MicroRNA regulatory networks in human adipose tissue and obesity

Peter Arner and Agné Kulyté

Abstract | MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression and, therefore, biological processes in different tissues. A major function of miRNAs in adipose tissue is to stimulate or inhibit the differentiation of adipocytes, and to regulate specific metabolic and endocrine functions. Numerous miRNAs are present in human adipose tissue; however, the expression of only a few is altered in individuals with obesity and type 2 diabetes mellitus or are differentially expressed in various adipose depots. In humans, obesity is associated with chronic low-grade inflammation that is regulated by signal transduction networks, in which miRNAs, either directly or indirectly (through regulatory elements such as transcription factors), influence the expression and secretion of inflammatory proteins. In addition to their diverse effects on signalling, miRNAs and transcription factors can interact to amplify the inflammatory effect. Although additional miRNA signal networks in human adipose tissue are not yet known, similar regulatory circuits have been described in brown adipose tissue in mice. miRNAs can also be secreted from fat cells into the circulation and serve as markers of disturbed adipose tissue function. Given their role in regulating transcriptional networks, miRNAs in adipose tissue might offer tangible targets for treating metabolic disorders.

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Introduction

An excess of adipose tissue is associated with several medical conditions such as type 2 diabetes mellitus (T2DM), hypertension, cardiovascular disease and cancer.[1] The global obesity epidemic has been paralleled by a similar increase in incidence of T2DM.[2,3] Adipose tissue is an important contributor to the pathophysiology of obesity (Box 1). Although high energy intake and low physical activity are the predominant factors that drive the development of obesity, lifestyle-independent factors, such as genetic variance and hormone dysfunction, also contribute to some forms of obesity.[1,4] Adipose tissue expands by generating new small fat cells, a process known as hyperplasia, or by increasing the lipid content, and thereby, the volume of pre-existing fat cells, a process known as hypertrophic expansion.[5] Hypertrophic adipose tissue has stronger associations with the metabolic complications of obesity than hyperplastic adipose tissue.[6] Furthermore, adipose tissues can be differentially distributed across anatomical sites;[7] accumulation of intra-abdominal fat tissue is more strongly associated with an adverse metabolic profile (insulin resistance, hypertension, dyslipidaemia) than accumulation of subcutaneously distributed adipose tissue.[7]

Adipose tissue also directly contributes to development of other complications associated with obesity. The lipolytic activity of fat cells is increased in individuals with obesity, which results in accelerated release of fatty acids from these cells into the circulation, which leads to inhibition of insulin action and insulin resistance.[8] In individuals with obesity, increased insulin resistance is a key factor that contributes to development of T2DM.[9] Adipokine activity within adipose tissue has also been implicated in the pathogenesis of T2DM. Production and release of adiponectin is decreased in individuals with obesity, in particular when accompanied by insulin resistance.[10] Finally, inflammatory adipokines might contribute to the metabolic complications associated with obesity.[11] Adipose tissue in individuals with obesity is associated with a chronic state of low-grade inflammation that is characterized by increased local production of inflammatory proteins, which can alter the lipolytic activity and adipokine functions of fat cells.[8,9,11]

A direct role of adipocytes in regulating whole-body energy balance and, therefore, development of obesity has been established.[8,9,12] The rate of lipid turnover in fat cells is decreased within the white adipose tissue (WAT) of individuals who are overweight or have obesity.[13,14] Moreover, decreased oxidation of fatty acids in brown adipose tissue (BAT) has been shown to result in obesity in rodent models.[15] Although adult humans have considerable amounts of BAT, whose regulation *in vivo* is similar to that in other species, the contribution of this tissue to energy expenditure in the context of development of obesity remains to be established.[16] The transition from healthy body weight to obesity can lead to dysregulation of normal adipose tissue functions.

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Key points

- MicroRNAs (miRNAs) are important for fat cell formation (adipogenesis) and for regulating the metabolic and endocrine functions of these cells
- Obesity influences the expression of miRNAs in adipose tissue, but altered expression of only a few of these miRNAs has been experimentally verified in humans
- Regional variations in expression of miRNAs in human adipose tissues have been demonstrated
- miRNAs signal through complex networks involving transcription factors, which has been demonstrated in the context of regulation of inflammation in human adipose tissue
- Extracellular miRNAs have specific expression profiles in obesity

Box 1 | Characteristics of adipose tissue

- Adipose tissue exists in two forms, white adipose tissue (WAT) and brown adipose tissue (BAT). The major function of WAT is to store and release energy-rich lipids, which form a single droplet within a fat cell and constitute >90% of its volume. Fatty acids are metabolized to triglycerides within adipocytes in response to caloric intake. Depending on energy demands, the triglycerides are hydrolyzed to fatty acids (lipolysis), which are released to the blood stream to be used for oxidation in muscle tissues.
- White adipocytes produce adipokines, which have local functions in adipose tissue, for example inflammatory adipokines. Some adipocyte-specific adipokines act peripherally in an endocrine fashion, such as leptin, which controls appetite and energy expenditure, and adipsin, which has insulin-like effects.
- When adipose tissue expands in individuals who are overweight or have obesity, metabolic and adipokine functions are altered, which contributes to several complications such as dyslipidaemia, hypertension and type 2 diabetes mellitus. The altered WAT function associated with obesity is, at least in part, caused by local chronic low-grade inflammation.
- Regional aspects of excess fat accumulation have been described. Central obesity, in particular intra-abdominal deposition of adipose tissue, has a stronger link to metabolic disease than peripheral subcutaneous fat deposition.
- Many small lipid droplets and an abundance of mitochondria characterize the fat cells in BAT. These mitochondria in these cells can undergo uncoupling, which enables them to oxidize fatty acids so that heat is produced. In many mammals, BAT is essential for energy metabolism and decreased BAT function in rodents causes obesity. The role of BAT in humans is not well established. The amount of BAT in humans is relatively abundant at the onset of life, but gradually reduces during ageing. The reduction in levels of BAT also seems to be accelerated in individuals with obesity.

Consequently, increased understanding of the molecular mechanisms that underlie normal and pathophysiological regulation of WAT and BAT is essential to identifying new targets and prognostic markers for treating obesity and its complications.

