RESEARCH

Selenoprotein S regulates adipogenesis through IRE1 α -XBP1 pathway

Lili Men^{1,*}, Junjie Yao^{1,*}, Shanshan Yu¹, Yu Li¹, Siyuan Cui¹, Shi Jin², Guixin Zhang³, Decheng Ren⁴ and Jianling Du¹

¹Department of Endocrinology, The First Affiliated Hospital of Dalian Medical University, Dalian, China

²Department of Laparoscopic Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian, China

³Department of General Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian, China

⁴Department of Medicine, The University of Chicago, Chicago, Illinois, USA

Correspondence should be addressed to J Du or D Ren: dujianling63@163.com or rendckent@yahoo.com

*(L Men and J Yao contributed equally to this work)

Abstract

The induction of endoplasmic reticulum (ER) stress is associated with adipogenesis, during which the inositol-requiring enzyme 1 α (IRE1 α)-X-box-binding protein 1 (XBP1) pathway is involved. Selenoprotein S (SelS), which is an ER resident selenoprotein, is involved in ER homeostasis regulation; however, little is known about the role of SelS in regulating adipogenesis. *In vivo* studies showed that SelS protein levels in white adipose tissue were increased in obese subjects and high-fat diet (HFD)-fed mice. Moreover, we identified that SelS protein levels increased in the early phase of adipogenesis and then decreased in the late phase during adipogenesis. Overexpression of SelS promoted adipogenesis. Conversely, knockdown (KD) of SelS resulted in the inhibition of adipogenesis, which was related to increasing cell death, decreased mitotic clonal expansion, and cell cycle G1 arrest. *In vivo* studies also showed that ER stress markers (p-IRE1 α /IRE1 α , XBP1s, and Grp78) were significantly increased with upregulating of SelS expression in subcutaneous and visceral adipose tissues in the obese subjects and HFD-fed mice. Furthermore, in SelS KD cells, the levels of Grp78 were increased and the levels of p-IRE1 α /IRE1 α were unchanged, but mRNA levels of *spliced XBP1* (*XBP1s*) produced by IRE1 α -mediated splicing were decreased, suggesting a role of SelS in the modulation of IRE1 α -XBP1 pathway. Moreover, inhibition of adipogenesis by SelS suppression can be rescued by overexpression of XBP1s. Thus, SelS appears to function as a novel regulator of adipogenesis through the IRE1 α -XBP1 signaling pathway.

Key Words

- ▶ SelS
- ▶ adipogenesis
- ▶ endoplasmic reticulum stress
- ▶ XBP1
- ▶ IRE1 α

Journal of Endocrinology
(2020) **244**, 431–443

Introduction

Obesity has been recognized as one of the most blatantly visible, yet most neglected, public-health problem which increases the risk of numerous diseases such as cardiovascular diseases, type 2 diabetes, hypertension, and depression. Despite measures of lifestyle intervention, pharmacotherapy, and surgery that have been taken to manage obesity, the prevalence of obesity

remains out of control and increased world-wide from 3.2% in 1975 to 10.8% in 2014 in men and from 6.4 to 14.9% in women (NCD Risk Factor Collaboration 2016). Thus, new scientific understanding on the pathogenesis of obesity needs to be investigated which in turn might help to explore a novel approach to tackle this problem.

Understanding the molecular basis of adipogenesis is important for controlling obesity. Adipogenesis is the process during which fibroblast-like preadipocytes develop into mature adipocytes. This process involves a few stages, including mesenchymal precursors, committed preadipocytes, growth-arrested preadipocytes, mitotic clonal expansion (MCE), terminal differentiation, and mature adipocytes (Lefterova & Lazar 2009, Tang & Lane 2012). Adipogenesis is governed by a multifaceted transcriptional regulatory cascades, CCAAT/enhancer-binding protein β (C/EBP β) is induced in the very early stage. Then, expression of C/EBP α and peroxisome proliferator-activated receptor γ (PPAR γ), two crucial pro-adipogenic transcription factors, is activated, which subsequently activates the transcription of lipogenic-specific genes, such as fatty acid-binding protein 4 (FABP4), adiponectin, and adipsin for the maturation of adipocytes (Lefterova & Lazar 2009, Tang & Lane 2012, Moseti *et al.* 2016).

Mounting evidence indicates that endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) are related to adipogenesis (Zha & Zhou 2012, Cha *et al.* 2014, Han & Kaufman 2014). When ER homeostasis is disrupted, unfolded proteins accumulate in the ER lumen, subsequently activating the UPR. In eukaryotic cells, the UPR has three branches, inositol-requiring enzyme 1 α (IRE1 α)-X-box-binding protein 1 (XBP1), protein kinase RNA-like ER kinase - eukaryotic translation initiation factor 2 α - activating transcription factor 4 and transcription factor 6 α , which contribute to the transcription of chaperones related to protein folding and ER-associated degradation (ERAD) (Zha & Zhou 2012). Among these branches, the IRE1 α -XBP1 pathway is the most conserved and has been reported previously to be required for adipogenesis by regulating the transcription of adipogenic genes (Sha *et al.* 2009, 2011). Some studies showed an increase in ER stress in high-fat diet (HFD)-induced obese animals (Castro *et al.* 2013, Deldicque *et al.* 2013). However, as obesity and adipogenesis are increasingly complex, the mechanism remains unclear.

Selenoprotein S (SelS), also known as Tanis or VCP-interacting membrane selenoprotein (VIMP), is responsible for ERAD by forming a complex with ubiquitin ligase E3, Derlin-1, selenoprotein K and p97ATPase (Bettigole *et al.* 2015); this complex is closely associated with ER stress by participating in unfolded and misfolded proteins degradation. Studies have shown that overexpression of SelS protected the astrocytes, vascular smooth muscle cells and macrophages from ER stress damage

(Kim *et al.* 2007, Fradejas *et al.* 2011, Ye *et al.* 2016). These findings suggest a regulatory effect of SelS during ER stress. Positive correlations of SelS gene expression in subcutaneous adipose tissue with body mass index (BMI) and waist circumference (WC) were observed in human subjects (Olsson *et al.* 2011), which further indicate a possible association of SelS with adipogenesis and obesity. Additionally, our study demonstrated that SelS promotes 3T3-L1 adipocyte survival through the IRE1 α -XBP1 pathway (Men *et al.* 2018b). However, the precise role of SelS and its underlying mechanisms associated with IRE1 α -XBP1 pathway during adipogenesis remains to be validated. In this study, we examined the role of SelS in adipogenesis and its association with UPR.

