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Mechanistic Role of MicroRNAs in Coupling Lipid Metabolism and Atherosclerosis

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Abstract

MicroRNAs (miRNAs, miRs) represent a group of powerful and versatile posttranscriptional regulators of gene expression being involved in the fine control of a plethora of physiological and pathological processes. Besides their well-established crucial roles in the regulation of cell cycle, embryogenesis or tumorigenesis, these tiny molecules have also been shown to participate in the regulation of lipid metabolism. In particular, miRs orchestrate cholesterol and fatty acids synthesis, transport, and degradation and low-density and high-density lipoprotein (LDL and HDL) formation. It is thus not surprising that they have also been reported to affect the development and progression of several lipid metabolism-related disorders including liver steatosis and atherosclerosis. Mounting evidence suggests that miRs might represent important “posttranscriptional hubs” of lipid metabolism, which means that one miR usually targets 3'-untranslated regions of various mRNAs that are involved in *different* steps of *one* precise metabolic/signaling pathway, e.g., one miR targets mRNAs of enzymes important for cholesterol synthesis, degradation, and transport. Therefore, changes in the levels of one key miR affect *various* steps of *one* pathway, which is thereby promoted or inhibited. This makes miRs potent future diagnostic and even therapeutic tools for personalized medicine. Within this chapter, the most prominent microRNAs involved in lipid metabolism, e.g., miR-27a/b, miR-33/33*, miR-122, miR-144, or miR-223, and their intracellular and extracellular functions will be extensively

discussed, in particular focusing on their mechanistic role in the pathophysiology of atherosclerosis. Special emphasis will be given on miR-122, the first microRNA currently in clinical trials for the treatment of hepatitis C and on miR-223, the most abundant miR in lipoprotein particles.

Keywords

Lipid metabolism; HDL; LDL; Cholesterol; Atherosclerosis; miR-33; miR-122; miR-223

Introduction

Lipid metabolism is a multifaceted process including lipid synthesis from their basic components (i.e., basically acetyl-CoA), accumulation, distribution to specific tissues of the organism, and also degradation and excretion [1]. The complex machinery of lipid metabolism is driven by a set of step-limiting enzymes and transporters that are finely modulated according to the needs of the organisms [2]. The discovery that these crucial components are targeted by microRNAs (miRNAs, miRs) grabbed our attention towards the exploiting of miRs as regulators of lipid metabolism with subsequent potential use in the treatment of lipid metabolism-related disorders, including dyslipidemia, atherosclerosis, and nonalcoholic fatty liver disease [1, 3, 4]. Within this chapter, we will provide a basic overview of the most important components of lipid and lipoprotein metabolism and then we will more deeply focus on the functional role of key miRs.

Lipid Metabolism in the Nutshell

Acetyl-CoA represents a well-established precursor molecule for both cholesterol and fatty acids synthesis. Depending on the cholesterol status, i.e., low- or high- cholesterol levels, metabolic pathways leading to cholesterol or fatty acids synthesis, respectively, are activated alongside with pathways leading to cholesterol uptake or efflux. Sterol regulatory element binding proteins (SREBPs) [5, 6] and liver X receptors (LXRs) [7] are among the most important modulators of such processes. Three SREBPs have been identified hitherto, namely SREBP-1a, SREBP-1c (two isoforms originating from *SREBP-1* gene), and SREBP-2 (originating from *SREBP-2* gene), whereas two LXRs isoforms (LXR α and LXR β) are known [5–7].

If the cholesterol level is low, SREBP-2 is activated, leading to upregulation of critical enzymes involved in cholesterol synthesis (about 20 various steps and reactions), with 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMGCoA-R) representing the rate-limiting enzyme. Furthermore, the expression of low-density lipoprotein receptor (LDLr) is upregulated both by SREBP-2 and SREBP-1a, thus enhancing cholesterol uptake [8].

Conversely, high cholesterol level triggers the activation of LXRs, which forms heterodimers with retinoid X receptors (RXRs) and subsequently activates genes involved in cholesterol efflux and also SREBP-1c, which further promotes formation of triglycerides and phospholipids with, e.g., fatty acid synthase (FAS) being one of the designed targets [7].

Furthermore, if there is too much cholesterol in the cell, HMGCoA-R and LDLr levels are downregulated in order to prevent cholesterol accumulation [7, 8].

Another way to affect cholesterol uptake is via scavenger receptors that bind LDL and modified oxidized LDL (oxLDL) [9]. Last but not least, cholesterol can be partly excreted from the organism as bile acids. Intriguingly, bile acids themselves were recently shown to act as signaling molecules via specific binding of farnesoid X receptor (FXR) [10]. Once FXR is activated, it creates heterodimers with RXRs which in turn further activate expression of enzymes involved in cholesterol degradation and excretion [10].

