Lipin Family Proteins – Key Regulators in Lipid Metabolism

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Key Words

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Abstract

Background: Proteins in the lipin family play a key role in lipid synthesis due to their phosphatidate phosphatase activity, and they also act as transcriptional coactivators to regulate the expression of genes involved in lipid metabolism. The lipin family includes three members, lipin1, lipin2, and lipin3, which exhibit tissue-specific expression, indicating that they may have distinct roles in mediating disease. To date, most studies have focused on lipin1, whereas the roles of lipin2 and lipin3 are less understood. **Summary:** This review introduces the structural characteristics, physiological functions, relationship to lipid metabolism, and patterns of expression of the lipin family proteins, highlighting their roles in lipid metabolic homeostasis. © 2014 S. Karger AG, Basel

and have been attributed to alterations in the expression or function of distinct sets of genes [1] associated with inappropriate lipid accumulation in tissues. Both lipodystrophy and obesity contribute to insulin resistance, hypertension, dysglycemia, and premature atherosclerosis [2–4]. Therefore, it is of interest to identify the factors that influence adipocyte differentiation and lipid biosynthesis and storage and to determine how genetic variation in the activity of these factors contributes to metabolic dysfunction.

This review focuses on the lipin family of proteins, which function as phosphatidate phosphatase enzymes required for lipid synthesis and as transcriptional coactivators that affect fatty acid oxidation. Because of the key roles that lipin proteins play in lipid homeostasis, it is important to better define their molecular and physiological functions. The elucidation of lipin proteins, particularly lipin1, may lead to novel approaches to improve lipid storage and metabolic dysfunction.

Introduction

The control of lipid metabolism is critical to maintaining systemic energy homeostasis. Obesity and conditions involving adipose-deficiency, such as lipodystrophy, are two opposite extremes of lipid metabolic dysfunction

The Lipin Proteins Family

In 1989, Langner et al. [5] first described a mutation in mice that caused a form of fatty liver dystrophy (fld). This mutation is associated with tissue-specific deficiency in the neonatal expression of lipoprotein lipase (LPL) and hepatic lipase (HL). The mutation was presumed to affect

a regulatory gene that plays an important role in lipid metabolism. In 2001, Peterfy et al. [6] identified the mutant gene in the fld mouse by positional cloning and named it lipin1. The fld mouse is characterized by lipodystrophy, hypertriglyceridemia, insulin resistance, neonatal fatty liver, and peripheral neuropathy due to a null mutation in the lipin1 gene. Unlike the fld mouse, lipin1 mutations in humans, including Arg800X, c.297 + 2T>C, and c.1259 + 2T>C, and a ~2 kb genomic deletion that includes exons 18 and 19, cause recurrent muscle pain, weakness, recurrent rhabdomyolysis [7], and myoglobinuria in childhood, but they do not cause lipodystrophy [8]. However, one study showed that HIV patients with lipodystrophy exhibit dramatically lower expression of total and individual lipin isoforms compared with patients without lipodystrophy, consistent with the data collected from fld mice [9]. The different effects of lipin1 deficiency in humans and mice remain to be explored.

In addition, Peterfy et al. [10] found that alternative mRNA splicing gives rise to two lipin isoforms, designated as lipin1 α and lipin1 β , which differ in their temporal expression patterns and subcellular localization, but have complementary functions. Lipin1 α is primarily formed in pre-adipocytes and functions during the early stages of adipocyte differentiation, whereas lipin1 β predominates in mature adipocytes and induces the expression of lipogenic genes and lipid storage [9, 10]. Similarly, the human lipin1 has three isoforms (lipin1 α , lipin1 β , lipin1 γ) derived from alternative mRNA splicing [11]. Recent studies have demonstrated that lipin1 γ is a novel lipin isoform that is localized to lipid droplets and alters lipid droplet morphology without affecting the triacylglycerol level. Lipin1 γ is highly expressed in normal human brain; conversely, the expression of lipin1 α and lipin1 β in the brain is very low, indicating that lipin1 γ may be a specialized regulatory protein in brain lipid metabolism [12].

