MicroRNAs in dysfunctional adipose tissue: cardiovascular implications

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Abstract

In this review, we focus on the emerging role of microRNAs, non-coding RNAs that regulate gene expression and signaling pathways, in dysfunctional adipose tissue. We highlight current paradigms of microRNAs involved in adipose differentiation and function in depots such as white, brown, and beige adipose tissues and potential implications of microRNA dysregulation in human disease such as obesity, inflammation, microvasculature dysfunction, and related cardiovascular diseases. We highlight accumulating studies indicating that adipocyte-derived microRNAs may not only serve as biomarkers of cardiometabolic disease, but also may directly regulate gene expression of other tissues. Finally, we discuss the future prospects, challenges, and emerging strategies for microRNA delivery and targeting for therapeutic applications in cardiovascular disease states associated with adipocyte dysfunction.

Keywords

MicroRNA • Adipocytes • Brown adipose tissue • White adipose tissue

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1. Introduction

Type 2 diabetes (T2D) is a worldwide problem that is observed both in developed and developing countries. Hallmark events in T2D are elevated blood glucose that stems from impaired responsiveness to insulin and insufficient insulin production.1 Insulin is a hormone that is produced by the pancreas, plays a fundamental and evolutionary conserved role in maintaining energy homeostasis.2,3 In response to food intake, there is a rapid increase in circulating insulin levels where it exerts its effect on three main target tissues: fat, liver, and skeletal muscle.4 Under homeostatic conditions, cellular signaling by insulin is initiated by binding to insulin receptors that help modulate pathways controlling lipid uptake, lipolysis, and lipogenesis.5,6

Insulin resistance, a major risk factor for both type 2 diabetes (T2D) and ischaemic cardiovascular disease, is defined as decreased responsiveness to insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscle, reduced expression of insulin signaling components in skeletal muscle as well as impaired suppression of hepatic glucose output.7–13 Insulin resistance is a key component in the development of T2D and obesity, which in turn significantly contributes to the development of cardiovascular disease and diabetic-associated macrovascular complications such coronary artery disease (CAD), peripheral vascular disease (PVD), and stroke, and microvascular complications such as retinopathy, neuropathy, and nephropathy.14 Diabetes can also contribute to cardiomyopathy resulting in diabetes-associated changes in the structure and function of the myocardium.

Adipose tissue plays a central role in health and disease. Under periods of excess energy intake, its primary function is to store energy in the form of triacylglycerides (TGs) and release energy during starvation or fasting.15 In addition to being an energy reservoir, adipose tissue is also an endocrine organ relaying hormonal signals that regulate the hypothalamic-pituitary-gonadal axis.16 Distinct roles have emerged for white, brown, and beige (brite) adipose tissue depots in regulating human energy expenditure and metabolism. It is a thermogenic tissue that is important, especially in small mammals, to pose dysfunction and obesity.17 For example, there is a complex relationship between increased energy intake, increased insulin levels followed by activation of biochemical pathways by insulin that regulate expansion of white adipose tissue (WAT) through adipocyte hypertrophy, or adipogenesis.18,19 Deletion of insulin receptors from WAT protects mice from obesity pointing out the key role insulin plays in adipocyte differentiation or adipocyte hypertrophy. However, the mechanisms controlling these pathways are not fully understood.20,21

Brown adipose tissue (BAT) has garnered significant attention in recent years in regulating human energy expenditure and metabolism. It is a thermogenic tissue that is important, especially in small mammals, to regulate their body temperature and keep the core body temperature constant under cold ambient temperatures. This is due to its high content of mitochondria, a property that is unique from WAT.22 MiRNAs, evolutionarily conserved small non-coding RNAs of ~22 nucleotides in length, are involved in a range of developmental and...
pathophysiological processes in metazoans and collectively target over one-half of protein-coding transcripts. MicroRNAs are important mediators in adipose function, T2D, obesity, and cardiovascular pathologies. Accumulating studies provide novel molecular and cellular insights into their impact on pathophysiological pathways in adipocyte differentiation, function, and insulin resistance and obesity.

MicroRNAs have garnered considerable attention not only for their ability to regulate adipogenesis and adipocyte function, but also for their extracellular presence, such as in circulating blood or urine, raising their potential use as biomarkers for diagnosis or prognosis. In this review, we highlight emerging roles for miRNAs and their target genes in regulating adipocyte function both in a cell intrinsic and extrinsic manner with a special emphasis on links to cardiovascular risk and disease states. Finally, we discuss opportunities and challenges of relevant miRNA delivery strategies that may impact adipocyte function, T2D, obesity, and cardiovascular disease.

