ATVB in Focus
New Developments in Hepatic Lipoprotein Production and Clinical Relevance
Series Editor: Zemin Yao

CIDE Proteins and Lipid Metabolism
Li Xu, Linkang Zhou, Peng Li

Abstract—Lipid homeostasis is maintained through the coordination of lipid metabolism in various tissues, including adipose tissue and the liver. The disruption of lipid homeostasis often results in the development of metabolic disorders such as obesity, diabetes mellitus, liver steatosis, and cardiovascular diseases. Cell death–inducing DNA fragmentation factor 45-like effector family proteins, including Cidea, Cideb, and Cidec (or Fsp27 in mice), are emerging as important regulators of various lipid metabolic pathways and play pivotal roles in the development of metabolic disorders. This review summarizes the latest cell death–inducing DNA fragmentation factor 45-like effector protein discoveries related to the control of lipid metabolism, with emphasis on the role of these proteins in lipid droplet growth in adipocytes and in the regulation of very low-density lipoprotein lipidaidsion and maturation in hepatocytes. (Arterioscler Thromb Vasc Biol. 2012;32:1094-1098.)

Key Words: cell death–inducing DNA fragmentation factor 45-like effector ■ lipid droplet ■ lipid metabolism ■ metabolic disorders ■ very low-density lipoprotein

The cell death–inducing DNA fragmentation factor 45-like effector (CIDE) proteins, including Cidea, Cideb, and Cidec (or Fsp27 in mice), have been shown to play important roles in the development of metabolic disorders.1,2 Mice with a deficiency in CIDE proteins exhibit a lean phenotype, resistance to high-fat diet–induced obesity, improved insulin sensitivity, and an increased whole-body metabolic rate.3-6 Cidea and Cidec are expressed in the white adipose tissue of humans, and their expression positively correlates with the development of insulin sensitivity in obese people.7,8 A patient who expresses a prematurely terminated Cidec protein develops ketosis-prone insulin resistance and partial lipodystrophy.9 In addition, increased Cidea and Fsp27 expression have been observed in the livers of high-fat diet-fed and ob/ob mice and under the condition of hepatic steatosis in humans.10,11 The levels of hepatic expression of Cidea and Cidec also positively correlate with body mass index.12 Furthermore, hepatic Cidec mRNA levels are decreased in obese patients who lose weight as a result of gastric bypass surgery.12 Fsp27 has been identified as a peroxisome proliferator–activated receptor-γ target and plays a crucial role in peroxisome proliferator–activated receptor-γ–induced hepatic steatosis.10 In addition, Cidea is identified as a sensor of dietary saturated fatty for the development of hepatic steatosis.13 Cideb is expressed at high levels in the liver, and modulates very low-density lipoprotein (VLDL) lipidadion and cholesterol homeostasis in hepatocytes.4,14-15 Recently, the levels of Cideb and Cidec have been found to be upregulated in macrophages in the presence of oxidized low-density lipoprotein, suggesting potential roles of Cideb and Cidec in foam cell formation and the development of atherosclerosis.16 Intriguingly, Cidea is observed to be highly expressed in lactating mammary gland and regulates milk lipid secretion by acting as a potential cofactor of CCAAT/enhancer-binding protein beta.17 Therefore, CIDE proteins are crucial factors to control multiple lipid metabolic pathways and maintain lipid homeostasis. The roles of CIDE proteins in cell death, energy metabolism and metabolic disorders, their transcriptional regulation, and their posttranslational modification have been extensively discussed previously.1,2 We will summarize the roles of CIDE proteins in the regulation of lipid metabolism, focusing on lipid droplet (LD) growth and VLDL lipidaidsion.