Materials and methods

SelS and ER stress markers (p-IRE1 α /IRE1 α , XBP1s, Grp78) protein levels were evaluated in human white adipocyte tissue (WAT)

This study designated two male and four female human subjects with obesity undergoing bariatric surgery as the obese group (BMI ≥ 28.0 kg/m²), one male and four female human subjects who suffered from congenital choledochal cyst or cholecystolithiasis as the N-obese group (BMI < 28.0 kg/m²) according to Chinese BMI standard (Wang *et al.* 2007). Subcutaneous and omental fat were obtained during surgery, immediately stored in liquid nitrogen and then stored at -80°C prior to use.

The exclusion criteria were as follows: (1) systemic or local inflammation in last 3 months; (2) coronary heart disease; (3) renal or hepatic dysfunction; (4) cerebrovascular disease or (5) tumours.

The protocol for this study was approved by the Ethics Committee of the First Affiliated Hospital of Dalian Medical University and the informed consents of all participants were obtained.

Animal study

Male C57BL/6 mice from the Jackson Laboratory were maintained on a 12 h darkness/light cycle at 21°C . Animals were randomly divided into three groups (six mice in each group) according to diet, including normal chow (NC), 45% HFD, and 60% HFD. Mice were fed for 16 weeks, and then the WAT including subcutaneous fat, omental fat, perirenal fat, and epididymal fat were obtained for Western blotting analysis and/or immunohistochemistry

(IHC). All experiments were approved by the Ethics Committee on Animal Experiments of Dalian Medical University.

3T3-L1 preadipocytes culture, induction of differentiation and lentivirus infection

Murine 3T3-L1 preadipocytes were cultured in DMEM and induced by MDI medium as previously reported (Men *et al.* 2018b). MDI medium consists of DMEM containing 10% (v/v) fetal bovine serum (Gibco), 500 nM 3-isobutyl-1-methyl-xanthine, 250 nM dexamethasone, and 167 μ M insulin (Sigma-Aldrich). pLKO.1-SelS short hairpin RNA (shRNA) lentivirus and pLV5-XBP1s lentivirus were prepared as previously described (Ren *et al.* 2014, Men *et al.* 2018b).

Recombinant SelS plasmid transfection

Human recombinant pCMV6-XL5-SelS was purchased from OriGene. For SelS overexpression (OE), 3T3-L1 preadipocytes were plated into six-well plates 16–18 h before transfection with pCMV6-XL5-SelS by using X-treme GENE 9 reagent (Roche) following the manufacturer's protocol. Briefly, adding X-treme GENE 9 in Opti-MEM medium to pCMV6-XL5-SelS plasmid and incubating for 30 min at room temperature. Then, 3T3-L1 cells culture medium was changed to complete DMEM medium prior to adding the above transfection mixture to cells.

RNA isolation and real-time quantitative PCR analysis

Total RNA was isolated using Trizol reagent (Invitrogen) and reverse transcription was performed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. TaqMan assay numbers as follows: *SelS* (Mm01318786_m1), *SREBP1* (Mm00550338_m1), *PPAR γ* (Mm01184322_m1), *C/EBP α* (Mm00514283_s1), *C/EBP β* (Mm00843434_s1), *FABP4* (Mm00445878_m1), *FASN* (Mm00662319_m1), *Adiponectin* (Mm01143935_g1), and *AdipQ* (Mm00456425_m1) were from Invitrogen. Relative expression levels of mRNA were determined using the comparative CT method and were normalized to actin levels. The *XBP1* mRNA splicing assay was performed as previously described (Bettigole *et al.* 2015). In brief, *Xbp1s* and *unspliced Xbp1* (*Xbp1u*) transcripts were amplified by semiquantitative RT-PCR using the pair primers forward, 5'-GAA CCA GGA GTT AAG AAC ACG-3', and reverse,

5'-AGG CAA CAG TGT CAG AGTCC-3'. PCR conditions were as follows: 95°C for 5 min, and 30 amplification cycles of 95°C for 30 s, 50°C for 45 s, and 72°C for 1 min, after which the products were separated by electrophoresis on a 2.5% agarose gel. Band intensities were measured using Image J software, and the splice percentage of *Xbp1s* was calculated using the following formula: intensity of *Xbp1s* band/(intensity of *Xbp1s* band + intensity of *Xbp1u* band) $\times$ 100%.

Immunoblotting

Tissues or cells were lysed with RIPA Buffer (Pierce) consisting of 25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate and 0.1% SDS. The extracts were immunoblotted with indicated antibodies. Immunoblotting was performed as previously described (Song *et al.* 2010). The blots were probed with primary antibodies (Supplementary Table 1, see section on supplementary materials given at the end of this article). After immunoblotting with primary antibodies, membranes were incubated with secondary antibodies (Supplementary Table 1). Then, the target proteins were visualized using an enhanced chemiluminescence kit and LAS-3000 imaging system (FUJIFILM). Western blotting results were analysed to quantify signal intensities using ImageJ and normalized to actin levels.

Immunohistochemistry (IHC)

Briefly, tissue sections were dried at 60°C, then were put in three changes of xylene 15 min each before passed through decreasing concentrations of alcohol, washed and covered with 3% H₂O₂ and then undertaken through EDTA/citrate antigen repair. Sections were then washed in 1% PBS, blocked for 20 min with serum and incubated with primary antibodies (Supplementary Table 1) overnight at 4°C. After PBS washing, HRP-coupled PV-9000 (ZSGB-BIO, China) secondary antibody was incubated for 40 min before covering the sections in DAB stained with hematoxylin, differentiated in acid-alcohol, blued in ammonia water and then dehydrated in graded alcohol. Sections were finally cleared in three changes of xylene 10 min each and mounted in neutral balsam for microscopy.

Oil Red O Staining

In order to visualize the intracellular lipid accumulation during differentiation, Oil Red O staining was performed on day 6 or day 7 in 3T3-L1 cells. Cells were fixed with

10% formalin. After washes with sterile water and incubation in 60% isopropanol for 2–5 min, cells were stained with freshly prepared Oil Red O working solution for 5 min, followed by washing with water. Then cells were stained with hematoxylin for 30 s prior to observation under the inverted phase contrast microscope. Oil Red O working solution was prepared by mixing 3 mg/mL Oil Red O (Sigma-Aldrich) in 99% isopropanol with ddH₂O at a ratio of 3:2, followed by filtration through filter paper.

Terminal dUTP nick end labeling (TUNEL) assay

3T3-L1 cells were seeded on glass coverslips in four-well dishes, infected with recombinant shRNAs lentivirus and subsequently induced by MDI. Then the apoptosis of differentiated adipocytes at day 5 was analyzed via TUNEL assay as previously reported (Men *et al.* 2018b). Cells treated with tunicamycin (Tm) or thapsigargin (Tg) for 36 h for the induction of cell death was used as positive control. Percentage of TUNEL-positive cells was expressed as the number of positively stained apoptotic cells/the total number of cells counted $\times 100\%$.