MicroRNAs (miRNAs) are short noncoding RNA molecules that act post-transcriptionally to regulate protein expression (Box 2).[17] Frequently, miRNAs act to finely tune the levels of mRNA transcripts within a cell,[18,19] but they can also function as repressors of protein production.[20] Only a few hundred miRNAs are estimated to regulate ~30–80% of the genes encoded in the human genome, with each miRNA targeting up to 100 genes, and multiple miRNAs having the potential to act on one gene.[21] A single miRNA can have multiple target sites within the 3' untranslated region of a particular mRNA transcript, and bioinformatic predictions suggest that a single miRNA family can regulate up to ~400 targets in human protein-coding genes.[22] Although the use of high-throughput expression analysis techniques has improved the accuracy of predictions of miRNA targets[23,24] and the identification of gene networks controlled by miRNAs,[25–29] only a small number of predicted targets and their corresponding miRNAs have been experimentally validated.[30]

This Review examines the role of miRNAs in human adipose tissue and their effects on signalling networks in patients with obesity and associated complications. The functions of extracellular miRNAs in patients with obesity are also explored.

Obesity

Several studies have compared miRNA expression profiles in obese and lean WAT from mice and from humans. In fat cells from mice with diet-induced obesity, which is phenotypically similar to obesity in humans, 35 of the 574 detected miRNAs were differentially expressed.[31] Microarray screening of human WAT tissues identified a number of miRNAs that are potentially dysregulated in patients with obesity, both in those who also have T2DM and in those who do not have T2DM.[32–38] However, validation experiments, such as investigations of additional fat-cell models, studies with increased sample sizes and analysis with quantitative RT-PCR,[39–42] have confirmed a role for only a small number of miRNAs in obesity (Table 1). Thus, although a large number of miRNAs are expressed in human WAT,[34,37] very few seem to be differentially expressed in the obese state and expression of many of these miRNAs is reduced in obese WAT compared with lean WAT.

By contrast, studies of expression levels of mRNAs in humans have detected numerous differentially expressed mRNA transcripts in WAT from patients with obesity compared with WAT from healthy individuals.[43,44] Notably, this seeming discrepancy does not exclude an important role for miRNAs in obesity, as each miRNA has the potential to regulate the levels of numerous mRNAs. The lack of congruency between findings reported by different studies of miRNAs in obesity[32–42] is, however, difficult to explain. Across these studies, only miR-221 was consistently found to be differentially expressed in human obese WAT compared with lean WAT; although it is important to note that this miRNA was found to be upregulated or downregulated depending on the study. Technical issues, such as differences in platforms and analysis methods, might, in part, explain the divergence of the reported results.[45,46] The small sample sizes of the studied cohorts might also contribute to the observed discrepancies, as many miRNAs are not uniformly expressed across individuals.[32] Additionally, sex is known to influence miRNA expression in human WAT,[35] and the source and mode of tissue preparation could also be confounding factors. Only ~50% of cells within adipose tissue are mature fat cells. Comparison of miRNA expression in intact WAT with that of isolated fat cells showed only 11 of 20 selected miRNAs with fully overlapping expression patterns, which suggests that these transcripts are similarly regulated in adipocytes and in the obese state.[32] As so many conflicting data exist, future expression studies should be standardized

Box 2 | MicroRNAs

- Originally discovered in *C. elegans*, microRNAs (miRNAs) are involved in regulatory networks of various biological processes including metabolism, differentiation, inflammation and energy homeostasis in health and disease states.
- miRNAs are evolutionary conserved 20–24 nucleotide RNAs that post-transcriptionally regulate gene expression. miRNAs are encoded in either their own genes or in introns.
- miRNAs are processed from miRNA precursors in the nucleus by Drosha (also known as RNA endonuclease type III) and later in the cytosol by endonuclease Dicer (also known as RNA endonuclease type II) into ~22 nucleotide miRNAs.
- One strand of miRNAs is incorporated into the multiprotein nuclease complex (RNA-induced silencing complex). Depending on the complementarity of the miRNA sequence to the target, genes can be regulated by either cleavage of mRNA or inhibition of protein translation.
- miRNAs contain a seed sequence (position 2–8 from the 5' end) that hybridizes with the 3' untranslated region of the target mRNA.
- The abundance of miRNAs varies from 1 to 10,000 copies per cell, with an estimated average of ~500 copies per cell.[163]

to enable reliable estimates of the number of miRNAs that are affected by obesity in human WAT.

Obesity is regulated by genetic factors; however, in human WAT the degree of variation in expression of miRNAs is substantially smaller than that of mRNAs.[47] Furthermore, obesity-induced changes in miRNA expression levels identified in humans correlate strongly with those in WAT from mice with genetic or diet-induced obesity.[31] These data suggest that altered miRNA expression in WAT is secondary to obesity; however, these alterations might occur at early stages of adipocyte differentiation. In differentiated adipocytes, 21 of the 40 differentially expressed miRNAs were similarly regulated in donors with obesity and healthy individuals.[37]

Exactly how many miRNAs are differentially expressed in obese human WAT remains to be established but the numbers are likely to be very small. To date, <10 upregulated miRNAs and only ~30 downregulated miRNAs have been described in this tissue type (Table 1). Although the functional roles of these miRNAs in development of obesity are not yet known, the actions of several of these transcripts in human fat cells have, at least in part, been characterized (Table 1).

Distribution in white adipose tissue

Global mRNA profiling of human subcutaneous WAT and omental WAT (a visceral depot) has demonstrated that numerous genes are differentially expressed in these adipose regions.[43,44,48] Obesity-induced changes in mRNA expression are similar in the two depots, but differences in gene expression that are associated with T2DM are much stronger in omental than in subcutaneous WAT.[43] Profiling of expression of 155 miRNAs in omental and subcutaneous WAT from individuals who are overweight or have obesity detected 106 transcripts in both depots, of which only 16 displayed a fat-depot-specific expression pattern (all more highly expressed in omental than in subcutaneous WAT).[49] 12 of these miRNAs were found in WAT from individuals with both obesity and T2DM, and expression of four miRNAs was dysregulated in patients with obesity and normal glucose tolerance (miR-20, miR-98, miR-197 and miR-331).[49] The idea that the anatomical region can influence miRNA expression is supported by most miRNAs having lower levels of expression in omental than in subcutaneous WAT.[50] The effects of exposure to a thiazolidinedione, which stimulates fat-cell differentiation, on miRNA expression in human WAT revealed that the treatment changed expression of 182 miRNAs in subcutaneous adipocytes but of only 46 miRNAs in visceral cells.[50]

Additionally, regional differences in levels of miRNA expression within subcutaneous WAT (abdominal versus gluteal) have been described.[51] Thus, although only a few miRNAs display differences in expression between subcutaneous and visceral WAT under steady-state conditions, regulation of miRNA expression in response to adipocyte differentiation in individuals with obesity might be influenced by regional variations. The importance of these regional differences, however, cannot be established until additional studies are performed.

