Estrogen suppresses adipogenesis by inhibiting S100A16 expression

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Abstract

The aim of this study is to determine the effects of E2 on metabolic syndrome and the molecular mechanisms involving S100A16. Ovariectomized (OVX) rat models and mouse embryonic fibroblasts cell models were used. E2 loss in OVX rats induced body weight gain and central abdominal fat accumulation, which were ameliorated by E2 treatment under chow and high-fat diet (HFD) conditions. E2 decreased the expression of the adipocyte marker genes PPARγ, aP2, C/EBPα, and S100A16. E2 inhibited adipogenesis. Overexpression of S100A16 reversed the E2-induced adipogenesis effect. A luciferase assay showed that E2 inhibited the expression of S100A16. E2 treatment decreased body weight gain and central abdominal fat accumulation under both chow and HFD conditions. Also, E2 suppressed adipogenesis by inhibiting S100A16 expression.

Key Words
- estrogen
- metabolic syndrome
- S100A16

Introduction

In postmenopausal women, estrogen loss is an independent risk factor for more severe menopausal symptoms, such as metabolic disease, insulin resistance, and type 2 diabetes (Carr 2003, Manrique et al. 2012). Studies on mouse models have shown that oophorectomy results in obesity, altered fat distribution, adipose tissue inflammation, and development of fatty liver (Rogers et al. 2009, Stubbins et al. 2012). Estrogen therapy is believed to have beneficial effects on abdominal fat accumulation, insulin resistance, and development of type 2 diabetes in postmenopausal women (Sørensen et al. 2001, Stubbins et al. 2012). However, there are reports that estrogen treatment does not improve insulin action in humans and rodents (Basu et al. 2007); furthermore, it is associated with a higher risk of insulin resistance and type 2 diabetes in postmenopausal women (Ryan et al. 2002, Ding et al. 2007). The longitudinal data from the Study of Women’s Health Across the Nation (SWAN), which included five ethnic groups in the USA – African-Americans, Caucasians, Chinese, Hispanic, and Japanese – indicated that early use of hormone therapy was a risk factor for obesity (Sutton-Tyrrell et al. 2010), and there was little evidence to suggest that estrogen application was beneficial for insulin resistance and stroke (Hu & Grodstein 2002,
Therefore, the relationship between estrogen use and metabolic disorders in postmenopausal women remains controversial. Moreover, the therapeutic effects of estrogen may be influenced by many factors, such as the dosage, the duration of treatment, and the type of estrogen used. Another disadvantage of the clinical application of estrogen is its reported growth-promoting effects on the uterus or mammary gland and tumor-promoting actions (Król & Milnerowicz 2012, Nichols et al. 2012). Further studies are therefore needed to re-evaluate the benefits of estrogen treatment and to determine the estrogen dose at which the risk of metabolic diseases is increased.

The mechanisms via which estrogen affects metabolism are complicated and not well known. It is reported that the anti-obesity effect of estrogen depends on leptin and Stat3 activation (Gao et al. 2007). In our previous study (Liu et al. 2011), we found that a novel adipogenesis-promoting factor, the S100A16 protein, which is a member of the S100 protein family, plays a role in the action of estrogen. We analyzed the S100A16 promoter using AliBaba2 (Grabe 2002), and found four estrogen response elements (EREs) within the 1500-bp promoter region. Therefore, we think that estrogen may regulate lipid metabolism by mediating S100A16 expression.

In this study, we used normal rats and ovariectomized (OVX) rats that received estrogen treatment or control treatment under chow and high-fat diet (HFD) conditions. Therefore, we think that estrogen may regulate lipid metabolism by mediating S100A16 expression.

**Materials and methods**

**Animals**

Fifty healthy female specific pathogen-free Sprague–Dawley (SD) rats 6 weeks of age (body weight, 150–200 g) were purchased from and housed at the Experimental Animal Center of Nanjing Medical University (Nanjing, China). The experiments were approved by the Nanjing Medical University Ethical Committee. Animals were housed at 23±1 °C with a 12 h light:12 h darkness cycle and 45±5% humidity and allowed free access to normal chow diet and water. Thirty-two female SD rats underwent bilateral oophorectomy at the age of 8 weeks under general anesthesia induced with ketamine (120 mg/kg, i.p.). After 2 weeks of recovery, the rats were allowed free access to water and were fed normal powder chow or HFD. The HFD was supplied by OpenSource DIETS (Research Diets, Inc., New Brunswick, NJ, USA, #D12451). Sixteen OVX rats received s.c. injections (at the back of the neck) of 200 μg/kg 17β-estradiol (Sigma, #E2758) twice a week for 16 weeks. Control rats (n=16) received PBS injections. We measured body weight every week after the first injection.

