A Novel Proangiogenic Function of Fsp27 in Endothelium: You Only Live Thrice?

Chieko Mineo, PhD

Fsp27 (fat-specific protein, 27 kD) also known as Cidec, is a member of the cell death–inducing DNA fragmentation factor 45–like effector (CIDE) family, which also includes Cidea and Cideb.[1,2] CIDE family proteins contain an N-terminal CIDE-N domain that shares homologous sequence to other DNA fragmentation factors and a unique C-terminal CIDE-C domain. Fsp27/Cidec was originally discovered as a gene highly expressed in both white and brown adipose tissues, the expression of which is upregulated during adipogenesis.[3] While the function of Fsp27 in the adipose tissue was yet to be clarified, the protein was rediscovered as an apoptotic protein through a screening to search for genes homologous to the CIDE-N domain of DNA fragmentation factor 45.[4,5] The apoptotic function of CIDE proteins was demonstrated through ectopic overexpression of the wild-type or mutant proteins in cultured mammalian cells.[4] However, it was subsequently shown that Fsp27 was not localized to mitochondria, where it would induce apoptosis and DNA fragmentation.[6] Instead, Fsp27 is localized to lipid droplets in adipocytes in a highly specific manner, where it has been shown to modulate storage of triglycerides in lipid droplets.[6–8] Fsp27 is required for enlargement of lipid droplets, and it attenuates lipolysis through negative regulation of adipose triglyceride lipase, the rate-limiting enzyme in the lipolytic pathway.[9,10] Depletion of Fsp27 in cultured adipocytes markedly increases the rate of lipolysis,[11] and mice lacking the protein globally have smaller lipid droplets in white adipose tissue and higher lipolysis rates, compared with wild-type mice.[8] As a result, the knockout mice are protected from obesity and insulin resistance induced by high-fat diet. Furthermore, Fsp27 deficiency leads to a reduction in fat accumulation and improved insulin sensitivity in ob/ob mice.[12] In contrast to animal models, in humans a homozygous nonsense mutation in the Fsp27 gene has been associated with partial lipodystrophy and insulin resistance.[13] Human studies further revealed that Fsp27 expression in adipose tissue is reduced with increases in adiposity and that it is associated with insulin sensitivity as indicated by lower homeostatic model assessment of insulin resistance in obese individuals.[1,14] One of the studies also found that bariatric surgery–induced weight loss results in an increase in Fsp27 expression in subcutaneous adipose tissue. Despite the discrepancies in manifestation of the metabolic phenotypes, these results in mouse models of obesity and in humans collectively indicate that Fsp27 plays a major role in lipid metabolism in adipose tissue affecting systemic metabolic health.

In the current issue of the *Journal of the American Heart Association* (*JAHA*), Karki et al have discovered Fsp27 in an unexpected place: in endothelial cells derived from adipose tissue microvasculature.[15] Using quantitative immunofluorescence, they have shown that Fsp27 is abundantly expressed in the primary endothelial cells isolated from human adipose tissues, and that the expression is lower in cells isolated from visceral fat depots compared with those from subcutaneous depots. They have further demonstrated that addition of recombinant Fsp27 ex vivo augments insulin-induced vasodilation in arterioles isolated from human visceral fat. Together with the previous finding by the authors' group indicating that insulin-induced vasodilation ex vivo is attenuated in arterioles from visceral fat compared with subcutaneous fat,[16] the data suggest that lower expression of Fsp27 may contribute to endothelial dysfunction in obese visceral fat depots. In culture, treatment of primary endothelial cells with recombinant Fsp27 enhanced stimulatory phosphorylation of Akt and endothelial nitric oxide synthase, as well as in vitro angiogenesis. Silencing of Fsp27 in endothelial cells showed opposite effects on the endothelial functions. The importance of these discoveries is further emphasized in the studies comparing microvascular endothelial cells isolated from fat depots extracted from the patients before and after weight

From the Department of Pediatrics, Center for Pulmonary and Vascular Biology, University of Texas Southwestern Medical Center, Dallas, TX.

