Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Estradiol plays a role in regulating the expression of lysyl oxidase family genes in mouse urogenital tissues and human Ishikawa cells*

Wen ZONG, Yan JIANG, Jing ZHAO†‡, Jian ZHANG†‡, Jian-gang GAO

(*Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, China*)

†E-mail: zhaojing@sdu.edu.cn; zhj8226@sdu.edu.cn

Received Feb. 26, 2015; Revision accepted May 7, 2015; Crosschecked Sept. 13, 2015

Abstract: The lysyl oxidase (*LOX)* family encodes the copper-dependent amine oxidases that play a key role in determining the tensile strength and structural integrity of connective tissues by catalyzing the crosslinking of elastin or collagen. Estrogen may upregulate the expression of *LOX* and lysyl oxidase-like 1 (*LOXL1*) in the vagina. The objective of this study was to determine the effect of estrogen on the expression of all *LOX* family genes in the urogenital tissues of accelerated ovarian aging mice and human Ishikawa cells. Mice and Ishikawa cells treated with estradiol (E2) showed increased expression of *LOX* family genes and transforming growth factor β1 (*TGF-β1*). Ishikawa cells treated with TGF-β1 also showed increased expression of *LOX* family genes. The Ishikawa cells were then treated with either E2 plus the TGF-β receptor (TGFBR) inhibitor SB431542 or E2 alone. The expression of *LOX* family genes induced by E2 was reduced in the Ishikawa cells treated with TGFBR inhibitor. Our results showed that E2 increased the expression of the *LOX* family genes, and suggest that this induction may be mediated by the TGF-β signal pathway. E2 may play a role in regulating the expression of *LOX* family genes.

Key words: Estradiol, Lysyl oxidase family genes, TGF-β signal pathway
doi:10.1631/jzus.B1500048 **Document code:** A **CLC number:** Q95

1 Introduction

Pelvic organ prolapse (POP) affects many women, leading to a decreased quality of life (Handa *et al*., 2008). There are multiple causes of POP, with aging, obesity, vaginal childbirth, and menopause as risk factors and with most patients being older and postmenopausal (Olsen *et al*., 1997; Hunskaar *et al*., 2005). Lower serum concentrations of estrogen and lower estrogen receptor (ER) expression in patients with POP have been observed (Lang *et al*., 2003; Bai *et al*., 2005). Estrogen deficiency may be an important factor in the pathogenesis of POP.

Lysyl oxidase (LOX) is a copper-dependent amine oxidase. Four paralogs, lysyl oxidase-like 1, 2, 3, and 4 (LOXL1, LOXL2, LOXL3, and LOXL4), have been identified. Most previous studies have focused on the role of LOX and LOXL1, whose main functions are catalyzing the crosslinking of elastin or collagen in the extracellular matrix (Hornstra *et al*., 2003; Liu *et al*., 2006). LOXL1-deficient mice display POP, thinner vaginal wall, and lower urinary tract dysfunction through an inability of urogenital tissues to replenish elastic fibers after parturition (Liu *et al*., 2006). LOX and LOXL3 protein levels are significantly decreased in POP patients (Alarab *et al*., 2010; Zhao and Zhou, 2012). *LOXL2* mRNA levels are significantly decreased in postmenopausal POP patients compared with asymptomatic postmenopausal controls (Drewes *et al*., 2005). There exists a close relationship between POP and the expression of *LOX* family members.

‡ Corresponding authors

* Project supported by the National Basic Research Program (973) of China (No. 2010CB945002), the Shandong Provincial Natural Science Foundation of China (No. ZR2013CQ041), and the Independent Innovation Foundation of Shandong University (No. IIFSDU 2013GN011), China

ORCID: Wen ZONG, http://orcid.org/0000-0001-7800-128X

High expression of *LOXL1* can be detected in the urogenital tract of adult female mice, but decreases during aging (Liu *et al*., 2006). The mRNA levels of the *LOX* family genes are lower in the urogenital tissues of naturally aging mice than in those of young control mice (Jiang *et al*., 2014). The level of estrogen is also significantly decreased during aging. Previous studies have revealed that the mRNA expression of *LOX* and *LOXL1* genes in the vaginal wall of mice can be upregulated by estradiol (E2) treatment and LOX protein expression and activity were increased by E2 treatment in rat cardiac fibroblasts (Drewes *et al*., 2007; Voloshenyuk *et al*., 2012). E2 can upregulate mRNA expression of *LOX* and *LOXL1* in the vaginal wall of humans (Drewes *et al*., 2005). E2 treatment also results in increases in *LOX* mRNA levels in the vagina of guinea pigs after an injury (Voloshenyuk *et al*., 2012; Balgobin *et al*., 2013).

Most of these studies focused on *LOX* and *LOXL1* in the vagina. The relationship between E2 and the other three *LOX* family genes remains unclear, and few studies of the relationship between *LOX* families and E2 in the uterus or bladder are reported. In our study, we used accelerated ovarian aging mice and Ishikawa cells derived from well-differentiated human endometrial adenocarcinoma as the experimental materials to investigate these questions. The accelerated ovarian aging mice express lower levels of E2 and *LOX* family genes. Furthermore, these mice can easily be developed, and this saves time compared with allowing mice to age naturally for use in investigating the role of estrogen (Jiang *et al*., 2013). We examined the expression of *LOX* family genes in the urogenital tissues of accelerated ovarian aging mice and Ishikawa cells following treatment with E2. Previous studies have shown that TGF-β family members may regulate *LOX* family genes (Sethi *et al*., 2011) and that E2 could significantly increase the expression of TGF-β family members in the uterus and vagina of mice (Takahashi *et al*., 1994). Thus, a preliminary investigation of the role of the TGF-β signal pathway in the relationship between E2 and the *LOX* family genes was included.

