The Role of Lipin-1 in the Pathogenesis of Alcoholic Fatty Liver

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Abstract — Aims: The aim of this review was to focus on the knowledge of the role of lipin-1 in the pathogenesis of alcoholic fatty liver. Methods: Systematic review of animal clinical and cell level studies related to the function of lipin-1 on alcoholic fatty liver, alcoholic hepatitis and alcoholic liver cirrhosis disease. Result: Ethanol could increase the expression of lipin-1 through the AMPK-SREBP-1 signaling and dramatically increase the ratio of Lpin1β to Lpin1α by SIRT1-SFRS10-Lpin1β/α axis in the liver. Moreover, research has shown that over-expression of lipin-1 could also remarkably suppress very low density lipoprotein-triacylglyceride secretion. Last, lipin-1 has potent anti-inflammatory property. Conclusion: In conclusion, lipin-1 has dual functions in lipid metabolism. In the cytoplasm, lipin-1β functions as a Mg2+-dependent phosphatidic acid phosphohydrolase (PAP) enzyme in triglyceride synthesis pathways. In the nucleus, lipin-1α acts as a transcriptional co-regulator to regulate the capacity of the liver for fatty acid oxidation and activity of the lipogenic enzyme. In hepatocytes of alcoholic fatty liver disease (AFLD), ethanol increases the expression of lipin-1 through the AMPK-SREBP-1 signaling and the Lpin1β/α ratio by SIRT1-SFRS10-Lpin1β/α axis. Of course, in addition to that, ethanol could also produce the PAP activity and interrupt the nucleus function of lipin-1. Furthermore, over-expression of lipin-1 could remarkably suppress very low-density lipoprotein-triacylglyceride (VLDL-TAG) secretion. In the end, endogenous lipin-1 has potent anti-inflammatory property. Increased synthesis of TAG, decreased fatty acid oxidation, impaired VLDL-TAG secretion and activated inflammatory factors act together to exacerbate the development of AFLD.

INTRODUCTION

According to statistics, alcoholism causes in about 2.5 million worldwide each year, accounting for 4% of all mortality. Although alcoholism is associated with >60 diseases, most mortality from alcoholism results from alcoholic liver disease (ALD) (Jaurigue and Cappell, 2014). Alcoholic fatty liver disease (AFLD), alcoholic hepatitis and alcoholic liver cirrhosis are the three main pathological types (Jaurigue and Cappell, 2014). Among all the drinkers, ~90% develop AFLD, 25% progress to alcoholic hepatitis, 15% suffer from developing alcoholic cirrhosis, and 10% are at risk for the development of hepatocellular carcinoma (Orman et al., 2013). The development of ALD results from many factors, including oxidative stress, cytokines, endotoxin, ischemia, immune regulators, metabolic disturbances, nutritional factors and genetic predisposition. AFLD, the earliest manifestation of ALD, is pathologically characterized by microvesicular and macrovesicular lipid accumulation within hepatocytes, minimal inflammatory reaction and no hepatic fibrosis (MacSween and Burt, 1986). AFLD is characterized by increased accumulation of lipid in the liver of patients who have consumed excessive amounts of alcohol for prolonged periods (Yin et al., 2012). AFLD results from liver lipid metabolism disorder, following lipid accumulation in the liver. There are four main pathogenic factors: (a) Nicotinamide adenine dinucleotide hydrogen (NADH) increased by alcohol oxidation not only inhibiting mitochondrial β-oxidation of fatty acids but also promoting fatty acid and triglyceride synthesis (European Association for the Study of the Liver, 2012). (b) Induced hepatic gather of free fatty acids from adipose tissue and of chylomicrons from the intestinal mucosa (European Association for the Study of the Liver, 2012). (c) Inhibition of adenosine monophosphate activated kinase (AMPK) activity by ethanol (You et al., 2005) resulting in decreased lipolysis and increased lipogenesis. (d) Damage to mitochondria and microtubules by acetaldehyde, resulting in a reduction of NADH oxidation and the accumulation of very low density lipoprotein (VLDL) (European Association for the Study of the Liver, 2012). Increased accumulation of lipid resulted from disordered lipid metabolism in the liver is associated with the increase in the rate of de novo lipogenesis (Hu et al., 2012) and the decrease in the rate of fatty acid oxidation (Finck et al., 2006) in animal liver. Recently, lipin-1 has been gaining recognition as one of the critical components in mediating hepatocyte steatosis signaling. The ample experimental evidence has shown that in hepatocyte of ALD, ethanol increases expression of lipin-1 through the AMPK-SREBP-1 signaling (Hu et al., 2012) and dramatically increases the ratio of Lpin1β to Lpin1α by SIRT1-SFRS10-Lpin1β/α axis in the liver (Yin et al., 2014). Moreover, it has been demonstrated ethanol can induce the activity of lipin-1 in cytoplasm and disturb the function of lipin-1 in nucleus (Yin et al., 2012). More importantly, over-expression of lipin-1 could also remarkably suppress very low-density lipoprotein-triacylglyceride (VLDL-TAG) secretion (Chen et al., 2008). Last, lipin-1 has strong anti-inflammatory property (Kim et al., 2010).