MicroRNAs Involvement in Lipid Metabolism

MicroRNAs act as negative posttranscriptional regulators of gene expression affecting also the expression of genes involved in lipid metabolism [1, 3]. One miR usually targets more mRNAs that are often connected in the same metabolic pathway and via their targets, miRs are also able to regulate expression of many other miRs [11, 12]. Moreover, each mRNA is usually targeted via several miRs, thus enabling a fine-tuning of the targeted mRNA expression [11, 13]. Some miRs show pleiotropic expression in almost all tissues, while others act more in a tissue-dependent manner [12, 14, 15]. Additionally, during their biogenesis, miRs can be transcribed as individual miRs from their genes having their own promoters, but quite often, they are embedded within the introns of the protein coding genes being transcribed together with their host [16–18], as outlined in detail in Chaps. 1 and 2 of this book. Moreover, during the last step of miR maturation, miR duplex is dissociated giving rise to mature miR and passenger miR* strand. Initially, only mature miRs were believed able to exert a function while passengers strands were simply degraded; however, it has been shown that even passenger miR* strand can exert crucial functions [19]. Furthermore, overcoming the borders of the cell membrane, miRs can be found also in extracellular fluids either bound to proteins or being packaged in microvesicles/ exosomes and also in lipoprotein particles—as such, miRs may serve as stable bio-markers and also as novel means of intercellular communication [20, 21].

The most abundant miR in the liver, miR-122, is involved in countless metabolic pathways within the hepatocytes, reflects liver damage if present in serum/plasma, and is mechanistically involved in the pathogenesis of hepatitis C virus infection by targeting its 5'-UTR [22–25]. Another key lipid metabolism miR, miR-33, was found to be hidden within the SREBP genes—miR-33a/33a* in the *SREBP-2* gene and miR-33b/33b* within the *SREBP-1* gene [18]. Interestingly, both mature and passenger strands of miR-33 have been shown to affect lipid and even glucose metabolism [19]. Last but not least, miR-223, besides affecting lipid metabolism on the cellular level, is the best example of miR highly abundant in HDL particles [26], microvesicles and exosomes [27] and even platelets [28]. The exact roles of the three abovementioned and other miRs will be described in more detail in this chapter. Returning to key regulatory molecules of lipid metabolism, i.e., SREBP, LXR, RXR, and FXR, various miRs have been shown to control their expression (Fig. 5.1). In particular, SREBP was predicted and confirmed to be targeted by miR-122 [4, 29]. In prostate cancer tumor cells, miR-185/342 was shown to target SREBP, which, alongside with a decrease in tumorigenicity, led also to downregulation of SREBP-targeted genes, i.e.,

fatty acid synthase (FASN) and HMGCoA-R [30]. Importantly, miR-185 was shown to significantly decrease SREBP-2 level, while expression of miR-185 itself is regulated by SREBP-1c [30], creating an autoregulatory loop. Other miRs targeting SREBP were also identified in osteosarcoma cell lines (miR-29a) and adrenal glands (miR-132) [31]. All of these miRs represent potentially strong modulators of lipid, and generally of the overall metabolism of the cell.

LXR α was revealed to be targeted by miR-613 whose upregulation caused inhibition of lipogenesis in HepG2 cells [32] and also triggered lipid accumulation, i.e., decreased cholesterol efflux, in THP1 macrophages (this effect was partly shown to be mediated by targeting ABCA1) [33]. Mice lacking miR-155, another miR targeting LXR α , exhibited increased cholesterol accumulation in the liver resulting in hepatic steatosis [34]. Conversely to miR-613, miR-206 has been shown to target LXR α ; however, miR-206 increase promotes cholesterol efflux from THP macrophages [35]. LXR increases the expression of miR-613 [36] and decreases the expression of miR-206 [35] in another autoregulatory feedback loop.

Looking one step above LXR and SREBP, expression of both genes was shown to be regulated by sirtuin 1 (SIRT1) [37, 38], which is a member of the sirtuin family of deacetylases involved in the regulation of innumerable cellular processes, including cellular metabolism [39]. SIRT1 increases the level of LXR [38] and decreases SREBP [40]; remarkably, SIRT1 itself is targeted by miR-34a [41, 42], miR-132 [43], miR-204-5p [44], miR-499 [45], and many others.