Insulin resistance and T2DM

miRNAs might induce insulin resistance in adipose tissue directly, by regulating adipocyte function, or indirectly, by eliciting effects on local inflammation. The majority of miRNAs involved in insulin-resistance pathways have been identified in studies of rodent models or in cell types other than adipocytes.[52,53] However, analysis of the relationship between miRNA expression in WAT and clinical parameters associated with insulin resistance (such as fasting glucose and insulin levels, circulating levels of HbA_{1c} and adiponectin, and an insulin sensitivity index) revealed significant positive correlations between these parameters and obesity-associated miRNAs, including the miR-25/93/106b[54] family and miR-221.[36,40] Decreased levels of miR-221 were associated with elevated levels of mRNA transcripts encoding tumour necrosis factor (TNF); miR-221 also directly downregulated expression of *ADIPOR1*, which might contribute to development of insulin resistance.[36,40] In women with polycystic ovary syndrome (which is an insulin-resistant condition), expression of miR-93 was inversely correlated with insulin sensitivity.[55] Finally, the expression levels of miR-17-5p, which are subjected to regional variation, were inversely correlated with circulating levels of HbA_{1c}.[49] However, these results[49,55] should be interpreted with caution because no correction for levels of body fat content, which in itself can influence insulin resistance,[9] were performed.

Associations between expression of miRNAs and adipocyte-related factors that influence insulin sensitivity have been investigated in human WAT and in mouse models of diabetes mellitus. Overexpression of miRNAs that are dysregulated in obese human WAT resulted in inhibition of lipolysis by miR-26a and miR-let7d and stimulation of lipolysis by miR-30c, miR-652, miR-193b and miR-145;[56] by contrast, miRNA-145 is a negative regulator of WAT lipolysis in mice.[57] Overexpression of miR-21 in a mouse adipocyte cell line resulted in expression of mRNA transcripts and increased protein levels

Table 1 | miRNAs dysregulated in WAT of humans with obesity

Study	WAT depot	Sex	Upregulated miRNAs	Downregulated miRNAs	Function in adipocytes
Studies using microarray analysis					
Heneghan *et al.* (2011)[33]	Visceral	Unknown	None	miR-17-5 p and miR-132	miR-132 regulates the immune system
Martinelli *et al.* (2010)[35]	Subcutaneous	Female and male	miR-519d	miR-150 and miR-659	Not established
Ortega *et al.* (2010)[37]	Subcutaneous	Female	miR-99a, miR-199a-5p, miR-125b, miR-221 and miR-1229	miR-130b, miR-139-5p, miR-185 and miR-484	Not established
Keller *et al.* (2011)[34]	Subcutaneous	Unknown	miR-21	miR-143	Not established
Arner *et al.* (2012)[32]	Subcutaneous	Female	miR-222 and miR-342-3p	let-7a, let-7d, let-7i, miR-16, miR-26a, miR-30c, miR-92a, miR-126, miR-139-5p, miR-143, miR-145, miR-151-5p, miR-193a-5p, miR-193b, miR-197, miR-484-5p, miR-378 and miR-652	let-7d, miR-26a, miR-30c, miR-145, miR-193 and miR-652 regulate lipolysis[56] Some of these miRNAs regulate production of CCL2[32] and TNF[56] miR-143, miR-145 and miR-378 influence adipocyte differentiation[31,45,76-80]
Meerson *et al.* (2013)[36]	Subcutaneous	Female and male	miR-221	miR-193a-3p and miR-193b-5p	Not established
Capobianco *et al.* (2012)[38]	Visceral	Female	None	miR-141 and miR-520	miR-144 and miR-520e might regulate glucose metabolism
Studies using a candidate gene approach					
Chou *et al.* (2013)[40]	Visceral	Female	None	miR-221	Not established
Diawara *et al.* (2014)[41]	Subcuatneous and visceral	Female and male	None	miR-125a	Not established
Oger *et al.* (2014)[42]	Visceral	Male	None	miR-200a and miR-200b	Not established
Chen *et al.* (2014)[39]	Subcutaneous and visceral	Unknown	miR-146b	None	Not established

Abbreviations: miRNA, microRNA; TNF, tumor necrosis factor; WAT, white adipose tissue.

of adiponectin.[58] miR-141 and miR-520 might influence insulin sensitivity in human WAT through their predicted effects on glucose metabolism (Table 1).[38]

Expression of obesity-associated miR-200a and miR-200b transcripts in WAT was lower in patients with obesity who also had T2DM than in patients with obesity but without T2DM.[42] Microarray studies identified 17 miRNAs that were differentially expressed in omental WAT from women with gestational diabetes mellitus who did not have obesity compared with omental WAT from healthy pregnant women.[59] These findings suggest that expression of miRNAs in WAT can be influenced by diabetes mellitus independently of the presence of obesity. This notion is supported by studies of WAT from nonobese diabetic mice (reviewed elsewhere[60]).

Insulin resistance and its associated metabolic variables can, therefore, affect expression of miRNAs in WAT, but the confounding effects of the levels of body fat tissue must be closely examined before any strong conclusions are reached. Notably, diabetes mellitus can be accompanied by dysregulation of some WAT miRNAs independently of obesity. miRNAs might regulate insulin sensitivity through actions on lipolysis, adipokines and insulin signalling pathways. However, a direct role in development of T2DM and gestational diabetes mellitus has yet to be established.