In addition to the lipin1 gene, two mutant alleles (lipin2 and lipin3) have been identified with 49 and 46% amino acid sequence similarity compared to lipin1 [13]. These three lipin genes present different tissue expression patterns, indicating that they may have unique physiological roles [14]. The lipin1 gene is highly expressed in adipose tissue, skeletal muscle, and testis, but it is observed at low levels in many other tissues, including the liver, kidney, brain, heart, and lungs. Lipin2 is expressed predominantly in the liver, and it is also expressed in the kidney, brain, and lungs. It is interesting that the hepatic levels of lipin2 are significantly increased in fld mice, suggesting that lipin2 may be able to partially compensate for the absence of lipin1 [15]. Lipin3 is

expressed at a low level in most tissues; however, it is highly expressed in the liver in pigs [16]. Several studies have reported that lipin3 and lipin1 cooperate in vivo to influence adipose tissue phosphatidate phosphatase (PAP) activity and adiposity [17]. The lipin genes are conserved from yeast to mammals. Yeast and most invertebrates have only a single lipin ortholog, while two orthologs have been detected in plants and fish, and three are present in most vertebrates.

Functions of Lipin Proteins

All lipin family proteins possess two evolutionarily conserved regions known as the N-terminal (N-LIP) and C-terminal (C-LIP) domains. In addition, a lysine- and arginine-rich nuclear localization signal (NLS) is present in the lipin proteins of most species, indicating that lipin can translocate into the nucleus and that this event may be affected by protein phosphorylation [18]. The C-LIP domain contains two key protein functional motifs: a haloacid dehalogenase (HAD)-like phosphatase motif (DXDXT), which is required for phosphatidate phosphatase enzyme activity [19]; and a nuclear receptor interaction motif (LXXIL), which has a transcriptional coactivator function (fig. 1) [20]. The function of the N-LIP domain is unclear; however, some studies have provided evidence that the N-LIP domain of lipin1 is important for its catalytic activity, nuclear localization, and binding to protein phosphatase-1 γ (PP-1 γ) [21].

Lipin Proteins as PAP Enzymes Required for Lipid Synthesis

Triacylglycerol (TAG) plays a key role in the maintenance of energy homeostasis, as the major energy storage molecule, and TAG accumulation in adipose tissue allows animals to survive under conditions of food deprivation. TAG is primarily synthesized via the sequential acylation of glycerol phosphate in the Kennedy pathway [22]. Phosphatidic acid (PA), which is a common precursor of glyceride synthesis, is dephosphorylated by phosphatidate phosphatase (PAP) enzymes catalysis to form diacylglycerol (DAG), which is a key substrate for the synthesis of TAG, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). The lipin proteins usually reside in the cytosol and translocate to the endoplasmic reticulum (ER) membrane in response to elevated fatty acid levels, where they bind to PA and catalyze its conversion to DAG (fig. 2) [23]. In addition, the lipin proteins present Mg²⁺-dependent activity specific for

Fig. 1. Domain structure and functional motifs of lipin. All members of the lipin proteins family contain two evolutionarily conserved regions known as the N-terminal (N-LIP) and C-terminal (C-LIP) domains. The C-LIP domain contains two key protein functional motifs: the DXDXT motif is the motif required for PAP enzyme activity, the LXXIL motif is the transcriptional coactivator motif required for lipin1 to interact with nuclear receptors and function as transcriptional regulator. The lipin1 β specific exon is derived from alternative splicing of the lipin1 gene. The NLS is a lysine and arginine-rich nuclear localization signal.

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Fig. 2. The dual functions of lipin as a PAP enzyme required for lipid synthesis and as a transcriptional coactivator that promotes fatty acid oxidation. Lipin proteins translocate from the cytosol to the ER and catalyze the conversion of PA to DAG, which is a key substrate for the synthesis of TAG, PE, and PC. In the nucleus, lipin1 also acts as a transcriptional coactivator, interacting with PGC-1 α , PPAR α , and other factors such as histone acetyltransferase (HAT) to stimulate the expression of genes involved in fatty acid oxidation.

