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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 b play a crucial role in controlling cholesterol and lipid metabolism in concert with their host genes, the SREBP transcription factors. Metabolic miRNAs such as miR-103 and miR-107 regulate insulin and glucose homeostasis, while others, such as miR-34a, may be 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 to ameliorate cardiometabolic disorders.

Introduction

Proper control of metabolic homeostasis is critical 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), Forkhead box protein O1 (FoxO1) and others, respond directly or indirectly to nutrients and metabolic cues such as cholesterol, lipids, glucose, and insulin, to rapidly alter gene expression programs governing metabolic homeostasis[1-5].

Small non-coding RNAs termed microRNAs (miRNAs) have recently been found to represent another critical 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 upon all aspects of biology. The biogenesis of microRNAs is described in Box 1[6, 7].

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By contrast with plants, where 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 mRNA[8]. 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-bodies[9].

While functional validation is frequently lacking, target prediction databases based primarily on Watson-Crick base-pairing (e.g., 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 fashion[7]. However, single miRNAs may have multiple target sites in the 3'UTRs of a particular mRNA, increasing repression efficiency, and mRNAs are predicted to be targets of many distinct miRNAs, suggesting that different miRNAs may act in a concerted manner to regulate mRNA translation and turnover[13]. As discussed further 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 effect[14]. MiRNAs are also frequently found to act in feed-forward and feed-back regulation that can amplify or dampen signal output[15], making timing of analysis after miRNA perturbation critical to an accurate assessment of regulatory impact. 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 (genetic or epigenetic) or extrinsic (environmental cues or stress) factors may contribute to aberrant gene expression patterns underlying abnormal developmental patterning or metabolic dysfunction.While 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 miR-122 liver-specific miRNA and the regulatory circuit comprised of the 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 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 as "endocrine" signalling molecules.

MiRNA regulation of cholesterol and lipid homeostasis

While roles for miRNAs were first described in developmental regulation in metazoans, recent studies have revealed that miRNAs also play key roles in controlling metabolic homeostasis. Below, we initially discuss the roles 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 required as structural components of cell membranes (e.g., cholesterol and phospholipids) and serve important roles in energy storage (e.g., triglycerides), but can also act as signalling molecules (e.g., steroid hormones). Lipids such as cholesterol and fatty acids are taken up in the diet, as well as 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 and is under tight feedback control to maintain proper homeostasis (reviewed in[16]). Cholesterol and other lipids are transported in the blood by association with lipoproteins such as very high-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoproteins (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). Aberrant cholesterol and lipid homeostasis represents a critical risk factor for cardiometabolic diseases prevalent in the developed world, such as metabolic syndrome (MetS), a constellation of metabolic disorders that include insulin resistance, obesity, abnormalities in circulating cholesterol and lipid profiles, and hypertension[17]. MetS is associated with increased risk of type 2 diabetes, and coronary artery disease (CAD). For example, the risk of atherosclerosis and CAD increases with a rise in circulating LDL levels, whereas HDL levels are inversely associated with cardiovascular disease risk[17]. 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), precursors to more severe liver diseases such as fibrosis and hepatocellular carcinoma[18].

Cholesterol and lipid regulation by the liver-specific miR-122

The first miRNA linked to metabolic control, miR-122, is expressed primarily in 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 [19]). Two early studies showed that antisense targeting of miR-122 results in significant (~25-30%) reduction in plasma cholesterol levels[20, 21]. First, a study employing cholesterol-conjugated antisense oligonucleotides (termed “antagomiRs”) showed that injection of antagomirs that target miR-122 into mice resulted in altered hepatic expression of a number of genes, including some involved in cholesterol biosynthesis, such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*Hmgcr*), 3-hydroxy-3-methylglutaryl-coenzyme A synthase 1 (*Hmgcs1*), and 7-dehydrocholesterol reductase *(Dhcr7*)[21]. Accordingly, they observed significant lowering of plasma cholesterol 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, and decreased hepatosteatosis in mice on a high-fat diet[20]. In subsequent studies, miR-122 was successfully targeted by antisense inhibitors employing Locked Nucleic Acid (LNA) chemistry[22] in non-human primates such as African green monkeys, resulting in lowered circulating cholesterol[23]. 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, and which would represent the first therapeutic targeting of a miRNA. However, the initial enthusiasms waned after it was found that miR-122 antagonism not only lowers LDL, 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 liver, they do not appear to be direct targets of miR-122[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 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 the hepatitis C virus, both directly at the level of viral replication and in controlling lipid co-factors 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 (discussed in 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, as well as in the production of phospholipids and triglycerides. The human *SREBF1* gene encodes SREBP-1a and -1c, alternative splice variants that primarily regulate lipogenic genes such as fatty acid synthase (*FASN*), acetyl-CoA carboxylase (*ACC*), and stearoyl-CoA desaturase (*SCD*). The SREBP-2 isoform produced by the *SREBF2* gene preferentially controls the expression of cholesterogenic genes, including *HMGCR* and *LDLR*[3].

