. miRNAs are small, approximately 23 nucleotides long RNAs with a crucial role in RNA interference (RNAi), a post-transcriptional gene silencing mechanism that exists in many eukaryotes [28–31]. miRNAs interact with partially complementary sites in the 3´UTR of mRNAs to diminish protein output via both mRNA destabilization and inhibition of translation [32]. MiRNAs are already well-known to play pivotal roles in numerous biological processes and diseases. Importantly, fat-selective inactivation of Dicer, an essential factor in miRNA biogenesis, resulted in mice which were almost devoid of WAT [33,34]. Moreover, adipose-specific ablation of Dgcr8 in mice, another crucial determinant in miRNA biogenesis, displayed enlarged but pale interscapular BAT, decreased expression of genes characteristic of brown fat and intolerance to cold exposure [35]. These findings suggest a pivotal role of miRNAs in the formation of white, brite and brown adipocytes. Indeed, a number of miRNAs have been discovered to govern fat cell formation, with a few candidates having an impact on obesity.

miRNAs in adipocyte formation

miRNAs in the formation of white adipocytes

miRNAs in human white adipogenesis

The identification of miRNAs with impact on mammalian adipocyte formation begun in 2004 with the discovery of miR-143 to promote human adipogenesis [36]. Surprisingly, its identified direct target ERK5 was not known before to influence adipocyte formation, thus miRNA research is also able to reveal protein-coding genes as novel regulatory players. The first human miRNA with repressive function on adipocyte formation, miR-27b, was identified in 2009 [37]. Interestingly, it could be shown that miR-27b directly targets and represses the expression of the master regulator in adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ). Subsequently, several anti-adipogenic miRNAs have been identified in human, e.g. miR-130 directly targets PPARγ [38], miR-138 governs EID-1, a nuclear receptor coregulator of the orphan nuclear receptor small heterodimer partner (SHP) which directly binds to PPARγ to increase its transcriptional activity [39], and miR-375, repressing AdipoR2, a receptor for globular and full-length adiponectin which mediates increased PPARα ligand activities [40]. Moreover, several pro-adipogenic miRNAs have been described so far. miR-30c has been described as the first miRNA-mediated regulation of an adipokine, namely PAI-1, identifying a co-repressive function for miR-30c on two identified and validated miR-30c targets in different pathways, PAI-1 and ALK2 [41]. Both miR-17 and miR-106a target BMP2, thus regulating the balance between osteogenesis and adipogenesis towards the latter [42]. The miR-26 family, consisting of miR-26a and miR-26b, targets the sheddase ADAM metalloprotease domain 17 (ADAM17/TACE) which cleaves Pref-1, an inhibitor of terminal adipocyte differentiation [43]. And last but not least miR-148a promotes adipogenesis via suppressing its direct target WNT1, an endogenous inhibitor of adipogenesis [44].

miRNAs in murine white adipogenesis

In 2008, the first murine miRNA, miR-17-92, was being elucidated to promote adipocyte differentiation [45]. Interestingly, with its validated direct target Rb2/p130, known to be involved in cell cycle regulation, miR-17-92 has an impact on the balance between proliferation and differentiation towards the latter [46,47]. Subsequently, further pro-adipogenic miRNAs have been elucidated, such as miR-204 and miR-
211 both able to repress Runx2 [48], miR-210 repressing anti-adipogenic Wnt signaling through targeting Tcf7l2 and activating the Pi3k/Akt pathway via targeting Ship1 [49,50], miR-103 activating Akt/mTor signaling by direct targeting of the anti-adipogenic Mef2d [51], and miR-125b for which a direct target that mediates the pro-adipogenic miRNA effect has not yet been identified [52].

