Adipocyte 1:3, 123–131; July/August/September 2012; © 2012 Landes Bioscience

Distinct roles for α-β hydrolase domain 5 (ABHD5/CGI-58) and adipose triglyceride lipase (ATGL/PNPLA2) in lipid metabolism and signaling

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Keywords: obesity, insulin resistance, triacylglycerol, lipolysis, lipase, phosphatidic acid, acyltransferase

Catabolism of stored triacylglycerol (TAG) from cytoplasmic lipid droplets is critical for providing energy substrates, membrane building blocks and signaling lipids in most cells of the body. However, the lipolytic machinery dictating TAG hydrolysis varies greatly among different cell types. Within the adipocyte, TAG hydrolysis is dynamically regulated by hormones to ensure appropriate metabolic adaptation to nutritional and physiologic cues. In other cell types such as hepatocytes, myocytes and macrophages, mobilization of stored TAG is regulated quite differently. Within the last decade, mutations in two key genes involved in TAG hydrolysis, α-β hydrolase domain 5 (ABHD5/CGI-58) and adipose triglyceride lipase (ATGL/PNPLA2), were found to cause two distinct neutral lipid storage diseases (NLSD) in humans. These genetic links, along with supporting evidence in mouse models, have prompted a number of studies surrounding the biochemical function(s) of these proteins. Although both CGI-58 and ATGL have been clearly implicated in TAG hydrolysis in multiple tissues and have even been shown to physically interact with each other, recent evidence suggests that they may also have distinct roles. The purpose of this review is to summarize the most recent insights into how CGI-58 and ATGL regulate lipid metabolism and signaling.

Introduction

We recently reported that CGI-58 generates signaling lipids that regulate inflammation and insulin action.[1] This review expands on the concepts presented in our initial report[1] and provides additional discussion of the mechanisms by which CGI-58 and the TAG hydrolase ATGL regulate lipid metabolism and signal transduction. We have shown that antisense oligonucleotide (ASO)-mediated knockdown of CGI-58 in mice results in severe hepatic steatosis, yet paradoxically improves hepatic insulin signaling.[1,2] CGI-58 knockdown: (1) prevents HFD-induced activation of hepatic stress kinases, despite elevated plasma T_H1 cytokines; (2) prevents the generation of phosphatidic acid (PA) species and other glycerophospholipids in the liver in response to TNFα, thereby attenuating downstream inflammatory signaling; and (3) alters the systemic inflammatory response to endotoxin.[1] Inhibition of inflammatory pathways may explain the dissociation of hepatic steatosis and insulin resistance in CGI-58 ASO-treated mice.[1,2] Importantly, lipidomic analysis shows several key changes in PA and other glycerophospholipid species with signaling potential in the liver.[1] Collectively, we propose that CGI-58 generates signaling lipids that can activate inflammatory pathways and downregulate hepatic insulin signaling (**Fig. 1B**).[1] ATGL/PNPLA2, also known as desnutrin[3] or inducible phospholipase A2ζ,[4] is a member of the patatin-like phospholipase domain containing (PNPLA) family of proteins, which have diverse lipid esterase and transacylase activities.[5] In addition to selective TAG hydrolase activity, ATGL has also been reported to possess phospholipase activity and acyl-CoA-independent acylglycerol transacylase activity, but the physiological functions of these activities are not currently known.[4] CGI-58/ABHD5 is a member of the α-β hydrolase domain (ABHD) family of proteins which share a highly conserved GXSXG active site motif. In CGI-58, the catalytic serine nucleophile is replaced by an asparagine,[6] and CGI-58 does not possess hydrolase activity to directly mobilize TAG. Rather, it is widely accepted that CGI-58 co-activates the TAG hydrolase activity of ATGL through an incompletely understood mechanism.[7-9] Lass and colleagues[7] first demonstrated that recombinant CGI-58, or extracts from CGI-58-overexpressing cells, increases the TAG hydrolase activity of both human and mouse ATGL in vitro. Purified CGI-58 increases TAG hydrolase activity in adipose tissue extracts from wild-type mice, but not in extracts from ATGL knockout ($ATGL^{-/-}$) mice.[8] This suggests that CGI-58 specifically co-activates ATGL-mediated lipolysis and that endogenous levels of CGI-58 in adipose tissue are limiting for maximal ATGL lipase activity.[8] In addition, CGI-58 possesses intrinsic coA-dependent lysophosphatidic acid acyltransferase (LPAAT) activity in vitro, generating PA.[10,11] Since either ATGL-mediated TAG hydrolysis or LPAAT activity could generate signaling lipids, the relative physiological importance of these functions becomes central to understanding the striking effects of CGI-58 knockdown on inflammation and insulin signaling.[1] Although CGI-58 co-activation of ATGL-mediated lipolysis has been well-established in vitro,[7-9] whether this is the primary physiological function

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Submitted: 02/21/12; Revised: 03/14/12; Accepted: 03/14/12
http://dx.doi.org/10.4161/adip.20035

