

MicroRNAs in metabolism and metabolic disorders

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Abstract | MicroRNAs (miRNAs) have recently emerged as key regulators of metabolism. For example, miR-33a and miR-33b have a crucial role in controlling cholesterol and lipid metabolism in concert with their host genes, the sterol-regulatory element-binding protein (SREBP) transcription factors. Other metabolic miRNAs, such as miR-103 and miR-107, regulate insulin and glucose homeostasis, whereas miRNAs such as miR-34a are emerging as key regulators of hepatic lipid homeostasis. The discovery of circulating miRNAs has highlighted their potential as both endocrine signalling molecules and disease markers. Dysregulation of miRNAs may contribute to metabolic abnormalities, suggesting that miRNAs may potentially serve as therapeutic targets for ameliorating cardiometabolic disorders.

P-bodies

Distinct cytoplasmic foci that contain a number of enzymes involved in mRNA turnover. They are important for mRNA degradation, storage of mRNA for translation and translational repression by microRNAs.

Proper control of metabolic homeostasis is crucial to the maintenance of human physiology and health. Accordingly, intricate and interwoven regulatory networks have evolved to monitor and respond to changes in environmental conditions and physiological states. Work over several decades has suggested that much of the orchestration of cellular and physiological responses to altered dietary and metabolic conditions occurs at the level of gene regulation in the cell nucleus. Indeed, a number of key transcription factors, including peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), sterol-regulatory element-binding proteins (SREBPs), carbohydrate response element-binding protein (CHREBP), CCAAT-enhancer-binding protein (C/EBP) and forkhead box protein O1 (FOXO1), respond directly or indirectly to nutrients and metabolic cues such as cholesterol, lipids, glucose and insulin to rapidly alter gene expression programmes governing metabolic homeostasis^{1–5}.

A class of small non-coding RNAs termed microRNAs (miRNAs) has recently been found to represent another crucial regulatory layer overlaying and intersecting with transcriptional control mechanisms in guiding metabolic homeostasis. Initially discovered in the nematode *Caenorhabditis elegans* as regulators of developmental timing, numerous miRNAs have subsequently been found in species from plants to humans, with regulatory roles touching on all aspects of biology. The biogenesis of miRNAs is described in BOX 1 (REFS 6,7).

In contrast to plants, in which miRNAs are often fully complementary to their mRNA targets and promote RNA cleavage and degradation, metazoan miRNAs typically

exhibit only partial sequence complementarity to their mRNA targets, and initial studies suggested that they promote translational repression rather than cleavage of the mRNA⁸. However, it has recently become apparent that metazoan miRNAs may also affect mRNA stability by promoting mRNA deadenylation and subsequent sequestration and turnover in P-bodies⁹.

Although functional validation is frequently lacking, target prediction databases based primarily on Watson–Crick base-pairing (for example, TargetScan, miRanda and Pictar^{10–12}) have suggested that miRNAs may have hundreds of mRNA targets, thereby rivalling transcriptional mechanisms in regulatory output complexity. However, whereas transcription factors may elicit profound changes in mRNA expression levels, single miRNAs typically exert relatively modest effects on individual mRNA targets and are thought to act primarily as ‘rheostats’ that modulate protein expression in a nuanced fashion⁷. However, single miRNAs can have multiple target sites in the 3′ untranslated regions (UTRs) of a particular mRNA, thereby increasing repression efficiency. Furthermore, mRNAs are predicted to be targets of many distinct miRNAs, suggesting that different miRNAs might act in a concerted manner to regulate mRNA translation and turnover¹³. As discussed below, certain miRNAs have also been shown to affect multiple targets in linear pathways or interconnected nodes in regulatory networks, thereby exerting a larger cumulative effect¹⁴. miRNAs are also frequently found to act in feedforward and feedback regulations that can amplify or dampen signal output¹⁵, making timing of analysis after miRNA perturbation crucial to an accurate assessment of the regulatory impact.

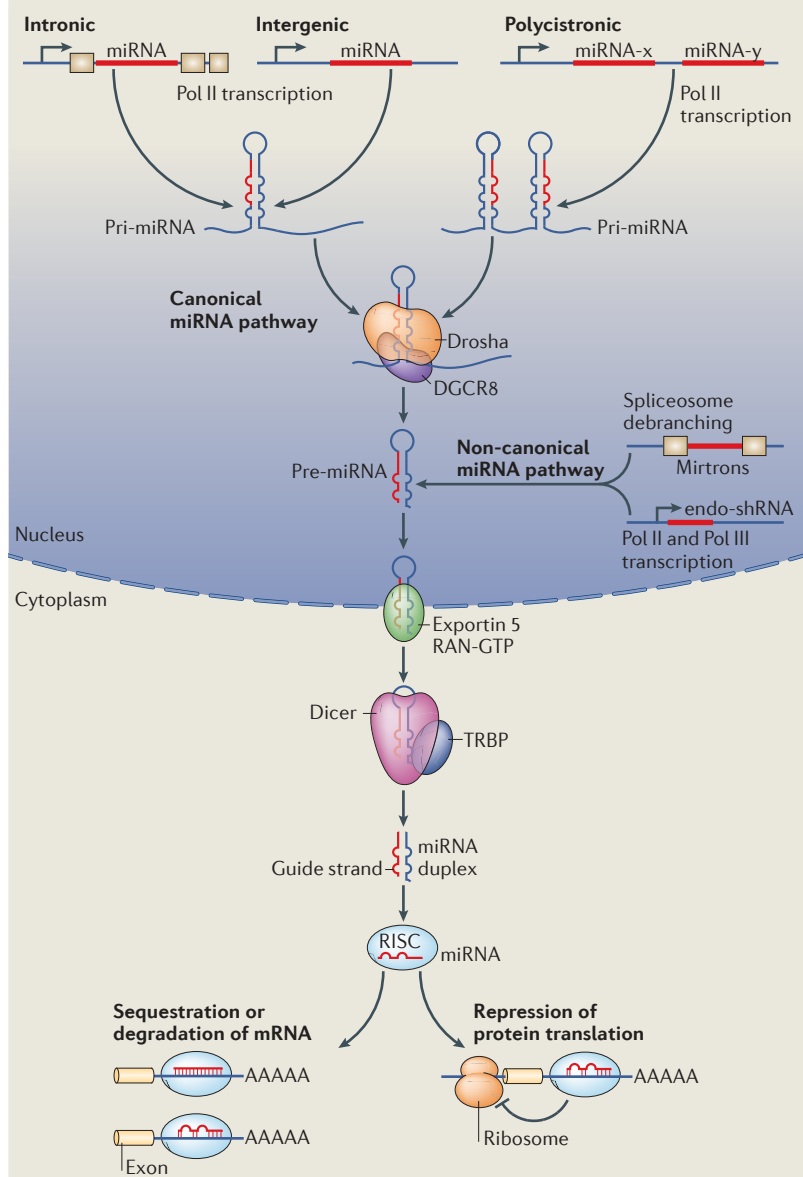
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Box 1 | The miRNA biogenesis pathway

MicroRNAs (miRNAs) generated by the canonical biogenesis pathway are transcribed as precursor RNAs from intergenic, intronic or polycistronic genomic loci by RNA polymerase II (Pol II)⁶. The primary miRNA (pri-miRNA) transcript forms a stem-loop structure that is recognized and processed by the Drosha and DGCR8 RNase III complex or the spliceosome apparatus in the nucleus. In the non-canonical miRNA pathway, miRNAs are transcribed directly as endogenous short hairpin RNAs (endo-shRNAs) or derive directly through splicing from introns that can refold into hairpins (mirtrons) (reviewed in REF. 134). The trimmed precursor (pre-miRNA) hairpins from both canonical and non-canonical miRNA pathways are then transported by an exportin 5 and RAN-GTP-dependent process to the cytosol, where they are typically further processed by the Dicer and transactivation-response RNA-binding protein (TRBP) RNase III enzyme complex to form the mature double-stranded ~22-nucleotide miRNA. Argonaute proteins (for example, AGO2, not shown) then unwind the miRNA duplex and facilitate incorporation of the miRNA-targeting strand (also known as the guide strand) into the AGO-containing RNA-induced silencing complex (RISC). The RISC-miRNA assembly is then guided to specific target sequences in mRNAs. The initial recognition of mRNAs by the RISC-miRNA complex is driven primarily by Watson-Crick base-pairing of nucleotides 2 to 8 in the mature miRNA (termed the seed sequence) with specific mRNA target sequences chiefly located in the 3' untranslated region, and additional base-pairing affords greater affinity and targeting efficiency⁷.