Since 2009, also several anti-adipogenic miRNAs have been discovered. For let-7 [53] and miR-24 [54,55] no direct targets have been validated yet which mediate the anti-adipogenic effect. Interestingly, murine miR-302a [56] has also been identified to target the adipogenic master regulator Pparγ as miR-27b does in human. miR-31 directly represses Cebpα, a key transcription factor in adipogenesis [54], miR-448 directly targets Klf5, a key regulator of adipocyte differentiation [57,58], and while miR-344 stabilizes the anti-adipogenic Wnt/β-catenin signaling pathway by targeting Gsk3β [59], miR-215 impairs adipocyte differentiation via co-repressing Fndc3 and Ctnnbip1 of which Fndc3 has been known to act as positive regulator of adipogenesis [60,61].

miRNAs with cross-species conserved function in white adipogenesis

So far, very few miRNAs have been demonstrated to govern adipocyte formation across species. Pro-adipogenic miRNAs with cross-species validated function in mouse and human are miR-21 which represses TGFBR2 and consequently the anti-adipogenic TGFβ signaling [62,63], and miR-342 which targets CtBP2 acting downstream of CEBPα as a transcriptional corepressor [64]. The miR-27 family has been identified as anti-adipogenic, with miR-27b in human and miR-27a in mouse both directly targeting PPARγ, the master regulator of adipocyte differentiation [37,65,66].

Table 1
MiRNAs with impact on the formation of white adipocytes.

<table>
<thead>
<tr>
<th>Organism</th>
<th>miRNA</th>
<th>Model used for functional characterization</th>
<th>Function</th>
<th>Validated direct target(s)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>human &amp; mouse</td>
<td>miR-21</td>
<td>human mesenchymal stem cells; murine adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>TGFBR2</td>
<td>Kim et al. [62]; Kang et al. [63]</td>
<td>2009, 2013</td>
</tr>
<tr>
<td></td>
<td>miR-27</td>
<td>human mesenchymal stem cells; murine adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>PPARγ</td>
<td>Karbiener et al. [37]; Lin et al. [65]; Kim et al. [66]</td>
<td>2009, 2009, 2010</td>
</tr>
<tr>
<td></td>
<td>miR-342</td>
<td>mesenchymal stem cells; adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>CtBP2</td>
<td>Wang et al. [64]</td>
<td>2015</td>
</tr>
<tr>
<td>human</td>
<td>miR-17</td>
<td>mesenchymal stem cells</td>
<td>pro-adipogenic</td>
<td>BMP2</td>
<td>Li et al. [42]</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>miR-106a</td>
<td>mesenchymal stem cells</td>
<td>pro-adipogenic</td>
<td>ALK2 &amp; PAI-1</td>
<td>Karbiener et al. [41]</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td>miR-138</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>EID-1</td>
<td>Yang et al. [39]</td>
<td>2011</td>
</tr>
<tr>
<td></td>
<td>miR-143</td>
<td>adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>ERK5</td>
<td>Esau et al. [36]</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>miR-148a</td>
<td>mesenchymal stem cells</td>
<td>pro-adipogenic</td>
<td>WNT1</td>
<td>Shi et al. [44]</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td>miR-375</td>
<td>mesenchymal stem cells</td>
<td>anti-adipogenic</td>
<td>AdipoR2</td>
<td>Kraus et al. [40]</td>
<td>2015</td>
</tr>
<tr>
<td>mouse</td>
<td>let-7</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>-</td>
<td>Sun et al. [53]</td>
<td>2009</td>
</tr>
<tr>
<td></td>
<td>miR-17-92</td>
<td>adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>Rb2/p130</td>
<td>Wang et al. [45]</td>
<td>2008</td>
</tr>
<tr>
<td></td>
<td>miR-24</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>-</td>
<td>Sun et al. [54]; Kang et al. [55]</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td>miR-31</td>
<td>mesenchymal stem cells</td>
<td>anti-adipogenic</td>
<td>Cebpα</td>
<td>Sun et al. [54]</td>
<td>2009</td>
</tr>
<tr>
<td></td>
<td>miR-103</td>
<td>adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>Mef2d</td>
<td>Li et al. [51]</td>
<td>2015</td>
</tr>
</tbody>
</table>
miRNAs in the formation of brite/brown adipocytes

