

*Thematic Review Series: Functional Regulation of Lipid Homeostasis
by microRNA*

Complexity of microRNA function and the role of isomiRs in lipid homeostasis

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Abstract MicroRNAs (miRNAs) are key posttranscriptional regulators of biological pathways that govern lipid metabolic phenotypes. Recent advances in high-throughput small RNA sequencing technology have revealed the complex and dynamic repertoire of miRNAs. Specifically, it has been demonstrated that a single genomic locus can give rise to multiple, functionally distinct miRNA isoforms (isomiR). There are several mechanisms by which isomiRs can be generated, including processing heterogeneity and post-transcriptional modifications, such as RNA editing, exonuclease-mediated nucleotide trimming, and/or nontemplated nucleotide addition (NTA). NTAs are dominant at the 3'-end of a miRNA, are most commonly uridylation or adenylation events, and are catalyzed by one or more of several nucleotidyl transferase enzymes. 3' NTAs can affect miRNA stability and/or activity and are physiologically regulated, whereas modifications to the 5'-ends of miRNAs likely alter miRNA targeting activity. Recent evidence also suggests that the biogenesis of specific miRNAs, or small RNAs that act as miRNAs, can occur through unconventional mechanisms that circumvent key canonical miRNA processing steps. The unveiling of miRNA diversity has significantly added to our view of the complexity of miRNA function. **In this review we present the current understanding of the biological relevance of isomiRs and their potential role in regulating lipid metabolism.**—Vickers, K. C., P. Sethupathy, J. Baran-Gale, and A. T. Remaley. **Complexity of microRNA function and the role of isomiRs in lipid homeostasis.** *J. Lipid Res.* 2013. 54: 1182–1191.

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Systemic lipid homeostasis is governed by one of the most complex and multilayered metabolic networks, involving

sensing and effector pathways, lipoproteins, and extracellular enzymes (1–5). Loss of cellular or plasma lipid control, generally referred to as dyslipidemia, can be caused by dysfunction in one or more of the multiple pathways responsible for lipid synthesis (6, 7), dietary fat utilization (8), lipid catabolism (9), fat mobilization (10–12), lipid storage (11, 13), lipoprotein transport (14–16), and cellular energetics (17–19). Hypercholesterolemia and hypertriglyceridemia are etiopathological causes of atherosclerosis, pancreatitis, fatty liver disease, and cancer (2, 5, 17, 20–22). Collectively, these diseases represent significant public health and financial burdens on all societies. Mainly due to societal changes in diet and lifestyle, as well as other recent environmental influences, dyslipidemia and related metabolic syndromes are currently rampant.

The liver is the epicenter of lipid and lipoprotein synthesis and metabolism (23, 24). Proper control and maintenance of cellular and plasma lipid content require precise hepatic gene regulation at transcriptional, posttranscriptional, and posttranslational levels. Multiple hepatic transcription factors (TF), including peroxisome proliferator activated receptors (PPAR family) (25, 26), liver X receptors (LXR family) (27), hepatocyte nuclear factors (HNF family) (1, 28, 29), cAMP response element binding protein (CREBP) (30), CCAAT enhancer binding proteins (C/EBP family) (31, 32), forkhead box transcription factors (FOX family) (33–35), and sterol-response element binding proteins 1 and 2 (SREBF1 and SREBF2) (36–39), have all been found to control gene networks that govern lipid synthesis, catabolism, storage, and secretion. Recent studies suggest that posttranscriptional microRNA (miRNA)-mediated

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Abbreviations: isomiR, miRNA isoform; miRNA, microRNA; NTA, nontemplated nucleotide addition; pre-miRNA, precursor miRNA; RISC, RNA-induced silencing complex; smRNA, small RNA; TG, triglyceride.

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gene regulation may also be highly relevant to the control of hepatic lipid metabolism (40–50). miRNAs have emerged not only as stable plasma biomarkers of metabolic states but also as attractive therapeutic targets for various cardio-metabolic disorders (46, 51–55). Recent advances in small RNA (smRNA) high-throughput sequencing have unveiled the enormous complexity of the repertoire of functional smRNAs. Here, we review the current state of knowledge regarding the diversity of smRNAs, including miRNA isomiRs, and their control of lipid metabolism.

