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MicroRNAs in brain cholesterol metabolism and their implications for Alzheimer's disease

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Abstract

Cholesterol is important for various neuronal functions in the brain. Brain has elaborate regulatory mechanisms to control cholesterol metabolism that are distinct from the mechanisms in periphery. Interestingly, dysregulation of the cholesterol metabolism is strongly associated with a number of neurodegenerative diseases. MicroRNAs are short non-coding RNAs acting as post-transcriptional gene regulators. Recently, several microRNAs are demonstrated to be involved in regulating cholesterol metabolism in the brain. This article reviews the regulatory mechanisms of cellular cholesterol homeostasis in the brain. In addition, we discuss the role of microRNAs in brain cholesterol metabolism and their potential implications for the treatment of Alzheimer's disease. This article is part of a special issue entitled: MicroRNAs and lipid/energy metabolism and related diseases edited by Carlos Fernández-Hernando and Yajaira Suárez.

Graphical Abstract

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Keywords

MicroRNA; Lipid metabolism; Cholesterol metabolism; Central nervous system; Alzheimer's disease

1. Introduction

MicroRNAs (miRNAs) are short non-coding RNAs that regulate expression of protein-coding genes [1]. It has been estimated that 60% of human protein-encoding genes contain at least one conserved miRNA-binding site and, with the inclusion of non-conserved miRNA binding sites, most protein-coding genes may be under the control of one or more miRNAs [2]. Indeed, miRNAs are involved in many biological processes and their dysregulations have been associated with human diseases [3]. Over the past decades, much progress has been made in understanding the roles of miRNAs in peripheral lipid metabolism [4-6]. However, lipid metabolism in the central nervous system (CNS) largely differs from the peripheral tissues, because the CNS is separated from the peripheral system by the blood-brain barrier (BBB) [7]. More importantly, several studies have demonstrated that some miRNAs have distinct temporal and spatial expression patterns and their function differs under different cellular contexts [8-10]. Therefore, it is important to keep in mind the differences in lipid metabolism between peripheral and central tissues when studying the role of miRNAs in lipid metabolism. Even within CNS, each cell type exhibits a differential miRNA expression profile and each has its own regulatory mechanism. In this article, we review the regulatory mechanisms of cholesterol homeostasis in the CNS compared to peripheral tissues. We also discuss the role of miRNAs in brain lipid, mainly cholesterol, metabolism and the therapeutic implications of miRNAs for Alzheimer's disease.

2. Regulation of Cholesterol Homeostasis in Periphery and CNS

The lipid components in the biological membranes play critical roles in membrane trafficking and signal transduction. Cholesterol, one of the three major membrane lipids, has a polar hydroxyl head and a nonpolar hydrocarbon tail on the planar ring. Given its

amphipathic property, cholesterol works as one of the main regulators for the lipid organization in the membrane by maintaining membrane integrity and fluidity [11]. Moreover, cholesterol is also the precursors to steroid hormones and bile acids [12]. Because cholesterol is involved in such diverse and critical biological processes, its intra- and extra-cellular levels are tightly regulated throughout the entire body. Perturbations in cholesterol regulatory system often lead to various human diseases [11, 13]. Notably, cholesterol regulatory mechanism in the brain is quite different to the ones in peripheral tissues and the impairment in brain cholesterol homeostasis markedly affects neuronal and glial functions.

2.1 Cholesterol Homeostasis in Periphery

Complex regulatory mechanisms maintain or adjust the intracellular cholesterol level by controlling biosynthesis, uptake, and efflux of cholesterol [13-15]. Cholesterol synthesis primarily takes place in endoplasmic reticulum (ER). There are two major transcriptional regulatory networks for coordinating cholesterol synthesis and uptake. One is regulated by sterol regulatory element-binding protein (SREBP) transcription factors and the other by liver X receptors (LXRs) in cooperation with peroxisome proliferator activated receptors (PPARs) and retinoid X receptor (RXR) [16, 17]. Cholesterol synthesis from acetyl-CoA is mediated by more than 20 enzymatic reactions and most key genes involved in these processes are controlled by the SREBP pathway [18, 19]. SREBPs are proteolytically activated and translocated to the nucleus in a cholesterol-dependent manner [19, 20]. Three major isoforms, SREBP1a, SREBP1c and SREBP2, are independently processed and regulated in response to different stimulation cues [21]. LXRs are nuclear hormone receptors that are activated by cellular cholesterol, in particular, oxysterol [17]. PPARs, on the other hand, are activated by fatty acids. LXRs have two isoforms, LXR α and LXR β . They make heterodimers with RXR isoforms and regulate the expression of genes involved in cholesterol biosynthesis and efflux. PPARs also become heterodimers with RXR isoforms and activate the transcription of genes involved in cholesterol uptake [13].