FXR was shown to be targeted by miR-92 [46] and miR-412 [47] in gastric cancer and hepatocellular carcinoma cells, respectively, promoting proliferation and invasiveness of both tumors. FXR negatively regulates the expression of several miRs (e.g., miR-199-3a [48] or 29a [49]) and such a negative regulation seems to be beneficiary—downregulation of FXR in human tumors is thus predictive for worse patient prognosis [46, 47]. In the context of lipid regulation, FXR was shown to upregulate the expression of miR-144, one of the ABCA1 regulators, resulting in cholesterol accumulation within the cells [50]. Further investigations are definitely needed to reveal all FRX-regulating and FXR-regulated miRs.

Lastly, RXR α is targeted by miR-34a [51] (similarly to LXR α [41, 42]) and by miR128-2 [52]. Looking at other fundamental molecules, also LDLR expression was shown to be targeted by miR-214 [53], while other miRs (miR-125a and miR-455 [54]) regulate scavenger receptor class B type I (SR-BI) expression. Other molecules involved in lipid metabolism besides those mentioned in previous paragraphs are also regulated by miRs (e.g., MTP, protein important for lipoprotein formation, is regulated by miR-30c [55], or Lectin-like oxidized LDL receptor-1 (LOX-1), one of the scavenger receptors, is targeted by let-7g [56]). Collectively, these data suggest a strong potential of miRs in the posttranscriptional regulation of lipid metabolism-related genes and prompt potential usage of selected miRs as tools to monitor and treat metabolic, cardiovascular and especially cardiometabolic disorders, including atherosclerosis [57].

miR-33a/b: In the Center of Metabolic Pathways

Expression of miR-33a/b

As mentioned above, miR-33 family comprises two members named miR-33a and miR-33b, embedded within the intron 16 of *SREBP-2* and intron 17 of *SREBP-1* genes [58, 59], respectively. Similarly to the majority of intronic miRs, miR-33a/b expression is coordinated with the expression of the host genes [60]. However, unlike many other miRs, in the case of miR-33 both strands are used in mRNA targeting; indeed following pre-miR-33 dicing, miR-33 and miR-33* are formed and both miRs create RISCs with Ago proteins, subsequently resulting in suppression of genes involved in lipid metabolism [19].

miR-33a/b in Cholesterol Metabolism and Atherosclerosis

miR33a/b have been shown to target multiple genes involved in individual steps of cholesterol metabolism, particularly in reverse cholesterol transport from peripheral tissues and cholesterol excretion into the bile. ATP-binding cassette (ABC) transporter A1, ABCA1, was confirmed as miR-33 target in various animal models and cell lines. As a direct consequence of ABCA1 targeting, cholesterol accumulation or cholesterol efflux into ApoA1 (with subsequent HDL formation) occurs, depending on whether miR-33 levels are upregulated or downregulated, respectively [18, 19, 58–65]. Antagonism of miR-33 in experimental animal models generally results in increased cholesterol efflux to ApoA1, thus increasing serum HDL and decreasing cholesterol levels in peripheral tissues, especially in peripheral macrophages (and atherosclerotic plaques), where also ABCG1 targeting has been ascertained to affect reverse cholesterol transport [58, 59]. The overall result is increased plaque stability, improved blood lipid profile, and inhibition of atherosclerosis progression [19, 62–64, 66, 67]. Strikingly, the effects of miR-33 antagonism were observed not only in mice, that have only one miR-33 isoform (specifically miR-33b) [62, 64, 66, 67], but also in nonhuman primates, which, similarly to human, express both miR-33 isoforms [19, 63].

Together with ABCA1 and ABCG1, the latter one being targeted only in murine models and not in humans [58, 59], other important cholesterol-related targets have been identified for miR-33: Nieman-Pick Protein C1 (NPC1), lysosomal protein involved in the intracellular distribution of cholesterol between lysosomes and other parts of the cell [19, 68] and ABCB11/ATP8B1 transporters that are both responsible for cholesterol excretion in the bile [65]. Inhibition of miR-33 thus also causes increased cholesterol secretion into the bile, thereby lowering cholesterol levels—a strategy that would be of potential use in the clinical scenario in patients with hypercholesterolemia.