SMALL RNA DIVERSITY

Small noncoding RNAs can be stratified into one of several categories based on their biogenesis mechanism, parent RNA type, and length: miRNAs (~22 nt in length) (56, 57), Piwi-interacting RNAs (piRNA, ~26–31 nt) (58–60), small nucleolar RNAs (snoRNAs, 70–120 nt) (60), small RNAs derived from snoRNA (sdRNA, ~17–19 nt) (61–63), endogenous small interfering RNAs (endo-siRNA, ~21 nt) (59, 64), small RNAs derived from vault RNA (svRNA, ~17–19 nt) (65, 66), small RNAs derived from tiny RNA (tiRNA, ~17–19 nt) (67, 68), and tRNA-derived RNA fragments (tRFs, ~17–19 nt) (61, 69). Although each of these classes of smRNA is thought to regulate gene expression through diverse mechanisms, miRNAs are currently the most widely studied and are known to be involved in posttranscriptional control of lipid homeostasis (56, 57, 70).

The human genome encodes for over 1,000 miRNAs, either within host protein-coding genes or as independent transcription units (56, 71). Canonical biogenesis of a miRNA commences with RNA Polymerase II-mediated transcription, yielding a primary miRNA transcript (pri-miRNA) of variable length depending on the locus (56, 59, 72). The pri-miRNA is cleaved by a nuclear RNase complex DROSHA/DGCR8, generating a precursor miRNA (pre-miRNA) that has a hairpin-like secondary structure. The pre-miRNA is then exported via the exportin 5-dependent pathway to the cytoplasm, where it is subject to further enzymatic processing by DICER and its cofactors, producing a short dsRNA duplex (~22 bp) (56, 57, 59, 72). One strand of the duplex (mature miRNA) is loaded onto the RNA-induced silencing complex (RISC), which it guides and tethers to target mRNAs in order to regulate their stability and/or translational efficacy (57, 73).

Some miRNAs are generated by alternative mechanisms, but they may still play a significant role in lipid homeostasis. For example, certain miRNA clusters that are interspersed among Alu repeats in the genome have been shown to be transcribed by RNA Polymerase III (74). Also, short-length introns can form hairpin-like structures upon nascent transcription and be processed by RNA splicing machinery, rather than by DROSHA, and cleaved into pre-miRNA products. This class of miRNAs is referred to as miRtrons, due to their unique biogenesis from intron-exon junctions (75–78). Although very few miRtrons are extensively conserved, they are present in both invertebrates and mammals, suggesting that they may have evolved

independently along multiple lineages. A recent large-scale meta-analysis of hundreds of smRNA data sets has systematically identified ~240 human miRtrons, many of them evolutionarily recent (76). Finally, one highly conserved pre-miRNA (pre-miR-451) has been demonstrated to bypass DICER processing altogether and is instead cleaved directly by Argonaute 2 (Ago2) within the RISC (79–81).

MIRNAS CONTROL HEPATIC LIPID METABOLISM

miRNAs bind to target RNAs through complementary base pairing, most critically in the seed region of the miRNA (nucleotides 2 through 7 at the 5'-end). One of the first miRNAs shown to have a role in lipid biology is miR-33, which is cotranscribed with the SREBF family of genes and is a critical regulator of lipoprotein metabolism and fatty acid oxidation (44, 46, 49, 82). The functional relevance of miR-33 to lipid homeostasis has been established in both mice and in nonhuman primates with systemic administration of chemically modified locked nucleic acid (LNA), which mediates safe and potent inhibition of endogenous miRNAs in vivo (46, 55, 83). Most recently, miR-33 has also been suggested as a key link between lipid metabolism and hepatocyte cell proliferation, particularly in the context of response to liver injury (47, 84).