With the new paradigm 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, a better understanding of the regulatory network that allows the formation and activation of thermogenic, both brite and brown, fat cells is of high interest [27]. The adipocyte recruitment can be achieved by different means which include de novo biogenesis of brown or/and brite adipocytes as well as conversion of mature adipocytes from white to brite [67].

miRNAs involved in human brite/brown adipogenesis

So far, the first and only in depth analysis of miRNAs in human brite/brown adipogenesis revealed the miR-26 family, consisting of miR-26a and miR-26b, being able to shift adipocyte differentiation from white to brite via induction of UCP1 expression, increase in mitochondrial density, morphological changes in mitochondria towards brown adipocyte characteristics, and via an increase in energy expenditure [43]. The identified and validated target that at least partially mediates the miR-26 effects in adipocytes is ADAM17, also known as TNFα converting enzyme (TACE), which upon knockdown causes a lean, hypermetabolic phenotype in mice [68].

miRNAs involved in murine brown adipogenesis

The first described murine miRNAs in brown adipocyte formation are miR-193b-365 which were shown to be essential for brown adipogenesis by targeting Runx1t1 [69], a key adipogenic signaling molecule that blocks PPARγ transcription [70], as well as by targeting Bace1 and Gprc5b [71]. However, another study challenged these in vitro results by demonstrating that mice with an inactivated miR-193b-365 locus had normal development, differentiation and function of BAT [72]. miR-378/378* is able to increase classical BAT mass and suppress the formation of brite adipocytes in WAT [73]. This effect is mediated by direct miRNA targeting of Pde1b in BAT but not WAT, a cyclic nucleotide phosphodiesterase that catalyzes the turnover of the signaling molecules cAMP and cGMP. In contrast, knock down of miR-106b-93 cluster leads to induced expression of brown-fat-specific genes in brown adipocytes [74]. miR-328 has recently been identified to promote the shift in cell commitment from muscle to BAT [71] by targeting the β-secretase Bace1 which is known to decrease body weight, to protect against diet-induced obesity and to enhance insulin sensitivity in mice [75], and by controlling the G-protein coupled receptor 5b (Gprc5b) a known link between diet-induced obesity and type 2 diabetes [76].

miRNAs involved in murine brite adipogenesis

<table>
<thead>
<tr>
<th>miR</th>
<th>adipocyte precursors</th>
<th>pro-adipogenic</th>
<th>-</th>
<th>Ouyang et al. [52]</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-125b</td>
<td>adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>Runx2</td>
<td>Huang et al. [48]</td>
<td>2010</td>
</tr>
<tr>
<td>miR-204</td>
<td>adipocyte precursors; mesenchymal stell cells</td>
<td>pro-adipogenic</td>
<td>Ship1</td>
<td>Qin et al. [49]</td>
<td>2010</td>
</tr>
<tr>
<td>miR-210</td>
<td>adipocyte precursors</td>
<td>pro-adipogenic</td>
<td>Tcf7l2</td>
<td>Liang et al. [50]</td>
<td>2013</td>
</tr>
<tr>
<td>miR-211</td>
<td>adipocyte precursors; mesenchymal stell cells</td>
<td>pro-adipogenic</td>
<td>Runx2</td>
<td>Huang et al. [48]</td>
<td>2010</td>
</tr>
<tr>
<td>miR-215</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>Fndc3b, Ctnnbip1</td>
<td>Peng et al. [60]</td>
<td>2016</td>
</tr>
<tr>
<td>miR-302a</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>Pparγ</td>
<td>Jeong et al. [56]</td>
<td>2014</td>
</tr>
<tr>
<td>miR-344</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>Gsk3β</td>
<td>Chen et al. [59]</td>
<td>2014</td>
</tr>
<tr>
<td>miR-448</td>
<td>adipocyte precursors</td>
<td>anti-adipogenic</td>
<td>Klf5</td>
<td>Kinoshita et al. [58]</td>
<td>2010</td>
</tr>
</tbody>
</table>
The first miRNA involved in murine brite adipocyte formation is miR-196a. miR-196a induces browning of white adipocytes by directly targeting Hoxc8, a repressor of Cebpβ, a master switch of the brown fat gene program [77]. Moreover, a recent study revealed miR-182 and miR-203 as positive regulators of brite adipocyte formation [35]. In contrast, miR-150 attenuates brite adipocyte differentiation by directly targeting Prdm16 and Pgc1α, two important regulators of brite adipogenesis [78].