Correspondence to: Chieko Mineo, PhD, Department of Pediatrics and Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9063. E-mail: chieko.mineo@utsouthwestern.edu

J Am Heart Assoc. 2019;8:e013042. DOI: 10.1161/JAHA.119.013042.

loss by bariatric surgery. There was a significant increase in expression levels of Fsp27 and an enhancement in angiogenic capacity after weight loss. A positive correlation was observed between cellular Fsp27 levels and angiogenic capacity. Mechanistically, the study found that in the cultured endothelial cells, Fsp27 co-immunoprecipitates with vascular endothelial growth factor (VEGF)-A and that silencing of Fsp27 results in loss of VEGF-A, suggesting that Fsp27 may regulate vasodilation and angiogenesis through the VEGF-A/VEGFR2 pathway. Collectively, the work by Karki et al has uncovered a novel function of Fsp27 in endothelial cells that may contribute to maintenance of normal vascular functions in the adipose tissue.

As is often the case with novel discoveries, the work by Karki et al raises many interesting questions.[15] First, the human epidemiological studies indicate that Fsp27 levels in adipocytes are positively associated with insulin sensitivity, whereas in mice, global loss of Fsp27 is associated with protection from obesity-induced insulin resistance.[1,2,8] Interestingly, adipocyte-specific Fsp27 deletion in mice, in which endothelial Fsp27 expression is presumably unaffected, results in lower adiposity with smaller adipocytes and resistance to high fat diet–induced body weight gain; however, it leads to hepatosteatosis and systemic insulin resistance.[17] Therefore, it is of great importance to determine whether an endothelium-specific manipulation of Fsp27 alters systemic metabolic health. Does an elevation of endothelial Fsp27 provide protection against obesity-induced vascular dysfunction and insulin resistance? Does a deletion of endothelial Fsp27 result in aggravation of metabolic abnormalities? Future experiments in mice with selective Fsp27 overexpression or deletion in endothelium will answer these questions. Secondly, what is the molecular basis of Fsp27 actions on VEGF-A? The results by Karki et al have shown that Fsp27 interacts with VEGF-A and that Fsp27 silencing causes marked downregulation of VEGF-A. In parallel to the actions of Fsp27 in endothelium, Fsp27 regulates lipolysis in adipocytes through 2 independent modes of action; it can directly interact with adipose triglyceride lipase to inhibit lipolytic function and it can also suppress adipose triglyceride lipase transcription by facilitating the inhibitory effect of transcription factor Egr1.[9,10] In endothelium, is the Fsp27 interaction with VEGF-A required for Fsp27 actions? Does Fsp27 regulate VEGF-A expression at the transcriptional level? Does Fsp27-VEGF-A interaction modulate the VEGF-A/VEGFR2 signaling independent of its regulation of expression? Additionally, it is well known that adipocytes abundantly produce VEGF-A.[18] VEGF expression may be regulated by Fsp27 in a similar manner in adipocytes. Further mechanistic investigations in both cell types are warranted. Third, what are the sources of Fsp27 that modulate endothelial functions? The work by Karki et al showed exogenous addition of recombinant Fsp27 and manipulation of Fsp27 production by the endothelial cells are both effective in modulating the cellular functions. Does Fsp27 produced by endothelium act on VEGF-A intracellularly or in an autocrine manner? Alternatively, does Fsp27 produced by neighboring adipocytes play a role? A recent finding has revealed that there is a close communication between endothelium and adipocytes in the adipose tissue via exosomes.[19] Tissue-specific deletion of Fsp27 in mice will also answer some of these questions. Fourth, recent attempts to silence Fsp27 using anti-sense oligonucleotides in high-fat-fed mice or ob/ob mice successfully decreased visceral adiposity, improved insulin sensitivity, and lowered liver steatosis.[20,21] The current study by Karki et al suggests that increasing Fsp27 in endothelium may provide protection against vascular dysfunction induced by obesity. As such, potential interventions to boost Fsp27 expression need to be specific for endothelium, which would require an endothelial cell–specific delivery system.

