Fibronectin Overexpression Modulates Formation of Macrophage Foam Cells by Activating SREBP2 Involved in Endoplasmic Reticulum Stress

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Key Words
Fibronectin • ER stress • SREBP2 • Foam cells

Abstract
Aims: To explore the explicit role of fibronectin (FN) isoforms in atherosclerotic lesions and the underlying mechanisms. Methods and Results: Inducible stable expression was performed, and similar results were observed between EDA\textsuperscript{+}FN (FN containing EDA domain) and EDA\textsuperscript{-}FN (FN devoid of EDA domain). FN isoforms could trigger endoplasmic reticulum (ER) stress, thereby leading to lipid accumulation in cultured Raw264.7 cells. FN isoforms-induced gene expression and lipid accumulation were inhibited by a chemical chaperone 4-phenyl butyric acid (PBA) or by overexpression of the ER chaperone, GRP78/BiP, demonstrating a direct role of ER stress in activation of cholesterol/triglyceride biosynthesis. Moreover, activation of the sterol regulatory element binding protein-2 (SREBP2) was found to be downstream of ER stress, and this activation was affirmed to account for the intracellular accumulation of cholesterol using RNAi technique. Conclusion: our study suggests that enhanced FN in lesions facilitates foam cell formation due to dysregulation of the endogenous sterol response pathway by activation of ER stress, and confirms that EDA\textsuperscript{+}FN has no more pro-atherogenic role than EDA-FN in triggering ER stress.

Introduction

In eukaryotic cells, the endoplasmic reticulum (ER) provides a contained environment for the synthesis and modification of membrane proteins and proteins destined to be secreted

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Agents or pathological conditions that adversely affect ER proteins folding cause the accumulation of unfolded or misfolded proteins in the ER, which is defined as ER stress. ER stress can be induced by insults that interfere with protein glycosylation, disulfide bond formation, Ca$^{2+}$ balance and/or the general overloading of the ER with proteins. A coordinated adaptive program called the Unfolded Protein Response (UPR) is activated in response to ER stress [1-3]. The UPR in mammalian cells consists of three signaling branches initiated by three ER transmembrane sensors including inositol-requiring protein 1 (IRE1), double-stranded RNA-dependent protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF-6). Recent studies have suggested that activation of the ER stress/UPR is implicated in the pathophysiology of various human diseases, such as cardiovascular disease, neurodegenerative disease, diabetes mellitus, and liver disease [4-7]. Among these, UPR and atherosclerosis were documented well [8-11]. Feng et al. reported that accumulation of free cholesterol (FC) in the endoplasmic reticulum membrane induced ER stress and activated the UPR in macrophages, and consequently led to apoptotic cell death, contributing to atherosclerotic lesion instability, rupture and thrombogenicity [8]. Zhou et al. found that hyperhomocysteine triggered the UPR activation, in addition to eliciting oxidative stress and inflammation [9], which became a key cellular stress mechanism for hyperhomocysteine to accelerate atherogenesis. But, Zhou et al. also found that the UPR occurred at all stages of atherosclerotic lesion development even in very early intimal macrophages in ApoE−/− mice fed with normal diet [10], suggesting that additional endogenous factors triggered the UPR, especially in the initial stages.

Fibronectin (FN) is a large glycoprotein found in plasma, extracellular matrix and cell surface. FN isoforms, generated by alternative splicing at three sites comprising the Extra Domain B (EDB), Extra Domain A (EDA), and the Type III Homologies Connecting Segment (IIICS) [12-14], have been associated with a variety of cellular processes such as embryogenesis, malignancy, hemostasis, wound healing and maintenance of tissue integrity [12, 15, 16]. In the last few years, the development of mouse models produced conclusive evidences for FN and its isoforms in these processes [14, 17-21].

