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FSP27 and PLIN1 interaction promotes the formation of large lipid droplets in human adipocytes

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Abstract

Human adipocytes express high levels of two distinct lipid droplet proteins, Fat Specific Protein 27 (FSP27; also called CIDEC), a member of the CIDE family, and perilipin1 (PLIN1), a member of the PAT family. Both proteins play a role in fat metabolism in adipocytes, but how they interact is not known. Our present study demonstrates that FSP27 and PLIN1 co-localize and interact in cultured human primary adipocytes. We also found that the C-terminal domain of FSP27, aa 120–220, interacts with PLIN1. Individual expression of exogenous FSP27 or PLIN1 increased triglyceride content and decreased glycerol release (a measure of lipolysis), but co-expression of both proteins did not further increase triglyceride content or decrease lipolysis in human adipocytes. However, the combination of PLIN1 and FSP27 increased the average size of lipid droplets or caused the formation of unilocular adipocytes. Our data suggest that FSP27 interacts with PLIN1 to regulate lipid droplet size in human adipocytes in a concerted manner.

Keywords

Adipocytes; fat metabolism; fatty acids; glycerol; fat; lipolysis

1. Introduction

Cellular lipid droplets (LDs), now considered to be dynamic intracellular organelles, are composed of a core of neutral lipids surrounded by a phospholipid monolayer and associated proteins [1; 2; 3; 4; 5]. Proteins associated with the surface of LDs contribute to the biogenesis, maturation and stability of these organelles [1; 6]. Of the LD-associated proteins, the best-characterized are members of the PAT family, also called the perilipin (Plin) family of proteins [7; 8; 9; 10; 11]. They act as a scaffold at the LD surface and are suggested to have a structural and/or regulatory role in LD formation and function [8; 10; 12].

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PLIN1 (also called Perilipin1) is highly expressed in adipocytes and plays a crucial role in regulating basal and stimulated lipolysis [8; 13]. Under basal conditions, PLIN1 prevents excess lipolysis by limiting access of hormone sensitive lipase, ATGL and its co-activator CGI-58 to LDs [14; 15; 16; 17; 18]. Upon β -adrenergic stimulation, protein kinase A (PKA) phosphorylates PLIN1 and causes the release of CGI-58 so it can bind and stimulate ATGL and also allows HSL to translocate to the LD surface [18; 19]. PLIN1 knockout increases basal lipolysis and decreases LD size in adipocytes and causes resistance to diet induced obesity in mice [20; 21]. In humans, lower PLIN1 expression is associated with higher rates of lipolysis [21; 22], and mutation in PLIN1 [23; 24], or its expression in obese human adipose tissue [25], correlates positively with insulin sensitivity.

FSP27 (also called CIDEC in humans) is also abundantly expressed on the LD surface [26; 27; 28] and has been shown to be crucial for the fusion of smaller LDs into larger ones [29; 30] and to promote triglyceride (TG) accumulation [26; 27; 28; 31; 32]. A mutation in CIDEC results in multilocular adipocytes associated with partial lipodystrophy and insulin resistance in a human subject [33]. Also, CIDEC expression was higher in visceral fat from insulin sensitive compared to insulin resistant obese humans [25]. These studies suggest that FSP27 plays an important role in regulating LD morphology and fat metabolism in adipocytes.

In 3T3-L1 adipocytes, exogenously expressed FSP27 co-localizes with endogenous PLIN1 at the LD surface [27]. However, the interaction of these proteins and its consequences for LD morphology and TG accumulation have not yet been reported. In the present study we analyzed the distribution of endogenous FSP27 and PLIN1 in cultured human primary adipocytes and tested the possibility that PLIN1 and FSP27 interact with each other. Furthermore, we analyzed the role of their interaction in regulating the LD morphology and TG accumulation in human adipocytes.

2. Materials and Methods

2.1. Materials

All chemicals were purchased from Sigma (St Louis, MO), except Rosiglitazone (Merck, Rahway, NJ), recombinant human insulin (Lilly, Indianapolis, IN), HCS LipidTOX-Deep Red (Invitrogen, CA). Fetal bovine serum and culture media were obtained from Invitrogen (Carlsbad, CA).

2.2. Cell culture

Human primary preadipocytes, procured from the Boston Nutrition Obesity Research Center adipocyte core, were cultured and differentiated as previously described [34].

2.3. Immunostaining

For determination of PLIN1 and FSP27 localization, cells were cultured on coverslips. The immunostaining was performed using guinea pig anti-perilipin polyclonal antibody (1:1000 dilution; Research Diagnostics Inc., Flanders, NJ) and FSP27 monoclonal antibodies (1:1000 dilution) as described [27].

