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CIDE Family-Mediated Unique Lipid Droplet Morphology in White Adipose Tissue and Brown Adipose Tissue Determines the Adipocyte Energy Metabolism

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White adipose tissue (WAT) stores energy as triacylglycerol in preparation for fasting state. In contrast, brown adipose tissue (BAT) consumes energy and produces heat in a cold environment. One of the major differences between these two adipose tissues is the morphology of the intracellular lipid droplet (LD), which is large and unilocular in WAT and small and multilocular in BAT. Although the fat-specific protein 27 alpha (FSP27 α), belonging to the cell death-inducing DNA fragmentation factor A (DFFA)-like effector (Cide) family, was known to be indispensable for large unilocular LD formation in WAT, the mechanism that regulated small multilocular LD formation in BAT remained unknown. We recently uncovered that FSP27 β , a novel isoform of FSP27 abundantly expressed in BAT, plays a crucial role in small multilocular LD formation by inhibiting the homodimerization of CideA in BAT. We speculate that unilocular LD formation is ideal for efficient lipid storage in WAT because lipolysis from the LD surface is restricted due to the minimum LD surface area. In addition, hydrolyzed free fatty acid (FFA) and glycerol can efficiently flow out into the circulation from the cell surface. In contrast, small multilocular LD formation is ideal for efficient intracellular lipolysis from the LD surface and the subsequent facilitation of FFA transport to mitochondria that are adjacent to LDs for β -oxidation in BAT. Thus, intracellular LD morphology is closely related to the functions and characteristics of adipose tissues. Given that the browning of adipose tissue leads to enhanced energy expenditure and the prevention of obesity, clarification of the mechanism with respect to intracellular LD formation is very meaningful.

Key words: Lipid droplet, Adipocyte, Cell death-inducing DNA fragmentation factor A (DFFA)-like effector A (CideA), Fat-specific protein 27 (FSP27), Brown adipose tissue (BAT), White adipose tissue (WAT)

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Introduction

Obesity and obesity-related disorders, including type 2 diabetes, dyslipidemia, hypertension, fatty liver, and atherosclerosis, are increasing worldwide¹⁾. Obesity is the result of energy imbalance caused by sufficient nutrient intake and poor energy expenditure and is characterized by excessive lipid accumulation in adi-

pose tissues²⁾. In particular, visceral adiposity is believed to cause insulin resistance and is associated with a variety of diseases including diabetes and cardiovascular diseases^{3, 4)}. Furthermore, the adipose tissue plays crucial roles in the regulation of whole-body energy homeostasis. Adipose tissue is not only highly adapted to store surplus energy as triacylglycerol (TAG) but also secretes various mediators and cytokines (adipokines) that affect whole-body metabolism⁵⁾ and heart diseases⁶⁾. Excessive TAG storage in adipose tissues causes adipose tissue inflammation and the deregulation of adipokines, leading to insulin resistance in remote insulin-sensitive tissues, for example, the liver, skeletal muscle and heart. In addition, surplus lipid that exceeds the storage capacity of adipocytes induces ectopic TAG

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accumulation in other tissues, such as the liver, skeletal muscle, heart, or pancreas. It induces not only insulin resistance in insulin-sensitive tissues but also impairment of insulin secretion in β cells⁷. These imply that TAG storage in adipocyte is associated with obesity, diabetes, and cardiovascular diseases. Adipocytes can be classified into energy-storing white adipocytes and energy-consuming brown ones according to their distinct function and morphology⁸. It is important to unveil the mechanism for efficient TAG storage in white adipocytes and for efficient energy expenditure in brown adipocytes. Although the transcriptional factors controlling the differentiation between white and brown adipocytes have been extensively investigated^{9, 10}, it is not enough to examine the difference of lipid droplet (LD) formation between the two adipocytes. We assume that the functions of these adipocytes are associated with the intracellular LD morphology. Thus, it is crucial to disclose the mechanism for lipid storage and consumption in white and brown adipocytes. We describe the recent progress regarding the relationship between LD morphology and energy metabolism in white and brown adipocytes.