In addition to *in situ* cholesterol synthesis, cholesterol is also provided from dietary lipids or lipoprotein-mediated cellular cholesterol exchange. Lipids from food intake are packaged into chylomicrons in the intestine then transported to other locations. For cellular cholesterol exchange, cholesterol is incorporated into lipoprotein particles and then taken up in the recipient cells. Lipoprotein particle contains triacylglycerols (TG) and cholesterol ester (CE) in the core, while the surface of the particle is covered by phospholipids and free cholesterol (FC). Therefore, the lipoprotein particles can transport cholesterol and other lipids across the polar environment. Lipoprotein particles are generally classified into four types, based on their size and density [22]. Chylomicron is the largest lipid particle and carries dietary lipids. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) are large and TG-rich particles. High density lipoprotein (HDL) is the smallest particle and is involved in the reverse cholesterol transport (RCT). Those lipoprotein particles are regulated through ligand-receptor mediated endocytosis by LDL receptor (LDLR) family members [22].

Cholesterol levels are also regulated by removing excessive cholesterols from cells. Excessive cholesterols are either stored in the form of lipid droplets or transported to the liver by RCT. ATP-binding cassette (ABC) transporter protein families play critical role in

the biogenesis of HDL particles. They transfer excess cellular cholesterol onto lipid-poor lipoprotein particles containing apolipoprotein A1 (APOA-I) and apolipoprotein E (APOE) [23]. ABC transporter A1 (ABCA1) mediates cholesterol efflux to the nascent HDL, whereas ABCG1 mediates efflux to the more matured HDL [24, 25] (Figure 1). ABCA1 has been extensively studied, because it is a rate-limiting protein in HDL formation. ABCA1 and ABCG1 expression are regulated by LXR and RXR in the liver [26, 27]. In addition, SREBP activates the transcription of ABC transporters by increasing LXR activity [28]. Scavenger receptor class B type 1 (SR-B1) takes up cholesterol-loaded HDL and facilitates the cholesterol transfer from other peripheral tissues to the liver. Taken together, peripheral cholesterol levels are intricately regulated by multiple feedback mechanisms between synthesis, efflux and uptake of cholesterol.

2.2 Cholesterol Homeostasis in CNS

Most cholesterol in CNS is synthesized *in situ*, because the transport of lipoprotein-mediated peripheral cholesterol to brain is blocked by BBB [29, 30]. Therefore, it is not surprising that brain has its own cholesterol regulatory mechanisms that are distinct from the ones in periphery. Using deuterium containing heavy water, a pioneering study demonstrated that the brain synthesizes its own cholesterol *in situ* [31]. Similarly, human ApoE overexpressed in mouse peripheral tissues was not detected in the mouse CSF [32]. Figure 1 summarizes the cholesterol regulatory mechanisms in periphery and in the brain.

