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miRNA and cholesterol homeostasis

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Abstract

MicroRNAs (miRNAs) have recently emerged as a novel class of epigenetic regulators of gene expression. They are systemically involved in the control of lipid metabolism through a complex interactive mechanism that involves gene regulatory networks. Hence, they can contribute to defective lipid metabolism and metabolic diseases. Here, we review recent advances in the roles of lipid-sensing transcription factors in regulating miRNA gene networks, as well as miRNA expression and function in the regulation of cholesterol metabolism.

Keywords

MicroRNA; Cholesterol metabolism; Transcription factor; Metabolic disease

1. Introduction

As one of the two major cellular lipid species, cholesterol is an essential structural component of eukaryotic cell membranes. It is a precursor for the synthesis of steroid hormones and bile acids, which are natural detergents and participate as signaling molecules that contribute to a wide variety of cellular functions [1,2]. Given these diverse yet fundamental roles, it is not surprising that small perturbations in regulatory networks that modulate cholesterol homeostasis can lead to severe clinical conditions, such as atherosclerosis, heart disease, and diabetes [3]. Hence, exquisite regulatory mechanisms have evolved to balance both intracellular and membrane levels of cholesterol in response to changing physiologic demand. Cholesterol homeostasis is maintained through the feedback regulation of de novo biosynthesis, lipoprotein uptake, sterol mobilization through lipophagy, and efflux/transport, which are regulated at the transcriptional and posttranscriptional levels [4]. A key transcriptional pathway for regulating cholesterol metabolism is through the sterol regulatory element-binding proteins (SREBPs). There are three major SREBP isoforms; SREBP-1a and 1c are encoded from the *SREBF1* gene on

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Conflict of interest

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chromosome 11 from 2 separate promoters to generate mRNAs with unique 5′ termini that encode proteins that only differ at their amino-temini. A singular SREBP-2 is encoded from the *SREBF2* gene that is encoded on chromosome 15 [4]. SREBPs coordinately regulate cholesterol and fatty acid biosynthesis and the published data suggest that SREBP-1c, which is a relatively weak transcriptional activator, preferentially activates genes involved in fatty acid metabolism whereas SREBP-1a activates genes of both cholesterol and fatty acid metabolism. SREBP-2 preferentially activates genes of cholesterol metabolism [5].

The different SREBP isoforms are independently regulated at the transcriptional level through different tissue-specific signaling pathways and the proteins are expressed as large 1100 amino acid precursors that are tethered to the endoplasmic reticulum (ER) membrane. When cellular sterol levels are low, the full-length endoplasmic reticulum (ER) membrane-associated SREBP precursor forms a complex with SREBP cleavage-activating protein (SCAP) and the complex trafficks to the Golgi apparatus where the amino-terminal mature SREBP transcription factor is proteolytically released from its membrane tether. The active transcription factor then translocates to the nucleus, where it induces the transcription of numerous genes involved in cholesterol biosynthesis, lipoprotein uptake, and lipophagy [6]. Evidence suggests that ER to golgi trafficking of all SREBPs is regulated by cholesterol but additional signals including polyunsaturated fatty acids also regulate the maturation of SREBP-1 isoforms [4].

A thorough review of the exquisite feedback mechanism for SREBP processing and nuclear translocation is beyond the scope of the current review and can be found elsewhere [4]. Instead, this review is focused on the emerging roles for micro-RNAs (miRNAs) in regulating cholesterol metabolism. miRNAs are a unique class of small noncoding RNAs that function as key regulators of fundamental cellular processes that modulate protein expression by hybridizing to their respective mRNA targets and increasing mRNA turnover or inhibiting translation or both [7]. miRNAs have been shown to regulate key proteins of cholesterol homeostasis, and altered expression of the implicated miRNAs is highly associated with metabolic disorders [3]. Studies have revealed that single miRNAs cannot only have multiple target sites in the 3′-un-translated region (UTR) of an mRNA, but a single mRNA molecule is also predicted to be the target of many distinct miRNAs, suggesting that miRNAs act in a concerted manner to regulate gene expression [8]. Thus, this posttranscriptional regulation action by miRNAs represents another critical layer of intricate regulatory networks, in addition to the complex regulatory layer of transcription factors and co-activators, for maintaining the intracellular cholesterol homeostasis effectively.