Finally, whereas miRNA functions under normal physiological conditions might be integrated into multi-layered control circuits ensuring proper development and homeostasis, dysregulation of miRNA expression or function in response to intrinsic factors (genetic or epigenetic) or extrinsic factors (environmental cues or stress) may contribute to aberrant gene expression patterns underlying abnormal developmental patterning or metabolic dysfunction. Although it is clear that the complex mechanisms of action and impact of miRNAs on animal development, physiology and disease need much further study, progress has been made in elucidating the individual roles of certain miRNAs in specific biological contexts.

In this Review, we discuss recent advances in our understanding of the emerging roles of miRNAs in controlling cholesterol and lipid homeostasis, with particular emphasis on the well-characterized liver-specific miRNA miR-122 and the regulatory circuit comprising miR-33a and miR-33b miRNAs and their SREBP host genes. The role of miRNAs such as the related miR-103 and miR-107 in controlling insulin signalling and glucose homeostasis is also highlighted. The potential pathological functions of miRNAs such as miR-33 and miR-34a in conditions associated with metabolic syndrome (MetS) are discussed, and we cover current efforts to therapeutically target specific metabolic miRNAs. Finally, we briefly discuss circulating miRNAs, with emphasis on new data linking miRNAs with lipoproteins and possible functions of miRNAs as endocrine signalling molecules.

miRNA regulation of lipid homeostasis

Although roles for miRNAs were first described in the regulation of metazoan development, recent studies have revealed that miRNAs also play key parts in controlling metabolic homeostasis. Below, we discuss the functions of the liver-specific miRNA miR-122 and the miR-33-SREBP host gene circuit in cholesterol and lipid homeostasis and highlight new and exciting data pointing to important physiological and therapeutic implications of these miRNAs in metabolic diseases.

Lipids are structural components of cell membranes (for example, cholesterol and phospholipids) and are important for energy storage (for example, triglycerides) but can also act as signalling molecules (for example, steroid hormones). Lipids such as cholesterol and fatty acids are taken up in the diet and are synthesized *de novo*, predominantly in the liver. Regulation of the biosynthesis of cholesterol, fatty acids and phospholipids is mediated by transcription factors such as SREBPs, the activity of which is under tight feedback control to maintain proper homeostasis (reviewed in REF. 16). Cholesterol and other lipids are transported in the blood by association with lipoproteins such as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL primarily transports cholesterol to peripheral tissues, where the LDL receptor (LDLR) mediates LDL uptake. HDL is important for the removal of cholesterol from peripheral tissues to the liver through a system called reverse cholesterol transport (RCT).

Box 2 | miR-122 and its function in hepatitis C virus propagation

Apart from its function in metabolic control, the microRNA miR-122 has also been shown to promote the propagation of hepatitis C virus (HCV) by multiple mechanisms^{28–30}. In addition to binding the HCV 5' untranslated region to facilitate proper folding of the HCV RNA for translation and replication, miR-122-dependent increase in cholesterol and lipid synthesis may stimulate the production of endoplasmic reticulum-associated lipid droplets and cholesterol and lipid-rich membrane domains (termed 'lipid rafts') that are central sites of viral replication and production¹³⁵. Hence, miR-122 antagonism could inhibit HCV both by preventing direct enhancement of viral RNA folding or replication and by lowering required cholesterol or lipid cofactors for viral packaging or extrusion, suggesting that antisense-based targeting of miR-122 could represent a powerful approach for treating chronic HCV infections, which affect up to 3% of the world's population. Accordingly, weekly subcutaneous injections of miR-122-targeting locked nucleic acid (LNA)-based antisense oligonucleotides (miravirsin, Santaris Pharma A/S) have been found efficacious in decreasing HCV titres in chimpanzees¹³⁶, and early indications from Phase II studies in humans with chronic HCV infection seem promising¹³⁷. Indeed, a 4-week therapy with miravirsin in treatment-naïve patients with chronic HCV genotype 1 infection provides long-lasting suppression of viraemia. This suppressive effect was maintained for more than 4 weeks after the last dose, did not reveal viral resistance and was well tolerated. These studies also showed lowered total cholesterol, and further assessment of miravirsin in humans should provide important information as to whether miR-122 therapeutic targeting might have a positive impact on circulating cholesterol and lipid abnormalities linked to metabolic syndrome. HCV infection is associated with non-alcoholic fatty liver disease¹⁰¹, and inhibition of viral propagation by miR-122-targeting antisense approaches might also ameliorate HCV-associated fatty liver disease. However, the emerging link of miR-122 to hepatocyte differentiation or cell fate determination¹³⁸ may also suggest that attention should be paid to potential deleterious long-term effects, such as the development of hepatocellular carcinoma²⁶.

Aberrant cholesterol and lipid homeostasis represents a crucial risk factor for cardiometabolic diseases prevalent in the developed world, such as MetS¹⁷. For example, the risk of atherosclerosis and coronary artery disease increases with a rise in circulating LDL levels, whereas HDL levels are inversely associated with cardiovascular disease risk¹⁷. Insulin resistance and other metabolic abnormalities can result in excess accumulation of hepatic triglycerides and fatty acids, which is associated with fatty liver diseases (hepatosteatosis) such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which are precursors to more severe liver diseases such as fibrosis and hepatocellular carcinoma¹⁸.

Cholesterol and lipid regulation by miR-122. miR-122 was the first miRNA to be linked to metabolic control. It is expressed primarily in the liver and was initially shown to affect hepatic cholesterol and lipid metabolism but has also been implicated in the maintenance of liver cell differentiation (reviewed in REF. 19). Two early studies showed that antisense targeting of miR-122 results in a vast (~25–30%) reduction in plasma cholesterol levels^{20,21}. First, a study showed that injection of miR-122-targeting antagomirs into mice resulted in altered hepatic expression of several genes, including genes involved in cholesterol biosynthesis, such as 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*Hmgcs1*) and 7-dehydrocholesterol reductase (*Dhcr7*)²¹. Accordingly, they observed significant lowering of plasma cholesterol levels in response to miR-122 antagonism. In a separate study, antisense-based silencing of miR-122 led to decreased hepatic cholesterol and fatty acid

biosynthesis and an increase in fatty acid β -oxidation associated with a reduction in circulating total cholesterol and triglycerides as well as decreased hepatosteatosis in mice on a high-fat diet²⁰. In subsequent studies, miR-122 was successfully targeted by antisense inhibitors using locked nucleic acid (LNA) chemistry²² in non-human primates such as African green monkeys, resulting in lowered circulating cholesterol²³. Antisense targeting of miR-122 was not associated with hepatotoxicity or adverse liver histopathology in mice or non-human primates, indicating the apparent safety of this therapeutic approach.

Taken together, these pioneering studies pointed to the exciting possibility of miR-122 antisense oligonucleotides as a novel treatment strategy for lowering circulating cholesterol, which would represent the first therapeutic targeting of an miRNA. However, the initial enthusiasm waned after it was found that miR-122 antagonism not only lowers LDL levels but also causes a decline in the levels of HDL, both in mice and in non-human primates^{20,23,24}. This presumably deleterious effect has called into question the therapeutic value of miR-122 as a target for the treatment of cholesterol-related disorders. Moreover, although genes involved in cholesterol and lipid metabolism are affected by miR-122 in the liver, these genes do not seem to be direct targets of miR-122 (REFS 19–21, 25). This lack of a mechanistic understanding of the effects of miR-122 on cholesterol homeostasis, and the possibilities of other adverse consequences, such as hepatocellular carcinoma^{26,27}, has also dampened the enthusiasm for the development of miR-122 antisense technologies as a therapeutic approach for the long-term management of cholesterol disorders.