Intriguingly, both SREBP-encoding genes in human were recently found to be host genes to highly conserved miRNAs[31-35]. The h*SREBF1* gene on chromosome 17 harbors miR-33b in intron 17, whereas h*SREBF2* on chromosome 22 contains miR-33a in intron 16. Mature miR-33a and miR-33b only differ in 2 nucleotides and are predicted to have largely overlapping target gene sets. Of note, rodents lack miR-33b in the corresponding intron in the *Srebp1* gene[35]; the importance of this will be discussed later. In humans, miR-33a and miR-33b appear 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 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.

miR-33a and SREBP-2 cooperate to control cholesterol homeostasis

Several recent studies reported the discovery of the miR-33a and SREBP-2 miRNA-host gene circuit, demonstrating that miR-33a functions in concert with the SREBP-2 cholesterogenic transcription factor to boost intracellular cholesterol levels[31-35]. MiR-33a and b were found to play a crucial role in the post-transcriptional repression of the ATP-binding cassette transporter (subfamily A, member 1) ABCA1, which promotes the efflux of free cholesterol from within the cell to ApoA1 and is critical for the formation of high-density lipoproteins (HDL)[37]. 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 disease[38]. ABCA1 is regulated by 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 upon inhibition or KO ablation of miR-33a[32-35]. Moreover, antisense inhibition of miR-33a in LDL-receptor KO mice, a well-validated model for hypercholesterolemia and cardiovascular disease, caused reduced high-fat diet-induced atherosclerotic plaque size and lipid content and promoted RCT, verifying the therapeutic potential of miR-33 targeting for the treatment of cardiovascular disease[39]. However, as noted above, rodents lack miR-33b, complicating the translation and relevance of these results to humans. This difference is important because expression of SREBP-1c (and miR-33b embedded within the *SREBF1* gene) is highly upregulated upon insulin stimulation[36]. Under elevated circulating insulin conditions (such as in insulin resistance), increased SREBP-1c and miR-33b levels in the liver could thus potentially contribute to both high VLDL levels as well as low HDL levels found in individual suffering from metabolic syndrome[40]. Recent studies have indeed verified that hepatic miR-33b levels are elevated in non-human primates in response to a high-carbohydrate diet, and show that antisense oligonucleotides targeting miR-33a and b 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 miR-33a and b for the treatment of cardiometabolic disorders[41] (V.R., A.M.N., et al., unpublished data). 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 complex.

Other miR-33 targets involved in cholesterol, fatty acid and lipid homeostasis

The discovery of coordinated regulation of cholesterol levels by miR-33a miRNA and the SREBP-2 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 b also regulate intracellular fatty acid and lipid levels in concert with their SREBP host gene products[14, 31, 36].

First, it was shown that miR-33a and b 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/energy generation[14, 31, 36]. These include carnitine O-octanoyltransferase (CROT) which helps to break down very long-chain fatty acids (>20 carbons) in peroxisomes, as well as carnitine palmitoyltransferase 1A (CPT1A), the rate-limiting transporter of fatty acids into mitochondria, and hydroxyacyl-coenzyme A-dehydrogenase (HADHB), a subunit of the trifunctional enzyme directly involved in mitochondrial fatty acid β-oxidation[14, 31, 36].

In addition to regulating fatty acid degradation, miR-33a and b have been shown to control critical upstream regulators of fatty acid and lipid homeostasis. For example, the sirtuin SIRT6, an NAD^+-dependent histone deacetylase which has been shown to be a crucial regulator of glucose metabolism and stress resistance[44, 4546, 47], was found to be targeted by miR-33a and b[14, 36]. Interestingly, a recent study of liver-specific SIRT6 knock-out mice revealed increased hepatic glycolysis, lipogenesis and triglyceride production, resulting in hepatosteatosis[48]. SIRT6 was shown to directly regulate SREBP-1 target genes relevant for fatty acid production such as acetyl-CoA carboxylase 1 (*Acc1*), stearoyl-CoA desaturase 1 (*Scd1*), and *Fasn*. This suggests that miR-33 inhibition of SIRT6 expression may result in increased chromatin acetylation and de-repression of SREBP-dependent fatty acid biosynthesis genes, and elevated lipogenesis.

The α1 subunit of the AMP-dependent kinase (AMPKα1) has also been found to be targeted by miR-33a and b[14, 36, 41]. AMPK is a master regulator of cellular energy levels in response to energy stress[49]. It responds to low cellular energy levels (increased AMP-to-ATP ratio) and acts to reduce energy-consuming processes such as protein, fatty acid and cholesterol synthesis, as well as 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 LXR [50], and through direct phosphorylation [51]. Thus, negative regulation of AMPKα1 by miR-33a and b 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 (IRS-2) was also recently shown to be a miR-33 target[14, 36]. IRS-2 is one of two intracellular adaptor proteins for the insulin receptor that relay insulin signaling to downstream effectors (such as phosphatidylinositol 3-kinase (PI3K) and Protein Kinase B (AKT)). In mouse models, reduction of IRS-2, but not IRS-1, has been found to increase SREBP-1c expression and activity [52-54]. Interestingly, in mouse NAFLD models, decreased hepatic levels of IRS-2 has been shown to result in a compensatory elevation in IRS-1 levels, which in turn activates SREBP-1[55]. Thus, miR-33 antisense targeting could potentially result in a reversal of the hepatic changes in IRS-2 and IRS-1 expression, thereby decreasing SREBP-1c 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 b to regulate cholesterol and lipid homeostasis (Figure 1).