2 Materials and methods

2.1 Animals and treatments

Mice with a CD-1 background were used in these studies. The mouse model of accelerated ovarian aging was generated according to the study previously described (Jiang *et al*., 2013). Twelve female mice at two months of age were injected with busulfan (Bu) in dimethyl sulfoxide (DMSO; 12 mg/kg, subcutaneously) and cyclophosphamide (Cy) in 0.9% (9 g/L) sterile sodium chloride solution (120 mg/kg, intraperitoneally). The Bu/Cy-treated mice showed a significant reduction of primordial and primary follicles by 30 d after the treatment. The treated mice showed lower levels of estrogen and decreased expression of *LOX* family genes and elastin (Jiang *et al*., 2013). We randomly divided the Bu/Cy-treated mice into two groups: an E2-treated group and a control group (n=6 each group). The E2-treated group was injected with E2 in soybean oil (0.5 mg/(kg·d), subcutaneously) for 12 d. The control group was injected with soybean oil. Twelve days after the onset of treatment, the mice in both groups were weighted, anesthetized with sodium pentobarbital (5 μg/g), and sacrificed. Urogenital tissues (vaginas, uteri, and bladders) were obtained for morphological and real-time polymerase chain reaction (PCR) analyses.

2.2 Cells and treatments

Ishikawa cells were human endometrial adenocarcinoma cells cultured in minimum essential medium (MEM; Gibco, USA) supplemented with 10% (0.1 g/ml) heat-inactivated fetal bovine serum (JBI, Daegu, Korea) and 1% (0.01 g/ml) penicillin streptomycin (Gibco) at 37 °C in a 5% CO_2 atmosphere. Before treatment, the cells were cultured in MEM without phenol red (Gibco), supplemented with 10% charcoal stripped fetal bovine serum (JBI) and 1% penicillin streptomycin, at 37 °C in a 5% CO_2 atmosphere for 24 h. The cells were then treated with TGF-β1 (Peprotech; 0 and 10 ng/ml), E2 (Sigma; 0 and 10 nmol/L), or E2 (0 and 10 nmol/L) plus TGFBR inhibitor SB431542 (Abcam; 0 and 10 μmol/L).

2.3 Real-time PCR

Tissues and cells were analyzed for *LOX* family gene, *TGF-β1*, and housekeeping gene mRNA using real-time PCR as previously described by Jiang *et al*. (2014). Primers are listed in Tables 1 and 2 (Kenyon *et al*., 2003; Liu *et al*., 2004; 2006; Guo *et al*., 2014).

2.4 Data analyses

All data are presented as the mean±standard error of the mean (SEM), and data analyses were performed using the SPSS statistical program, version

Table 1 Primers for quantification of gene expression in mouse*

Gene	GenBank accession No.	Sequence	
		Forward primer (location)	Reverse primer (location)
mLOX	NM_010728.2	5'-TGCCAGTGGATTGATATTACAGATGT-3'	5'-AGCGAATGTCACAGCGTACAA-3'
mLOXL1	NM_010729.3	5'-AAGGCACAGCGGACTTTCTC-3'	5'-GAACTCGTCCATGCTGTGGTAA-3'
mLOXL2	NM_033325.2	5'-CAACCCCAAAGCCTATAAAACCT-3'	5'-GCCCGTGCAGTTCATAGAAAA-3'
mLOXL3	NM_013586.4	5'-GGGTGGACTCATAGTGCCAAATA-3'	5'-TCCCCCTGCAGCTCAGATT-3'
mLOXL4	NM_053083.3	5'-TGGTGACCTGTCGGCAACT-3'	5'-TCCCCCTGCAGCTCAGATT-3'
mTGFβ1	NM_011577.1	5'-TGAGTGGCTGTCTTTTGACG-3'	5'-TCTCTGTGGAGCTGAAGCAA-3'
mGAPDH	NM_008084.2	5'-TGAAGGTCGGTGTGAACGGATTTGGC-3'	5'-CATGTAGGCCATGAGGTCCACCAC-3'

* Data refer to those reported previously (Kenyon *et al*., 2003; Liu *et al*., 2004; 2006; Guo *et al*., 2014)

Table 2 Primers for quantification of gene expression in human cells*

Gene	GenBank accession No.	Sequence	
		Forward primer (location)	Reverse primer (location)
hLOX	NM_001178102.1	5'-TGCCAGTGGATTGATATTACAGATGT-3'	5'-AGCGAATGTCACAGCGTACAA-3'
hLOXL1	NM_005576.2	5'-TGAGGCCACCGACTACGATG-3'	5'-GAACTCGTCCATGCTGTGGTAA-3'
hLOXL2	NM_002318.2	5'-GATGACGACTTCTCCATCCACG-3'	5'-GTCGCCTCGTTGCCAGTACAG-3'
hLOXL3	NM_032603.2	5'-CGATGATGACTTCACGCTGC-3'	5'-CTCAAGTTGTCCAGCCAGATGC-3'
hLOXL4	NM_032211.6	5'-GCCTGTCGACAGCCACTACTACAG-3'	5'-ACACAGCTGACCACAGCGTGCATG-3'
hTGFβ1	NM_000660.5	5'-CGCGTGCTAATGGTGGAA-3'	5'-CGCTTCTCGGAGCTCTGATG-3'
hGAPDH	NM_002046.3	5'-CACATCGCTCAGACACCATGG-3'	5'-AATGAAGGGGTCATTGATGGCAAC-3'