Recently, miR-122 was found to be required for the propagation of hepatitis C virus (HCV), both directly at the level of viral replication and in controlling lipid cofactors required for virus replication and assembly^{28–30}. The therapeutic targeting of miR-122 in this context is very intriguing and represents the first miRNA-targeting drug in human clinical trials (BOX 2).

The SREBP–miR-33 regulatory circuit. The SREBP family of basic helix–loop–helix leucine zipper transcription factors controls the expression of numerous genes involved in cholesterol and fatty acid biosynthesis and uptake and in the production of phospholipids and triglycerides. The human *SREBF1* gene encodes SREBP1A and SREBP1C, which are alternative splice variants that primarily regulate lipogenic genes such as fatty acid synthase (*FASN*), acetyl-CoA carboxylase (*ACC*; also known as *ACAC*), and stearoyl-CoA desaturase (*SCD*). The SREBP2 isoform produced by the *SREBF2* gene preferentially controls the expression of cholesterologenic genes, including *HMGCR* and *LDLR*³.

Intriguingly, both human SREBP-encoding genes were recently found to be host genes to highly conserved miRNAs^{31–35}. The *SREBF1* gene on chromosome 17 harbours *mir-33b* in intron 17, whereas *SREBF2* on chromosome 22 contains *mir-33a* in intron 16. Mature miR-33a and miR-33b only differ in two nucleotides and are predicted to have largely overlapping target gene sets. Of note, rodents lack *mir-33b* in the corresponding intron

Metabolic syndrome (MetS). A combination of metabolic disorders characterized by insulin resistance, obesity, abnormalities in circulating cholesterol and lipid profiles, non-alcoholic fatty liver disease and hypertension. MetS is associated with increased risk of type 2 diabetes and cardiovascular disease (coronary artery disease and stroke).

Endocrine signalling
Hormonal signal, secreted from a specific cell and causing a specific effect in distal target cells. Hormones produced by endocrine cells can travel through the blood to reach all parts of the body.

Triglycerides
Esters of glycerol and fatty acids, used for the storage of energy.

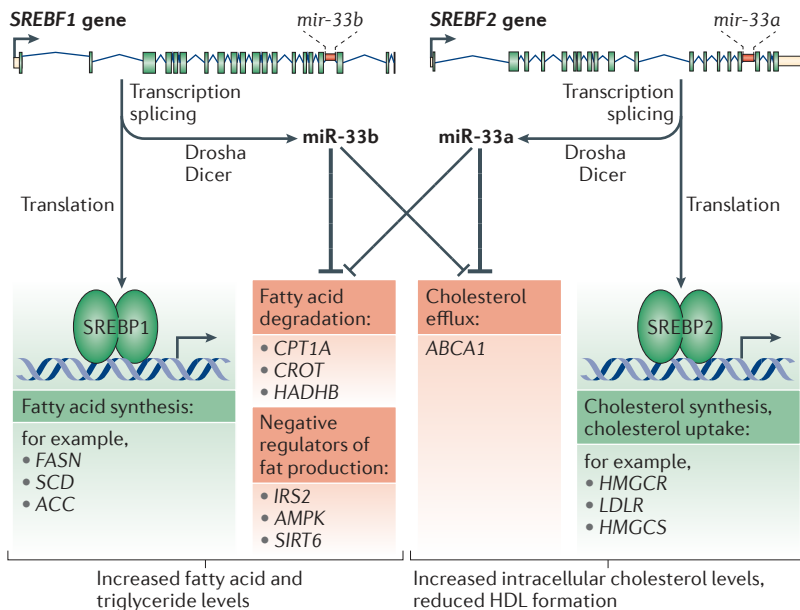


Figure 1 | Model of the SREBP and miR-33 circuit. The sterol-regulatory element-binding protein (SREBP) transcription factors act coordinately with their intronic microRNAs (miRNAs) miR-33a and miR-33b to regulate fatty acid, triglyceride and cholesterol homeostasis. Transcription of the *SREBF1* and *SREBF2* loci gives rise to SREBP1 and SREBP2 and to the miRNAs miR-33b and miR-33a. SREBP1 activates genes involved in fatty acid, phospholipid and triglyceride synthesis (for example, *FASN* (fatty acid synthase), *SCD* (stearoyl-CoA desaturase) and *ACC* (acetyl-CoA carboxylase)), whereas SREBP2 activates genes involved in cholesterol synthesis and uptake (such as *HMGCR* (3-hydroxy-3-methylglutaryl-CoA reductase), *LDLR* (low-density lipoprotein receptor) and *HMGCS* (3-hydroxy-3-methylglutaryl-CoA synthase)). miR-33a and miR-33b act to repress genes functioning in fatty acid β -oxidation (for example, *CROT* (carnitine *O*-octanoyltransferase), *HADHB* (hydroxyacyl-CoA dehydrogenase-3-ketoacyl-CoA thiolase-enoyl-CoA hydratase (trifunctional protein) beta subunit) and *CPT1A* (carnitine palmitoyltransferase 1A)), cholesterol efflux (for example, *ABCA1* (ATP-binding cassette subfamily A member 1)) and negative regulators of SREBPs (for example, *IRS2* (insulin receptor substrate 2), *AMPK1* (AMP-activated protein kinase alpha 1 subunit; also known as *PRKAA1*) and *SIRT6* (sirtuin 6)). HDL, high-density lipoprotein.

in the *Srebp1* gene³⁵; the importance of this will be discussed later. In humans, *mir-33a* and *mir-33b* seem to be extensively co-expressed with their host genes in many cells and tissues^{34,36}, suggesting that they derive from the same primary transcript. This also pointed to a potential coordinated function of the miR-33 isoforms and their host gene products. Indeed, several studies have now revealed extensive collaboration of miR-33a and miR-33b and the SREBP gene regulators in controlling cholesterol and lipid homeostasis (FIG. 1).

miR-33a and SREBP2 control cholesterol homeostasis. Several recent studies reported the discovery of the miR-33a and SREBP2 miRNA–host gene circuit, demonstrating that miR-33a cooperates with the SREBP2 cholesterol transcription factor to boost intracellular cholesterol levels^{31–35} (FIG. 1). miR-33a and miR-33b were found to have a crucial role in the post-transcriptional repression of ATP-binding cassette transporter subfamily A member 1 (*ABCA1*), which promotes the efflux of free cholesterol from within the cell to apolipoprotein A1 (*APOA1*) and is essential for the formation of HDL³⁷. Plasma HDL levels show a strong inverse relationship

with cardiovascular disease risk, and intensive efforts are ongoing to find effective pharmacological approaches to increase HDL levels for the treatment of cardiovascular disease³⁸. *ABCA1* is regulated by the miR-33 isoforms through its 3' UTR, confirming *ABCA1* as a direct miR-33 target. In accord with regulation of *ABCA1* by miR-33, modulation of miR-33a levels also results in changes in cholesterol efflux in mouse macrophages^{32–35}, suggesting that miR-33-targeting antisense approaches might promote RCT from atherogenic macrophages and decrease atherosclerosis. Importantly, in mouse models, hepatic and macrophage *ABCA1* expression and circulating HDL levels increase following inhibition or knockout ablation of miR-33a^{32–35}. Moreover, antisense inhibition of miR-33a in *LDLR*-knockout mice, a well-validated model for hypercholesterolaemia and cardiovascular disease, caused a reduction in atherosclerotic plaque size and lipid content in mice fed a high-fat diet and also promoted RCT, verifying the therapeutic potential of miR-33 targeting for the treatment of cardiovascular disease³⁹.

As noted above, rodents lack miR-33b, complicating the translation and relevance of these results to humans. This difference is important because expression of SREBP1C (and *mir-33b* embedded within the *SREBF1* gene) is highly upregulated following insulin stimulation³⁶. Under high circulating insulin conditions (such as in insulin resistance), increased SREBP1C and miR-33b levels in the liver could thus potentially contribute to both high VLDL levels and low HDL levels found in individuals suffering from MetS⁴⁰. Recent studies have indeed verified that hepatic miR-33b levels are increased in non-human primates in response to a high-carbohydrate diet and show that antisense oligonucleotides targeting miR-33a and miR-33b are effective in increasing HDL levels and lowering VLDL triglycerides, paving the way for future human clinical trials to evaluate the safety and efficacy of antisense targeting of miR-33a and miR-33b for the treatment of cardiometabolic disorders⁴¹ (V.R. and A.M.N., unpublished observations). It is interesting to note that *ABCA1* is also targeted by other miRNAs, such as miR-758 and miR-106b, which were also shown to modulate cellular cholesterol efflux^{42,43}, indicating that *ABCA1* and HDL regulation by miRNAs is likely to be complex.