Using in silico strategies for miRNA target prediction and enrichment analysis, we recently identified several miRNAs, including miR-27, miR-223, and miR-125, as candidate regulatory hubs in the pathways that govern lipid homeostasis (85). We demonstrated in vitro in human hepatocytes that miR-27b represses the expression of glycerol-3-phosphate acyltransferase 1 (GPAM), angiopoietin-like 3 (ANGPTL3), peroxisome proliferator-activated receptor γ (PPAR γ), and heparan sulfate N-deacetylase/N-sulfotransferase 1 (NDST1) (15, 85–91). Inhibition of miR-27b in hepatocytes resulted in a significant increase in GPAM mRNA and protein levels. GPAM-mediated acyl esterification of glycerol-3-phosphate (sn-1 position) is the first committed (enzymatic) step in triglyceride (TG) biosynthesis, and thus, miR-27b upregulation in a short-term high-fat diet is likely an adaptive response to mitigate TG production and attenuate hyperlipidemia and hepatosteatosis. Likewise, elevated hepatic miR-27b also represses *ANGPTL3*, which is produced and secreted from the liver to inhibit plasma TG lipolysis (92, 93). Heparin sulfate proteoglycans on hepatocyte membranes contribute to the cellular uptake of TG-rich lipoproteins, and loss of NDST1, the heparin sulfate biosynthetic enzyme, results in decreased TG-rich and cholesterol-rich lipoproteins in the liver (88). Although diet-induced dyslipidemia results in elevated plasma cholesterol and TG, miR-27b upregulation in the liver likely protects the liver from extensive lipid accumulation by repressing NDST1 activity and heparin sulfate biosynthesis and, thus, reducing TG-rich lipoprotein uptake in the liver. Subsequent in vivo experimentation in *ApoE*^{-/-} mice showed that in response to a high-fat/high-cholesterol diet, miR-27b is also significantly

upregulated, while its target genes (GPAM and ANGPTL3) are significantly downregulated (85). miR-27b overexpression in hepatocytes resulted in the significant downregulation of 27 lipid-related genes (85). These findings lead to the hypothesis that miR-27b plays an adaptive role in controlling systemic lipid levels.

miRNAs can simultaneously repress hundreds of genes and directly influence the output of many interconnected biological networks. Furthermore, miRNAs can also indirectly influence network output by targeting key network hubs, such as transcription factors (94). For example, critical lipid-sensitive transcription factors controlling hepatic metabolism, such as the LXR and PPAR families, have been shown to be functional targets of multiple miRNAs (LXR α : miR-613; PPAR α : miR-21, miR-10b, miR-141; PPAR γ : miR-23a, miR-27b, miR-130) (85, 94–99). Moreover, complex feedback loops between transcription factors and miRNAs appear to control hepatocyte differentiation and possibly other aspects of liver biology (100–102). Several other canonical miRNAs have been shown to influence lipid metabolic pathways, including miR-122, miR-370, miR-378, and miR-758, and they are comprehensively discussed in numerous recent review articles (43–45, 94, 103).

Although the discovery of alternative modes of miRNA biogenesis is relatively recent, several noncanonical miRNAs have already been shown to be relevant to lipid metabolism. For example, a human miRtron, miR-1224, was found to be induced by lipopolysaccharide (LPS) and to directly target and regulate Sp1 transcription factor activity, which controls the expression of many lipid-related genes (77, 104) including the LDL receptor (105–107). Another recent study found that miR-451, which bypasses DICER processing, is significantly reduced in the livers of rats on high-fat diets (108), supporting an earlier study that found hepatic miR-451 to be downregulated in humans with non-alcoholic steatohepatitis (NASH) (109). The relevant targets of miR-451 remain unknown and merit further investigation. Also, two miRNAs generated from the human mitochondrial (mt) genome (miR-1974 and miR-1978), most likely smRNA fragments derived from mt tRNAs, were found to be significantly upregulated in human coronary artery endothelial cells (HCAEC) treated with oxidized low-density lipoprotein (oxLDL) (110). Most interestingly, putative mRNA targets for these miRNA-like small RNAs are significantly downregulated upon oxLDL treatment in HCAECs (110).

ISOMIRS OF CANONICAL MIRNAS

Irrespective of the biogenesis mechanism, a single genomic locus can give rise to multiple distinct isoforms (isomiR; Fig. 1) (111–113). Initially, isomiRs were either undetected by conventional profiling methods, such as qPCR, or dismissed as experimental artifacts with limited biological relevance. Nevertheless, sophisticated new technologies and computational analyses of smRNA sequencing datasets have demonstrated that they are often present in far higher frequencies than previously appreciated. Moreover, subsequent biochemical experiments have revealed