miR-33a/b in Fatty Acids Metabolism

Various studies revealing the role of miR-33 in lipid metabolism further focused on miR-33 targets involved in fatty acids synthesis and oxidation [18, 19, 63]. The first identified targets include three important enzymes critical for β -oxidation: carnitine- O- octanoyltransferase (CROT), carnitine palmitoyltransferase 1A (CPT1A), and hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase beta subunit (HADHB) [18, 19]. Other targets were then identified in studies conducted on nonhuman primates, where

therapeutic inhibition of miR-33, except of its known effect on HDL and LDL levels, also affected VLDL and circulating triglycerides level, a phenotype that was not observed in mice [63]. This was partly caused by activation of β -oxidation (through previously identified CROT, CPT1A, HADHB, and the novel target protein kinase AMP-activated α 1 (PRKAA1)) and inhibition of fatty acid synthesis (by targeting fatty acid synthase (FAS), ATP citrate lyase (ACLY), or Acetyl-CoA carboxylase 1 (ACACA)) [63]. Hypothetically, targeting Sirtuin-6 (SIRT6), a protein known to be involved in triglycerides metabolism, by miR-33 may also affect triglycerides metabolism [69], similarly to targeting AMPK α , which is known to stimulate β -oxidation and even ketogenesis in the liver [69].

To sum it up, inhibition of miR-33a increases β -oxidation and decreases fatty acid synthesis, eventually resulting in ameliorated circulating lipids profile, slowing down the progression of atherosclerosis. These findings may result in the introduction of anti-miR-33 specific treatment in the future.

miR-33 in Glucose and Energy Metabolism

Captivatingly, also glucose metabolism, insulin signaling, and energy metabolism were shown to be affected in anti-miR-33 treated cells. Ramirez and colleagues identified phosphoenolpyruvate carboxykinase (PCK1) and glucose-6-phosphatase (G6PC) as miR-33 putative targets. Both of these enzymes are essential for hepatic gluconeogenesis [70]. Another putative target, insulin receptor substrate-2 (IRS-2), was confirmed as miR-33 target by Fernandez-Hernando's group [19]—IRS-2, together with IRS-1, are both important for transduction of insulin signaling over the cell membrane and their blockade thus interferes with insulin effects on the cells [71].

Recently, miR-33 function was also studied in pancreatic islets, unveiling that it induces cholesterol accumulation by targeting ABCA1 [72]. Cholesterol accumulation further leads to a decrease in glucose-stimulated insulin secretion, supporting a connection among miR-33 function, hypercholesterolemia, pancreatic β cell dysfunction, and insulin resistance [72]. A thorough overview of miR and β cell dynamics is presented in Chap. 6. Most recently, miR-33 was clearly shown to be involved in the pathophysiology of atherosclerosis (as expected considering its roles in lipid metabolism and macrophage function) [73]. Moreover, it represents a major regulator of cell cycle [74] and is also involved in the self-renewal of hematopoietic stem cells via p53 targeting [75].

miR-122: Liver-Specific miR with Pleiotropic Effect on Hepatic Functions

Regulation of miR-122 Expression

In 2002, miR-122 was identified as a liver-specific miR, representing approximately 70 % of all liver miRs [14]. During embryogenesis, level of miR-122 rapidly increases and around 50,000 copies are present in the average hepatic cell before birth [76]. In humans, miR-122 is located on chromosome 18 (hr18:54269286–54269370, NCBI36/hg1) and initially it was suggested that miR-122 was transcribed from the *hcr* gene which encodes for the noncoding RNA in the woodchuck (gi: 51212); however, a 7506 bps long primary transcript was further described as a primary sequence for miR-122 [77]. Furthermore, hepatocyte nuclear factor

4 α (HNF4 α) was shown to regulate miR-122 expression by binding to the promoter region of this primary transcript [77]. Other regulatory factors include Rev-ERB2 α , an orphan nuclear receptor that is responsible for circadian variation of miR-122 levels; however, circadian rhythmicity does not seem to significantly affect miR-122 function [78]. Of note, miR-370, which transfection is known to alter triglycerides accumulation in the liver, positively regulates miR-122 expression [29]. Last but not least, miR-122 is regulated at posttranscriptional level by 3' adenylation. Indeed, germ line development-2 (GLD-2) knockout animals present with low hepatic miR-122 levels, a phenotype mainly attributable to the absence of GLD-2-mediated adenylation and stabilization [79].

Targets of miR-122 and Lipid Metabolism

Heretofore, studies investigating the roles of miR-122 in lipid metabolism used either antisense oligonucleotides (ASO) [22, 80, 81] or gene knockout approaches [25, 82]. Moreover, just indirect measurements (usually gene expression profiling) are performed to quantify the effects of miR-122 loss. Thus, only a few targets have been validated and more analyses are needed to reveal whether miR-122 targets lipid metabolism directly through many targets or indirectly through a few key targets, such as SREBP [83].