MiRNA regulation of insulin signalling and glucose homeostasis

A number of miRNAs have recently been implicated in controlling both insulin signalling and glucose metabolism at multiple levels[56]. Insulin is a hormone synthesized in and secreted by the pancreatic β-cells in response to increased nutrient levels (e.g. glucose) in the blood. It acts in concert with glucagon (a hormone produced by the 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 protein[57]. Binding of insulin to the insulin receptor (INSR) promotes an intracellular signalling cascade involving the insulin receptor tyrosine kinase activity, the adaptor proteins IRS-1 and IRS-2, and a cascade of kinases including PI3K, 3-phosphoinositide-dependent protein kinase-1 (PDPK1) and AKT[58]. These kinases affect a complex downstream network of proteins such as glycogen synthase kinase 3β (GSK-3β), mammalian target of rapamycin complex 1 (mTORC1), and the FOXO family transcription factors[59]. Insulin also activates *SREBF1* transcription and SREBP-1c processing and transport, stimulating lipogenesis[60]. 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, all components of metabolic syndrome[59]. While 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 (Table 1, Figure 2).

MiRNA regulation of pancreatic insulin production

Insulin is produced from pancreatic isletβ-cells, and the pancreatic miRNA miR-375 was shown to be required for pancreatic island development in zebrafish [61], and for the maintenance of pancreatic α and β-cell mass in mice [62]. Interestingly, miR-375 may have a dual role as miR-375 was also found to decrease insulin exocytosis/secretion at least in part through the repression of myotrophin (Mtpn), a gene involved in actin depolymerization, and potentially, vesicular fusion[63]. It may additionally affect downstream insulin signalling through direct repression of the insulin signalling intermediate kinase PDPK1[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 miRNAs[65]. MiR-124a is also involved in pancreatic islet development, potentially through regulation of the FoxA2 transcription factor involved in β-cell differentiation, as well as Rab27a, a GTPase required for insulin secretion[66, 67]. Other miRNA 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 (Onecut2) as well as through SIRT1[68, 69],

whereas miR-29a and b, which are highly expressed in the pancreatic islets of diabetic mice, inhibit the expression of monocarboxylate transporter 1 (Mct1) and its function in insulin release[70]. Taken together, these findings indicate that miRNAs play key roles in controlling insulin secretion through effects on both pancreatic development and insulin exocytosis.

MiRNA modulation of insulin sensitivity, signalling and glucose metabolism in target tissues

Other miRNAs act in target tissues to regulate responses to insulin and glucose homeostasis. For example, miR-29a and b, which are upregulated in muscle, white adipose tissue, and liver of diabetic Goto-Kakizaki rats[71], have been linked to insulin resistance, at least in cell culture experiments. The effects of miR-29a and b were suggested to be mediated through downregulation of proteins that promote insulin signalling such as Caveolin 2, a lipid raft-associated protein that responds to insulin levels[72], as well as Insulin-induced gene 1 (Insig1), a negative regulator of SREBPs, and the insulin signalling intermediate PI3 kinase subunit p85α (PIK3R1)[71, 73]. Another layer of insulin regulation by miRNAs is provided by miR-126, which promotes insulin resistance through its inhibition of IRS-1[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 of the glucose transporter GLUT4[75]. As discussed above, miR-33a and b may also influence insulin signaling and glucose regulation by targeting IRS-2, SIRT6 and AMPKα1[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 1-3 (PANK1-3) genes)[83] were recently shown to be upregulated in livers of leptin-deficient (*ob/ob*) and diet-induced obese (DIO) mice[81]. Interestingly, antisense-mediated silencing 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 resistance[84]. These miRNAs were proposed to exert their functions on insulin and glucose regulation at least in part through the inhibition of caveolin-1 (CAV1), which influences lipid rafts and affects insulin receptor availability[81]. However, it is also possible that some of the effects are mediated through other miRNAs since miR-103 and miR-107 also strongly inhibit the miRNA-processing enzyme Dicer[85]. Indeed, partial knockdown of Dicer or pancreas-specific knockout experiments have revealed that this enzyme (and, presumably, miRNAs) is required for both the development and maintenance of the pancreas and insulin signalling[86, 87].

Several additional miRNAs have been found to be elevated in obesity models and exert regulatory effects on insulin signalling and glucose homeostasis. Similar to miR-103 and miR-107, miR-143 is overexpressed in the obese leptin receptor-mutant mice (*db/db*) and DIO mice[77]. 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, a miRNA that may play 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 mice[81]. let-7 has well-studied tumor suppressor roles in cancer biology but was recently also shown to be involved in regulation of glucose metabolism, where its overexpression in skeletal muscle resulted in insulin resistance and impaired glucose tolerance[91]. This effect may be at least partially mediated by let-7 targeting of the insulin-like growth factor receptor 1 (IGF-1R), INSR, and IRS-2.

In conclusion, a number of miRNAs have been suggested 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 adipogenesis, obesity, and non-alcoholic fatty liver disease