Almost 25% of total cholesterol in the whole body is found in the brain. Given the small mass proportion of brain over the whole body, 25% is a substantial amount of cholesterol. Neurons and glial cells are main cell types in the brain and both of them require high levels of cholesterol to maintain high membrane surface area [33]. To maintain their structural and functional properties, high membrane surface area is essential for neurons and glial cells. Neurons transmit chemical signals by forming synapses that require extensive branching of dendrites and axons. Glial cells, such as astrocytes or microglial cells, also require high membrane surface areas, because they need to surround neurons to provide nutrients and support neuronal functions [34, 35]. In rodent brain, cholesterol synthesis rates are variable in different brain sub-regions. The synthesis rate is the highest during postnatal stage then declines after brain development [36, 37]. Importantly, several experiments showed that the loss of sterol biosynthesis function in neuronal precursors causes apoptosis in newly generated neurons, suggesting that autonomous cholesterol synthesis is critical for neuronal cell survival during development [38, 39]. In mature brain, on the other hand, cellular cholesterol transfer is a major source for cholesterol supply. Although there are some conflicting results, strong evidence suggests that astrocytes synthesize cholesterol at a higher rate than neurons and transfer their excess cholesterol to neurons [38, 40].

In general, mechanisms of cholesterol transfer are similar between brain and the peripheral tissues. However, apolipoproteins in brain have several distinct features, compared to the ones in the periphery. APOE, APOJ and APOA-I are three major apolipoproteins in the brain, while APOB is not present in CNS. APOE in the brain has a slightly higher molecular weight and has more acidic isoforms [41]. Almost all lipoprotein particles in the cerebrospinal fluid (CSF) have a size and density similar to the HDL particles in plasma.

While APOE in plasma is primarily found in the large lipoprotein particles, such as chylomicron, APOE in CSF is predominantly found in the HDL-like particles [42]. Astrocytes and microglia are the major sources of APOE expression in the brain, but neurons also produce APOE under certain conditions [43, 44]. Cell-type specific gene ablation and new metabolic labeling techniques dramatically increased our understanding of cell-type specific cholesterol metabolism. Mechanisms of cell-type specific cholesterol synthesis, uptake and efflux are beyond the scope of this review and were discussed in other review [33].

3. Implications of Cholesterol in CNS Diseases

3.1 Functions of cholesterol in the brain

Cholesterol is required for diverse biological processes; maintaining membrane integrity and fluidity, synthesis of steroid hormones and bile acids, formation of lipid rafts, and cell signaling [11, 12]. In the brain, cholesterol is involved in nerve growth factor signaling, axonal guidance, synaptic formation, myelination and synaptic transmission [45, 46]. For example, Maunch et al. reported the critical role of glial cell-derived cholesterol for synaptogenesis in retinal ganglion cell (RGC) during postnatal neuronal development [47]. Similarly, Goritz et al. demonstrated that cholesterol secreted from glial cells promoted presynaptic differentiation in murine RGC [48]. Conversely, removal of cholesterol diminished the size of evoked excitatory postsynaptic currents, suggesting that cholesterol is essential for maintaining synaptic activity [48]. Extensive membrane reorganization is required during synaptic regeneration after brain injury. To increase cholesterol dynamics, genes responsible for cholesterol synthesis, uptake, and efflux are modulated after brain injury [49]. Cholesterol is also essential for myelin formation. Conditional disruption of cholesterol synthesis in oligodendrocytes caused severe hypomyelination in white matter [50]. Taken together, maintaining cholesterol homeostasis is critical for brain function and alteration of brain cholesterol level may lead to various brain diseases.

Because cholesterol is critical for neuronal function, dysregulation of cholesterol metabolism has been associated with several neurodegenerative diseases, such as Alzheimer's disease (AD), Huntington's diseases (HD), Parkinson's disease (PD) and Niemann-Pick type C (NPC) disease. Among them, AD has been extensively studied because APOE4 genotype is the strongest genetic risk factor for AD and APOE isoforms differentially affect synaptic plasticity [51, 52]. Recent studies on microRNAs involved in brain lipid metabolism and AD pathogenesis will be further described below. The roles of cholesterol in other neurodegenerative diseases were discussed elsewhere [53-56].