2. MiRNA biogenesis and function

MiRNAs are transcribed by RNA polymerase II in the nucleus to form primary-miRNAs (pri-miRNAs) with a stem-loop structure that harbors the mature miRNA sequences. Next, the nuclear ribonuclease (RNase)-III enzyme, Drosha, cleaves pri-miRNA into precursor-miRNAs (pre-miRNAs), hairpin-shaped RNAs of ~70 base pairs (bp) harboring an imperfect stem-loop structure. Drosha then forms a protein complex with the essential cofactor DGCR8 (also known as Pasha; a protein containing two double-stranded (ds)RNA-binding domains) which directs the cleavage of the stem-loop of the hairpin RNA structure of pri-miRNA. This complex, also known as microprocessor, plays an important role in the post-transcriptional cross-regulation between Drosha and DGCR8. Pre-miRNAs are then exported by a Ran-guanosine triphosphate (GTP)-dependent nucleo/cytoplasmic transporter named exportin 5 (Exp5), which mediates the export of the mature miRNA sequences.

After cleavage by Dicer, the resulting 21-24 nt miRNA duplex unwinds to release one of the strands for entering the RNA-induced silencing complex (RISC) and binding to Argonaute which facilitates its function. Two strands are capable of being loaded onto Argonaute and repressing target mRNAs. Typically, the more thermodynamically stable strand of the two becomes the mature miRNA strand that is loaded into the RISC, whereas the other strand of this short-lived duplex disappears. Subsequently, the miRNA directs the RISC to the 3’ UTR of the target mRNA to negatively regulate mRNA expression by promoting mRNA cleavage and/or translational repression. In animals, the 7 nt ‘seed region’, mapping to positions 2 - 8 at the 5’ end, of miRNA serves an essential role for target recognition. Indeed, the seed region is highly conserved across species. Upon a perfectly matched complementary sequence between the miRNA and its target, the target mRNA is cleaved. However, miRNA partial sequence complementarity may also exert target gene translational repression.

3. MicroRNAs in adipose tissue

Adipose tissue is known to have two major roles in the body: a storage depot for triglyceride for times of caloric restriction and an endocrine organ that regulates whole body homeostasis. However, excess adipose tissue, especially in the abdomen area increases the risk for a number of conditions including type 2 diabetes, hypertriglyceridemia, low grade chronic inflammation, hypertension, and coronary artery disease (CAD). While adipocytes account for the majority of fat pad volume, the non-adipocyte cell subsets, such as endothelial cells, fibroblasts, immune cells, among others, predominate by overall cell number.

Accumulating studies indicate that miRNAs play an integral role in adipose tissue formation and function. Initial findings demonstrated that inhibition of Drosha and Dicer in human mesenchymal stem cells inhibited differentiation into adipocytes, and inhibition of Drosha in 3T3-L1 cells inhibited adipogenesis. Interestingly, adipocyte-specific deletion of Dicer in mice resulted in severe depletion of white adipose tissue with reduced adipoogenic-associated transcripts. Although Dicer deletion was not required for brown fat lipogenesis, the expression of genes involved in thermoregulation were reduced. Adipose-specific ablation of Dicer in mice resulted in enlarged but pale interscapular brown adipose tissue (BAT), decreased expression of signature brown fat genes, and cold intolerance. Collectively, these studies of regulators of miRNA biogenesis suggest an important role for miRNAs in adipose tissue homeostasis.

3.1 White adipose tissue (WAT)

There are a growing number of miRNAs that are expressed and regulated in WAT in response to obesity and T2D (Figure 1). WAT is distributed throughout the body and is a vascularized organ. Adipocytes are the main constituents of adipose tissue by volume and play an important role in insulin resistance and T2D. Important contributors to WAT dysfunction include adipocytes and their cross-talk between the microvasculature in adipose tissue (also known as perivascular adipose tissue (PVAT)) and macrophages that release cytokines such as TNF-α, an effect promoting low grade chronic inflammation. Indeed, inflammation in WAT can be quantified histologically by staining for the number of macrophages surrounding adipocytes in what has been termed as ‘crown-like structures’.

Since there is complex relationship between the many different cell types involved in the adipose tissue and their involvement in adipogenesis and obesity, a recent study focused on identifying a miRNA signature profile following 6 weeks of endurance training in obese, human male subjects. Interestingly enough, there were no significant changes in the miRNA expression profiles in adipocytes isolated pre- or post-training from abdominal subcutaneous (ABD) and gluteofemoral (GF) adipose tissue, raising the possibility that endurance exercise does not dynamically change adipose tissue miRNA expression compared to other pathophysiological stimuli.

