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Aromatase as a target for treating endometriosis

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Abstract

Endometriosis is a common gynecological disease that causes various clinical symptoms, such as chronic pelvic pain, dysmenorrhea and infertility, seriously affecting women's health and their quality of life. The symptoms and endometriotic lesions are relieved, in many cases, after menopause, when estrogen levels are lowered. Therefore, endometriosis is considered to be estrogen-dependent. Aromatase, the enzyme responsible for the last step of estrogen biosynthesis converting testosterone and androgen to estrogen, was previously reported to be more abundant in endometriotic tissues than in the normal endometrium, leading to an increased local estrogen concentration. Therefore, aromatase is considered a key therapeutic target for regulating local estrogen biosynthesis in endometriosis. A more complete understanding of the mechanisms that modulate aromatase and its activity is required to develop novel estrogen-targeted therapies for endometriosis. In this review article, we outline the current understanding of the pathological processes involved in estrogen production in endometriosis and propose novel strategies to treat this disorder.

Key words: aromatase, endometriosis, estrogen, PGC-1α, treatment.

Introduction

Endometriosis is a common gynecological disease characterized by the presence and growth of endometrium-like tissues at extrauterine sites. The morbidity of endometriosis reaches approximately 6–10% in women of reproductive age and causes various clinical symptoms, such as chronic pelvic pain, dysmenorrhea and infertility, seriously reducing women's health and their quality of life.[1] Although studies regarding the histological origin of endometriosis have been conducted, the exact etiology of the disease remains poorly understood.

Accumulating evidence has shown that local estrogen production plays a key role in the pathogenesis of endometriosis.[2] Indeed, the symptoms and endometriotic lesions are relieved, in many cases, after menopause. Therefore, endometriosis is considered to be estrogen-dependent. Existing treatment options for endometriosis include gonadotropin-releasing hormone (GnRH) agonists, oral contraceptives (OC) and progestogens, as well as androgens and nonsteroidal anti-inflammatory agents.[3,4] Currently, the successful treatment of endometriosis-associated pain is based on suppressing estrogen production. Despite the benefits of these hormonal medications, GnRH agonists can be only used for a limited time due to unacceptable side-effects, such as osteoporosis and climacteric disorders. In addition, hormonal medications, including GnRH agonists and OC, are not suitable for patients attempting to conceive because they suppress ovulation. Accordingly, it is crucial to develop a novel therapeutic strategy that is more effective and has fewer side-effects in patients with endometriosis. To achieve this purpose, a more detailed understanding of the molecular and pathological significance of endometriosis is required.

Aromatase is the enzyme responsible for the last step of estrogen biosynthesis. Our group, and other investigators, demonstrated that aromatase is more

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abundant in endometriotic tissues than normal endometrium, leading to an increased local estrogen concentration.[5–7] Developing therapeutic strategies that target local estrogen formation may be a promising approach to treat this disorder. Here, we outline the current understanding of the pathological process via estrogen production in endometriosis.

Expression of Aromatase in Endometriosis

Aromatase converts testosterone and androstenedione to estradiol (E_2) and estrone (E_1), respectively. Aromatase is expressed in a number of human tissues and cells, such as ovarian granulosa cells, adipose tissue, skin fibroblasts, placental syncytiotrophoblasts, osteoblasts and brain. In women of reproductive age, aromatase is most potently and periodically secreted by the ovary. Ovarian granulosa cells express high levels of aromatase under the influence of follicle-stimulating hormone (FSH).[8,9] On the other hand, in postmenopausal women, estrogen formation occurs in extraglandular sites such as adipose tissue and skin.[10] The main substrate of aromatase in adipose and skin tissues is androstenedione secreted from adrenal tissues. In postmenopausal women, approximately 2% of circulating androstenedione is converted by aromatase to E_1, which is further converted to E_2 by 17ß-hydroxysteroid dehydrogenase type 1 (17ß-HSD1) in peripheral tissues. High levels of E_2 in serum lead to endometrial hyperplasia and endometrial cancer.[11]