2. Basic biogenesis and transcription of miRNA

In mammalian miRNA biogenesis, most miRNA genes are transcribed into long primary miRNA transcripts (pri-miRNA, often several kb long) by RNA polymerase II (RNA Pol II) then capped, polyadenylated, and spliced to generate a pre-miRNA [9]. Some miRNAs are also transcribed by pol III [10]. The transient pre-miRNA intermediate is then cleaved into a hairpin-containing stem-loop precursor (pre-miRNAs, ~70–90 nucleotides [nt]) by the nuclear RNase-III DROSHA. This is followed by export to the cytoplasm, which is mediated

by EXPORTIN-5, a Ran-GTP-dependent nuclear transport receptor, and further processing occurs by the cytosolic DICER, another RNase-III related enzyme, to generate the mature miRNA (~22 nt). Mature miRNAs assemble with an Argonaute family protein (AGO) to form the RNA-induced-silencing-complex (RISC), which then binds to specific sites usually within the 3'UTR of protein-coding mRNA transcripts by complementary base-pairing through a small “seed” region in the miRNA followed by enhanced mRNA turnover and/or translational repression [11].

Current mammalian genome annotation records suggest that miRNA genes are one of the most abundant gene classes, many of which are highly conserved even between distantly related species, indicating miRNAs have important roles throughout animal evolution. miRNAs are classified either as intragenic (intronic or exonic) or intergenic, based on their genomic location. Intergenic miRNAs are transcribed from their own promoters, whereas, intragenic miRNAs can be expressed from their own promoters or processed from the primary transcript of the host gene and are dependent on the transcriptional regulatory mechanisms that govern expression of the host gene. In the latter case, there is a strong correlation between expression of the host mRNA and the intragenic miRNA expression [12,13].

miRNAs gene expression is controlled through tissue-specific or developmental-stage-specific processes [14]. Since transcription of most miRNA genes is mediated by RNA Pol II, their promoters are perceived to have sequence features and transcriptional regulatory mechanisms like protein-coding genes [15]. Indeed, high throughput sequencing and bioinformatics approaches have revealed that the proximal upstream region of miRNA genes has a TATA box, initiator element, TFIIB recognition element (BRE), and a large number of transcription factor-binding motifs [12,16,17]. Moreover, recent studies have predicted miRNA promoter regions close to transcription start sites (TSSs) by the presence of CpG islands and the trimethylation of Lys4 of histone 3 (H3Kme3), suggesting the contribution of epigenetic mechanisms, such as DNA methylation and histone modification to the regulation of miRNA expression [12,18]. More detailed information on alternative promoters, splicing, and processing of miRNA genes are required for a better understanding of the diverse structures, functions, and biogenesis of miRNA genes.

3. Roles of miRNAs in cholesterol biosynthesis

3.1. Regulatory loop for cholesterol homeostasis: SREBP and miRNAs

As mentioned above, SREBP-1a and SREBP-1c modulate the expression of genes involved in energy metabolism, including fatty acid and glucose metabolism, whereas SREBP-2 is more specific to the transcriptional regulation of cholesterol metabolism genes [4]. The inactive SREBP precursor in the ER interacts directly with SCAP, which is the primary membrane cholesterol sensor. When the ER membrane is above a threshold level of 5 mol/%, SCAP binds directly to cholesterol and maintains a conformation that binds a third protein, INSIG, and this interaction traps SREBP in the ER membrane. However, when the ER membrane cholesterol falls below 5%, the conformation of SCAP changes and no longer interacts with INSIG [19]. This allows SCAP to interact with the COP II trafficking complex which moves the SCAP-SREBP complex to the golgi apparatus where a concerted two-step