3.2 Cholesterol homeostasis and Alzheimer's diseases

AD is the most common cause of dementia in the elderly. AD is clinically characterized by progressive decline in memory and cognitive function. The pathological hallmark of AD is the formation of amyloid plaques and neurofibrillary tangles in the brain. Sequential proteolytic cleavages of amyloid β precursor protein (APP) by β - and γ -secretases generate A β peptides. Accumulation of A β peptide is hypothesized to initiate pathogenic cascades leading to AD [57]. Genetic and biochemical studies strongly suggest that dysregulation of

A β production or clearance is critical for familial or sporadic AD pathogenesis, respectively. Notably, the majority of extracellular A β is produced by proteolytic cleavage of APP within endocytic compartment. Moreover, the two essential APP proteolytic enzymes (β - and γ -secretases) and its substrate APP are membrane proteins [58]. Therefore, their trafficking and proteolytic activities are influenced by cholesterol homeostasis in the membrane. Multiple studies have demonstrated that the alterations in cholesterol level or key cholesterol regulators lead to change in A β metabolism [51, 59-61].

ABC transporter protein families regulate A β production and secretion *in vitro* [62-69]. For example, Koldamova et al. and Sun et al. reported that ABCA1 induction decreases A β levels in cells by inhibiting both β - and γ -cleavage of APP [62-64]. Similarly, Kim et al. also demonstrated that ABCA1 as well as ABCG1 increased cholesterol efflux to APOE, leading to a reduction in A β generation [65]. Furthermore, these *in vitro* findings are consistent with *in vivo* studies using genetically modified mouse models [63, 66-69]. ABCA1 gene ablation in mice decreased APOE lipidation and levels and increased A β accumulation in APP transgenic mouse models. In contrast, ABCA1 overexpression or LXR/RXR agonist treatment decreased amyloid deposition [66-69].

The role of APOE in AD pathogenesis has been extensively studied, because APOE4 is the strongest genetic risk factor for AD. Three major APOE isoforms in human are APOE2 (cys112, cys158), APOE3 (cys112, arg158), and APOE4 (arg112, arg158). APOE4 allele increases the risk of developing AD, whereas APOE2 allele is protective [70, 71]. Importantly, the amino acid differences in APOE isoforms dramatically change the structure of APOE protein and differentially affect the degradation and clearance of A β , mitochondrial function, and other downstream pathways [51, 52, 72]. In addition to isoform, the levels of APOE protein and its lipidation status also influence A β metabolism. Several studies demonstrated that treatment of LXR/RXR agonists increase the levels of ABCA1 and APOE and decrease amyloid deposition in APP overexpressing cells and mice [73-77]. For example, Cramer et al. demonstrated that RXR activation facilitate A β clearance in an ApoE dependent manner [74]. Subsequently, several groups also investigated the role of RXR in AD pathogenesis [75-78]. Given the critical role of APOE in regulating A β degradation and clearance, it is likely that the induction of APOE contributes to A β clearance after LXR/RXR agonist treatment. However, genetically reducing APOE3 or APOE4 levels decreased amyloid accumulation in APP transgenic mouse models [79, 80]. Therefore, it is still possible that other mechanisms, such as changes in APOE lipidation or microglia activation state, also contribute to the effect of LXR/RXR agonist. Because the activation of transcription factor LXR/RXR can regulate the levels of many genes, it will be important to explore other possible mechanisms.

Extracellular A β in interstitial fluid or CSF undergoes cellular uptake, proteolytic degradation, and other clearance. APOE was reported to facilitate the degradation of A β by microglia through phagocytosis and increase astrocyte-mediated A β clearance as well [81, 82]. Apolipoprotein receptors also regulate A β production and/or clearance by modulating endocytosis of lipoprotein or by directly interacting with A β [83]. Receptor related protein1 (LRP1), a member of LDLR family, works as a major efflux transporter for A β across the

BBB. Binding of A β to LRP1 is known to initiate A β clearance [84]. The impairment in A β clearance is believed to cause vascular A β deposition [85].