* Data refer to those reported previously (Guo *et al*., 2014)

17.0. Student's *t* test was used for single factor experiments involving two groups. A one-way analysis of variance (ANOVA) with Bonferroni testing was used for single factor experiments involving more than two groups. A significance level was set to $P<0.05$ for all statistical analyses.

3 Results

3.1 Increased expression levels of *LOX* family genes and *TGF-β1* in urogenital tissues of accelerated ovarian aging mice treated with E2

Mice in the E2 and control groups were weighed and sacrificed, and their uteri, vaginas, and bladders were harvested for morphological analysis. After E2 treatment for 12 d, a small increase of 9% in the body weight of the E2 group was observed compared with the soybean oil vehicle control group ($P<0.05$; Fig. 1a). The uterine and vaginal walls of the E2 group were thicker than those of the control group (Fig. 1b). The uterine wet weight of the E2 group increased by 60% ($P<0.01$; Fig. 1c). Moreover, a 30% increase was observed in the ratio of uterus to body weight of E2 mice relative to that of the control group ($P<0.05$; Fig. 1d).

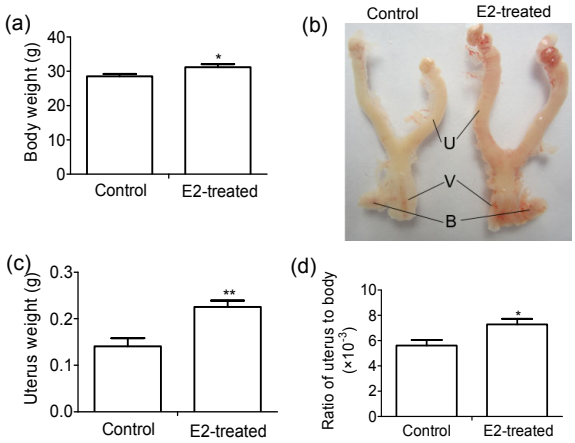

Fig. 1 Histological differences between the genitourinary organs of E2-treated and control mice

(a) E2 group showed a 9% increase in the body weight compared with the control group (* $P<0.05$); (b) The uterine and vaginal walls of the E2 group were thicker than those of the control group (U: uterine; V: vaginal; B: bladder); (c) E2 group showed a 60% increase in the wet weight of the uterus compared with the control group (** $P<0.01$); (d) The ratio of the uterus weight to the body weight of the E2 group showed a 30% increase compared with the control group (* $P<0.05$). Data are expressed as mean±SEM ($n=6$)

Uteri, vaginas, and bladders were obtained from the accelerated ovarian aging mice treated with E2 (0.5 mg/(kg·d), subcutaneously) or with the soybean oil (control). The mRNA levels of the *LOX* family and

the *TGF-β1* genes were determined in these tissues. In the uteri, mRNA levels of *LOX*, *LOXL1*, *LOXL2*, *LOXL3*, *LOXL4*, and *TGF-β1* were significantly higher in the E2-treated group than in the control group, with increases of 297%, 51%, 155%, 51%, 594%, and 176%, respectively (Fig. 2). Furthermore, the mRNA levels of those genes in the vaginas of the E2-treated group were upregulated by 242%, 498%, 157%, 287%, 12%, and 285%, respectively (Fig. 3). The expression levels in the bladders of the E2-treated group increased by 286%, 120%, 274%, 28%, 31%, and 177%, respectively (Fig. 4).

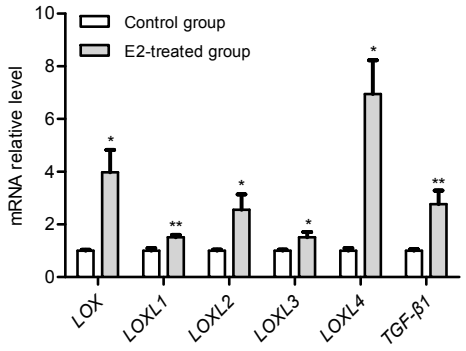

Fig. 2 Effect of E2 treatment on *LOX* family and *TGF-β1* mRNA levels in the uterus

The blank columns represent the control group (E2-untreated accelerated ovarian aging mice) and the grey columns represent the E2 group (0.5 mg/(kg·d) E2-treated accelerated ovarian aging mice). Each bar represents the mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, E2-treated group vs. control group