proteolytic attack by site-1 and site-2 proteases (S1P and S2P) on SREBP releases the amino-terminal domain representing the mature transcription factor that migrates to the nucleus and activates the expression of SREBP target genes. Nuclear SREBPs are unstable and are degraded by the ubiquitin-proteasome system via targeting of the E3 ubiquitin ligase F-box and WD repeat domain containing 7 (FBXW7) [6]. Recently, our group revealed that hepatic expression of the miR-183/96/182 operon is directly activated by SREBP-2 in mice and human cells. SREBP-2 interacts with a conserved binding site (E-box) that is located near the TSS for the miR-183/96/182 transcription unit and promotes polycistronic transcription of the three miRs. miR-182 targets FBXW7 and miR-96 targets INSIG2 thereby creating a positive-feedback loop to regulate SREBP activity for cholesterol homeostasis [20,21] (Fig. 1a).

Another recent report suggests that miR-24 targets INSIG1 which would also have a positive effect on SREBP levels [22] but how regulation of miR-24 might be linked to SREBP homeostasis remains to be determined. Even so, the authors showed that antagonizing miR-24 in diet-induced obese mice significantly reduced plasma and hepatic lipid levels through down-regulation of lipogenic gene expression. Higher levels of miR-24 and lower levels of INSIG1 were also observed in non-alcoholic fatty liver disease (NAFLD) or steatohepatitis (NASH) patients [23,24], suggesting that crosstalk between miR-24 and INSIG1 may be important for controlling lipid homeostasis in metabolic diseases [22].

Expression of another microRNA, miR-185, is regulated by SREBP-1c, which binds to a specific motif within the promoter region of miR-185. In this case, gain-of-function results in decreased expression of cholesterol metabolism genes, such as hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and low density lipoprotein receptor (LDLR) by targeting the 3'UTR of SREBP-2 both in vitro and in vivo. SREBP-1c mainly regulates the production of fatty acids that are needed for cholesteryl ester (CE) synthesis, suggesting that the SREBP-1c/miR-185 axis constitutes a cholesterol-responsive feedback loop that maintains the ratio of free cholesterol and CE [25]. Further studies are needed for understanding the physiological role of miR-185 in metabolic diseases.

3.2. Other miRNAs in cholesterol biosynthesis

Knockdown studies on the liver-restricted miRNA, miR-122, suggests that it has a major effect on lipogenic and fatty acid oxidation genes, thereby causing reduction in plasma and hepatic cholesterol and triglyceride levels in mice [26,27]. Follow-up studies also showed that reduced miR-122 reduced circulating cholesterol levels in non-human primates [28]. Thus, miR-122 is considered a potential novel lipid-lowering agent suggesting it may be a therapeutic target. However, another study showed that miR-122 expression is significantly downregulated in NASH patients, and inhibition of miR-122 promotes the expression of fatty acid and cholesterol biosynthesis genes in human liver cell lines [23]. These seemingly conflicting studies are likely due to the fact that miR-122 is one of the most highly expressed miRNAs in liver, and likely has major effects on many pathways that are critical to normal hepatic function.

miR-34a levels are elevated in livers of humans with NASH and this is associated with increased SREBP-2 and HMGCR [29]. This may be through miR34a regulation of sirtuin-1

NAD-dependent deacetylase (SIRT1) as an earlier study reported that miR-34a also suppresses SIRT1 [30] which could lead to stabilization of SREBP-2 as reported previously [31]. Lee et al. [32] reported that the farnesoid X receptor (FXR) inhibits miR-34a expression through activation of the transcriptional repressor, small heterodimer partner (SHP), which results in regulation of SIRT1. Elevated p53 and miR-34a are associated with suppressed SIRT1 in NAFLD [33,34]. Ursodeoxycholic acid is a natural bile acid that reduces lipotoxicity and NAFLD possibly through activation of the bile acid receptor FXR, and Castro et al. reported that this may be through effects of FXR on the miR-34a/SIRT1/p53 axis [35] (Fig. 1d). Hence, upregulation of miR-34a may contribute to the development and progression of hepatosteatosis, suggesting anti miR-34a compounds may be beneficial for NAFLD and NASH.