Endothelial cell dysfunction in adipose tissue results in suppression of angiogenesis during adipose expansion, resulting in local hypoxia and inflammation that contribute to systemic metabolic abnormality.[18] Manipulations to enhance angiogenesis facilitate healthy adipose tissue expansion, associated with attenuated inflammation and fibrosis of adipose tissue. In contrast, inadequate angiogenesis leads to adipose tissue hypoxia, enlarged adipocyte size, increased inflammation, and fibrosis, representing an unhealthy pattern in adipose tissue expansion. As such, promoting angiogenesis during early adipose tissue expansion has beneficial effects on systemic metabolic homeostasis. Several studies have proven that local activation of the VEGF-A/VEGFR2 signaling in adipose tissue results in improved vascularization and resistance to high-fat-diet-induced metabolic dysfunction.[22,23] The current study by Karki et al provides a novel mechanism that is intrinsic to endothelial cells, by which Fsp27 regulates VEGF-A expression to potentially influence metabolic phenotypes of the adipose tissue during fat expansion associated with obesity.

Disclosures

None.

References

1. Gao G, Chen FJ, Zhou L, Su L, Xu D, Xu L, Li P. Control of lipid droplet fusion and growth by CIDE family proteins. *Biochim Biophys Acta Mol Cell Biol Lipids*. 2017;1862:1197–1204.
2. Slayton M, Gupta A, Balakrishnan B, Puri V. CIDE proteins in human health and disease. *Cells*. 2019;8:E238.
3. Danesch U, Hoeck W, Ringold GM. Cloning and transcriptional regulation of a novel adipocyte-specific gene, FSP27. CAAT-enhancer-binding protein (C/EBP) and C/EBP-like proteins interact with sequences required for differentiation-dependent expression. *J Biol Chem*. 1992;267:7185–7193.