White Adipose Tissue and Brown Adipose Tissue

Energy storage as droplets containing neutral lipids (mainly TAG and steryl esters) in the cytoplasm in preparation for starvation is a common and widespread feature among eukaryotic cells. LDs of all types of cells share a general structure. A hydrophobic core of neutral lipids in LD is surrounded by a membrane monolayer of phospholipids (phosphatidylcholine and phosphatidylethanolamine)^{11, 12}. LDs are believed to derive from the endoplasmic reticulum (ER). Neutral lipids are synthesized in the interior of the bilayer of the ER and enlarge into spheres between the bilayer; they eventually bud from the ER into the cytoplasm surrounded by a phospholipid monolayer derived from the ER¹³. In addition, the phospholipid monolayer of LDs contains a variety of proteins involved in the appropriate regulation of LD formation and degradation¹⁴. Among them, perilipin 1 was identified as the first LD protein expressed abundantly in adipocytes¹⁵. It is a major phosphorylated protein by protein kinase A in adipocytes, and its phosphorylation is essential for catecholamine-stimulated lipolysis¹⁶. Furthermore, perilipin 1 was also found to regulate cellular lipid metabolism¹⁷. LDs exist ubiquitously in various organs. In non-adipocyte cells, the LD is very small, and its size is usually smaller than 1 μ m in diameter, except for extreme pathological states such as hepatocytes in steatosis¹⁸. However, adipocytes have large LDs because

adipocytes are cells in which the lipid storage function has specifically developed. There are two types of adipocytes that play different roles in energy metabolism⁸. One is the white adipocyte that stores lipid as a large unilocular lipid droplet that occupies almost all the cytoplasmic space and can be in the 100 μ m range¹⁸. The other is the brown adipocyte that stores lipid as small multilocular lipid droplets. Both adipocytes have the common characteristic of storing lipid efficiently for each tissue-specific purpose. White adipocyte uptakes glucose and free fatty acids (FFA), synthesizes TAG, and stores it as lipid in the postprandial period. In the fasting, it hydrolyzes TAG to FFA and glycerol. The former is utilized in skeletal muscles and the heart as an energy source instead of glucose. The latter is used in the liver as a substrate for the production of glucose. Conversely, brown adipocytes dissipate energy for heat production by using FFA generated by hydrolyzing intracellular LD through the proton leak via the activation of the BAT-specific protein, uncoupling protein 1 (UCP1)^{19, 20}.

WAT is mainly located in the visceral or subcutaneous space, whereas BAT is mainly located in the interscapular and perirenal spaces in rodents. Visceral fat accumulation has been shown to increase with age²¹. BAT was traditionally thought to exist only in the neonatal and early childhood periods in the interscapular region and to disappear with age in humans²². However, it was revealed that adult humans also have BAT depots, for example, in the cervical, supraclavicular, and paravertebral areas²³⁻²⁸.

WAT and BAT originate by a distinct developmental program. White adipocytes originate from myogenic lineage marker Myf5-negative mesenchymal precursor cells. On the contrary, brown adipocytes arise from precursors that express Myf5, and the transcriptional profiles of brown adipocytes are similar to those of the skeletal muscle^{29, 30}. Several factors have been identified as brown fat-specific gene induction factors, such as peroxisome proliferator-activated receptor (PPAR) γ -coactivator-1 α (PGC-1 α)³¹, PR domain-containing protein-16 (PRDM16)³², bone morphogenic protein family (BMP)³³, CCAAT/enhancer binding protein β (C/EBP β)³⁴, and lysine-specific demethylase 1 (Lsd1)³⁵.

Recently, another type of thermogenic adipocyte, designated as "beige" or "brite" cells, was demonstrated to be induced in the WAT of rodents and humans^{36, 37}. Beige adipocytes derive from Myf5-negative cells in WAT³⁰, and their development is induced in response to environmental stimulation, such as cold exposure, PPAR γ agonists, and exercise³⁸. They are highly energy expending and characterized by plenty of mitochondria, small multilocular LDs, and increased expression

of UCP1 similar to brown adipocytes³⁹). In recent years, brown and beige adipocytes are suggested as therapeutic targets for weight loss⁴⁰.