The association between adipose tissue and the cardiovascular disease (CVD) is a complex one. Obesity-related hyperlipidemia is well-studied where obesity-induced elevation of triglycerides and cholesterol leads to atherosclerosis. However, there is also a strong correlation between obesity, inflammation, and CVD. Although adipose tissue is not considered an immune organ, it is an endocrine organ and many pro-inflammatory genes such as TNF-α, IL-6, IL-1β, PAI-1, angiotensinogen, C-reactive protein or adipokines are highly expressed in dysfunctional adipose tissue, resulting in both local and systemic inflammation that is characterized by infiltration of macrophages and T cells to the vessel wall and thereby increasing the risk for CVD such as atherosclerosis. Another important factor predisposing patients to CVD is the location of the adipose tissue. Obese human subjects with excess visceral adipose tissue (VAT), or abdominal obesity, are at higher risk for T2D and CVD.
3.1.1 Adipocyte

The role of miRNAs in adipogenesis is well established and have been shown to act as either pro- and anti-adipogenesis regulators. Examples of pro-adipogenic microRNAs include miR-143, miR-103, miR-146b, miR-148, and miR-33b. Overexpression of miR-143 or miR-103 in preadipocytes accelerated adipogenesis. This process was associated with increased expression of transcription factors such as PPARγ2, cell cycle regulators such as G0/G1 switch 2 (G0S2), FABP4, GLUT4, and adiponectin. Interestingly, several studies demonstrate an inverse correlation between miRNAs that are implicated in adipogenesis and obesity. Consistent with this notion, in contrast to their pro-adipogenic role, expression of miR-143 and miR-103 were significantly decreased in adipocytes of obese mice. Mir-146b expression is increased in HFD-induced obese mice and in ob/ob and db/db mice, whereas the expression of its target gene SIRT1 was decreased in WAT. Mir-146b induced adipogenesis by inhibiting SIRT1-dependent acetylation of the transcription factor FOXO1. Indeed, in vivo neutralization of miR-146a expression increased SIRT1 expression and ameliorated diet-induced obesity. Expression of another miRNA, miR-148, increased in both obese human subjects and mice fed a HFD. Furthermore, miR-148 targeted an inhibitor of adipogenesis, Wnt1, thereby acting as a pro-adipogenic miRNA regulating the differentiation of human adipose-derived mesenchymal stem cells (hMSCs-Ad). Interestingly, the pro-moter region of miR-148a contains a functional cAMP-response element-binding protein (CREB), which was required for miR-148a expression in hMSCs-Ad. Finally, the intronic miRNA miR-33b and its host gene SREBP-1 are highly induced upon preadipocyte differentiation. Inhibition of miR-33b suppressed preadipocyte differentiation and lipid droplet accumulation, whereas its overexpression promoted differentiation. These effects were mediated in part by targeting of HMGA2 and cyclin-dependent kinase 6 (CDK6), an important regulator of cell cycle progression, specifically the G1-S transition. These findings have translational relevance as mutations in miR-33 binding sites of the 3'-UTR of HMGA2 cause liposarcomes in humans. Taken together, these

Figure 1  Example of microRNAs involved in the differentiation of white, beige, and brown adipocytes. These microRNAs are involved in regulating adipocyte differentiation through effects on their targets that control expression of metabolic genes, inflammation, or insulin resistance. Activation of brown and/or beige adipocytes may contribute to favorable effects on energy expenditure, insulin resistance, and potentially cardiovascular remodeling. Solid lines with arrows followed by (+) indicate positive regulation of the indicated adipocyte differentiation pathway. Dashed lines followed by (+) or (++) indicate that the adipocyte differentiation pathway may be favorably implicated in regulating cardiovascular disease.
examples highlight that miRNAs may exhibit pro-adipogenic activity by regulating divergent transcriptional and epigenetic targets known to be involved in adipogenesis.

In contrast to the above miRNAs, miR-34a, miR-125-5p and miR-200b/a/429 cluster are examples of miRNAs that inhibit adipogenesis. MiR-34a is an excellent example of an anti-adipogenic miRNA in which its expression is increased in serum of patients with T2D.93 There is a positive correlation with increased miR-34a expression in human subcutaneous WAT (scWAT) and increased miR-34a expression during adipocyte differentiation in vitro.94 Compared to controls, miR-34a KO mice gained more weight at baseline and in response to HFD. In addition, miR-34a KO epididymal white adipose tissue (eWAT) had a smaller adipocyte area that increased with HFD accompanied with increased expression of metabolic genes such as CD36, HMGCR, LXRα, PGC1α, and FASN. The eWAT from miR-34a KO mice also showed increased inflammation reflected by higher accumulation of F4/80 positive macrophages.95 The expression of another miRNA, miR-125b-5p, increased during human adipocyte differentiation. Overexpression of miR125b-5p reduced adipogenesis; however, the target gene(s) involved in this process remain unclear.96 Adipocyte-specific knockout of the miR-200b/a/429 cluster in mice resulted in HFD-induced weight gain, decreased glucose tolerance and insulin sensitivity, and impaired lipolysis compared to the WT control, effects mediated in part through targeting the EP58 and GLIS2 genes.97