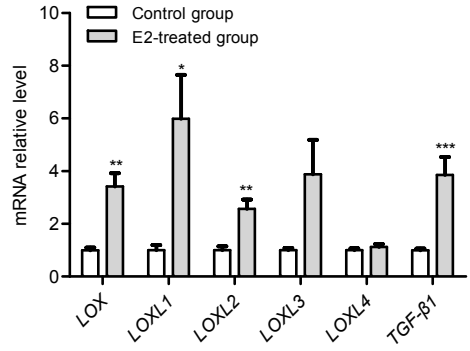

Fig. 3 Effect of E2 treatment on *LOX* family and *TGF-β1* mRNA levels in the vagina

The blank columns represent the control group (E2-untreated accelerated ovarian aging mice) and the grey columns represent the E2 group (0.5 mg/(kg·d) E2-treated accelerated ovarian aging mice). Each bar represents the mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, E2-treated group vs. control group

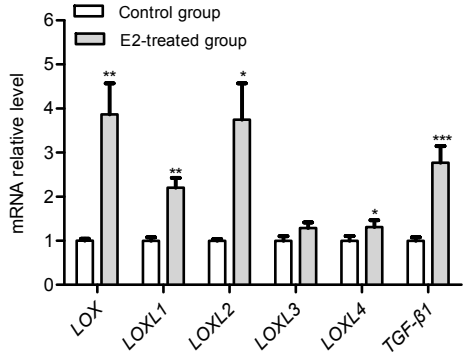

Fig. 4 Effect of E2 treatment on *LOX* family and *TGF-β1* mRNA levels in the bladder

The blank columns represent the control group (E2-untreated accelerated ovarian aging mice) and the grey columns represent the E2 group (0.5 mg/(kg·d) E2-treated accelerated ovarian aging mice). Each bar represents the mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, E2-treated group vs. control group

3.2 Increased expression levels of *LOX* family genes and *TGF-β1* in Ishikawa cells treated with E2

We also checked the expression of all five members of the *LOX* gene family in cultured cells. Ishikawa cells were used because our previous study showed that all the *LOX* family genes were expressed in this type of cell (data unpublished). We examined the levels of *LOX* and *LOXL* mRNA in the Ishikawa cells treated with E2. The mRNA levels of *LOX*, *LOXL1*, *LOXL2*, *LOXL3*, *LOXL4*, and *TGF-β1* were significantly higher in the E2 (10 nmol/L)-treated cells than in the control cells, with increases of 832%, 218%, 436%, 629%, 569%, and 99%, respectively (Fig. 5).

3.3 Increased expression levels of the *LOX* family genes in Ishikawa cells treated with TGF-β1

We examined the effect of TGF-β1 (10 ng/ml) on the expression of the *LOX* family genes in Ishikawa cells. TGF-β1 significantly increased the mRNA expression levels of *LOX*, *LOXL1*, *LOXL2*, *LOXL3*, and *LOXL4* in the Ishikawa cells, with increases of 45%, 50%, 123%, 70%, and 109%, respectively, compared with controls (P<0.05; Fig. 6).

3.4 Decreased expression levels of *LOX* family genes in Ishikawa cells treated with TGFBR inhibitor

To test the role of TGF-β1 in the relationship between E2 and *LOX* family genes, Ishikawa cells were treated either with E2 (10 nmol/L) plus TGFBR

inhibitor SB431542 (10 μmol/L) or with only E2 (10 nmol/L). The mRNA expression of the *LOX* family genes in the E2 plus TGFBR inhibitor-treated cells was lower than that in the E2-treated cells, with decreases of 815%, 142%, 350%, 553%, and 426%, respectively (Fig. 5).

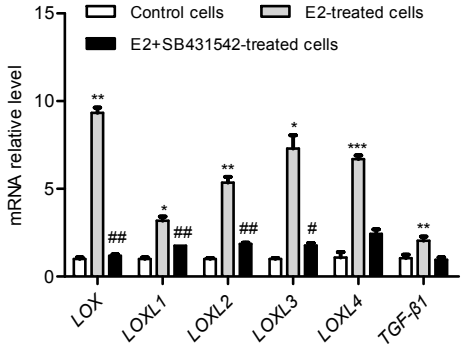

Fig. 5 Effect of E2 and E2 plus TGFBR inhibitor treatments on *LOX* family and *TGF-β1* mRNA levels in Ishikawa cells

The blank columns represent the control cells (E2-untreated Ishikawa cells), the grey columns represent the 10 nmol/L E2-treated Ishikawa cells, and the black columns represent 10 nmol/L E2 plus 10 μmol/L SB431542-treated Ishikawa cells. Each bar represents the mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, E2-treated cells vs. control cells; $^{\#}P$<0.05, $^{\#\#}P$<0.01, E2+SB431542-treated cells vs. E2-treated cells

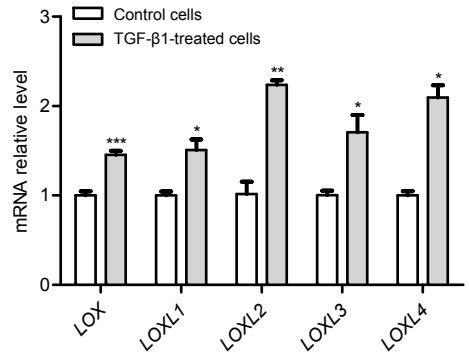

Fig. 6 Effect of TGF-β1 treatment on *LOX* family mRNA levels in Ishikawa cells

The blank columns represent the control cells (TGF-β1-untreated Ishikawa cells) and the grey columns represent the 10 ng/ml TGF-β1-treated Ishikawa cells. Each bar represents the mean±SEM (n=6). $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001, TGF-β1-treated cells vs. control cells

4 Discussion

Estrogen plays an important role in the development and growth of the urogenital system. The term "estrogen" includes a group of chemically similar hormones: estradiol (E2, the most abundant in women of reproductive age), estrone, and estriol (Shimizu, 2010). Estrogen, specifically E2, is secreted mainly by the ovaries in premenopausal women (Shimizu, 2010). Estrogen circulates in the bloodstream and affects many tissues, including the urogenital system (Yasui *et al*., 2009).