Other miR-33 targets involved in lipid homeostasis. The discovery of coordinated regulation of cholesterol levels by miR-33a and the SREBP2 host gene product has prompted investigation into whether the SREBP host genes and their intronic miRNAs might act together more broadly to control not only cholesterol but also fatty acid and lipid homeostasis in an integrated manner. Indeed, we and others have now found that miR-33a and miR-33b also regulate intracellular fatty acid and lipid levels in concert with their SREBP host gene products^{14,31,36} (FIG. 1).

First, it was shown that miR-33a and miR-33b directly control the expression of several proteins involved in fatty acid β -oxidation, the process by which fatty acids are degraded in mitochondria and peroxisomes to produce acetyl-CoA destined for the citric acid cycle and ATP for energy generation^{14,31,36}. These proteins include: carnitine *O*-octanoyltransferase (*CROT*), which helps to

Antagomirs

Small, synthetic, cholesterol-conjugated DNA oligonucleotides that are complementary to an endogenous microRNA (miRNA) of interest. The cholesterol moiety allows antagomirs to enter most cell types efficiently, where they specifically bind and sequester endogenous miRNAs.

Fatty acid β -oxidation

Degradation process of fatty acids to acetyl-CoA primarily in the mitochondria. Long chain fatty acids need to be transported via binding to carnitine.

break down very long-chain fatty acids (>20 carbons) in peroxisomes; carnitine palmitoyltransferase 1A (CPT1A), which is the rate-limiting transporter of fatty acids into mitochondria; and hydroxyacyl-CoA dehydrogenase–3-ketoacyl-CoA thiolase–enoyl-CoA hydratase (trifunctional protein) β -subunit (HADHB), which is directly involved in mitochondrial fatty acid β -oxidation^{14,31,36}.

In addition to regulating fatty acid degradation, miR-33a and miR-33b have been shown to control crucial upstream regulators of fatty acid and lipid homeostasis. For example, sirtuin 6 (SIRT6), an NAD⁺-dependent histone deacetylase that has been shown to be a crucial regulator of glucose metabolism and stress resistance^{44–47}, was found to be targeted by miR-33a and miR-33b^{14,36}. Interestingly, a recent study of liver-specific *Sirt6*-knockout mice revealed increased hepatic glycolysis, lipogenesis and triglyceride production, resulting in hepatosteatosis⁴⁸. SIRT6 was shown to directly regulate SREBP1 target genes relevant for fatty acid production, such as *Acc1*, *Scd1* and *Fasn*. This suggests that miR-33-mediated inhibition of SIRT6 expression may result in increased chromatin acetylation and derepression of SREBP-dependent fatty acid biosynthesis genes and increased lipogenesis.

The $\alpha 1$ subunit of AMP-activated protein kinase (AMPK $\alpha 1$) has also been found to be targeted by miR-33a and miR-33b^{14,36,41}. AMPK is a master regulator of cellular energy levels in response to energy stress⁴⁹. It responds to low cellular energy levels (increased AMP/ATP ratio) and acts to reduce energy-consuming processes such as protein, fatty acid and cholesterol synthesis, in addition to activating mitochondrial biogenesis, fatty acid β -oxidation and glucose uptake to promote ATP synthesis. AMPK directly phosphorylates and deactivates several key SREBP lipogenic and cholesterogenic targets, such as ACC1 and HMGCR. AMPK also inhibits SREBPs themselves both indirectly, through LXR⁵⁰, and through direct phosphorylation⁵¹. Thus, negative regulation of AMPK $\alpha 1$ by miR-33a and miR-33b may relieve AMPK inhibition of both SREBPs and their target genes to coordinately boost intracellular levels of cholesterol, fatty acids and other lipids.

Insulin receptor substrate 2 (IRS2) was also recently shown to be a miR-33 target^{14,36}. IRS2 is one of two intracellular adaptor proteins for the insulin receptor (INSR) that relay insulin signalling to downstream effectors (such as phosphoinositide 3-kinases (PI3Ks) and protein kinase B (PKB; also known as AKT)). In mouse models, reduction of IRS2, but not IRS1, has been found to increase SREBP1C expression and activity^{52–54}. Interestingly, in mouse NAFLD models, decreased hepatic levels of IRS2 have been shown to result in a compensatory increase in IRS1 levels, which in turn activates SREBP1 (REF. 55). Thus, miR-33 antisense targeting could potentially result in a reversal of the hepatic changes in IRS2 and IRS1 expression, thereby decreasing SREBP1C levels or activity associated with hepatosteatosis.

Taken together, these results reveal an extensive and integrated network of functional interactions between the SREBP transcription factors and their intronic miRNAs miR-33a and miR-33b to regulate cholesterol and lipid homeostasis (FIG. 1).

miRNA regulation of insulin signalling

Several miRNAs have recently been implicated in controlling both insulin signalling and glucose metabolism at multiple levels⁵⁶. Insulin is a hormone synthesized in and secreted by pancreatic β -cells in response to increased nutrient levels (for example, glucose) in the blood. Insulin acts in concert with glucagon (a hormone produced by pancreatic α -cells, with opposite functions to insulin) to maintain glucose homeostasis. Insulin secretion causes uptake of glucose in muscles and adipose cells (through glucose transporters such as GLUT4), blocks *de novo* production of glucose in the liver and increases storage of nutrients in form of fat, glycogen and protein⁵⁷. Binding of insulin to INSR promotes an intracellular signalling cascade involving INSR tyrosine kinase activity, the adaptor proteins IRS1 and IRS2 and a cascade of kinases including PI3Ks, 3-phosphoinositide-dependent protein kinase 1 (PDK1) and AKT⁵⁸. These kinases affect a complex downstream network of proteins such as glycogen synthase kinase 3 β (GSK3 β), mammalian target of rapamycin complex 1 (mTORC1) and the FOXO family transcription factors⁵⁹. Insulin also activates *SREBF1* transcription and SREBP1C processing and transport, thereby stimulating lipogenesis⁶⁰.

Insulin resistance (a condition where tissues are no longer able to respond adequately to the action of insulin) is one of the most prevalent metabolic abnormalities in the developed world and is strongly associated with obesity, circulating cholesterol and lipid abnormalities and NAFLD, which are all components of MetS⁵⁹. Whereas the mechanisms underlying this complex metabolic disease remain to be fully elucidated, an increasing number of miRNAs have now been implicated in the control of insulin signalling and glucose homeostasis at all steps of regulation, from pancreatic islet development, β -cell differentiation and insulin secretion to target tissue insulin sensitivity and intracellular insulin signalling to downstream effectors (FIG. 2; TABLE 1).

miRNA regulation of pancreatic insulin production. The pancreatic miRNA miR-375 was shown to be required for pancreatic islet development in zebrafish⁶¹ and for the maintenance of pancreatic α -cell and β -cell mass in mice⁶². Interestingly, miR-375 may have a dual role, as miR-375 was also found to decrease insulin exocytosis and secretion at least in part through the repression of myotrophin (*Mtpn*), a gene involved in actin depolymerization and, potentially, in vesicular fusion⁶³. It may additionally affect downstream insulin signalling through direct repression of the insulin signalling intermediate kinase PDK1 (REF. 64). The miR-124a miRNA is co-expressed with miR-375 and, at least in cultured cells, also targets *Mtpn*, suggesting coordinated control of *Mtpn* by several miRNAs⁶⁵. miR-124a is also involved in pancreatic islet development, potentially through regulation of the FOXA2 transcription factor, which is involved in β -cell differentiation, and RAB27A, a GTPase required for insulin secretion^{66,67}. Other miRNAs have also been implicated in controlling pancreatic insulin exocytosis. For example, miR-9 may regulate insulin secretion through its inhibition of the transcription factor one cut homeobox 2

Locked nucleic acid (LNA). A modified DNA oligonucleotide analogue that is locked in an 'N-type conformation'. LNA is capable of recognizing DNA and RNA with high affinity and is resistant to degradation.