The role of miRNA, miR-155 in adipogenesis is somewhat unclear. In human obese subjects and in 3T3-L1 cells, increased miR-155 expression correlated to body mass index (BMI) and TNF-α expression, respectively. Moreover in vitro studies performed in 3T3-L1 cells linked induced expression of miR-155 to activation of the NF-κB signaling pathway. The anti-adipogenic and anti-lipogenic function ascribed to miR-155 may be mediated through direct targeting of PPAR-γ.98 However, in another study, miR-155 was found to function as a pro-adipogenic miRNA. Female miR-155 knock out (KO) mice were protected from high fat diet (HFD)-induced weight gain, obesity, and insulin resistance through decreased WAT accumulation, adipose size, inflammation, and increased energy expenditure.99 While it is unclear how to reconcile the systemic KO studies in mice with the in vitro studies performed using human adipocytes, it is likely that miR-155 may regulate non-adipocyte cell types that may indirectly influence adipocyte function, or miR-155 may have a species or gender-specific role.99 MiR-155 also plays an important role in the regulation of lipid metabolism in liver. Interestingly, deficiency of miR-155 may lead to hepatic steatosis in mice through targeting Nr1h3 (LXRα).100 Collectively, miRNAs can either positively or negatively regulate adipogenesis and thereby having an impact on glucose tolerance, insulin sensitivity, or obesity.

3.1.2 The microvasculature

There is a functional and anatomical relationship between adipose tissue and the microvasculature. The crosstalk between the two organs is essential in controlling vascular homeostasis. However, the mechanism regulating this relationship is poorly understood. Pervascular adipose tissue (PVAT) generates vasorelaxants and vasoconstriction factors under physiological conditions or inflammation, respectively, and thereby regulates vessel contractility. Under inflammatory conditions, PVAT can also augment vascular dysfunction by secreting pro-inflammatory adipokines such as adipocyte fatty acid binding protein or TNF-α, hormones, and reactive oxygen species (ROS), which are all known to contribute to endothelial activation in both the macro- and microvasculature leading to vascular wall inflammation and dysfunction.101 A study by our group identified that in response to HFD-induced insulin resistance, the anti-inflammatory microRNA, miR-181b,102,103 is significantly reduced in adipose tissue endothelial cells (ECs), but not adipocytes, from eWAT after 1 week. In the light of this finding, we delivered via tail-vein injection liposomally encapsulated miR-181b to HFD-induced insulin resistant mice. Compared to controls, these miR-181b injected mice exhibited markedly improved glucose homeostasis and insulin sensitivity.104 From a mechanistic perspective, miR-181b overexpression in adipose ECs enhanced insulin-mediated Akt phosphorylation at Ser473, and reduced endothelial dysfunction, an effect that shifted macrophage polarization toward an M2 anti-inflammatory phenotype in eWAT. Importantly, these effects were associated with induction of endothelial nitric oxide synthase (eNOS), nitric oxide activity, and FoxO1 phosphorylation specifically in eWAT, but not in liver or skeletal muscle. In contrast, overexpression of miR-181b in peripheral blood mononuclear cells (PBMCs) had no effect on macrophage activation, proliferation, or recruitment to visceral fat. Similarly, adipocytes overexpressing miR-181b had no intrinsic effect on glucose uptake. Using complementary bioinformatics, gene profiling studies, and siRNA-mediated knockdown approaches, pleckstrin homology domain leucine-rich repeat protein (PHLPP2), a phosophatase known to dephosphorylate Akt at Ser473, was revealed as a bona-fide target of miR-181b and PHLPP2 inhibition recapitulated the effects of miR-181b. Interestingly, PHLPP2 is highly expressed in eWAT, whereas it is barely detectable in liver and skeletal muscle, which may help explain the miR-181b tissue-specific effect in eWAT. Taken together, these findings solidified the role of 181b in the maintenance of homeostasis in the microvasculature of visceral fat to control EC inflammation and insulin resistance (Figure 2).

A unique fat depot that has recently garnered attention is epicardial adipose tissue (EAT) that surrounds the heart and epicardial vessels; however, its role is not fully understood. Under physiological conditions, EAT is thought to be cardioprotective and is composed of a mixed lineage of fat cells that is close to a beige phenotype.105 However, under pathological conditions, its thickness is associated with insulin resistance,105 and is a risk factor for CAD, atrial fibrillation, and heart disease, suggesting it may play a role in inflammation.106-108 Through a whole genome microarray analysis of patients with CAD, miR-103-3p was shown to be overexpressed in EAT. Furthermore, miR-103-3p targeted the 3'UTR of the pro-inflammatory chemokine CCL13 suggesting that it may regulate EAT inflammation and may be important as a biomarker for CAD.109 Finally, microarray profiling of EAT from hyperglycemic pigs revealed increased expression levels of miR-193a-3p and miR-675-5p, whereas the expression of miR-144-3p was reduced.110 The functional roles for these miRNAs in EAT will require further study.