Importance of LD Morphology in the Characteristics of Adipocytes with Respect to Energy Metabolism

In mammals, white adipocytes play an important role as the primary reservoir of excess energy. Large unilocular LDs in white adipocytes are thought to be the ideal structure to store TAG. In this form, the surface area of the LD becomes minimum. Thus, the area of the contact site of the LD with lipase becomes small, resulting in the restriction of lipolysis. In addition, white adipocytes need to supply FFA and glycerol to other tissues in case of energy demand, such as the fasting state. In such cases, FFA and glycerol generated on the LD surface that is close to the plasma membrane can efficiently flow out of the cells into the circulation. On the other hand, brown adipocytes possess small multilocular LDs in their cytoplasm and effectively conduct thermogenesis by uncoupling substrate oxidation and adenosine triphosphate production through the proton leak. Small multilocular LD formation increases the contact area of the LD with lipase, which efficiently promotes lipolysis and facilitates FFA transport to the mitochondria adjacent to LDs for β -oxidation and heat production in mitochondria (**Fig. 1**). As just described, these differences in the lipid accumulation pattern between the two adipocytes are thought to reflect their functional characteristics. However, the mechanism responsible for the formation of LDs in white and brown adipocytes has remained unknown.

Cide Family Proteins Regulate Whole-Body Energy Homeostasis

Cell death-inducing DNA fragmentation factor A (DFFA)-like effector (Cide) family proteins, containing CideA, CideB, and the fat-specific protein 27 (FSP27) (CideC in humans), are among the LD-associated proteins and have been shown to play a crucial role in lipid and energy metabolism, including lipolysis, lipid oxidation, and LD formation⁴¹. They share homology with the N-terminal region of the DNA fragmentation factor DFF45 (DFFA), which regulates apoptosis⁴².

CideA is strongly and almost exclusively expressed in BAT in mice⁴³. CideA-deficient mice show lean phenotype and are resistant to diet-induced obesity and diabetes. CideA appears to be a mitochondrial protein that suppresses UCP1 activity and regulates thermo-

genesis and lipolysis⁴³. Lipid accumulation and LD size in brown adipocytes decreased in CideA-deficient mice stimulated by cold exposure⁴³. Increased expression level of CideA was also observed in the livers of high-fat diet (HFD)-fed mice and leptin-deficient (ob/ob) mice^{44, 45}. In recent years, CideA was demonstrated as mediating LD fusion at the LD-LD contact site between two LDs in white and brown adipose tissues^{46, 47}. CideB is highly expressed in the liver and kidney^{42, 48}. Energy expenditure was increased and insulin sensitivity was improved in CideB-null mice⁴⁸. CideB is also localized on the LD-LD contact sites and promotes LD fusion and growth in hepatocytes⁴⁹. Moreover, CideB plays an important role in mature very-low-density lipoprotein secretion⁵⁰⁻⁵².

FSP27 was initially reported to be one of the LD proteins in 3T3-L1 adipocytes⁵³. Thereafter, FSP27 was found to be predominantly expressed in adipose tissues, and it contributes to the efficient storage of TAG and the large unilocular LD formation in white adipocytes of mice⁵⁴. FSP27 knockout (KO) mice showed reduction of lipid storage in WAT and were protected from HFD-induced obesity and insulin resistance^{54, 55}. Interestingly, WAT in FSP27 KO mice displayed multilocular LD formation that is similar to BAT⁵⁴. In addition, insulin sensitivity was also improved in CideA and FSP27 double-KO mice, and their white and brown adipose tissues showed smaller LDs than those of CideA or FSP27 single deficient mice⁴⁶. These results suggest that in the Cide family, CideA, CideB, and FSP27 are all involved in the enlargement of LD. In particular, FSP27 is essential for unilocular LD formation that is the characteristic LD morphology of WAT.

In humans, the results reported on CideA and CideC are inconsistent with those in mice. CideA and CideC are both expressed in WAT in humans; however, CideA is abundantly expressed in BAT, but not in WAT, in mice. The expression levels of CideA and CideC correlate positively with the insulin sensitivity in obese people⁵⁶. In addition, a patient with homozygous nonsense mutation in CideC was reported to be characterized by lipodystrophic phenotype, i.e., white adipocytes partially with multilocular LDs and insulin resistance supposedly caused by fatty liver⁵⁷. Increased hepatic expressions of CideA and FSP27 were also observed under the condition of liver steatosis in humans⁵⁸. In the mice model, FSP27-deficient mice appeared to be protected against weight gain and insulin resistance^{54, 55}. However, small LDs in WAT were observed, and hepatosteatosis and insulin resistance developed on HFD feeding in adipocyte-specific FSP27 KO mice⁵⁹. Furthermore, insulin resistance and hepatic steatosis developed in FSP27 KO mice that