Three groups of rats were on the Chow diet: normal control rats (n=9); OVX rats (n=8); and OVX rats given E2 treatment (n=8). There were also three groups of rats on HFD: normal control rats (n=9); OVX rats (n=8); and OVX rats given E2 treatment (n=8). All procedures were approved by the Experimental Animal Center of Nanjing Medical University.

To assess the effects of E2 levels on body weight gain, we monitored the body weight of all rats every week and measured visceral fat weight when the rats were anesthetized with Nembutal (100 mg/kg). All protocols involving the use of animals have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Nanjing Medical University. In addition, we investigated the morphology of visceral fat cells from all rats fed normal chow and HFD by using hematoxylin and eosin staining.

**Intraperitoneal glucose tolerance test**

Fourteen weeks after the E2 treatment, IPGTT was conducted. All rats were starved for 12 h, and then their tail blood glucose concentrations (mM) were monitored using a handheld glucometer (ACCU-CHEK Performa, Roche). Blood was collected from the inner canthus to detect insulin concentration. Next, all rats received i.p. injections of glucose (2 g/kg body weight); tail blood glucose concentrations were measured using the handheld glucometer and blood was collected from the inner canthus at 15, 30, 60, and 120 min. Blood samples were analyzed for insulin at every time point using an immunoradiometric assay kit (#KAP1251) supplied by DIAsource Immuno-Assays S.A (Louvain-La-Neuve, Belgium).

**Measurement of E2 and liver and kidney function indicators**

At the end of 16 weeks of E2 treatment, all rats were anesthetized with ketamine (120 mg/kg i.p.), and blood was collected by cardiac puncture from the left ventricle into tubes precoated with potassium-EDTA, and centrifuged at 3000 g and 4 °C for plasma preparation. The plasma concentrations of E2 were quantified using immunoradiometric assay kits supplied by DIAsource ImmunoAssays S.A. (#KIP0629). The plasma levels of
total cholesterol (TC), triglyceride (TG), LDL, HDL, alanine transaminase (ALT), lactate dehydrogenase (LDH), creatinine (Cr), urea, and uric acid (UA) were determined by the Medical Laboratory of Jiangsu Province Hospital, The First Affiliated Hospital of Nanjing Medical University. All assays were performed according to the manufacturer’s instructions.

Visceral fat was removed rapidly; part of the samples was frozen in liquid N2 and stored at −80°C for the extraction of protein, and part of the samples was formalin fixed and paraffin embedded for the production of pathological sections. The same procedure was performed on the rest of the tissues.

Mouse embryonic fibroblast isolation and differentiation

Mouse embryonic fibroblasts (MEFs) were isolated from the embryos of C57BL/6 and S100A16+Tg/+ mice at 13.5 days post coitum. The construct was generated by inserting S100A16 cDNA into a vector with the PCAG promoter. We then obtained transgenic mice by the microinjection method. This F0 transgenic mouse was bred with the C57BL/6 mouse to obtain F1 transgenic mouse lines. S100A16-positive transgenic mice and their genotype were confirmed by PCR, and the expression of S100A16 mouse lines.

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TG GPO-POD assay

Cellular TG content was determined using a TG GPO-POD assay kit (Sigma). MEFs were cultured and induced in a 10-cm well by MIX to differentiate into adipocytes (10 days), washed with PBS twice, scraped into 500 μl PBS, sonicated to homogenize the suspension, and then assayed for total TG content.

Protein extraction and western blotting

Tissues and cells were washed twice with ice-cold PBS. Then, 100 mg tissue was lysed with 1 ml lysis buffer (50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% (v/v) Nonidet-P40, 1 mM EDTA, 1 mM NaF, 10 μg/ml aprotinin, 10 μM leupeptin, and 1 mM phenylmethylsulfonyl fluoride). The cells were scraped into the lysis buffer, and then tissues and cells were allowed to stand on ice for 30 min. After centrifugation at 4°C, the proteins in the supernatant were extracted and separated on SDS–polyacrylamide gels before being subjected to a standard western blot assay and imaged using Molecular Imager ChemiDoc XRS+ with the Image Lab Software (Version 4.0.1, Bio-Rad Laboratories, Hercules, CA, USA).