Recently, FN is regarded as a candidate gene in atherosclerosis via influencing cholesterol [22]. As we known, in the normal artery wall, an amount of fibronectin (FN) is present, strictly devoid of the EDA and EDB domains. In atherosclerotic lesions, there is a remarkable increase in FN and EDA+FN, and this increase can be discovered in an early stage [12, 14, 23]. Tang et al. reported that EDA-FN facilitated macrophage foam cell formation [14]. However, Babaev and his colleges in their study found that there were no difference between EDA-FN and EDA FN in affecting foam cell formation [24]. The explicit role of FN and the underlying mechanism in the development of atherosclerotic lesions remains unknown.

To explore the explicit role of FN isoforms in atherosclerotic lesions and the underlying mechanisms, we established inducible stable clones of fibronectin isoforms in Raw264.7 cells. We found that overexpression of EDA-FN or EDA FN promoted cholesterol accumulation in macrophage, and surmised that the markedly enhanced fibronectin is involved in altering cholesterol/triglyceride metabolism in macrophage cells by eliciting ER stress and consequently activating the SREBP2.

Materials and Methods

Materials, cell cultures and plasmids

293T cells and mouse macrophage cell line Raw264.7 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). 293T and Raw264.7 cells were cultured in DMEM medium (GIBCO) containing 10% fetal bovine serum and supplemented with penicillin and streptomycin (both at 1%). Cells were maintained in a humidified incubator at 37°C, in the presence of 5% CO$_2$, and were subcultured following enzymatic digestion by 0.025% trypsin/EDTA. EDA-FN were cloned by RT-PCR from embryonic brain tissues, the sequences were confirmed and subcloned into a retroviral vector containing a Tet response element, pTRE vector (Clontech). Plasmids expressing retroviral Gag-Pol (GP) and VSV-G
proteins were from Stratagene. Primers from flanking EDA exon that distinguish included versus excluded forms were: 5′-CTGCCTTCACCTTCTCCCCGGTG-3′ and 5′-GATTCTTTTCATTGCGTCCCCGTG-3′.

**Tissue**

Female apoE/- mice on a C57BL/6J background (Department of laboratory animal science, Peking University Health Science Center, Beijing, China) were fed with a high fat diet and euthanized at 18 weeks of age. The heart (including the aorta) was removed, cut transversely, and embedded in paraffin. All procedures were approved by Huazhong University of Science & Technology Animal Research Ethics Board.

**Retroviral infection**

Retroviral infection was performed as described previously [25]. Briefly, retroviral plasmid DNA was co-transfected into 293T cells with the GP and VSV-G plasmids, using a calcium phosphate method. After 48h, a virus-containing medium was then harvested and utilized to infect target cells in the presence of 10 µg/ml of polybrene (Sigma). To determine the retrovirus titer, a LacZ retrovirus without a mammalian selection marker was co-packaged with a retroviral vector expressing the targeted cDNA at a ratio of 1:5. The virus was used to infect Raw264.7 cells in a period of 36h before staining for LacZ. Comparable levels of staining among individually packed retroviruses indicate comparable titers.

**Generation of stable EDA-FN/EDA+FN inducible clones**

Stable EDA-FN/EDA+FN inducible clones were obtained using a modified tetracyclin-regulated inducible expression system (Tet-on system, Clontech) according to the manufacturer's protocol. In short, Raw264.7 cells were infected with a pTet-on retrovirus that contains the reverse doxycyclin repressor (rtTA) expression cassette (Clontech) in the first round to generate Raw264.7/Tet-on cells. The selection of infectants was performed in the presence of G418 (800 µg/ml). Stable Raw264.7/Tet-on clones were screened to using the Promega Luciferase Assay System after transient transfection with pTRE-Luc plasmid. The second round of infections was infected with pTRE-EDA-FN/pTRE-EDA+FN. The infections were selected in a medium containing hygromycin (200 µg/ml).