Cell labeling with 5-bromo-2'-deoxyuridine (BrdU)

Cells were seeded on glass coverslips and induced with MDI after 2 days of postconfluence. After differentiation for 16 h, cells were incubated with 3 $\mu\text{g}/\text{ml}$ BrdU labeling solution for 2 h to label DNA synthesis. Cells were fixed with 10% formalin for 30 min, washed with PBS, and resuspended in 2 N HCl for 30 min at room temperature. After blocking in 5% BSA for 1 h, cells were incubated with anti-BrdU primary antibody (Proteintech) overnight in cold room. Then the samples were washed and incubated with FITC-labeled secondary antibody, and subsequently stained with DAPI for 5 min at room temperature prior to immunofluorescence microscopic analysis.

Analysis of cell cycle distribution

Control and SelS KD 3T3-L1 cells after induction for 12, 20, 24, and 28 h were harvested, cell cycle was analysed by flow cytometry as previously reported (Men *et al.* 2018a).

Triglyceride assay

After differentiation for 6 days, cell lysates was assayed for total triglyceride content using Serum Triglyceride Quantification Kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's protocol, and for cellular

protein using the Pierce BCA protein assay kit (Thermo Scientific). The results were expressed as mg of triglyceride per mg of cellular protein.

Statistical analysis

Unpaired Student's *t* test was used to assess the statistical significance of differences between two sets of data, and ANOVA was performed to analyze results among multiple groups. $*P < 0.05$ were considered statistically significant. Variables were presented as mean $\pm$ s.d.

Results

The expression of SelS in human/mice WAT and 3T3-L1 preadipocytes during adipogenesis

First, we examined the protein levels of SelS in WAT from N-obese and obese patients. Western blotting showed that the SelS levels of subcutaneous fat and omental fat were increased in the obese group compared with the N-obese group ($P < 0.001$) (Fig. 1A and B).

Second, we examined the protein levels of SelS in WAT from mice fed NC or HFD. Feeding 45% HFD or 60% HFD for 16 weeks resulted in an increase in mice body weight (Supplementary Fig. 1). The protein levels of SelS in the subcutaneous fat, omental fat, perirenal fat, and epididymal fat were determined by immunoblot analysis, and they were significantly increased compared to mice fed NC (Fig. 1C, E and F) ($P < 0.001$), especially in the 60% HFD groups.

Third, to further examine the role of SelS in adipogenesis, the mRNA and protein levels of SelS were monitored at different stages of adipogenesis in 3T3-L1 preadipocytes. The mRNA levels of *SelS* were significantly increased from day 2 to day 8 compared with day 0 (Supplementary Fig. 2). Interestingly, the protein levels of SelS were dramatically increased at day 2 but decreased during the middle and late phases of adipogenesis (Fig. 1G). Adipogenesis of these cells was confirmed by monitoring the expression patterns of PPAR γ (Fig. 1G). Collectively, these results imply that SelS is expressed during adipogenesis.

SelS is essential for adipocyte differentiation

First, we investigated the effect of SelS KD on 3T3-L1 preadipocytes adipogenesis. SelS KD in 3T3-L1 preadipocytes by four different lentiviral SelS shRNAs

Figure 1

The expression levels of SelS in white adipose tissue (WAT) and 3T3-L1 preadipocytes during adipogenesis. The protein levels of SelS in human subcutaneous fat (A) and omental fat (B). SelS protein levels in human subcutaneous and omental fat from the N-obese group ($n = 5$) and obese group ($n = 6$) were determined by immunoblotting analysis. Data represent the means $\pm$ s.d. ** $P < 0.01$ versus N-obese. (C, D, E and F) The protein levels of SelS in mice WAT. Subcutaneous fat, omental fat, perirenal fat, and epididymal fat samples were from mice fed NC, 45% HFD, or 60% HFD. Sixteen weeks after HFD feeding, SelS protein levels in WAT were determined by immunoblotting analysis ($n = 6$ each). *** $P < 0.01$, **** $P < 0.001$ versus NC. (G) Western blots showed the levels of SelS and PPAR γ at different stages during adipogenesis. Post-confluent 3T3-L1 preadipocytes were treated with differentiation medium containing MDI. Cells were harvested on days 0, 2, 4, 6, and 8 after induction. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus N-obese group or NC group or day 0, # $P < 0.05$, ## $P < 0.01$ versus day 2.

showed that number 1, 2 and 4 of shRNAs significantly decreased SelS protein levels. The protein levels of SelS were decreased by 95 and 93% ($P < 0.001$) after infection with lentiviral SelS shRNA 1# and 2# for 2 days, respectively (Fig. 2A). The accumulation of intracellular lipids monitored by Oil Red O staining was decreased in SelS KD cells (Fig. 2B). In addition, the triglyceride content was significantly decreased in SelS KD cells during adipogenesis (Fig. 2C). Expression of adipogenic proteins PPAR γ and C/EBP α rapidly decreased in SelS KD cells, as compared with that of control cells (Fig. 2D). Consistently, *FABP4* and *sterol-regulatory-element-binding-protein-1* (*SREBP1*) expressions were markedly decreased in SelS KD cells at various phases of adipogenesis (Supplementary Fig. 3). We also found that mRNA levels of *fatty acid synthase* (*FA SN*), *PPAR γ* , *C/EBP α* , *Adipsin* and *AdipQ* were significantly lower in SelS KD cells at intermediate and late phases of adipogenesis (Supplementary Fig. 3). Surprisingly, *C/EBP β* expression was only decreased at late phase of adipogenesis (Supplementary Fig. 3).

To examine whether SelS OE increases adipogenesis, SelS was overexpressed in 3T3-L1 cells. Accumulation of intracellular lipid droplets was increased in SelS OE cells, as shown by Oil Red O staining (Fig. 2E). The expressions of the adipogenic markers *FABP4*, *PPAR γ* and *C/EBP α* were

increased by SelS OE in 3T3-L1 cells during differentiation (Fig. 2F). Taken together, these results indicate that SelS promotes adipogenesis.

SelS KD induces cell death during adipogenesis

Consistent with our previously results (Men *et al.* 2018b), SelS KD dramatically increased cell death (Fig. 3A and B) and cleaved caspase3 protein levels (Fig. 3C) which were related to decreasing anti-apoptotic members (Bcl-2, Bcl-xL and Mcl-1) and increasing pro-apoptotic Bcl-2 family members (Bax, Bak and Bad) (Fig. 3C and D) in 3T3-L1 adipocytes during adipogenesis.

SelS KD causes cell-cycle arrest in 3T3-L1 adipocytes

As an important early event, MCE is required for 3T3-L1 adipocyte differentiation, so we further tested whether SelS affected the progression of MCE and the expression levels of cell cycle regulators.

