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MicroRNAs and Noncoding RNAs in Hepatic Lipid and Lipoprotein Metabolism: Potential Therapeutic Targets of Metabolic Disorders

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Abstract

Noncoding RNAs and microRNAs (miRNAs) represent an important class of regulatory molecules that modulate gene expression. The role of miRNAs in diverse cellular processes such as cancer, apoptosis, cell differentiation, cardiac remodeling, and inflammation has been intensively explored. Recent studies further demonstrated the important roles of miRNAs and noncoding RNAs in modulating a broad spectrum of genes involved in lipid synthesis and metabolic pathways. This overview focuses on the role of miRNAs in hepatic lipid and lipoprotein metabolism and their potential as therapeutic targets for metabolic syndrome. This included recent advances made in the understanding of their target pathways and the clinical development of miRNAs in lipid metabolic disorders.

Keywords

noncoding RNAs; microRNAs; dyslipidemia; metabolic syndrome; drug-development

INTRODUCTION

Dyslipidemia is characterized by increased plasma triglyceride (TG) and free fatty acids (FAs), owing to elevated production of very-low-density lipoprotein (VLDL), as well as increased small dense low density lipoprotein (LDL) and decreased high-density lipoprotein (HDL) [Christian and Su, 2014]. This type of lipid profile is associated with increased risk of cardiovascular disease which is currently one of the major causes of death in North America [Pagidipati and Gaziano, 2013]. The increased biosynthesis of lipids, overproduction of VLDL and altered biogenesis of HDL initiate dyslipoproteinemia that is

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frequently accompanied with metabolic syndrome [Su et al., 2009; 2010; 2014]. Emerging evidence has elucidated the critical regulatory roles of microRNAs (miRNAs) and noncoding RNAs in controlling cellular lipid and lipoprotein metabolism via cross-talk with metabolic signaling within mitochondria and the ER (endoplasmic reticulum) [Christian and Su, 2014]. Here we review recent advances on the role(s) of miRNA in lipid and lipoprotein metabolism and their potential as therapeutic approaches to treating metabolic syndrome.

BIOGENESIS AND FUNCTION OF LONG NON-CODING RNAs AND MIRNA

Long non-coding RNAs (lncRNAs) are RNA molecules longer than 200 nucleotides that lack protein-coding ability. They have been understudied till now, but with the development of novel RNA sequencing and high throughput technology, it is becoming a progressively expanding area of research. Emerging evidence indicates that lncRNAs are transcribed by RNA polymerase II (RNAPII) due to their ability to be capped, spliced and polyadenylated [Guttman and Rinn, 2012]. The ability to be transcribed from different loci of the genome gives rise to various type of lncRNAs including, but not limited to intronic, antisense, intergenic as well as mitochondrial lncRNAs [Rackham et al., 2011; Rinn and Chang, 2012]. lncRNAs can either be cis- or trans-acting by regulating the expression of genes located on the same chromosome from which they are transcribed or from a different chromosome respectively. Cis-acting lncRNA acts by binding and recruiting epigenetic modifiers to the target genes while remaining attached to the elongating RNAPII, which can be described as 'tethering' [Guttman and Rinn, 2012; Magistri et al., 2012]. Alternatively, trans-acting lncRNA do not tether but still target RNAPII activity to modify transcriptional output. The cellular localization of lncRNAs remains unclear as some studies show that most lncRNAs are confined to the nucleus [Derrien et al., 2012; Zhang et al., 2014] while another indicates lncRNA to be present in all subcellular fractions, with the majority of expressed lncRNAs being enriched in the cytosol and in ribosomes [van Heesch et al., 2014].