Apolipoprotein A1 (APOA1). The major protein component of high-density lipoprotein (HDL) in plasma. It promotes the efflux of cholesterol from tissues to the liver, and defects cause low HDL levels, which are often associated with cardiovascular disease.

Atherogenic macrophages Cholesterol-loaded macrophages that accumulate in the arterial wall and can lead to atherosclerotic lesions.

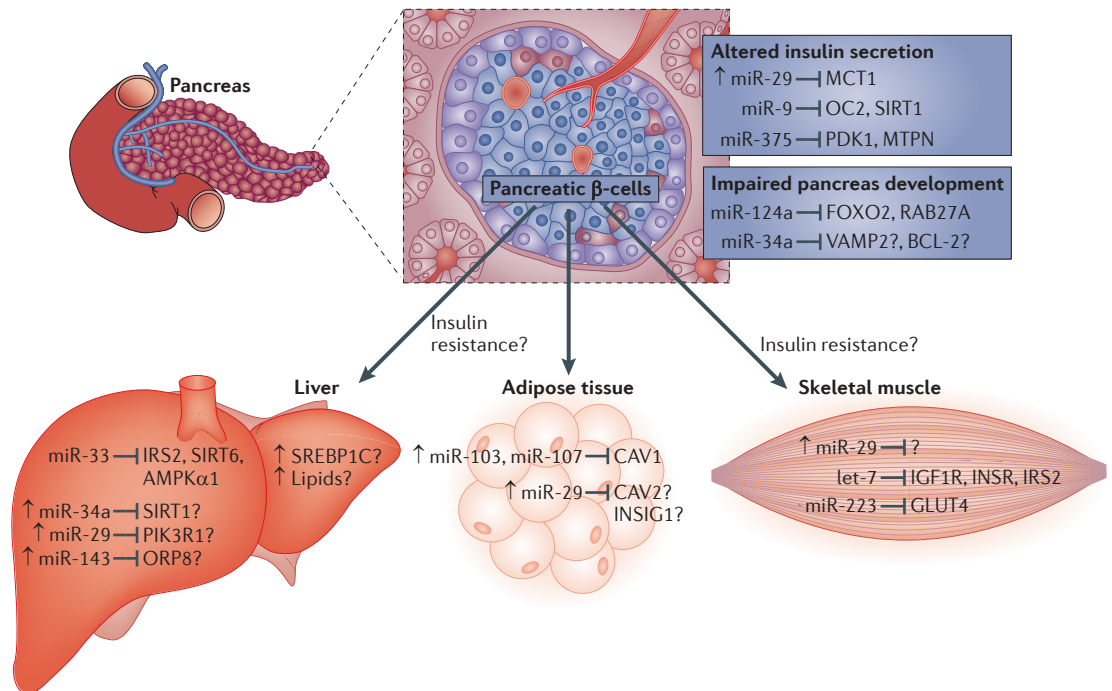


Figure 2 | miRNA regulation of insulin signalling and glucose homeostasis. Normally, following feeding, insulin is produced in pancreatic β -cells and after release will reach target tissues such as the muscle, liver and adipose to cause uptake of glucose, reduce the production of glucose and activate fat production and storage. MicroRNAs (miRNAs) that affect diverse parts of insulin signalling in the pancreas, liver, muscle and adipose tissue have been identified. miR-124a and miR-34a are involved in pancreatic development (through effects on FOXO2 (forkhead box protein O2), RAB27A, VAMP2 (vesicle-associated membrane protein 2) and BCL-2 (B cell lymphoma 2)). miR-29, miR-9 and miR-375 are involved in insulin secretion (miR-29 activates insulin secretion, whereas miR-9 and miR-375 inhibit insulin secretion), acting through MCT1 (monocarboxylate transporter 1), OC2 (one cut homeobox 2), SIRT1 (sirtuin 1), PDK1 (phosphoinositide-dependent kinase 1) and MTPN (myotrophin). miR-33, miR-34a, miR-29 and miR-143 act in the liver on targets involved in insulin signalling and its regulation (such as IRS2 (insulin receptor substrate 2), SIRT6, AMPK α 1 (AMP-activated protein kinase- α subunit 1), SIRT1, PIK3R1 (phosphatidylinositol 3-kinase subunit- α) and ORP8 (oxysterol-binding protein-related protein 8)). Insulin signalling in adipose tissue is modulated by miR-103 and miR-107 (through CAV1 (caveolin 1)) and miR-29 (through INSIG1 (insulin-induced gene 1) and CAV2). The miR-29, let-7 and miR-223 miRNAs may act in muscle to modulate insulin signalling (IGF1R (insulin-like growth factor receptor 1), INSR (insulin receptor) and IRS2) and glucose uptake (GLUT4 (glucose transporter type 4)). Known and predicted targets that lack *in vivo* evidence are marked with a question mark. In disease conditions, such as impaired insulin secretion or insulin resistance, several miRNAs are upregulated (marked with an arrow).

(OC2; also known as ONECUT2) and through SIRT1 (REFS 68,69), whereas miR-29a and miR-29b, which are highly expressed in the pancreatic islets of diabetic mice, inhibit the expression of monocarboxylate transporter 1 (MCT1) and its function in insulin release⁷⁰. Taken together, these findings indicate that miRNAs have key roles in controlling insulin secretion through effects on both pancreatic development and insulin exocytosis.

miRNAs regulate insulin signalling in target tissues. Other miRNAs act in target tissues to regulate responses to insulin and glucose homeostasis. For example, miR-29a and miR-29b, which are upregulated in the muscle, white adipose tissue (WAT) and the liver of diabetic Goto-Kakizaki rats⁷¹, have been linked to insulin resistance, at least in cell culture experiments. The effects of miR-29a and miR-29b were suggested to be mediated through downregulation of proteins that promote insulin signalling such as caveolin 2 (CAV2), a lipid raft-associated protein that responds to insulin levels⁷², insulin-induced gene 1 (INSIG1), a negative regulator of SREBPs, and the

insulin signalling intermediate phosphatidylinositol 3-kinase regulatory subunit- α (PIK3R1)^{71,73}. Another layer of insulin regulation by miRNAs is provided by miR-126, which promotes insulin resistance through the inhibition of IRS1 (REF. 74). Intracellular glucose levels can also be directly regulated by miRNAs. For example, in skeletal muscle, miR-223 was found to inhibit glucose uptake through targeting GLUT4 (REF. 75). As discussed above, miR-33a and miR-33b may also influence insulin signalling and glucose regulation by targeting IRS2, SIRT6 and AMPK α 1 (REFS 14,36). miRNAs are thus acting to control insulin signalling and glucose uptake at multiple levels in target cells and tissues.

A number of miRNAs have also been implicated in metabolic disorders associated with aberrant insulin response, including obesity and NAFLD^{76–82}. For example, the related *mir-103* and *mir-107* (which are located in introns in the pantothenate kinase 1 (*Pank1*), *Pank2* and *Pank3* genes)⁸³ were recently shown to be upregulated in livers of leptin-deficient (*ob/ob*) and diet-induced obese (DIO) mice⁸¹. Interestingly, antisense-mediated silencing

Lipid raft

Microdomain of the cell plasma membrane with high cholesterol and sphingolipid content that compartmentalizes cellular processes by acting as a platform to colocalize proteins such as signalling molecules and receptors.