4. miRNAs in CNS Lipid Metabolism

4.1 miRNAs: their biogenesis and regulation

MicroRNAs (miRNAs) are short non-coding RNAs, approximately 22 nucleotides in length. They are involved in post-transcriptional regulation of gene expression [1]. miRNA genes are located within various regions of the genome, including introns of protein-coding genes [86]. Although promoter of most miRNA genes is yet to be identified, miRNA gene residing within introns of protein-coding genes sometimes shares the promoter of the host gene [87]. miRNA genes, transcribed by RNA polymerase II, produce a hairpin structure called primary-miRNA, from which Drosha/DGCR8 cleave it to produce the precursor-miRNA (pre-miRNA) [88, 89]. The pre-miRNA is then exported out of the nucleus by Exportin 5 and is further processed by Dicer to generate a mature miRNA duplex [90, 91]. The seed sequence, the nucleotides at position 2-7 near the 5' end of miRNAs, is crucial for target recognition. The partial complementary base pairing between the 3' UTR of the target mRNA and the miRNA seed sequence is a critical determinant in miRNA target selection. However, noncanonical binding outside of the seed sequence also contributes to miRNA-mRNA interaction [92]. Following miRNA-mRNA pairing, RNA-induced silencing complex (RISC) promotes mRNA degradation or interferes with protein translation step [93, 94]. Usually, only one arm of the miRNA duplex is functional while the other arm is degraded [93, 94]. However, both arms (termed as 5p and 3p) can also have functional effects on target mRNAs in certain cases. More detailed discussion on the biogenesis and functions of miRNAs can be found in other review [87].

4.2 Potential role of miRNAs in the brain cholesterol metabolism

It is estimated that most protein-coding genes may be under the control of one or more miRNAs. Moreover, it has been reported that many apparently unrelated miRNAs may have a common target gene, or sometimes one miRNA may target multiple genes in one pathway [95, 96]. Such redundancy indicates that miRNAs may act as an important biological buffering system [97]. As mentioned earlier, cholesterol homeostasis requires elaborate regulatory mechanisms to maintain the cholesterol levels. Given the critical role of miRNAs in various cellular homeostasis processes, multiple groups have investigated whether miRNAs regulate lipid metabolism in the peripheral tissues [98, 99]. Because CNS has an independent cholesterol circulation system in the brain that is different from the periphery, findings from peripheral tissues do not translate to the brain. In addition, many miRNAs exhibit temporal and spatial expressions under different cellular contexts and brain has a quite distinct miRNA expression pattern [8, 9, 100, 101]. Here, we summarize the function of miRNAs involved in brain lipid metabolism. The detail discussion on the miRNAs in periphery is discussed in other reviews [4-6, 102, 103].

4.3 Regulation of ceramide synthesis by miR-137 and miR-181c

Ceramide is a sphingolipid composed of sphingosine and a fatty acid. Along with cholesterol, it is one of main membrane components. Ceramide levels are increased in

sporadic AD patients [104, 105]. Several studies suggest that ceramide may directly contribute to AD pathogenesis by modulating APP processing. Inhibition of ceramide synthesis pathway decreased A β production, whereas ceramide treatment increased A β production [106, 107]. One possible mechanism may be related to the increase of β - and γ -secretase activity [108, 109]. Given these studies, targeting ceramide synthesis may be a promising therapeutic strategy for treating AD [110]. Serine palmitoyltransferase (SPT) regulates the *de novo* ceramide synthesis pathway [111]. It is composed of SPT long chain (SPTLC) 1 and either SPTLC2 or SPTLC3. Inhibition of SPT activity decreased ceramide levels and attenuated neuronal cell death triggered by A β [104]. To determine whether SPTLC1 or SPTLC2 expression is regulated by miRNAs, Geekiynage et al. screened multiple miRNAs using luciferase reporter assays [112]. They found that miR-137 and miR-181c decrease the levels of SPTLC1 and ceramide in primary astrocytes. In addition, overexpression of miR-9 and miR-29a/b-1 decreased the levels of SPTLC2 and ceramide [112]. Conversely, inhibition of those miRNAs increased SPTLC levels, leading to more A β production. Interestingly, the levels of those miRNAs were negatively correlated with the levels of SPTLC1 and SPTLC2 in sporadic AD brains. Taken together, these data suggest that miRNAs regulating ceramide levels may be potential therapeutic targets for AD. Further *in vivo* studies are required to determine functional effects of these miRNAs in AD pathogenesis.