**Real time RT-PCR analysis**

Total RNA was extracted from cells using an RNeasy kit (Qiagen, German), based on the manufacturer's protocols. The concentration and purity of RNA were determined by a spectrophotometer. cDNA was synthesized with MMLV reverse transcriptase (Promega, Madison, WI, USA) and then subjected to real-time RT-PCR (ABI PRISM 7700) using SYBR Green Real-Time PCR Master Mix (ABI, USA). Primers were sense: 5′-TGGAGTTCCCCAGATTGAAG-3′, antisense: 5′-GCCGCTTTTGAGCTTTTTGG-3′ for GRP78; sense: 5′-TCCCTGCCTTTCACCTTG-3′, antisense: 5′-GCTTTGGGATGTGCGTGT-3′ for CHOP; and sense: 5′-TCATTGACCTCAACTCAGTTG-3′, antisense: 5′-GAAGATGGTGATGGGATTTC-3′ for GAPDH. A standard curve analysis using the primers was not done, and to normalize the amount of total RNA present in each reaction, the GAPDH gene was used as an internal standard.

**Overexpression of GRP78/Bip in EDA-FN/EDA+FN inducible clones**

For transient overexpression of GRP78/Bip, cells were seeded 24h before transfection in six-well plates at a density of 5×10⁵ cells/well. The transfection with pcDNA3.1 containing GRP78/Bip (a gift from Dr. Austin Richard, McMaster University, Canada) was carried out using Lipofectamine 2000 according to the manufacturer's instructions. Cells were used for experiments after a 48h recovery period. Transfection efficiency was determined in parallel plates by transfection of the pEGFP-N1 control vector.

**Western blotting analysis**

Cells were lysed on ice with lysis buffer containing 1mM phenylmethylsulfonyl fluoride, 1µg/ml leupeptin and 2µg/ml aprotinin. Lysates were centrifuged at 4°C, 14,000 rpm for 10min to pellet cell debris. Extractions of nuclear protein were completed with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce), according to the manufacturer's instructions. Protein concentrations were determined using an assay kit (Bio-Rad, Hercules, CA, USA). Supernatant was separated on a 10% SDS-PAGE gel and transferred onto a polyvinylidene fluoride membrane. Membranes were incubated in blocking buffer (Tris-buffered saline [TBS], 0.1% Tween 20, and 5% non-fat dry milk) for 1h at room temperature, followed by
hybridization with anti-EDA-FN antibody (F6140 or F7387, Sigma Aldrich, Saint Louis, Missouri, USA), anti-CHOP antibody (sc-575, Santa Cruz, CA, USA), anti-GRP78 antibody (610978, BD Transduction Laboratories, NJ, USA), anti-SREBP1 or SREBP2 antibody (ab3259 and ab30682, respectively, Abcam Ltd. Hongkong, China) or anti-β-actin antibody (sc-47778, Santa Cruz, CA, USA) at 4°C overnight. After washed in TBS/0.1% Tween 20 three times, the membranes underwent hybridization with a horseradish peroxidase-conjugated secondary antibody (Life Technologies Inc.) for 1h at room temperature, and were formed using the ECL reagent (Pierce Chemical Co., Rockford, Illinois, USA).

**Uptake of BODIPY FL LDL**

Cells pretreated with or without chaperone 4-phenyl butyric acid (PBA) for 6h and further cultured in the presence or absence of doxycycline (2µg/ml) for 48h, were washed with PBS and incubated in medium containing 10µg/ml BODIPY FL LDL (Molecular Probes, Eugene, Oregon, USA). After incubation at 37°C for 2h, cells were washed with PBS, fixed in 3% formaldehyde in PBS, and the uptake of LDL was detected by fluorescence microscopy.

**Total cholesterol and triglyceride levels**

Cultured cells were homogenized in lysis buffer containing 0.1% Triton X-100. Lysates were saponified, and lipids were extracted with hexane/isopropanol (3:2) [26]. Colorimetric cholesterol and triglyceride assays were carried out using the BioVision Cholesterol and Triglyceride Quantification Kit (BioVision Research Products, CA, USA).