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PA, but they have no activity against other phospholipids including lysoPA, ceramide-1-phosphate, or sphingosine-1-phosphate.

Lipin in Yeast

In 2006, Carman purified the PAP1 enzyme from *Saccharomyces cerevisiae*, obtained its amino acid sequence, and identified it as Pah1p, a yeast ortholog of lipin [24]. Sequence analysis demonstrated that the PAP1 activity is associated with the DXDXT motif in the C-LIP domain of Pah1p, which is conserved from yeast to mammals. Studies of yeast Pah1p mutants have shown a significant increase in PA accumulation, accompanied by a decrease in TAG synthesis [25]. The activity of Pah1p is regulated by membrane association with the amino-terminal amphipathic alpha helix. Phosphorylation of Pah1p prevents

the amino-terminal amphipathic alpha helix from binding to membranes [26]. Phosphorylation-deficient Pah1p mutant exhibits elevated PAP1 activity, suggesting that phosphorylation inhibits the PAP activity of this enzyme [27].

Studies in yeast have shown that lipins play an important role in nuclear organization. Smp2, the yeast homologue of mammalian lipin, regulates nuclear membrane growth by influencing lipid biosynthesis during the cell cycle. Deletion of Smp2 or its dephosphorylated form causes transcriptional upregulation of genes involved in phospholipid biosynthesis concurrent with a massive expansion of the nuclear envelope. Conversely, constitutive dephosphorylation of Smp2 represses de novo phospholipid synthesis [28] and inhibits cell division [29], although the underlying mech-

anisms remain to be determined. In addition, RNAi-mediated down regulation of lipin1 in *C. elegans* causes nuclear envelope breakdown and alterations in the organization of the endoplasmic reticulum membrane, resulting in the appearance of large membrane sheets [30, 31]. These studies suggest that lipins may not only regulate lipid metabolism, but also be involved in membrane dynamics.

Lipin in Mammals

There are several key differences in lipin proteins biology between yeast and mammals. For instance, lipin phosphorylation alters the intrinsic PAP activity in yeast but not in mammals [27]. Furthermore, many of the key phosphorylation sites that regulate lipin activity in yeast are not found in the mammalian lipins [32]. As mentioned above, all lipin proteins contain a C-LIP domain; therefore, all members of the lipin family exhibit PAP1 activity. However, the PAP1 activity of lipin1 β is higher than that of lipin1 α ; lipin1 γ has lower activity but a greater affinity for the PA; the activity of lipin-2 is similar to that of lipin1 α ; and lipin-3 has a lower relative activity [11, 14]. Studies performed on lipin1-deficient fld mice have determined that lipin1 accounts for all of the PAP1 activities in white and brown adipose tissue and skeletal muscle [14]. The fld mice exhibits impaired adipose tissue development; in contrast, lipin1 overexpression in adipocytes leads to increased TAG accumulation [1]. Interestingly, the PAP1 activity in the liver tissue of fld mice is normal [14] or downregulated by half of the measured PAP1 activity compared with wild-type mice [18]. Lipin2 is expressed at similar levels in wild-type and fld mice, but the expression of lipin3 is increased by 4-fold in fld mice compared with wild-type mice [14]. In addition, knocking down the lipin2 gene significantly decreases hepatic PAP1 activity [15], suggesting that lipin2 and lipin3 expression may account for PAP1 activity in the livers of lipin1-deficient mice.

As described previously, lipin1 mutations in humans do not cause lipodystrophy, as was observed in fld mice [8, 33]. However, studies have shown that lipin1 deficiency could cause severe cardiac muscle damage and rhabdomyolysis in early childhood, possibly because of the accumulation of PA and lysophospholipids in the muscle tissue, which was revealed in an analysis of muscle lipids [8, 34]. In skeletal muscle, lipin1 accounts for almost all of the PAP activity, and lipin2 and lipin3 are expressed at much lower levels [14]. Furthermore, muscle lipin1 levels affect energy metabolism and mitochondrial enzymes [35].