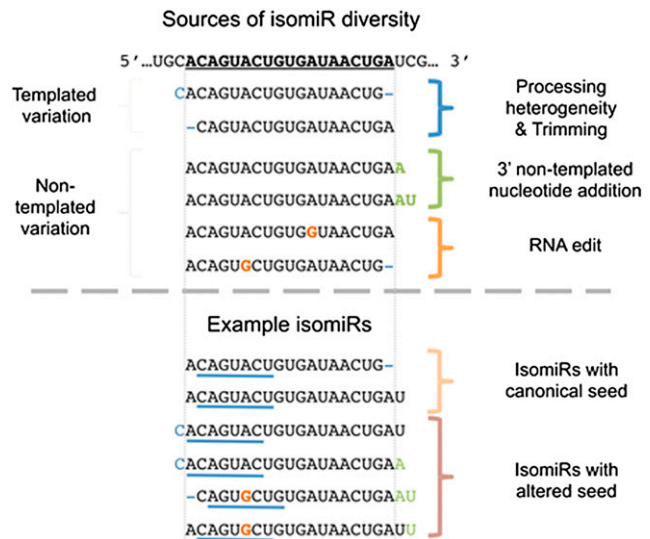


Fig. 1. Sources of isomiR diversity. The top panel illustrates sources of isomiR diversity, which stratify into two classes: templated and nontemplated variations. As illustrated by the bottom panel, an isomiR may contain one or both types of variations, and both templated (e.g., 5'-shifts) and nontemplated (e.g., RNA edits) variations can create an isomiR with an altered seed. The seed region of each isomiR is underlined in blue.

that isomiRs are loaded onto the RISC and recognize target mRNAs for gene repression (61, 113, 114). Some isomiRs actually match the genomic template, but differ in their 5'-start and/or 3'-end positions (Fig. 1). This is likely due to variable enzymatic processing during biogenesis and/or exonuclease-mediated nucleotide trimming. Changes to the 5'-start position of a miRNA are expected to alter the canonical seed sequences, thereby reshuffling the profile of target mRNAs (Fig. 2). Recent studies have used gene reporter (luciferase) assays to demonstrate that two seed-shifted isomiRs have different targeting effects relative to their canonical counterparts (115, 116). Some isomiR sequences may diverge from the genome due to posttranscriptional enzymatic editing and/or tailing of specific nucleotides (Figs. 1, 2). The most dominant type of RNA editing is adenosine deamination, which is commonly referred to as adenosine-to-inosine (A-to-I) editing, and occurs with highest frequency in noncoding regions (e.g., regulatory RNAs such as pre-miRNAs, 3' untranslated regions, etc.) (117, 118). RNA editing events in the 5'-end region of miRNA have been shown to redirect miRNA targeting activity and function (119, 120). For example, A-to-I editing in the seed region of miR-376a results in a new pool of mRNA targets for miR-376a, which includes a new target (enzyme) in the uric acid metabolism pathway (121).

Posttranscriptional tailing, also referred to as nontemplated nucleotide addition (NTA), is carried out by a diverse set of nucleotidyl transferases (Fig. 2). At least 7 out of the 12 known nucleotidyl transferases are thought to mediate 3' NTA (19). For example, the PAP-associated domain family (PAPD4/GLD2, PAPD5) and the mitochondrial poly(A) polymerase (MTPAP) have been found to add adenines to the 3'-end of miRNAs, including several that are highly relevant to hepatic function, such as miR-122

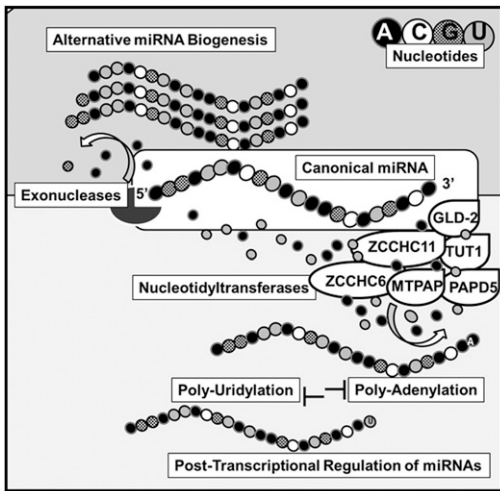


Fig. 2. Schematic of microRNA isomiRs. Nucleotidyl transferases add nontemplated nucleotides to the 3' terminal end of miRNAs and alter miRNA loading onto Argonaute complexes and general miRNA stability. Poly-adenylation inhibits poly-uridylation and vice versa. Exonucleases trim 5' terminal bases from mature miRNAs and generate novel miRNAs or "isomiRs" of canonical mature miRNAs. GLD-2, PAP-associated domain containing 4; MTPAP, mitochondrial poly(A) polymerase; PAPD5, PAP-associated domain containing 5; TUT1, terminal uridylyl transferase 1, U6 snRNA-specific ZCCHC11, zinc finger, CCHC domain containing 11; ZCCHC6, zinc finger, CCHC domain containing 6.