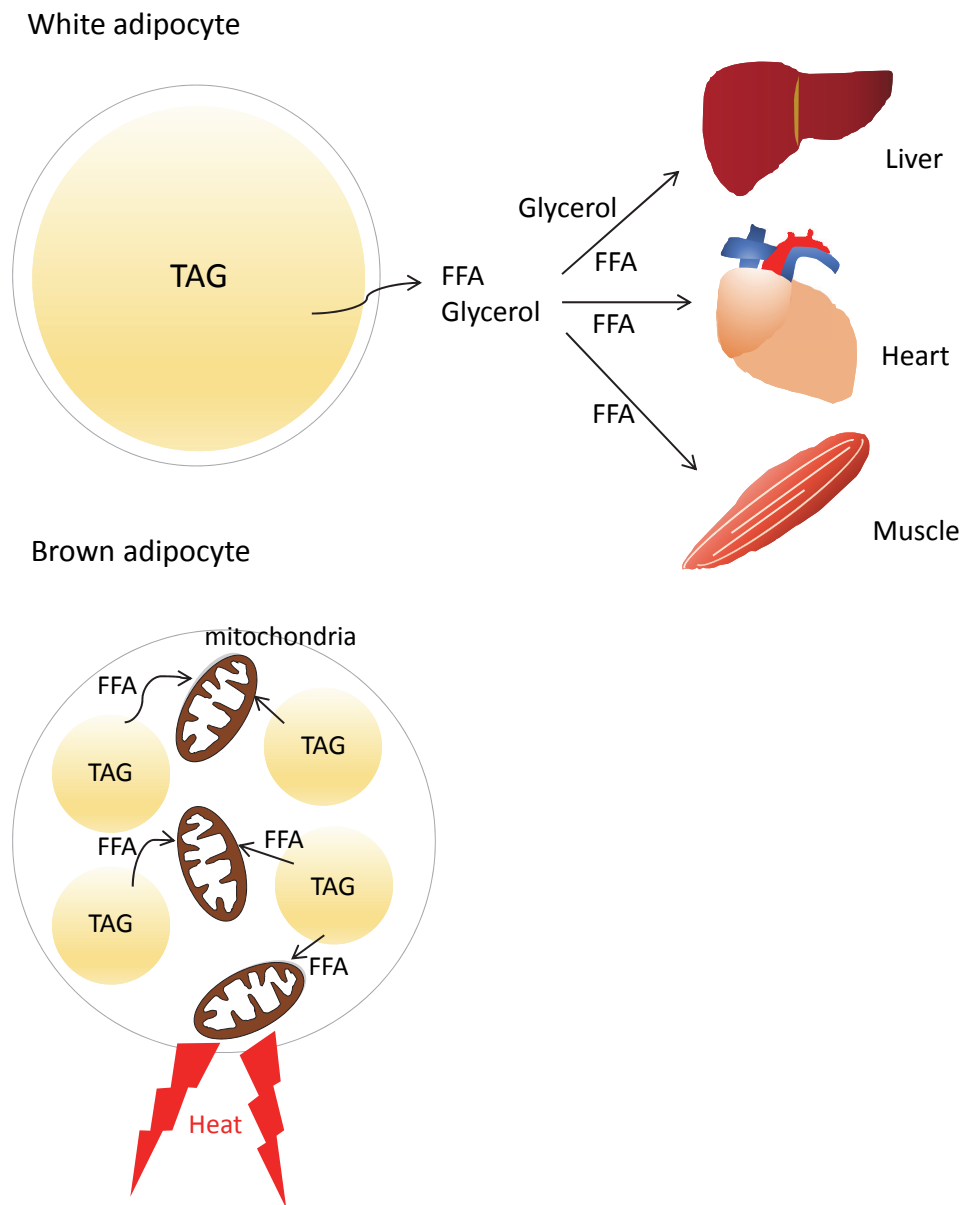


Fig. 1. Lipid droplet formation and function in white and brown adipocytes

White adipocyte stores TAG as a unilocular large LD. In a fasting state, stored TAG is hydrolyzed to FFA and glycerol, which are delivered to the skeletal muscle, heart, and liver. They are utilized as substrates for ATP production in the muscles and heart or for glucose production in the liver. Brown adipocyte forms small multilocular LDs and effectively induces lipolysis from the enlarged total surface area of multilocular LDs. Generated FFAs flow into adjacent mitochondria and are utilized as a source for β -oxidation and the production of heat by uncoupling.