Inflammation

Chronic inflammation is a hallmark of many features of the metabolic syndrome, including obesity and T2DM. Obese WAT is characterized by increased infiltration of macrophages and increased release of inflammatory adipokines, such as CCL2, TNF and IL-6.[11] The inflammatory environment within WAT impairs insulin signalling and induces oxidative stress and endothelial dysfunction, which leads to systemic insulin resistance and cardiovascular disease.[61]

miRNAs have important roles in inflammation,[37,49,62] and several individual miRNAs that control inflammation in WAT have been described (Table 2).[63,64] miR-132, which is downregulated in omental WAT from individuals with obesity,[33] activates the nuclear factor κ-light-chain activator of B cells (NF-κB) protein complex, which results in transcription of *IL-8* and *CCL2* in human primary preadipocytes and in adipocytes that have been differentiated *in vitro*.[65] 10 miRNAs have been identified as regulators of inflammation in human WAT owing to their effects on CCL2 release from

Table 2 | miRNAs associated with inflammation in human adipose tissue

miRNA	Expression pattern and/or function	Tissue or cell	Reference
miR-26b	Expression is associated with the number of macrophages infiltrating the fat depot	Subcutaneous and omental WAT	Kloting *et al.* (2009)[49]
	Affected by levels of TNF, leptin and resistin	Preadipocytes	Xu *et al.* (2013)[156]
miR-95	Expression is associated with serum concentrations of adiponectin and negatively correlated with levels of C-reactive protein and IL-6	Subcutaneous WAT	Kloting *et al.* (2009)[49]
miR-99a	Negative correlation with levels of free fatty acids	Subcutaneous and omental WAT	Kloting *et al.* (2009)[49]
miR-99a and miR-325	Negative correlation with secretion of IL-6	Subcutaneous and omental WAT	Kloting *et al.* (2009)[49]
miR-125a–5p	miRNA partly regulates the inflammatory response, lipid uptake and ORP-9 expression	Monocytes and macrophages	Chen *et al.* (2009)[157]
miR-132	Expression levels are associated with the number of macrophages infiltrating the fat depot	Subcutaneous and omental WAT	Kloting *et al.* (2009)[49]
	Activates NF-κB signalling and the transcription of *IL8* and *CCL2*	Primary adipocytes differentiated *in vitro*	Strum *et al.* (2009)[65]
	Decreased expression is associated with increased secretion of IL-6	Visceral WAT from patients with NAFLD	Estep *et al.* (2010)[158]
miR-143	Expression is downregulated by resistin, leptin and free fatty acids	Preadipocytes differentiated *in vitro*	Zhu *et al.* (2013)[159]
miR-146b-5p	Bidirectional relationship between levels of globular adiponectin and expression of miRNA	Monocytes	Hulsmans *et al.* (2012)[160]
	Expression of the miRNA and promoter activity is increased by exposure to TNF and IL-6	Mature adipocytes	Shi *et al.* (2014)[67]
miR-155	Expression levels are associated with the number of macrophages infiltrating the fat depot	Subcutaneous WAT	Kloting *et al.* (2009)[49]
	Silencing expression enhances the inflammatory response and lipid uptake	Oxidized-LDL-stimulated macrophage cell line	Huang *et al.* (2010)[161]
miR-181a	Expression is negatively correlated with levels of adiponectin	WAT	Kloting *et al.* (2009)[49]
miR-221	Decreased expression is associated with high levels of TNF	hASC from women with obesity	Chou *et al.* (2013)[40]
miR-221 and miR-222	Inhibit cell migration, proliferation and angiogenesis	Endothelial cell line	Urbich *et al.* (2008)[162]
miR-335	Expression is upregulated by exposure to leptin, resistin, TNF and IL-6	Adipocytes	Zhu *et al.* (2014)[68]
miR-26a, miR-92a, miR-126, miR-143, miR-145, miR-193a, miR-193b, miR-652, let-7a and let-7d	Affects secretion of CCL2	Cultured preadipocytes	Arner *et al.* (2012)[32]
miR-26a, let-7d, miR-143, miR-92a, let-7a, miR-193a-5p, miR-193b and miR-145	Affects secretion of TNF	Cultured preadipocytes	Lorente-Cebrian *et al.* (2014)[56]
miR-17-5p and miR-132	Expression is inversely associated with markers of hyperglycaemia and insulin resistance	Adipose tissue	Kloting *et al.* (2009)[49]
miR-132, miR-150, miR-433, miR-28-3p, miR-511, miR-517a and miR-671	Levels of IL-6 in the serum are negatively correlated with expression levels of miRNAs	Visceral adipose tissue from patients with NAFLD	Estep *et al.* (2010)[158]
miR883b-5p	Upregulated by adiponectin and represses LBP and TLR4 signalling	WAT	Ge *et al.* (2012)[70]

Abbreviations: hASC, human adipose stem cells; LBP, lipopolysaccharide-binding protein; NAFLD, non-alcoholic fatty liver disease; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; WAT, white adipose tissue.

adipocytes and macrophages.[32] Of these miRNAs, eight modulated release of TNF from adipocytes by either direct or indirect mechanisms.[32,56] miR-223 abolished proinflammatory activation of macrophages via inhibition of expression of *Pknox1*, which, in mice, results in protection from diet-induced inflammation in adipose tissue and systemic insulin resistance.[66] Decreased expression of miR-221 was positively correlated with increased expression of *TNF* in differentiated adipocytes obtained from women with obesity.[40] miR-146b regulates inflammatory processes by attenuating NF-κB-mediated cytokine signalling. Furthermore, expression

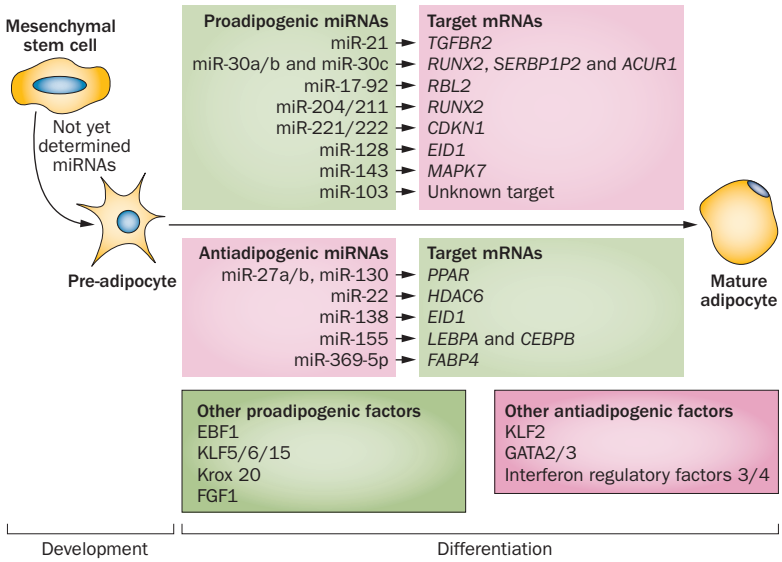

Figure 1 | miRNAs and verified targets that contribute to adipogenesis in humans. Adipogenesis is driven by the transcription factors C/EBPβ/δ, C/EBPα and PPARγ, as well as by SREBP1, which interacts with other factors and miRNAs. Additional factors can act either as either drivers or inhibitors of adipogenesis (proadipogenic and antiadipogenic factors, respectively). Abbreviations: miRNA, microRNA; qPCR, quantitative PCR.