Lipin-1 is one member of the lipin family (Reue, 2009). Recent studies indicate that lipin-1 exhibit dual distinct functions in lipid metabolism depending on their subcellular localization. In the cytoplasm, lipin-1 functions as a Mg2+-dependent phosphatidic acid phosphohydrolase (PAP) enzyme in the triglyceride synthesis pathway. When lipin-1 enters the nucleus, it acts as a transcriptional co-regulator to regulate lipid metabolism (Csaki et al., 2013). On one hand, it interacts with the nuclear factor peroxisome proliferator-activated receptor α (PPARα) and the transcriptional coactivator PPAR gamma coactivator-1α (PGC-1α) to increase hepatic capacity for mitochondrial fatty acid oxidation (Finck et al., 2006). On the other hand, it inhibits the nuclear abundance and promoter activity of sterol regulatory element binding protein 1 (SREBP-1) lipogenic transcription factor (Xu et al., 2013). In conclusion, lipin-1 plays an important role in lipid metabolism.
THE LIPIN-1 PROTEIN

Lipin-1 protein molecular structure

Lipin-1 protein is encoded by the gene LPIN1 (Reue, 2009). Gene-expression studies have indicated that lipin-1 has a unique tissue expression pattern, and unequal expression of lipin-1 gene is present in many tissues (Donkor et al., 2007). Lipin-1 is expressed at the highest level in white and brown adipose tissue, skeletal muscle, and whole brain, and at lower level in other tissues, including liver, bone, lung, heart, cerebellum, kidney and muscle (Csaki et al., 2013). The mouse lipin-1 gene undergoes alternative mRNA splicing to generate two isoforms, known as lipin-1α and lipin-1β, which have 891 and 924 amino acids in length, respectively (Peterfy et al., 2005). Lipin-1 protein contains two highly conserved regions, known as the N-terminal lipin (N-LIP) and C-terminal lipin (C-LIP) domains, based on their locations at opposite ends of the protein (Peterfy et al., 2001). There is a stretch of positively charged residues near the N-LIP domain which is required not only for the translocation of lipin-1 to the nucleus (Peterfy et al., 2010), but also for lipin-1 binding to phosphatidic acid (PA) (Ren et al., 2010). The C-LIP domain contains a DIDGT motif, which is present in all species and constitutes the catalytic site for a Mg2+−dependent PAP enzyme (Csaki and Reue, 2010). Other researches indicate that lipin-1 can be identified as a member of the haloacid dehalogenase superfamily (Han et al., 2006).

Regulation of LPIN1 in transcription, translation and modification

Lipin-1 is regulated by many elements in gene transcription, alternative mRNA splicing, protein phosphorylation, as well as subcellular protein localization (Csaki and Reue, 2010).