Interestingly, aromatase is expressed at higher levels in breast cancer than normal breast tissues.[12–14] The estrogens produced *in situ*, due to overexpression of aromatase in breast cancer cells, is thought to play a more crucial role in stimulating cancer cell growth than circulating estrogen.[15] In endometrial cancer, aromatase is also highly expressed, although the expression is low or not detected in normal endometrium and endometrial hyperplasia.[16,17] Furthermore, the expression of aromatase in stromal cells is associated with unfavorable survival in patients with endometrial cancer.[18]

Like estrogen-dependent cancers, accumulating evidence demonstrates high levels of aromatase expression in endometriosis.[5,19–22] Noble *et al*. first reported that aromatase was detected in endometriotic implants in much larger amounts than in eutopic endometrium, although it was not detected in normal endometrium in disease-free women. Using immunohistochemistry analysis and a catalytic activity assay, in addition to the reverse transcription polymerase chain reaction, our group showed that local estrogen production by aberrantly elevated aromatase takes place in endometriotic and adenomyosis, not in normal endometrium.[5] Another group recently examined tissue estrogen concentrations in normal endometrium throughout the menstrual cycle and in different types of endometriotic lesions, including peritoneum, ovarian endometrium and deep endometriosis.[22] They showed that ovarian endometriotic lesions presented with markedly higher intratissue estrogen concentrations than normal endometrium and peritoneal and deep endometriosis. They also demonstrated that the expression of aromatase mRNA was significantly higher in the proliferative/secretory menstrual phase of ovarian endometrioma and in the proliferative phase of deep endometriosis.[22] These findings confirmed that aromatase played a critical role in local estrogen production, especially in ovarian endometriosis. This suggests the existence of autocrine and paracrine sources for estrogens in local lesions.

Regulation of Aromatase Expression in Endometriosis

The human aromatase gene was mapped to chromosome 15, band q21 by *in situ* hybridization studies and was confirmed by recent human genome analysis.[23] The gene consists of nine coding exons. The ATG translation start site is located in coding exon II, and upstream of the gene, there are a number of promoters; at least eight untranslated exons/promoters (I.1, I.2, I.3, I.4, I.5, I.6, I.7, and PII) have been identified.

A complex mechanism is involved in the control of human aromatase expression. Various exon I-containing mRNAs are present at different levels in different tissues and cells. Aromatase expression in tissues is driven by the promoters situated upstream of the exons. To determine tissue-specific promoter usage, the real-time polymerase chain reaction using exon I-specific promoters was performed. Transcripts of exon I.I, located most distally upstream from the coding region, were elevated in placental trophoblast cells to maintain a high level of aromatase expression.[24] In breast cancer specimens, exons I.3 and PII were the major exons present in aromatase mRNA transcripts.[12,14,25] In contrast, exons/promoter I.3 and II were used only minimally in normal breast adipose

tissues, and a low level of aromatase was expressed via exons/promoter I.4 in the tissue.[26]. These findings suggest that there is a shift of the regulatory mechanism of aromatase expression from normal breast adipose tissue to cancer tissues.

In endometriotic tissues, exons/promoter I.3 and PII are considered the main promoters. This was recently confirmed by our group[21,27] (Fig. 1). Levels of aromatase are stimulated markedly by cyclic adenosine monophosphate (cAMP) analogs or prostaglandin (PG)E_2 in endometriotic stromal cells.[20] PGE_2 formation is stimulated by the cyclooxygenase type 2 (COX-2) enzyme in endometriotic stromal cells.[28] Furthermore, estrogen has been linked directly to the promotion of inflammation, as evidenced by the estrogen-mediated induction of various cytokines in cells.[29] These cytokines, including interleukin (IL)-6 and IL-8, as well as PGE_2 and COX-2, can stimulate hormone synthesis and regulate the activation of pro-survival signaling pathways.[30] PGE_2 also enhances the expression of steroidogenic acute regulatory protein, which is an enzyme elevated in endometriotic stromal cells that could facilitate the entry of cholesterol into mitochondria, leading to the production of steroid hormones. These findings suggest that there is a feed-forward mechanism in the overproduction of estrogen, PGE_2 and cytokines that promotes the persistence of endometriotic lesions.[31]