Estrogen has been demonstrated to affect the expression of *LOX* and *LOXL1* genes in the vagina (Drewes *et al*., 2005; 2007; Balgobin *et al*., 2013), though little is known about its effects on other members of the *LOX* family. Thus, to investigate the roles of estrogen and *LOX* family genes in the vagina, we examined the expression of *LOX* family genes in accelerated ovarian aging mice undergoing treatment with E2. The expression of *LOX* and *LOXL1* genes was significantly higher in the E2-treated group than in the control group, which is consistent with previous reports (Drewes *et al*., 2005; 2007; Balgobin *et al*., 2013). The expression of the other *LOX* family genes (*LOXL2*, *LOXL3*, and *LOXL4*) was also increased in the vaginas of the mice treated with E2. These results suggest that E2 may improve the expression of all *LOX* family genes in vagina of mice.

To investigate whether E2 affected the expression of *LOX* family genes in other urogenital tissues, we used accelerated ovarian aging mice to determine the expression of *LOX* family genes in the uterus and the bladder of mice treated with E2. The expression of *LOX* family genes was significantly increased in the uterus and the bladder of mice in the E2-treated group compared with controls. We used Ishikawa cells that express all five *LOX* family genes to confirm similar results *in vitro*. The mRNA levels of *LOX*, *LOXL1*, *LOXL2*, *LOXL3*, and *LOXL4* in the 10 nmol/L E2-treated group increased to variable degrees. The results showed that in addition to affecting expression in the vagina, E2 can affect the expression of *LOX* family genes in other urogenital tissues (uterus and bladder).

Takahashi *et al*. (1994) showed a strong link between E2 and TGF-β family members. E2 was able to stimulate the mRNA and protein expression of TGF-β family members in the uterus and vagina of mice prior to initiation of DNA synthesis. *TGF-β1* mRNA levels were dramatically increased in the vaginas of E2-treated guinea pigs (Balgobin *et al*., 2013). Topical application of E2 can upregulate the

expression of tropoelastin by activating the TGF-β signaling pathway in aged human skin *in vivo* (Son *et al*., 2005). E2 induces the formation and secretion of TGF-β (Soares *et al*., 2003; Gantus *et al*., 2011; Yu *et al*., 2011) and is able to increase TGF-β mRNA level in mice (Lindberg *et al*., 2002). Consistent with the above reports, our results showed that E2 induced TGF-β1 mRNA expression in the urogenital tissues of accelerated ovarian aging mice and Ishikawa cells.

TGF-β family members are primary regulators of many cellular processes including proliferation, growth, differentiation, and other functions in many cell types (Maurya *et al*., 2013). The functions of TGF-β family members are executed mainly through binding to type I and type II receptors, thus initiating a post-receptor signaling cascade (Derynck *et al*., 1996). The TGF-β signaling pathway has been shown to play an important role in many biological functions including the regulation of the extracellular matrix components and enzymes (Herpin *et al*., 2004). The TGF-β signaling pathway can regulate the amount and activity of the *LOX* family (*LOX*, *LOXL1* to *LOXL4*) in human trabecular meshwork cells (Sethi *et al*., 2011). All five *LOX* family members can be upregulated by TGF-β1 in anterior cruciate ligament and medial collateral ligament fibroblasts after mechanical injury in humans (Xie *et al*., 2013). In our study, the E2 induction of *LOX* family gene expression was reduced by TGFBR inhibitors in the Ishikawa cells. Also, we found that the expression of all five *LOX* genes in Ishikawa cells was increased by treatment with TGF-β1. Thus, we suggest that the increased expression of *LOX* family genes by E2 treatment may occur through TGF-β signaling. In our results, the increase in mRNA expression of *LOX* family members induced by TGF-β1 was not as significant as the increase induced by E2 treatment. The reason is mainly that TGF-β family members exist in at least three isoforms, TGF-β1, TGF-β2, and TGF-β3. The regulation of *LOX* family gene expression by E2 was potentially via TGF-β1, TGF-β2, and TGF-β3, not only TGF-β1.

There has been no systematic study of the molecular mechanism of E2-induced *TGF-β1* gene expression. However, Guo *et al*. (2006) showed that E2 binds to the ER and induces subsequent nuclear translocalization of the receptor dimmers. In the nucleus, E2 modulates the PI3K/Akt signal pathway via the action of ER at transcriptional level in Ishikawa cells (Guo *et al*., 2006). E2 also elicits non-transcriptional effects to activate the PI3K/Akt signal pathway in Ishikawa cells. In summary, E2 promptly activates the PI3K/Akt signal pathway in Ishikawa cells via ER-dependent and ER-independent mechanisms (Guo *et al*., 2006). The expression of TGF-β family members is reduced by blockade of the PI3K/Akt signal pathway in mouse osteosarcoma (Tsubaki *et al*., 2011). Activated Akt leads to upregulation of TGF-β1 in mesangial cells (Wu *et al*., 2009). In relation to E2-induction of *TGF-β1* gene expression, it is likely that E2 stimulates the PI3K/Akt signal pathway, and activated Akt accumulation prompts the expression of TGF-β family members.