miRNAs involved in murine brite and brown adipogenesis

The miRNAs miR-30b and miR-30c were found to promote brown and brite adipocyte differentiation by targeting Rip140, a corepressor of genes implicated in fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation in fat [79], which upon knockout in mice generates a lean phenotype with resistance to diet-induced obesity [80]. Another miRNA that promotes brown and brite adipocyte formation in vitro and in vivo is miR-455 which also targets Runx1t1 and, in addition, Necdin, two key adipogenic repressors [81]. Conversely, miRNAs which are repressors of brite and brown adipocyte differentiation are the muscle-enriched miR-133 directly repressing Prdm16, a key regulator of the browning [82], miR-155 via targeting Cebpβ in a bistable loop [83], miR-27 by controlling several transcriptional regulators such as Prdm16, Paprα, Pgc1β, and Creb1 [84], and miR-34 directly targeting Fgf21 signaling through repression of Fgfr1 [85].

miRNAs with cross-species conserved function in brite adipogenesis

So far, only two miRNAs have been identified very recently to be involved in the recruitment of brite adipocytes in mouse and human. First, let-7i is able to repress the conversion of adipocytes from white to brite, [86], and second, miR-125b impairs brite adipocyte conversion via targeting mitochondrial biogenesis [87]. However for both miRNAs there are no direct targets known so far that could function as mediators of the miRNA effect.

Table 2

miRNAs with impact on the formation of brite/brown adipocytes.