Luciferase assay

Luciferase reporter plasmid construction. The S100A16 promoter (1500 bp) was amplified by PCR using primers terminating in an XhoI and HindIII recognition sequence. The PCR product was digested and ligated into a pGL3-basic vector.

Transient transfection and luciferase assay. The day before transfection, CHO cells were plated on 12-well cell culture plates at 3 × 10⁵ cells/well. Transfection was performed using the X-tremeGENE HP DNA Transfection Reagent (#06365752001, Roche), following the manufacturer’s protocol. Six hours later, cells were treated with E2 (400 pg/ml) and/or tamoxifen (0.05 μM) for 24 h. The Renilla luciferase reporter plasmid pRL-SV40 was used as an internal control. Luciferase activity was measured using the dual luciferase assay system (Promega, E1910) with a luminometer (GloMax 20/20 Luminometer, Promega (Turner)). Luciferase assays were performed in triplicate and repeated at least three times to confirm their reproducibility.

Statistical analyses

The in vitro and in vivo results were analyzed using one-way ANOVA. P<0.05 was considered to indicate statistical significance. The results are presented as mean ± S.D. of the values from three to six replicates per group.
Results

The effect of E2 on body weight gain, visceral fat weight, and biochemical traits under both the chow and HFD conditions

We measured E2 concentrations at the time the animals were killed. Under the chow conditions, in comparison to the control rats, E2 concentrations increased in the OVX rats treated with E2 (759.11 ± 53.22 vs 77.09 ± 5.21 pg/ml) and decreased in the OVX rats not treated with E2 (42.0 ± 3.02 vs 77.09 ± 5.21 pg/ml); also under the HFD conditions, E2 concentrations increased in the OVX rats treated with E2 (1209.08 ± 198.02 vs 100.82 ± 14.09 pg/ml) and decreased in the OVX rats not treated with E2 (34.0 ± 3.15 vs 100.82 ± 14.09 pg/ml) (Table 1).

Under the chow and HFD conditions, the high level of E2 was more efficacious at lowering the percentage of body weight gain and visceral fat weight than control treatment. Under both diet conditions, low levels of E2 had the opposite effect. Under the chow conditions, the percentage of body weight gain was 5.32% in the high-E2 group, 64.71% in the low-E2 group, and 38.23% in the control group. Visceral fat weight was 12.06 g in the control group (Table 1).

Under both diet conditions, the size of visceral fat cells was decreased in the E2-treated OVX rats, and it was increased in the OVX rats. These results were similar to the body weight changes and confirm that E2 inhibited body weight gain (Fig. 1A).

The plasma levels of TC and TGs increased in OVX rats, and returned to normal levels with E2 treatment under both conditions (Table 2). There were no changes in HDL and LDL levels under these conditions, which was decreased using E2 treatment in the two conditions (Table 2).

Table 1 E2 inhibited body weight gain and visceral fat weight increase under both the chow and HFD conditions. Plasma concentration of E2, body weight gain, and visceral fat weight in the different groups of rats. Results are expressed as mean ± s.d.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma E2 (pg/ml)</th>
<th>Body weight gain (%)</th>
<th>Visceral fat weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>OVX</td>
<td>OVX + E2</td>
</tr>
<tr>
<td>Chow</td>
<td>77.09 ± 5.21</td>
<td>42.3 ± 3.02*</td>
<td>759.11 ± 53.22</td>
</tr>
<tr>
<td>HFD</td>
<td>100.82 ± 14.09</td>
<td>34.3 ± 3.15*</td>
<td>1209.08 ± 198.02*</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with the corresponding control group. OVX, ovariectomized; HFD, high-fat diet; E2, estrogen.

The levels of ALT and LDH, which are common biomarkers for liver damage, were determined in plasma samples. Under both conditions, the level of ALT and LDH were increased, which were reduced by E2 treatment (Table 3). Morphological analysis of liver cells showed that compared with chow-fed rats, the livers of rats fed HFD appeared to be filled with macrovesicular fat within the hepatocytes. Importantly, E2 treatment reduced lipid accumulation in the liver under both conditions (Fig. 1B).

The plasma levels of UA in the OVX group was increased but the difference was not significant. Whilst the levels for the E2-treated OVX group were lower than those for the OVX group, in the HFD group the levels were higher than in the control group and in the Chow group the levels were lower. Both these differences were significant, implying that the E2-treated groups differed from the control group in opposite directions (Table 3).