Studies have shown that deficient synthesis and degradation of elastic fibers are associated with POP (Liu *et al*., 2006). Decreased expression of elastin fibers in POP has been found (Karam *et al*., 2007; Goepel, 2008; Klutke *et al*., 2008; Zong *et al*., 2010). Functional elastin fibers are formed through a complex process, whereby one or more members of the LOX family of enzymes crosslink tropoelastin monomers to form polymers in the extracellular matrix (Liu *et al*., 2004). Human studies have revealed a significant decreased protein levels of LOX, LOXL1 and LOXL3 in the uterosacral and cardinal ligaments of POP patients (Kobak *et al*., 2005; Zhang *et al*., 2008; Zhou *et al*., 2013). A significantly decreased elastin content and suppression of mRNA expression from *LOX*, *LOXL1*, and *LOXL2* are found in the uterosacral ligament tissue of women with prolapse compared with women in control groups (Klutke *et al*., 2008). In addition, LOXL1 knockout mice have a failure of elastic fiber homeostasis in the uterine tract postpartum and develop abnormalities, leading to POP (Liu *et al*., 2004; 2006). Thus, a lack of expression of *LOX* family genes may be a causal factor for POP in humans. In this study, we found that E2 could increase the expression of *LOX* family genes, and that this induction may be mediated by the TGF-β signal pathway. Thus, we suggest that E2 may potentially be used in interventions for POP in humans.

Compliance with ethics guidelines

Wen ZONG, Yan JIANG, Jing ZHAO, Jian ZHANG, and Jian-gang GAO declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Alarab, M., Bortolini, M.A., Drutz, H., *et al*., 2010. LOX family enzymes expression in vaginal tissue of premenopausal women with severe pelvic organ prolapse. *Int. Urogynecol. J.*, **21**(11):1397-1404. [doi:10.1007/s00192-010-1199-9]

Bai, S.W., Chung, D.J., Yoon, J.M., *et al*., 2005. Roles of estrogen receptor, progesterone receptor, p53 and p21 in pathogenesis of pelvic organ prolapse. *Int. Urogynecol. J.*, **16**(6):492-496. [doi:10.1007/s00192-005-1310-9]

Balgobin, S., Montoya, T.I., Shi, H., *et al*., 2013. Estrogen alters remodeling of the vaginal wall after surgical injury in guinea pigs. *Biol. Reprod.*, **89**(6):138. [doi:10.1095/biolreprod.113.112367]

Derynck, R., Gelbart, W.M., Harland, R.M., *et al*., 1996. Nomenclature: vertebrate mediators of TGFβ family signals. *Cell*, **87**(2):173. [doi:10.1016/S0092-8674(00)81335-5]

Drewes, P.G., Marinis, S.I., Acevedo, J., *et al*., 2005. Paper 17: regulation of lysyl oxidase in the vaginal wall: role of estrogen, progesterone, and pregnancy. *J. Pelvic Med. Surg.*, **11**:S8-S9. [doi:10.1097/01.spv.0000176091.92426.fa]

Drewes, P.G., Yanagisawa, H., Starcher, B., *et al*., 2007. Pelvic organ prolapse in fibulin-5 knockout mice: pregnancy-induced changes in elastic fiber homeostasis in mouse vagina. *Am. J. Pathol.*, **170**(2):578-589. [doi:10.2353/ajpath.2007.060662]

Gantus, M.A.V., Alves, L.M., Stipursky, J., *et al*., 2011. Estradiol modulates TGF-β1 expression and its signaling pathway in thyroid stromal cells. *Mol. Cell. Endocrinol.*, **337**(1-2):71-79. [doi:10.1016/j.mce.2011.02.001]

Goepel, C., 2008. Differential elastin and tenascin immunolabeling in the uterosacral ligaments in postmenopausal women with and without pelvic organ prolapse. *Acta Histochem.*, **110**(3):204-209. [doi:10.1016/j.acthis.2007.10.014]

Guo, R.X., Wei, L.H., Tu, Z., *et al*., 2006. 17β-Estradiol activates PI3K/Akt signaling pathway by estrogen receptor (ER)-dependent and ER-independent mechanisms in endometrial cancer cells. *J. Steroid Biochem. Mol. Biol.*, **99**(1):9-18. [doi:10.1016/j.jsbmb.2005.11.013]

Guo, X., Zhou, G., Guo, M., *et al.*, 2014. Adiponectin retards the progression of diabetic nephropathy in db/db mice by counteracting angiotensin II. *Physiol. Rep*., **2**(2):e00230. [doi:10.1002/phy2.230]

Handa, V.L., Cundiff, G., Chang, H.H., *et al*., 2008. Female sexual function and pelvic floor disorders. *Obstet. Gynecol.*, **111**(5):1045-1052. [doi:10.1097/AOG.0b013e31816bbe85]

Herpin, A., Lelong, C., Favrel, P., *et al*., 2004. Transforming growth factor-β-related proteins: an ancestral and widespread superfamily of cytokines in metazoans. *Develop. Comp. Immunol.*, **28**(5):461-485. [doi:10.1016/j.dci.2003.09.007]

Hornstra, I.K., Birge, S., Starcher, B., *et al*., 2003. Lysyl oxidase is required for vascular and diaphragmatic development in mice. *J. Biol. Chem*., **278**(16):14387-14393. [doi:10.1074/jbc.M210144200]

Hunskaar, S., Burgio, K., Clark, A., *et al*., 2005. Epidemiology of urinary (UI) and faecal (FI) incontinence and pelvic organ prolapse (POP). WHO-ICS International Consultation on Incontinence, 3rd Ed. Health Publications Ltd., Paris, p.255-312.