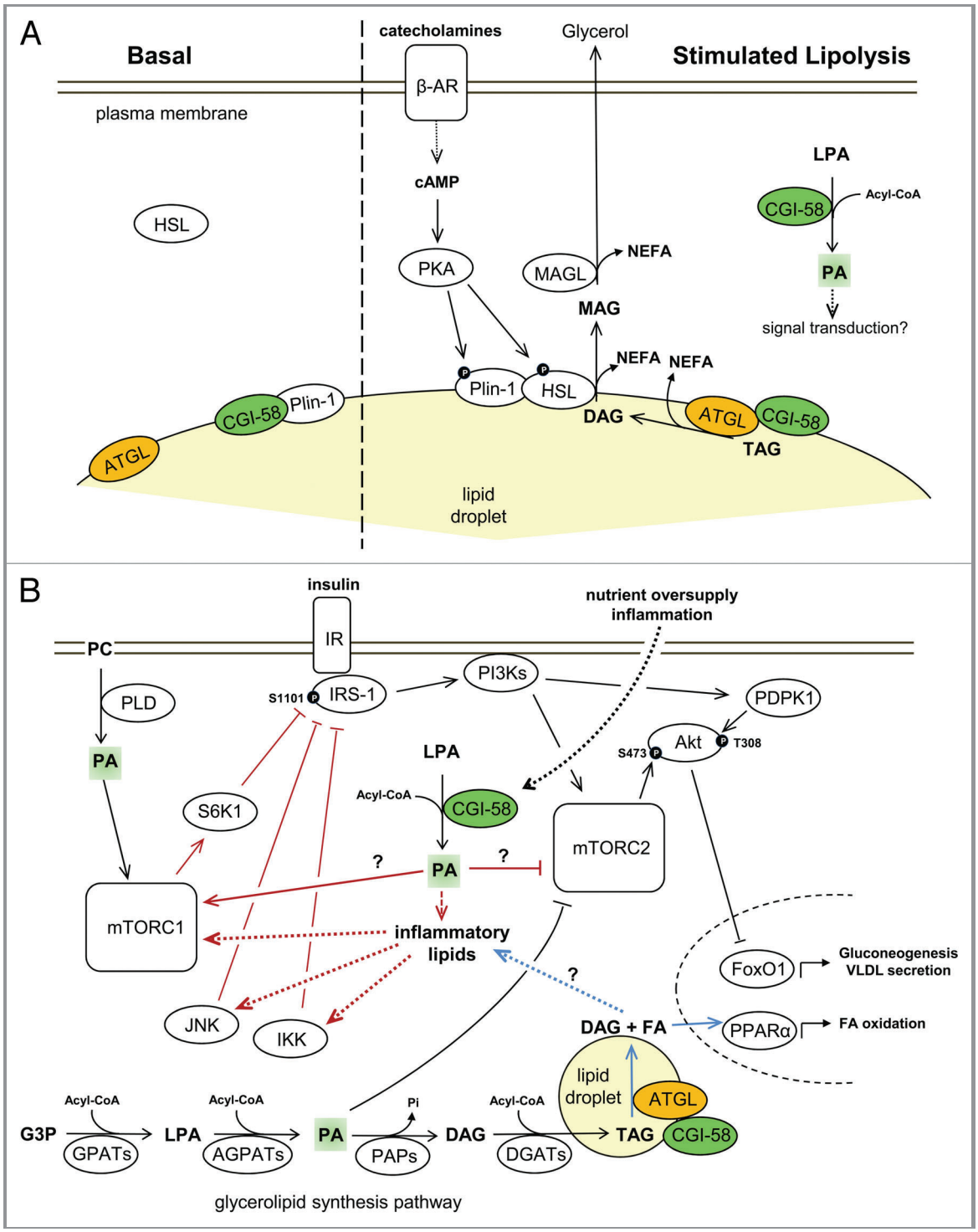

Figure 1. For figure legend, see page 125.

of CGI-58 in vivo remains to be verified. Despite many similarities between CGI-58 and ATGL deficiency, several key differences seem to indicate additional, ATGL-independent functions of CGI-58 (**Table 1**). Here, we summarize recent insights into the function(s) of CGI-58 and ATGL in vivo, based on studies in humans and mice.

CGI-58 and ATGL Mutations Cause Neutral Lipid Storage Disease in Humans

Neutral lipid storage disease (NLSD) is a rare autosomal recessive, nonlysosomal disorder characterized by ectopic TAG deposition in multiple tissues, including the hallmark accumulation of lipid