of miR-146b in human mature adipocytes was markedly increased in response to exposure to the inflammatory cytokines TNF and IL-6.[67]

Levels of miRNA transcripts in WAT could also be affected by increased local inflammation. Treatment of a mouse adipocyte cell line with TNF resulted in changes in miRNA expression that were similar to those observed in WAT of obese mice.[31] In human preadipocytes, expression of miR-221 was downregulated in response to treatment with either leptin or TNF.[40] Expression of miR-335, which is associated with adipogenesis, was considerably upregulated in response to treatments with leptin, resistin, TNF and IL-6 in human mature adipocytes.[68] TNF treatment of a mouse mature adipocyte cell line resulted in an increase in levels of miR-130 (a regulator of adipogenesis) through enhanced binding of transcription factor p65 to the promoter region of miR-130.[69] Furthermore, exposure to TNF also induced downregulation of miR-103 and miR-143, as well as upregulation of miR-221 and miR-222 in mouse adipocytes.[31]

The anti-inflammatory adipokine adiponectin regulates expression of several miRNAs in WAT with chronic low-level inflammation in both mice and individuals with obesity.[70] Moreover, this adipokine induces increased expression of miR-155 via JNK–NF-κB-dependent mechanisms in a mouse macrophage cell line.[71] Expression of miR-221 and miR-222 is positively correlated with TNF and negatively correlated with adiponectin expression in WAT of mice in response to different dietary regimes and to treatment with conjugated linoleic acid associated with fat loss.[72] Adipose-tissue-specific overexpression of adiponectin in mice results in upregulation of miR883b-5p, which leads to repression of lipopolysaccharide-binding protein and toll-like receptor 4 signalling, thus this miRNA acts as a mediator of the anti-inflammatory action of adiponectin.[70]

Thus, miRNAs might mediate inflammation in WAT by regulating the activation of macrophages and/or the production of adipokines. This miRNA-mediated regulation is relayed via both direct and indirect activation of signalling pathways and cell-specific transcriptional networks in WAT.

Adipogenesis

Growth arrest, clonal expansion and terminal differentiation of preadipocytes are required for generation of mature adipocytes.[73] These processes are controlled by a complex network of transcription factors, including peroxisome proliferator-activated receptor γ (PPARγ), CCAT/enhancer binding proteins, Krupple-like factors and sterol regulatory element-binding proteins, as well as extracellular hormones.[5,12] Inhibition of enzymes involved in miRNA biogenesis, such as ribonuclease 3 (commonly known as Drosha) and endoribonuclease Dicer, repressed the differentiation of human mesenchymal stem cells into adipocytes,[74] which supports a role for miRNAs in adipocyte development. Ablation of endonuclease Dicer from mouse preadipocytes resulted in blockade of induced adipogenesis that was characterized by impaired lipogenesis and downregulated expression of adipocyte markers such as PPARγ, TNF receptor superfamily member 6 (also known as FAS), solute carrier family 2 facilitated glucose transporter member 4 (GLUT4) and fatty acid-binding protein, adipocyte (FABP4).[75]

The functions of miRNAs as stimulators or inhibitors of murine and/or human adipocyte differentiation programmes have been reviewed in detail elsewhere.[31,45,76–80] miRNAs such as let-7,[81,82] miR-21,[83] miR-22,[84] miR-27,[83,85,86] miR-31,[87,88] miR-130,[89] miR-138,[90] miR-145,[91] miR-155,[92] miR-221/222,[36,72] miR-224-5p,[93] miR-369-5p[94] and miR-448[95] function to inhibit adipogenesis in human, mouse and porcine cells. Here, we highlight miRNAs with verified targets in human cells (Figure 1). In human stem cells derived from subcutaneous WAT (hASC), miR-21 antagonizes TGFβ signalling and increases adipogenesis.[83] Overexpression of miR-22 inhibits adipogenesis by targeting *HDAC6*.[84] The miR-17–92 cluster functions to drive adipogenesis by negatively regulating *Rbl2* transcripts.[96] Ectopic expression of miR-143 and miR-103 in mouse and human adipocytes enhances adipogenesis, possibly by targeting genes such as *ARNT*, *FZD1*, *RUNX1T1* and *ERK5* that encode anti-adipogenic factors.[31,97] Interestingly, in adipose precursor cells isolated from rats, miR-143 seems to have a dual role in adipogenesis. Overexpression of miR-143 during clonal expansion inhibits differentiation of the cells, whereas miR-143 overexpression during terminal differentiation promotes adipogenesis via the MAPKK5–MAPK 7 signalling pathway.[98]

In hASCs, adipocyte differentiation is increased by overexpression of miR-371, as evidenced by an increase in expression of adipogenic genes such as *ADIPOQ* and *FABP4*.[94] miR-138 inhibits adipogenesis at least in part

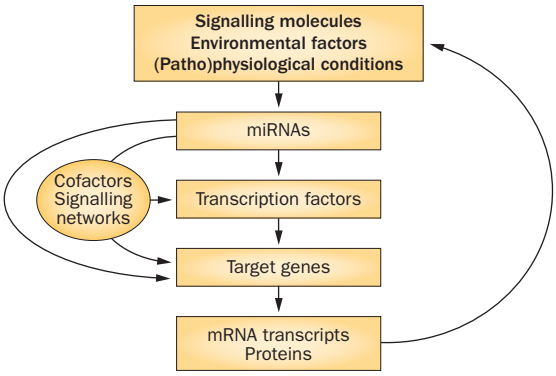

Figure 2 | miRNA signalling in adipose tissue. miRNAs influence the expression of target genes in multiple ways: directly, by binding to target gene transcripts to alter (usually reduce) mRNA transcript levels; indirectly, by altering the expression of transcription factors, which, in turn, regulates the expression of target genes; and by acting on cofactors for transcription factors or on genes in signalling networks that control transcription factors, which forms an additional layer of indirect regulation. These different miRNA regulatory pathways enable a vast diversity of signal transduction mechanisms, which can be amplified through interactions between the different components. Target proteins, in turn, can function to regulate feedback loops that control expression of miRNAs. External factors such as diet or pathological states such as obesity might also influence miRNA expression. Abbreviation: miRNA, microRNA.

via inhibition of the nuclear receptor coregulator *EID1* gene.[90] The miR-30 family promotes adipocyte differentiation via suppression of *SERBP1P2*, *ACVR1* and *RUNX2* transcripts.[99,100] However, miR-27 and miR-130 directly inhibit *PPARG* in mouse and human cells.[85,89,101]