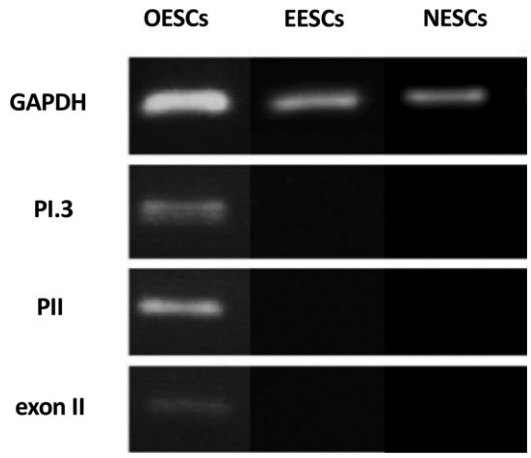

Figure 1 Aromatase exon Is specific RT-PCR. The bands of PI.3, PII and exon II without detection of PI.1, I.4 and I.6 amplifications in endometrial stromal cells from ovarian endometrioma (OESC) suggested that the main aromatase promoters used in endometriosis were PI.3 and PII. In contrast, basal aromatase transcription in endometrial stromal cells from eutopic endometrium (EESC) and the cells from normal endometrium (NESC) were not detected.

Interaction of Transcription Factors with the Regulation of Aromatase in Endometriosis

It is important to understand the regulatory mechanism of aromatase in normal ovarian granulosa cells during the menstrual cycle. As described before, aromatase converts androstenedione and testosterone to E_1 and E_2, respectively. The serum E_2 level reaches a peak just before ovulation. Thereafter, estrogen continue to be secreted from the corpus luteum at a lower level. Once FSH secreted from the pituitary gland binds to G protein-coupled receptors in the granulosa cell membrane, the intracellular level of cAMP is strongly elevated. Steroidogenic factor-1 (SF-1) and the cAMP response element-binding protein are then recruited to bind to aromatase promoter II, leading to estrogen production at the preovulatory phase.[32,33]

In endometriotic lesions, a previous report showed that aromatase expression, stimulated by cAMP analogs and PGE_2, was regulated by the binding of SF-1 to the nuclear receptor half-site upstream of the aromatase gene promoter II.[27] On the other hand, chicken ovalbumin upstream promoter-transcription factor (COUP-TF) is an inhibitor of aromatase expression. COUP-TF is expressed in both eutopic and normal endometrium, whereas SF-1 is expressed in endometriosis but not in normal eutopic endometrial cells. COUP-TF competes for the same binding site as SF-1 in promoter II of aromatase. In addition, promoters I.3 and II in endometriosis are regulated by many other transcription factors, including Wilms' tumor-1, CCAAT-enhancer binding protein (C/EBP)α and C/EBPβ, DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) and liver receptor homolog-1.[6] Wilms' tumor-1 functions as a corepressor of SF-1 on the nuclear half-site of aromatase promoters I.3 and II, similar to COUP-TF. C/EBPα and C/EBPβ bind to the −211/−197 cAMP response element site located just upstream of the nuclear half-sites of aromatase PI.3 and II. C/EBPα functions as an enhancer, whereas C/EBPβ is an inhibitor of aromatase expression.[6] C/EBPβ is also downregulated selectively in endometriosis but not in eutopic endometrium. DAX-1 acts as a dominant-negative regulator of SF-1 transcription.[34]

Gurates *et al*. demonstrated that it inhibited SF-1-dependent expression of aromatase in endometriosis.[35,36] Thus, these findings indicate that transcription factors play important roles in the differential modulation of aromatase expression between endometriotic tissues and eutopic/normal endometrium.