miRNAs are RNA molecules ~18–25 nucleotides long, that bind to mRNA sequences to regulate gene expression. miRNAs were first discovered in the nematode *C. elegans* [Lee et al., 1993] and are evolutionarily conserved [van Rooij et al., 2007]. miRNA genes are generally transcribed by RNA polymerase II (RNAPII) into a long primary transcript (pri-miRNA) up to several kilobases in length that forms a stem-loop structure [Bartel, 2004]. Pri-miRNA is processed in the nucleus to pre-miR (~70 nucleotides) by *Drosha* and DGCR8 RNase III complex [Lee et al., 2003]. The pre-miRNA hairpins are then exported from the nucleus to the cytosol by an exportin 5/RAN-GTP-dependent process [Bohnsack et al., 2004]. In the cytosol, pre-miRNA binds to the RNase III enzyme Dicer [Bernstein et al., 2001] and the RNA-induced silencing complex (RISC), which consists of the transactivation-responsive RNA-binding protein (TRBP) and Argonaute 2 (Ago2). Twelve nucleotides are cleaved from 3' end of the pre-miRNA by Ago2 and further cleavage is completed by Dicer to result in a ~22 nucleotide double-stranded miRNA duplex. The miRNA duplexes are loaded into an Argonaute protein in the miRNA-induced silencing complex (miRISC) and are rapidly unwound. The mature miRNA is retained in the miRISC and the complementary strand, known as miRNA star (miR*), is released. miRNAs mainly bind to the 3' untranslated region (UTR) of their target mRNAs [Delay et al., 2011]. However, they can also bind to 5'UTR, or open reading frame (ORF) of the target mRNA

[Moretti et al., 2010]. By binding to their target mRNA, they can regulate the translation of proteins from mRNA or cleave the mRNA [Bartel, 2004].

Targeting *de novo* Lipogenic Signalling by miRNAs

Lipid metabolism is governed by variety of cellular regulators, including the transcription factors involved in the synthesis and oxidation of fatty acids (FA) and cholesterol. The sterol response element binding proteins (SREBPs) are transcription factors that are involved in the regulation of cholesterol uptake and FA synthesis [Brown and Goldstein, 1997]. SREBP1a and SREBP2 activate the transcription of cholesterol-metabolism genes, including 3-hydroxy-3 methylglutaryl coenzyme A reductase (HMGCoAR), the rate-limiting enzyme of cholesterol biosynthesis, and the LDL receptor (LDLr) that acts as a scavenger of circulating LDL from the bloodstream. SREBP1c mainly upregulates the transcription of genes involved in FA metabolism which results in increased *de novo* lipogenesis. Similar to SREBPs, liver X receptors (LXRs) are transcription factors that also play key role in regulating cholesterol, FA and lipoprotein metabolism [Mak et al., 2002; Repa et al., 2000; 2002]. LXRs regulate cholesterol absorption and transport by targeting ATP binding cassette transporters A1 (ABCA1), ABCG1 and ABCG5/G8. LXRs regulate levels of the enzyme Cyp7a, involved in bile acid biosynthesis, and levels of apolipoprotein E (ApoE), involved in lipid transport. LXR α also regulates lipogenesis via increased expression of the lipogenic genes SREBP-1c, FAS, acyl-CoA carboxylase (ACC) and stearoyl-CoA desaturase 1 (SCD1). Both SREBPs and LXRs are involved in the regulation of lipogenesis; however other transcription factors known as retinoid X receptors (RXRs) are involved in the activation of the acyl-CoA oxidase gene promoter encoding the rate-limiting enzyme of peroxisomal β -oxidation of FAs [Guo et al., 2007]. Thus, transcription factors can act to either increase oxidation of lipids or increase *de novo* lipogenesis highlighting their importance in various metabolic disorders.

Transcription factors are key players in regulating both oxidation and synthesis of lipids. Peroxisome proliferator-activated receptors (PPARs) are enzymes that act critical regulators of lipid oxidation and transport. PPAR α activates peroxisomal β -oxidation of FAs and upregulates expression of long-chain acyl-CoA synthetase gene [Guo et al., 2007; Schoonjans et al., 1995]. The PPAR γ coactivator (PGC)1- α can increase FA oxidation by increasing carnitine palmitoyltransferase 1 α (CPT1 α) expression and has a positive feedback to increase PPAR α levels [Mulvihill and Huff, 2012]. Alternatively, PGC-1 β coactivates SREBP and LXR families of transcription factors and thus controls two major pathways of lipid metabolism, namely *de novo* lipogenesis and lipoprotein secretion. PGC-1 β coactivates LXR and LXR target genes (ie/CYP7a1 and ABCA1) involved in the regulation of lipoprotein secretion and also directly interacts with SREBPs to activate the expression of SREBP target genes (ie/FAS, SCD-1, HMG-CoA reductase, DGAT, and GPAT) [Lin et al., 2005]. Key enzymes regulating *de novo* synthesis of FAs are under the regulatory control of SREBP. In the process of *de novo* lipogenesis, ACC catalyzes the conversion of acetyl-CoA to malonyl-CoA acting as the rate-limiting enzyme for FA synthesis. Malonyl-CoA is then used in the formation of long-chain FAs via FAS. Diacylglycerol O-acyltransferase (DGAT) enzymes are also under the regulatory control of SREBP and catalyze the final step in TG synthesis. DGAT1 catalyzes the terminal and only committed step in TG synthesis while