(122) and the let-7 family (21). Other enzymes, such as zinc finger CCHC domain containing 11 (ZCCHC11) and terminal uridylyl transferase 1 (TUT1), catalyze either 3'-uridylation or 3'-adenylation, depending on the miRNA (19, 31). Interestingly, knockdown of PAPD5 and MTPAP also resulted in the reduced frequency of 3' nontemplated "GA" for miR-1246 (19). While it is evident that nucleotidyl transferases preferentially modify specific miRNAs, the sequence or structure features that determine how they select their targets remains unclear.

Currently, multiple strategies and platforms are available to detect and quantify miRNA isomiRs. A comprehensive and exhaustive review on miRNA profiling, methods, and approaches is found in Ref. 123. The most widely used technique for the quantification of canonical miRNAs is real-time PCR using individual assays or low-density arrays. However, this method is not well-suited for isomiR quantification, as reverse transcription primers and probes are not designed to detect 3'-end variability. A significant advantage of an alternate approach, high-throughput smRNA sequencing, is the ability to precisely resolve the 5'- and 3'-ends of miRNA sequences (124). Massive parallel sequencing, often referred to as high-throughput sequencing, takes advantage of amplified clusters of small length cDNA prepared from small RNAs or DNA fragments, which are then simultaneously sequenced by imaging. smRNA sequencing can be labor intensive and time-inefficient, and it also may not provide a means of linear quantification (125). Despite these limitations, the smRNA sequencing method is currently the most sensitive and, as such, has been the method of choice for isomiR analyses. A new, promising approach for isomiR analysis is digital

PCR and NanoString technology (125). This high-throughput technique directly detects miRNA sequences, provides high precision, and allows for linear range of quantification of a large number of samples (125). Another recent study has used mass spectrometry (MS) to observe and characterize miRNA isomiRs (122).

Several studies have reported that 3' NTAs alter miRNA stability and activity (34–40). As such, NTAs represent a means by which miRNAs themselves are posttranscriptionally regulated, which adds another layer of biological complexity to miRNA-mediated gene regulation. The most prevalent 3' NTAs are adenylation and uridylation, which appear to confer different functions for different miRNAs (31). One seminal study reported that the same miRNA can have varying 3'-NTA frequencies across different developmental stages, indicating for the first time that isomiR production is biologically regulated. For a detailed discussion of the biogenesis and functional significance of isomiRs, we direct the reader to two comprehensive review articles (Refs. 112 and 126).

ISOMIRS IN LIPID METABOLISM

We recently performed smRNA sequencing to profile hepatic miRNA changes in response to diet-induced dyslipidemia, and we identified miR-27b as a candidate regulatory hub in lipid metabolism (85). To characterize miR-27b isoform diversity, we requantified miRNA isomiRs in the same smRNA sequencing libraries generated in the aforementioned study. We found that ~20% and ~16% of the hepatic miR-27b reads from C57BL/6J mice were 3'-adenylated and 3'-uridylylated, respectively (Fig. 3A, B). In comparison, miR-122, another key hepatic lipid miRNA, was found to be preferentially adenylylated at its 3'-end (~18% adenylation versus ~3% uridylation) (Fig. 3B). miR-27b, miR-24-1, and miR-23b are poly-cistronic miRNAs, processed from the same primary transcript. In our dataset, all three miRNAs were upregulated in dyslipidemic conditions (6); however, their NTA patterns were very different (Fig. 3B, C). The overall 3' NTA frequency for miR-27b was decreased in the livers of mice on high-fat diets, as both 3'-adenylation and 3'-uridylation were reduced by ~1.6 and ~1.8-fold, respectively (Fig. 3C, Table 1) (85). Contrastingly, miR-23b was found to be more highly 3'-adenylated (1.54-fold) and 3'-uridylylated (1.48-fold) in response to high-fat diet (Fig. 3C, Table 1). As in the case of miR-23b, the 3'-adenylation frequency for miR-24 was also increased by high-fat diet, but substantially more so (~105-fold) (Table 1). Recently, another study using a panel of nine human tissues and cells demonstrated that miR-24 is among the most frequently 3'-modified miRNA (125). In healthy WT mice, 81% of the top 100 expressed miRNAs in the liver have some percentage of isomiRs with poly-adenylation (81/100 miRNAs had isomiRs with poly-adenylation), as opposed to 67% with poly-uridylation. Strikingly, the number of miRNAs (in top 100) with poly-adenylation and poly-uridylation in high-fat diet increased to 94% and 85%, respectively. Although miR-27b and miR-24 isomiRs