Figure 1 (See opposite page). CGI-58 and ATGL functions in adipocytes (A) and hepatocytes (B). (A) Within the adipocyte, triacylglycerol (TAG) hydrolysis is dynamically regulated by hormones to ensure appropriate metabolic adaptation to nutritional and physiologic cues. In the basal state, CGI-58 is primarily sequestered by perilipin-1(Plin-1) at the lipid droplet, ATGL is localized at the lipid droplet, and hormone sensitive lipase (HSL) resides in the cytoplasm. Downstream of catecholamine stimulation through β-adrenergic receptors (βAR), cAMP-activated protein kinase A (PKA) phosphorylates Plin-1 and HSL. Plin-1 phosphorylation results in the release of CGI-58. CGI-58 reversibly disperses to the cytoplasm, while a small amount remains at the lipid droplet, interacting with ATGL to co-activate TAG hydrolysis. Meanwhile, phosphorylated HSL translocates to the lipid droplet and interacts closely with Plin-1, preferentially hydrolyzing diacylglycerol (DAG) to monoacylglycerol (MAG). In the final step of lipolysis, MAG is hydrolyzed by MAG lipase (MAGL), leading to glycerol release from the adipocyte. Based on the localization of CGI-58 in the cytoplasm during stimulated lipolysis, CGI-58 may have a physiological function distinct from the co-activation of ATGL-mediated lipolysis. The role of CGI-58 LPA acyltransferase (LPAAT) activity in adipocytes is not known, but CGI-58-derived PA and downstream products might participate in signal transduction. (B) Within the hepatocyte, CGI-58 generates signaling lipids to activate inflammatory kinases and dampen insulin signaling. Downstream of nutrient oversupply or systemic inflammation, CGI-58-derived PA could serve as a precursor to pro-inflammatory signaling lipids that lead to the activation of stress kinases such as IκB kinase (IKK), c-jun N-terminal kinase (JNK) and mammalian target of rapamycin (mTOR). CGI-58-derived PA might directly activate mTOR complex 1(mTORC1), similar to PA generated by phospholipase D (PLD). mTORC1 activates S6 kinase 1 (S6K1) which phosphorylates insulin receptor substrate-1 (IRS-1) at Ser1101 and other residues to inhibit insulin signaling through the insulin receptor (IR). mTOR complex 2 (mTORC2) mediates insulin action by phosphorylating Akt at Ser473. PA generated within the glycerolipid synthesis pathway can impair insulin signaling through mTORC2. CGI-58-derived PA might also inactivate mTORC2 to prevent Akt phosphorylation. Phosphorylated Akt inactivates forkhead box protein O1 (FoxO1), a transcription factor that drives expression of genes involved in gluconeogenesis and very low density lipoprotein (VLDL) secretion. In addition to signaling lipids derived through CGI-58 LPAAT activity, it is possible that ATGL-mediated lipolysis could produce precursors to signaling lipids. Products of ATGL-mediated lipolysis can serve as signaling lipids to activate PPARα, a transcription factor that drives oxidative gene expression. CGI-58's role in hepatic TAG mobilization and PPARα target gene expression is likely ATGL-dependent (blue lines). Unlike CGI-58, ATGL does not significantly affect hepatic insulin signaling. Thus, CGI-58's role in the regulation of insulin signaling is proposed to be independent of ATGL (red lines). Direct effects are denoted by solid lines. Potential pathway effects are indicated by dotted lines. CGI-58, comparative gene identification-58; ATGL, adipose triglyceride lipase; LPA, lysophosphatidic acid; PA, phosphatidic acid; PI3Ks, phosphoinositide 3 kinases; PDPK1, phosphoinositide dependent protein kinase 1; GPAT, glycerol-3-phosphate acyltransferases; AGPAT, *sn*-1-acylglycerol-3-phosphate acyltransferase; PAP, phosphatidic acid phosphatase; DGAT, diglyceride acyltransferase.

vacuoles in blood leukocytes (Jordan anomaly).[12] NLSD patients can be divided into two subsets: NLSD with ichthyosis (NLSDI), also known as Chanarin-Dorfman syndrome, and NLSD with myopathy (NLSDM).[12,13] Mutations in CGI-58 cause NLSDI,[14] while ATGL mutations cause NLSDM.[15] All known CGI-58 mutations cause ichthyosis, whereas ATGL mutations always cause cardiomyopathy yet do not result in ichthyosis.[13] Hepatomegaly and hepatic steatosis occur more frequently with CGI-58 mutations than with ATGL mutations.[13] CGI-58 mutations are also commonly associated with neurological disorders, which are not seen with ATGL mutations.[13] Interestingly, obesity has not been observed in patients with NLSDI and NLSDM,[12,13] suggesting either that adipose lipolysis is not significantly decreased or that other factors compensate to prevent obesity in these patients. In skin fibroblasts from a NLSDI patient lacking functional CGI-58, TAG accumulation appears to be secondary to a defect in phospholipid metabolism.[16,17] The phenotypic differences between patients with NLSDI and NLSDM clearly indicate distinct roles for CGI-58 and ATGL, at least in the skin and nervous system. Additional studies in patients with primary CGI-58 and ATGL mutations will be extremely important to clarify the shared and distinct functions of these proteins in the context of human physiology and pathology.