DGAT2 plays a key role in VLDL assembly by esterifying exogenous FAs to glycerol. Substantial evidence has shown that various miRNAs directly or indirectly target and regulate lipid and lipoprotein signaling at multiple metabolic levels. Herein, we summarized recent studies that contribute to the advancement of our knowledge in this field.

miRNAs Target Transcription Factors in Lipid Metabolism

Emerging evidence has shown that lipid metabolism is regulated by miRNAs that target transcription factors involved in lipid and lipoprotein metabolism. Below we highlight the role that miRNAs play in regulating *de novo* lipogenesis by targeting and modulating SREBP and LXR as well as the role in regulating FA oxidation via modulation of PPARs.

SREBPs—MiR-33a and miR-33b are intronic miRNAs located within intron 16 of the *SREBP-2* gene and intron 17 within the *SREBP-1* gene, respectively [Najafi-Shoushtari et al., 2010]. Metabolic stimuli that activate *SREBP* gene expression also regulate miR-33a and miR-33b expression, indicating that miR-33 is co-expressed with its host genes. miR-33 knockout mice have elevated hepatic SREBP1 expression causing increased FA synthesis and lipid accumulation in both the liver and adipose tissues. However they do not show any changes in SREBP-2 expression levels [Horie et al., 2013]. SREBP-2 levels are modulated by cholesterol depletion which enhances expression of miR-33a and *Srebp-2* while excess cholesterol downregulates the expression of miR-33a and *Srebp-2* [Rayner et al., 2010]. Both miR-122 and miR-370 also regulate SREBP levels as shown in a study where overexpression of either miR-370 or miR-122 upregulated SREBP-1c expression in HepG2 cells while inhibition resulted in decreased SREBP-1c expression [Iliopoulos et al., 2010]. *In vivo* studies have further shown that increased expression of miR-370 and miR-122 in ApoE^{-/-} mice increases hepatic cholesterol and TG levels [Iliopoulos et al., 2010]. Finally, SREBP transcription factors can also be modulated indirectly via changes in insulin-induced gene 1 (Insig1), an ER polytopic membrane protein that retains SREBPs in the ER to prevent their proteolytic activation in the Golgi bodies [Yang et al., 2002]. miR-24 represses Insig1 thus facilitating SREBP activation and subsequent induction of lipogenic genes resulting in hepatic lipid accumulation in the human hepatoma cell line, HepG2 [Ng et al., 2014]. Alternatively, miR-24 inhibitors augment Insig1 expression thus reducing hepatic lipid accumulation [Ng et al., 2014]. SREBPs can be thus be modulated both directly and indirectly by miRNAs to either increase or decrease hepatic FA synthesis.

LXR α —LXR α plays a key role in regulating FA metabolism by regulating SREBP1-c as well as the downstream targets involved in FA synthesis. LXR α is regulated and repressed by miR-613 to reduce expression levels of genes involved in *de novo* lipogenesis (ie/ SREBP-1c, FAS, ChREBP and ACC) and also reduced LXR α -induced lipid droplet accumulation in HepG2 cells [Zhao et al., 2014]. Similarly, miR-155 represses LXR α and thus plays a protective role in the prevention of non-alcoholic hepatosteatosis in mice fed a high fat diet (HFD) [Miller et al., 2013].