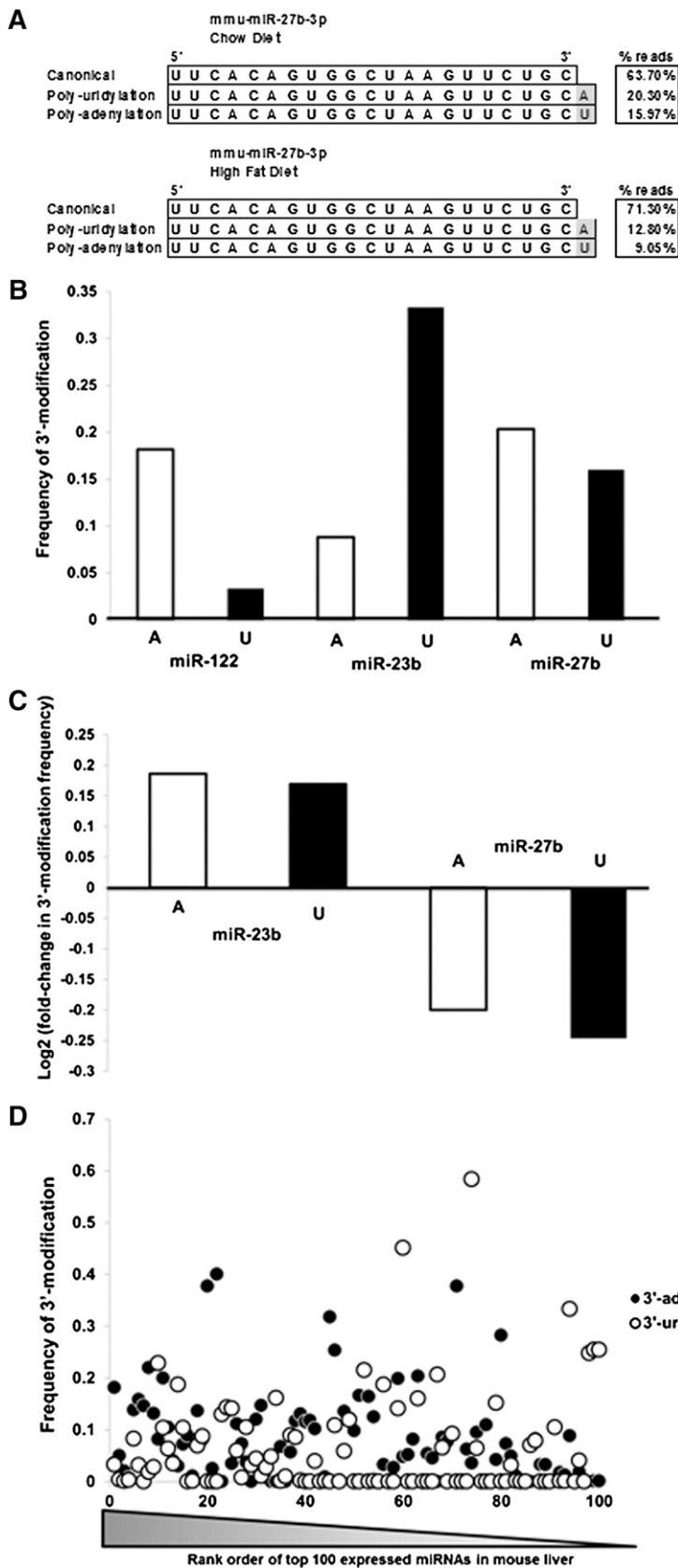


Fig. 3. Hepatic miRNA isomiRs are differentially altered in diet-induced hyperlipidemia. (A) mmu-miR-27b canonical, 3'-adenylation and 3'-uridylation reads (frequency) in normal chow and high-fat diet fed mouse livers, as determined by small RNA high-throughput sequencing. (B) Frequency of mmu-miR-122 (miR-122), mmu-miR-23b (miR-23b), and mmu-miR-27b-3p (miR-27b) 3' terminal modifications in normal mouse liver. (C) Differential 3' modifications to mature miR-23b and miR-27b in high-fat diet mouse livers compared with normal chow diet at three weeks (21% fat, Western diet). Presented as Log_2 (fold-change). (D) Frequency of miRNA non-templated additions are not linked to miRNA expression levels. Frequency of mature miRNA 3' terminal adenylation and uridylation in top 100 expressed miRNAs in mouse liver, as determined by small RNA high-throughput sequencing. Filled circles, 3' adenylation; Open circles, 3' uridylation.