CGI-58 and ATGL in the Skin

Whole-body CGI-58 knockout (CGI-58$^{-/-}$) mice die within hours after birth due to a skin barrier defect.[9] The skin of CGI-58$^{-/-}$ mice shows characteristics of lamellar ichthyosis, similar to NLSDI in humans.[9] In contrast, ATGL$^{-/-}$ mice do not develop ichthyosis and survive to 12–14 weeks of age before dying of cardiomyopathy.[18] Dermal TAG hydrolase activity is lower in both CGI-58$^{-/-}$ mice and ATGL$^{-/-}$ mice compared with wild-type mice.[9] However, epidermal TAG hydrolase activity is 80% lower in CGI-58$^{-/-}$ mice but is unchanged in ATGL$^{-/-}$ mice.[9] This difference clearly supports an ATGL-independent function of CGI-58 in the epidermis. Perhaps CGI-58 co-activates an unknown lipase to hydrolyze TAG for the production of skin barrier lipids. Alternatively, CGI-58 could function in glycerophospholipid metabolism to generate signaling lipids that are important for keratinocyte differentiation and skin barrier formation. Interestingly, PNPLA1, a protein that shares substantial homology with ATGL/PNPLA2, was recently discovered to be mutated in autosomal recessive congenital lamellar ichthyosis in humans and dogs.[19] Unlike ATGL, PNPLA1 does not exhibit CGI-58-driven TAG hydrolase activity, and PNPLA1 mutations do not cause neutral lipid accumulation in isolated keratinocytes.[19] Furthermore, PNPLA1 mutations do not result in neutral lipid vacuoles in blood leukocytes (Jordan anomaly),[19] a hallmark of NLSD.[12] Rather, levels of PA and phosphatidylethanolamine (PE) are significantly lower in PNPLA1 mutant keratinocytes, suggesting that PNPLA1 may possess acyltransferase activity and could function in glycerophospholipid synthesis or remodeling.[19] Based on CGI-58's LPAAT activity,[10,11] it is possible that altered glycerophospholipid metabolism in the skin could contribute to NLSDI. In fact, altered phospholipid metabolism appears to be the primary defect in human NLSDI skin fibroblasts.[16,17] However, the role of CGI-58 LPAAT activity in the skin remains unclear, since human CGI-58 mutations associated with ichthyosis do not change the LPAAT activity of the protein in vitro.[10] In addition, total levels of PE are not altered in the epidermis of CGI-58$^{-/-}$ mice.[9] Further work is needed to elucidate the ATGL-independent function of CGI-58 in the skin. It would be interesting to explore the possibility that mutations in CGI-58 and PNPLA1 may cause ichthyosis through similar glycerophospholipid pathways in the skin. Since

Table 1. Comparison of CGI-58 and ATGL mouse models

Endpoint	Mouse model	Phenotype vs. control mice
Obesity and adipose tissue	ASO-mediated CGI-58 knockdown[2]	Complete prevention of HFD-induced obesity 50% lower fat mass on both chow and HFD No difference in BW on chow diet
	Adipose-specific CGI-58 overexpression[36]	No difference in BW or HFD-induced obesity No difference in lipolysis No difference in fat mass or adipocyte size
	Whole-body ATGL knockout[18,45]	Higher rate of HFD-induced obesity Higher fat mass Larger adipocyte size Blunted lipolysis
	Adipose-specific ATGL knockout[32]	Higher rate of HFD-induced obesity Higher fat mass Larger adipocyte size Blunted lipolysis
	Adipose-specific ATGL overexpression[33]	Attenuated HFD-induced obesity Lower fat mass Smaller adipocyte size Lower adipocyte TAG content Increased lipolysis
Liver lipid metabolism	Whole-body CGI-58 knockout[9]	Higher TAG Lower TAG hydrolase activity
	ASO-mediated CGI-58 knockdown[1,2]	Higher TAG, DAG, and ceramides Lower TAG hydrolase activity Lower VLDL-TAG secretion Lower β-oxidation
	Whole-body ATGL knockout[9,18,40]	Higher TAG Lower TAG hydrolase activity
	Liver-specific ATGL knockout[41]	Higher TAG Lower TAG hydrolase activity No difference in VLDL-TAG secretion No difference in DAG and ceramides
	Adenovirus-mediated ATGL knockdown[42]	Higher TAG Lower TAG hydrolase activity No difference in VLDL-TAG secretion
	Adenoviral ATGL overexpression[21,44]	Lower TAG No difference in VLDL-TAG secretion Higher rate of NEFA release into plasma Higher rate of FA oxidation
Insulin signaling	ASO-mediated CGI-58 knockdown[1,2]	Improved whole-body glucose tolerance on chow and HFD Improved whole-body insulin tolerance on HFD Improved hepatic insulin signaling
	Whole-body ATGL knockout[18,40,44,45]	Improved whole-body glucose tolerance on chow and HFD Improved whole-body insulin tolerance on chow and HFD Unchanged or slightly impaired hepatic insulin signaling No difference in insulin signaling in primary hepatocytes
	Adenoviral ATGL overexpression[44]	Slight improvement in insulin signaling
Lipid signaling	ASO-mediated CGI-58 knockdown[1,2]	Lower PPARα target gene expression in liver Altered hepatic glycerophospholipids Prevention of hepatic stress kinase activation and inflammatory signaling
	Whole-body ATGL knockout[60]	Lower PPARα target gene expression in liver Steatosis and cardiomyopathy prevented by PPARα agonist
	Adenovirus-mediated ATGL knockdown[42]	Lower PPARα target gene expression in liver

ASO, antisense oligonucleotide; HFD, high fat diet; BW, body weight; TAG, triacylglycerol; DAG, diacylglycerol; FA, fatty acid; NEFA, non-esterified fatty acid; VLDL, very low density lipoprotein.

ASO-mediated knockdown of CGI-58 does not result in ichthyosis, future studies of CGI-58 in the skin will require conditional knockout mouse models.