PPAR α —PPARs play a key role in the oxidation and transport of lipids and PPAR α regulates peroxisomal β -oxidation. PPAR α is under the regulatory control of both miR-27b and miR21 which have been shown to decrease PPAR α protein levels when overexpressed

in Huh7 cells [Kida et al., 2011]. Furthermore, PPAR α protein levels are increased by inhibition of miR-21 and miR-27b; whether this leads to downstream changes in hepatic lipid oxidation is yet to be investigated.

miRNAs Target Lipogenic Enzymes in Lipid Metabolism

Similar to the regulation of transcription factors, lipogenic enzymes are also under the regulatory control of various miRNAs. miR122 accounts for 70% of liver total miRNAs and targets multiple lipogenic genes in the liver including FA synthase (FASN), ACC-1 and ACC-2 [Esau et al., 2006]. miR-122 inhibition in mice using antisense oligonucleotides (ASO) reduced plasma TG and cholesterol levels via a reduced rate of FA and cholesterol synthesis in hepatocytes. Expression levels of *FASN*, *ACC2* and *SCD1* involved in FA synthesis and *HMG-CoAR* involved in cholesterol synthesis were indirectly reduced by anti-miR-122 treatment [Esau et al., 2006]. This suggests that inhibition of miR-122 may act as an important therapeutic target in treating hepatic steatosis and dyslipidemia. Gene expression profiling following liver-specific knockout of miR-122 (*Mir122a*-LKO) revealed upregulation of the genes involved in the TG biosynthesis pathway in the liver, including 1-Acylglycerol-3-Phosphate O-Acyltransferases (*Agpat1*, *Agpat3*, *Agpat9*), Monoacylglycerol O-Acyltransferase 1 (*Mogat1*) and phosphatidic acid phosphatases (*Ppap2a*, *Ppap2c*). Furthermore, there was an upregulation of the lipid droplet protein *Cidec* that functions by inhibiting lipolysis thereby augmenting TG accumulation [Hsu et al., 2012]. In accordance with this altered gene expression, miR-122 KO resulted in increased hepatic TG accumulation due to higher TG synthesis and reduced TG secretion. It is unclear why miR-122 inhibition by ASO and miR-122 KO resulted in completely opposite lipogenic effects. However, additional studies showed that miR-370 overexpression caused an increase and miR-370 inhibition caused a decrease in expression of miR-122 and expression of lipogenic genes in HepG2 cells supporting the concept that miR-370 stimulates lipogenesis indirectly via increasing the expression of miR-122 [Iliopoulos et al., 2010]. Increased plasma levels of miR-122 and miR-370 are associated with coronary artery disease in hyperlipidemia patients [Gao et al., 2012]. Further investigation is required to clarify the role of miR-122 in regulating hepatic lipid homeostasis.

Abnormal lipid metabolism in hepatocellular carcinoma (HCC) is regulated by miRNAs, particularly miR-205. miR-205 targets and decreases acyl-CoA synthetase long-chain family member 1 (ACSL1) mRNA expression levels in hepatoma cells leading to decreased lipogenesis, while antisense miR-205 increased lipogenesis. Low levels of hepatic miR-205 resulted in elevated hepatic TG accumulation due to higher ACSL1 expression [Cui, M et al., 2014]. Interestingly, low levels of miR-205 were observed in clinical HCC tissues, which also displayed elevated expression of ACSL1. Lastly, miR-205 can directly downregulate ACSL4 in hepatoma cells by Hepatitis B virus X protein (HBx) leading to hepatic cholesterol accumulation [Cui, M et al., 2014]. Thus, miR-205 may act as an important therapeutic agent for HCC patients in the regulation of hepatic lipid metabolism.

miRNAs Target Enzymes in FA Oxidation

Mitochondria play a crucial role in hepatic FA oxidation and impairment in mitochondrial lipid oxidation has been related to the onset and progression of obesity. CPT1 α is a

mitochondrial enzyme that plays a crucial role in FA oxidation by mediating the transport of long-chain FAs and has been shown to be inhibited by miR-370 leading to a reduction in hepatic lipid oxidation [Iliopoulos et al., 2010]. Cpt1 α is also targeted and inhibited by miR-33a/b as shown by a study in which overexpression of miR-33a and miR-33-b reduced both FA oxidation in hepatic cell lines and increased cellular nonesterified FAs and TG [Davalos et al., 2011; Gerin et al., 2010]. Other transcripts encoding enzymes of FA oxidation pathway, namely, carnitine O-octanoyltransferase (CROT) and hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein) β subunit (HADHB) are also targeted and downregulated by miR-33 [Davalos et al., 2011]. CROT is a peroxisomal enzyme that couples short-chain FAs to carnitine to facilitate their transport into the mitochondria while HADHB is involved in the final three steps of the mitochondrial β -oxidation pathway [Mannaerts et al., 2000]. Thus, downregulation of CROT and HADHB by miR-33 results in decreased hepatic FA oxidation and may lead to increased hepatic steatosis. miR-122 has also been implicated in dysregulation of FA oxidation. ASO mediated inhibition of miR-122 increased the hepatic FA oxidation rate and these changes paralleled with an inhibition of ACC2; a key enzyme in FA oxidation [Esau et al., 2006]. Another miRNA, miR199a-5p is associated with impaired FA- β -oxidation in mitochondria and abnormal lipid accumulation in hepatocytes [Li et al., 2014]. MiR-199a-5p is co-transcribed with miR-214 within the Dynamin3 gene (*Dnm3*) as a common primary transcript in mouse, human and zebrafish [Loebel et al., 2005]. The hypoxia-inducible microRNA cluster of miR-199a and miR-214 (miR-199a~214) inhibits mitochondrial FA oxidation by targeting and repressing myocardial PPAR δ , which targets and decrease FA oxidation genes [el Azzouzi et al., 2013].