Firstly, the expression of lipin-1 is regulated by SREBP-1 (Pittner et al., 1985), the major activator of hepatic lipogenesis. The activity of SREBP-1 is upregulated by insulin through multiple regulatory steps (Zhu et al., 2014). A sterol regulatory element (SRE) and nuclear factor-Y (NF-Y) bind to sequence motifs upstream of the LPIN1 gene and both appear to induce LPIN1 gene transcription (Hu et al., 2012). Moreover, glucocorticoid can regulate the expression of LPIN1 through combination with a glucocorticoid response element within the Lipin-1 promoter region, which has been shown to bind the glucocorticoid receptor upon addition of dexamethasone (Csaki and Reue, 2010). Furthermore, fasting and diabetes induce PGC-1α gene expression, which in turn activates expression of lipin-1 (Finck et al., 2006). In contrast, lipin-2 and cytokines negatively regulate the expression of the lipin-1 gene (Csaki and Reue, 2010). First of all, Grimsley et al. have demonstrated that siRNA knockdown of either lipin-1 or lipin-2 could cause an increase in mRNA and protein levels of the other lipin in HeLa cells (Grimskey et al., 2008), so we can predict that lipin-2 is a negative regulator of the lipin-1 gene expression. Secondly, in terms of cytokines, we would point out that inflammatory conditions cause lipin-1 gene expression (Csaki and Reue, 2010). In the end, lipin-1 is immediately suppressed by tumor necrosis factor (TNF)-α and interleukin-1β treatment in adipocytes (Lu et al., 2008).

As mentioned above, the lipin-1 gene undergoes alternative splicing to generate two protein isoforms, lipin-1α and lipin-1β in mouse (Han and Carman, 2010). However, the lipin-1 mRNA alternative splicing is regulated by SFRS10 (splicing factor, arginine/serine-rich 10) (Yin et al., 2012). The SFRS10 splicing factor has been implicated in the inclusion of the lipin-1β exon (Csaki et al., 2013). Down-regulation of SFRS10 in the HepG2 cells of liver leads to increased lipin-1β expression relative to lipin-1α (Yin et al., 2012). Evidence suggests that lipin-1β mainly resides in the cytoplasm as a PAP type enzyme in lipid synthesis. Lipin-1α is a nucleocytoplasmic shuttling protein and it functions as a transcriptional co-activator in the nucleus (Yin et al., 2012). The posttranslational modification and subcellular localization of lipin-1 are regulated by phosphorylation, sumoylation and 14-3-3 proteins. Dephosphorylated forms of lipin-1 are abundant in the cytosolic fraction, whereas phosphorylation forms are enriched in the endoplasmic reticulum (ER) membrane fraction (Han and Carman, 2010; Hu et al., 2012). More importantly, lipin-1 phosphorylation /dephosphorylation appears not to affect the activity of PAP enzyme, but rather only influences subcellular localization of the protein (Csaki and Reue, 2010). Sumoylation induces the nuclear localization of lipin-1α where lipin-1α acts as a transcriptional co-regulator (Csaki et al., 2013). Interaction of lipin-1α with 14-3-3 proteins determines its cytoplasmic localization in 3T3-L1 adipocytes (Bou Khalil et al., 2010). Even, the nuclear localization of lipin-1 is blocked by its interaction with 14-3-3 proteins in the cytosol (Han and Carman, 2010). Only because of the posttranslational modification and subcellular localization, does lipin-1 have complex functions in lipid metabolism.