2.4. Lentivirus production and transduction

293T cells were seeded in 10 cm plates. Recombinant lentiviruses were produced by a five-plasmid transfection procedure as described [35]. The packaged recombinant lentiviruses were harvested from the supernatant of cell cultures 48 h after transfection and filtered through 0.45- μ m filters. 500 μ l supernatant and 10 μ g/ml Polybrene was added to each well of a 12 well plate containing differentiated human adipocytes; after overnight incubation, the

medium was changed to a regular maintenance medium. Protein expression was observed after 4 days of transduction.

2.5. Adenovirus transduction

PLIN1-Flag tagged and FSP27-HA tagged adenoviruses were generated at the Adenoviral Vector Core Facility at Tufts Medical Center. Virus was added at m.o.i of 100 to the human adipocytes. Cells were analyzed for protein expression after 24 or 48 h of infection.

2.6. Immunoprecipitation

Fully differentiated human adipocytes in 10 cm plates were transduced with recombinant adenovirus or lentivirus. Immunoprecipitations were carried out using antibodies as we described previously [36].

2.7. Lipid droplet staining

Cells plated on glass cover slips were washed twice with PBS, fixed in 4% formaldehyde for 20 minutes and quenched with 0.1 M glycine. Cells were then incubated with 0.5 μ g/ml of Nile Red or HCS LipidTOX-Deep Red stain for 30 minutes and then washed with PBS.

2.8. Microscopy

Microscopy was performed using a Zeiss LSM 710-Live Duo scan (Carl Zeiss, Oberkochen, Germany) with a 100X oil immersion objective. Images were processed using Metamorph imaging software, version 6.1 (Universal Imaging, Downingtown, PA).

2.9. Lipolysis and Triglyceride Determination

The cultured adipocytes were washed twice with PBS and incubated in Krebs-Ringer bicarbonate HEPES buffer supplemented with 4% bovine serum albumin. The buffer was collected after 2.5 hr of incubation for assaying glycerol as a measure of lipolysis. For triglyceride measurement the cells were lysed with cell lysis buffer (CellSignal). Glycerol and triglyceride were quantified using the Triglyceride Determination Kit (Sigma) according to the manufacturer's instructions.

3. Results

3.1. FSP27 co-localizes with PLIN1 in human adipocytes

Our previous study showed that exogenously expressed GFP-FSP27 co-localizes with endogenous PLIN1 in 3T3-L1 adipocytes [27]. Whether endogenous FSP27 and PLIN1 co-localize is not yet known. Therefore, in the present study the distribution of endogenous proteins was studied by immunofluorescence using monoclonal anti-FSP27 and polyclonal anti-perilipin antibodies. Fig. 1A shows localization of endogenous FSP27 and PLIN1 on the surface of a single LD in a cultured human adipocyte. Although FSP27 and PLIN1 did not completely overlap, the distribution pattern suggested that the two proteins might be in the same complex at the surface of the LD. Interestingly, both FSP27 and PLIN1 were also distributed apart from LDs (Fig. 1B). It could be that besides LDs these proteins are present in the endoplasmic reticulum or on minute LDs. In fact, recent studies have shown that FSP27 [37] and PLIN1 (Skinner et al. Adipocyte Journal (in press)) also localize to the endoplasmic reticulum in adipocytes.

3.2. FSP27 interacts with PLIN1

Based upon the distribution pattern of endogenous FSP27 and PLIN1, we hypothesized that these proteins might interact with each other. To study their interaction, PLIN1-Flag and FSP27-HA constructs were used to produce lenti-viral preparations and infect mature human

adipocytes. Anti-Flag and anti-HA antibodies were used to pull down the proteins. As shown in Fig. 1(C–D), endogenous PLIN1 co-immunoprecipitated with FSP27-HA (Fig. 1C) and PLIN1-Flag co-immunoprecipitated endogenous FSP27 (Fig. 1D) in human adipocytes. We therefore could pull down PLIN1 with FSP27 and vice versa. In order to confirm that endogenous PLIN1 and endogenous FSP27 interact with each other, we immunoprecipitated PLIN1 using PLIN1 antibodies and immunoblotted for FSP27. As expected, endogenous FSP27 co-immunoprecipitated with endogenous PLIN1 in human adipocytes (Fig. 1E).