We previously focused on the expression and function of estrogen-related receptors (ERR). ERRα, one of the subtypes, is an orphan nuclear receptor without known endogenous ligands. Interestingly, Sasano *et al*. reported microarray expression profiling and clustering analysis using specimens from breast cancer tissues that showed a significant positive correlation between ERRα and aromatase expression.[37] We examined the expression patterns of ERRα and aromatase in endometriotic tissues. However, ERRα was not highly expressed. Rather, peroxisome proliferator-activated receptor gamma (PPARγ) and coactivator-1α (PGC-1α), known as a representative coactivator of ERRα, were elevated significantly in endometriosis and were coexpressed with aromatase.[21]

PGC-1α is a coactivator that interacts with a broad range of transcription factors involved in various biological responses, including adaptive thermogenesis, mitochondrial biogenesis, oxidative metabolism and steroidogenesis.[38–40] For example, in brown adipose tissue, PGC-1α is induced by exposure to cold and coactivates PPARγ to stimulate adipocyte differentiation.[41] In addition, PGC-1α regulates progesterone production in ovarian granulosa cells as a coactivator of SF-1 and liver receptor homolog-1.[40] Other investigators showed that, in skeletal muscle cells, PGC-1α downregulated the expression of insulin-sensitive glucose transporter type 4 and was involved in glucose uptake.[42] Thus, PGC-1α is differentially expressed in different tissues and functions as a coactivator interacting with tissue-specific transcription factors.

Our previous studies on endometriosis found that PGC-1α was elevated aberrantly in ovarian endometrioma, correlating with the localization of aromatase in tissues (Fig. 2). We also showed that PGC-1α overexpression increased aromatase promoter I.3/II activity and the aromatase mRNA expression level in stromal cells from ovarian endometrioma (Fig. 3). The chromatin immunoprecipitation assay revealed the recruitment of endogenous PGC-1α to the nuclear receptor half-site, 5′-AGGTCA-3′. Our preliminary results also showed that retinoid X receptor α was one nuclear receptor able to regulate aromatase in endometriosis in cooperation with PGC-1α. It is also interesting that TNF-α,