were overloaded with excess lipid in the context of leptin deficiency or long-term exposure to HFD⁶⁰⁾. Anyway, the effects of FSP27 on whole-body energy metabolism may be different between mice in which CideA is expressed exclusively in BAT and humans in whom CideA is also expressed in WAT. However, at least in humans, adipose FSP27 is supposed to improve insulin sensitivity by increasing lipid accumulation in

adipose tissues and, resultantly, minimizing ectopic fat accumulation in other insulin-sensitive tissues, for example, the liver or skeletal muscle. Therefore, it is unclear whether the silencing of FSP27 that resulted in the reduction of WAT mass and the enhanced insulin sensitivity without leading to hepatosteatosis in obese mice⁶¹⁾ can also be of therapeutic application to obese people.

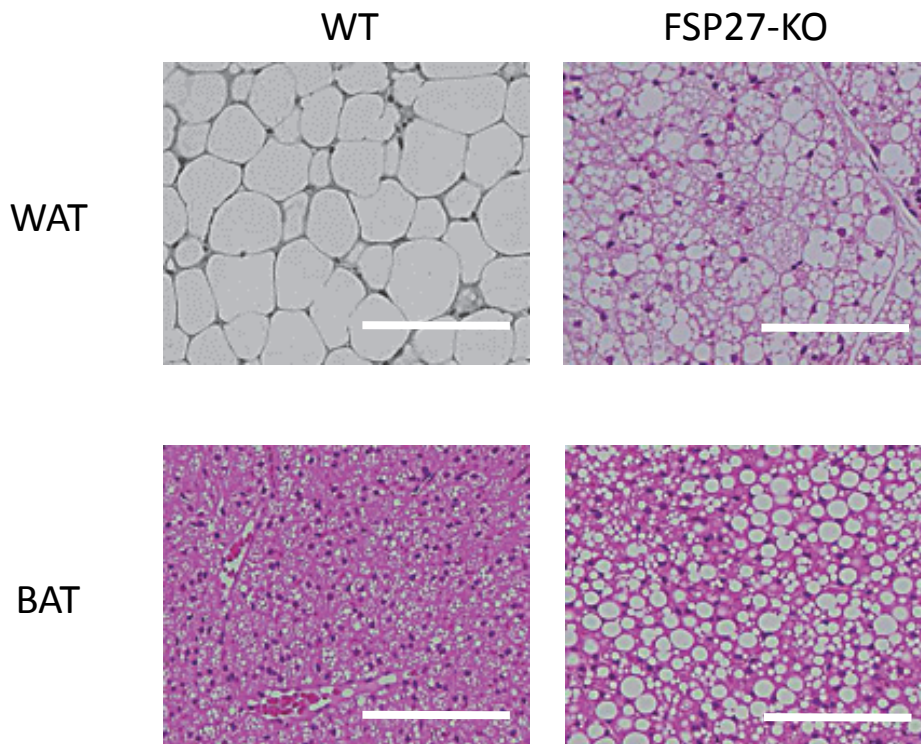


Fig. 2. WAT and BAT of FSP27 KO mouse

Hematoxylin and eosin staining of epididymal WAT and BAT (Scale bar: 100 μ m). LDs are smaller and multilocular in the epididymal WAT of an FSP27 knockout (KO) mouse. In contrast, LDs in the BAT of an FSP27 KO mouse are larger than those of a wild-type (WT) mouse.