The role of miRNAs in adipogenesis has also been explored in human mesenchymal stem cells. Expression of miR-637 enhanced adipocyte differentiation in these cells through inhibition of Osterix, which is an important transcription factor of osteoblasts.[102] miR-369-5p directly targets *FABP4* mRNA transcripts and inhibits upregulation of *ADIPOQ* during adipogenesis.[94] Ectopic expression of miR-155 and miR-221/222 in primary human mesenchymal stem cells resulted in inhibition of adipogenesis through targeting of *PPARG*, *CEBPA* and *CDKN1B* transcripts.[103]

Numerous studies have identified individual miRNAs that affect adipogenesis by targeting genes that encode various adipogenic transcription factors and signalling molecules; however, little is known about their role in the regulation of fat-cell size and number, and about their function in obesity in humans. Additionally, many miRNAs that are upregulated in obesity are downregulated in adipogenesis and vice versa.[31,37] This inverse regulation might be mediated by high levels of TNF.[31]

Differentiation of brown adipose tissue

Fat cells exist in three forms: white, beige and brown. Beige adipocytes have an intermediate phenotype and, in the resting state, their function is similar to cells in WAT.[104] However, following activation by a stimulus such as cold exposure, these cells rapidly increase the expression of genes that encode uncoupling proteins and other proteins essential for thermogenesis in brown fat cells. Brown precursor cells can transdifferentiate into white or beige fat cells and, conversely, white precursor cells can differentiate into brown and beige adipocytes.[104]

Numerous studies in rodent models show that transfection of preadipocytes with miRNA mimetics or inhibitors results in marked changes in the differentiation programmes of white, brown and beige fat cells.[105,106] The molecular mechanisms by which miRNAs drive adipocyte differentiation in humans have not yet been fully explored, but in mice, miRNAs form networks that can either directly or indirectly target mRNA transcripts encoding transcription factors, which in turn regulate the expression of genes that control adipocyte differentiation and/or thermogenesis.[106] The miRNA signalling network in brown fat tissue is, therefore, similar to that regulating inflammation in WAT. Overexpression of miR-26a/b and miRNA-196a in human adipocyte precursors can induce differentiation of human subcutaneous stem cells towards a brown adipocyte phenotype.[107,108]

The role of miRNAs in formation of BAT has been studied in mice. For example, miR-155 inhibits formation of BAT and enhances transition of WAT to a beige phenotype.[109] Similar findings have been obtained with experiments in which miR-133a was overexpressed;[110] however, to date, no studies have been published that directly examine the role of miRNAs in human BAT.

miRNA regulatory networks

The regulatory actions of miRNAs are complex (Figure 2). miRNAs can act directly on target mRNA transcripts or indirectly by first regulating intermediate components such as transcripts that encode transcription factors, which, in turn, control the expression of downstream genes. Ultimately, miRNAs alter levels of protein expression, which influence cell function and can also affect miRNA expression. This complexity is difficult to study experimentally. However, using bioinformatics, genome-wide miRNA–mRNA networks in specific tissues in various health and disease states can be elucidated.[111,112] Correlation of clinical data with the expression status of various network components allows for creation of integrative networks. In turn, changes in the expression of genes that can perturb signal networks might have causal roles in development of disease.[113] Thus, the molecular networks involving genes, mRNAs and noncoding regulatory units such as miRNAs offer new tools for dissecting the complex regulation of disease states.

miRNA and other cell signalling networks have been reviewed extensively elsewhere,[114–117] and miRNA–gene regulatory networks have been described in many diseases and pathophysiological processes;[116,118] however, little is known about WAT-specific miRNA regulatory networks. We have developed and applied an experimental pipeline to study miRNA–gene regulatory networks in human adipose tissue (Figure 3).[32] Using this analysis pipeline, we have identified and characterized a

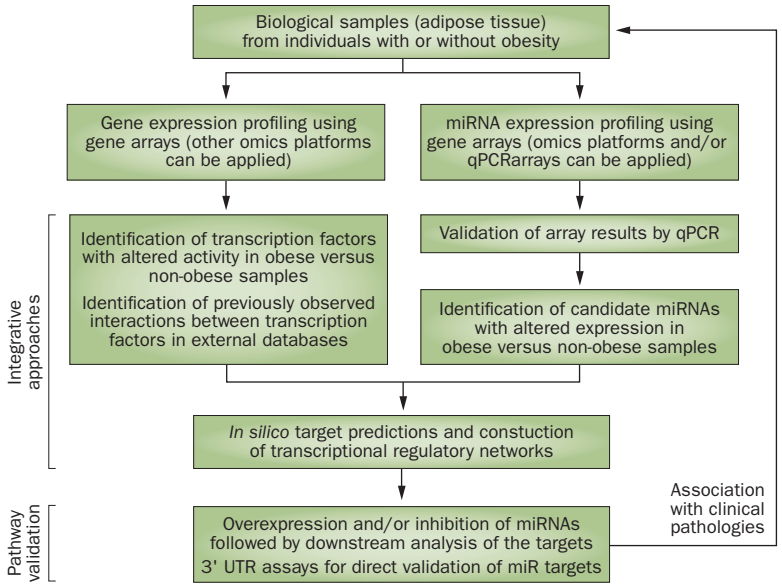

Figure 3 | Experimental pipeline implemented to elucidate integrative miRNA–gene regulatory networks in human adipose tissues. Identification of obesity-regulated candidate miRNAs and transcription factors is indicated in the upper part of the figure. Integrative analysis of miRNAs, miRNA targets and mRNAs are indicated in the middle. Experimental validations are indicated in the lower part of the figure. Abbreviations: miRNA, microRNA; qPCR, quantitative PCR; UTR, untranslated region.

transcriptional regulatory network in human WAT that demonstrates that specific miRNAs regulate inflammation in human WAT through effects on CCL2 secretion (Figure 4a).[37] The regulation of secretion of CCL2 is mediated by miRNA interactions with *CCL2* mRNA and/or indirect effects on transcription factor circuits.[32] The possibility exists that miRNAs targeting various adipogenesis-regulating pathways (for example, pathways that stimulate insulin signalling or repressing pathways such as WNT or TGFβ signalling) can affect adipogenesis in different ways. The function of miR-30c as a promoter of adipocyte differentiation via direct targeting of the interconnected transcripts, *SERPINE* and *ACVR1* is one such example.[85] These findings demonstrate that a specific miRNA can target two pathways, which can be interconnected, to promote adipogenesis, and support the idea that miRNAs might coordinate larger regulatory networks than previously anticipated.[99] Various computational methods for miRNA–gene network creation and experimental approaches for pathway validation can be adapted from other biological contexts and applied to identify miRNAs in adipose tissues that are regulated by obesity or other metabolic states.[119,120]