**RNA Interference**

Mouse SREBP2 On-Target Plus Smart Pool siRNA and control nontargeting siRNA were purchased from Dharmacon (Lafayette, CO). Cells were transfected with 100nM using GeneEraser siRNA reagent (Stratagene) at 60% confluence. After 48h, cells were treated with doxycycline for 24h, and cell lysate was harvested as described before. The expression of SREBP2 protein was used to assess efficacy of downregulation by RNAi.

**Statistical Analysis**

Statistical analyses were performed with SPSS 14 for Windows using one-way ANOVA, with Tukey HSD for post hoc analysis. P<0.05 was considered significant. Data are presented as the means±SD.

**Results**

**FN elicits ER stress and activates UPR in Raw264.7 Cells**

To investigate whether ER stress/UPR is activated by FN isforms, we established stable, EDA-FN/EDA-FN-inducible clones in Raw264.7 cells using the doxycycline-inducible expression system (Tet-on). EDA-FN/EDA-FN was cloned by RT-PCR from embryonic brain tissues of mouse (Fig. 1A). In EDA-FN/EDA-FN-inducible clones, a strong expression of EDA-FN/EDA-FN was detected after 24h of doxycycline induction, and the expression could still be observed after 72h of continuous induction. On the contrary, no expression of EDA-FN was inspected in the uninduced state (Fig. 1B).

GRP78 that is commonly used as a hallmarker of ER stress and UPR activation [7, 10, 27] was characterized by western blotting. In these cells, a significant expression of GRP78 was observed when EDA-FN/EDA-FN was induced by doxycycline for 24h, and its expression level approached the maximum at 48h (Fig. 2A). Another ER stress response protein, C/EBP homologous protein-10 (CHOP), was also determined. CHOP is a proapoptotic factor and plays a critical role in the apoptosis by ER stress. We found that there was an apparent expression of CHOP after 72h of doxycycline induction (Fig. 2A). In addition, real time RT-PCR was used to evaluate mRNA levels of these two ER stress response proteins. As shown in Figure 2B, after 48h or 72h of doxycycline induction, the mRNA levels of GRP78 or CHOP was obviously increased. Altogether, these results demonstrate that the enhanced expression of FN elicits ER stress, activates the UPR and increases expression of ER stress response genes such as GRP78 and CHOP.
FN promotes the formation of macrophage foam cells

To directly identify the role of FN isforms in formation of macrophage foam cells, we detected the intracellular cholesterol and triglycerides in the first step. Raw264.7 cells were cultured in the absence or presence of doxycycline for 48h, and total cholesterol and triglycerides were measured. As displayed in Figure 3A, there was an obvious elevation of cholesterol upon EDA-FN/EDA-FN induction in Raw264.7 cells, whereas the expression levels of triglycerides was not affected significantly (data not shown). Additionally, doxycycline per se has no effect on levels of cholesterol.

To further determine the influence of FN isforms on expression and activity of LDL-receptor, we measured the ability of cultured cells treated with doxycycline to bind and internalize fluorescently-labeled LDL. Our results demonstrated that LDL uptake was enhanced upon FN isforms expression induced by doxycycline (Fig. 3B).

Inhibition of ER stress prevents foam cells formation induced by FN

Because ER stress can induce lipogenesis and promote hepatic steatosis [28-31] and FN isforms elicited ER stress, it is reasonable to hypothesize that FN isforms-induced foam cell formation depends on its activation of ER stress. To test this, the chemical chaperone...
4-phenyl butyric acid (PBA) was used. PBA can alleviate ER stress, and hence, provide an experimental opportunity to approach the role of ER stress in atherosclerosis [32, 33]. We first measured whether PBA could alter ER stress in Raw264.7 cells upon FN isoforms overexpression. Treatment with doxycycline triggered ER stress in macrophages (Fig. 2 A, B), as determined by GRP78 and CHOP. However, application with PBA resulted in essentially complete protection against FN-induced ER stress (Fig. 4 A, B and C). We next determined
whether modification of ER stress in this setting could impede foam cells formation in Raw264.7 cells. The findings showed that the cell ability of binding and internalizing fluorescently-labeled LDL was markedly decreased in the presence of PBA (Fig. 5) and indicating that PBA can protect cultured Raw264.7 cells against FN-induced ER stress and foam cells formation in vitro.