CIDE Proteins in Lipid Storage and LD Growth
Despite their diverse tissue expression patterns, deficiencies in all CIDE proteins result in 1 common phenotype: the accumulation of small LDs in the respective cell types.3,4,8,16-21 For example, brown adipocytes with a Cidea deficiency contain significantly smaller LDs.3 In addition, white adipocytes from Fsp27-deficient mice5,6 or from humans with Cidec mutations9 all exhibit the accumulation of smaller LDs. Therefore, CIDE proteins appear to play a unique role in the control of the sizes of cytosolic LDs in various cell types. Interestingly, CIDE proteins are localized to the surface of cytosolic LDs and the endoplasmic reticulum (ER),2 where nascent LDs are synthesized. LDs are important subcellular organelles responsible for neutral lipid storage in all cell types. Recently, LDs were shown to regulate viral assembly and intracellular protein and lipid trafficking.22,23 More importantly, white adipocytes contain a giant unilocular LD, and the sizes of LDs in adipocytes reflect the lipid storage capacity and have been linked to the development of obesity and insulin resistance.9,24-26 Genetic screens in Drosophila and yeast cells have uncovered links between factors involved in phospholipid synthesis,27,28 vesicular transport,29,30 and seipin/Fldp28,31,32 and regulation of LD size and morphology. However, the mechanisms of LD...
growth and the formation of unilocular LDs in adipocytes remain elusive.

Our recent research on the roles of Fsp27 and Cidea in the control of LD growth uncovered a novel molecular mechanism for LD growth and the formation of unilocular LDs in adipocytes. We have observed that both Fsp27 and Cidea are enriched at a particular sub-LD location: the LD-LD contact site (LDCS). The enrichment of Fsp27 at LDCSs is a critical first step for Fsp27-mediated LD growth. Interestingly, we observed that once Fsp27 and Cidea are enriched at LDCSs, rapid lipid exchange among contacted LD pairs is detected. In addition, a directional net transfer of neutral lipids from smaller to larger LDs occurs at Fsp27-positive LDCSs, resulting in the merging of smaller LDs to form large LDs. The phenomenon of Fsp27 facilitating LD clustering was also observed in another study. Intriguingly, Fsp27-mediated directional lipid transfer can be observed in vitro in an autonomous or cytosolic factor-independent manner. In addition, we observed a 50-fold higher lipid exchange activity in differentiated adipocytes than in preadipocytes overexpressing Fsp27, suggesting that additional adipocyte-specific factors are required to accelerate Fsp27-mediated LD growth in adipocytes.

Fsp27- and Cidea-mediated lipid transfer and LD growth is a unique process that is distinct from other membrane fusion processes, including vesicle fusion, ER fusion, and mitochondria fusion. Vesicle fusion involves the merging of lipid bilayers, the formation of an interconnected membrane structure and a fusion pore in a short period of time, and the mixing of the internal contents of the 2 vesicles. However, Fsp27- and Cidea-mediated lipid transfer and LD growth require these proteins to first be clustered and enriched at LDCSs. The clustering of Fsp27 and Cidea may provide a tethering force for stable LD attachment. In addition, the clustering of Fsp27 at LDCSs may recruit other proteins that deform the monolayer phospholipids at the LDCS to form a pore or a lipid transfer channel to initiate neutral lipid exchange among LDs that are in contact, resulting in net lipid transfer from smaller to larger LDs due to the difference in internal pressure (Figure). Fsp27-mediated LD growth is a relatively slow process that depends on the size of the LDs that are in contact, whereas vesicle fusion usually proceeds very rapidly, and a size requirement is not observed. Therefore, Fsp27-mediated lipid transfer and LD growth can be defined as a special LD fusion process that involves a localized and defined boundary (LDCS) and the directional transfer of lipids gradually from smaller to larger LDs. Such a slow process is likely favorable in adipocytes because the 1-step mixing of the LD cores of large LDs due to fusion pore expansion may generate mechanical stress in cells. The presence of phospholipid monolayer in LDs may lower the energy barrier for Fsp27-mediated LD growth relative to the energy barriers for other bilayer membrane fusion mechanisms. Further analyses including the complete identification of protein complex that facilitates Fsp27- or Cidea-mediated lipid transfer at the LDCS, structural characterization of Fsp27 and its associated proteins, and in vitro biochemical reconstitution of Fsp27-mediated lipid transfer will be needed to elucidate the molecular basis of CIDE proteins in controlling LD growth in adipocytes.