FSP27 Regulates the Formation of Large Unilocular LD in White Adipocytes

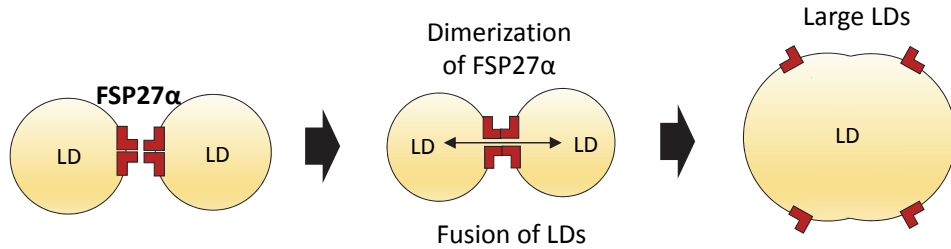
Ectopic expression of FSP27 led to the enlargement of LDs and TAG accumulation in non-adipose cells^{54, 62-65}, whereas depletion of FSP27 in cultured adipocytes resulted in small LD formation and increased lipolysis^{54, 62, 63}. Moreover, depletion of FSP27 by siRNA in HW adipocytes that show the morphological characteristics of white adipocytes resulted in the formation of many small LDs similar to brown adipocytes⁵⁴. In fact, WAT mass was reduced, and the LDs in WAT were also of a small multilocular pattern in FSP27 KO mice (**Fig. 2**)^{54, 55}. Energy expenditure was significantly increased in FSP27 KO mice due to enhanced mitochondrial biogenesis and FFA oxidation in WAT⁵⁴. These results suggest that FSP27 plays essential roles in large unilocular LD formation in WAT. In addition, the mechanism by which FSP27 promotes LD enlargement is also clarified. FSP27 is highly enriched at an LD-LD contact site and forms homodimers that are involved in directional lipid transfer from small to large LDs between adjacent LDs due to the higher internal pressure in small LDs^{66, 67}. Furthermore,

structure-function analysis reveals that the carboxy-terminal domain of FSP27 (amino acids 131–239) plays a crucial role in LD expansion, possibly by homodimerization, whereas the amino-terminal domain (amino acids 1–130) has a supportive role⁶⁵. On the other hand, there are studies showing the importance of the amino-terminal region of FSP27 in promoting LD growth⁶⁷. However, considering the report that a patient carrying a homozygous nonsense mutation in CIDE-C, the human homologue of FSP27, which is predicted to truncate the protein at amino acid 186, shows small and multilocular LDs in white adipocytes⁵⁷, the carboxy-terminal domain of FSP27 is essential for large unilocular LD formation in human WAT. Besides, FSP27 was found to store TAG efficiently in cooperation with several proteins: perilipin 1^{67, 68}, adipose triacylglycerol lipase⁶⁹, and Egr1⁷⁰.

CideA and FSP27 β Coordinately Regulate LD Formation in Brown Adipocytes

Recently, a new isoform of FSP27, FSP27 β , was identified in the liver⁷¹. It contains 10 additional amino acids at the amino-terminal domain of the conventional

White adipocyte



Brown adipocyte

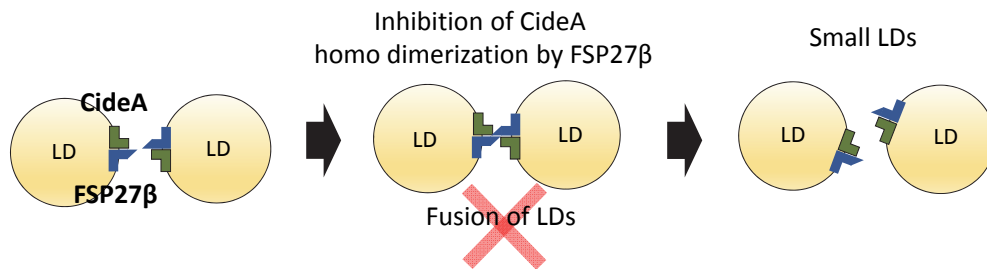


Fig. 3. Proposed mechanisms by which the Cide protein family regulates LD size in WAT and BAT

In WAT, FSP27 α on neighboring LDs forms homodimerization, resulting in the fusion of LD, subsequent lipid exchange, and formation of larger LDs. CideA also promotes large LD formation by forming homodimerization. However, in BAT, FSP27 β inhibits the homodimerization of CideA and suppresses the formation of large LDs, resulting in the formation of multilocular LDs.

isoform of FSP27, designated as FSP27 α . Both isoforms of FSP27 are alternatively transcribed from the same gene and driven by distinct promoters. Although FSP27 α is mainly expressed in WAT, FSP27 β is expressed at a high level in the liver and BAT in mice^{71, 72}. FSP27 α is transcriptionally regulated by PPAR γ , and FSP27 β is regulated by the liver-enriched transcription factor cyclic-AMP-responsive-element-binding protein H (CREBH) in the liver⁷¹. CREBH is activated by ER stress and proinflammatory stimuli and induces the expression of acute phase response genes⁷³. CREBH expression increases during fasting through FFA and PPAR α ⁷⁴. It regulates glucose and lipid metabolism in the liver⁷⁵⁻⁷⁷. CREBH-deficient mice have been reported as developing hepatic steatosis due to increased lipolysis in adipose tissues when fasted or fed a ketogenic diet⁷⁸. A recent study revealed that the loss of CREBH decreases the hepatic expression level of FSP27 β in fasted mice, and the overexpression of CREBH induces LD growth and TAG accumulation through FSP27 β by suppressing lipolysis in the liver of mice⁷¹. In the liver, FSP27 β is thought to be associated with LD growth in the same manner as FSP27 α ⁷¹. FSP27 β is also expressed in BAT along with the simultaneous