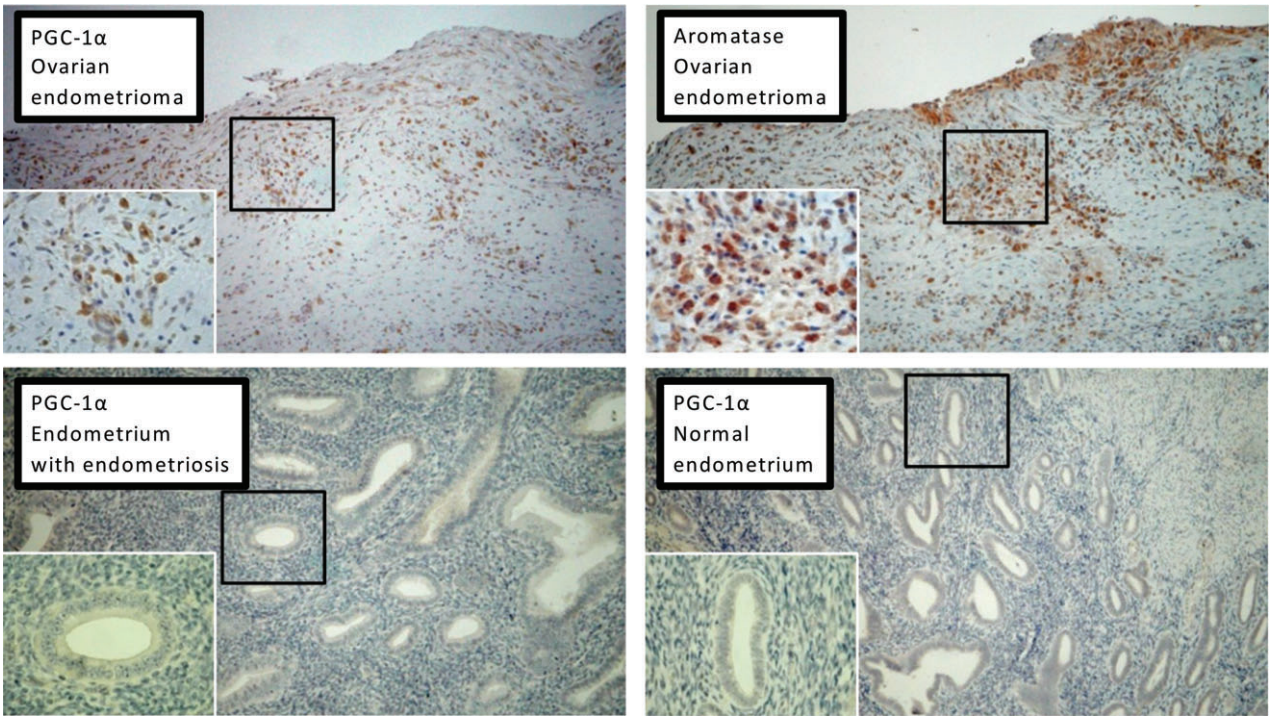

Figure 2 Immunohistochemical expression of PGC-1α and aromatase in specimens from ovarian endometrioma and normal endometrium.

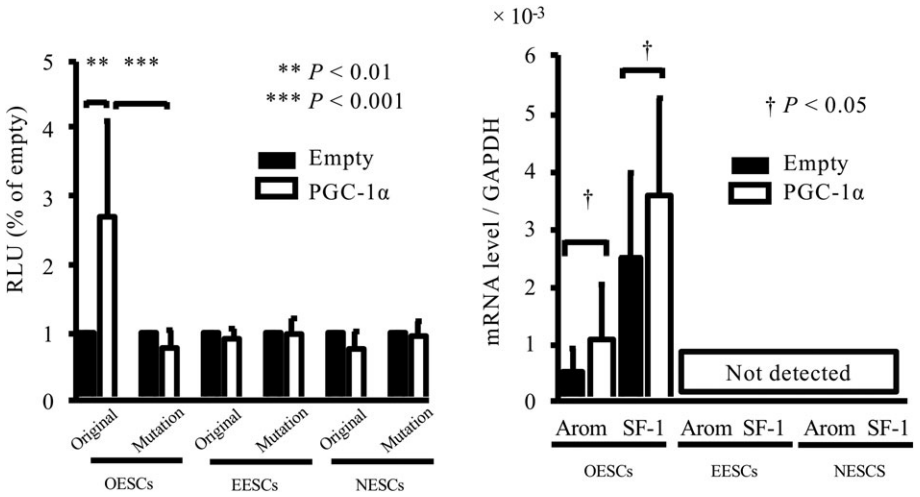

Figure 3 Luciferase activities of aromatase promoter PI.3-II with/without real-time PCR analysis of aromatase and SF-1 with/without PGC-1α overexpression. Luciferase activities were determined by the Dual Luciferase Reporter assay system. The RLU represents the relative ratio of luminescence intensity with PGC-1α overexpression to without overexpression. The nuclear receptor half-site (5′-AGGTCA-3′) in aromatase promoter reporter plasmid (original) was changed to 5′-ACGACT-3′ (mutation) using QuickChange Lightning (Agilent Technologies).

produced by peritoneal macrophage and endometriotic tissue, stimulates PGC-1α transcription in endometrial stromal cells from ovarian endometrioma (Fig. 4). Further investigations are needed to understand the regulation of aromatase in endometriosis.