Conditions of nutritional excess and lack of physical activity can result in excessive fat storage manifesting in obesity and non-alcoholic fatty liver disease, and strongly increase the risk for cardiovascular disease and type 2 diabetes. Normal adipogenesis involves regulation by transcriptional factors such as the PPARγ and its coactivators (e.g peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)), as well as members of the C/EBP family and Kruppel-like factors (KLFs) (reviewed in [92]). MiRNAs have also recently been implicated in adipogenesis[93]. 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 ERK5[94]. Several others, including miR-204, miR-141, miR-200a-c, and miR-429, are involved in early adipocyte cell fate determination, whereas miR-17-92, miR-130, miR-27a and b, and miR-378 and 378* have been suggested to be involved in terminal differentiation and mature white adipocyte function (recently reviewed in [95]). Brown adipose tissue (BAT), a highly metabolically active adipose tissue type involved in thermogenesis and energy expenditure, has been suggested to correlate inversely with obesity[96]. A recent study reported a key role for the miR-193b-365 miRNA cluster in brown fat differentiation, in part by repressing myogenesis[97]. The accumulating data thus indicate that miRNAs are acting as central modulators of normal white adipose tissue (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. showed that certain miRNAs involved in adipogenesis exhibit inverse expression patterns in obesity[82]. While 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 role in hepatic metabolic diseases. Both NAFLD and NASH patients exhibit highly elevated hepatic expression of miR-34a[101, 102], and miR-34a levels were also higher in type 2 diabetic subjects as compared to healthy controls[103]. A plausible molecular mechanism for the function(s) of miR-34a in metabolic control has recently been suggested (reviewed in [104], summarized in Figure 3). This involves an intricate regulatory network of miR-34a, SIRT1, Farnesoid X receptor (FXR) and tumor protein 53 (p53). SIRT1 is a key sensor and regulator of metabolic states since 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 PGC-1α, PPARs, p53, FOXO1, LXR, FXR and SREBPs to alter the expression of transcriptional programs 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 cells[113]. Subsequent work showed that miR-34a also inhibits SIRT1 expression in the liver[114]. 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). SHP sequesters p53 away from the promoter of miR-34a, again reducing 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, PGC-1α, LXR, and FOXO1, potentially further contributing to hepatosteatosis (Figure 3). A crucial next step is to investigate the effect of antagonizing elevated miR-34a levels in models for NAFLD and obesity on SIRT1 expression, and deacetylation of SIRT1 downstream targets. SIRT1 activators, such as resveratrol and other more potent synthetic inducers, improve hepatosteatosis and insulin resistance in DIO mice[111, 119-121]. If verified *in vivo*, activation of SIRT1 through antisense inhibition of miR-34a could potentially represent an alternative therapeutic avenue for the treatment of NAFLD and other obesity-related diseases.

Circulating miRNAs as disease biomarkers and “endocrine signals”

Recent studies have found that miRNAs can be readily detected in human plasma, suggesting possibilities for novel disease biomarker discovery (reviewed in [122] and [123]), as well as the intriguing notion that they may serve regulatory purposes (endocrine-miRs) in target cells. Plasma fractionation schemes initially isolated circulating miRNAs as part of microvesicles and exosomes derived from plasma membranes of “donor” cells[124].

Subsequent findings indicate that miRNAs can also be found in lipid-free protein assemblies, such as in association with the miRNA-processing enzyme 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 have been found to traffic from donor cells to distant sites and alter the gene expression output of recipient cells[131]. This suggests that circulating miRNAs are not merely “vesicular passengers”, but may rather act as signaling molecules in an endocrine or paracrine fashion to deliver a regulatory “message” from donor to target cells. By contrast with the highly specific and potent action of classical endocrine hormones such as steroids[132], circulating miRNAs may have only modest effects on mRNA and protein output. Similar to mRNAs taken up by cells from microvesicles[133], miRNAs presumably do not require specific receptors for their action and are therefore only limited by the fact that the mRNA needs to be expressed in target cells for the miRNA to have an impact, and it is unclear whether they 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 highly abundant in certain vesicles and lipoprotein particles exert “mass action” effects on gene expression programs in target cells or tissues, thereby acting in a more potent manner akin to classic endocrine cues?

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 RNA cargo conveying specific regulatory information[128]. Vickers et al. found that certain endogenous miRNAs (e.g., miR-135a*, miR-375 and miR-223) are exported from peripheral cells in HDL particles[128]. Export of miRNAs to HDL was shown to be controlled by neutral sphingomyelinase II (nSMase2), the rate-limiting enzyme in ceramide biosynthesis, suggesting that plasma membrane lipids such as sphingomyelin (SM) and ceramides are involved in controlling miRNA incorporation into HDL.

Once miRNAs are embedded in HDL, they are likely trafficked to the liver by the classic reverse cholesterol transport pathway for uptake in a scavenger receptor B type I (SR-BI)-dependent manner, and may exert effects on hepatic gene expression programs (Figure 4). Consistent with this notion, Vickers et al. showed that treatment of hepatic Huh-7 cells with HDL loaded with exogenous miRNAs (e.g., miR-223) could indeed impact target mRNA expression[128]. 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 heart

disease patients 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 have recently come to the fore as critical regulators of numerous aspects of animal development, physiology, and disease. As discussed in this review, a rapidly growing number of miRNAs have been implicated in regulation of genes and proteins involved in the control and maintenance of metabolic homeostasis. While the best-characterized roles for miRNAs in metabolic control concerns the normal maintenance of cholesterol and lipid homeostasis (e.g., miR-122 and miR-33), new data has also revealed contributions of miRNAs to insulin signaling and glucose homeostasis, as well as 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 signaling molecules with impact on the gene expression output in distant target organs and cells (e.g., liver in the case of HDL-associated miRNAs), akin to classical endocrine cues.

MiRNA therapeutics for the treatment of metabolic disorders

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 centered on inhibition of what is perceived as “druggable” targets, such as enzymes (e.g., HMG-CoA reductase targeted by cholesterol-lowering statins). However, the recent discovery by us and others that certain miRNAs may represent critical 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 b 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 b for the treatment of metabolic syndrome and cardiovascular disease should be explored further. It is likely that other miRNAs with regulatory roles in human metabolism (e.g., miR-103 and miR-107 in insulin resistance) may also serve as suitable candidates for therapeutic intervention.