The function of lipin-1 in lipid metabolism of hepatocyte

Lipin-1 is a multifunctional protein that has both enzymatic activity and the ability to regulate transcription, which is dependent on its subcellular localization. In cytoplasm, lipin-1 acts as a Mg2+−dependent PAP enzyme converting phosphatidate to diacylglycerol, an essential precursor in triglyceride and phospholipid synthesis (Finck et al., 2006; Yin et al., 2014). Lipin-1 does not contain DNA-binding domains but instead regulate gene expression indirectly by interacting with transcription factors and regulating their activity (Kim et al., 2010). For instance, when lipin-1 enters the nucleus after sumoylation, it can directly interact with transcription factors to act as a transcriptional co-regulator enhancing PPARα and PGC1-α (Csaki and Reue, 2010) and suppressing the function of SREBP-1 (Xu et al., 2013). Consequently, the activity of fatty acid oxidation is increased (Finck et al., 2006), but the levels of gene encoding lipogenic enzymes are decreased (Xu et al., 2013). Lipin-1 serves as an inducible coactivator of the PGC-1α-PPARα circuit to increase expression of genes involved in fatty acid β-oxidation, the TCA cycle, and the mitochondrial respiratory chain. As transcription factors, SREBPs regulate the expression of genes involved in fatty acid, TAG and cholesterol metabolism in the liver (Horton et al., 2002; Jeon and Osborne, 2012). The SREBP family consists of SREBP-1a, SREBP-1c and SREBP-2 (Xu et al., 2013). SREBP-1c is mostly responsible for the expression of genes involved in fatty acid biosynthesis, whereas SREBP-2 activates cholesterol metabolism genes (Horton et al., 2002; Xu et al., 2013). SREBP-1c is synthesized as an inactive form that is bound to the ER in a complex with SREBP cleavage activating protein. Then it is transported to the Golgi where it continues to be cleaved by sites 1 and 2 proteases to liberate the mature protein. The mature SREBP-1c
translocates to the nucleus and activates transcription factors that promote lipogenesis (Esfandiari et al., 2010; Fang et al., 2013; Xu et al., 2013). In spite of a nuclear localization signal in the HLH-Zip domain, the nuclear entry of processed SREBP-1 and SREBP-2 could be regulated by the mechanistic target of rapamycin complex 1 (mTORC1) (Peterson et al., 2011; Xu et al., 2013). Lipin-1 carrying a mutation in the mTORC1 phosphorylation site suppresses the nuclear entry of processed SREBP-1c and the expression of lipogenic target genes, indicating that lipin-1 suppresses the transcriptional function of SREBPs by inhibiting their nuclear localization (Xu et al., 2013). Recent studies demonstrate that lipin-1α appears to be predominantly nuclear as a transcriptional co-regulator to regulate the capacity of the liver for fatty acid oxidation and activity of the lipogenic enzyme, while lipin-1β resides mostly in the cytoplasm as PAP enzyme in the triglyceride synthesis pathways. In the nucleus, lipin-1α activates mitochondrial fatty acid oxidative metabolism via transcriptional activation of the gene encoding PPARα and direct cooperative interactions with PPARα and PGC-1α (Yin et al., 2012). Moreover, lipin-1α can inhibit the function of SREBP-1 in nucleus, which leads to reduce lipogenesis. Herein, we can draw a conclusion that lipin-1 plays a significant role in balancing the lipid metabolism in hepatocytes.

ETHANOL INCREASES THE EXPRESSION OF LIPIN-1 BY AMPK-SREBP-1-LIPIN-1 SIGNALING SYSTEM

AMPK is a serine/threonine protein kinase that functions as a sensor of cellular energy homeostasis (Harmel et al., 2014). In the liver, it also functions as a key mediator in the regulation of β-oxidation (Shearn et al., 2014). As a heterotrimer, it consists of one catalytic α subunit and regulatory β and γ subunits (Harmel et al., 2014). It is complex to regulate the activity of AMPK. The molecular and cellular mechanisms by which ethanol regulate AMPK are multiple and still incompletely understood. It is worth noting that ethanol can inhibit the dephosphorylation of p-AMPK, decreasing the activity of AMPK (Hu et al., 2012). Moreover, the AMPK can also regulate fatty acid oxidation, fatty acid synthesis, cholesterol synthesis and glucose synthesis through multiple pathways in the liver (Jeong et al., 2014). For example, AMPK phosphorylates SREBP-1, preventing its proteolytic processing and translocation into the nucleus, thus inhibiting transcription of lipogenic genes (Li et al., 2011). As mentioned above, the SREBPs family contains SREBP-1a, SREBP-1c and SREBP-2 (Xu et al., 2013). SREBP-1c is mostly in charge of the expression of fatty acid biosynthesis genes, whereas SREBP-2 activates cholesterol metabolism genes (Horton et al., 2002). Moreover, in nucleus, SREBP-1 can also induce the expression of LPIN1 through SRE and NF-Y bind to sequence motifs upstream of the LPIN1 gene (Hu et al., 2012).