Aromatase-Targeting Treatment in Endometriosis

Endocrine preparations currently recommended for the treatment of endometriosis include OC, progestogens and GnRH agonists. OC are widely used in women with chronic pelvic pain and clinically suspected endometriosis. These drugs inhibit ovulation and substantially reduce the volume of menstrual flow. Progestins have also been used in the management of symptomatic endometriosis. Progestins occasionally inhibit the hypothalamic-pituitary-ovarian axis, leading to anovulation, reduction of the serum E_2 level and atrophy of eutopic/ectopic endometrium.

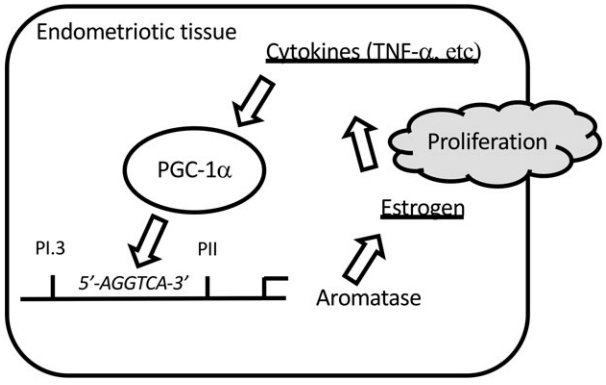

Figure 4 The vicious cycle of local estrogen production during endometriosis through PGC-1α and TNF-α.

Dienogest, a fourth-generation progestin, has potent oral progesterone activity without any systemic androgenic activity.[43] Previous randomized trials demonstrated that long-term use of dienogest exerted a potent effect in relieving endometriosis-associated pelvic pain.[44,45] Accumulating evidence showed that dienogest directly inhibited progesterone receptor-mediated cell proliferation[46] and the production of inflammatory cytokines,[47,48] toll-like receptor 4[49] and nerve growth factor.[50] Our group showed, using spheroid cultures of endometriotic stroma cells, that dienogest reduced the expression of aromatase and the expression and enzyme activity of 17ß-HSD1, which catalyze the conversion of less potent E_1 to the more potent E_2 form in tissues.[51,52] These findings suggest that dienogest comprehensively inhibits abnormal estrogen production in endometriosis.

GnRH agonists induce pharmacological menopause by decreasing the production of gonadotropins and suppressing ovulation to reduce ovarian

steroidogenesis. GnRH agonists can achieve relief of moderate to severe pelvic pain of endometriosis.[53] However, long-term use of GnRH agonists is associated with hypoestrogenic side-effects and a substantial reduction in bone mineral density.[54] Consequently, pelvic pain often recurs after completion of GnRH agonist treatment; the median interval until recurrence of pain is 6.1 months.[55] There are two main reasons for the failure of GnRH agonist treatment of endometriosis. One explanation is that local estrogen secretion from the ovary resumes after completion of the treatment and contributes to the development of endometriotic lesions. Another is that GnRH agonists affect only the hypothalamo-pituitary-gonadal axis but do not cover extraglandular sites of estrogen biosynthesis in women. Therefore, estrogen production occurs in adipose tissue, skin and local endometriotic lesions during the treatment.

Aromatase inhibitors (AI) are another therapeutic option for patients with endometriosis. Third-generation AIs, such as anastrozole, letrozole and exemestane, are currently used widely for postmenopausal patients with hormone-dependent breast cancers.[56] Side-effects of the AI include vaginal dryness, hot flushes, headache, numbness in lower extremities and arthralgia. It is considered that AI are likely to maintain low estrogen levels in extraovarian sites during the treatment. However, AI administered in premenopausal women increase FSH levels through positive feedback of the hypothalamo-pituitary-gonadal axis, sometimes leading to the development of ovarian cysts. Therefore, it is necessary to use AI in combination with progestins, OC and GnRH agonists for women of reproductive age with endometriosis.