Under normal physiological conditions, multiple miRNAs seem to act synergistically.[25,121] miRNAs can also act through feedback and feed-forward loops that can amplify or reduce their effects.[122] Indeed, miRNAs regulate disease-relevant mRNA modules in complex human diseases (such as T2DM) in which most miRNAs act in a complementary fashion (that is, on different mRNAs) in disease states, but function mostly synergistically (that is regulating the same gene) in a healthy state. In type 2 T helper cells, complementary regulation of *IL5* and *IL13* by miR-223 and miR-139-3p has been demonstrated.[123] Given that individual miRNAs only moderately repress their targets, expression of families and/or clusters of miRNAs that affect multiple targets within a pathway might have increased effects on that pathway. We have also shown that CCL2 production in human WAT can be regulated directly or indirectly in an additive mode within adipocyte-specific or macrophage-specific miRNA–gene networks (Figure 4b);[124] however, this study was mainly focused on adipocyte-specific miRNA–transcription-factor regulatory circuits. miRNAs can also be transcriptionally or post-transcriptionally regulated by proteins that bind upstream of miRNA genes or post-transcriptionally by RNA-binding proteins.[125] However, to the best of our knowledge, no studies have been performed on the regulation of miRNA biogenesis in adipocytes at this level.

Integrative miRNA–gene networks provide tools for identification of disease-related miRNA signatures and the functions of these miRNAs in disease pathogenesis. However, new experimental strategies and additional investigations are needed to describe the various miRNA signalling networks in human adipose tissue.

Extracellular miRNAs

Circulating miRNAs are present in extracellular membrane-covered microvesicles (exosomes, shedding microvesicles and apoptotic bodies), as well as in extracellular protein complexes such as HDL and the RNA-induced silencing complex.[126,127] Microvesicles can contain common protein markers, as well as unique proteins that are reflective of the cells from which they originate.[128] These structures protect the circulating miRNAs from ribonuclease activity and degradation.[129] Evidence from studies of healthy individuals and patients with various diseases, such as cancer and pregnancy-related diseases, suggests that 100–500 different miRNAs can be present in the circulation.[130–133]

Microvesicles can be secreted by adipocytes. Approximately 7,000 adipocyte-specific mRNAs and 140 miRNAs were detected in exosomes derived from a mouse adipocyte cell line.[134] In adipose tissue, adipocytes might communicate with each other through microvesicles, and transferred miRNAs seems to be involved in the transcription of multiple genes that are required for lipid synthesis and cell growth.[135] miRNAs in adipocyte-derived exosomes could also control lipid storage in adipocytes and the size of these cells.[136,107] In a mouse model of obesity, extracellular vesicles released from adipose tissue were incorporated into macrophages that secreted increased levels of IL-6 and TNF, and contributed to development of insulin resistance.[137] Exposure of cultured liver cells to adipocyte-derived microvesicles containing miRNAs that were obtained from individuals with obesity resulted in disruption of TGFβ signalling.[138]

Distinct serum and plasma miRNA expression signatures have been reported in individuals with obesity and T2DM.[139,140] Dysregulation of miR-197, miR-23a, miR-509-5p, miR-130a-b, miR-195, miR-27a and

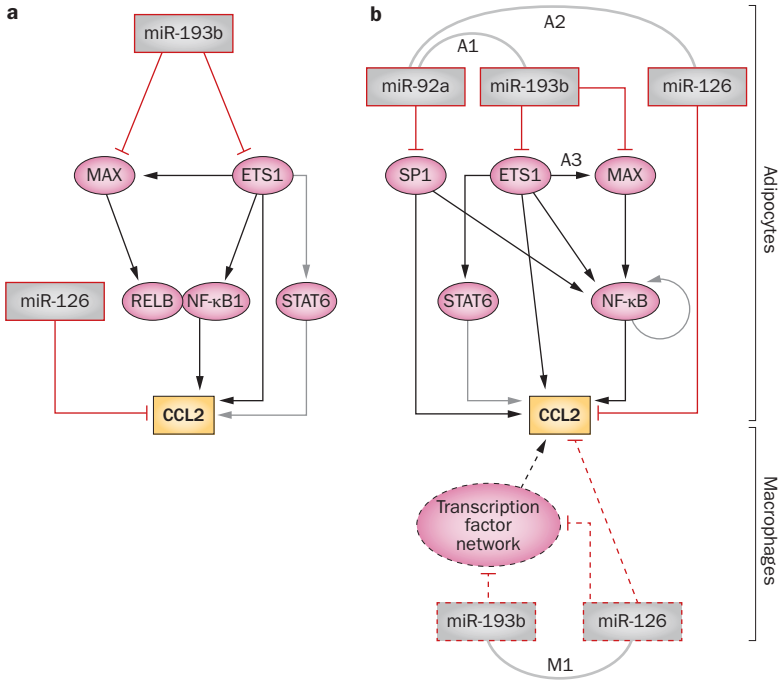

Figure 4 | miRNA regulatory circuits that control levels of CCL2 in human white adipose tissue. **a** | The experimentally verified transcriptional network directly or indirectly regulating CCL2 production in human adipocytes includes miR-126 and miR-193b and five transcription factors (ETS1, MAX, NF-κB, RELB, and STAT6). Experimentally validated or previously described interactions between network members are indicated in bold. Thin lines indicate prediction by network analyses.[32] **b** | Transcription factors (ETS1, MAX, SP1 and an NF-κB subnetwork) and miRNAs (miR-92a, miR-126 and miR-193b) regulate CCL2 production in a combinatorial manner in human adipocytes and macrophages.[124] The experimentally verified network in adipocytes is shown in the upper part. The partly validated model in macrophages is shown in the lower part. miRNAs might regulate CCL2 in human adipose tissue in an additive manner in at least three ways: two miRNAs acting through a transcriptional regulatory network (A1); a single miRNA acting through transcription factors in a transcriptional regulatory network with other miRNAs acting directly on CCL2 (A2); a single miRNA acting on a few transcription factors in a transcriptional regulatory network (A3). In macrophages, two miRNAs could act either directly on CCL2 or through a network of transcription factors (M1). T bars indicate inhibition; arrows indicate stimulation. Bold lines represent interactions between network players in adipocytes and thin lines are interactions predicted by network analyses and/or shown in other cell types. Dashed lines in the lower part represent possible interactions. The networks presented do not include transcription factors without known DNA-binding motifs and coregulators of transcription factors. Permission for part a obtained from American Diabetes Association © Arner, E. *et al.* Adipose tissue microRNAs as regulators of CCL2 production in human obesity. *Diabetes* **61**, 1986–1993 (2012). Permission for part b obtained from American Diabetes Association © Kulyté, A. *et al.* Additive effects of microRNAs and transcription factors on CCL2 production in human white adipose tissue. *Diabetes* **63**, 1248–1258 (2014).