To identify the domain of FSP27 interacting with PLIN1, we first tested if either the N- or C-terminus of FSP27 could pull down PLIN1. HA tagged aa 1–120 (N-terminus) and aa 120–239 (C-terminus) were co-expressed with PLIN1 in COS7 cells, which do not have endogenous expression of FSP27 or PLIN1. HA antibodies were used for immunoprecipitation. Only the C-terminal domain co-immunoprecipitated PLIN1 (data not shown), suggesting that C-terminus of FSP27 is responsible for its interaction with PLIN1. In a recent study we showed that amino acids 173–220 of FSP27 target its localization to LDs and play a role in LD clustering, whereas a fusogenic domain of FSP27 (aa 120–210) is sufficient for both clustering and LD fusion [29]. Therefore, we tested the domain aa 120–220, which spans both functional domains of FSP27 and belongs to the C-terminus region, for its interaction with PLIN1. HA-FSP27(120–220) was expressed in human adipocytes using a lentivirus. HA antibodies were used to immunoprecipitate FSP27. As shown in Fig. 1F, endogenous PLIN1 co-immunoprecipitated with FSP27(120–220), showing that at least aa 120–220 of FSP27 are involved in its interaction with PLIN1.

3.3. Effect of exogenous FSP27 and PLIN1 co-expression on triglyceride accumulation and lipolysis in human adipocytes

Overexpression of FSP27 or PLIN1 in adipocytes enhances TG storage [26; 27; 38], whereas FSP27 or PLIN1 depletion increases basal lipolysis in adipocytes [27; 38; 39; 40]. Therefore, in order to test if FSP27 and PLIN1 have additive or synergistic effect on TG accumulation or lipolysis in human adipocytes, we first examined expression levels of FSP27 and PLIN1 after adenoviral transfection of cultured human adipocytes. As shown in Fig. 2A, there was a 3- to 4-fold increase in the expression of both proteins. We then measured glycerol release into the medium, as a measure of lipolysis, and total TG in the cells. As shown in Fig. 2B, 48 hr after adenoviral-mediated overexpression of FSP27 or PLIN1 in human adipocytes, the total TG amount increased by about 40% and 70 %, respectively, but there was no significant further increase in total TG after 48 hrs of FSP27 and PLIN1 co-overexpression. Similar results were obtained after 72 hr of overexpressing FSP27 and/or PLIN1, that is, no difference in lipolysis or TG after co-overexpressing exogenous FSP27 and PLIN1 compared to the individually overexpressed proteins (data not shown). Consistent with this observation, FSP27 and PLIN1 individually decreased the accumulation of glycerol in the medium by about 30% and 60%, respectively (Fig. 2C). However, there was no additional effect of FSP27 and PLIN1 co-overexpression on glycerol release.

3.4. Effect of FSP27 and PLIN1 co-overexpression on lipid droplet size in adipocytes

FSP27 knockdown causes fragmentation of LDs in adipocytes [27; 40]. Also, we and others recently demonstrated the role of FSP27 in regulating LD morphology [29; 30]. Therefore, we hypothesized that FSP27-PLIN1 interaction might facilitate the increase in LD size in human adipocytes. To test our hypothesis, we infected mature human adipocytes with FSP27 and/or PLIN1 adenovirus and studied the LD morphology. At 48–72 h after infection almost 25% of the FSP27 and PLIN1 co-expressing adipocytes showed unilocular droplets (Fig. 3A). Other cells showed either enlarged multiple droplets with tiny droplets

surrounding them, or a much enlarged single droplet with almost 3–4 times the average radius of other droplets. A similar increase in LD size was observed when the PLIN1 interacting domain of FSP27, aa 120–220, was co-expressed with PLIN1 (data not shown). Quantitatively, the range of LD size was increased (Fig. 3B) in agreement with the average decrease in number of LDs (Fig. 3C) in adipocytes transduced with FSP27, PLIN1 or FSP27+PLIN1, with a larger effect in FSP27+PLIN1. These results suggest that both FSP27 and PLIN1 regulate the morphology of LDs in a concerted manner.

4. Discussion

We here highlight the role of FSP27-PLIN1 interaction in regulating LD morphology in human adipocytes. We established that endogenous FSP27 and PLIN1 co-localize at the surface of LD in human adipocytes. Co-IP studies showed that these two proteins interact either directly or indirectly. Furthermore, we identified that the C-terminus domain, aa 120–220, of FSP27 interacts with PLIN1. The ability of FSP27 to increase LD size is further enhanced by PLIN1. After 48–72 h of co-expressing FSP27 and PLIN1 in cultured human adipocytes, unilocular LDs were formed in at least 25% of the cells. This morphological change was not associated with further accumulation of TG or a further decline in lipolysis when compared to the individually expressed proteins.