As is known, overexpression of GRP78 has been reported to protect cells from ER stress [34, 35]. In this context, transient transfected cells overexpressing GRP78 were generated to further evaluate whether blocking FN isoforms-induced ER stress in this way could prevent cholesterol biosynthesis. As shown in Figure 6A, the transfection efficiency was more than 80% in all experiments, which can be determined in parallel plates by transfection of the pEGFP-N1 control vector. Moreover, cells overexpressing GRP78 exhibited almost a 6.0-fold enhancement of protein levels, compared with wild type or vector-transfected cells (Fig. 6B). Incubation with doxycycline for 24h, the levels of cellular cholesterol were not increased in Raw264.7 cells transfected with pcDNA3.1 containing GRP78 gene (Fig. 6C). Together with the results from PBA, these findings vigorously indicate that ER stress plays a key role in forming macrophage foam cells by FN isoforms, although it is not clear BiP overexpression and PBA work to alleviate ER stress whether by enhancing the ER functional capacity at large, or by specifically acting on FN.

**Fig. 3.** Effect of FN isoforms on formation of foam cells. A, FN isoforms increase intracellular total cholesterol in cultured Raw264.7 cells. Raw264.7 cells were incubated without or with doxycycline (2µg/ml) for 24h. Cells were harvested and lipids were extracted as described in Methods. Total cholesterol was normalized for protein content, and values were expressed relative to the control sample (i.e. Raw264.7 cells in the absence of doxycycline). Data are presented as the mean ± SD from three separate experiments (*p<0.05 con

vs others). B, Effect of FN isoforms on LDL uptake. Cells treated in the absence or presence of 2µg/ml doxycycline for 48 hours were washed with PBS followed by incubation for an additional 2 hours at 37°C in media containing 10µg/ml BODIPY FL LDL. After washing with PBS, cells were fixed, and LDL binding/uptake was detected by fluorescence microscopy.
Next, we asked which was involved in the connection between ER stress and lipids accumulation induced by FN. Since the sterol regulatory element binding proteins (SREBPs), ER-resident transcription factors, are responsible for the induction of genes in the cholesterol/triglyceride biosynthesis and uptake pathways [36, 37], and they were induced by some ER stressors and could stimulate lipid accumulation in hepatocytes and human aortic smooth muscle cells [38-40], we investigated SREBPs protein levels upon FN induction. Western blotting analysis showed that expression levels of mature form of SREBP2 (mSREBP2) were increased in Raw264.7 cells when FN isforms were overexpressed (Fig. 7A), whereby the same increment was not found for SREBP1 (data not shown). In Raw264.7 cells, the enhancement of mSREBP2 induced by FN could be blocked by PBA. In addition, the expression of SREBP2-targets, the 3-hydroxy-3-methyl glutaryl CoA reductase (Hmgcr, the key enzyme of de novo cholesterol biosynthesis), is the same as mSREBP2 (Fig. 7B). These results suggest that SREBP2 and de novo cholesterol biosynthesis were activated by FN overexpression and it triggered-ER stress was involved in this activation. The RNAi technique was further used

**Effect of SREBPs on FN isforms-induced foam cells formation**

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**Fig. 5.** Effect of PBA on LDL uptake. Cells were pretreated without or with 3mM PBA for 6 hours, and were cultured in the absence or presence of 2µg/ml doxycycline for 48h. Samples were washed with PBS followed by incubation for an additional 2 hours at 37°C in media containing 10µg/ml BODIPY FL LDL. After washing with PBS, cells were fixed, and LDL binding/uptake was detected by fluorescence microscopy.