Recent studies have revealed that miR-223 transcription is increased by cellular cholesterol levels. Overexpression of miR-223 in human liver cell lines suppresses cholesterol uptake and biosynthesis by inhibiting the scavenger receptor BI (SR-BI), hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1), and methylsterol monooxygenase 1 (SC4MOL), as well as enhances cholesterol efflux by promoting ABCA1. Moreover, miR-223 knockout mice exhibit high plasma and hepatic cholesterol levels by inducing cholesterol biosynthesis genes, including HMGCS1 [36]. These results suggest that miR-223 plays a critical role in the regulation of systemic cholesterol homeostasis.

4. miRNA regulation of reverse cholesterol transport

4.1. Cholesterol efflux and miRNAs

In addition to biosynthesis, regulated efflux of excess cholesterol is also essential for maintaining cholesterol homeostasis. The cholesterol transporter, ATP binding cassette transporter A1 (ABCA1) promotes cholesterol efflux to extracellular acceptors such as lipid-poor apolipo-proteins A-I (APOA1) to form nascent high-density lipoprotein (HDL) particles. This process is the initial step in reverse cholesterol transport (RCT), a process by which excess cholesterol is transported from peripheral cells, such as macrophages to the liver for excretion into bile and feces [37]. Mutation of the ABCA1 gene causes Tangier disease, which is characterized by a physiological absence of HDL-cholesterol (HDL-C) [38], and inactivation of hepatic ABCA1 leads to a severe reduction of total HDL-C [39] in mice. Since plasma HDL-C levels are inversely related to cardiovascular disease (CVD), ABCA1-mediated cholesterol efflux is critical in the maintenance of cholesterol homeostasis and protection against atherosclerosis. Recent studies have demonstrated that several miRNAs target ABCA1, thereby regulating plasma levels of HDL-C. Among them, the most extensively studied miRNA, miR-33a, is located within an intron of the *SREBF2* gene, and because of co-expression, miR-33a and SREBP-2 function coordinately to regulate cholesterol transport and synthesis/uptake to maintain cellular cholesterol homeostasis [40–44] (Fig. 1b). Moreover, inhibition of miR-33a leads to a significant increase in hepatic ABCA1 expression, thereby increasing circulating HDL-C in mice [40–43,45]. miR-33a is highly conserved across species, whereas miR-33b, which is encoded within an intron of the human *SREBF1* gene is absent in rodents. Since hyperinsulinemia caused by insulin resistance persistently induces SREBP-1c expression, miR-33b would markedly increase

under these conditions in humans. This could contribute to hyperlipidemia or CVD through dysregulation of lipid biosynthesis and cholesterol efflux. Indeed, both miR-33b and SREBP-1c are significantly increased in insulin resistant non-human primates [46], and miR-33 antagonism remarkably lowers VLDL and increases HDL through modulation of miR-33 target genes, including ABCA1 in these animals [46,47]. Although the effect of antagonists on glucose homeostasis or insulin sensitivity has not been observed, these results suggest a therapeutic potential for miR-33a/b targeting in the treatment of human metabolic disease. The roles of miR-33a and miR-33b are discussed in detail in other companion reviews in this volume.

miR-758 was also identified as a post-transcriptional regulator of ABCA1 by bioinformatics-based target prediction tools and genome-wide miRNAs analysis. This miRNA, similar to the regulation of miR-33a/b, is significantly downregulated by a cholesterol overload, increasing cholesterol efflux to APOA1 in macrophages, hepatocytes, and astrocytes [48]. In the brain, ABCA1 regulates cholesterol homeostasis and apolipoprotein metabolism, and is highly associated with the pathogenesis of Alzheimer's disease (AD) [49]. ABCA1 deficiency increases accumulation of amyloid β (Aβ), which leads to AD, and a Liver X receptor (LXR) agonist treatment reduced Aβ deposition at least partly through ABCA1 induction in mice [50]. However, human studies on the association of ABCA1 function and the risk of AD are complicated and have provided conflicting results [51]. Another recent study reported that miR-106b regulates Aβ metabolism by targeting ABCA1 in neuronal cells, but the physiological or pathological relevance of miR-106b remains to be determined [52]. Interestingly, miR-33a/b, miR-758, and miR-106b are all highly expressed in the brain, suggesting that these miRNAs may coordinately target ABCA1 regulation of neuronal cholesterol efflux with significant physiological effects on neuronal development and disease.