<table>
<thead>
<tr>
<th>Organism</th>
<th>miRNA</th>
<th>Model used for functional characterization</th>
<th>Function</th>
<th>Validated direct target(s)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>human &amp; mouse</td>
<td>let-7i</td>
<td>mature adipocytes; wildtype mice injected with miRNA mimics</td>
<td>prevents conversion of white to brite adipocyte formation and brite adipocyte function</td>
<td>-</td>
<td>Giroud et al. [86]</td>
<td>2016</td>
</tr>
<tr>
<td>mouse</td>
<td>miR-125b</td>
<td>mature adipocytes; wildtype mice injected with miRNA mimics or inhibitors</td>
<td>responsive to beta-adrenergic stimulation, mimic injection inhibits conversion of white to brite adipocytes, while inhibition promotes brite adipocyte formation</td>
<td>-</td>
<td>Giroud et al. [87]</td>
<td>2016</td>
</tr>
<tr>
<td>human</td>
<td>miR-26a/b</td>
<td>mesenchymal stem cells; adipocyte precursors</td>
<td>promotes brite adipocyte formation and function by shifting adipogenesis from white to brite</td>
<td>ADAM17</td>
<td>Karbiener et al. [43]</td>
<td>2014</td>
</tr>
<tr>
<td>mouse</td>
<td>miR-27</td>
<td>adipocyte precursors</td>
<td>directly represses components of the brown transcriptional network and decreases brown differentiation</td>
<td>Prdm16, Pparα, Pgc1β, Creb1</td>
<td>Sun &amp; Trajkovski [84]</td>
<td>2014</td>
</tr>
<tr>
<td>mouse</td>
<td>miR-30b/c</td>
<td>adipocyte precursors</td>
<td>forced expression increases brite and brown adipocyte differentiation, while miR-30b/c inhibition impairs brite/brown characteristics</td>
<td>Rip140</td>
<td>Hu et al. [79]</td>
<td>2015</td>
</tr>
<tr>
<td>miRNA</td>
<td>Precursors/Origin</td>
<td>Effect</td>
<td>Marker/Condition</td>
<td>Authors/Year</td>
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<tr>
<td>miR-34</td>
<td>adipocyte precursors; lentiviral-mediated miR-34a repression in wildtype mice upon HFD</td>
<td>Inhibition promotes brite and brown marker expression and decreases adiposity</td>
<td>Fgf1</td>
<td>Fu et al. [85] 2014</td>
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<tr>
<td>miR-106b-93</td>
<td>adipocyte precursors; wildtype mice upon HFD</td>
<td>Impairs brown adipocyte differentiation, while inhibition promotes brown adipogenesis</td>
<td>-</td>
<td>Wu et al. [74] 2013</td>
<td></td>
<td></td>
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<tr>
<td>miR-133</td>
<td>adipocyte precursors</td>
<td>Prevents differentiation to brown adipocytes in both BAT and SAT precursors</td>
<td>Prdm16</td>
<td>Trajkovski et al. [82] 2012</td>
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<tr>
<td>miR-150</td>
<td>Ksrp +/- mice; adipocyte precursors</td>
<td>Forced expression attenuates the elevated expression of brown fat genes caused by KSRP deletion</td>
<td>Prdm16, Pgc1α</td>
<td>Chou et al. [78] 2014</td>
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<tr>
<td>miR-155</td>
<td>adipocyte precursors; mice overexpressing miR-155 globally under the control of the PGK promoter; mice overexpressing miR-155 BAT-specific under the control of the UCP1 promoter</td>
<td>Inhibition enhances brown and brite adipocyte differentiation, while forced expression reduces brown adipocyte recruitment and function</td>
<td>Cebpβ</td>
<td>Chen et al. [83] 2013</td>
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</tr>
<tr>
<td>miR-182</td>
<td>adipocyte precursors</td>
<td>Inhibition causes a reduction of brown fat but not common adipogenic markers</td>
<td>-</td>
<td>Kim et al. [35] 2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-193b-365</td>
<td>murine adipocyte precursors; murine myoblasts</td>
<td>Inhibition in vitro impairs brown adipocyte adipogenesis, while forced expression blocks myogenesis in myoblasts and induces myoblasts to differentiate into brown adipocytes; however miR-193 and miR-365 inactivation in vivo does not affect brown fat in mice</td>
<td>Runx1t1, Bace1, Gpcre5b</td>
<td>Sun et al. [69], Oliverio et al. [71], Feuermann et al. [72] 2011, 2013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-196a</td>
<td>adipocyte precursors; mice overexpressing miR-196a fat-enriched under the control of the aP2 promoter</td>
<td>Inhibition prevents the induction of thermogenic markers during white adipocyte differentiation, while forced expression induces the recruitment of brite adipocytes and prevents diet-induced obesity</td>
<td>Hoxc8</td>
<td>Mori et al. [77] 2012</td>
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<tr>
<td>miR-203</td>
<td>adipocyte precursors</td>
<td>Inhibition causes a reduction of brown fat but not common adipogenic markers</td>
<td>-</td>
<td>Kim et al. [35] 2014</td>
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<tr>
<td>miR-328</td>
<td>adipocyte precursors</td>
<td>Inhibition blocks preadipocyte commitment, while overexpression triggered BAT differentiation and impaired muscle progenitor commitment</td>
<td>Bace1, Gpcre5b</td>
<td>Oliverio et al. [71] 2016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-378/378*</td>
<td>mice overexpressing miR-378/378* fat-enriched under the control of the aP2 promoter; adipocyte precursors; mesenchymal stem cells</td>
<td>Forced expression increases classical BAT mass, suppresses formation of brite adipocytes in subcutaneous WAT, and prevents diet-induced obesity</td>
<td>Pde1b in BAT</td>
<td>Pan et al. [73] 2014</td>
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<td></td>
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<tr>
<td>miR-455</td>
<td>adipocyte precursors; mesenchymal stem cells</td>
<td>Forced expression induces the recruitment of brown adipocytes incl. mitochondrial biogenesis and prevents diet-induced obesity</td>
<td>Runx1t1, Necdin</td>
<td>Zhang et al. [81] 2015</td>
<td></td>
<td></td>
</tr>
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</table>