| pTRE-EDA-FN | Dox | PBA |
|-------------|-----|-----|
| -           | -   | +   |
| -           | +   | -   |
| -           | -   | +   |
| +           | -   | +   |
| +           | +   | +   |

**Fig. 6.** Effect of overexpression of GRP78 on intracellular total cholesterol. A, cells were transfected with pcDNA3.1 containing GRP78/Bip by means of Lipofectamine 2000 and transfection efficiency was determined in parallel plates by transfection of the pEGFP-N1 control vector and detected by fluorescence microscopy. B, Western-blotting analysis of GRP78 expression in empty (lane 1), pcDNA transfected (lane 2), or GRP78 over-expressing (lane 3) cells (*p<0.05 lane 1, 2 vs lane 3). C, Cells non-overexpressing GRP78 or overexpressing GRP78 were incubated without or with doxycycline (2µg/ml) for 24h. Cells were harvested and lipids were extracted as described in Methods. Total cholesterol was normalized for protein content, and values were expressed relative to the control sample (i.e. Cells in the absence of doxycycline and non-overexpressing GRP78). Data are presented as the mean ± SD from three separate experiments (*p<0.05 con vs others).
to determine whether SREBP2 was involved in stimulating foam cells formation related to FN. As displayed in Figure 8, transfection of siRNA for SREBP2 decreased the expression of SREBP2 protein (Fig. 8A), and doxycycline failed to increase the levels of cholesterol in cells knockdown of SREBP2 (Fig. 8B). Taken together, these findings imply that ER stress induced by FN upregulates the expression of mSREBP2 and subsequently contributes to cholesterol accumulation in cells. The Figure 8C showed that knockdown of SREBP2 did not affect the expression of FN.

Discussion

A growing body of evidence indicates that ER stress plays a crucial role in both atherosclerosis and plaque rupture, yet the previous studies on triggers of ER stress have mostly focused on oxidized lipids, hyperhomocysteine, inflammation or metabolic stress. Herein, we report a new stressor, FN, a kind of ECM protein can induce ER stress/UPR. FN, the large glycoprotein, is synthesized, processed and secreted from ER, it suggests that ER
stress will be elicited when FN is overexpressed and loaded in the endoplasmic reticulum. Secondly, FN knockdown affected Ca\(^{2+}\) release from the endoplasmic reticulum and induced mitochondria-dependent apoptosis [41], which suggests that FN is involved in maintaining Ca\(^{2+}\) homeostasis. Therefore, the overexpressed FN in atherosclerosis may enable to induce ER stress through affecting the release of Ca\(^{2+}\) and disturbing Ca\(^{2+}\) homeostasis in ER.

Although the specific molecular mechanisms by which FN lead to ER stress need to be well clarified, our results is the first to demonstrate that the FN overexpression in atherosclerotic lesion could be a new inducer of ER stress in macrophages, and elucidate the corresponding mechanism of FN in the progression of atherosclerosis. In addition, no difference between EDA\(^+\)FN and EDA\(^-\)FN in accelerating accumulation in macrophages was observed in our study, which is consistent with Babaev’s results [24]. It implicates that EDA domain does not affect foam cell formation from the perspective of triggering ER stress.

Prolonged or severe ER stress can result in apoptotic cell death through the activation of multiple ER-specific proapoptotic factors, such as CHOP, Caspase-12 and TDAG51 [7, 10]. CHOP is most characteristic among these factors, and it plays a crucial role in macrophages death and plaque rupture in atherosclerotic lesions [7]. In this paper, it was observed that CHOP was markedly elevated after 72h of doxycycline treatment in cells transfected with FN isforms, but this expression is posterior to GRP78 maximum expression for 24h (Fig. 2A). Although we did not determine whether Raw264.7 cells apoptosis occurred after CHOP expression, we found that the cultured cells transfected with FN were in poor conditions treated with doxycycline after 72h. It means that prolonged overexpression of FN in macrophages would lead to cell apoptotic death. Recent studies showed that ER-initiated cell apoptosis were derived from FC and/or oxidized lipids accumulation in macrophages or aortic smooth muscle cells [8, 10, 39]. Intriguingly, our results indicate that highly expressed FN in atherosclerotic lesions may contribute to macrophages death and plaque rupture by activation of ER stress as well.