CGI-58 and ATGL in White Adipose Tissue

Both CGI-58 and ATGL are highly expressed in white adipose tissue (WAT).[2,20,21] ATGL and hormone sensitive lipase (HSL) together account for more than 90% percent of TAG hydrolase activity in mouse WAT.[8] In WAT of ATGL$^{-/-}$ mice, TAG hydrolase activity is 80% lower than in wild-type mice, suggesting that ATGL is the rate-limiting enzyme for TAG hydrolysis in WAT, whereas HSL functions primarily as a diacylglycerol hydrolase.[18,22] A general model of lipolytic trafficking in adipocytes is depicted in **Figure 1A**. In the basal state, CGI-58 is primarily sequestered by perilipin-1 (Plin-1) at the lipid droplet (LD),[23-25] and ATGL is localized to the LD.[26] During hormone-stimulated lipolysis, cAMP-activated protein kinase A (PKA) phosphorylates Plin-1,[27-29] resulting in the release of CGI-58 to interact with ATGL at the LD[30] or to reversibly disperse to the cytoplasm.[23,25] Meanwhile, phosphorylated HSL moves from the cytoplasm to the LD[28,31] and interacts closely with Plin-1[30] to preferentially hydrolyze DAG[22] generated by ATGL. In agreement with a rate-limiting role in TAG hydrolysis,[8] both whole-body and adipose-specific ATGL knockout mice are mildly obese, with higher fat pad weight and larger adipocyte size, and have blunted lipolysis.[18,32] Conversely, adipose-specific ATGL overexpression in HFD-fed mice results in higher TAG hydrolase activity in WAT, attenuated HFD-induced obesity, lower fat pad weight, smaller adipocyte size, and lower TAG content in adipocytes.[33] ATGL overexpression has no effect on fat mass in chow-fed mice.[33] In addition, ATGL overexpression in WAT increases lipolysis but does not result in elevated serum NEFA or ectopic TAG accumulation, possibly indicating increased FA oxidation in adipose.[33] If CGI-58 functions as the co-activator of ATGL-mediated lipolysis in WAT,[8] the phenotype of CGI-58 knockdown should be similar to ATGL deficiency. However, in striking contrast to ATGL deficiency, 80–95% knockdown of CGI-58 expression in WAT results in 50% lower fat mass in both chow-fed and HFD-fed mice.[2] CGI-58 knockdown does not change body weight in chow-fed mice but completely prevents HFD-induced obesity.[2] Neither ATGL nor CGI-58 appears to have an effect on food intake.[2,18,32] Although the prevention of adiposity resembles overexpression of ATGL,[33] ATGL mRNA levels in WAT are not higher in CGI-58 ASO-treated mice.[2] In HFD-fed CGI-58 ASO-treated mice, plasma NEFA levels are 35% lower in both the fed and fasted states, compared with control ASO mice,[2] possibly reflecting less ATGL-mediated lipolysis in WAT. Similarly, Radner and colleagues reported 60% lower plasma NEFA in newborn CGI-58$^{-/-}$ mice.[9] It is conceivable that CGI-58 could co-activate ATGL to drive TAG hydrolysis, yet also promote expansion of WAT through an ATGL-independent mechanism. For example, ATGL-mediated lipolysis might provide lipid substrates for a separate function of CGI-58 in glycerophospholipid synthesis or signaling lipid generation in adipocytes. Interestingly, Yang and colleagues reported the existence of an alternatively-spliced isoform of mouse CGI-58 lacking a putative lipid binding domain.[34] This isoform retains LPAAT activity yet is predominately cytoplasmic and does not co-activate ATGL lipase activity.[34] A similar isoform of CGI-58 has not been identified in humans, and the physiological function of this cytoplasmic isoform in mice remains unknown.[34] It is noteworthy that in 3T3-L1 adipocytes, the vast majority of CGI-58 disperses from the LD to the cytoplasm following lipolytic stimulation,[23,25] while only a small amount remains at the LD, presumably interacting with ATGL.[30,35] The fact that the vast majority of CGI-58 moves away from the LD during stimulated lipolysis, and the finding that CGI-58 knockdown prevents HFD-induced obesity seem to suggest that CGI-58 has a physiological function in WAT apart from the co-activation of ATGL-mediated TAG hydrolysis (**Fig. 1A**). At the same time, it is important to note that CGI-58 knockdown is not adipocyte-specific.[2] CGI-58 knockdown in other ASO-targeted tissues, namely the liver and macrophages, could possibly influence adiposity. However, overexpression of CGI-58 using the aP2 promotor (adipose-selective) in mice has no effect on diet-induced obesity, fat pad weight, or adipocyte size.[36] Lipolysis in vivo is not altered in response to fasting or treatment with a β3-adrenergic receptor agonist.[36] Likewise, neither basal nor isoproterenol-stimulated lipolysis is affected in isolated adipocytes.[36] These data suggest that CGI-58 is at least not limiting for ATGL-mediated lipolysis. Although ATGL is clearly a rate-limiting enzyme in adipocyte lipolysis, additional work is needed to clarify the physiological function of CGI-58 in adipocytes and to determine the basis for the opposite effects of CGI-58 and ATGL deficiency on adiposity.