Jiang, Y., Zhao, J., Qi, H.J., *et al*., 2013. Accelerated ovarian aging in mice by treatment of busulfan and cyclophosphamide. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **14**(4):318-324. [doi:10.1631/jzus.B1200181]

Jiang, Y., Zong, W., Luan, H., *et al*., 2014. Decreased expression of elastin and lysyl oxidase family genes in urogenital tissues of aging mice. *J. Obstet. Gynaecol. Res.*, **40**(8):1998-2004. [doi:10.1111/jog.12425]

Karam, J.A., Vazquez, D.V., Lin, V.K., *et al*., 2007. Elastin expression and elastic fibre width in the anterior vaginal wall of postmenopausal women with and without prolapse. *BJU Int.*, **100**(2):346-350. [doi:10.1111/j.1464-410X.2007.06998.x]

Kenyon, N.J., Ward, R.W., McGrew, G., *et al*., 2003. TGF-β_1 causes airway fibrosis and increased collagen I and III mRNA in mice. *Thorax*, **58**(9):772-777. [doi:10.1136/thorax.58.9.772]

Klutke, J., Ji, Q., Campeau, J., *et al*., 2008. Decreased endopelvic fascia elastin content in uterine prolapse. *Acta Obstet. Gynecol. Scand.*, **87**(1):111-115. [doi:10.1080/00016340701819247]

Kobak, W., Lu, J., Hardart, A., *et al*., 2005. Expression of lysyl oxidase and transforming growth factor β2 in women with severe pelvic organ prolapse. *J. Reprod. Med.*, **50**(11):827-831.

Lang, J.H., Zhu, L., Sun, Z.J., *et al*., 2003. Estrogen levels and estrogen receptors in patients with stress urinary incontinence and pelvic organ prolapse. *Int. J. Gynaecol. Obstet.*, **80**(1):35-39. [doi:10.1016/S0020-7292(02)00232-1]

Lindberg, M.K., Moverare, S., Eriksson, A.L., *et al*., 2002. Identification of estrogen-regulated genes of potential importance for the regulation of trabecular bone mineral density. *J. Bone Miner. Res.*, **17**(12):2183-2195. [doi:10.1359/jbmr.2002.17.12.2183]

Liu, X., Zhao, Y., Gao, J., *et al*., 2004. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat. Genet.*, **36**(2):178-182. [doi:10.1038/ng1297]

Liu, X., Zhao, Y., Pawlyk, B., *et al*., 2006. Failure of elastic fiber homeostasis leads to pelvic floor disorders. *Am. J. Pathol.*, **168**(2):519-528. [doi:10.2353/ajpath.2006.050399]

Maurya, V.K., Jha, R.K., Kumar, V., *et al*., 2013. Transforming growth factor-beta 1 (TGF-B1) liberation from its latent complex during embryo implantation and its regulation by estradiol in mouse. *Biol. Reprod.*, **89**(4):84. [doi:10.1095/biolreprod.112.106542]

Olsen, A.L., Smith, V.J., Bergstrom, J.O., *et al*., 1997. Epidemiology of surgically managed pelvic organ prolapse

and urinary incontinence. *Obstet. Gynecol.*, **89**(4):501-506. [doi:10.1016/S0029-7844(97)00058-6]

Sethi, A., Mao, W., Wordinger, R.J., *et al*., 2011. Transforming growth factor-β induces extracellular matrix protein cross-linking lysyl oxidase (*LOX*) genes in human trabecular meshwork cells. *Invest. Ophthalmol. Vis. Sci*., **52**(8):5240-5250. [doi:10.1167/iovs.11-7287]

Shimizu, Y., 2010. Estrogen: estrone (E1), estradiol (E2), estriol (E3) and estetrol (E4). *Nihon Rinsho*, **68**(7): 448-461 (in Japanese).

Soares, R., Guo, S., Gartner, F., *et al*., 2003. 17β-Estradiol-mediated vessel assembly and stabilization in tumor angiogenesis requires TGFβ and EGFR crosstalk. *Angiogenesis*, **6**(4):271-281. [doi:10.1023/B:AGEN.0000029413.32882.dd]

Son, E.D., Lee, J.Y., Lee, S., *et al*., 2005. Topical application of 17β-estradiol increases extracellular matrix protein synthesis by stimulating TGF-β signaling in aged human skin *in vivo*. *J. Invest. Dermatol.*, **124**(6):1149-1161. [doi:10.1111/j.0022-202X.2005.23736.x]

Takahashi, T., Eitzman, B., Bossert, N.L., *et al*., 1994. Transforming growth factors β1, β2, and β3 messenger RNA and protein expression in mouse uterus and vagina during estrogen-induced growth: a comparison to other estrogen-regulated genes. *Cell Growth Differ.*, **5**(9):919-935.