Table 1 | **MicroRNAs involved in metabolism and metabolic disorders**

miRNA	Target tissue(s)	Function	Target genes*	Refs
miR-103 and miR-107	Adipose, liver	Insulin and glucose homeostasis, adipogenesis	CAV1, DICER	81,82,85
miR-122	Liver	Hepatic lipid metabolism	SLC7A1 (also known as CAT1), ADAM17	20,139,140
miR-124a	Pancreas	Pancreatic islet development	FOXA2, RAB27A	66,67
miR-143	Adipose, liver, pancreas	Adipocyte differentiation, insulin resistance	ERK5 (also known as BMK1 or MAPK7), OSBPL8 (also known as ORP8)	77,80,94
miR-223	Muscle	Glucose uptake, insulin resistance	SLC2A4 (also known as GLUT4)	75
miR-27a	Adipose	Adipogenesis	(PPARG, CEBPA) [†]	141,142
miR-29	Muscle, adipose, liver	Glucose transport	INSIG1, CAV2, SLC16A1 (also known as MCT1), PIK3R1	70,71,73
miR-33a and miR-33b	Liver, macrophage	Cholesterol, lipid and energy homeostasis	ABCA1, NPC1, CPT1A, HADHB, CROT, IRS2, SIRT6, PRKAA1 (also known as AMPKA1)	31–36,39
miR-335	Pancreas, liver, adipose	Insulin production, fatty acid and triglyceride biosynthesis	STXBP1	76,79
miR-34a	Liver, pancreas	Lipid metabolism, B cell exocytosis	SIRT1, VAMP2, ACSL1	88,113,143
miR-375	Pancreas	Insulin secretion, pancreatic islet development	MTPN, USP1, JAK2, ADIPOR2, PDPK1	62–64
miR-378 and miR-378*	Adipose	Adipocyte differentiation, lipid synthesis	ESRRG, GABPA, (ribosomal genes) [†]	144–146
miR-9	Pancreas	Insulin secretion	ONECUT2, SIRT1	68,69
miR-130	Adipose	Adipogenesis	PPARG	147
let-7	Muscle, adipose	Insulin sensitivity	IGF1R, INSR, IRS2, HMGA2	91,148

ABCA1, ATP-binding cassette subfamily A member 1; ACSL1, acyl-CoA synthetase 1; ADAM17, ADAM metalloproteinase domain 17; ADIPOR2, adiponectin receptor protein 2; CEBPA, CCAAT-enhancer-binding protein alpha; CAT1, cationic amino acid transporter 1; CAV, caveolin; CPT1A, carnitine palmitoyltransferase 1A; CROT, O-octanoyltransferase; ERK5, extracellular signal-regulated kinase 5; ESRRG, oestrogen-related receptor gamma; FOXA2, forkhead box A2; GABPA, GA-binding protein alpha; HADHB, hydroxyacyl-CoA dehydrogenase-3-ketoacyl-CoA thiolase-enzyme (trifunctional protein) beta subunit; HMGA2, high-mobility group protein A2; IGF1R, insulin-like growth factor receptor 1; INSIG1, insulin-induced gene 1; INSR, insulin receptor; IRS2, insulin receptor substrate 2; JAK2, Janus activated kinase 2; MTPN, myotrophin; NPC1, Niemann-Pick C1 protein; ONECUT2, one cut homeobox 2; OSBPL8, oxysterol binding protein-like 8; PDPK1, 3-phosphoinositide-dependent protein kinase 1; PIK3R1, phosphatidylinositol 3-kinase subunit p85 alpha; PPARG, peroxisome proliferator-activated receptor gamma; PRKAA1, protein kinase, AMP-activated, alpha 1; SIRT, sirtuin; SLC, solute carrier; STXBP1, syntaxin-binding protein 1; USP1, ubiquitin-specific protease 1; VAMP2, vesicle-associated membrane protein 2.*Human names are used. [†]Genes in parentheses have not been shown to be direct targets.

of miR-103 and miR-107 improved insulin sensitivity and glucose homeostasis, whereas overexpression (predominantly in adipose tissue) was sufficient to cause defects in glucose homeostasis in these mouse models. If confirmed in non-human primates and humans, this suggests that miR-103 and miR-107 may represent therapeutic targets to ameliorate obesity-associated insulin resistance⁸⁴. These miRNAs were proposed to exert their functions on insulin and glucose regulation at least in part through the inhibition of CAV1, which influences lipid raft signalling and affects INSR availability⁸¹. However, it is also possible that some of the effects are mediated through other miRNAs as miR-103 and miR-107 also strongly inhibit the miRNA-processing enzyme Dicer⁸⁵. Indeed, partial knockdown of Dicer and pancreas-specific knockout experiments have revealed that this enzyme (and, presumably, its target miRNAs) is required for both the development and maintenance of the pancreas and insulin signalling^{86,87}.

The expression of several additional miRNAs is increased in obesity models, and these miRNAs exert

regulatory effects on insulin signalling and glucose homeostasis. Similarly to miR-103 and miR-107, miR-143 is overexpressed in obese leptin receptor-mutant (*db/db*) mice and DIO mice⁷⁷. Overexpression of miR-143 reduces insulin sensitivity, presumably through its target oxysterol-binding protein-related protein 8 (ORP8), a protein involved in AKT activation. miR-34a, an miRNA that may have important roles in cancer development through its function in a network with SIRT1 and p53, has additionally been implicated in diabetes as a target to prevent pancreatic β -cell death^{88–90}. Finally, several members of the let-7 miRNA family have been found to be upregulated in *ob/ob* and DIO mice⁸¹. The tumour suppressor roles of let-7 are well studied in cancer biology, but let-7 was recently also shown to be involved in the regulation of glucose metabolism, as overexpression of let-7 in skeletal muscle resulted in insulin resistance and impaired glucose tolerance⁹¹. This effect may, at least partially, be mediated by let-7-targeting of insulin-like growth factor receptor 1 (IGF1R), INSR and IRS2.

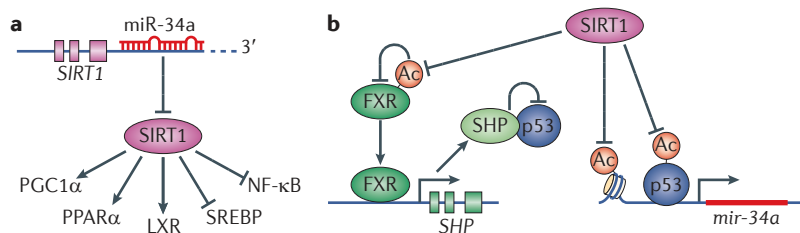


Figure 3 | The regulatory loop of miR-34a, SIRT1, FXR and p53. The microRNA miR-34a is highly expressed in patients with non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and type 2 diabetes. At the molecular level, miR-34a has been shown to exert its function through its effect on sirtuin 1 (SIRT1). **a** | miR-34a inhibits SIRT1 expression and reduces its protein levels, thereby preventing the activation of peroxisome proliferator-activated receptor- α (PPAR α), PPAR γ co-activator 1 α (PGC1 α) and liver X receptor (LXR) (key regulators of cholesterol, lipid and energy homeostasis). Furthermore, miR-34a-mediated SIRT1 repression results in inhibition of sterol-regulatory element-binding proteins (SREBPs) and nuclear factor- κ B (NF- κ B) (activators of lipogenesis and cholesterol synthesis and of inflammation, respectively). **b** | miR-34a is in turn inhibited by SIRT1 in a regulatory loop that includes miR-34a, SIRT1, farnesoid X receptor (FXR) and p53 to affect cholesterol, lipid and energy homeostasis and inflammation. SIRT1 feedback inhibits miR-34a in several ways: it deacetylates p53 and inhibits p53-dependent transcriptional activation of *mir-34a*. In addition, SIRT1 inhibits the *mir-34a* promoter through histone deacetylation. Finally, SIRT1 deacetylates and activates FXR. FXR transcriptionally activates small heterodimer partner (SHP), which sequesters p53 and thus inhibits miR-34a transcription.