The effect of E2 on systemic glucose homeostasis

The effect of E2 on systemic glucose homeostasis was evaluated by the IPGTT. Under the chow conditions, blood glucose reached its maximum at 15 min in all rats. Interestingly, the maximum blood glucose level was 53.3 ± 4.3 and 54.7 ± 3.2 mM in the normal and OVX rats, respectively, but only 28.4 ± 2.3 mM in the OVX rats treated with E2. Subsequently, the blood glucose level gradually decreased in all rats. Importantly, the blood glucose of control and OVX rats treated with E2 dropped to low levels at 120 min, but the blood glucose of the OVX rats not treated with E2 was higher than physiological concentrations at 120 min (32.2 ± 2.2 vs 6.1 ± 1.1 mM; Fig. 2A). The OVX rats had already become glucose-intolerant at this time. When compared with control rats, glucose-stimulated insulin secretion was abolished and insulin concentration remained low in the OVX rats but not E2-treated OVX rats (Fig. 2B).

Under the HFD conditions, blood glucose reached its maximum at 30 min in normal and OVX rats, at 88.6 ± 7.7 and 87.2 ± 6.5 mM, respectively, but the maximum was decreased in all groups of rats. Results are expressed as mean ± s.d.
71.1 ± 4.3 nM at 15 min in the OVX rats treated with E2. The blood glucose of the normal and the OVX rats decreased but did not drop to normal levels (42.2 ± 3.1 vs 6.1 ± 1.1 nM). The blood glucose of the OVX rats treated with E2 dropped to close to the normal levels, at 20.4 ± 5.7 nM at 120 min (Fig. 2C). Glucose-stimulated insulin secretion was abolished and insulin concentration remained low in the OVX rats but not E2-treated OVX rats (Fig. 2D). The OVX rats had already become glucose-intolerant at this time.

**The effect of E2 on the expression of adipogenesis marker genes PPARγ, C/EBPα, and aP2 in fat tissues**

Differentiation of preadipocytes to adipocytes requires coordinated action of a large repertoire of transcription factors, including PPARγ and C/EBPα (6). The expression of these genes is often induced by E2, which can promote adipogenesis (7). To investigate the effect of E2 on adipogenesis in fat tissues, we measured the expression levels of PPARγ, C/EBPα, and aP2 in the fat tissues of control rats, OVX rats, and OVX rats treated with E2.

**Table 2** The effect of E2 on TC, TG, LDL, and HDL under both the chow and the HFD conditions. Plasma level of TC, TGs, LDL, and HDL in the different groups of rats. Results are expressed as mean ± S.D.

<table>
<thead>
<tr>
<th>Diet</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>LDL (mmol/l)</th>
<th>HDL (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>OVX</td>
<td>OVX + E2</td>
<td>Normal</td>
</tr>
<tr>
<td>Chow</td>
<td>2.16 ± 0.16</td>
<td>2.84 ± 0.09*</td>
<td>2.18 ± 1.12*</td>
<td>0.67 ± 0.26</td>
</tr>
<tr>
<td>HFD</td>
<td>2.23 ± 0.27</td>
<td>3.3 ± 0.15*</td>
<td>2.32 ± 0.29*</td>
<td>1.11 ± 0.08</td>
</tr>
</tbody>
</table>

*P < 0.05 compared with the corresponding control group. TC, total cholesterol; TGs, triglycerides; OVX, ovariectomized; HFD, high-fat diet; E2, estrogen.
factors, such as PPARγ, C/EBPα, and aP2. S100A16 is a novel adipogenesis-promoting factor. In order to study the mechanism of E2-suppression of body weight gain, we detected the expression of these proteins in the fat tissue of all groups. According to the results of western blotting, the expression of S100A16, PPARγ, C/EBPα, and aP2 was significantly elevated in fat tissues from non-treated OVX rats fed chow or HFD. In sharp contrast to these observations, these proteins only showed a slight increase in the fat tissues from OVX rats treated with E2 under both conditions (Fig. 3A, B, C, D, E, F, G and H).