miR-320a in the circulation has been associated with features of the metabolic syndrome.[136,141] Additionally, circulating levels of miR-17-5p and miR-132 were decreased in obesity and this change was also reflected in the expression of miRNAs in omental fat tissue from the same individuals.[33] An altered circulating miRNA profile (mostly reduced expression) is linked to T2DM, and miR-15a, miR-29b, miR-126, miR-223 and miR-150 have been proposed to be biomarkers for T2DM.[142,143] Levels of miRNAs in plasma are dysregulated in men with morbid obesity and this expression signature can change with extensive weight loss.[142] On the basis of these findings, miR-142-3p, miR-140-5p, miR-15a, miR-520c-3p and miR-423-5p have been proposed as biomarkers for risk estimation and classification of patients with morbid obesity.[144] Furthermore, the association of circulating miRNAs with the metabolic syndrome is sex-dependent,[145] and levels of these miRNAs are dysregulated in prepubertal children with obesity.[146]

Extracellular miRNAs might act as endocrine or paracrine signalling molecules to deliver regulatory messages inside recipient cells. Circulating levels of miRNAs are influenced by obesity and associated metabolic abnormalities. However, circulating miRNAs exist in low concentrations in serum and might only have modest effects on target genes. Nevertheless, detailed studies are needed to reveal the functions of extracellular adipose miRNAs and how they affect gene functions in recipient cells, as well as their endocrine and paracrine roles and their potential use as biomarkers.

Targeting miRNAs in obesity and T2DM

Given that miRNAs can target numerous genes, miRNA mimetics and inhibitors offer an attractive option for development of tissue-specific therapeutic interventions. Antisense nucleotides (antagomirs) were intravenously injected into mice and blocked miRNA functions.[147] Antagomirs coupled to cholesterol can specifically inhibit miRNAs in the liver and have been investigated in rodent models.[148] Locked nucleic acids (also known as inaccessible RNAs) have been shown to inhibit the liver-expressed miR-122 in primates.[149] A proprietary compound that targets miR-122 for treatment of hepatitis C virus infection in humans is undergoing phase II trials.[150] Delivery of a mimetic of miR-34 (which is downregulated in many cancers) is currently undergoing phase I trials.[151] To our knowledge, several other miRNAs that either inhibit or mimic miRNA actions are currently undergoing preclinical testing in rodent models. Of these, targeting miR-103/107 and miR-155 is of particular interest as these miRNAs target metabolic and inflammatory pathways, respectively.[152] However, adipose-specific miRNA targeting has not yet been tested in trials. For adipose tissue, it might be of particular interest to target the regulatory network of miRNAs that contribute to local inflammation in WAT.[32,64,124] Targeting the angiogenesis that is crucial for adipose tissue function and expansion could be used as a therapeutic strategy for obesity.[153] It might also be possible to directly interfere with the macrophages resident in adipose tissue by administration of miRNA mimetics or inhibitors in lipophilic carriers.[154] Furthermore, the possibility of 'browning' of WAT by miRNAs as part of novel anti-obesity therapies to increase thermogenesis is a tantalising speculation. We anticipate that clinical trials that test the applicability of circulating miRNAs as novel diagnosis tools for metabolic diseases will come in the near future. Of note, other forms of miRNA therapeutics have been reviewed elsewhere.[79,155] In summary, a number of issues, such as gene selectivity, delivering of miRNA therapeutics, adverse effects, cellular context and

treatment efficacy, need to be resolved before adipose-specific miRNA agents that have been revealed in experimental research on mice or humans can be thoroughly tested in clinical trials.

Conclusions

miRNAs regulate adipogenesis and cell-specific functions of fat cells. In addition, miRNAs might have pathophysiological functions and have been associated with impaired adipogenesis, insulin resistance and obesity-related inflammation. Several miRNAs are present in human adipose tissue; however, only a few are differentially expressed in obesity or display regional variation in their expression. The challenge remains to determine the biological importance of many of these miRNAs and how they are regulated and/or function in human adipose tissue. For example, their actions in fat cells compared with other cell types within adipose tissue must be understood. If miRNAs have a causative role in metabolic complications associated with obesity or in the development of hypertrophic and/or hyperplastic WAT needs to be elucidated. In addition, the role of adipose miRNA in regulating extracellular factors that are altered in obesity, such as hormone levels and circulating metabolites, remains largely unknown.

Given that a single miRNA might fine-tune the expression of hundreds of mRNA transcripts and that each mRNA might be targeted by hundreds of miRNAs, either directly or indirectly through networks involving transcription factors and their co-regulators, miRNA regulatory networks have enormous capacity and complexity. Inflammation in human WAT is, at least in part, controlled by such miRNA networks that also amplify the effects of miRNAs on the metabolic and endocrine function of fat cells. However, regulation of human adipose tissue functions by miRNA signalling networks remains to be established.

The discovery of stable, reproducible and tissue-specific miRNAs in serum supports their potential for use as novel biomarkers. Extracellular miRNAs originating from adipose tissue could be used for classification and management of obesity. Moreover, miRNAs are likely to be involved in communication between fat cells, as well as between adipose and other tissues, which makes them attractive as therapeutics that can be specifically targeted to adipose tissue. Finally, specific phenotypes of adipose tissue, such as chronic inflammation, might be viable targets for miRNA therapy to combat T2DM and other metabolic complications that are associated with obesity.

Review criteria

We systematically searched PubMed using different combinations of the following words: “microRNA”, “obesity”, “diabetes”, “adipose tissue”, “inflammation”, “adipogenesis”, “circulating miRNAs”, “extracellular vesicles”, “regulatory/gene networks” and “exosomes”. Articles published until October 2014 were selected. Whenever possible, the most recent and complete reviews on the topic were chosen. All selected articles are in English.

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