**miRNAs with impact on obesity**

Despite numerous miRNAs which have been identified in the recruitment of thermogenic brite and brown adipocytes, only a subset of miRNAs has been proven so far to prevent or ameliorate obesity in mice. This set of miRNAs includes first miR-196a which enhances energy expenditure in transgenic mice overexpressing miR-196a predominantly in the adipose tissue and results in resistance to obesity indicating that the induced brite adipocytes are metabolically functional [77]. Second, miR-26a ameliorates high fat diet (HFD)-induced obesity in mice upon global overexpression, but not in mice.
overexpressing miR-26a specifically in the liver [88]. This indicates that the miR-26a effect improving obesity resistance is dependent on its function in another organ than the liver. Conversely, there seems to be no adipocyte-specific genetic miRNA knockout yet that affects obesity. Global miR-155 deletion improves resistance to HFD-induced weight gain in female but not male mice by inducing the browning program in white adipocytes and abrogating HFD-induced hypertrophy of white adipocytes [89]. However, if these effects of miR-155 knockout derives from the adipose tissue still needs to be elucidated, as miR-155 is an immunomodulatory miRNA that might effect obesity from outside the adipocyte [90]. miR-378 has been shown to ameliorate obesity in mice globally overexpressing miR-378 [91]. Mechanistically this phenotype was determined by impairing glucose metabolism which was caused by an activated pyruvate-PEP futile cycle in skeletal muscle and enhanced lipolysis in adipose tissues which was mediated by Stearoyl-CoA desaturase 1 (Scd1) known to protect against obesity [92]. For miR-34a, controversial studies have been published. While lentiviral miR-34a repression in mice reduced diet-induced obesity by targeting the Fgfr21 receptor components Fgfr1 and βKl [85], a recent study with global miR-34 knockout mice showed susceptibility to diet-induced obesity [93].

Table 3
miRNAs with impact on obesity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>miRNA</th>
<th>Model used for functional characterization</th>
<th>Function</th>
<th>Validated direct target(s)</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>mouse</td>
<td>miR-26a</td>
<td>mice overexpressing miR-26a globally under the control of the Hprt promoter</td>
<td>Tg mice had less weight gain upon HFD compared to wildtype mice</td>
<td>Acs1, Acs4, Gsk3β, Pck1, Pkcδ, Pkcθ, Tcf7l2</td>
<td>Fu et al. [88]</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td>miR-34a</td>
<td>global miR-34 KO mice</td>
<td>Lentiviral-mediated miR-34a repression in wildtype mice reduces diet-induced obesity; However KO mice fed a HFD are susceptible to diet-induced obesity</td>
<td>Fgfr1, βKl</td>
<td>Fu et al. [85]</td>
<td>2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lavery et al. [93]</td>
<td>2016</td>
</tr>
<tr>
<td></td>
<td>miR-155</td>
<td>global miR-155 KO mice</td>
<td>KO in female mice prevents HFD-induced obesity via abrogated adipocyte hypertrophy and WAT inflammation</td>
<td>-</td>
<td>Gaudet et al. [89]</td>
<td>2016</td>
</tr>
<tr>
<td></td>
<td>miR-196a</td>
<td>mice overexpressing miR-196a fat-enriched under the control of the ap2 promoter</td>
<td>forced expression induces the recruitment of brite adipocytes and prevents diet-induced obesity</td>
<td>Hoxc8</td>
<td>Mori et al. [77]</td>
<td>2012</td>
</tr>
<tr>
<td></td>
<td>miR-378</td>
<td>mice overexpressing miR-378 globally under the control of the β-actin promoter</td>
<td>Tg mice display reduced fat mass and increased energy expenditure, while systemic administration of miR-378 mimics prevents and ameliorates obesity</td>
<td>Scd1</td>
<td>Zhang et al. [91]</td>
<td>2016</td>
</tr>
</tbody>
</table>