Targeting Lipoprotein Metabolism by miRNAs

Lipoprotein particles are a combination of both lipid and protein complexes which transport cholesterol and TGs to various tissues. They consist of a core of cholesteryl esters and TGs surrounded by outer layer of phospholipids, cholesterol and apolipoproteins. VLDL particles are formed in hepatocytes by the assembly of TGs and cholesteryl esters with apoB100. The proper assembly and secretion of VLDL is dependent upon the availability of its lipid substrates and the proper functioning of proteins such as apoB and microsomal TG transfer protein (MTP). MTP is an intracellular lipid-transfer protein which transfers TGs onto apoB during its translation in the liver and intestine [Christian and Su, 2014; Hussain et al., 2003]. miR-30c targets MTP mRNA by inducing its degradation and reduces plasma lipid concentration. MiR-30c also reduces *de novo* lipogenesis by targeting lysophosphatidylglycerol acyltransferase (LPGAT1), an enzyme involved in phospholipid synthesis [Soh et al., 2013]. Expression of miR-30c in Huh-7 cells reduced mRNA levels of LPGAT1 while anti-miR-30c showed the opposite effect. This data was supported in an *in vivo* model in apoE KO mice showing reduced plasma lipid levels and decreased atherosclerotic plaques in both the aorta and the aortic root following lentiviral overexpression of miR-30c. Alternatively, overexpression of anti-miR-30c elevated plasma TG and cholesterol levels in apoE KO mice fed a Western diet. This study highlights the therapeutic potential of miR-30c in the prevention of dyslipidemia and hepatic steatosis. miRNAs that target and decrease hepatocellular lipid synthesis can lead to decreased fasting plasma lipid levels due to less lipid substrate being available for VLDL assembly. miR-33 is

involved in decreasing plasma lipid levels due to its ability to target hepatic FA and cholesterol biosynthesis thereby leading to a decrease in VLDL. In African green monkeys, anti-miR-33 increased expression of FA oxidation genes (CROT, CPT1A, HADHB and PRKAA1) and reduced expression of genes involved in FA synthesis (SREBF1, FASN, ACLY and ACACA), thus lowering plasma levels of VLDL-associated TGs [Rayner et al., 2011]. miR-155 is also implicated in the regulation of VLDL metabolism as shown in a study where lack of miR-155 in mice fed a HFD increased hepatic steatosis and serum VLDL/LDL cholesterol levels. The mechanism by which miR-155 increases hepatic lipid levels involved the targeting of LXR α [Miller et al., 2013]. miRNAs that target and modulate VLDL synthesis may serve as a novel therapeutic approach to prevent and treat metabolic disorders associated with dyslipidemia.