A meta-analysis of eight trials that enrolled 137 women showed that AI, in combination with progestins, OC or GnRH agonists, relieved endometriosis-associated pain, reduced the size of extrauterine endometrial lesions and improved the quality of life.[57] A more recent meta-analysis of 10 trials that enrolled 251 women reported similar results.[58] Taken together, the European Society of Human Reproduction and Embryology (ESHIRE) guidelines recommend the concomitant use of AI with OC, progestins or GnRH agonists for patients with pain from rectovaginal endometriosis, refractory to other medical or surgical treatments.[59]

Isoflavones are plant-derived nonsteroidal compounds that possess weak estrogenic activity. Isoflavones exert estrogen-like activity due to their structural similarities to E_2, but also exert antiestrogenic effects in reproductive-aged women with high estrogen levels.[60] Previous studies investigated the effect of isoflavones on endometriosis. Puerarin and parthenolide, two flavonoids, inhibited the proliferation of oral epithelial stem cells.[61,62] Other investigators showed that genistein caused regression of an endometriotic implant using a rat model.[63] Our group recently demonstrated that daidzein-rich isoflavone aglycones (DRIA) suppressed cell proliferation in oral epithelial but not in neuroepithelial stem cells at clinically feasible concentrations.[64] The effect was most likely mediated by PGE_2 formation via the inhibition

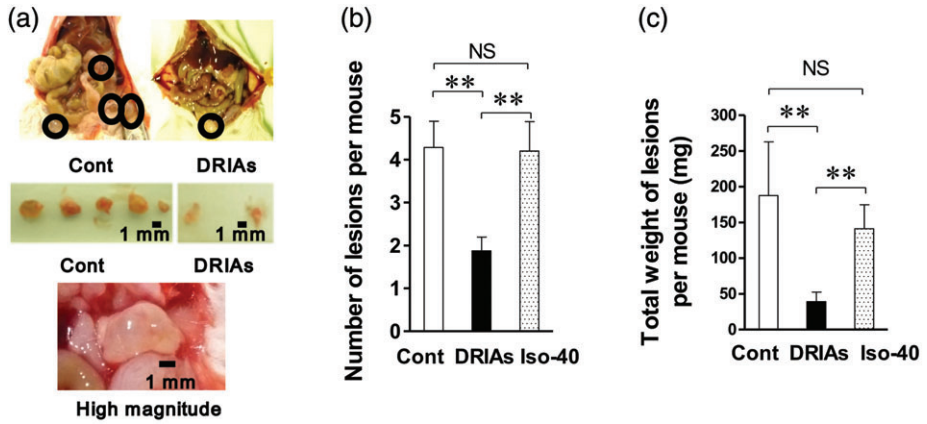

Figure 5 (a) Representative endometriosis-like cystic lesions in the abdominal cavity of recipient mice fed with normal food (Cont), DRIA-containing food (DRIAs) or Isoflavone-40-containing food (Iso-40). Comparison of lesions in total number (b), weight (c) between the control (*open bars*), DRIA (*solid bars*) and Isoflavone-40 treatments (*dotted bars*). $^{**}P < 0.01$. NS indicates not significant.

of aromatase expression/activity and COX-2 expression. In addition, DRIA inhibited the formation of endometriosis-like lesions in an in vivo mouse model, indicating that they might be useful for the treatment of endometriosis[64] (Fig. 5). Clinical trials will be necessary to clarify the effect of DRIA in patients with endometriosis.

Conclusions

Endometriosis is apparently an estrogen-dependent disease. Suppression of in situ estrogen biosynthesis can be achieved by preventing aromatase expression and activity in patients with endometriosis. An understanding of the regulatory mechanism for the expression of aromatase in endometriosis will provide useful information concerning aberrant estrogen production in local lesions. Novel treatment strategies to control estrogen biosynthesis in endometriosis tissues could be designed to more effectively control this disorder.

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Disclosure

None declared.

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