Lipin1 Functions as a Transcriptional Coactivator of Lipid Metabolism Genes

As described above, lipin1 serves as a transcriptional coactivator and requires an LXXIL motif located within the C-LIP domain. This domain enables lipin1 to interact with nuclear receptors and function as a transcriptional regulator. In 2006, Finck et al. first identified lipin1 transcriptional coactivator activity in studies of peroxisome proliferator-activated receptor (PPAR γ) coactivator-1 α (PGC-1 α) knockout mice. These authors demonstrated that the expression of lipin1 is induced by PGC-1 α , and lipin1 forms a physical complex with PGC-1 α and PPAR α to control the expression of genes involved in fatty acid oxidation and mitochondrial metabolism (fig. 2) [36]. Studies have suggested that depletion of nuclear lipin1 by ethanol is likely to lead to impairment of the PPAR α /PGC-1 α axis, thus contributing to the decrease of fatty acid oxidation and the development of alcoholic liver steatosis [37, 38]. In addition to PGC-1 α and PPAR α , lipin1 can also interact with other nuclear receptors, such as PPAR γ [39], hepatocyte nuclear factor-4 α (HNF-4 α) [40], and the glucocorticoid receptor (GR) [41], as well as non-nuclear receptor transcription factors, including nuclear factor of activated T-cells c4 (NFATc4) [42] and myocyte enhancer factor 2 (MEF2) [43]. Lipin1 not only co-activates these transcription factor complexes, but it also functions as a transcriptional repressor in some cases. For example, lipin1 inhibits the activity of NFATc4 by participating in the recruitment of histone deacetylases to target promoters [42]. Therefore, lipin1 can serve as a molecular scaffold to activate or repress gene expression depending on the cellular context. Both lipin2 and lipin3 contain the LXXIL motif, and some studies have indicated that lipin-2 can act as a transcriptional co-activator for PPAR γ , similar to lipin1 in hepatocytes [19]. Lipin3 also interacts with PPAR α [36]. It remains to be determined whether members of the lipin family function as transcriptional coactivators in tissues other than the liver.

Lipin Proteins and Lipid Metabolism in Different Tissues

Initial studies by Langner reported that the lipin1-deficient fld mice exhibit fatty liver and hypertriglyceridemia during the neonatal period [5], suggesting that the PAP activity of lipin1 may be not necessary for hepatic TAG synthesis. However, studies have also suggested that

the loss of coactivator activity of lipin1 and dysregulation of genes related to hepatic fatty acid oxidation contribute to the neonatal fatty liver in fld mice [20]. Lipin1 is expressed at low levels in the livers of adult mice, and it contributes little to the total hepatic PAP activity. Lipin2 is expressed at high levels in the liver, and livers of lipin1-deficient mice exhibit normal PAP activity [14], indicating that lipin2 may account for most of the PAP activity in the liver and affect hepatic TAG synthesis.

The rates of TAG synthesis are not impaired in hepatocytes isolated from adult lipin1-deficient fld mice and are actually increased in neonatal fld mice. In addition, the hepatic very low density lipoprotein (VLDL) secretion rates were markedly increased in fld mice of both neonates and adults. Lipin1 overexpression in the mouse did not alter TAG synthesis rates, but it significantly suppressed VLDL secretion [44]. Conversely, studies in a rat hepatocyte cell line have demonstrated that enhanced expression of lipin1 stimulates VLDL secretion, and knock-down of endogenous lipin1 decreases VLDL secretion [45]. These results suggest that lipin1 is closely associated with the synthesis and secretion of VLDL, but the specific mechanism remains to be determined.