Regulation of LD size and lipid storage by Fsp27 and Cidea may also be mediated by its activity in controlling lipolysis, as Fsp27 and Cidea deficiencies result in an increased lipolysis, whereas overexpression of Cidea and Fsp27 leads to reduced lipolysis. The mechanism by which CIDE proteins control lipolysis is unclear. Several sequence homologous regions between CIDE proteins and perilipin are identified, raising the possibility that CIDE proteins could play dual roles in lipolysis as perilipin does. Alternatively, CIDE proteins may act as physical barriers to protect LDs from lipolysis, similar to that of adipose differentiation-related protein and TIP47 in contributing to LD stabilization.

Interestingly, Cidea and Fsp27 are also reported to be markedly upregulated in the livers of high-fat diet–fed and ob/ob mice. Overexpression of Cidea and Fsp27 in hepatocytes promotes the formation of larger LDs in hepatocytes. In contrast, Cidea and Fsp27 deficiency leads to reduced hepatic triacylglycerol (TAG) levels, the accumulation of smaller sized LDs, and alleviation of ob/ob or high-fat diet-induced hepatic steatosis. Therefore, Cidea and Fsp27 are likely to play similar roles in promoting LD growth in hepatocytes. However, the expressions of Cidea and Fsp27 in hepatocytes are differentially regulated upon fat challenge as Cidea is upregulated in the presence of dietary saturated fatty acids, which is mediated by sterol-regulatory-element-bindin g protein-1c, whereas Fsp27 gene expression is controlled by peroxisome proliferator–activated receptor-γ pathway. Therefore, these 2 proteins may play different roles at the LD contact site.
different stages of LD growth in hepatocytes. In addition, the sizes of LDs in hepatocytes are much smaller than those of adipocytes, possibly due to the lack of some crucial cofactors required to accelerate the activity of Fsp27 and Cidea.

CIDE Proteins in VLDL Lipidation and Maturation in Hepatocytes

The assembly and maturation of VLDL in hepatocytes is generally thought to involve 2 steps.43,48 The first step of VLDL assembly occurs in the ER with the formation of lipid-poor lipoprotein particles (pre-VLDL),46 which is dependent on MTP to transfer locally synthesized TAG to apoB-100.47 Lipid-poor pre-VLDL particles are then further lipidated to generate TAG-rich and mature VLDL particles for secretion.43,44 The subcellular location of VLDL lipidation and maturation, the source of the lipids, and the factors promoting VLDL lipidation remain subjects of intense debate and investigation. Some researchers suggest that the ER may be the site of VLDL lipidation because the majority of TAG is synthesized in the ER, and apoB-100 has been shown to be localized to the ER membrane.49-51 Other researchers have demonstrated that pre-VLDL particles exit the ER and that VLDL lipidation occurs primarily in the Golgi apparatus or post-ER compartments.53-55 The factors affecting VLDL assembly and maturation in hepatocytes have recently been reviewed.55

Two types of LDs, cytosolic LDs and ER-luminal LDs, have been identified using the biochemical method.56-59 Two hypotheses regarding the source of the lipids involved in VLDL lipidation have been proposed. First, TAG is directly transferred from cytosolic LDs to ER-luminal LDs to TAG-poor VLDL particles.51,60 Second, TAG from cytosolic LDs has been reported to be hydrolyzed to yield free fatty acids that are then re-esterified on the luminal side of secretory apparatuses, such as the Golgi, generating lipid-rich VLDL particles.61 The fusion of ER-luminal LDs with VLDL particles during VLDL lipidation and maturation is proposed to be mediated by ApoC III.62,63 One remaining question is whether TAG from cytosolic LDs, which are the most abundant source of neutral lipids, can be directly transferred to TAG-poor VLDL particles to form lipid-rich VLDL particles. Many factors, including ADP ribosylation factor 1 (ARF1, a small GTPase involved in vesicular trafficking),64 phospholipase D,65 the phospholipase iPLA2,66 phosphoinositide-3 kinase,66 and coat protein complex II,67 are involved in VLDL lipidation and secretion. In addition, the synthesis of phosphatidylcholine and phosphatidylethanolamine, and their ratio regulate the VLDL assembly and secretion.68