In nonalcoholic steatohepatitis [4] and hepatocellular carcinoma (HCC, [84]), miR-122 level was found to be downregulated, which contributed to the pathophysiology of both disorders. In knockout animals, the deletion of miR-122 led to accumulation of lipids in the liver (resembling steatohepatitis) and also to increased fibrosis (resembling cirrhosis) and to formation of tumor-like lesions (resembling HCC) [25, 82]—the absence of miR-122 thus definitely alters liver metabolism and makes liver more susceptible to the development of mentioned diseases.

On the other hand, even in the gene knockout animals, level of circulating HDL and VLDL is lower compared to control animals and this is essentially due to altered hepatic secretion of these lipoproteins [25, 82]. Similarly, cholesterol levels decrease after the introduction of ASO [22, 80, 81], even in the African green monkeys in which also no signs of toxicity connected with locked nucleic acid (LNA) administration was observed [81]. Moreover, it was shown that after ASO introduction, fatty acids and cholesterol synthesis was decreased and liver steatosis was reversed in high-fat diet-induced obesity model [22].

Indirect targets of miR-122 involved in lipid metabolism were repeatedly suggested including SREBP-1c and SREBP-2 [4, 29], which further makes identification of miR-122-related lipid metabolism targets more difficult due to SREBPs pleiotropic effects on lipid metabolism. Among others let us name fatty acid synthase [4, 22, 29], aldolase-A [80], ATP-citrate lyase (ACLY), acyl-CoA carboxylase (ACC1, ACC2) [22, 29], HMGCR [4], and many others. Altogether, miR-122 is predicted to have more than 100 various targets and potential usage of miR-122 antagonism in the treatment of lipid disorders may represent a novel therapeutic approach in the future after all security issues are solved.

miR-122 in HCV Infection: Miravirsen

Besides the roles of miR-122 in the normal liver functions, its involvement in the replication of hepatitis C virus resulted in the development of the first miR based therapeutics called miravirsen, i.e., miR-122 suppression therapy [85, 86].

Within the genome of HCV, there is a conserved 5'UTR region that was shown to be targeted by miR-122 [87]. However, although miR binding usually leads to the degradation of targeted mRNA, in case of HCV RNA, miR-122 binding is necessary to stabilize this RNA molecule and to promote its replication [86]. Miravirsen was designed as a 15 nct long LNA-modified anti-miR, and it binds to the 5'UTR of miR-122 thus inhibiting its function [88].

In the recently performed phase 2a clinical trial involving seven international centers, miravirsen application led to a decrease in HCV RNA levels and after 14 weeks, viral RNA was even not detected in five patients (out of 36). Moreover, no adverse events were observed and no escape mutation in HCV genome was detected [86]. As long as miR-122 binding site in HCV genome seems to be conserved across HCV genotypes, these results make miravirsen a very promising therapeutic tool against HCV infection [24].

Circulating miR-122 as a Disease/Damage-Related Biomarker

Besides the therapeutic potential in HCV or dyslipidemia treatment, it was shown that circulating miR-122 levels are increased in obese patients and that there are correlations connecting miR-122 levels to BMI, levels of ALT, TG, and HDL-cholesterol and moreover, after adjustment for confounding factors, elevated miR-122 levels were shown to be a risk factor for insulin resistance [89]. Similarly, miR-122 levels are increased in plasma of patients with nonalcoholic steatohepatitis [90, 91]. Moreover, since circulating miRs may be found in the protein complexes or in exosomes, Bala and colleagues studied the different liver injury models and revealed that in inflammatory diseases, miR-122 levels are increased in exosomal fraction of the plasma, while in drug-induced injury, miR-122 levels are higher predominantly in protein-fraction suggesting exosomal miR-122 to act as a mean of intercellular communication [91]. Last but not least, circulating levels of miR-122 are also increased in hyperlipidemia patients and they correlate with the levels of cholesterol, triglycerides, and LDL-c; in the subset of patients with hyperlipidemia and coronary artery disease (CAD), miR-122 levels also correlated with CAD severity [92]. Specificity for circulating miR-122 increase still definitely needs to be determined; however, in the combination with altered extracellular expression of other miRs, miR-122 may serve even as a diagnostic or prognostic tool in the future.

miR-223: “The Messenger”

Regulation of miR-223 Expression

The gene encoding for miR-223 is located on the X-chromosome; miR-223 was originally connected with the hematopoiesis and the function of granulocytes [93]. Transcription factors regulating miR-223 expression have already been identified and include transcription factor binding to PU-box 1 (PU.1), CCAAT-enhancer-binding proteins α and β (C/EBP α

and β), and nuclear factor I-A (NFI-A) [94, 95]. The first two mentioned transcription factors increase miR-223 expression whereas the third one decreases miR-223 expression; moreover, NFI-A itself is an miR-223 target, thus creating a negative regulatory feedback loop [94, 95]. In relation to lipid metabolism, high intracellular cholesterol levels were also shown to increase miR-223 expression [96].