In adipose tissue, lipin1 accounts for virtually all of the PAP activity. The loss of lipin1 PAP activity in fld mice contributes to the failure of adipose tissue to store lipids and causes lipodystrophy [5]. In contrast, enhanced lipin1 expression targeted to adipose tissue in transgenic mice results in obesity [1]. Studies have shown that lipin1 expression was induced during the late stage of differentiation of 3T3-L1 preadipocytes, and it was maintained at high levels in mature adipocytes. Knockdown of expression of lipin1 in 3T3-L1 preadipocytes almost completely inhibited their differentiation into adipocytes, whereas lipin1 overexpression accelerated adipocyte differentiation [46]. Lipin1 expression in differentiating preadipocytes is required for adipogenic transcription factors, such as PPAR γ and CCAAT/enhancer binding protein α (C/EBP α), which are induced during lipogenesis and lipid accumulation [47]. These findings suggest that lipin1 plays a critical role in adipocyte differentiation. In addition, lipin2 may also have an important effect on adipocyte differentiation. Lipin2 and lipin1 exhibit reciprocal expression in differentiating 3T3-L1 adipocytes. Lipin2 levels increase in lipin1-depleted 3T3-L1 cells without rescuing the adipogenic defects, whereas depletion of lipin2 results in increased lipin-1 levels and more rapid and robust induction of the adipocyte fatty acid binding protein (aP2), a marker of mature adipocytes [32]. These results suggest that lipin1 and lipin2

have distinct and non-redundant roles in adipocyte differentiation.

In skeletal muscle, the lipin1 expression levels are similar to those in the adipose tissue. Transgenic mice expressing the muscle-specific lipin-1 gained more weight than adipose-specific transgenic mice. The muscle-specific lipin-1 transgenic mice became obese on a chow diet, and this weight gain was accelerated further on a high-fat diet [1]. The obesity is associated with reduced energy expenditure and decreased fatty acid oxidation in the muscle. The fld mice exhibit the opposite phenotype, with increased energy expenditure and fatty acid oxidation in muscle. Restoration of lipin1 expression in the skeletal muscle of fld mice normalizes energy expenditure, but does not restore adipose tissue function [1]. These results suggest that the lipin levels in the skeletal muscle have a key role in energy metabolism. Studies also have shown that lipin1 could activate mitochondrial fatty acid oxidative metabolism via interactions with PPAR δ in skeletal muscle [35]. Moreover, enhanced lipin expression in adipose tissue and skeletal muscle has distinct effects on glucose homeostasis and insulin sensitivity. Adipose-specific lipin1 transgenic mice exhibit greater insulin sensitivity, whereas muscle-specific lipin-1 transgenic mice develop insulin resistance [1]. This result may be due to increased TAG accumulation and altered metabolism in the muscle.

Regulation of Lipin Proteins Activity and Expression

The lipin proteins activity is regulated at several levels, including mRNA transcription, protein phosphorylation, and subcellular localization (fig. 3). As mentioned above, lipin1 is primarily cytosolic and translocates to the ER membrane in response to fatty acids. In 2002, Huffman first demonstrated that lipin1 phosphorylation induced by insulin or amino acid treatment caused the emergence of multiple lipin1 electrophoretic variants in adipocytes [48]. Lipin1 phosphorylation by insulin is dependent on phosphatidylinositol-3-kinase activity and the mammalian target of rapamycin (mTOR) signaling pathways. On the other hand, treatment with the phosphatase inhibitor okadaic acid or epinephrine displaces PAP1 from the ER membrane, suggesting that PAP1 phosphorylation influences PAP1 activity by modulating its subcellular localization [18, 49]. In the liver, PAP1 activity is increased during starvation [50], diabetes [51], hypoxia, and alcoholic fatty liver [52], as well as in response to elevated glucocorticoids. However, in the adipose tissue, PAP1 ac-

Fig. 3. Regulation of lipin1 expression. Glucocorticoids induce lipin1 expression through the glucocorticoid receptor (GR). The depletion of intracellular sterols modulates the expression of lipin1 through the sterol regulatory response element binding protein 1 (SREBP-1) and nuclear factor Y (NF-Y). PGC-1 α induces lipin1 expression through hepatocyte nuclear factor-4 α (HNF-4 α). Lipin1 expression is also positively correlated with insulin sensitivity. The expression of lipin1 is observably suppressed by estrogen in the uterus and liver. LPS and zymosan inhibit lipin1 expression in mouse adipose tissue through the activation of the Toll-like receptors TLR4 and TLR2, respectively. ER stress and pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interferon- γ (IFN- γ) down-regulate lipin1 expression.

tivity is decreased by starvation and diabetes, resulting in decreased TAG synthesis and increased lipolysis [53]. Therefore, the mechanisms that regulate PAP1 activity in specific tissues may be different.