abundant expression of CideA⁷². However, the roles of FSP27 β in BAT were not clarified.

Although CideA is known to be exclusively expressed in BAT and known to promote LD enlargement, the molecular mechanism of CideA that forms the small multilocular LD in BAT has remained unknown. We recently demonstrated that CideA and FSP27 β coordinately regulate LD formation in brown adipocytes⁷². We found that the overexpression of FSP27 α or CideA promoted the formation of large LDs in COS cells, as was reported previously; however, the sole expression of FSP27 β did not induce the enlargement of LDs⁷². Interestingly, the simultaneous overexpression of FSP27 β and CideA in COS cells that imitated the expression pattern of the Cide family proteins in BAT resulted in smaller LD formation compared with the cells that overexpressed CideA only. RNAi-mediated FSP27 β knockdown in HB2 adipocytes, which possess the characteristics of brown adipocytes, resulted in enlarged LDs. Furthermore, FSP27 KO mice in which FSP27 β was not expressed in BAT had larger LDs in BAT compared with wild-type mice (**Fig. 2**). FSP27 β and CideA formed a complex in the BAT of mice⁷². FSP27 β inhibited the homodimeriza-

Table 1. Characteristics and functions of two FSP27 isoforms

	FSP27 α	FSP27 β
Structure	1-239 amino acids	10 amino acids longer at the N-terminus of FSP27 α
Major expression tissues	WAT	Liver, BAT
Transcription factor	PPAR γ	CREBH (liver), Unknown (BAT)
Function in adipose tissue	Promote the formation of large lipid droplet in white adipocytes ↓ Effective energy storage in WAT	Maintain the formation of small lipid droplets in brown adipocytes ↓ Increase energy expenditure in BAT

tion of CideA by binding to CideA in COS cells⁷². Co-expressed Cide proteins, including CideA, CideB, and FSP27, are shown to localize on the LD surface and form a complex at the LD-LD contact site in non-adipose cells⁴⁹. These results indicate that FSP27 β negatively regulates CideA-promoted enlargement of LD size by inhibiting the homodimerization of CideA on the LD surface of brown adipocytes (**Fig. 3**). β 3-adrenergic agonist-stimulated oxygen consumption was increased in isolated white adipocytes from FSP27 KO mice that show multilocular LD. In contrast, oxygen consumption was reduced in isolated brown adipocytes of FSP27 KO mice that showed large LDs compared with those of wild-type mice⁵⁴. Given that the multiloculization of LDs resulted in increased oxygen consumption in white adipocytes and the enlargement of LDs resulted in decreased oxygen consumption in brown adipocytes, cellular LD morphology can affect cellular energy metabolism. Thus, FSP27 β , which is essential for small multilocular LD formation, may be a potential target for application in therapies aiming to switch adipocytes into energy-dissipating adipocytes.

Summary

FSP27 α and FSP27 β are indispensable when regulating the morphology of LDs in adipocytes. FSP27 α promotes unilocular LD growth in white adipocytes, and FSP27 β inhibits the enlargement of LDs induced by CideA and contributes to the multilocularization of LDs in brown adipocytes (**Table 1**). Consequently, Cide family proteins regulate energy metabolism efficiently through the modulation of intracellular LD morphology in WAT and BAT.

Conflict of Interest Statement

The authors have no conflicts of interest.

Footnotes

The following abbreviations are used: WAT, white adipose tissue; BAT, brown adipose tissue; LD, lipid droplet; FSP27, fat-specific protein 27; Cide, cell death-inducing DFF45-like effector; TAG, triacylglycerol; ER, endoplasmic reticulum; FFA, free fatty acids; KO, knockout; HFD, high-fat diet; CREBH, cyclic-AMP-responsive-element-binding protein H.

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