miR-223 and Lipid Metabolism

Within the cells, miR-223 was described as an important regulator of cholesterol homeostasis [96] cooperating side by side with miR-122 and miR-33a/b. As mentioned above, miR-223 level is increased in the presence of high intracellular cholesterol; in order to prevent cholesterol overload, miR-223 (a) inhibits HDL cellular uptake via targeting scavenger receptor IB (SR-IB), (b) inhibits cholesterol synthesis by targeting HMGCoA-R and methylsterol monooxygenase 1 (MSMO1), and (c) increases cholesterol efflux by targeting Sp3, thus indirectly upregulating levels of ABCA1 [96]. Similar results were obtained by Wang and colleagues who demonstrated that SR-IB is targeted not only by miR-223, but also by miR-185 and miR-96 [97].

Intriguingly, miR-223 was shown to be transferred within microvesicles [27, 98] and within HDL and LDL particles [99]. In particular, miR-223 is one of the most abundant miRs in HDL particles [99] and is also present in LDL particles, where one of the most abundant miRs seems to be the inflammation-related miR-155 [26]. From HDL, miR-223 can be transferred to endothelial cells, where miR-223 is not commonly expressed [99, 100], which may at least partly explain the anti-inflammatory properties of HDL [101–105]. Namely, miR-223 targets NOD-like receptor pyrin domain containing 3 (NLRP3) [106] and intercellular adhesion molecule I (ICAM-1) [100] and downregulation of both these molecules suppresses endothelial inflammation and reactivity, thereby preventing leukocyte infiltration and inflammation connected with atherosclerosis [107, 108].

Involvement of miR-223 in Inflammation, Insulin Resistance, and Platelet Activation

Macrophages and platelets were shown to produce microvesicles/exosomes containing miR-223 and miR-223 was again shown to be transferred to various cells, regulating posttranscriptionally gene expression [27, 98, 109]. For example, miR-223 transferred to endothelial cells (HUVECs) increases their apoptosis induced by advanced glycation products [98]. Interestingly, also gap junctions were described to enable miR-223 transfer from macrophages to hepatocellular carcinoma cells, which resulted in inhibition of proliferation [110]. Concerning the potential involvement of miR-223 in the development of insulin resistance, there is evidence proving that miR-223^{-/-} mice receiving high-fat diet display profound insulin resistance and increased adipose tissue inflammation compared with wild-type animals on high-fat diet [111]. One of the potential miR-223 targets that may be involved in the development of insulin resistance is glucose transporter 4 (GLUT4) as validated in neonatal rat necrosis cytes [112].

Lastly, miR-223 was shown to be involved in platelet function [28] and reactivity to anti-aggregation treatment—in patients with low-response to clopidogrel therapy, platelet miR-223 levels were significantly lower compared to normal-responders [113]. Lower levels

of miR-223 were also observed in plasma of low-responders [114]; however, most recently Chyrchel and coworkers presented a study that argues against the notion of low plasma miR-223 as a marker of platelet responsiveness to dual antiplatelet therapy [115]. Further studies are definitely necessary to reveal the true potential of miR-223 in platelets [116–118], since it has already been shown that circulating miR-223 levels negatively correlate with the susceptibility to myocardial infarction [116].

miR-27a/b and Other Essential Transcriptional Hubs

As shown above, miR-33, miR-122, and, miR-223 can target many mRNAs translated in proteins involved in the regulation of lipid metabolism and they can thus be considered as “transcriptional miR hubs,” i.e., important miRs targeting more proteins affecting transcription (and thus function) of lipid metabolism-related proteins. A similar “hub” role was predicted and confirmed for miR-27a/b. In particular, miR-27a targets RXR α , ABCA1 (see below), FAS, both SREBP-1 and SREBP-2, both PPAR α (regulator of ABCA1) and PPAR γ (regulator of adipocyte differentiation), and last but not least proteins involved in lipoprotein formation: ApoA1, ApoB100, and ApoE3 [119–122]. Similarly to miR-27a, miR-27b was predicted to target 27 various targets involved in lipid metabolism and out of these, PPAR γ , angiopoietin-like 3 (ANGPTL3), N-deacetylase/N-sulfotransferase 1 (NDST1), mitochondrial glycerol-3-phosphate acyltransferase (GPAM) and C/EBP α have been already validated [123–128]. Recently, sequential regulation of cholesterol metabolism by miR-27 was reported by Zhang and colleagues—their study elegantly demonstrated that cholesterol efflux (by targeting ABCA1 and apoA1), influx (by targeting LPL and CD36), and esterification (by targeting ACAT1) are affected at one time by miR-27 [129]. Notably, predicted targets of miR-27a and other miRs that are profoundly dysregulated in metabolic syndrome include genes that partake in pathways related to fatty acid and sphingolipid metabolism and are also involved in vascular signaling [3], suggesting thereby a key role for miR-27 in the maintenance of overall metabolic homeostasis.