Future directions and open questions

A number of open questions remain concerning the function of miRNAs in metabolic control (and more broadly). For example, while it is apparent that several miRNAs, including miR-33a and b, have important regulatory impact on cholesterol and lipid metabolism *in vivo* by themselves, 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 action on multiple functionally related targets in linear pathways or key nodes in regulatory networks is 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 employing systems biology methodologies may be required to determine composition and regulation of such putative miRNA networks, and to elucidate how miRNA networks are coordinated with other regulatory systems (e.g., 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 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? Additionally, 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 impact on metabolic homeostasis will undoubtedly be topics for intensive and exciting research for years to come.

Biography

Anders M. Näär received his PhD at the University of California, San Diego, School of Medicine, Howard Hughes Medical Institute, USA, with Michael G. Rosenfeld, and performed his postdoctoral research at the University of California, Berkeley, Howard Hughes Medical Institute, USA, with Robert Tjian. He is currently an associate professor in the Department of Cell Biology at Harvard Medical School and the Massachusetts General Hospital Cancer Center, where his laboratory studies mechanisms of gene regulation.

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Glossary terms

Antagomir a small synthetic cholesterol-conjugated DNA oligonucleotide that is complementary to an endogenous miRNA of interest. The cholesterol

	moiety allows antagomirs to enter most cell types efficiently, where they specifically bind and sequester endogenous miRNAs.
LNA	Locked Nucleic Acid, a modified DNA oligonucleotide analogue, locked in a “N-type confirmation” capable of recognizing DNA and RNA with high affinity and is resistant to degradation.
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).
Lipid rafts	microdomains of the cell plasma membrane with high cholesterol and sphingolipid content that compartmentalize cellular processes by acting as platforms to co-localize proteins such as signalling molecules and receptors.
Fatty acid β-oxidation	degradation process of fatty acids to acetyl-CoA primarily in mitochondria. Long chain fatty acids need to be transported via binding to carnitine.
Triglycerides	esters of glycerol and fatty acids, used for the storage of energy.
Atherogenic macrophages	cholesterol-loaded macrophages that accumulate in the arterial wall and can lead to atherosclerotic lesions.
Exosome	small (30-90 nm) vesicle secreted from the plasma membrane of mammalian cells. They contain proteins, RNA and miRNA molecules and can be transported from cell to cell.
Endocrine/ paracrine	both are hormonal signals, secreted from a specific cell and causing a specific effect. Endocrine responses occur over large distances in distant target, paracrine signals have only local effects.

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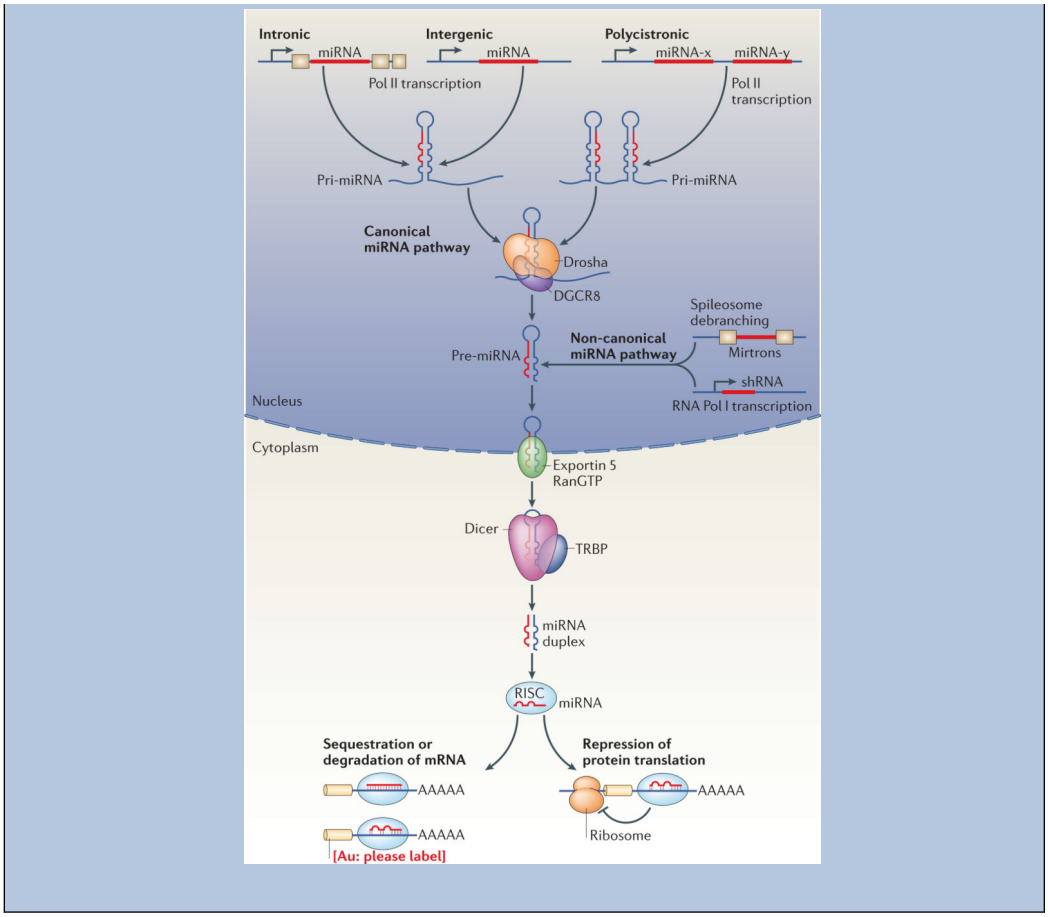
Box 1: The miRNA biogenesis pathway