4.4 Regulation of ABCA1 expression by miR-106b-5p

As previously mentioned in the section 3.2, ABCA1 has an important role in lipid homeostasis and A β metabolism. Because ABCA1 has a long 3'UTR, there is a possibility that ABCA1 can be regulated by miRNAs. Multiple studies demonstrated that several miRNAs regulate ABCA1 levels in peripheral tissues [113-115]. Among them, only three miRNAs (miR-106b-5p, miR-758-5p and miR-33a) were validated to directly regulate ABCA1 in neurons. miR-106b-5p directly targets the 3' UTR of ABCA1 and significantly decreases the ABCA1 protein level in a neuronal cell line as well as in fully differentiated primary hippocampal neurons [116]. The reduction of ABCA1 levels by miR-106b-5p was sufficient to decrease cellular cholesterol efflux and increase A β secretion. Based on APP processing analysis, it appeared that miR-106b-5p increases the β -secretase cleavage of APP. Increase of A β levels by miR-106b-5p was completely rescued by the miR-106b-5p-resistant ABCA1 construct that lacks the 3' UTR. This result suggests that ABCA1 is the likely target gene responsible for the effect of miR-106b-5p on A β level. Notably, miR-106b-5p also targets APP in the HeLa cell line [117]. This data suggests that miR-106b-5p may have opposing effects on A β level depending on whether which gene is predominantly regulated by miR-106b-5p. Therefore, the *in vivo* effect of miR-106b-5p on A β metabolism will need further study using APP transgenic mouse models.

4.5 Regulation of ABCA1 expression by miR-758-5p

Ramirez et al demonstrated that miR-758-5p negatively regulates ABCA1 expression in both human and mouse macrophages and hepatic cell lines [118]. Moreover, miR-758-5p overexpression inhibited cholesterol efflux to ApoA1. Interestingly, excess cholesterol in macrophages decreased the level of miR-758-5p *in vivo* and *in vitro*. These data suggest that miR-758-5p and cholesterol have a feedback mechanism. miR-758-5p expression survey

experiment indicated that it is highly enriched in mouse brain. This data suggests that miR-758-5p may affect neurological functions through cholesterol modulation. Indeed, miR-758-5p overexpression significantly decreased ABCA1 protein levels and cellular cholesterol efflux in cells, whereas inhibition of endogenous miR-758 caused the opposite effects. Furthermore, miR-758-5p overexpression repressed several genes involved in myelination, neuronal migration, and neurite outgrowth in human neuroglioma cells. Therefore, miR-758-5p may be involved in regulating neurological functions, although its role in the brain has not been tested *in vivo* yet. Given the critical role of brain cholesterol homeostasis in several neurodegenerative diseases, further studies are warranted to determine whether miR-758-5p can be modulated as a therapeutic target.

4.6 Regulation of ABCA1 and ABCG1 expression by miR-33a

Humans have two miR-33 genes, miR-33a and miR-33b. miR-33a is homologous to mouse miR-33, which is evolutionarily well conserved in mammals [119]. As an intronic miRNA, miR-33a gene is located within the SREBF-2 gene that codes SREBP2 protein, whereas miR-33b gene is located within the SREBF-1 gene that codes SREBP1 protein. As we discussed in the section 2.1, SREBP is a transcriptional regulator for many cholesterologenic and lipogenic genes [20, 120]. The genetic location of miR-33 provided an important clue for the pioneering studies that demonstrated the role of miR-33 in peripheral lipid metabolism [119, 121-124]. Among the potential targets investigated, ABCA1 is the most consistently regulated target gene. According to the peripheral tissue studies, miR-33a/b and its host genes SREBP2/1 were coexpressed and coordinately regulated by the alteration of cellular cholesterol status. In addition, miR-33a levels correlated with the levels of cholesterol, ABCA1, and SREBF2 [134,136]. Particularly, miR-33 decreased cholesterol efflux to ApoA1 and nascent HDL in mouse macrophages [119, 123]. Moreover, overexpression of miR-33 decreased ABCA1 expression and plasma HDL levels, whereas the pharmacological or genetic inhibitions of miR-33 increased ABCA1 levels, cholesterol efflux, and plasma HDL levels *in vivo* [119, 121-126].