Therapeutic approaches for miRNA-based targeting of obesity

In other diseases, therapeutic approaches that aim at antagonizing or restoring miRNA function are already on their way from bench to bedside [94–96]. To treat obesity, there are no known miRNA therapeutics designed to reduce fat mass in obesity so far. This might be caused by the fact that miRNA-based therapeutic approaches require the identification on miRNAs which exert resistance to obesity, ideally combined with a conserved function across species, minimal long-term side effects, and validated direct targets and mediators. And it is worth to note that also the latter is not a trivial task [97]. So far the miR-26 family gets closest to these prerequisites, because its miRNA candidates demonstrate cross-species beneficial metabolic effects in human adipocytes [43,98] and in obesity in mouse [88], with no physiological and pathological side effects at least in liver-specific transgenic mice up to two years of age [88], and with several direct targets validated with impact on metabolism [43,88,98]. Thus future research is in demand to further explore these and further miRNAs and their molecular actions on energy.
expenditure and metabolic benefits in obesity and its sequelae that hopefully results in novel and more effective anti-obesity therapeutics.

Summary (250 words)

The worldwide epidemic of obesity is inexorably progressing and thus demands the development of novel and more effective therapeutic approaches. Adipocytes are the core unit in energy metabolism trying either to cope with a positive energy balance by hypertrophy and hyperplasia of white adipocytes or to counteract obesity by an increase in energy expenditure via brite/brown adipocytes. However, the comprehensive regulatory network of adipocyte formation remains to be elucidated. In this context, miRNAs are an emerging class of important regulatory determinants in biological processes and diseases. Indeed, nowadays there are several miRNAs known to govern white, brite or brown adipocyte formation. However, only a few of those that are involved in brite/brown adipocyte recruitment have been shown so far to prevent or ameliorate also diet-induced obesity. This collection of candidates shrinks even more when criteria for therapeutic applications are applied, such as cross-species conserved function, minimal long-term side effects, and validated direct targets and mediators. However, this does not mean that miRNAs are not appropriate drugs and drug targets to fight obesity. On the very contrary, this is an invitation to enforce the research efforts to elucidate therapeutically promising anti-obesity miRNA candidates, as already successfully applied for other diseases.

Practice points

• Common-sense approaches aimed at the prevention and treatment of overweight and obesity have failed thus novel anti-obesity mechanisms and approaches are in demand.
• Adipocyte formation is pivotal in the growth of adipose tissue during childhood and takes place throughout life, as approximately 10% of fat cells are renewed annually at all adult ages.
• An increase in white adipocyte number (hyperplasia) is a response to obesity with continuous and excessive fat accumulation and correlates with obesity severity.
• The recent rediscovery of brown adipose tissue (BAT) which can dissipate excess energy via non-shivering thermogenesis in adult humans fuels the paradigm that recruitment and activation of thermogenic adipocytes contribute to anti-obesity strategies.
• Thermogenic adipocytes occur as brown adipocytes in BAT and as brite or beige adipocytes in white adipose tissue (WAT).
• miRNAs are an emerging class of potent regulatory determinants in many biological processes and diseases, including adipocyte formation and obesity, which have potential to serve as drug targets and drugs in therapeutic anti-obesity applications.

Research agenda

• A better understanding of the regulatory network in the formation of fat cells, in particular of white, brite and brown adipocytes, and their impact on obesity is in demand.
• More miRNAs with cross-species conserved function in adipocyte formation, function and obesity are needed for further translational research and clinical applications.
For better understanding miRNA effects, the identification and validation of direct miRNA targets and mediators is an essential asset to specify and evaluate the miRNA’s beneficial as well as adverse effects in future pharmacological and therapeutic applications.

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References