MiRNAs generated by the canonical biogenesis pathway are transcribed as precursor RNAs from intergenic, intronic, or polycistronic genomic loci by RNA polymerase II[6]. The primary miRNA (pri-miRNA) transcript forms a stem-loop structure that is recognized and processed by the complex of RNase III, Drosha and DGCR8 or the spliceosome apparatus in the nucleus. In the non-canonical miRNA pathway, miRNAs are transcribed directly as short endogenous hairpin RNAs (shRNAs) or derive directly through splicing from introns that can refold into hairpins (mirtrons) (reviewed in [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 enzyme complex of RNase III, Dicer and TRBP to form the mature double-stranded ~22 nucleotide miRNA. Argonaut proteins (e.g., Ago2) then unwind the miRNA duplex and facilitate incorporation of the miRNA-targeting strand (also known as *guide* strand) into the Argonaut-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 2-8 in the mature miRNA (termed the *seed* sequence) with specific mRNA target sequences chiefly located in the 3'UTR, with additional base-pairing affording greater affinity and targeting efficiency [7].



Box 2: miR-122 and its function is hepatitis C virus propagation

Apart from its function in metabolic control 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'UTR 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 production[135]. Hence, miR-122 antagonism could inhibit HCV both by preventing direct enhancement of viral RNA folding or replication as well as by lowering required cholesterol or lipid co-factors for viral packaging or extrusion, suggesting that antisense-based targeting of miR-122 could represent a powerful approach to treat chronic HCV infections which plague up to 3% of the world's population. Accordingly, weekly subcutaneous injections of miR-122-targeting LNA-based antisense oligonucleotides (miravirsen) have been found efficacious in decreasing HCV titers in chimpanzees[136], and early indications from Phase II studies in humans with chronic HCV infection appear promising[137]. Indeed, a four-week therapy with miravirsen in treatment-naïve patients with chronic HCV genotype 1 infection provides long-lasting suppression of viremia that was maintained for more than four 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 miravirsen in humans should provide important information as to whether miR-122 therapeutic targeting might have a positive impact on circulating cholesterol/lipid abnormalities linked to metabolic syndrome. HCV infection is associated with NAFLD[101], and inhibition of viral propagation by miR-122-targeting antisense approaches might also ameliorate HCV-associated fatty liver disease. On the other hand, the emerging link of miR-122 to hepatocyte differentiation or cell fate determination[138] may also suggest that attention should be paid to potential deleterious long-term effects, such as the development of hepatocellular carcinoma[26].

"At a glance" online summary

- MiRNAs such as miR-33a/b are key regulators of cholesterol/lipid homeostasis, and represent attractive therapeutic target to raise HDL and lower triglycerides.
- A role for miRNAs (e.g., miR-103 and miR-107, and let-7) in regulation of insulin signaling and control of glucose homeostasis has recently been elucidated.
- An emerging link of certain miRNAs to metabolic dysregulation in adipogenesis and obesity suggests that this class of non-coding RNAs may play 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 microRNAs (e.g., miR-122 and miR-33a/b).