3.1.3 Macrophage

Studies to date indicate that monocytes are recruited to peripheral tissues such as pancreas, liver, and adipose tissue to become resident macrophages where they may contribute to local inflammation and development of insulin resistance.111 During the pathogenesis of insulin resistance, macrophage populations can switch from an anti-inflammatory (M2) population to an inflammatory (M1) population, a process termed macrophage polarization.112 This event results in the release of proinflammatory cytokines and chemotactic factors that exacerbate the local inflammatory environment, thereby increasing the risk for atherosclerosis and CVD.113

There are a number of miRNAs that regulate monocyte/macrophage activation, differentiation, or recruitment. For example, oxidized low
density lipoprotein (oxLDL) increased the expression of miR-155 and promoted macrophage-derived foam cell formation. Interestingly, inhibition of miR-155 decreased lipid-loading in macrophages and reduced atherosclerotic plaques in ApoE-/- mice.114 However, when ApoE-/-/miR-155-/- mice were placed on a HFD, they surprisingly developed obesity, adipocyte hypertrophy, and fatty liver but did not develop glucose intolerance or insulin resistance.115 Overexpression of a different miRNA, miR-144-3p, increased the secretion of inflammatory cytokines such as IL-6, IL-1β, and TNF-α to promote THP-1 macrophage differentiation into foam cells through targeting the ATP-binding cassette transporter A1 (ABCA1). Conversely, inhibition of miR-144-3p in ApoE-/- mice increased macrophage accumulation in atherosclerotic lesions suggesting that miR-144-3p accelerated the progression of atherosclerosis.114 Furthermore, miR-144 was shown to regulate cholesterol metabolism via suppressing ABCA1 expression.116 Moreover, in response to activation of the bile acid receptor farnesoid X receptor (FXR), miR-144 was found to regulate ABCA1 and plasma HDL-cholesterol.117 A role of this miRNA in diet-induced obesity and insulin resistance will require further investigation. Activation of Liver X receptors (LXRs) in macrophages promotes cholesterol efflux118,119 and protects against the development of insulin resistance and atherosclerosis.120 Overexpression of miR-206 in Tamm Horsfall Protein 1 (THP-1) cells increased LXRα expression and enhanced cholesterol efflux. Furthermore, LXRα activation reduced miR-206 expression, indicating the presence of a feedback loop between miR-206 and LXRα in regulating cholesterol efflux, insulin resistance, vascular inflammation, and atherosclerosis.121

3.2 Brown adipose tissue (BAT)

Brown adipose tissue (BAT) is one of the major fat depots. It has a unique function of converting stored chemical energy into heat. The uncoupling protein-1 (UCP-1), a BAT-specific protein located within the mitochondria, plays an important role in this process. UCP-1 ‘uncouples’ fuel oxidation from ATP synthesis to generate heat without muscle energy.17 Brown adipocytes are located in the interscapular and perirenal regions of rodents and are highly innervated, vascularized and made up of cells with multilocular lipid droplets.22,122 Although initially BAT was thought only to be active in rodents and human infants, studies employing PET/CT scans where healthy subjects were exposed to ambient room temperature or 18 °C confirmed the presence of metabolically active BAT in adult humans.123 Its activity is significantly decreased in obesity in humans by mechanisms that are not fully understood yet.
Interestingly, although miRNAs are known to regulate brown adipocyte differentiation such as miR-133,124 miR-378,125 miR-155,126 miR-455,127 miR-26,128 and miR-34,129 activation of BAT can also regulate miRNA expression profiles.124 BAT activation may also play an important role in reducing CVD events through its ability to increase energy expenditure and regulate glucose and lipid metabolism.130 However, in APOE and LDL KO mice, cold activation of BAT increased lipolysis that resulted in an increase of LDL cholesterol levels thereby potentially increasing their risk of cardiovascular events in severe hypercholesterolemic states.131