In conclusion, some miRNAs seem to affect multiple aspects of insulin signalling and glucose homeostasis, from pancreatic insulin production to insulin sensitivity and regulation of glucose uptake in target tissues. However, much work remains to elucidate the *in vivo* contribution of specific miRNAs to prevalent pathological conditions, such as obesity-associated insulin resistance, and to evaluate their potential for therapeutic targeting.

miRNAs in obesity and hepatosteatosis

Conditions of nutritional excess and lack of physical activity can result in excessive fat storage manifesting in obesity and NAFLD and strongly increase the risk for cardiovascular disease and type 2 diabetes. Normal adipogenesis involves regulation by transcriptional factors such as peroxisome proliferator-activated receptor- γ (PPAR γ) and its co-activators (for example, PPAR γ co-activator 1 α (PGC1 α)) as well as members of the C/EBP family and Krüppel-like factors (KLFs) (reviewed in REF. 92). miRNAs have also recently been implicated in adipogenesis⁹³. For instance, miR-143 has been shown to be important in adipocyte differentiation of cultured mouse 3T3-L1 pre-adipocytes, perhaps through its regulation of extracellular signal-regulated kinase 5 (ERK5; also known as MAPK7)⁹⁴. Several other miRNAs, including miR-204, miR-141, miR-200a, miR-200b, miR-200c and miR-429, are involved in early adipocyte cell fate determination, whereas the miR-17–miR-92 miRNA cluster, miR-130, miR-27a and miR-27b as well as miR-378 and miR-378* have been suggested to be involved in terminal differentiation and mature white adipocyte function (recently reviewed in REF. 95). Brown adipose tissue (BAT), which is a highly metabolically active adipose tissue type involved in thermogenesis and energy expenditure, has been suggested to correlate inversely with obesity⁹⁶. A recent study reported a key role for the miR-193b–miR-365 miRNA

cluster in brown fat differentiation, in part by repressing myogenesis⁹⁷. The accumulating data thus indicate that miRNAs are acting as central modulators of normal WAT and BAT differentiation and biology. A growing number of studies have also found obesity-associated alterations in expression levels of miRNAs in WAT^{82,98–100}. For example, Xie *et al.*⁸² showed that certain miRNAs involved in adipogenesis exhibit inverse expression patterns in obesity. Although many miRNAs have been linked to adipogenesis *in vitro*, or have altered expression in WAT in obese individuals and in animal obesity models, evidence for a causative role in obesity-related diseases is still lacking, and much additional detailed *in vivo* work is needed to clarify the regulatory roles of individual miRNAs in modulating energy balance and adipose biology and their potential contribution to obesity.

The hepatic miR-34a and SIRT1 regulatory circuit. As mentioned above, several studies have measured miRNA expression in metabolic tissues in rodent models of obesity, type 2 diabetes and NAFLD^{78,81,82}. Interestingly, a number of miRNAs that were dysregulated in these models were also changed in human patients with obesity-related NAFLD and NASH^{81,101–103}. Among these, miR-34a may play a particularly relevant part in hepatic metabolic diseases. Patients with NAFLD and NASH exhibit increased hepatic expression of miR-34a^{101,102}, and miR-34a levels were also higher in subjects with type 2 diabetes as compared to healthy controls¹⁰³. A plausible molecular mechanism for the function (or functions) of miR-34a in metabolic control has recently been suggested (reviewed in REF. 104, summarized in FIG. 3). This involves an intricate regulatory network of miR-34a, SIRT1, farnesoid X receptor (FXR) and p53. SIRT1 is a key sensor and regulator of metabolic states, as it responds to NAD⁺ levels in the cell and directly deacetylates and modulates both histone and non-histone targets, including important metabolic regulators such as PGC1 α , PPARs, p53, FOXO1, LXR, FXR and SREBPs, to alter the expression of transcriptional programmes governing cholesterol, lipid and energy homeostasis^{105–112}. SIRT1 was recently shown to be regulated by miR-34a, causing p53-dependent apoptosis in human colon cancer cells¹¹³. Subsequent work showed that miR-34a also inhibits SIRT1 expression in the liver¹¹⁴. The current data suggest that miR-34a, SIRT1, p53 and FXR constitute a regulatory loop: miR-34a inhibits SIRT1 and is itself regulated by SIRT1 through several mechanisms. First, SIRT1 directly inactivates the *mir-34a* promoter through histone deacetylation. Second, SIRT1 deacetylates and inactivates p53, a transcriptional activator of *mir-34a*^{115–118}. Finally, SIRT1 deacetylates FXR, which then activates the liver repressor small heterodimer partner (SHP; also known as NR0B2). SHP sequesters p53 away from the promoter of miR-34a, which results in reduced miR-34a levels^{105,114}. Low hepatic levels of SIRT1 and high levels of miR-34a are associated with fatty liver disease. We speculate that miR-34a regulation of SIRT1 could affect other key metabolic targets of SIRT1, such as SREBPs, PPARs, PGC1 α , LXR and FOXO1, potentially further contributing to hepatosteatosis (FIG. 3). A crucial

next step is to investigate whether antagonizing increased miR-34a levels in models for NAFLD and obesity has an effect on SIRT1 expression and deacetylation of SIRT1 downstream targets. SIRT1 activators, such as resveratrol and other more potent synthetic inducers, improve hepato-steatosis and insulin resistance in DIO mice^{111,119–121}. If verified *in vivo*, activation of SIRT1 through anti-sense inhibition of miR-34a could potentially represent an alternative therapeutic avenue for the treatment of NAFLD and other obesity-related diseases.

Circulating miRNAs as ‘endocrine signals’?

Recent studies have found that miRNAs can be readily detected in human plasma, suggesting possibilities for novel disease biomarker discovery (reviewed in REFS 122,123) and the intriguing notion that some miRNAs (endocrine-miRNAs) may serve regulatory purposes in target cells. Plasma fractionation schemes initially isolated circulating miRNAs as part of microvesicles and exosomes derived from plasma membranes of ‘donor’ cells¹²⁴. Subsequent findings indicate that miRNAs can also be found in lipid-free protein assemblies, such as in association with the miRNA-processing enzyme Argonaute 2 (AGO2)^{125–127}. The discovery of circulating miRNAs represents an important advance for biomarker discovery, and altered circulating miRNA profiles have already been linked to disease states, including NAFLD, NASH and hepatic injury (miR-122)¹⁰², atherosclerosis (miR-223)¹²⁸, type 2 diabetes (miR-126)¹²⁹ and hypertension (let-7e)¹³⁰. It is likely that additional circulating miRNAs will be identified as biomarkers for metabolic diseases, and this represents a rapidly evolving area of research.

Endocrine miRNAs? Interestingly, circulating miRNAs traffic from donor cells to distant sites and alter the gene expression output of recipient cells¹³¹. This suggests that circulating miRNAs are not merely ‘vesicular passengers’ but may rather act as endocrine or paracrine signalling molecules to deliver a regulatory ‘message’ from donor to target cells. However, in comparison with the highly specific and potent action of classical endocrine hormones such as steroids¹³², circulating miRNAs may have only modest effects on mRNA and protein output. In a similar way to mRNAs taken up by cells from microvesicles¹³³, miRNAs presumably do not require specific receptors for their action and are therefore only limited by the fact that their target mRNAs need to be expressed in target cells for the miRNA to have an impact, and it is unclear whether miRNAs are regulated by intercellular feedback mechanisms. Nevertheless, this intriguing notion should be thoroughly investigated. Could specific classes of miRNAs be enriched in different types of vesicles and lipoprotein particles, with distinct cellular targeting potential depending on surface lipid and protein content, thus allowing selective messaging to specific organs or cell types? If so, might specific miRNAs that are highly abundant in certain vesicles and lipoprotein particles exert ‘mass action’ effects on gene expression programmes in target cells or tissues, thereby acting in a more potent manner akin to classic endocrine cues?

Exosomes

Small (30–90 nm) vesicles secreted from the plasma membrane of mammalian cells. They contain proteins, RNA and microRNA molecules and can be transported from cell to cell.

Paracrine signalling

Signalling mediated by secreted molecules that act locally.