Our recent study identified the LD fusogenic potential of FSP27 [29]. Although direct evidence is lacking, it is likely that PLIN1 acts as a scaffold for FSP27 at the LD surface where FSP27 facilitates the fusion of LDs. A similar study from another group showed that exogenously expressed FSP27 concentrates at the contact site of droplets and promotes LD growth by lipid transfer between the droplets [30], whereas in our studies we did not observe a distinct distribution of endogenous FSP27 or PLIN1 at the contact site of droplets in human adipocytes (Fig. 1B). Probably a more complex process of membrane dynamics than simply a lipid transfer between droplets is involved in their fusion. Proteins like SNARE's, which have been shown to mediate fusion between cytosolic LDs, could be involved in this integrated process [41]. Further studies are required to establish a mechanism of LD fusion in adipocytes. Our present study suggests a concerted action of FSP27 and PLIN1 in promoting the enlargement of LDs. The expression of exogenous FSP27 in COS7 cells, which do not express endogenous FSP27 or PLIN1, increases LD size [29]. These enlarged droplets in COS7 cells are much smaller than the droplets in adipocytes, suggesting that the presence of both PLIN1 and FSP27 is required for the formation of enlarged LDs in adipocytes. Our observations are further supported by *in vivo* studies showing that FSP27 knockout mice have multilocular white adipocytes [31; 32; 42], and a mutation in human FSP27 (CIDEA) also mimics the multilocular phenotype in white adipose tissue [33].

It is commonly believed that the decrease in relative surface area on increasing LD size decreases the access of lipases and thus decreases lipolysis. While both PLIN1 and FSP27 overexpression increased triglyceride accumulation, PLIN1 overexpression increased triglyceride accumulation to a greater extent than FSP27. However, co-expression of both proteins had no additional effect on triglyceride content and lipolysis as compared to PLIN1 despite causing formation of larger and in many cases unilocular LDs. This strongly suggests that inhibition of lipolysis is a direct function of these LD surface proteins themselves and not an indirect result of the change in LD morphology, though clearly these two proteins also have important and synergistic roles in controlling LD morphology in adipocytes.

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Abbreviations

FSP27	Fat specific protein 27
PLIN1	Perilipin 1
TG	triglyceride
aa	Amino acids
LD	lipid droplet

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Highlights

- FSP27 interacts with PLIN1, and aa 120–220 of FSP27 play a role in this interaction.
- FSP27 and PLIN1 co-localize at the lipid droplet surface in human adipocytes.
- FSP27 and PLIN1 interaction regulates lipid droplet morphology.
- This study reveals important players in lipid droplet dynamics in adipocytes.

Figure 1. FSP27 and PLIN1 co-localize and co-immunoprecipitate in human adipocytes
 (A) A single LD in a human adipocyte showing the distribution of endogenous FSP27 (green) and PLIN1 (red) at its surface. Nucleus was stained with DAPI (blue). Bar, 10 μ m. (B) 4- μ m confocal Z-section of a human adipocyte showing localization of endogenous FSP27 (green) and PLIN1 (red). Bar, 10 μ m. (C) HA-FSP27 was immunoprecipitated with anti-HA antibodies and immunoblotted with PLIN1 and HA antibodies. (D) Flag-PLIN1 was immunoprecipitated with Flag antibodies and immunoblotted with FSP27 or Flag antibodies. (E) Endogenous PLIN1 was immunoprecipitated with PLIN1 antibodies and immunoblotted with PLIN1 and FSP27 antibodies. (F) HA tagged FSP27(120–220) was immunoprecipitated with HA-antibodies and immunoblotted with PLIN1 or HA antibodies. In panels C, D, E and F, input represents whole cell lysate and control represents the beads conjugated with Flag or HA antibodies which were incubated with the uninfected human adipocytes.

Figure 2. Expressing exogenous FSP27 or PLIN1 increases total TG content while decreasing lipolysis in human adipocytes, but FSP27+PLIN1 has no additional effect on TG content or lipolysis

(A) Adenovirus-mediated expression of HA-FSP27 and Flag-PLIN1 in human adipocytes using FSP27 and PLIN1 antibodies, respectively. GFP-containing adenovirus was used as a control. (B, C) Biochemical quantification of total triglyceride (B) and lipolysis (C) in human adipocytes infected with control, FSP27, PLIN and FSP27+PLIN1 adenoviruses; *, $p < 0.05$ (paired t-test). Control represents GFP-containing empty virus. For lipolysis, glycerol released in 2.5 h was measured and normalized to total triglycerides and total proteins. The data show an average of three independent experiments. Values are means $\pm$ standard error.

Figure 3. Transducing FSP27+PLIN1 for 72 hr induces formation of unilocular LDs in cultured human adipocytes

(A) Phase contrast image showing LD morphology after 72 h of transduction of FSP27, PLIN1 or FSP27+PLIN1 adenoviruses in cultured human adipocytes. Control represents GFP-containing empty virus. Bar, 10 μ m. (B) Range of the LD size. (C) Average number of LDs per cell. LD size and number was measured in more than 15 cells in each condition from three independent experiments. Values are means $\pm$ standard deviation. *, $p < 0.01$; **, $p < 0.05$ (paired t-test).