3.2.1 MiRNA regulation of brown fat adipocyte differentiation
Because of BAT’s unique ability to increase energy expenditure rather than store it in the form of WAT, a major interest in recent years has been how to expand BAT. Several miRNAs have been implicated in differentiating adipocytes into brown fat. For example (Figure 1), transgenic overexpression of miR-378, a miRNA that is enriched in BAT and induced with cold temperature exposure, promoted BAT adipogenesis and increased BAT, but not WAT. MiR-378 directly targeted PDE1b, a phosphodiesterase that catalyses the turnover of cAMP and cGMP.125 Overexpression of members of the miR-26 family, miR-26a and miR-26b, in adipose-derived stem cells (hMADS cells) upregulated the BAT marker UCP-1 both at the mRNA and protein level, an effect that may be mediated by targeting ADAM metallopeptidase domain 17 (ADAM17) thereby allowing increased UCP-1 expression in hMADS cells.128 MiR-455, induced by cold temperature exposure and bone morphogenetic protein (BMP7), regulates BAT differentiation by targeting the hypoxia-inducible factor 1α inhibitor (HIIFα), a hydroxylase that modifies AMP-activated kinase α1 subunit (AMPKα1) by hydroxylation and AMPK, thereby promoting thermogenesis. Furthermore, adipose-specific overexpression of miR-455 induced UCP-1 expression and brown adipogenesis.127 MiR-155 is a miRNA with a dual role of brown adipocyte differentiation and browning of WAT. MiR-155 KO mice have increased UCP-1 and PGC-1α expression and an activated thermogenic program in WAT thereby allowing for the increased ability to adapt to cold exposure by targeting CCAAT/enhancer-binding protein β (C/EBPβ).126 Finally, miR-133 is a negative regulator of BAT adipogenesis through direct targeting of PRDM16, inhibiting its expression and reducing brown adipogenesis in white adipocyte progenitor cells.124,132 Collectively, these studies suggest that microRNAs can effectively regulate brown fat differentiation, providing potential targets for modulating energy expenditure.

3.2.2 MiRNA regulation of skeletal muscle progenitor brown fat metabolism
A brown fat enriched miRNA cluster, miR-193b-365 located on chromosome 6 as a ~5 kb gene, is significantly increased during brown fat adipogenesis. Furthermore, in obese mice, expression levels of miR-193 and miR-365 are significantly reduced in BAT. Blocking of miR-193a/b and miR-365 reduced lipid accumulation in brown adipocytes. Furthermore, miR-193 directly targets RUNX1T1. Interestingly, due to the common development origin of brown adipocytes and skeletal muscle, the authors hypothesized that blockade of the brown adipocyte lineage by miR-193 or miR-365 may switch brown fat preadipocyte fate to the muscle lineage. Indeed, expression of miR193 and miR-365 in C2C12 skeletal muscle myoblasts induced their differentiation into brown adipocytes. Mechanistically, this is partially controlled by the induction of miR-193 and miR-365 by PRDM16 through peroxisome proliferator-activated receptor alpha (PPARα).133 Activation of PPARα, a member of the nuclear hormone receptor family, was previously shown to prevent inflammation in WAT.134 Similarly, miR-133, a miR that is decreased in mice in response to cold exposure, regulates the lineage commitment between myogenic and brown adipocytes by targeting the 3’UTR of PRDM16. In diet-induced obese mice, inhibition of miR-133 during muscle regeneration increased uncoupled energy expenditure, glucose uptake, thermogenesis in locally-treated muscle, uncoupled oxygen consumption, glucose tolerance, and induced metabolically active brown adipocytes in skeletal muscle.132 Inhibition of miR-328, a regulator of BAT differentiation, blocked preadipocyte commitment, whereas miR-328 overexpression promoted BAT differentiation and impaired muscle progenitor commitment through inhibition of the β-secretase BACE1. In vivo BACE1 inhibition delayed diet induced obesity and improved glucose tolerance and insulin sensitivity.135 Collectively, these miRNAs highlight the dynamic relationship between brown fat preadipocytes and skeletal muscle progenitors.

3.3 beige (brite) adipose tissue
Adipose tissue is a complex endocrine organ. Islands of thermogenic adipocytes, termed ‘brite’ (brown-in-white) or beige are a subtype of adipose depot that has recently attracted attention. These adipocytes emerge within WAT with positive expression of UCP-1 and the capability to burn fat and carbohydrates. Beige adipocytes emerge as a physiological response to cold temperature exposure136 by chronic PPARγ activation followed by the activation of the classical brown adipose tissue transcriptional regulator, PRDM16, in subcutaneous WAT (scWAT)137 or by stimulation with β3-adrenoceptor agonists such as CL 316,243 hydrate.138–140 The precise origin of brite adipocytes is still under debate as they are thought to either transdifferentiate from white adipocytes in scWAT136,141 or arise from de-novo differentiation from precursor adipocytes.142 Brite adipocytes are considered to be potential therapeutic targets in the settings of energy expenditure, exercise, and metabolism as they are considered as an ‘inducible’ brown fat subtype.