First, we observed the effect of SelS on cell mitosis. Control and SelS KD cells were harvested, the cell number was counted. As shown in Fig. 4A, the cell number was unchanged from day 0 to day 2 in the SelS KD group after MDI induction, but the cell number was increased by

Figure 2

Sels is essential for adipocyte differentiation. (A) Effect of Sels knockdown (KD) was assessed by Western blotting. After infection with recombinant lentivirus containing scrambled shRNA as a control or four different lentiviral Sels shRNAs for 2 days, immunoblotting analysis was used to verify Sels KD efficiency. (B) Oil Red O staining was performed in control and Sels KD 3T3-L1 cells after a 6-day induction of differentiation. (C) The triglyceride content was determined in control or Sels KD 3T3-L1 cells on day 6 after induction of differentiation. (D) Immunoblots showing the levels of Sels and key adipogenic markers (C/EBPα and PPARγ) in control and Sels KD 3T3-L1 cells during adipogenesis at the indicated times. Post-confluent 3T3-L1 preadipocytes were induced to differentiate with MDI and harvested on day 0, 2, 4, 6, and 8. $^{***}P < 0.01$ versus day 0, $^{*}P < 0.05$, $^{**}P < 0.01$ versus day 2. (E) Oil Red O staining was performed on 3T3-L1 cells on day 6 after induction of differentiation. Cells were transfected with recombinant lentivirus containing scrambled shRNA as a control, Sels shRNA for KD of Sels, and pCMV6-XL5-Sels for overexpression (OE) of Sels on day -4. After confluence for 2 days, 3T3-L1 preadipocytes were induced to differentiate and harvested on day 6. (F) Protein levels of adipogenic markers (C/EBPα, PPARγ, FABP4, C/EBPβ, adiponectin) were assessed by Western blotting in control, Sels KD, and Sels OE 3T3-L1 cells after 3 days of adipogenesis, $^{*}P < 0.05$, $^{***}P < 0.01$ versus Sels KD. Values in panels (A), (C), (D) and (F) are means $\pm$ s.d. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control.

two-fold in the control group. Compared with the control condition, Sels KD also significantly reduced cell number in proliferating 3T3-L1 preadipocytes on day 1, 2, 4, and 6 (Fig. 4A). Moreover, the cell cycle was analysed by flow cytometry, as shown in Fig. 4B, the cell cycle analysis clearly indicated that MDI initiated cell cycle progression in control cells, whereas Sels KD induced cell cycle arrest at the G1-S-phase transition. Consistently with this, the ratio of BrdU incorporation decreased from 20.8% in control cells to 6.8% in Sels KD cells (Fig. 4C).

Second, we checked the cell cycle-related proteins levels to assess the inhibitory mechanism of Sels KD on cell cycle block. As shown in Fig. 4D, the level of cyclin D1, synthesized during the early G₁ phase, and cyclin A2, synthesized during the S-phase transition, were increased in control cells during MCE, but this increase was inhibited by Sels KD. No dramatic effect on cyclin E1 in the late G₁ phase or on cyclin B1 levels in the M phase of the cell cycle were observed in Sels KD cells. CDK6 and CDK2 were also inhibited by Sels KD. Interestingly, Sels KD increased CDK4 protein levels (Fig. 4D). During MCE, Sels protein levels were increased at 4, 8, and 12 h after MDI treatment in control cells and then decreased beginning at 18 h.

To further identify the mechanism of cell cycle arrest, cell cycle inhibitors such as Rb, p21 and p27

were checked. Indeed, the phosphorylation of Rb at three sites was strongly increased in the late G₁ phase in control cells, whereas Sels KD inhibited the phosphorylation of Rb (Fig. 4E). As expected, the Rb level was decreased in control cells and increased in Sels KD cells (Fig. 4E). Thus, E2F1, which is repressed by active hypophosphorylated Rb, was increased in control cells but decreased in Sels KD cells (Fig. 4E). p27 was markedly increased, but p21 levels were not affected in Sels KD cells during MCE (Fig. 4E). According to these findings, induction of negative regulators of the cell cycle and remarkable reductions in cyclin D1, cyclin A2 and CDK6 are in part responsible for the cell cycle G₁ arrest in Sels KD cells.

The expression of ER stress markers in human and mice WAT

First, we examined the protein levels of ER stress markers (p-IRE1α/IRE1α, XBP1s, and Grp78) in WAT from N-obese and obese human subjects. Western blotting showed that the p-IRE1α/IRE1α, XBP1s, and Grp78 levels of subcutaneous fat and omental fat were increased with up-regulation of Sels in the obese group compared with the N-obese group ($P < 0.05$) (Fig. 5A and B).

Figure 3

Sels KD induces cell death during adipogenesis. (A) 3T3-L1 cells were observed under inverted phase contrast microscope after 5-day induction of differentiation. Arrows show the dead cells. Scale bar = 20 μm. (B) TUNEL labeling for apoptotic cell nuclei and subsequent DAPI staining for all the nuclei (blue) were performed on control and Sels KD 3T3-L1 cells after 5-day induction. Tunicamycin (Tm) and thapsigargin (Tg) were used as positive control. Percentage of TUNEL-positive cells was expressed as the number of positively stained apoptotic cells/the total number of cells counted × 100%. Scale bar = 20 μm. (C) Immunoblots showed the protein levels of Cleaved Casp3, Bim, Bcl-2, Bcl-xL, and Sels in control and Sels KD 3T3-L1 cells on day 6 after induction of differentiation. (D) Western blotting analysis of Bim, Puma, Bad, Bcl-2, Bcl-xL, Mcl-1, Bax, Bak, FABP4, and Sels was performed in control and Sels KD 3T3-L1 cells during adipogenesis on day 0, 2, 4, 6, and 8. Values in panel (B) and (C) are means ± s.d. ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control.

Second, we examined the protein levels of ER stress markers (p-IRE1α/IRE1α, XBP1s, and Grp78) in the subcutaneous fat and epididymal fat from mice fed NC or HFD by immunoblot analysis and IHC. The results showed that ER stress markers were significantly increased with upregulation of Sels in the HFD group compared with NC group (Fig. 5C, D, E and F) ($P < 0.05$).

Sels modulates IRE1α-XBP1 pathway

Whether Sels regulated adipogenesis is associated with the IRE1α-XBP1 pathway was assessed. First, we examined the effect of ER stress inducers, such as tunicamycin (Tm) and thapsigargin (Tg), on Sels protein levels. 3T3-L1 cells treated with 2.5, 5, and 10 μg/mL Tm for 12 h or 2.5 μg/mL Tm for 12, 24, and 36 h significantly increased protein levels of the Sels and ER stress markers (Grp78 and XBP1s) (Fig. 6A). In addition, 3T3-L1 cells treated with 0.1 and 1 μM Tg or 0.1 μM Tg for 12, 24, and 36 h significantly increased the Sels, Grp78 and XBP1s protein levels (Fig. 6B). Then, we determined the role of endogenous Sels in Tm- or Tg-induced ER stress in 3T3-L1 cells. As shown in Fig. 6C, Sels KD increased Grp78 levels but dramatically decreased XBP1s levels induced by Tm or Tg.