Tsubaki, M., Yamazoe, Y., Yanae, M., *et al*., 2011. Blockade of the Ras/MEK/ERK and Ras/PI3K/Akt pathways by statins reduces the expression of bFGF, HGF, and TGF-β as angiogenic factors in mouse osteosarcoma. *Cytokine*, **54**(1):100-107. [doi:10.1016/j.cyto.2011.01.005]

Voloshenyuk, T.G., Larkin, K., Fournett, A., *et al*., 2012. Estrogen receptor dependence of lysyl oxidase expression and activity in cardiac fibroblasts. *FASEB J.*, **26**:1059.16.

Wu, D., Peng, F., Zhang, B., *et al*., 2009. PKC-β1 mediates glucose-induced Akt activation and TGF-β1 upregulation in mesangial cells. *J. Am. Soc. Nephrol.*, **20**(3):554-566. [doi:10.1681/ASN.2008040445]

Xie, J., Wang, C., Huang, D.Y., *et al*., 2013. TGF-β_1 induces the different expressions of lysyl oxidases and matrix metalloproteinases in anterior cruciate ligament and medial collateral ligament fibroblasts after mechanical injury. *J. Biomech.*, **46**(5):890-898. [doi:10.1016/j.jbiomech.2012.12.019]

Yasui, T., Saijo, A., Uemura, H., *et al*., 2009. Effects of oral and transdermal estrogen therapies on circulating cytokines and chemokines in postmenopausal women with hysterectomy. *Eur. J. Endocrinol.*, **161**(2):267-273. [doi:10.1530/EJE-09-0063]

Yu, L., Wang, C.Y., Shi, J., *et al*., 2011. Estrogens promote invasion of prostate cancer cells in a paracrine manner through up-regulation of matrix metalloproteinase 2 in prostatic stromal cells. *Endocrinology*, **152**(3):773-781. [doi:10.1210/en.2010-1239]

Zhang, S.Q., Zhang, L.L., Yu, H., 2008. Expression of elastin, lysyl oxidase and elafin in the cardinal ligament of women with pelvic organ prolapse. *Chin. J. Obstet. Gynecol.*, **43**(9):675-679 (in Chinese).

Zhao, B.H., Zhou, J.H., 2012. Decreased expression of elastin, fibulin-5 and lysyl oxidase-like 1 in the uterosacral ligaments of postmenopausal women with pelvic organ prolapse. *J. Obstet. Gynaecol. Res*., **38**(6):925-931. [doi:10.1111/j.1447-0756.2011.01814.x]

Zhou, Y., Ling, O., Bo, L., 2013. Expression and significance of lysyl oxidase-like 1 and fibulin-5 in the cardinal ligament tissue of patients with pelvic floor dysfunction. *J. Biomed. Res.*, **27**(1):23-28. [doi:10.7555/JBR.27.20110142]

Zong, W., Stein, S.E., Starcher, B., *et al*., 2010. Alteration of vaginal elastin metabolism in women with pelvic organ prolapse. *Obstet. Gynecol.*, **115**(5):953-961. [doi:10.1097/AOG.0b013e3181da7946]

中文概要

题　目：雌激素对赖氨酰氧化酶（*LOX*）家族基因表达的影响

目　的：探讨雌激素对小鼠泌尿生殖系统以及人子宫内膜癌细胞系中的 *LOX* 家族基因表达的影响。

创新点：系统地研究了雌二醇在小鼠泌尿生殖系统（子宫、阴道和膀胱）以及人子宫内膜癌细胞系中对 *LOX* 家族基因表达的影响；提出了转化生长因子-β（TGF-β）信号通路可能在雌二醇调节*LOX*家族中发挥作用。

方　法：使用雌二醇对卵巢快速衰老小鼠和人子宫内膜癌细胞系进行处理，然后通过荧光实时定量聚合酶链式反应（PCR），分别从体内和体外研究雌二醇对 *LOX* 家族基因表达的影响（图 2~5）。在人子宫内膜癌细胞系中加入 TGF-β1，通过荧光实时定量 PCR，检测 *LOX* 家族基因表达的变化（图 6）。利用人子宫内膜癌细胞系为对象，进行分组研究：一组无任何处理，为对照组；一组只加雌二醇；一组则同时加入雌二醇和 TGF-β 受体抑制剂（SB431542）。24 小时后，检测 *LOX* 家族基因及 *TGF-β1* 的表达（图 6）。

结　论：雌二醇可以促进卵巢快速衰老小鼠和人子宫内膜癌细胞系中 *LOX*、*LOXL1*、*LOXL2*、*LOXL3*、*LOXL4* 和 *TGF-β1* 表达的升高，其中雌二醇很有可能是通过 TGF-β 信号通路影响 *LOX* 家族基因的表达量。由于 *LOX* 家族基因与盆腔疾病关系密切，所以我们的发现可能为临床上盆腔器官疾病的预防和治疗提供一些理论基础。

关键词：赖氨酰氧化酶（*LOX*）家族基因；雌二醇；TGF-β 信号通路