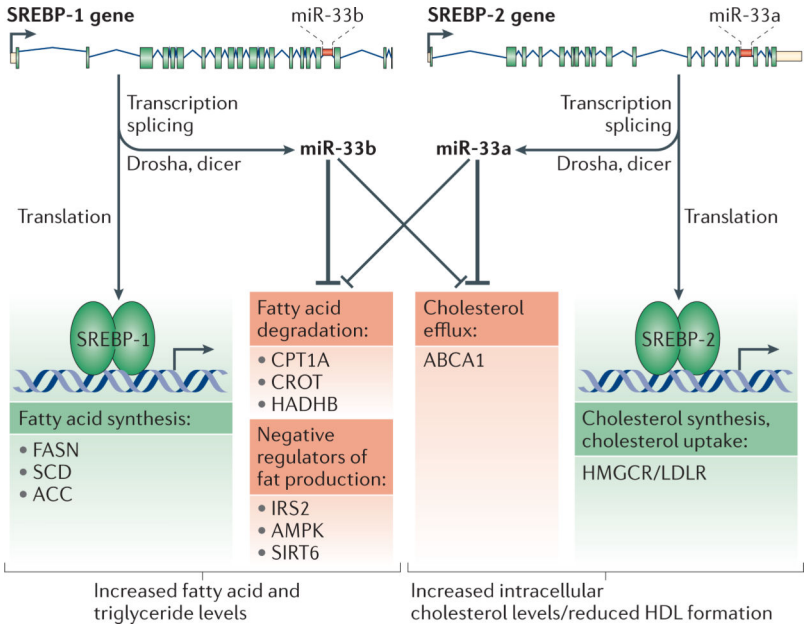


Figure 1. Model of the SREBP and miR-33 circuit

The sterol regulatory element-binding protein (SREBP) transcription factors act coordinately with their intronic miRNAs miR-33a and b to regulate fatty acid, triglyceride, and cholesterol homeostasis. Transcription of the *SREBF1* and *SREBF2* loci gives rise to the SREBP-1 and SREBP-2 transcription factors and the miR-33a/b miRNAs. SREBP-1 activates genes involved in fatty acid, phospholipid and triglyceride synthesis (e.g., FASN (fatty acid synthase), SCD (stearoyl-CoA desaturase) and ACC (Acetyl-CoA carboxylase)) whereas SREBP-2 activates genes involved in cholesterol synthesis/uptake, such as HMGCR (3-hydroxy-3-methylglutarylcoenzyme A reductase) and LDLR (Low-Density Lipoprotein Receptor). miR-33a and b act to repress genes functioning in fatty acid β-oxidation (e.g., CROT (carnitine O-octanoyltransferase), HADHB (hydroxyacyl– coenzyme A dehydrogenase/3-ketoacyl–coenzyme A thiolase/enoyl–coenzyme A hydratase [trifunctional protein], β subunit) and CPT1A (carnitine palmitoyltransferase 1A)), cholesterol efflux (e.g., ATP-binding cassette, subfamily A member 1 (ABCA1)), as well as negative regulators of SREBPs (e.g., IRS-2 (insulin receptor substrate 2), AMPKα1 (AMP-activated protein kinase α1 subunit) and SIRT6 (sirtuin 6)).

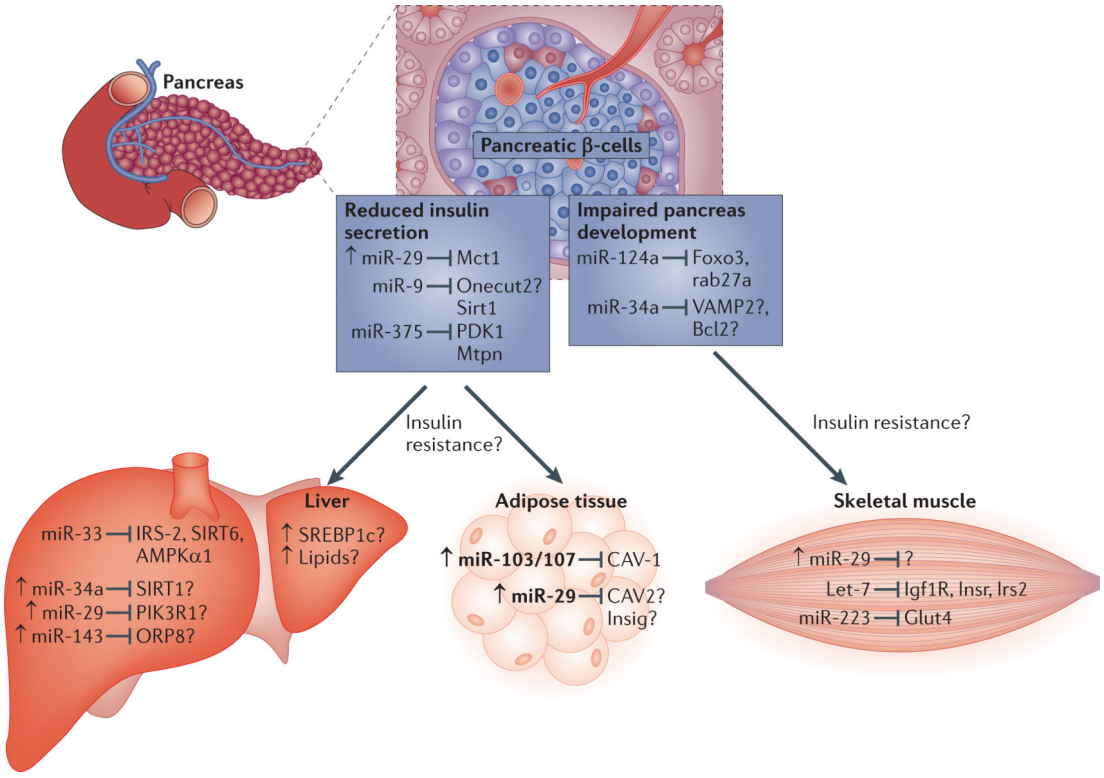


Figure 2. MiRNA regulation of insulin signalling and glucose homeostasis

Normally, upon feeding, insulin is produced in the pancreatic β-cells and upon release will reach target tissues such as the muscle, liver and adipose to cause uptake of glucose, reduce the production of glucose and to activate fat production and storage. MiRNAs have been identified that affect diverse parts of insulin signalling in pancreas, liver, muscle and adipose tissue. miR-124a and miR-34a are involved in pancreatic development (through effects on Foxo2, Rhab27a, VAMP2, and Bcl2), whereas miR-29, miR-9 and miR-375 are involved in insulin secretion (through Mct1, Onecut2, Sirt1, PDK1, and Mtpn). miR-33, miR-34a, miR-29 and miR-143 act in the liver on targets involved in insulin signalling and its regulation (such as IRS-2, SIRT6, AMPKa1, SIRT1, PIK3R1 and ORP8). miR-103 and miR-107 and miR-29 act to modulate insulin signalling in adipose tissue (through CAV-1 and Insig1, respectively). miR-29, let-7 and miR-223 act in the muscle on insulin uptake (Glut4) and insulin signalling (Igf1R, Insr and Irs2). 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 arrow).