To further determine whether the IRE1α-XBP1s pathway modulated by Sels KD is related to adipocyte differentiation defects, the protein levels of p-IRE1α, IRE1α, XBP1s and Grp78 were determined in differentiation-induced 3T3-L1 cells. The protein expression levels of Grp78 were increased, p-IRE1α/IRE1α levels were not changed, but those of XBP1s were decreased in Sels KD cells through the entire adipogenic programme (Fig. 6D and E). Furthermore, the mRNA levels of XBP1s produced by IRE1α-mediated splicing were dramatically decreased by Sels KD compared with control levels after stimulation with Tm or Tg (Fig. 6F).

XBP1 OE rescued the phenotype of adipogenesis defect induced by Sels KD in 3T3-L1 cells

To further observe the effect of XBP1s on adipocyte differentiation in Sels KD/XBP1 OE cells, XBP1s was overexpressed. XBP1s OE significantly increased the reduced accumulation of intracellular lipids induced by Sels KD in Sels KD/XBP1 OE cells, as shown by Oil Red O staining (Fig. 7A). XBP1s OE also rescued the mRNA and protein levels of the adipogenic markers FABP4, PPARγ, C/EBPα and adiponectin in Sels KD/XBP1 OE cells

Figure 4

Sels KD causes G1 arrest in differentiation-induced 3T3-L1 cells. (A) Cell counts were determined in control and Sels KD 3T3-L1 cells from day -4 to day 6 of adipogenesis. (B) Cell cycle was analysed by flow cytometry in control and Sels KD 3T3-L1 cells after induction for 12, 20, 24, and 28 h. (C) BrdU labelling was performed on control and Sels KD 3T3-L1 cells for 2 h. The results were detected by fluorescence microscopy. Scale bar = 20 μ m. (D) Expression levels of cell cycle-related proteins (Cyclin D1, CDK4, CDK6, Cyclin E1, CDK2, Cyclin A2, Cyclin B1) were assessed by Western blotting analysis in control and Sels KD 3T3-L1 cells after induction for 0, 4, 8, 12, 18, and 24 h. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus day 0. (E) Immunoblots showed the levels of cell cycle inhibitors (p-Rb, Rb, p21, p27, E2F1) in control and Sels KD 3T3-L1 cells after induction for 0, 4, 8, 12, 18, and 24 h. Values in panel (A), (C), (D), and (F) are means $\pm$ s.d. ($n = 3$). $^*P < 0.05$, $^{**}P < 0.01$.

(Fig. 7B and C). Collectively, these findings demonstrate that suppression of fat accumulation induced by Sels KD can be inhibited by restoration of XBP1s.

To further identify the mechanism underlying the effect of XBP1s OE on the restoration of adipocyte differentiation defects induced by Sels suppression, the cell number was counted, and the cell cycle was analyzed by flow cytometry. The cell number increased from 18.9×10^4 in the Sels KD group to 56.0×10^4 in the Sels KD/XBP1 OE group ($P < 0.001$) (Fig. 7D). TUNEL assay showed that the percentage of TUNEL-positive cells were decreased from 8.98% in the Sels KD group to 3.4% in the Sels KD/XBP1s

OE group (Fig. 7E). As shown in Fig. 7F, the cell cycle analysis indicated that G0/G1 stage cells were decreased from 63.75% in the Sels KD group to 49.14% in the Sels KD/XBP1s OE group. In an effort to assess the mechanism underlying the effect of XBP1s on cell cycle block induced by Sels KD, cell cycle-related proteins and inhibitors were assessed. XBP1s OE significantly inhibited the decrease in Cyclin D1 and p-Rb807/Rb and the increase in P27 protein levels induced by Sels KD in Sels KD/XBP1 OE cells (Fig. 7G). According to these findings, XBP1 increases cycle-related proteins and decreases cell cycle inhibitors to promote progression of the cell cycle inhibited by Sels KD.

Figure 5

The expression of ER stress markers in human/mice WAT (A and B) Western blotting showed the levels of ER stress markers (p-IRE1α/IRE1α, XBP1s, and Grp78) in subcutaneous and omental fat of human subjects. * $P < 0.05$, ** $P < 0.01$ versus N-obese. The protein levels of ER stress markers (p-IRE1α/IRE1α, XBP1s, and Grp78) in mice subcutaneous and epididymal fat were determined by immunoblotting analysis (C and D), and Immunohistochemistry (E and F), Scale bar = 20 μm . * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus NC group.

Fig. 7H shows the model in which SelS deficiency blocks adipocyte differentiation. SelS KD induced cell cycle arrest through disruption of the IRE1α-XBP1s branch.

Discussion

SelS is a transmembrane protein that is localized primarily in the cell membrane and ER membrane (Zhang *et al.* 2011, Bettigole *et al.* 2015). It has been reported that SelS is closely associated with inflammation, oxidative stress and ER stress (Shchedrina *et al.* 2010, Fradejas *et al.* 2011, Liu *et al.* 2013), and its biological characteristics suggest a role in the pathogenesis and development of metabolic syndrome (Du *et al.* 2008, Olsson *et al.* 2011). This study showed that SelS protein levels in the obese group were significantly higher than in the N-obese group. Additionally, similar results were obtained in mice. These results revealed that SelS plays a role in the pathogenesis and development of obesity.

Furthermore, SelS was shown to act as a positive regulator of adipogenesis by regulating the cell cycle and

increasing cell survive. However, a study showed that SelS was decreased throughout the entire adipogenic program (Kim & Kim 2013). It seems not easy to pinpoint the reason to explain the discrepancy between these results because the multiple factors in the experimental conditions may contribute to the difference. Of note, one of the factors was the concentration of dexamethasone. In our study, we used 0.25 μM rather than 5 μM dexamethasone to induce adipogenesis. Because 5 μM dexamethasone can induce SelS degradation (Kim & Kim 2013), SelS protein levels were decreased during adipogenesis after higher dexamethasone stimulation. We also observed a significant decrease in SelS during the middle and late phases of adipogenesis, whether the decrease is also dexamethasone-dependent or not warrants further investigation.