Previous studies have confirmed that ethanol-induced overexpression of lipin-1 gene is associated with activation of SREBP-1, which is mediated through the inhibition of AMPK (You et al., 2004). In order to investigate the molecular and cellular mechanism by which ethanol causes overexpression of lipin-1, investigators set up an experiment to study how ethanol regulated expression of lipin-1. In the experiment, the immortalized mouse hepatocyte cell line (AML-12) was exposed to ethanol or other reagents (Hu et al., 2012). First of all, they demonstrated that the lipin-1 reporter activity was significantly increased in a concentration-dependent manner by incubation with ethanol in AML-12 hepatocytes (Hu et al., 2012). Moreover, LPIN1 promoter was transactivated by SREBP-1 together with NF-Y though SRE and NF-Y-binding sites (Hu et al., 2012). In order to further explore the function of AMPK-SREBP-1 signaling on the expression of lipin-1 gene, they measured the mRNA levels of lipin-1 through activating and inhibiting AMPK and SREBP respectively in AML-12 cells exposed to ethanol. As a result, pretreatment with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), an activator of AMPK, or over-expression of a constitutively active form of AMPK significantly abolished ethanol-dependent increases in LPIN1 promoter activity and mRNA levels (Hu et al., 2012). On the contrary, inhibition or silencing of AMPK with either Compound C or AMPKα siRNA slightly enhanced the effect of ethanol on LPIN1 (Hu et al., 2012). They stimulated SREBP-1 activity by over-expression of the active nuclear form of SREBP-1c in AML-12 cells. Results showed that over-expression of SREBP-1c prevented the ability of AICAR to inhibit ethanol-mediated induction the expression of LPIN1. Conversely, inhibition of SREBP-1 expression by SREBP-1 siRNA further enhanced the effect of AICAR on LPIN1 (Hu et al., 2012). In summary, inhibition of AMPK and activation of SREBP-1 were involved in ethanol-induced up-regulation of lipin-1 gene expression.

As we all know, alternative splicing of the LPIN1 transcript gives rise to two distinct lipin-1 proteins, lipin-1α and lipin-1β. It is the high time to know that which one or two are upregulated by ethanol. Therefore, we pay attention to how ethanol affects the ratio of lipin-1α to lipin-1β in hepatocytes of AFLD.

ETHANOL INCREASES THE RATIO OF LPIN1β/α BY SIRT1-SFRS10-LPIN1β/Α SIGNALING SYSTEM

Sirtuin1 (SIRT1) is a nicotinamide adenine dinucleotide dependent protein deacetylase, and it can regulate lipid metabolism by modifying histones and transcription factors in the liver (Yin et al., 2014). Accumulating evidence has demonstrated that SIRT1 regulates genes encoding lipogenic or fatty acid oxidation enzyme and that chronic ethanol exposure decreases SIRT1 gene and protein expression levels (Yin et al., 2012). Further studies reveal that over-expression of microRNA-217 (miR-217) in AML-12 cells promotes ethanol-mediated decreases in SIRT1 and SIRT1-regulated genes encoding enzymes (Yin et al., 2012). MicroRNAs are small, non-coding RNAs that inhibit gene expression by binding to the 3'-untranslated region of target mRNAs, resulting in mRNA cleavage or suppression of translation (Yin et al., 2012). For instance, miR-217, a particular miRNA, is a target of ethanol in the liver and significantly exacerbates the ethanol-mediated suppression of SIRT1 (Yin et al., 2012). SFRS10, the homolog of Drosophila transformer-2, belongs to the SR-like protein family of splicing factors (Pihlajamaki et al., 2011). The alternatively spliced exon 6 of LPIN1 has a GGAA sequence motif that binds to SFRS10 (Yin et al., 2014), which regulates the alternative splicing of LPIN1.