CGI-58 and ATGL in the Liver

In human NLSD, hepatomegaly and hepatic steatosis are more common with CGI-58 mutations than with ATGL mutations.[13] The regulation of CGI-58 and ATGL subcellular localization and trafficking is less clearly defined in the liver and other non-adipose tissues that do not express Plin-1. In these tissues, it is likely that other perilipin family proteins such as perilipin-2[24,37] and perilipin-5[38,39] play an important role in regulating the interaction of CGI-58 and ATGL. In mice, ATGL deficiency (whole-body knockout,[9,18,40] liver-specific knockout[41] and adenovirus-mediated knockdown[42]) has been reported to result in elevated hepatic TAG, although the effect has been variable, ranging from 62% higher[40] to 4-fold higher levels of TAG.[9] CGI-58 deficiency likewise results in markedly higher hepatic TAG levels compared with control mice.[1,2,9] Radner and colleagues[9] reported 4-fold higher hepatic TAG in newborn CGI-58$^{-/-}$ mice, and we have measured 2 to 4-fold more hepatic TAG in CGI-58 ASO-treated mice.[1,2] Based on more frequent liver complications in humans with NLSDI,[13] CGI-58 deficiency might be expected to result in more severe steatosis than ATGL deficiency. However, the variable range of steatosis observed in ATGL studies and the limited number of CGI-58 studies make it difficult to interpret the relative degree of steatosis. Comparison of actual hepatic TAG values is further complicated by experimental differences

in the background strains, diets, and ages of the mice. Clearly, both proteins have an important role in TAG metabolism in the liver. Although expression of ATGL is much lower in liver than in WAT,[21] ATGL deficiency results in significantly lower total TAG hydrolase activity in the liver.[9,18,21,41,42] Likewise, TAG hydrolase activity and intracellular NEFA levels are significantly lower in the liver of CGI-58 ASO-treated mice.[2] Interestingly, newborn CGI-58$^{-/-}$ mice have 73% lower TAG hydrolase activity in the liver compared with wild-type mice, while newborn ATGL$^{-/-}$ mice have only 46% lower activity.[9] This could indicate a role for CGI-58 in hepatic TAG mobilization beyond the co-activation of ATGL-mediated hydrolysis. In CGI-58 ASO-treated mice fed HFD, fasting-induced plasma levels of β-hydroxybutyrate are significantly lower compared with control mice, suggesting less FA oxidation in the liver.[2] In addition, CGI-58 knockdown results in a significantly lower rate of very low density lipoprotein (VLDL) secretion from the liver,[2] suggesting that CGI-58-driven TAG mobilization is linked to the secretion of TAG-rich lipoproteins by the liver. This dual role for CGI-58 in promoting FA oxidation and VLDL-TAG secretion has been previously reported using gain-of-function and loss-of-function cell models.[20,43] In contrast, ATGL mobilization of hepatic TAG appears to preferentially lead to FA oxidation, without changing VLDL secretion. Haemmerle and colleagues reported that ATGL$^{-/-}$ mice have lower plasma VLDL levels, accounting for lower fasting plasma TAG.[18] However, liver-specific ATGL deletion[41] or knockdown[42] does not alter the rate of VLDL secretion from the liver. This makes it tempting to speculate ATGL deficiency may alter VLDL turnover or clearance instead of hepatic production. In addition, liver-specific overexpression of ATGL in obese mice attenuates hepatic steatosis, but does not change TAG or ApoB secretion from the liver.[21] Rather, ATGL mobilization of hepatic TAG is associated with FA oxidation and NEFA release into plasma, as well as elevated fasting-induced ketogenesis.[21,42,44] Another important observation is that CGI-58 knockdown results in the accumulation of DAG and ceramides in the liver,[1,2] whereas ATGL deficiency has been associated with normal or slightly lower DAG and ceramide content in the liver.[40,44] In sum, whether CGI-58 and ATGL have distinct roles in hepatic TAG metabolism remains inconclusive based on complex and subtle differences. However, an ATGL-independent function of CGI-58 in the liver is more clearly suggested by differences in hepatic insulin signaling.