It has been reported that SelS are involved in various cells survival during ER stress and oxidative stress (Lee *et al.* 2015, Men *et al.* 2018a,b). Consistent with these results, we also found that SelS deficiency induced an augmentation in 3T3-L1 adipocyte apoptosis after induction of differentiation by disruption of the IRE1α-XBP1 pathway, which was associated with an

Figure 6

Sels KD disrupts the IRE1 α -XBP1s branch. (A) Western blotting showed the levels of Sels and ER stress markers (Grp78, XBP1s) in 3T3-L1 preadipocytes treated with different concentrations of Tm for 12 h or 2.5 μ g/mL Tm for different times. (B) Protein levels of Sels and ER stress markers (Grp78 and XBP1s) were analysed by Western blotting analysis in 3T3-L1 preadipocytes treated with different dosages of Tg for 12 h or 0.1 μ M Tg for different times, 0: no treatment control, D: treat with DMSO, Tm: treated with Tm, Tg: treated with Tg. (C) Immunoblots showing the protein levels of the ER stress markers Grp78 and XBP1s in control and Sels KD 3T3-L1 cells treated with or without Tm and Tg. At 2 days postconfluence, cells were treated with 2.5 μ g/mL Tm or 0.1 μ M TG for 12 h before harvesting. (D) Protein levels of IRE1 α , XBP1s, and Grp78 were estimated by immunoblots in control and Sels KD 3T3-L1 cells after induction for 0, 4, 8, 12, 16, 20, and 24 h. (E) Protein levels of IRE1 α , XBP1s, and Grp78 were estimated by immunoblotting in control and Sels KD 3T3-L1 cells on day 0, 2, 4, 6, and 8. Values in panel (A), (B), (C), (D), and (E) are means $\pm$ s.d. ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus '0' or control. (F) RT-PCR analysis of *spliced Xbp1* (*Xbp1s*) and *unspliced Xbp1* (*Xbp1u*) mRNA in control and Sels KD 3T3-L1 cells treated with Tm or Tg. Values are means $\pm$ s.d. ($n = 3$), ** $P < 0.01$ versus control-Tm, # $P < 0.05$ versus control-Tg.

imbalance of the Bcl-2 family in favor of the apoptotic pathway. On the other hand, we found that Sels KD induced a decrease in cell number, suggesting that Sels KD inhibited cell proliferation by inducing cell death and cell cycle arrest.

MCE is necessary for the progression through subsequent steps in differentiation programs. Moreover, the cell cycle and its regulation play a critical role in the completion of MCE. It has been shown that some selenoproteins, including selenoprotein H and selenoprotein W, have a function in controlling

cell cycle (Alkan *et al.* 2015, Bertz *et al.* 2018). However, the role of Sels on cell cycle is still unclear. In view of MCE arrest induced by Sels KD, we performed cell cycle regulation studies. Because the G1/S-phase transition is a critical point of MCE, understanding the role of Sels in regulating G1/S-phase transition is very important. In this study, we found that Sels KD induced cell cycle arrest in the G1/S-phase transition by decreasing cyclin D1/CDK4/6 complexes and phosphorylation of Rb and by increasing p27. Regarding the mechanisms by which Sels regulates adipocyte differentiation,

Figure 7

XBP1s OE rescued the phenotype of Sels KD-induced adipogenesis defects in 3T3-L1 cells. (A) Oil Red O staining was performed in Sels KD/XBP1s OE cells. Four days after Sels KD and/or XBP1 OE in 3T3-L1 cells, 3T3-L1 preadipocytes were induced to differentiate, and Oil Red O staining was performed on day 5. Scale bar = 20 μ m. (B) Quantitative PCR of Sels KD/XBP1s OE cells. Sels KD and/or XBP1s OE in 3T3-L1 cells on day -4, the mRNA levels of adipogenic markers PPAR γ , C/EBP α , FABP4, and adiponectin were determined by quantitative PCR on day 6. (C) Western blotting of Sels KD/XBP1s OE cells. Immunoblotting of the adipogenic markers PPAR γ , C/EBP α , FABP4, adiponectin, XBP1s, and Sels proteins were performed on day 6. (D) Cell counts were determined in Sels KD/XBP1s OE 3T3-L1 cells on day 2. (E) TUNEL labeling of Sels KD/XBP1s OE 3T3-L1 cells after 5-day induction. Quantitative TUNEL data were shown. Scale bar = 20 μ m. (F) Cell cycle was analyzed by flow cytometry in Sels KD/XBP1s OE cells after induction for 18 h. (G) Immunoblotting of Cyclin D1, P27, p-Rb807, Rb, XBP1s and Sels in Sels KD and/or XBP1s OE 3T3-L1 cells after induction for 18 h. (H) The model showing that Sels deficiency blocks preadipocyte proliferation and differentiation through disruption of the IRE1 α -XBP1 branch. Values are means $\pm$ s.d. ($n = 3$), * $P < 0.05$, ** $P < 0.01$ versus control, # $P < 0.05$, ## $P < 0.01$ versus Sels KD, \$ $P < 0.05$, \$\$ $P < 0.01$ versus XBP1s OE.

our results suggest that Sels promotes cell cycle transition during MCE.

In obese rodent and human models, some studies have demonstrated that ER stress and related signaling pathways activation are the most common and important cellular mechanism underlying obesity and insulin resistance (Gregor & Hotamisligil 2007, Zha & Zhou 2012). It also has been reported that obesity increases the levels of ER stress in adipocytes, IRE1 activation with upregulation of XBP1s mRNA were found in subcutaneous and visceral adipose tissue of obese subjects (Ozcan *et al.* 2004, Boden *et al.* 2008, Vendrell *et al.* 2010, Cnop *et al.* 2012). Moreover, ER stress markers (Grp78, XBP1s) were reduced in adipose tissue of obese subjects after weight loss (Gregor *et al.* 2009). Consistent with these results, our study demonstrated that ER stress was present and IRE1 α -XBP1 pathway was activated with upregulating of Sels

expression in visceral and subcutaneous adipose tissue of obese subjects.

Furthermore, the results of present study showed that ER stress inducers significantly increased protein levels of the Sels, proved that ER stress could regulate Sels expression. XBP1 regulates a subset of UPR genes that promote ERAD of misfolded proteins, Sels is responsible for ERAD by forming a complex with ubiquitin ligase E3, Derlin-1, selenoprotein K and p97ATPase (Bettigole *et al.* 2015), suggesting that Sels levels were increased in WAT of obesity maybe relate to XBP1s increased. As an ER resident protein, Sels is largely known to participate in modulating ERAD, a ubiquitin-proteasome-dependent cellular survival mechanism, against overloading misfolded proteins (Curran *et al.* 2005). It has been demonstrated that Sels was involved in Alzheimer disease by regulating ER stress (Jang *et al.* 2017) and plays a critical role in the expression of XBP1, which spliced

by IRE1 α and required for initiation of the adipogenesis (Sha *et al.* 2009, Cho *et al.* 2014, Lee *et al.* 2014). Some studies suggested that XBP1-deficient cells or animals exhibit profound defects in adipogenesis and adipocyte differentiation (Lee *et al.* 2005, Sha *et al.* 2009), which are similar to the phenotypes of SelS-deficient 3T3-L1 cells. Thus, the mechanism of adipogenesis defects in SelS KD cells via disruption of the IRE1 α -XBP1 branch was identified.