are frequent, diverse, and sensitive to metabolic conditions, literature currently purports that 3'-end NTAs do not mediate changes in direct miRNA targeting but, rather, alter miRNA stability and/or expression. Nevertheless, 3'

NTAs have been demonstrated to alter AGO2-RISC loading; therefore, some 3' NTAs may also indirectly alter miRNA function. Here we describe for the first time isomiR changes associated with high-fat diets or dyslipidemia;

TABLE 1. Differential expression of hepatic miRNA isomiRs and NTA in high-fat diet mice

miRNA isomiR	isomiR Fold Change (HFD/Chow)	NTA Fold Change A	NTA Fold Change U
mmu-mir-194-2-5p	7.44	1.16	0.90
mmu-mir-24-1-3p	5.90	105.07	1.90
mmu-mir-24-2-3p	5.90	105.07	1.90
mmu-mir-194-1-5p	5.55	17.36	1.18
mmu-mir-26a-2-5p	5.42	6.41	119.64
mmu-mir-148a-3p	5.08	0.14	33.84
mmu-mir-16-1-5p	4.92	1.24	0.79
mmu-mir-26a-1-5p	4.44	7.82	146.06
mmu-mir-22-3p	3.37	0.73	0.44
mmu-mir-29c-3p	3.27	0.43	0.08
mmu-mir-21-5p	3.22	2.28	6.13
mmu-mir-29a-3p	3.14	3.34	0.19
mmu-let-7f-1-5p	3.09	1.97	200.55
mmu-mir-25-3p	3.03	0.75	0.25
mmu-mir-23a-3p	2.87	0.00	0.45
mmu-mir-27b-3p	2.85	0.63	0.57
mmu-mir-92a-1-3p	2.63	0.32	3788.75
mmu-mir-23b-3p	2.46	1.54	1.48
mmu-mir-126-3p	2.42	0.99	1.02
mmu-mir-142-3p_-1	2.25	10.71	0.25
mmu-mir-140-3p	2.08	112.57	61845.86
mmu-mir-21-5p_+2	2.05	0.34	9.58
mmu-mir-192-5p	1.81	1.05	0.54
mmu-mir-126-3p	1.69	1.14	1.13
mmu-mir-125b-2-5p	1.67	0.76	25979.11
mmu-mir-125b-1-5p	1.63	0.78	25979.11
mmu-mir-139-5p	1.47	0.71	0.00
mmu-mir-378-3p	1.30	0.00	0.65
mmu-let-7a-2-5p	1.04	0.83	77.53
mmu-let-7d-5p	1.02	1.10	0.00
mmu-mir-126-5p	0.98	0.87	0.50
mmu-mir-122-5p	0.95	1.29	0.64
mmu-mir-122-5p_-1	0.88	0.93	0.00
mmu-let-7c-1-5p	0.86	1.18	0.42
mmu-mir-122-5p_+1	0.73	1.05	0.14
mmu-mir-122-5p_-1	0.72	1.36	0.59
mmu-mir-101b-3p	0.63	237.01	1.20
mmu-mir-29b-2-3p	0.60	1.05	17499.10
mmu-mir-29b-1-3p	0.59	1.08	19026.28
mmu-mir-122-5p_+2	0.56	4.43	1.39
mmu-mir-122-5p_+3	0.48	1.56	9.04
mmu-mir-130a-3p	0.48	1.39	0.55
mmu-let-7b-5p	0.46	1.05	0.28
mmu-mir-193-3p	0.38	1.92	1.46
mmu-mir-126-5p_+2	0.34	0.86	1.42
mmu-mir-126-5p_+1	0.32	0.87	2.47
mmu-mir-122-5p_+4	0.29	1.43	1.12
mmu-mir-221-3p	0.26	1.38	0.37

+1 represents loss of 1 nucleotide on 5'-end of mature canonical miRNA, and -1 represents additional nucleotide on 5' end of canonical mature miRNA. HFD, high-fat diet.


however, the mechanism responsible for miRNA variability in the context of dyslipidemia is currently unknown. To our knowledge, none of the known NTA enzymes has been reported to be altered with high-fat diets or hyperlipidemia.