LXRs are master regulators of cholesterol metabolism because they activate many genes involved in cholesterol transport including ABCA1, ABCG1, ABCG5, and ABCG8 in response to endogenous oxysterol ligands [53]. A recent study showed that part of this regulation is through miR-26 which is activated by oxysterols and targets ABCA1 and the intracellular cholesterol trafficking protein ADP-ribosylation factor-like 7 (ARL7), in macrophages [54]. Conversely, miR-144 is up-regulated by LXR under similar conditions and targets ABCA1, suggesting the presence of negative-feedback regulation in the cholesterol efflux process [55]. LXR upregulation of hepatic miR-613 involves a negative auto-regulatory feedback loop by targeting the 3'UTR of LXR [56] (Fig. 1c). Interestingly, the activation of nuclear receptor FXR promotes miR-144 expression, which controls genes of bile acid synthesis, excretion, and transport. miR-144 also suppresses hepatic ABCA1 and reduces plasma HDL-C levels. Treatment with an FXR agonist also increases the expression of hepatic SR-BI, which mediates uptake of HDL-C into the liver, thereby repressing plasma HDL-C, suggesting that hepatic FXR induction of miR-144 is a complementary pathway for efficient channeling of cholesterol into bile in the RCT process [57] (Fig. 1d).

4.2. Uptake of HDL-C and miRNAs

SR-BI, is highly expressed in liver as mentioned above and in steroidogenic tissues, where it delivers cholesterol derived from HDL-C for steroid hormone synthesis [58]. Although low plasma HDL-C level is a hallmark of atherosclerosis, the benefit of raising HDL-C is still controversial. Indeed, hepatic overexpression of SR-BI in mice reduces atherosclerosis despite extremely low levels of plasma HDL-C. Gain-of-function hepatic SR-BI increases selective uptake of HDL-C and increases levels of remnant HDL particles that could be more efficient acceptors of cholesterol efflux from macrophages, thereby increasing the cholesterol excretion in bile and the overall rate of macrophage cholesterol efflux. On the other hand, SR-BI knockout mice exhibit markedly increased HDL-C levels due to impaired hepatic catabolism of HDL cholesteryl ester, but these mice are more susceptible to atherosclerosis in APOE- or LDLR-deficient mice because of low rates of macrophage RCT [59, 60]. These results suggest that hepatic SR-BI can influence overall rates of RCT, and regulation of its expression is significantly important in the maintenance of cholesterol homeostasis. Recent studies indicate that miR-185, miR-96, and miR-223 all repress SR-BI and HDL-C uptake by coordinately targeting its 3'UTR in human hepatic cell lines. Moreover, hepatic expressions of miR-96 and miR-185 were significantly downregulated in high-fat diet-fed APOE knockout mice, whereas SR-BI expression was increased, suggesting that both miRNAs may regulate plasma HDL-C levels and RCT [61]. Consistent with this finding, our recent study showed a dramatic downregulation of miR-96 in livers of high cholesterol diet-fed mice, and thus repression of SREBP-2-dependent cholesterol synthesis [20]. Therefore, miR-96 inhibition could potentially result in a reversal of atherogenic progression through suppression of cholesterol synthesis and activation of RCT. Other studies showed that miR-125a, miR-455, and SR-BI are inversely regulated by the adrenocorticotropic hormone (ACTH) in steroidogenic cells, and gain-of-function for both miR-125a, miR-455 blunts the ACTH dependent stimulation of SR-BI expression and HDL-C uptake, and thus attenuates HDL-stimulated progesterone production in steroidogenic cells [62].