Our results showed downregulation of mRNA and protein levels of XBP1s in SelS KD cells during Tm or Tg treatment, but the levels of Grp78 were increased, the levels of p-IRE1 α /IRE1 α were not changed, indicating that SelS is required for the splicing of XBP1 by IRE1 α . Moreover, XBP1s OE rescued the phenotype of SelS KD cells, further suggesting that the IRE1 α -XBP1 pathway is involved in the modulation of adipogenesis defects induced by SelS KD. Our results suggest that SelS KD blocks preadipocyte differentiation through disruption of the IRE1 α -XBP1 branch. Therefore, SelS is a novel pro-adipogenic selenoprotein and may be a potential target for the treatment of obesity and associated disorders.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-19-0292>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants from National Natural Science Foundation of China (81700747, 81570727, and 81700726) and the Natural Science Foundation of Liaoning Province (20170540289). This work was also supported by the University of Chicago and Diabetes Research and Training Center (P30-DK020595).

Author contribution statement

Lili Men and Junjie Yao performed experiments, analyzed data and wrote the paper; Shanshan Yu performed experiments and wrote the paper; Yu Li, Jin Shi, Guixin Zhang, and Siyuan Cui performed experiments; Jianling Du and Decheng Ren were the guarantor of this work, who have full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analyses. All authors discussed the results. All authors read and approved the final manuscript.

Acknowledgements

The authors thank Dr Pixu Liu and Dr Huihua Li for insightful suggestions, advice and discussions.

References

- Alkan Z, Duong FL & Hawkes WC 2015 Selenoprotein W controls epidermal growth factor receptor surface expression, activation and degradation via receptor ubiquitination. *Biochimica et Biophysica Acta* **1853** 1087–1095. (<https://doi.org/10.1016/j.bbamcr.2015.02.016>)
- Bertz M, Kuhn K, Koeberle SC, Muller MF, Hoelzer D, Thies K, Deubel S, Thierbach R & Kipp AP 2018 Selenoprotein H controls cell cycle progression and proliferation of human colorectal cancer cells. *Free Radical Biology and Medicine* **127** 98–107. (<https://doi.org/10.1016/j.freeradbiomed.2018.01.010>)
- Bettigole SE, Lis R, Adoro S, Lee AH, Spencer LA, Weller PF & Glimcher LH 2015 The transcription factor XBP1 is selectively required for eosinophil differentiation. *Nature Immunology* **16** 829–837. (<https://doi.org/10.1038/ni.3225>)
- Boden G, Duan X, Homko C, Molina EJ, Song W, Perez O, Cheung P & Merali S 2008 Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. *Diabetes* **57** 2438–2444. (<https://doi.org/10.2337/db08-0604>)
- Castro G, C Areias MF, Weissmann L, Quaresma PG, Katashima CK, Saad MJ & Prada PO 2013 Diet-induced obesity induces endoplasmic reticulum stress and insulin resistance in the amygdala of rats. *FEBS Open Bio* **3** 443–449. (<https://doi.org/10.1016/j.fob.2013.09.002>)
- Cha BH, Kim JS, Ahn JC, Kim HC, Kim BS, Han DK, Park SG & Lee SH 2014 The role of tauroursodeoxycholic acid on adipogenesis of human adipose-derived stem cells by modulation of ER stress. *Biomaterials* **35** 2851–2858. (<https://doi.org/10.1016/j.biomaterials.2013.12.067>)
- Cho YM, Kwak SN, Joo NS, Kim DH, Lee AH, Kim KS, Seo JB, Jeong SW & Kwon OJ 2014 X-box binding protein 1 is a novel key regulator of peroxisome proliferator-activated receptor gamma2. *FEBS Journal* **281** 5132–5146. (<https://doi.org/10.1111/febs.13052>)
- Cnop M, Foufelle F & Velloso LA 2012 Endoplasmic reticulum stress, obesity and diabetes. *Trends in Molecular Medicine* **18** 59–68. (<https://doi.org/10.1016/j.molmed.2011.07.010>)
- Curran JE, Jowett JB, Elliott KS, Gao Y, Gluschenko K, Wang J, Abel Azim DM, Cai G, Mahaney MC, Comuzzie AG, *et al.* 2005 Genetic variation in selenoprotein S influences inflammatory response. *Nature Genetics* **37** 1234–1241. (<https://doi.org/10.1038/ng1655>)
- Deldicque L, Cani PD, Delzenne NM, Baar K & Francaux M 2013 Endurance training in mice increases the unfolded protein response induced by a high-fat diet. *Journal of Physiology and Biochemistry* **69** 215–225. (<https://doi.org/10.1007/s13105-012-0204-9>)
- Du JL, Sun CK, Lü B, Men LL, Yao JJ, An LJ & Song GR 2008 Association of SelS mRNA expression in omental adipose tissue with Homa-IR and serum amyloid A in patients with type 2 diabetes mellitus. *Chinese Medical Journal* **121** 1165–1168.
- Fradejas N, Serrano-Pérez Mdel C, Tranque P & Calvo S 2011 Selenoprotein S expression in reactive astrocytes following brain injury. *Glia* **59** 959–972. (<https://doi.org/10.1002/glia.21168>)
- Gregor MF & Hotamisligil GS 2007 Thematic review series: adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. *Journal of Lipid Research* **48** 1905–1914. (<https://doi.org/10.1194/jlr.R700007-JLR200>)
- Gregor MF, Yang L, Fabbrini E, Mohammed BS, Eagon JC, Hotamisligil GS & Klein S 2009 Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes* **58** 693–700. (<https://doi.org/10.2337/db08-1220>)