We found that the overexpressed FN increased the levels of cholesterol in Raw264.7 cells. Furthermore, we demonstrated that the positive influence of FN on cholesterol accumulation
is due to its activation of ER stress/UPR in macrophages with the methods of overexpression of GRP78 or PBA. Additionally, SREBP2 is found to be the downstream factor of ER stress, which regulates lipid metabolism in this setting.

SREBPs are transcription factors synthesized as inactive precursors bound to the ER membrane [42] and consist of three different members: SREBP1a, SREBP1c and SREBP2. SREBP-1c is responsible for genes controlling FA and TG synthesis pathways, while SREBP2 is predominantly involved in regulation of the cholesterol biosynthetic pathway [43]. In the present study, activation was only observed for SREBP2 (by detecting the expression of mSREBP2) in Raw264.7 cells treated with doxycycline for 48h, which is in accordant with our finding: there was an apparent increase of cholesterol upon FN induction in Raw264.7 cells, but no changes were found for the levels of triglycerides.

SREBPs localize in the ER via their interactions with SREBP cleavage activating protein (SCAP) and Insig. SCAP forms a complex with the SREBPs, which is essential for their activation. Insig is an ER-resident protein anchor that binds to SCAP and further maintains the ER localization of the SCAP-SREBP complex. Upon cellular sterol depletion, the SCAP-SREBP is released from Insig, and then, SCAP assists in the transport of SREBP from the ER to the Golgi for cleavage by site-1-serine protease (S1P) and site-2-zinc metalloproteinase (S2P) [44]. Subsequently, the active mature form of SREBP was transported to the nucleus where it induces genes required for lipid biosynthesis and uptake [36, 37]. Interestingly, SREBPs are also controlled by an ER connecting, and they have the common activating machinery components with the UPR [5, 39]. For example, PERK-mediated eIF2 phosphorylation exposed to ER stress conditions upregulates the expression of mSREBP2 by accelerating Insig degradation [39, 47]; ER stress induces cleavage of membrane-bound ATF-6 by the proteases that were involved in SREBPs activation (S1P and S2P) [45, 46], although it seems that ATF6 antagonizes SREBP2 via interacting with its mature form in the nuclear. The previous results about ER stress/UPR and SREBP2 are seemingly contradictory that ER stress was found to activate SREBP2 [47, 48] as well as diminish its activation [46, 49]. Austin and co-workers implicated that these paradoxical results could represent a negative feedback loop to dampen cholesterol biosynthesis following ER stress [47]. We hypothesize that these contradictions may result from the varied agents or triggers of ER stress owing that UPR components display distinct sensitivities toward different forms of ER stress and ER stress sensors can confer different responses as well [50]. For instance, compared with the intensive and rapid response of both IRE1 and PERK to ER stress induced by thapsigargin, the response of ATF6 was very weak and remarkable delayed. It implies that SREBP2 will be activated upon the ER stress condition of thapsigargin without suppress of ATF6 in the cells.

To conclude, our results indicate that FN appears to activate SREBP2 in a cholesterol-independent manner. Although we do not currently understand how FN dysregulates SREBP activation, the mechanism appears to involve FN-induced ER stress. Identifying the components of FN-induced ER stress signaling cascade may provide more information for us to understand the relationship between FN and the molecular events that result in increased lipid biosynthesis.

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Disclosure Statement

No conflicts of interest, financial or otherwise, are declared by the authors.
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