The role of S100A16 in E2-induced suppression of adipogenesis

When compared with control cells, E2-treated S100A16Tg/C−/− MEFs (preadipocytes) showed a significant decrease in S100A16 expression in a dose-dependent manner (Fig. 4A and C). Stimulation of S100A16Tg/C−/− MEFs with 100 pg/ml E2 (Fig. 4B and D) showed a significant change in S100A16 protein expression after 24 h. Therefore, E2 inhibited S100A16 expression in a dose- and time-dependent manner. We also detected the effect of E2 on endogenous S100A16 expression using normal MEFs from C57BL/6 mice. The results indicated that E2 inhibited S100A16 expression (Fig. 4E and F). We examined the effect of E2 on the levels of S100A16 mRNA in normal MEFs and S100A16Tg/C−/− MEFs. The results showed that E2 suppressed the S100A16 mRNA expression in normal MEFs, but had no effect on the S100A16 mRNA expression in S100A16Tg/C−/− MEFs (Fig. 4G and H).

To confirm whether E2 suppressed adipogenesis by inhibiting the expression of S100A16, MEFs isolated from S100A16Tg/C−/− and C57BL/6 mice were treated with different concentrations of E2 and stained with Oil Red O. E2 treatment resulted in sparse staining, which was ameliorated by the overexpression of S100A16 (Fig. 4I). Consistent with the staining patterns, quantitative analysis of cellular TGs showed that TG accumulation was significantly lower in the C57BL/6 MEFs treated with E2 (400 pg/ml) than the S100A16Tg/C−/− cells (Fig. 4J). The results indicate that overexpression of S100A16 reversed reduction of TG accumulation induced by E2.

Effect of E2 on S100A16 transcription

We predicted the transcription factors that may bind to the promoter region of S100A16, using bioinformatics (http://www.cbrc.jp/htbin/nph-tfsearch). The S100A16 promoter has numerous transcription factor-binding
sites, and also four estrogen receptor (ER)-binding sites (Fig. 5A).

The 1.44-kb upstream region of mouse S100A16 was constructed into a luciferase reporter (pGL3-basic). In CHO cells, E2 reduced the transactivation of this reporter, which was partly reversed by tamoxifen (an inhibitor of ER; Fig. 5B). The data indicated the possibility that E2 inhibited S100A16 expression, at least in part, by acting as a negative transcriptional regulator of S100A16.

**Discussion**

In the present study, we studied the effect of different estrogen dosages on weight gain, insulin sensitivity, glucose and lipid metabolism, differentiation of MEFs, and liver and renal function in OVX and control female rats fed chow and HFD.

Our data indicated that estrogen depletion induced body weight gain and central abdominal fat accumulation, which were ameliorated by estrogen therapy under both diet conditions (Table 1). We also found that E2 loss induced glucose metabolism disorder and insulin resistance under different diet conditions (Fig. 2), and subsequently led to the development of metabolic syndrome. This is in agreement with results of studies which showed that E2 loss was positively associated with insulin resistance (Kalish et al. 2003). The features of the metabolic syndrome are related to the accumulation of visceral adiposity, insulin resistance, and disorder of biomarkers of liver and kidney. Although high amounts

![Figure 2](http://jme.endocrinology-journals.org)
of abdominal fat are thought to promote insulin resistance (Ryan 2000, Soares et al. 2006), in our study, E2 treatment inhibited body weight and visceral fat gain, and alleviate insulin resistance. With regard to diet, we found that E2 inhibited body weight and visceral fat gain, and alleviate insulin resistance. With regard to diet, we found that E2 also reduced lipid accumulation in the liver under both conditions (Fig. 2). Moreover, E2 normalized the levels of TC, TG, and UA that had been increased in the OVX rats, under the chow and the HFD conditions. HDL and Cr displayed no changes, while LDH and ALT displayed no changes in the OVX rats but decreased in E2-treated rats (Tables 2 and 3). E2 treatment reduced lipid accumulation in the liver under both conditions (Fig. 1B). UA is an independent risk factor for metabolic syndrome. Our results showed that E2 loss in OVX rats obviously increase the level of UA whether under

**Figure 3**
The effect of E2 on the expression of adipogenesis marker genes of PPARγ, C/EBPα, PPARγ, and aP2 in fat tissue. The protein expression of S100A16 (A), PPARγ (B), C/EBPα (E), and aP2 (G) was analyzed with western blotting. (C, D and F) Relative expression of S100A16 based on grayscale analysis. α-Tubulin was used as the control. Lanes 1 and 2, normal control rats on the chow diet; lanes 3 and 4, normal control rats on the HFD; lanes 5 and 6, OVX rats on the chow diet; lanes 7 and 8, OVX rats on the HFD; lanes 9 and 10, OVX rats treated with E2 on the chow diet; lanes 11 and 12, OVX rats treated with E2 on the HFD. Results are expressed as the mean ± s.d. *P<0.05 compared with the corresponding control group.