Regulation of HDL metabolism is important in controlling circulating cholesterol levels. Reverse cholesterol transport (RCT) is a process which increases HDL-cholesterol levels and decreases the risk of atherosclerosis. In RCT, excess cholesterol from atherosclerotic macrophages is transferred and carried by HDL to the liver for excretion as bile acids or free cholesterol [Ghosh, 2012]. In the first stage of RCT, hydrolysis of intracellular cholesterol esters is released as free cholesterol via cholesterol transporter ABCA1 onto apolipoprotein AI (apoAI) to form HDL particles. ABCA1 regulates the rate of cholesterol efflux to apoAI during synthesis of nascent HDL [Chung et al., 2011]. miRNAs regulate different steps of RCT including HDL biogenesis, cellular cholesterol efflux, and HDL cholesterol uptake in the liver. miR-33 regulates the initial steps of RCT by inhibiting expression of the ABCA1 and thus attenuating cholesterol efflux to apoAI [Rayner et al., 2010]. Circulating HDL levels were reduced following lentiviral delivery of miR-33 in mice due to reduced hepatic ABCA1 expression [Rayner et al., 2010]. Alternatively, silencing of miR-33 *in vivo* increased both circulating HDL levels and hepatic expression of ABCA1. Inhibitors of miR-33 also enhanced cholesterol efflux from hepatocytes to apoAI *in vitro* and elevated levels of HDL-cholesterol by 30%. Moreover, increases in plasma HDL-cholesterol of 25% in male, 40% in female miR-33 null mice was detected following targeted deletion of miR-33 [Horie et al., 2013]. To determine the potential clinical relevance of miR-33 lipid regulation, systemic delivery of an anti-miRNA oligonucleotide targeting both miR-33a and miR-33b in African green monkeys over 12 weeks was carried out. This led to increased hepatic expression of ABCA1, increased plasma HDL levels, increased expression levels of FA oxidation genes (CROT, CPT1A, HADHB and PRKAA1) and reduced expression FA synthesis genes (SREBF1, FASN, ACLY and ACACA). These data suggest that therapeutic inhibition of miR-33a and miR-33b is a useful strategy to increase plasma HDL cholesterol levels and potentially decrease associated dyslipidemias and atherosclerotic risk [Rayner et al., 2011].

ABCA1 and of ABCG1 are hepatic cholesterol transporters under the regulatory control of LXR. These regulate cholesterol efflux and are upregulated in states of cholesterol excess [Ramirez et al., 2013]. miR-144 targets ABCA1 with overexpression inhibiting ABCA1 levels in both hepatocytes and monocyte/macrophages [Ramirez et al., 2013]. *In vivo* delivery of miR-144 reduced ABCA1 liver expression and further reduced circulating HDL-cholesterol levels regulating both macrophage cholesterol efflux and hepatic HDL

biogenesis. LXR ligands induce miR-144 resulting in a negative feedback loop to provide a tight regulation of cholesterol homeostasis. Additionally, miR-128-2 also inhibits the expression of ATP-binding cassette transporters, ABCA1, ABCG1 and RXR α directly via a miR-128-2-binding site within their respective 3' UTR.

Attenuation of cholesterol efflux and reduction of protein and mRNA levels of ABCA1, ABCG1 and RXR α have been observed with miR-128-2 overexpression. Conversely, anti-miRNA treatment leads to increased ABCA1, ABCG1 and RXR α expression and stimulation of cholesterol efflux, thus establishing miR-128-2 as a regulator of cholesterol efflux [Adlakha and Saini, 2013]. Other miRNAs directly target and repress ABCA1 and cholesterol efflux including miR-758 [Ramirez et al., 2011], miR-26 [Sun et al., 2012] and miR-106b [Kim et al., 2012]. MiR-758 decreased the expression of ABCA1 in mouse and human cells *in vitro* and reduced cellular cholesterol efflux to apoAI in mice while anti-miR-758 enhanced cholesterol efflux. Interestingly, miR-758 is decreased in peritoneal macrophages and in liver following a high fat diet. Finally, miR-26, miR-106b, miR-19b and miR-145 target and decrease ABCA1 levels in hepatoma cells potentially inhibiting cholesterol efflux and promoting cholesterol accumulation and atherosclerosis [Kang et al., 2013; Kim et al., 2012; Lv et al., 2015; Sun et al., 2012].

Scavenger receptor class B type I (SR-BI) mediates selective HDL-cholesterol uptake by hepatocytes and thus plays a critical role in regulating circulating cholesterol homeostasis. MiR-185, miR-96, and miR-223 have been implicated in repression of selective HDL cholesterol uptake through posttranscriptional inhibition of SR-BI [Wang et al., 2013]. In THP-1 macrophage derived foam cells, miR-27a/b reduced HDL-mediated cholesterol efflux by inhibiting SR-BI, ABCG1 and ABCA1 [Zhang et al., 2014] and miR-223 has also been shown to inhibit SR-BI and repress HDL cholesterol uptake [Vickers et al., 2014]. TakemiRNAs that target any level of the RCT pathway including HDL-cholesterol uptake may act have benefit in treating hypercholesterolemia.