3.3.1 MiRNA regulation of beige (brite) fat differentiation
In response to a range of stimuli, global miRNA expression profiling of adipocytes has identified a number of miRNAs that are regulated in the browning of WAT (Figure 1). For example, let-7i-5p, a member of the one of the first described let-7 miRNA family was identified from gene profiling of hMSCs-Ad cells that were differentiated from white adipocytes to a brite phenotype using the PPARγ agonist rosiglitazone. Expression of let-7i-5p was reduced in brite adipocytes that negatively correlated with UCP1 expression in mouse and human cells and tissues. Furthermore, let-7i-5p regulated brite adipocyte function in vitro through the specific inhibition of UCP1 expression, which in turn impaired mitochondrial oxygen consumption. Overexpression of let-7i-5p in vivo through injection into scWAT impaired the formation and function of brite adipocytes through partial inhibition of β3-adrenergic activation of the browning process.143 Activation of β3-adrenergic receptors by epinephrine, norepinephrine, or specific agonists typically results in the GS-dependent activation of adenylate cyclase, increased intracellular cAMP, and stimulation of protein kinase A (PKA) and several downstream kinases including p38 MAPK.144,145 Activation of p38 MAPK induces a transcriptional program including PGC-1α, ATP-2, and UCP-1 leading to brown fat activation and thermogenesis.146 MiR-125-5p is another example of a miRNA whose expression is reduced during brite
adipocyte formation in hMSCs-Ad. In response to β3-adrenergic receptor stimulation in vivo, miR-125b-5p expression was reduced in scWAT as well as in interscapular BAT. Overexpression of miR-125-5p inhibited brite adipocyte formation in scWAT as evidenced by decreased expression of brite adipocyte markers such as UCP1, OPT1M, CIDEA, and a defect in the formation of multilocular lipid droplet-containing adipocytes representative of activated brite/brown adipocytes. In contrast, loss-of-function studies in mouse scWAT promoted activated brite adipocytes indicative of activated brite adipocytes. Collectively, a number of miRNAs that contribute to browning also participate in beige adipocyte differentiation. It will be of interest to elucidate their adipocyte-specific roles in cardiovascular pathophysiological states such as heart failure, atherosclerosis, exercise, and a range of vascular disease states.

4. Adipose-associated circulating microRNAs

Emerging studies indicate that adipocytes can release miRNAs in exosomes. Indeed, adipose-derived circulating miRNAs may regulate gene expression in other tissues highlighting their 'endocrine-like' systemic effects. Another study highlighted that exosomes isolated from both human and mouse serum may harbor miRNAs such as miR-92a. Interestingly, there was an inverse correlation of miR-92a expression and BAT activity in healthy individuals as measured by PET/CT scans. The ability to detect circulating miRNAs and their correlation with adipose tissue activity holds promise that they may serve as metabolic biomarkers. Plasma samples collected from a European cohort of children who were healthy, overweight, or obese were screened for differentially expressed circulating miRNAs. Of the 372 miRNAs that were screened for, miR-31-5p, miR-2355-5p, and miR-206 were differentially expressed and correlated with obesity. miRNA profiling of 85 lean vs. 40 obese children revealed increased concentrations in plasma of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p—all of which associated with factors such as body mass index (BMI), percent fat mass, waist and regional fat distribution, insulin resistance, high-molecular-weight adiponectin, C-reactive protein, and circulating lipids. Plasma concentrations of some of these circulating miRNAs changed significantly during the 3-year follow-up in children who showed an increase or decrease in their weight. These results suggest that the circulating miRNAs are promising candidates as potential biomarkers in the detection of metabolic syndrome even in childhood. Additional studies will be required to determine whether adipose-derived circulating miRNAs will have a major impact in cardiovascular pathologies. However, these findings raise the possibility that similar paradigms may exist for miRNAs to be derived from other cells (e.g. endothelial cells, platelets, leukocytes) or tissues (e.g. liver, skeletal muscle) that are also prominently involved in homeostatic control of cardiometabolism.

5. Future directions and conclusion

Accumulating studies demonstrate that microRNAs serve as critical players of gene regulation including the pathways involved in the regulation of adipocyte differentation and function. They regulate not only white adipose differentiation, but also beige (brite) and brown adipose differentiation and brown fat expansion. Consequently, miRNA-based therapeutics targeting these adipocyte depots may have the ability to reverse adipose tissue dysfunction, insulin resistance, diabetes, obesity, and a range of diabetes-associated cardiovascular complications including accelerated coronary and peripheral atherosclerosis, cardiac lipotoxicity, and microvascular disease in tissues.