You et al. have set up an experiment to examine the effects of miR-217-SFRS10 signaling on lipin-1 in AML-12 cells (Yin et al., 2012). The experimental results indicated that ethanol
dramatically increased the miR-217 levels in a concentration-dependent manner in AML-12 hepatocytes. Over-expression of miR-217 in AML-12 cells not only significantly induced total lipin-1 mRNA levels by nearly 2-fold but also induced the ratio of Lipin1β to Lipin1α compared with the control. In addition, it induced the Lpin1β/α ratio through significantly inhibiting the mRNA levels of SFRS10, a known splicing factor that regulated lipin-1 mRNA alternative splicing (Yin et al., 2012). Further study indicated that hepatic SIRT1-SFRS10-Lpin1β/α axis represented a major signaling route in ethanol-induced steatosis in hepatocytes (Yin et al., 2014). In order to clarify it, investigators compared mice that had liver-specific deletion of Sirt1 (Sirt1LKO) mice with their LOX littermates (controls) (Yin et al., 2014). Firstly, they examined whether SIRT1 was associated with lipin-1 signaling or not. They measured mRNA levels of SIRT1, SFRS10, and lipin-1β and lipin-1α in two liver samples of mice, Sirt1LKO and WT controls. Results showed that removal of hepatic SIRT1 significantly induced mRNA expression levels of total lipin-1 and the ratio of Lpin1β/α compared with controls (Yin et al., 2014). Moreover, subsequent analysis revealed that both mRNA and protein expression levels of SFRS10 were severely reduced in Sirt1LKO compared with WT controls (Yin et al., 2014). Collectively, these data indicated that hepatic SIRT1 deficiency disturbed hepatic lipin-1 mRNA alternative splicing in mice (Yin et al., 2014). Then, they explored the involvement of lipin-1 signaling in ALD. This time they measured mRNA levels of SIRT1, SFRS10, and lipin-1β to lipin-1α in four liver samples of mice, Sirt1LKO, ethanol-fed Sirt1LKO, ethanol-fed WT and WT controls. As a result, hepatic SIRT1 deficiency significantly enhanced the ethanol-mediated increase in Lpin1β/α compared with that in all other groups (Yin et al., 2014). Moreover, ethanol-mediated SFRS10 inhibition was slight but largely enhanced in the liver of ethanol-fed Sirt1LKO mice compared with that in all other groups (Yin et al., 2014). Collectively, removal of hepatic SIRT1 promoted ethanol-mediated impairment of lipin-1 signaling in mice. Last, they further indicated whether ethanol-induced interrupt lipin-1 signaling was mediated through inhibition of the SIRT1-SFRS10 axis using a cellular alcoholic steatosis model (Yin et al., 2014). Results showed that unsubdued SIRT1 and SFRS10 significantly blocked the ability of ethanol to disturb the Lpin1β/α axis. By contrast, when SIRT1 and SFRS10 were inhibited, these SIRT1 or SFRS10-mediated protective effects disappeared, augmenting the TAG levels in AML-12 hepatocytes. Conclusively, all of data demonstrated that increased Lpin1β/α ratio by ethanol was mediated by inhibition of the SIRT1-SFRS10 axis using a cellular alcoholic steatosis model (Yin et al., 2014). These findings suggest that nutritional or pharmacological modulation of SIRT1-SFRS10-Lpin1β/α could be potential therapeutic approaches for treating human ALD (Yin et al., 2014). Because You et al. have demonstrated that over-expression of miR-217 promoted ethanol-mediated impairment of SIRT1 expression and SIRT1-regulated genes encoding lipogenic or fatty acid oxidation enzymes (Yin et al., 2012). It is tempting to postulate that over-expression of miR-217 may promote the hepatic SIRT1-SFRS10-Lpin1β/α axis.