5. Conclusion

miRNAs regulate cholesterol homeostasis by coordinating pathways involved in, de novo biosynthesis, lipoprotein uptake, free cholesterol efflux, and biliary excretion (Fig. 1 and Table 1). Several miRNAs are involved in this pathway coordination so dysregulation of miRNA expression could contribute significantly to the pathogenesis of cholesterol metabolism disorders. In fact, the recent evidence reviewed here points to the existence of co-regulatory networks of transcription factors and miRNAs that balance cholesterol metabolism. In this regard, intracellular sterol sensors, such as SREBPs, LXR, and FXR have been identified as factors that directly regulate transcription of miRNAs, and many of these participate in the maintenance of cholesterol homeostasis by feedback or feedforward regulatory circuits. Understanding these transcription factor-miRNA co-regulatory networks will reveal complex homeostatic mechanisms and help more fully understand how they contribute to metabolic diseases.

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Abbreviations

miRNA	microRNA
SREBP	sterol regulatory element-binding protein
SCAP	SREBP cleavage-activating protein
Ago	Argonaute family protein
RISC	RNA-induced-silencing-complex
Fbxw7	F-box and WD repeat domain containing 7
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
HMGCR	hydroxy-3-methylglutaryl-CoA reductase
LDLR	low density lipoprotein receptor
CE	cholesteryl ester
FXR	farnesoid X receptor
SHP	small heterodimer partner
SR-BI	scavenger receptor BI
HMGCS1	hydroxy-3-methylglutaryl-CoA synthase 1
SC4MOL	methylsterol monooxygenase 1
ABCA1	ATP binding cassette transporter A1
ApoAI	apolipoproteins A-I
HDL	high-density lipoprotein
RCT	reverse cholesterol transport
Aβ	amyloid β
LXR	Liver X receptor
ARL7	ADP-ribosylation factor-like 7
PPAR	peroxisome proliferator-activated receptors