The Role of CGI-58 and ATGL in Insulin Sensitivity

Despite hepatic accumulation of TAG, DAG and ceramides, CGI-58 knockdown improves whole-body glucose tolerance and insulin sensitivity.[2] In fact, CGI-58 ASO-treated mice are completely protected against HFD-induced hyperglycemia, glucose intolerance and systemic insulin resistance.[2] ATGL$^{-/-}$ mice also have significant improvement in whole-body glucose tolerance and insulin sensitivity,[18,40,45] but the underlying mechanisms likely differ significantly. In both ATGL$^{-/-}$ mice and CGI-58 ASO-treated mice, plasma insulin levels are very low compared with wild-type controls.[2,18,45] It has been shown that ATGL is necessary for glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells in mice.[46] In agreement, plasma insulin does not increase in response to a glucose challenge in ATGL$^{-/-}$ mice.[45] Although a role for CGI-58 in GSIS has not been examined, defective GSIS is unlikely in CGI-58 ASO-treated mice because ASOs do not knock down CGI-58 in the pancreas (unpublished data). Furthermore, plasma insulin increases in response to a glucose challenge in CGI-58 ASO-treated mice,[2] suggesting that enhanced insulin sensitivity, rather than impaired GSIS, likely accounts for reduced plasma insulin levels. In fact, we have shown that CGI-58 knockdown results in significantly more Akt phosphorylation (Ser473 and Thr308) in the liver in response to insulin injected into the portal vein.[1] Insulin-stimulated Akt phosphorylation is not altered in skeletal muscle or WAT,[1] suggesting that the liver primarily accounts for enhanced whole-body insulin sensitivity in CGI-58 ASO-treated mice.[2] In contrast, Kienesberger and colleagues[45] reported that chow-fed ATGL$^{-/-}$ mice have diminished hepatic Akt phosphorylation at Thr308 and impaired Akt activity in response to intraperitoneally administered insulin, while Akt phosphorylation at Ser473 is not affected. The same study showed that insulin-stimulated Akt phosphorylation (Ser473) is increased in skeletal muscle of ATGL$^{-/-}$ mice, suggesting that skeletal muscle, rather than the liver, primarily contributes to whole-body insulin sensitivity in ATGL$^{-/-}$ mice in vivo.[45] On the other hand, Hoy and colleagues[40] found that in conjunction with insulin administration, glucose uptake was significantly higher in the liver and cardiac muscle of HFD-fed ATGL$^{-/-}$ mice compared with wild-type mice, but was not altered in skeletal muscle. Although this suggested improved hepatic insulin signaling in ATGL$^{-/-}$ mice, insulin-stimulated Akt phosphorylation (Ser473) in the liver was not different compared with wild-type mice.[40] Turpin and colleagues[44] also reported that Akt phosphorylation is not affected in primary hepatocytes isolated from ATGL$^{-/-}$ mice. In addition, these authors showed that adenoviral overexpression of ATGL in obese mice significantly attenuates hepatic steatosis but only mildly improves hepatic insulin sensitivity.[44] Interestingly, both adipose-specific ATGL deficiency[32] and overexpression[33] have been associated with enhanced hepatic insulin sensitivity, but in both cases the improvement is likely due to an amelioration of hepatic steatosis. In contrast to ATGL deficiency, ASO-mediated CGI-58 knockdown significantly improves Akt phosphorylation in the liver despite marked accumulation of TAG, DAG and ceramides.[1,2] Thus, although ATGL and CGI-58 are both implicated in regulating systemic insulin sensitivity, only CGI-58 knockdown improves hepatic insulin signaling.[1,2] Whereas liver-specific ATGL deficiency[41] or overexpression[44] does not alter hepatic inflammation in HFD-fed mice, CGI-58 knockdown in the liver significantly blunts phosphorylation of IκBα, JNK and S6 in response to inflammatory cytokines (TNFα, IL-1β and IL-6).[1] In addition, CGI-58 knockdown in the liver is associated with the prevention of HFD-induced stress kinase activation (IKK, mTOR, S6K1 and JNK) and prevention of serine phosphorylation of insulin receptor substrate-1 (IRS1) at Ser1101.[1] It is well-known that phospholipase D (PLD)-derived PA can activate

the mammalian target of rapamycin complex 1 (mTORC1).[47-50] mTORC1 then activates S6 kinase 1 (S6K1) which phosphorylates IRS-1 on serines residues and inhibits insulin signaling.[51] Since CGI-58 knockdown prevents HFD-induced phosphorylation of IRS1 (Ser1101) in the liver,[1] it is possible that CGI-58-derived PA can directly activate mTORC1 to dampen insulin signaling (**Fig. 1B**). Recently, Zhang and colleagues elegantly demonstrated that PA in the glycerolipid synthetic pathway can impair mTORC2-mediated phosphorylation of Akt (Ser473) in mouse primary hepatocytes.[52] Although we did not examine mTORC2 activity in the context of CGI-58 knockdown, this mechanism could also contribute to improved Akt phosphorylation (Ser473) in the liver of CGI-58 ASO-treated mice.[1] Taken together, it is possible that CGI-58-derived PA could both activate mTORC1 and diminish mTORC2 activity (**Fig. 1B**). Improved insulin signaling could also alter hepatic TAG metabolism in CGI-58 ASO-treated mice. Insulin suppresses VLDL secretion,[53] likely through apoB degradation[54] and reduced FoxO1-driven MTP expression.[55-57] It is tempting to speculate that reduced VLDL secretion in CGI-58 ASO-treated mice[2] might partially result from improved Akt-mediated inactivation of FoxO1.[1] Collectively, these studies suggest that CGI-58 regulates insulin action in the liver through an ATGL-independent mechanism, either the generation of PA through LPAAT activity or perhaps an unknown function of CGI-58 in signaling pathways.