Lipin-1α is a nucleocytoplasmic shuttling protein localized in both the cytoplasm and the nucleus of hepatocyte (Peterson et al., 2011). Over-expression of miR-217, ethanol treatment, or the combination of miR-217 and ethanol resulted in lipin-1α being localized to the cytoplasm and impaired nuclear function (Yin et al., 2012). However, ethanol exposure could not affect the subcellular localization of lipin-1β (Hu et al., 2013). Another study showed that it also could impair lipin-1α-inhibited SREBP-1 transcriptional activity, following increased lipogenesis. We can draw a conclusion that lipin-1 signaling impaired by ethanol through SIRT1-SFRS10-Lpin1β/α axis not only promotes triglyceride synthesis by over-expression of lipin-1β, but also inhibits the capacity of the liver for fatty acid oxidation and activity of the lipogenic enzyme by an interrupted transcriptional co-regulator, lipin-1α (Yin et al., 2012). Increased triglyceride synthesis and decreased fatty acid oxidation promote lipid accumulation in the liver and development of AFLD.

**LIPIN-1 INHIBITS VLDL-TAG SECRETION IN HEPATOCYTE**

It was demonstrated that lipin-1 promoted TAG synthesis through PAP activity in hepatocyte (Chen et al., 2008). Then TAG was transported into other tissues from hepatocytes by association with VLDL. As above, lipin-1 played an important role in regulating TAG metabolism through its activity as a PAP enzyme and its transcriptional effects on fatty acid metabolic gene programs (Chen et al., 2008). So lipin-1 might be expected to stimulate hepatic VLDL-TAG secretion (Chen et al., 2008). Alteration in hepatic expression of lipin-1 and its compartmentalization controlled VLDL assembly/secretion (Bou Khalil et al., 2009). Therefore, the precise role of lipin-1 in promoting or attenuating hepatic VLDL secretion was still controversial (Hu et al., 2013). In 2008, scientists evaluated the direct effects of lipin-1 on hepatic TAG synthesis and secretion by gain-of-function and loss-of-function approaches in fatty liver dystrophic mice (Chen et al., 2008). The result suggested that over-express of lipin-1 functioned directly to suppress VLDL-TAG secretion in the liver, which was independent of PAP activity but required the domain that mediated its interaction with nuclear receptor transcription factors (Chen et al., 2008). It should be noted that the two obligatory components of the VLDL-TAG exporting machinery, apoB and MTP, were not inhibited (Chen et al., 2008). However, the expression of stearoyl-coenzyme A desaturase, which might promote TAG synthesis and VLDL, was remarkably increased (Chen et al., 2008). In short, overexpression of lipin-1 could suppress VLDL-TAG secretion. As above, ethanol could cause over-expression of lipin-1 in ALD. Therefore over-expression of lipin-1 by ethanol should also suppress VLDL-TAG secretion in the liver. Similarly, Yu et al. verified that ethanol administration remarkably decreased the rates of VLDL-TAG secretion in the livers of WT mice compared with WT controls (Hu et al., 2013). Moreover, VLDL-TAG secretion rates were remarkably increased in lipin-1-deficient (lipin-1LKO) mice fed a control diet compared with WT controls (Hu et al., 2013). Taking all these into consideration, we can draw a conclusion that lipin-1 regulated by ethanol exacerbates lipid accumulation in liver, promoting the development of AFLD.

**THE FUNCTION OF LIPIN-1 IN DEVELOPMENT AND PROGRESSION OF ALCOHOL-INDUCED STEATOHEPATITIS**

Lipin-1 regulates nuclear factor of activated NFATc4, an important inflammatory factor (Yin et al., 2014). Recent studies
have demonstrated that endogenous lipin-1 has potent anti-inflammatory property (Hu et al., 2013). Kim et al. demonstrated that lipin-1 repressed the transcriptional activity of NFATc4 through forming a complex with NFATc4 and DNA in vitro, which did not require lipin-1 lipid phosphatase activity (Kim et al., 2010). Subsequently, You et al. used a liver-specific lipin-ILKO mouse model to investigate the functional role of lipin-1 in the development of alcoholic steatohepatitis (Hu et al., 2013). Results clearly showed that deletion of lipin-1 led to activation of both NF-κB and NFATc4 and exacerbated inflammation in ethanol-fed lipin-ILKO mice (Hu et al., 2013). Then, it was easy for us to consider that over-expression of lipin-1 might induce the activity of inflammatory factors in AFLD. However, in liver-specific deletion of Sirt1LKO mice, although the level of lipin-1 was nearly 2.5-fold higher than controls, the activity of inflammation factor was promoted (Yin et al., 2014). Moreover, ethanol administration to Sirt1LKO mice significantly decreased the amount of NFATc4 in the cytoplasm and increased nuclear accumulation of NFATc4 (Yin et al., 2014). Ethanol-fed Sirt1LKO mice might be mediated by impairment of the hepatic SIRT1-lipin-1-NFATc4 axis (Yin et al., 2014). Because the ratio of lipin-1β to lipin-1α was disturbed and the anti-inflammatory property acted in nucleus (Kim et al., 2010). I boldly inferred that it might be the lipin-1α played a role in anti-inflammation.

