Biochimica et Biophysica Acta xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

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

Hyejin Yoon [a,c], Luis F. Flores [b], Jungsu Kim [a,c,*]

[a] Neurobiology of Disease Graduate Program, Mayo Graduate School, Jacksonville, FL, United States
[b] Biochemistry and Molecular Biology Graduate Program, Mayo Graduate School, Jacksonville, FL, United States
[c] Department of Neuroscience, Mayo Clinic, Jacksonville, FL, United States

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 28 April 2016
Accepted 29 April 2016
Available online xxxx

Keywords:
MicroRNA
Lipid metabolism
Cholesterol metabolism
Central nervous system
Alzheimer's disease

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.

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 precursor 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

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.

☆ 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.

* Corresponding author at: Department of Neuroscience, Mayo Clinic College of Medicine, 4500 San Pablo Rd S, Jacksonville, FL 32224, United States.
E-mail address: kim.jungsu@mayo.edu (J. Kim).

http://dx.doi.org/10.1016/j.bbalip.2016.04.020
1388-1981/

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 the 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, LXRα and LXRβ. 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 a 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] (Fig. 1). ABCA1 has been extensively studied, because it is a rate-limiting protein in HDL formation. The expression of ABCA1 and ABCG1 is 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 the brain is blocked by BBB [29,30]. Therefore, it is not surprising that the brain has its own cholesterol regulatory mechanisms that are distinct from the ones in the 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]. Fig. 1 summarizes the cholesterol regulatory mechanisms in the 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 subregions. 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 the brain and the peripheral tissues. However, apolipoproteins in the 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 reviews [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

Please cite this article as: H. Yoon, et al., MicroRNAs in brain cholesterol metabolism and their implications for Alzheimer's disease, Biochim. Biophys. Acta (2016), http://dx.doi.org/10.1016/j.bbalip.2016.04.020