Because miR-33 is highly enriched in the brain, it is tempting to speculate that it may have a critical role in brain cholesterol metabolism [119, 127]. Indeed, overexpression of miR-33 decreased ABCA1 levels and impaired cholesterol efflux in neuronal and glial cells [127]. More importantly, miR-33 increased extracellular A β levels in mouse and human neuronal cells. Furthermore, pharmacological inhibition of miR-33 significantly increased ABCA1 level [127, 128]. Both genetic and pharmacologic inhibition of miR-33 decreased cortical A β levels. [127]. This study suggests that targeting miR-33 and other miRNAs regulating ABCA1 may have therapeutic potential for AD treatment.

4.7 Other miRNAs that may be involved in brain lipid metabolism

Although their role in brain lipid metabolism is still unknown, miR-613, miR-1 and miR-206 merit further investigation because they are known to regulate the LXR transcription factor in peripheral tissues. miR-613 directly decreased LXRA level through a posttranscriptional inhibition, whereas miR-613 is also regulated by the activation of LXR in human hepatocytes [129]. miR-1 and miR-206 inhibited the production of lipid droplets in hepatocytes by targeting LXRA transcription factor [130]. Because they are expressed in the

brain, miR-613, miR-1 and miR-206 may affect brain lipid metabolism. Interestingly, several miRNA profiling studies reported that certain miRNAs were regulated in response to the alteration of cellular lipid contents. For example, the levels of miR-291b, miR-672 and miR-673 decreased in response to an increase in cellular cholesterol contents [118]. Therefore, these miRNAs might mediate cellular responses to high cholesterol. Identification of their upstream transcription regulators may increase our understanding of cholesterol homeostasis. Figure 2 illustrates the miRNAs-mediated cholesterol regulation mechanism and its link to A β metabolism in the brain.

5. Concluding Remarks and Future Therapeutic Applications

miRNAs can function as an expression switch or buffers. When miRNAs act as expression buffers, they fine-tune target gene expression in response to environmental and cellular changes. Cholesterols in the brain require elaborate regulatory mechanisms, where many miRNAs are involved in regulating cholesterol homeostasis. Among the proteins implicated in brain cholesterol metabolism, APOE and ABCA1 are the most extensively studied for AD pathogenesis. ABCA1 is redundantly targeted by miR-106b, miR-758, and miR-33 in the brain. Inhibition of these miRNAs was proposed as a potential therapeutic strategy [116, 118, 127]. However, there are several obstacles that need to be overcome for their use in clinical applications.

Because one miRNA can regulate multiple target genes, it is important to determine whether other target gene also has any implication to the disease. One important concept to keep in mind is that miRNA targets are generally not random genes. Recent studies demonstrated that miRNAs selectively regulate genes within the same pathway [97, 131-133]. Therefore, it is tempting to speculate that miRNA may not trigger significant nonspecific off targets. In addition, growing number studies indicate there was an overestimation in the miRNA target prediction algorithms. Previously, it was claimed that one miRNA can regulate hundreds or even thousands of downstream target genes based on the computation prediction algorithms. However, such algorithms failed to consider the biological context where miRNAs and their predicted target genes are expressed [134, 135]. Many miRNAs have a strong tissue-specific expression pattern and 3' UTR of target gene plays a critical role in determining the miRNA-target interaction [136, 137]. In fact, current target prediction algorithms often identify many false positive target genes and there is no strong overlap in the predicted target gene list among prediction algorithms [138-141]. Therefore, it is difficult to determine miRNA function primarily based on the predicted target genes. While *in silico* approach is easy to use as the first round of analysis, the bioinformatics results require careful wet lab validation experiments using cells and animals. In addition, to overcome this critical issue, systems biology approaches are required.

Delivering small molecules across BBB is one of the major obstacles in clinical application for brain diseases. Notably, intrathecal antisense oligonucleotides (ASO) injection successfully delivered ASO to the cortical cells in nonhuman primates [142]. In addition, intrathecal ASO injection to human was successfully carried out in the phase I and II clinical trials [143]. In both clinical trials, the intrathecal ASO injection was safe and effective. Besides ASO, other advanced techniques are being tested for the brain delivery. For

example, exosome- or liposome-based nanoparticles and conjugation with neurotrophic virus peptide have generated some promising data [144-147]. In addition, several small molecules have been identified to modulate miRNAs [148]. Whether these technical advancement will translate to therapy warrants further preclinical and clinical studies.