THE FUNCTION OF LIPIN-1 IN PROGRESSING FROM FATTY LIVER TO MILD FIBROTIC LIVER

You et al. investigated the role of SIRT1 on AFLD by pair-feeding WT and Sirt1LKO mice using a chronic-binge ethanol feeding protocol (Yin et al., 2014). Result indicated that the mRNA levels of early markers of hepatic fibrosis, such as collagen I, tissue inhibitor of metalloproteinase 1, or transforming growth factor β1, were increased in ethanol-fed Sirt1LKO mice compared with others (Yin et al., 2014). A smooth muscle actin and collagen revealed higher levels of those indicators of liver fibrosis in the livers of ethanol-fed Sirt1LKO mice compared with others (Yin et al., 2014). The mRNA levels of fibronectin were significantly increased in ethanol-fed WT and in Sirt1LKO mice with or without receiving ethanol when compared with controls. All results suggested that ethanol-fed Sirt1LKO mice partially progressed from fatty liver to mild fibrotic liver. In ethanol-fed mice, the expression of SIRT1 is inhibited, so the development of alcoholic fatty liver might also happen as well. Only the precise function of lipin-1 in promoting fatty liver to mild fibrotic liver is not entirely clear.

CONCLUSION

Lipin-1 has dual functions in lipid metabolism. In the cytoplasm, lipin-1β functions as a Mg2+-dependent PAP enzyme in triglyceride synthesis pathways. In the nucleus, lipin-1α acts as a transcriptional co-regulator to regulate the capacity of the liver for fatty acid oxidation and activity of the lipogenic enzyme. In hepatocytes of AFLD, ethanol increases the expression of lipin-1 through the AMPK-SREBP-1 signaling and the ratio of lipin-1β to lipin-1α through SIRT1-SFRS10- Lpin1β/α axis in liver. In cytoplasm, lipin-1β as PAP enzyme converts PA to DAG. In nucleus, lipin-1α directly interacts with PCG-1α and PPARα to induce fatty acid oxidation. Lipin-1α could also inhibit the activity of SREBP-1, decreasing the synthesis of fatty acid. Over-expression of lipin-1 could suppress VLDL-TAG secretion. Endogenous lipin-1 has potent anti-inflammatory property. AMPK, adenosine monophosphate activated kinase; miR-217, microRNA-217; SIRT1, sirtuin1; SREBP-1, sterol regulatory element binding protein 1; PAP, phosphatidic acid phosphohydrolase; SFRS10, splicing factor, arginine/serine-rich 10; VLDL-TAG, very low-density lipoprotein-triacylglyceride; PPARα, peroxisome proliferator-activated receptor α; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α.

Furthermore, over-expression of lipin-1 could remarkably suppress VLDL-TAG secretion. In the end, endogenous lipin-1 has potent anti-inflammatory property. Increased synthesis of TAG, decreased fatty acid oxidation, impaired VLDL-TAG secretion and activated inflammatory factors act together to exacerbate the development of AFLD (Fig. 1). So it is the high time for us to investigate the precise function of lipin-1 in development of AFLD.

We should also focus our attention on the relationship of between lipin-1 and alcoholic liver cirrhosis or hepatocellular carcinoma. Moreover, most studies of lipin-1 are based on an animal level so far, so perhaps they should be elevated to clinical levels. Furthermore, current researches have not found any activators and inhibitors of lipin-1, being beneficial for treatment of ALD clinically.

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