Canonical miR-122 and its isomiRs account for over 80% of miRNAs present in the liver. As such, miR-122 is the most studied hepatic miRNA and is directly involved in hepatitis C infection (127, 128), cellular proliferation (129, 130), differentiation (102), and tumor suppression (131). miR-122 is also a critical regulator of hepatic lipid metabolism and systemic lipid homeostasis (40, 132–134). miR-122 is thought to be transcribed only in hepatocytes; however, miR-122 is present in extracellular fluids (135, 136), including plasma, and may be transferred to other cells by extracellular RNA carriers (137). Liver-specific

deletion of miR-122 in mice (Mir122ko) was found to cause severe steatosis as a result of the significant accumulation of TG, but not cholesterol, in the liver (138). Specifically, de novo TG synthesis was found to be significantly elevated while TG secretion was significantly decreased in Mir122ko mice (138). Loss of liver miR-122 also resulted in decreased plasma total cholesterol, HDL-cholesterol, and LDL-cholesterol, but only a slight drop in plasma TG was reported (138). Most interestingly, 3'-end adenylation by GLD-2 was found to stabilize miR-122, as GLD-2-null mice were reported to have increased levels of 3'-uridylylated miR-122 (degradation signal), decreased levels of mature miR-122, and increased levels of miR-122 target genes (122). Although GLD-2 is quite possibly involved in the 3'-adenylation of other miRNAs, none other than miR-122 was reported to have significantly reduced 3'-adenylation

in GLD-2-null mice (122). Notably, 3'-adenylation may have different effects on different miRNAs, as 3'-adenylation of miR-26a failed to alter stability but, rather, significantly reduced targeting capacity (139). This apparent discrepancy could be due differential nucleotide transfer enzymes, as miR-122 is poly-adenylated by GLD-2 and miR-26a was found to be poly-adenylated by ZCCHC11 (122, 139). Although miR-122 is one of the most abundant miRNAs in the liver and one of the most highly modified miRNAs, we found that overall the frequency of 3' NTA is not correlated with miRNA expression (Fig. 3D).

SUMMARY

The study of miRNAs has provided insights into novel molecular mechanisms that underlie complex disease etiology. Accordingly, miRNAs have emerged as compelling drug targets for the treatment of numerous disorders, including cardiovascular disease and dyslipidemia. Recent technological advances, such as next-generation sequencing, small biologics, and integrative computational tools, have facilitated rapid progress in the profiling and functional characterization of miRNAs. For example, there are now several cost-efficient platforms and protocols for smRNA-seq, including those from Illumina, Life Technologies, and NanoString, that provide a high-resolution, digital representation of intracellular miRNA expression. Very recent computational analyses of smRNA-seq data have led to the discovery that many miRNAs are present in multiple isoforms, called isomiRs, indicating that the complete repertoire of functional miRNAs is likely more complex than previously appreciated. This review highlights the extensive diversity of miRNAs and the potential for complex posttranscriptional regulation of miRNAs as well as other noncoding smRNAs. In summary, miRNAs and other smRNAs control many facets of lipid metabolism, and this is not limited to the canonical forms but also likely includes related functional variants such as isomiRs. 

PERSPECTIVES

- A diverse group of miRNAs and miRNA-like smRNAs control lipid metabolism.
- miR-27b is a posttranscriptional regulatory hub for lipid metabolism and regulates 27 genes related to lipid homeostasis.
- Many miRNAs previously reported to target lipid-regulating genes are present in multiple isoforms, due to various types of 5' and 3' posttranscriptional modifications.
- Multiple sequencing and nonsequencing-based methods facilitate the quantification and analysis of miRNA isomiRs.
- 5' variability likely alters miRNA targeting activity and biological function.
- 3' nontemplated nucleotide additions are miRNA specific and alter miRNA stability and/or loading onto the RISC.

- Frequency of miRNA nontemplated nucleotide additions is not directly correlated with miRNA expression levels.

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