4. Liang L, Zhao M, Xu Z, Yokoyama KK, Li T. Molecular cloning and characterization of CIDE-3, a novel member of the cell-death-inducing DNA-fragmentation-factor (DFF45)-like effector family. *Biochem J*. 2003;370:195–203.
5. Inohara N, Koseki T, Chen S, Wu X, Nunez G. CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. *EMBO J*. 1998;17:2526–2533.
6. Puri V, Konda S, Ranjit S, Aouadi M, Chawla A, Chouinard M, Chakladar A, Czech MP. Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. *J Biol Chem*. 2007;282:34213–34218.
7. Keller P, Petrie JT, De RP, Gerin I, Wright WS, Chiang SH, Nielsen AR, Fischer CP, Pedersen BK, MacDougald OA. Fat-specific protein 27 regulates storage of triacylglycerol. *J Biol Chem*. 2008;283:14355–14365.
8. Nishino N, Tamori Y, Tateya S, Kawaguchi T, Shibakusa T, Mizunoya W, Inoue K, Kitazawa R, Kitazawa S, Matsuki Y, Hiramatsu R, Masubuchi S, Omachi A, Kimura K, Saito M, Amo T, Ohta S, Yamaguchi T, Osumi T, Cheng J, Fujimoto T, Nakao H, Nakao K, Aiba A, Okamura H, Fushiki T, Kasuga M. FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. *J Clin Invest*. 2008;118:2808–2821.
9. Grahn TH, Kaur R, Yin J, Schweiger M, Sharma VM, Lee MJ, Ido Y, Smas CM, Zechner R, Lass A, Puri V. Fat-specific protein 27 (FSP27) interacts with adipose triglyceride lipase (ATGL) to regulate lipolysis and insulin sensitivity in human adipocytes. *J Biol Chem*. 2014;289:12029–12039.
10. Singh M, Kaur R, Lee MJ, Pickering RT, Sharma VM, Puri V, Kandror KV. Fat-specific protein 27 inhibits lipolysis by facilitating the inhibitory effect of transcription factor Egr1 on transcription of adipose triglyceride lipase. *J Biol Chem*. 2014;289:14481–14487.
11. Ito M, Nagasawa M, Hara T, Ide T, Murakami K. Differential roles of CIDEA and CIDEC in insulin-induced anti-apoptosis and lipid droplet formation in human adipocytes. *J Lipid Res*. 2010;51:1676–1684.
12. Toh SY, Gong J, Du G, Li JZ, Yang S, Ye J, Yao H, Zhang Y, Xue B, Li Q, Yang H, Wen Z, Li P. Up-regulation of mitochondrial activity and acquirement of brown adipose tissue-like property in the white adipose tissue of fsp27 deficient mice. *PLoS One*. 2008;3:e2890.
13. Rubio-Cabezas O, Puri V, Murano I, Saudek V, Semple RK, Dash S, Hyden CS, Bottomley W, Vigouroux C, Magre J, Raymond-Barker P, Murgatroyd PR, Chawla A, Skepper JN, Chatterjee VK, Suliman S, Patch AM, Agarwal AK, Garg A, Barroso I, Cinti S, Czech MP, Argente J, O'Rahilly S, Savage DB. Partial lipodystrophy and insulin resistant diabetes in a patient with a homozygous nonsense mutation in CIDEC. *EMBO Mol Med*. 2009;1:280–287.
14. Puri V, Ranjit S, Konda S, Nicoloro SM, Straubhaar J, Chawla A, Chouinard M, Lin C, Burkart A, Corvera S, Perugini RA, Czech MP. Cidea is associated with lipid droplets and insulin sensitivity in humans. *Proc Natl Acad Sci USA*. 2008;105:7833–7838.
15. Karki S, Farb MG, Sharma V, Jash S, Zizza EJ, Hess DT, Carmine B, Carter CO, Pernar LI, Apovian CM, Puri V, Gokce N. Fat specific protein 27 regulation of vascular function in human obesity. *J Am Heart Assoc*. 2019;8:e011431. DOI: 10.1161/JAHA.118.011431.
16. Farb MG, Karki S, Park SY, Saggese SM, Carmine B, Hess DT, Apovian C, Fetterman JL, Breton-Romero R, Hamburg NM, Fuster JJ, Zuriaga MA, Walsh K, Gokce N. WNT5AJNK regulation of vascular insulin resistance in human obesity. *Vascular Medicine*. 2016;21:489–496.
17. Tanaka N, Takahashi S, Matsubara T, Jiang C, Sakamoto W, Chanturiya T, Teng R, Gavrilova O, Gonzalez FJ. Adipocyte-specific disruption of fat-specific protein 27 causes hepatosteatosis and insulin resistance in high-fat diet-fed mice. *J Biol Chem*. 2015;290:3092–3105.
18. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab*. 2013;18:478–489.
19. Crewe C, Joffin N, Rutkowski JM, Kim M, Zhang F, Towler DA, Gordillo R, Scherer PE. An endothelial-to-adipocyte extracellular vesicle axis governed by metabolic state. *Cell*. 2018;175:695–708.
20. Langhi C, Arias N, Rajamoorthi A, Basta J, Lee RG, Baldan A. Therapeutic silencing of fat-specific protein 27 improves glycemic control in mouse models of obesity and insulin resistance. *J Lipid Res*. 2017;58:81–91.
21. Rajamoorthi A, Arias N, Basta J, Lee RG, Baldan A. Amelioration of diet-induced steatohepatitis in mice following combined therapy with ASO-Fsp27 and fenofibrate. *J Lipid Res*. 2017;58:2127–2138.
22. Sung HK, Doh KO, Son JE, Park JG, Bae Y, Choi S, Nelson SM, Cowling R, Nagy K, Michael IP, Koh GY, Adamson SL, Pawson T, Nagy A. Adipose vascular endothelial growth factor regulates metabolic homeostasis through angiogenesis. *Cell Metab*. 2013;17:61–72.
23. Robciuc MR, Kivela R, Williams IM, de Boer JF, van Dijk TH, Elamaa H, Tigistu-Sahle F, Molotkov D, Leppanen VM, Kakela R, Eklund L, Wasserman DH, Groen AK, Alitalo K. VEGFB/VEGFR1-induced expansion of adipose vasculature counteracts obesity and related metabolic complications. *Cell Metab*. 2016;23:712–724.

Key Words: Editorials • adipose depot • adiposity • angiogenesis • endothelial cell • obesity • vascular biology