**Figure 4**
E2-induced suppression of adipogenesis via S100A16 inhibition.
(A) Western blot assay of S100A16 expression in the protein extracts collected from S100A16Tg+/+ MEFs treated with different concentrations of E2 (0, 50, 100, 200, 400, and 800 pg/ml) for 24 h. (B) Western blot assay of S100A16 expression in the protein extracts collected from S100A16Tg−/− MEFs treated with 100 pg/ml E2 for different times (0, 12, 24, 48, and 72 h). α-Tubulin was used as the control. (E) Western blot assay of S100A16 expression in the protein extracts collected from C57BL/6 MEFs treated with different concentrations of E2 (0, 50, 100, 200, and 400 pg/ml) for 24 h. (C, D and F) Relative expression of S100A16 based on grayscale analysis. (G and H) mRNA levels of S100A16 S100A16Tg−/− MEFs and normal MEFs, respectively, treated with E2. (I) Oil Red O staining patterns for MEFs from C57BL/6 and S100A16Tg+/+ mice treated with different concentrations of E2 and induced to differentiate into adipocytes. Photographs were taken under a light microscope with 200× magnification. (J) Triglyceride accumulation in the MEFs was significantly higher in the S100A16Tg−/− MEFs for all E2 concentrations. *P<0.05 compared with the corresponding control group.
the chow conditions or under the HFD conditions, but E2 replacement therapy could reverse this phenomenon (Table 3).

Little is known about the molecular mechanism underlying E2-induced weight loss. This complex process is regulated by many cell signals. For example, E2 binding to ERs (ERα and ERβ) resulted in the initiation of signal transduction. E2 may exert the effect of inhibiting body weight gain via leptin-like effects (Gao et al. 2007). In our previous study, we first found a new gene, S100A16, related to obesity, which is a member of the S100 protein family (Liu et al. 2011). It is interesting that we found four EREs in the promoter of S100A16 within the 1500 bp by using AliBaba2 analysis (Fig. 5A). Therefore, we speculated that estrogen might regulate metabolism progress by mediating S100A16 expression. To verify this hypothesis, we examined the expression of S100A16 as well as the adipocyte marker genes PPARγ, C/EBPα, and aP2 in the fat tissues from the different groups of rats mentioned earlier. The results showed that E2 treatment inhibited the expression of adipocyte marker genes, including PPARγ, C/EBPα, and aP2, and of S100A16 (Fig. 3), which was consistent with the previous studies (Alonso-Vale et al. 2009, Yim et al. 2011). To further determine whether E2 acts via the regulation of S100A16 expression, MEFs were extracted from the S100A16<sup>Tg</sup>C/C mice bred in our laboratory and incubated with E2 at different dosages and for different times, we found that the E2 inhibited S100A16 expression in a dose- and time-dependent manner (Fig. 4A, B, C and D). At the same time, we observed the role of E2 in the differentiation of MEFs extracted from S100A16<sup>Tg</sup>C/C mice and control C57BL/6 mice by Oil Red O staining and TG determination. It was significant that S100A16 promoted the differentiation of MEFs and that E2 inhibited the effects of S100A16 (Fig. 4E and F). Results of the S100A16 luciferase reporter assay also showed that S100A16 expression was reduced in the presence of E2, and this effect was ameliorated by the addition of tamoxifen (Fig. 5B).

So we think that estrogen might regulate metabolism by mediating S100A16 expression. Though many studies must be processed to reveal the molecular mechanisms of the effects of estrogen on metabolism via the S100A16 pathway. Indeed, our data showed that E2 inhibited adipogenesis and S100A16 expression, which indicates that E2 might decrease S100A16 expression by inhibiting...
S100A16 gene transcription. Our study has revealed the interaction of S100A16 with estrogen, and will be applicable to clinical treatment involving estrogen.

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.

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Author contribution statement
R Z, D S, and W Z conducted the molecular and the animal studies, carried out the data collection, and wrote this paper; Y X, D L, M L, Q H, and Y Z conducted the molecular studies; A Z conducted part of the molecular studies; and Y L designed this study.

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