ABCA1: Target of Multiple miRs

Protein levels of ABCA1, transporter that is crucial for cholesterol loading into ApoA1 and formation of HDL [13], are known not to correlate with its mRNA levels suggesting highly complex posttranscriptional regulation [130]. Besides other factors, due to its remarkably long 3'UTR, ABCA1 was revealed to be targeted by various miRs (Table 5.1), specifically by miR-10b [131], miR-19b [132], miR-26 [133], miR-106a [134], miR-106b [135], miR-128-2 [52], miR-143/145 cluster [136, 137], miR-144 [50, 138], miR-302a [140], miR-613 [33], and miR-758 [141, 142]. Among the miRs mentioned above, also miR-27 [129] and miR-33 [19, 72, 142] target ABCA1. Additional miRs will be certainly validated in the future, since more than 100 miRs have been predicted to bind ABCA1 3'UTR [143]. This probably reflects the different regulation of ABCA1 in particular tissues; studying ABCA1 regulation still represents a challenge since its involvement in insulin resistance/secretion [72, 136] and Alzheimer's disease [135] has also been demonstrated.

Regulation of miRs by miRs: miR-370 and miR-122

The fact that one single miR can target more mRNAs and one mRNA is commonly targeted by several miRs (e.g., as shown above for ABCA1) creates an incredibly complicated network orchestrating lipid metabolism. Even more complexity is added when realizing that each miR can be also directly regulated by another miR. This was described for miR-370 upregulating the expression of miR-122 [29]; using sense and antisense miR-122 and miR-370, the same set of targets was shown to be affected. However, if miR-122 is blocked with its antagomiR, miR-370 effect is blunted [29]. Interestingly, in patients with CAD, miR-122 and miR-370 levels correlate with each other and they were also shown to reflect CAD severity in patients with hyperlipidemia [92]. Both miRs may be involved in the so-called fetal reprogramming: if mice are fed with high-fat diet, levels of both miRs in the liver of their offspring are altered. miRs may thus represent novel mediators involved in transgenerational epigenetic settings of lipid metabolism [144].

Conclusion

MicroRNAs represent important regulators of lipid metabolism working either as important transcriptional hubs (affecting the expression of various proteins at one time), or by fine tuning the regulation of key genes (i.e., more miRs at time controlling the expression of crucial genes) participating in lipid metabolism. Multitarget function of miRs can be definitely exploited as future tool to study pathophysiology of disease and test potential targeted therapies [145]. We can almost say that miRs “show us” how everything is connected together and some connections never thought before may be revealed. Since miRs are highly evolutionary conserved, this can suggest us the way by which the research in the field can continue in order to reveal the true essence of some disorders. This may in the future result in identifying new and enhanced strategies for diagnostics and therapy of lipid metabolism-related disorders.

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Fig. 5.1.

MicroRNAs in lipid metabolism. The figure summarizes the main miRs involved in lipid metabolism. *Blunted arrows* represent inhibition, *classical arrows* represent activation. miRs are marked in *circles*, their targets are marked in *squares*. Detailed description is provided in the text. *FXR* Farnesoid X receptor, *SREBP* sterol regulatory element binding protein, *LXR* liver X receptor, *RXR* retinoid X receptor, *miR* microRNA, *RT* reverse transport; *ABCA1* ATP-binding cassette transporter A1, *ABCG1* ATP-binding cassette transporter G1, *ABCB11* ATP-binding cassette, subfamily B member 11, *LDLR* low density lipoprotein receptor, *SR-IB* scavenger receptor type I class B, *HMGCoA-R* hydroxymethylglutaryl coenzyme A reductase, *MSMO1* methylsterol monooxygenase 1, *FASN* fatty acid synthase, *NFYH* nuclear transcription factor Y, *RIP-140* receptor-interacting protein 140, *SRC* steroid receptor coactivator, *CPT1A* carnitine palmitoyltransferase 1A, *HADBDH* hydroxyacyl-CoA-dehydrogenase, *CROT* carnitine O-octaniltransferase, *AMPK* AMP-activated protein kinase, *ACC* acyl-CoA carboxylase, *GPAM* mitochondrial glycerol-3-phosphate, *GPC-6* glucose-6-

phosphatase, *PCK1* phosphoenolpyruvate carboxykinase; *IRS-2* insulin receptor substrate 2, *ALDO-A* aldolase A, *ApoE3* apolipoprotein E3, *ApoA1* apolipoprotein A1, *ANGPTL3* angiopoietin-like 3, *PPAR* peroxisome proliferator-activated receptor α , *CEBPCCAAT*/enhancer-binding protein α