- Han J & Kaufman RJ 2014 Measurement of the unfolded protein response to investigate its role in adipogenesis and obesity. *Methods in Enzymology* **538** 135–150. (<https://doi.org/10.1016/B978-0-12-800280-3.00008-6>)
- Jang JK, Park KJ, Lee JH, Ko KY, Kang S & Kim IY 2017 Selenoprotein S is required for clearance of C99 through endoplasmic reticulum-associated degradation. *Biochemical and Biophysical Research Communications* **486** 444–450. (<https://doi.org/10.1016/j.bbrc.2017.03.060>)
- Kim CY & Kim KH 2013 Dexamethasone-induced selenoprotein S degradation is required for adipogenesis. *Journal of Lipid Research* **54** 2069–2082. (<https://doi.org/10.1194/jlr.M034603>)
- Kim KH, Gao Y, Walder K, Collier GR, Skelton J & Kissebah AH 2007 SEPS1 protects RAW264.7 cells from pharmacological ER stress agent-induced apoptosis. *Biochemical and Biophysical Research Communications* **354** 127–132. (<https://doi.org/10.1016/j.bbrc.2006.12.183>)
- Lee AH, Chu GC, Iwakoshi NN & Glimcher LH 2005 XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO Journal* **24** 4368–4380. (<https://doi.org/10.1038/sj.emboj.7600903>)
- Lee JH, Kwon JH, Jeon YH, Ko KY, Lee SR & Kim IY 2014 Pro178 and Pro183 of selenoprotein S are essential residues for interaction with p97(VCP) during endoplasmic reticulum-associated degradation. *Journal of Biological Chemistry* **289** 13758–13768. (<https://doi.org/10.1074/jbc.M113.534529>)
- Lee JH, Park KJ, Jang JK, Jeon YH, Ko KY, Kwon JH, Lee SR & Kim IY 2015 Selenoprotein S-dependent selenoprotein K binding to p97(VCP) protein is essential for endoplasmic reticulum-associated degradation. *Journal of Biological Chemistry* **290** 29941–29952. (<https://doi.org/10.1074/jbc.M115.680215>)
- Lefterova MI & Lazar MA 2009 New developments in adipogenesis. *Trends in Endocrinology and Metabolism* **20** 107–114. (<https://doi.org/10.1016/j.tem.2008.11.005>)
- Liu J, Li F & Rozovsky S 2013 The intrinsically disordered membrane protein selenoprotein S is a reductase in vitro. *Biochemistry* **52** 3051–3061. (<https://doi.org/10.1021/bi4001358>)
- Men L, Sun J & Ren D 2018a Deficiency of VCP-interacting membrane selenoprotein (VIMP) leads to G1 cell cycle arrest and cell death in min6 insulinoma cells. *Cellular Physiology and Biochemistry* **51** 2185–2197. (<https://doi.org/10.1159/000495865>)
- Men L, Yu S, Yao J, Li Y, Ren D & Du J 2018b Selenoprotein S protects against adipocyte death through mediation of the IRE1alpha-XBP1 pathway. *Biochemical and Biophysical Research Communications* **503** 2866–2871. (<https://doi.org/10.1016/j.bbrc.2018.08.057>)
- Moseti D, Regassa A & Kim WK 2016 Molecular regulation of adipogenesis and potential anti-adipogenic bioactive molecules. *International Journal of Molecular Sciences* **17** E124. (<https://doi.org/10.3390/ijms17010124>)
- NCD Risk Factor Collaboration (NCD-RisC) 2016 Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* **387** 1377–1396. ([https://doi.org/10.1016/S0140-6736\(16\)30054-X](https://doi.org/10.1016/S0140-6736(16)30054-X))
- Olsson M, Olsson B, Jacobson P, Thelle DS, Bjorkegren J, Walley A, Froguel P, Carlsson LM & Sjöholm K 2011 Expression of the selenoprotein S (SELS) gene in subcutaneous adipose tissue and SELS genotype are associated with metabolic risk factors. *Metabolism: Clinical and Experimental* **60** 114–120. (<https://doi.org/10.1016/j.metabol.2010.05.011>)
- Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Gorgun C, Glimcher LH & Hotamisligil GS 2004 Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science* **306** 457–461. (<https://doi.org/10.1126/science.1103160>)
- Ren D, Sun J, Mao L, Ye H & Polonsky KS 2014 BH3-only molecule Bim mediates beta-cell death in IRS2 deficiency. *Diabetes* **63** 3378–3387. (<https://doi.org/10.2337/db13-1814>)
- Sha H, He Y, Chen H, Wang C, Zenno A, Shi H, Yang X, Zhang X & Qi L 2009 The IRE1alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis. *Cell Metabolism* **9** 556–564. (<https://doi.org/10.1016/j.cmet.2009.04.009>)
- Sha H, He Y, Yang L & Qi L 2011 Stressed out about obesity: IRE1alpha-XBP1 in metabolic disorders. *Trends in Endocrinology and Metabolism* **22** 374–381. (<https://doi.org/10.1016/j.tem.2011.05.002>)
- Shchedrina VA, Zhang Y, Labunskyy VM, Hatfield DL & Gladyshev VN 2010 Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. *Antioxidants and Redox Signaling* **12** 839–849. (<https://doi.org/10.1089/ars.2009.2865>)
- Song W, Ren D, Li W, Jiang L, Cho KW, Huang P, Fan C, Song Y, Liu Y & Rui L 2010 SH2B regulation of growth, metabolism, and longevity in both insects and mammals. *Cell Metabolism* **11** 427–437. (<https://doi.org/10.1016/j.cmet.2010.04.002>)
- Tang QQ & Lane MD 2012 Adipogenesis: from stem cell to adipocyte. *Annual Review of Biochemistry* **81** 715–736. (<https://doi.org/10.1146/annurev-biochem-052110-115718>)
- Vendrell J, Maymo-Masip E, Tinahones F, Garcia-Espana A, Megia A, Caubet E, Garcia-Fuentes E & Chacon MR 2010 Tumor necrosis-like weak inducer of apoptosis as a proinflammatory cytokine in human adipocyte cells: up-regulation in severe obesity is mediated by inflammation but not hypoxia. *Journal of Clinical Endocrinology and Metabolism* **95** 2983–2992. (<https://doi.org/10.1210/jc.2009-2481>)
- Wang Y, Mi J, Shan XY, Wang QJ & Ge KY 2007 Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China. *International Journal of Obesity* **31** 177–188. (<https://doi.org/10.1038/sj.ijo.0803354>)
- Ye Y, Fu F, Li X, Yang J & Liu H 2016 Selenoprotein S is highly expressed in the blood vessels and prevents vascular smooth muscle cells from apoptosis. *Journal of Cellular Biochemistry* **117** 106–117. (<https://doi.org/10.1002/jcb.25254>)
- Zha BS & Zhou H 2012 ER stress and lipid metabolism in adipocytes. *Biochemistry Research International* **2012** 312943. (<https://doi.org/10.1155/2012/312943>)
- Zhang N, Jing W, Cheng J, Cui W, Mu Y, Li K & Lei X 2011 Molecular characterization and NF-kappaB-regulated transcription of selenoprotein S from the Bama mini-pig. *Molecular Biology Reports* **38** 4281–4286. (<https://doi.org/10.1007/s11033-010-0551-y>)

Received in final form 3 December 2019

Accepted 16 December 2019

Accepted Manuscript published online 17 December 2019