Targeting Lipid Metabolism by lncRNAs

While thousands of lncRNAs have been discovered in recent years, their physiological role remains largely unknown. The identification of lncRNAs involved in lipid metabolism may provide insight into regulation of lipid metabolism pathways, which can provide new targets for therapeutics of dyslipidemia. We describe herein the recently identified lncRNAs that play a key role in maintaining lipid balance.

lncLSTR is a liver-enriched lncRNA which is a liver specific triglyceride regulator for maintaining lipid homeostasis [Li et al., 2015]. Its depletion can reduce triglyceride levels in a hyperlipidemia mouse. The gene that encompasses the non-coding RNA, *gadd7* (growth arrested DNA-damage inducible gene 7) is induced by reactive oxygen species (ROS) induced by palmitate and ER stress [Brookheart et al., 2009]. Depletion of *gadd7* by mutagenesis or short hairpin RNA knockdown reduces lipid and non-lipid mediated ROS. Highly Up-Regulated In Liver Cancer long non-coding RNA (HULC) upregulates the transcriptional factor PPAR α , which then activates the ACSL1 promoter in hepatoma cells [Cui et al., 2015]. HULC causes methylation of CpG islands in the miR-9 promoter and

suppress miR-9 targeting of PPAR α mRNA. HULC promotes lipogenesis, thereby augmenting intracellular triglycerides and cholesterol accumulation *in vitro* and *in vivo*.

SPRY4-IT1 is an LncRNA that binds to the enzyme lipin 2 that converts phosphatidate to diacylglycerol (DAG) [Mazar et al., 2014]. siRNA mediated knockdown of SPRY4-IT1 caused an accumulation of lipin 2 protein and induced changes in lipid species, including increased acyl carnitine, fatty acyl chains, and TG, changes that are known to cause apoptosis. LncRNAs are therefore promising drug target for diseases associated with aberrant lipid metabolism. However, given their low expression, it will be a challenging task to deduce their biological function [Derrien et al., 2012].

Clinical Development of miRNA Therapeutics

Several pharmacological approaches are being currently used to inhibit Proprotein convertase subtilisin/kexin type 9 (PCSK9), which functions by reducing LDL receptor expression causing elevated plasma LDL-cholesterol [Maxwell and Breslow, 2004]. In nonhuman primates a month long course of weekly subcutaneous therapy with locked nucleic acid (LNA) antisense oligonucleotides targeting PCSK9 decreased expression of both PCSK9 and circulating LDL cholesterol without reported toxicity [Lindholm et al., 2012]. Since PCSK9 functions to increase the rate of degradation of LDL receptor, a step that is critical for the removal of LDL from circulation, reduced PCSK9 expression thus prevents LDL clearance and increases circulating LDL levels. Moreover, another PCSK9 inhibitor, SPC5001 is an mRNA-based compound that targets PCSK9. SPC5001 oligonucleotide has phosphorothioate backbones and was designed as an LNA gapmers 13–16 bases long having two or three LNA modifications at the 5' end, three LNA modifications at the 3' end, and a DNA core (to facilitate RNase H-mediated cleavage of the complementary PCSK9 mRNA target). The first Phase I safety and tolerability clinical trial of SPC5001 (<http://clinicaltrials.gov/show/NCT01350960>) for the treatment of hypercholesterolemia was terminated due to its short therapeutic window since the compounds was intended for chronic use. In another study, 20 base-long 2'-O-methoxyethyl antisense gapmers were used to target apoB mRNA in liver tissue [Yu et al., 2009]. SPC4955 is an mRNA targeted drug candidate that inhibits apoB present in LDL-cholesterol and reduced LDL-cholesterol and triglycerides. It is in Phase I for hypercholesterolemia