The Role of CGI-58 and ATGL in Lipid Signaling

We have shown that the attenuation of hepatic inflammation in CGI-58 ASO-treated mice is associated with changes in multiple lipid species with signaling potential.[1] Signaling defects are apparent downstream of several cytokine receptors, and it is difficult to identify which lipid species are ultimately responsible for the effects on signaling.[1] Although LPAAT activity is the only enzymatic function of CGI-58 described to date,[10,11] it is possible that CGI-58 may also modify other lipid substrates in addition to LPA. CGI-58-generated PA could act directly in signaling pathways or serve as a precursor to other signaling glycerophospholipid species (**Fig. 1B**). In fact, the effects of CGI-58 knockdown on inflammation likely result from changes in multiple lipid species. We were not able to detect a change in total hepatic LPAAT activity in CGI-58 ASO-treated mice.[1] Similarly, total LPAAT activity is unchanged in whole liver lysates from CGI-58$^{-/-}$ mice.[9] However, this does not rule out an important role for CGI-58 as a signaling LPAAT in the liver. The levels of CGI-58-derived PA could be masked by total cellular PA from other sources and might act locally without a major impact on total glycerophospholipid levels. In addition, CGI-58 activity in vivo could be regulated by mechanisms not detectable in cell lysates. Collectively, these factors may explain why seemingly modest changes in hepatic PA and other glycerophospholipid species have a profound effect on inflammatory signaling in CGI-58 ASO-treated mice.[1] It is also possible that signaling lipids could derive from CGI-58's ability to co-activate ATGL-mediated TAG hydrolysis (**Fig. 1B**).[7-9] The effect of ATGL deficiency on hepatic inflammatory kinase activation has not been reported. However, liver-specific ATGL deficiency does not alter hepatic inflammatory cytokine expression in HFD-fed mice.[41] Interestingly, Fuchs and colleagues[58] recently reported that ATGL$^{-/-}$ mice are protected from hepatic ER stress and inflammatory gene expression (TNFα and iNOS) in response to tunicamycin. In macrophages, ATGL deficiency results in lower IL-6 expression and impaired migration.[59] Further studies are needed to clarify whether specific inflammatory pathways are altered by ATGL deficiency. Haemmerle and colleagues recently showed that ATGL$^{-/-}$ mice have severely blunted PPARα target gene expression in the liver and heart and that ATGL-mediated TAG hydrolysis is required for the activation of hepatic gene transcription by the PPARα-PCG-1 complex.[60] In agreement, Ong and colleagues[42] reported that adenoviral knockdown of ATGL in mice results in lower hepatic expression of PPARα and target genes. ATGL-mediated lipolysis can also activate PPARα in adipose tissue.[32,33] Adipose-specific ATGL knockout mice have reduced PPARα binding to genes involved in thermogenesis and show impaired thermogenesis similar to PPARα$^{-/-}$ mice.[32] Conversely, adipose-specific ATGL overexpression results in elevated thermogenic gene expression and FA oxidation in adipose tissue.[33] Collectively, these studies suggest that ATGL-mediated TAG hydrolysis generates endogenous ligands of PPARα (**Fig. 1B**). Remarkably, long-term treatment with a PPARα agonist prevents cardiomyopathy-related death and completely reverses hepatic TAG accumulation in ATGL$^{-/-}$ mice.[60] Hepatic PPARα target gene expression is also lower in CGI-58 ASO-treated mice[2] (unpublished data), possibly reflecting the loss of PPARα ligands derived from ATGL-mediated TAG hydrolysis. Alternatively, CGI-58 could generate endogenous PPARα ligands through glycerophospholipid pathways.[61] In addition to signaling in the liver,[1] it is possible that CGI-58 could function in lipid signaling in the nervous system, since neurological disorders are frequently observed in NLSDI patients.[12,13] Interestingly, ABHD4, which shares the highest sequence homology (55%) with CGI-58/ABHD5, has been shown to generate precursors for endocannabinoid signaling lipids in the central nervous system.[6] In sum, further studies will be needed to examine the role of ATGL in inflammatory signaling and to determine the source of CGI-58-derived signaling lipids in the liver,[1] as well as putative signaling functions in other tissues.

Conclusion

In summary, several recent studies provide insight into possible ATGL-independent functions of CGI-58 in humans and mice (**Table 1**). Despite significant advances in our understanding of the biochemistry of CGI-58, the primary physiological function of CGI-58 still remains unclear. An important next step will be clearly distinguishing which effects of CGI-58 deficiency result from decreased ATGL-mediated lipolysis vs. altered signal transduction. Based on the studies discussed here, several questions remain: does CGI-58 knockdown limit ATGL-mediated lipolysis in vivo? What is the function of CGI-58 in adipose tissue, and why are CGI-58 ASO-treated mice protected

from obesity while ATGL$^{-/-}$ mice are obese? Do ATGL and CGI-58 promote hepatic TAG mobilization through distinct or common mechanisms? Does CGI-58 regulate hepatic insulin signaling and inflammation through ATGL-mediated lipolysis or through LPAAT activity? Which CGI-58-derived signaling lipids promote inflammatory signaling in the liver? The answers to these and similar questions will greatly advance our understanding of how CGI-58 and ATGL are related to the development of metabolic disease. Tissue-specific CGI-58 and ATGL knockout mouse models will be essential to understanding cell-autonomous alterations in signaling and metabolism. In conclusion, both CGI-58 and ATGL are critical enzymes in the regulation of TAG metabolism and cellular signaling, yet many additional studies are required to clarify their individual contributions to NLSD, obesity and type 2 diabetes.

Acknowledgments

This work was supported in part by the National Heart, Lung, and Blood Institute (R00-HL096166) and the American Heart Association (11BGIA-7840072) to J.M.B.

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