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Abbreviations

AAV	adeno associated virus
ABC	ATP-binding cassette
ABCA1	ABC transporter A1
AD	Alzheimer's disease
APOA-I	apolipoprotein A1
APOE	apolipoprotein E
APP	amyloid β precursor protein
BBB	blood-brain barrier
CE	cholesteryl ester
CETP	cholesterylester transfer protein
CNS	central nervous system
CSF	cerebrospinal fluid
ER	endoplasmic reticulum
FC	free cholesterol
HD	Huntington's diseases
HDL	high density lipoprotein
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
LDLR	LDL receptor
LXR	liver X receptors
miRNAs	microRNAs
NPC	Niemann-Pick type C

PD	Parkinson's disease
PPARs	proliferator activated receptors
pre-miRNA	precursor miRNA
RCT	reverse cholesterol transport
RGC	retinal ganglion cell
RISC	RNA induced silencing complex
RXR	retinoid X receptor
SLOS	Smith-Lemli Opitz syndrome
SPT	serine palmitoyltransferase
SPTLC	SPT long chain
SR-B1	scavenger receptor class B type 1
SREBP	sterol regulatory element-binding protein
TG	triacylglycerols
VLDL	very low density lipoprotein

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Highlights

- Cholesterol homeostasis is critical for various neuronal functions in the brain.
- miRNAs are important regulators of cholesterol homeostasis in the brain.
- miR-137 and miR-181c regulate brain ceramide synthesis and A β production.
- miR-106b, miR-758 and miR-33a modulate ABCA1-mediated cholesterol efflux.
- miR-106b, miR-758 and miR-33a regulate A β metabolism.

Figure1. Regulation of cholesterol homeostasis in the periphery and CNS

Peripheral tissues and brain have their own cholesterol regulatory network, because brain is separated from the peripheral system by the blood-brain barrier. Peripheral cholesterol levels are maintained by feedback regulations between dietary intake, cholesterol synthesis (lipogenesis), cellular cholesterol efflux (lipidation) and uptake of lipoprotein particles. Green arrows indicate transcriptional activation. SREBP and LXR/RXR activate transcription of genes involved in cholesterol biosynthesis and efflux. Excessive cellular cholesterol is either stored in lipid droplets (illustrated as a yellow circle) or transported back to the liver. Black arrows represent movement of proteins and lipoprotein particles. ABCA1 and ABCG1 mediate cholesterol efflux to the nascent HDL and matured HDL, respectively. LCAT esterifies cholesterol in the nascent HDL to form matured HDL. CETP mediates CE and TG exchanges between lipoproteins. Most lipoprotein particles synthesized in the periphery cannot enter the brain (red arrow). Instead, CNS synthesizes cholesterol *in situ*. APOE, APOJ and APOA-I are three major apolipoprotein populations in the brain. In the fully developed mature brain, astrocytes transfer cholesterol to neurons by using mediated by lipoprotein particles and their receptors. Both in the CNS and periphery, miRNAs are involved in multiple cholesterol regulatory mechanisms.

Figure2. miRNAs-mediated cholesterol regulations and their link to Aβ metabolism in the brain
 Several miRNAs directly regulate cholesterol homeostasis in the brain (red bar). SPT is an important regulator for de novo ceramide synthesis. miR-137 and miR-187c regulate the level of SPT. ABCA1 mediates cholesterol efflux to lipoprotein particle. miR-106b, miR-758, and miR-33 directly target ABCA1. The levels of several miRNAs (highlighted in blue) are regulated by cellular cholesterol levels. miRNAs highlighted in green target LXR in periphery, but whether they also regulate LXR in the brain is unknown. Increased ABCA1 level facilitate APOE lipidation and decreased Aβ levels in the brain. Black arrows, green arrows, and red bars indicate the movement of proteins, transcriptional activation, and posttranscriptional inhibition, respectively.