Another therapeutically advanced miRNA targeting lipid metabolism is miR-33, which plays a regulatory role in cellular cholesterol transportation, including the inhibitory effect on ABCA1, ABCG1 and Niemann-Pick C protein (NPC1) [Marquart et al., 2010; Rayner et al., 2010]. An miRNA specifically targeting miR-33a/b is being evaluates for the treatment of atherosclerosis, obesity and hypertriglyceridemia (Donner, 2013). ASOs against miR-33 that are modified on 2' fluoro/methoxyethyl- and 2'F/MOE- groups increased circulation level of HDL-cholesterol by enhancing RCT to the plasma, liver, and feces, and reduced plaque size and lipid content in mice [Rayner et al., 2011]. However, this was also accompanied by hepatic lipid accumulation, increased plasma TG levels and upregulation of the genes involved in hepatic FA synthesis in mice fed a HFD compared to chow controls [Rayner et al., 2011]. The upregulation in hepatic FA synthesis seemed to be due to increased nuclear transcription Y subunit gamma (NFYC), a miR-33 target gene that is

required for DNA binding and full transcriptional activation of SREBP-responsive genes [Shimano, 2001]. While the therapeutic potential of miR-33 in lowering dyslipidemia is apparent, additional research is required to identify novel strategies for miRNA targeting and delivery to avoid associated adverse effects.

CONCLUSIONS

The discovery of microRNAs has provided new insight into the molecular mechanism of pathogenesis of hyperlipidemia, NAFLD (non-alcoholic fatty liver disease), obesity related fatty liver and type-2 diabetes. The data reviewed here demonstrates that a large array of miRNAs participate in lipid and lipoprotein metabolism (Table 1) having both positive or negative regulatory effects on lipid metabolism and lipoprotein assembly and secretion. This involves modulation of lipid biosynthesis, FA β -oxidation, and lipoprotein metabolism by targeting enzymes and proteins that are involved in these metabolic pathways. Future studies will provide more insights into miRNA biological function, which may pave the path for future directions in miRNA therapeutics. Moreover, understanding the regulatory roles of miRNAs in the modulation of other long noncoding RNAs will elucidate noncoding RNA function and will enhance utilization of miRNA mimics, antisense miRNAs and long noncoding RNAs as therapeutic tools to combat dyslipidemia and metabolic syndrome.

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Table 1

miRNAs regulating lipid and lipoprotein metabolism

miRNA	Lipid metabolism gene	Function of gene	Reference
miR-33a/33b	SRBEP HADHB CPT1a CROT AMP α ABCA1 NPC1	Cholesterol biosynthesis, uptake FA oxidation FA oxidation FA oxidation FA oxidation Cholesterol efflux Cholesterol metabolism	[Horie et al., 2013; Najafi-Shoushtari et al., 2010] [Davalos et al., 2011; Gerin et al., 2010] [Davalos et al., 2011; Gerin et al., 2010] [Davalos et al., 2011; Gerin et al., 2010] [Davalos et al., 2011; Gerin et al., 2010] [Najafi-Shoushtari et al., 2010] [Rayner et al., 2010]
miR-122	SREBP-1c FAS ACC-1 ACC-2 HMGCR SCD1 DGAT2	FA synthesis FA biosynthesis FA biosynthesis FA oxidation Cholesterol synthesis Cholesterol synthesis Triglyceride synthesis	[Esau et al., 2006] [Esau et al., 2006] [Esau et al., 2006] [Esau et al., 2006] [Esau et al., 2006] [Esau et al., 2006] [Esau et al., 2006]
miR 613	LXR α	Lipid synthesis	[Zhao et al., 2014]
miR-155	LXR α	Lipid synthesis	[Miller et al., 2013]
miR-370	Cpt1a	FA oxidation	[Iliopoulos et al., 2010]
miR-199a	PPAR δ	FA oxidation	[el Azzouzi et al., 2013 Li et al., 2014]
miR-27a/b	SR-BI, ABCG1 and ABCA1	Cholesterol metabolism	[Zhang, M et al., 2014] [Zhang, M et al., 2014]
miR-24	Insig1	Lipogenesis	[Ng et al., 2014]
miR-185, miR-96, and miR-223	SR-B1	Cholesterol uptake	[Wang et al., 2013]
miR-758, miR-26, miR-106b miR-144 miR-145 miR-19b	ABCA1 ABCA1 ABCA1 ABCA1 ABCA1 ABCA1	Cholesterol efflux	[Ramirez et al., 2011] [Sun et al., 2012] [Kim et al., 2012] [Ramirez et al., 2013] [Kang et al., 2013] [Lv et al., 2015]
MiR-128-2	ABCA1, ABCG1 and RXR α	Cholesterol efflux	[Adlakha and Saini, 2013]