LRH-1 liver receptor homolog 1

ACTH adrenocorticotropic hormone

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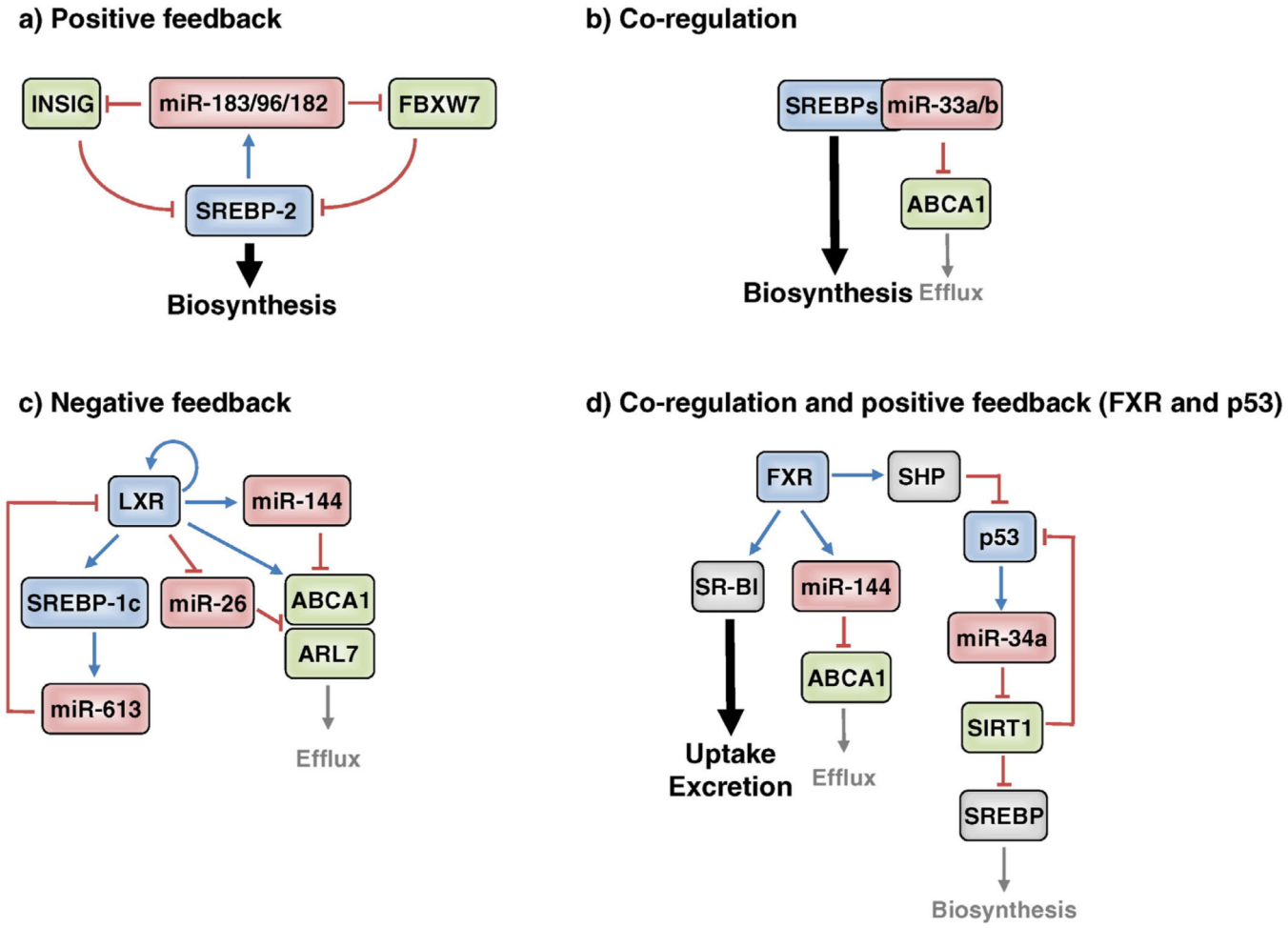

Fig. 1.

Transcription factors and miRNAs regulatory network in cholesterol homeostasis. a. SREBP-2 increased miR-183/96/182 cluster that targets INSIG2 and Fbxw7, constituting a positive-feedback loop to regulate SREBP activity. b. miR-33a/b locate within the introns of *SREBP* genes, and these transcripts coordinate to regulate cholesterol transport and synthesis/uptake to maintain cellular cholesterol homeostasis. c. LXR downregulates miR-26 that targets ABCA1 and ARL7, enhancing cholesterol transport, whereas miR-144 and miR613 are upregulated by LXR and target ABCA1 and LXR, suggesting the presence of negative-feedback regulation in the cholesterol efflux process. d. FXR-regulated miR-144 suppresses hepatic ABCA1 and plasma HDL-C levels, but increased hepatic *SR-BI* enhances biliary excretion in the RCT process. FXR also inhibits miR-34a through SHP activation that inactivates p53, which results in a positive regulation of *SIRT1*.

Table 1

miRNAs targeting genes of cholesterol homeostasis.

Biosynthesis		Efflux		Uptake		Trafficking	
miRNA	**Target gene**	**miRNA**	**Target gene**	**miRNA**	**Target gene**	**miRNA**	**Target gene**
■ 122	– Unknown	■ 33a/b	– ABCA1, ABCG1	■ 125a	– SR-BI	■ 33a/b	– NPC1
■ 34a	– SIRT1	■ 758	– ABCA1	■ 455	– SR-BI		
■ 183	– Unknown	■ 106b	– ABCA1	■ 185	– SR-BI		
■ 96	– INSIG2	■ 10b	– ABCA1, ABCG1	■ 96	– SR-BI		
■ 182	– FBXW7	■ 26	– ABCA1, ARL7	■ 223	– SR-BI		
■ 185	– SREBP-2	■ 144	– ABCA1				
■ 24	– INSIG1	■ 145	– ABCA1				
■ 223	– HMGCS, SC4MOL	■ 143	– ABCA1				