There are a number of challenges for miRNA delivery to human tissue. For example, naked miRNAs are readily degraded by endogenous circulating or tissue RNases. Consequently, chemical modifications have been employed to protect miRNAs and improve durability. Current paradigms for local or systemic delivery utilize a range of chemically modified anti-miRs (to inhibit miRNA) or miRNA mimics (to overexpress miRNA). Several strategies have been used to chemically modify anti-miR oligonucleotides to enhance target stability, affinity, or tissue uptake. For example, locked nucleic acids exhibit high binding efficiencies and improved stability with the addition of a methylene link between the 2'-oxygens and the 4'-carbon resulting in a locked position and reducing the flexibility of the ribose ring. There are examples of other modifications including ribose 2'-OH group modifications, such as 2'4'-constrained 2'0'-ethyl and 2'methoxethyl, and phosphorothioate modification. However, it has recently been reported that phosphorothioate modifications may facilitate nucleotide-based drugs to bind and activate platelets eliciting thrombus formation in response to carotid injury, pulmonary thromboembolism, and mesenteric artery injury in mice. Future studies in human subjects will require careful attention to potential toxicities.

In contrast to their double-stranded counterparts (miRNA mimics), single-stranded anti-miR oligonucleotides can be formulated in saline or phosphate buffered saline for subcutaneous or intravenous delivery and do not require lipid- or nanoparticle-based delivery systems. Following systemic delivery, these anti-miRs rapidly leave the plasma and are taken up by multiple tissues, most notably liver, spleen, kidney, adipose tissue, and bone marrow. Upon cellular uptake, the anti-miR generates a high-affinity, stable bond with the miRNA reducing the availability of the endogenous miRNA for binding to the 3'-UTR of the mRNA target(s).

Preclinical studies in nonhuman primates using naked anti-miR oligonucleotides have shown promise for targeting miRNAs expressed highly in the liver such as miR-122 or miR-33. Cholesterol analogs have been added to anti-miRs to facilitate cellular uptake, particularly in the liver, and this enhances their incorporation into HDL and LDL.
Alternative approaches for miRNA inhibition include the use of competitive miRNA inhibitors, such as miRNA sponges or decoy transcripts that contain miRNA binding sites complementary to the seed sequence of the miRNA of interest.\textsuperscript{161} Several miRNA inhibitors have already advanced into human clinical trials. For example, a human phase 2 study of miR-122 inhibitors (Miraviren) demonstrated dose-dependent antiviral activity when given as a 4-week monotherapy.\textsuperscript{162}

In disease states where there is a deficiency of a certain miRNA, the delivery of double-stranded therapeutic miRNA molecules in vivo has many of the same challenges as siRNAs. Therefore, drug delivery vehicles such as liposomes, polymeric micelles, and nanoparticle-based carriers are being developed to deliver these oligonucleotides into the cells and tissues. A few miRNA ‘mimics’ are being evaluated in clinical trials. For example, miR-29 mimics are being evaluated in phase 1 studies for safety and tolerability in the context of dermal remodeling (e.g. to prevent fibrous scar tissue formation); NCT02603224.

Another major challenges associated with miRNA mimics delivery is the difficulty to target miRNA to a specific cell type to bypass uptake by other tissues, and the potential requirement of multiple doses to achieve sustained levels for continued target repression. Viral vectors including short hairpin RNAs that can be locally delivered and processed in the target cell to the mature miRNA, are attractive candidates for such a task and this approach has been used in multiple preclinical studies.\textsuperscript{163,164}

However, viral delivery systems will require careful examination for clinical use. Cell-type specific ligands, peptides, and nanoparticles directed at white adipocytes, for example, are likely to provide novel delivery platforms enabling sustained miRNA expression or knockdown for targeted delivery and minimal toxicity to VAT. Collectively, adipocyte-specific miRNA targeting may facilitate beneficial metabolic effects on energy expenditure, insulin resistance, and subsequently cardiovascular remodeling, for example, via induction of browning markers while minimizing any potential non-adipocyte toxicity.

6. Conclusion

In summary, accumulating studies highlight the importance of miRNAs in adipocyte dysfunction and their impact on a range of cardiovascular disease states including diabetes, obesity, and atherosclerosis. Harnessing protective miRNAs associated with browning of VAT may provide a novel strategy to improve energy expenditure, insulin resistance, body fat mass, and cardiometabolic health. Conversely, targeting maladaptive miRNAs in adipocytes may also figure prominently to ameliorate a range of cardiovascular disease states. To this end, a number of miRNAs have been identified in white, beige, and brown adipose tissue depots that can positively or negatively regulate adipose cell differentiation and function. In addition, the function of adipocytes may be regulated by microRNA-mediated effects on neighboring cells, such as microvascular endothelial cells or leucocyte subsets, providing alternative non-adipocyte targeted strategies for regulating cardiometabolic disease. Finally, emerging studies highlight that circulating adipocyte-derived miRNAs may not only serve as potential biomarkers of metabolism, but also may directly impact gene expression and potentially function in remote tissues. Future studies will be informative to ascertain the relative importance of adipocyte-derived miRNAs in health and cardiovascular disease states.

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