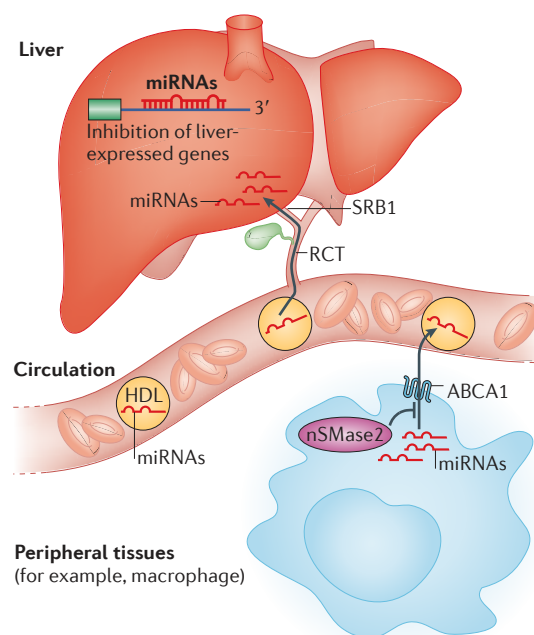


Figure 4 | Model for the function of circulating miRNAs associated with HDL. MicroRNAs (miRNAs) such as miR-375 and miR-223 are produced in peripheral tissues and incorporated into high-density lipoproteins (HDLs). This incorporation is controlled by the cholesterol transporter ATP-binding cassette subfamily A member 1 (ABCA1) and inhibited by neutral sphingomyelinase 2 (nSMase2). miRNAs associated with HDL are thought to be trafficked to the liver through the reverse cholesterol transport (RCT) pathway and taken up by a scavenger receptor class B member 1 (SRB1)-dependent mechanism. Once inside target cells, miRNAs can then exert inhibitory effects on a range of target genes.

miRNAs in HDL. An exciting new study revealed that miRNAs, such as miR-375 and miR-223, can also be found in HDL particles, suggesting new potential roles for HDL, and perhaps other lipoprotein assemblies, in transporting not only cholesterol and other lipids and proteins but also regulatory RNAs¹²⁸. Vickers *et al.*¹²⁸ found that certain endogenous miRNAs (for example, miR-135a*, miR-375 and miR-223) are exported from peripheral cells in HDL particles. Export of miRNAs to HDL was shown to be controlled by neutral sphingomyelinase 2 (nSMase2; also known as SMPD3), the rate-limiting enzyme in ceramide biosynthesis, suggesting that plasma membrane lipids such as sphingomyelin and ceramides are involved in controlling miRNA incorporation into HDL.

Once miRNAs are embedded in HDL, they are probably trafficked to the liver by the classic RCT pathway for uptake in a scavenger receptor class B member 1 (SRB1; also known as SCARB1)-dependent manner and may exert effects on hepatic gene expression programmes (FIG. 4). Consistent with this notion, Vickers *et al.*¹²⁸ showed that treatment of hepatic Huh-7 cells with HDL loaded with exogenous miRNAs (for example, miR-223) could indeed affect target mRNA expression. Interestingly, they also found that HDL miRNA profiles differ between normal subjects and atherosclerotic individuals, both in

mice and humans. This warrants follow-up studies to determine whether circulating miRNAs associated with HDL in patients with heart disease could participate in the disease process, or whether specific miRNAs associated with HDL might constitute part of the protective function against atherosclerosis ascribed to HDL. Finally, plasma HDL-miRNA profiling might serve as a novel biomarker tool for detecting or monitoring cardiovascular disease progression.

Concluding remarks

miRNAs are important regulators of numerous aspects of animal development, physiology and disease and their function in the control of metabolic homeostasis has recently emerged. The best-characterized metabolic role for miRNAs is in the maintenance of normal cholesterol and lipid homeostasis (for example, miR-122 and miR-33). New data reveal that miRNAs also contribute to insulin signalling and glucose homeostasis and highlight the potential pathological roles of aberrant miRNA expression in cardiometabolic disorders such as obesity, NAFLD, insulin resistance, type 2 diabetes and coronary artery disease. The exciting finding that miRNAs can be found circulating in the blood not only points to their potential use as biomarkers for disease states, but also suggests that circulating miRNAs may be actively secreted from donor cells and tissues to act as signalling molecules that affect the gene expression output in distant target organs and cells (for example, in the liver in the case of HDL-associated miRNAs), akin to classical endocrine cues.

The emergence of miRNAs as important regulators of metabolism has garnered much interest not only from a scientific point of view but also from a clinical perspective, with the potential contribution of aberrant miRNA expression and therapeutic implications for the treatment of cardiometabolic diseases raising considerable excitement. Therapeutic efforts to treat metabolic disorders have traditionally centred on the inhibition of what are perceived as 'druggable' targets, such as enzymes (for example, HMGCR targeted by cholesterol-lowering statins). However, the recent discovery by us and others that certain miRNAs may represent crucial regulators of metabolism has provided important insights into metabolic regulatory networks that are already yielding novel types of therapeutic targets and strategies for the treatment of metabolic diseases. For example, the central and concerted role of miR-33a and miR-33b and their SREBP host genes in controlling metabolic homeostasis, together with promising data from mice and non-human primate models, suggest that antisense targeting of miR-33a and

miR-33b for the treatment of MetS and cardiovascular disease should be explored further. It is likely that other miRNAs with regulatory roles in human metabolism (for example, miR-103 and miR-107 in insulin resistance) may also serve as suitable candidates for therapeutic intervention.

A number of open questions remain concerning the function of miRNAs in metabolic control. For example, although it is apparent that several miRNAs, including miR-33a and miR-33b, have an important regulatory impact on cholesterol and lipid metabolism *in vivo* by acting in concert with their SREBP host genes, it is unclear how widespread the contribution of individual miRNAs is to metabolic control. miRNAs typically have rather modest effects on target protein levels, and, as is the case for miR-33, combinatorial actions on multiple functionally related targets in linear pathways or key nodes in regulatory networks are probably required for single miRNAs to significantly influence a complex cellular and organismal process such as metabolic homeostasis. As mRNAs have multiple predicted miRNA target sites, it is likely that groups of miRNAs also act in concert to exert more potent effects on specific target mRNAs. New experimental strategies using systems biology methodologies may be required to determine the composition and regulation of such putative miRNA networks and to elucidate how miRNA networks are coordinated with other regulatory systems (for example, transcriptional and translational control circuits). Akin to the restrictions imposed by complex chromatin architectures on accessibility of DNA sequences to regulatory factors, could secondary and tertiary structural features of 3' UTR folding govern miRNA efficacy? Are other regulators that bind mRNAs — including proteins such as the ubiquitous member of the Hu family of RNA-binding proteins, HUR, and other non-coding RNAs — working together or in opposition to miRNAs in controlling mRNA folding and accessibility, stability and translational output? Additionally, individual miRNAs typically have many targets, which gives rise to the question of whether certain miRNAs may coordinately control metabolism with other cellular and organismal processes, such as cell growth and proliferation, cellular differentiation or organ growth. Moreover, given the likelihood that many miRNAs may regulate targets involved in multiple cellular processes, what are the possible long-term consequences of miRNA-directed therapeutics? These and many other unanswered questions concerning miRNA biology and the impact of miRNAs on metabolic homeostasis will undoubtedly be topics for intensive and exciting research for years to come.

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Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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ALL LINKS ARE ACTIVE IN THE ONLINE PDF

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Online 'at-a-glance' summary

- MicroRNAs (miRNAs) such as miR-33a and miR-33b are key regulators of cholesterol and lipid homeostasis and represent attractive therapeutic targets for raising high-density lipoprotein (HDL) levels and lowering triglyceride levels.
- A role has recently been elucidated for miRNAs (for example, miR-103, miR-107 and let-7) in regulation of insulin signalling and control of glucose homeostasis.
- An emerging link of certain miRNAs to metabolic dysregulation in adipogenesis and obesity suggests that this class of non-coding RNAs may have important roles in disorders associated with metabolic syndrome (MetS).
- Aberrant hepatic miRNA expression may also contribute to other aspects of MetS, such as non-alcoholic fatty liver disease (NAFLD).
- Circulating miRNAs have recently been identified in the blood, including as part of HDL. Data suggest that circulating miRNAs may exert effects on gene expression in target cells and tissues.
- The link of abnormal miRNA expression to metabolic disorders has highlighted the therapeutic potential of antisense targeting of specific miRNAs (for example, miR-122, miR-33a and miR-33b).

000 MicroRNAs in metabolism and metabolic disorders

Veerle Rottiers and Anders M. Näär

MicroRNAs (miRNAs) have recently emerged as key regulators of metabolism. For example, miR-33a and miR-33b control cholesterol and lipid metabolism in concert with their host genes, the sterol-regulatory element-binding protein (SREBP) transcription factors. miRNAs also regulate insulin and glucose homeostasis. Thus, miRNAs may be potential therapeutic targets for ameliorating cardiometabolic disorders.

Subject categories

Metabolism
Small RNAs
Cell signalling
Gene expression