Cideb, a member of the CIDE family of proteins, is expressed at higher levels in the liver and kidney.4 Cideb-/- mice exhibit an increased whole-body metabolic rate and reduced serum TAG levels and are resistant to diet-induced obesity.4 Further analyses reveal that the VLDL particles secreted from Cideb-/- mice or isolated Cideb-/- hepatocytes contain significantly less TAG but have similar levels of apoB-100/48, indicating an impairment in VLDL lipidation in Cideb-deficient hepatocytes. In addition, Cideb is localized to LDs and the ER and interacts with apoB-100/48. The interaction between Cideb and apoB-100 is required for VLDL lipidation in hepatocytes.13 Interestingly, the overexpression of Plin2, another LD-associated protein, has also been shown to decrease the production and secretion of lipid-rich VLDL particles and to increase cytosolic TAG accumulation in McARH7777 cells.69 In contrast, mice with Plin2 and leptin double deficiency exhibit increased VLDL lipidation,70 suggesting that Plin2 negatively controls VLDL lipidation. Therefore, LD-associated proteins appear to play important roles in controlling VLDL lipidation and maturation. However, the mechanisms of Cideb-mediated VLDL lipidation and maturation remain elusive. Future studies will focus on the following questions: (1) Where does Cideb-mediated VLDL lipidation occur? ER, post-ER compartments, or the Golgi apparatus? (2) Does Cideb-mediated VLDL lipidation require the cooperation of Cideb with other LD- or ER-associated proteins? (3) Does Cideb-mediated VLDL lipidation involve direct neutral lipid transfer from cytosolic (or ER-associated) LDs to TAG-poor VLDL particles? Alternatively, Cideb-mediated VLDL lipidation may require the hydrolysis of TAGs in cytosolic LDs. Genetically modified animal models and a well-established cell model will be good tools to address these questions.71 The function of Cideb in hepatocytes may be dependent on its subcellular localization. When localized to LDs, Cideb mediates lipid transfer and LD growth using similar mechanism to that of Cidea and Fsp27. When in close contact with pre-VLDL particles, Cideb promotes VLDL lipidation and maturation possibly by mediating direct transfer of TAG from cytosolic LDs to pre-VLDL particles or the second step, bulk TAG pool.71

Conclusion

CIDE proteins are important modulators of diverse lipid metabolic pathways, including lipolysis, fatty acid oxidation, VLDL lipidation, and LD growth in adipocytes and hepatocytes. By localizing to LDs and the ER, Cideb controls VLDL lipidation and maturation in hepatocytes. Furthermore, Fsp27 and Cidea are enriched at LDCs and promote lipid exchange and lipid transfer between LDs that are in contact, resulting in the final growth and enlargement of LDs in adipocytes. However, our understanding of the mechanistic details of CIDE protein functions in controlling lipid metabolism is still in its early stages. Much effort will be needed to determine how CIDE proteins modulate the processes involved in lipid homeostasis, including VLDL lipidation and LD growth in hepatocytes and adipocytes and perhaps in other cells. CIDE proteins may be new drug targets for the treatment of metabolic disorders.

Sources of Funding

The work that formed the basis for opinions expressed in this review was supported by grants from the National Basic Research Program (2011CB910800) and National High Technology Research and Development program (2010AA023002) from the Ministry of Science and Technology of China and National Natural Science Foundation of China (30800555 to L.X. and 31030038 to P.L.). Dr Li is a professor and investigator for Tsinghua-Peking Center for Life Sciences.

Disclosures

None.
References


52. Blasiole DA, Oler AT, Attie AD. Regulation of ApoB secretion by the low density lipoprotein receptor requires exit from the endoplasmic reticulum and interaction with ApoE or ApoB. J Biol Chem. 2008;283:11374–11381.


64. Asp L, Claesson C, Borén J, Olofsson SO. ADP-ribosylation factor 1 and its activation of phospholipase D are important for the assembly of very low density lipoproteins. J Biol Chem. 2000;275:26285–26292.


CIDE Proteins and Lipid Metabolism
Li Xu, Linkang Zhou and Peng Li

doi: 10.1161/ATVBAHA.111.241489
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/32/5/1094

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/