The expression of the lipin gene is regulated by several stimuli. Lipin1 expression, rather than lipin2 or lipin3, is activated by synthetic glucocorticoids during adipocyte differentiation. The stimulatory effect is enhanced by glucagon or cyclic adenosine monophosphate (cAMP), and it is antagonized by insulin [41]. A functional glucocorticoid response element in the lipin1 upstream sequence, which was shown to bind to the GR, allow for activation by synthetic glucocorticoid and dexamethasone [54]. Lipin1 expression is also positively correlated with insulin sensitivity, because lipin1 expression in the adipose tissue is induced by insulin-sensitizing compounds such as thiazolidinediones and harmine [55–57]. Several studies have demonstrated that intracellular sterols modulate the expression of human lipin1 via the sterol regulatory response element binding protein 1 (SREBP-1) and nu-

clear factor Y (NF-Y), and the expression of lipin1 induced by sterol depletion controls TG accumulation through its PAP1 activity in the cytosol [58]. Furthermore, ethanol increases hepatic lipin1 expression, largely through the activation of SREBP-1 and NF-Y [59]. Moreover, PGC-1 α induces lipin1 expression through HNF-4 α -dependent mechanisms in HepG2 cells [59].

Other negative regulators repress lipin1 expression. Studies have shown that lipin1 expression is inhibited by treatment with ER stress inducers (tunicamycin and thapsigargin) in 3T3-L1 adipocytes, while activation of PPAR- γ restores the ER stress-induced lipin1 suppression, suggesting the involvement of PPAR- γ in the inhibitory effect of ER stress on lipin1 expression [60]. In addition, ER stress induces lipin2 expression in the liver, but has a limited effect on lipin2 expression in adipocytes, indicating that the role of ER stress in the expression of lipin family genes is tissue-dependent [60, 61]. The expression of lipin1 in the uterus and liver is remarkably suppressed by estrogen, demonstrating that there is

a potential role for lipin1 in uterine function and reproductive biology [62]. Additionally, lipopolysaccharide (LPS) and zymosan inhibit lipin1 expression in mouse adipose tissue by activating the Toll-like receptors TLR4 and TLR2, respectively. Lipin1 expression is also down-regulated in response to pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interferon- γ (IFN- γ) in 3T3-L1 adipocytes [63]. Furthermore, lipin1 suppression by TNF- α is reversed by inhibition of Janus kinase 2 (Jak2) signaling [64].

The activity and expression of lipin2 have been less well studied compared to that of lipin1. As previously described, lipin2 also exhibits PAP activity, although it is lower than that of lipin1 and it is increased in fld mice through a possible compensatory mechanism [14]. Like lipin1, the hepatic expression of lipin2 is up-regulated during fasting [14]. However, unlike lipin1, lipin2 expression is not regulated by PGC-1 α , cAMP signaling, or glucocorticoids [18, 32]. Lipin2 and lipin1 exhibit reciprocal patterns of protein expression in differentiating 3T3-L1 adipocytes [32]. Lipin3 is the least studied of the lipin family members, and its activity and expression have not yet been determined.

Conclusions

The lipin family proteins are key regulators of lipid metabolism. Lipin1 has dual seemingly paradoxical effects on lipid metabolism. On the one hand, lipin1 acts as a PAP enzyme and is required for lipid synthesis; on the other hand, it serves as a transcriptional coactivator to promote fatty acid oxidation. The dual functions of lipin1 may allow it to maintain lipid metabolic homeostasis. However, many issues remain to be addressed. For instance, what are the specific physiological roles of lipin2 and lipin3 and by what mechanism is their activity and expression regulated? Why are different lipin family members expressed in different tissues, and is there any interrelationship among them? Do lipin proteins have other unknown functions in addition to their role in the regulation of lipid metabolism? These questions remain to be answered in the future studies.

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