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Table 5.1

MicroRNAs involved in ABCA1 regulation

miR	Details	References
miR-10b	Gut microbiota produces protocatechuic acid, which was shown to reduce levels of miR-10b in macrophages and thus increase cholesterol efflux exerting anti-atherogenic effect	[131]
miR-19b	Treatment of ApoE ^{-/-} mice with miR-19b resulted in decreased HDL (via ABCA1) and increased LDL levels which was shown to increase aortic plaques size	[132]
miR-26	miR-26 overexpression resulted in decreased cholesterol efflux by targeting ABCA1 and ARL7 levels. Moreover, in LDL loaded Hep2G cells, miR-26 levels decreased and ABCA1 levels increased suggesting the role of dietary cholesterol in decreasing miR-26 expression	[133]
miR-106a	miR-106a levels are higher in cisplatin resistant cell line A549 (non small cell lung cancer cell line) and downregulation of ABCA1 by miR-106a represents one of the novel mechanisms of cisplatin resistance	[134]
miR-106b	In Alzheimer disease, ABCA1 is known to affect metabolism of pathogenic amyloid- β ; miR-106b was shown to affect ABCA1 levels and thus increase amyloid- β production and decreased its clearance	[135]
miR-128-2	miR-128-2 attenuates cholesterol efflux by targeting ABCA1, ABCG1, and RXR α in HEK293T, HepG2, and MCF7 cells	[52]
miR-143/miR-145	Ldlr ^{-/-} and miR-143/145 ^{-/-} double knockout mice were fed with high-fat diet. Comparison of atherosclerosis plaque from these animals and Ldlr ^{-/-} mice showed that the absence of miR-143/145 resulted in smaller atherosclerotic plaques and decreased macrophage infiltration. This was accompanied by a decrease in circulating cholesterol levels in VLDL and LDL. ABCA1 upregulation was observed in liver and vessels	[136, 137]
	miR-145 levels were shown to be decreased in pancreatic cells after glucose stimulation. This results in an increase in cholesterol efflux and improvement in glucose-stimulated insulin secretion	
miR-144	LXR and FXR agonists increased miR-144 expression, which reduced ABCA1 expression and cholesterol efflux in macrophages and hepatic cells. miR-144 silencing increased HDL	[50, 138, 139]
	miR-144 mimic inhibited cholesterol efflux from macrophages via ABCA1 targeting and it further decreased HDL cholesterol levels and promoted atherosclerosis in ApoE ^{-/-} mice. Furthermore, it increased inflammatory cytokines expression (IL-1 β , IL6, TNF α) and circulating miR-144 levels correlated with serum CK, CK-MB, LDH, and AST in patients after acute myocardial infarction	
miR-302a	Ldlr ^{-/-} mice were treated with antagomiR against miR-302a, which resulted in a decrease in atherosclerotic plaques size and an increase in circulating HDL levels	[140]
miR-613	By targeting PPAR γ and ABCA1, miR-613 decreases cholesterol efflux from PPAR γ -activated macrophages	[33]
miR-758	High-fat diet reduced miR-758 levels in peritoneal macrophages and liver followed by increased in ABCA1, which led to increased cholesterol efflux	[141]
	In human atherosclerotic carotid plaques, expression of ABCA1 and ABCG1 was found to be increased, together with an increase in miR-33b and miR-758, which is probably a reason for no changes in the protein levels of mentioned proteins	[142]

ApoE apolipoprotein E, *Ldlr* low density lipoprotein receptor, *HDL* high density lipoprotein, *ARL7* ADP-ribosylation factor-like 7, *HEK293T* human embryonic kidney, *HepG2* liver hepatocellular carcinoma, *MCF7* breast cancer cell line, *ABCG1* ATP binding cassette G1, *RXR α* retinoid X receptor α , *IL* interleukin, *TNF* tumor necrosis factor; *CK* creatin kinase, *CK-MB* creatin kinase- muscle/brain subunits, *LDH* lactate dehydrogenase, *AST* aspartate aminotransferase, *PPAR γ* peroxisome proliferator-activated receptor gamma