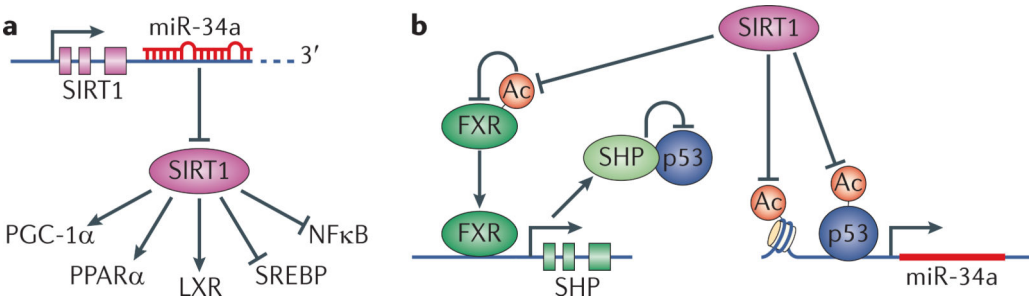


Figure 3. The regulatory loop of miR-34a, SIRT1, FXR and p53

miR-34a is highly expressed in patients with NAFLD and NASH and type 2 diabetes. On the molecular level, miR-34a has been shown to exert its function through its effect on SIRT1. miR-34a is then in turn inhibited by SIRT1 in a regulatory loop that includes miR-34a, SIRT1, FXR and p53 to affect cholesterol, lipid and energy homeostasis as well as inflammation. **A.** miR-34a inhibits SIRT1 and reduces its protein level and prevents its activation of PGC-1α, PPARα and LXR (key regulators of cholesterol/lipid/energy homeostasis), and inhibition of SREBP and NF-κB (activators of lipogenesis and cholesterogenesis, and inflammation, respectively). **B.** 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 SHP, which sequesters p53 and thus inhibits miR-34a transcription.

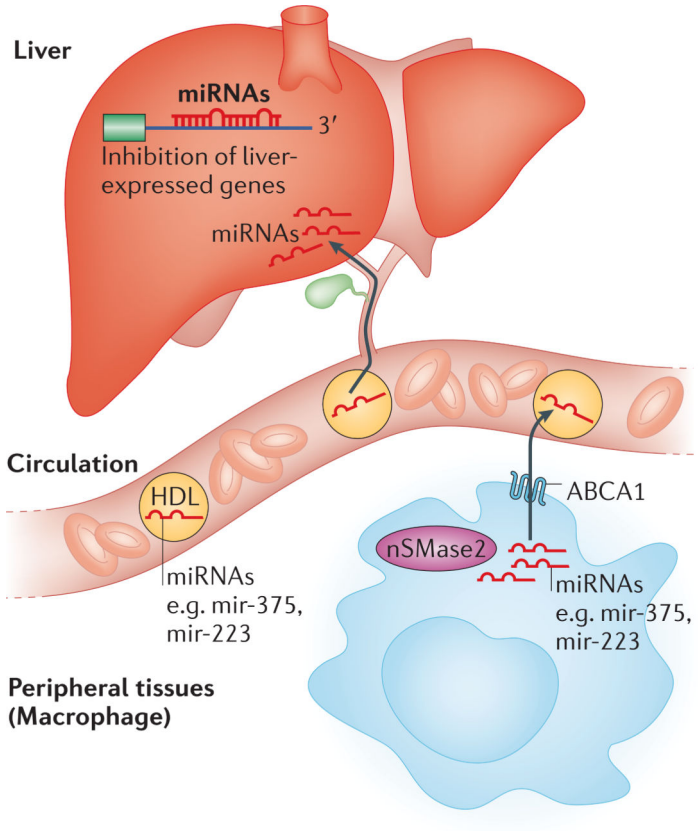


Figure 4. Model for the function of circulating miRNAs associated with HDL

MiRNAs such as miR-375 and miR-223 are produced in peripheral tissues and incorporated in exosomes through a mechanism controlled by nSMase2, the rate limiting enzyme in ceramide biosynthesis, and transported in the blood. Some miRNAs are also bound to high-density lipoproteins (HDL), incorporation of microRNAs into HDL is controlled by the cholesterol transporter ABCA and by nSMase. Uptake of RNA and miRNAs from exosomes in target cells does not require binding to a specific receptor. 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 B I-dependent mechanism. Once inside target cells, miRNAs can then exert inhibitory effects on a range of target genes.

Table 1

miRNA	Target tissue(s)	Function	Target genes	References
miR-103/107	Adipose, liver	Insulin/glucose homeostasis Adipogenesis	Cav1, Dicer	81, 82, 85
miR-122	Liver	Hepatic lipid metabolism	CAT-1, ADAM17	20, 139, 140
miR-124a	Pancreas	Pancreatic islet development	FoxA2, Rab27A	66, 67
miR-143	Adipose Liver Pancreas	Adipocyte differentiation Insulin resistance	ERK5/BMK1/MAPK7 ORP8	77, 80, 94
miR-223	Muscle	Glucose uptake, insulin resistance	Glut4	75
miR-27a	Adipose	Adipogenesis	[PPARγ, C/EBPα]	141, 142
miR-29	Muscle, Adipose Liver	Glucose transport	Insig1, Cav2, Mct1, PIK3R1	70, 71, 73
miR-33a/b	Liver Macrophage	Cholesterol/lipid/energy homeostasis	ABCA1, NPC1, CPT1A, HADHB, CROT, IRS2, SIRT6, AMPKα1	31-36, 39
miR-335	Pancreas Liver Adipose	Insulin production Fatty acid and TGA 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-378/378*	Adipose	Adipocyte differentiation lipid synthesis	ERRγ, ? [ribosomal proteins]	144-146
miR-9	Pancreas	Insulin secretion	Onecut2, Sirt1	68, 69
miR-130	Adipose	Adipogenesis	PPARγ	147
let-7	Muscle Adipose	Insulin sensitivity	IGF-1R, INSR, IRS2 HMGA2	91, 148