Aromatase and other steroidogenic genes in endometriosis: translational aspects

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Endometriosis is a common, chronic and estrogen-dependent gynaecological disorder associated with pelvic pain and infertility. In addition to, or perhaps as a consequence of, immune, environmental and genetic factors, endometriotic lesions show high estradiol (E₂) biosynthesis and low E₂ inactivation compared with normal endometrium. Current medical therapies of pain, which aim to lower circulating E₂ concentrations, are not effective in at least half of these patients. We and others recently demonstrated the expression of a few steroidogenic genes in endometriosis. The most important genes in this group are steroidal acute regulatory protein (StAR) and aromatase. Both are essential for E₂ production. Prostaglandin E₂ (PGE₂) is the most potent known stimulator of both StAR and aromatase. PGE₂ production in endometriosis is up-regulated by increased levels of the enzyme cyclo-oxygenase-2 (COX-2) in this tissue. COX-2 in turn is stimulated by E₂, interleukin-1β (IL-1β) and PGE₂ itself in endometrial and endometriotic cells. Thus, there is a positive feedback loop that favours continuous formation of E₂ and PGE₂ in endometriosis.

These basic findings led to recent phase-II studies employing aromatase inhibitors in the treatment of endometriosis. Aromatase inhibitors treat both postmenopausal and premenopausal endometriosis at least as effectively as the existing medical treatments. In premenopausal women, we and others administered aromatase inhibitors in combination with ovarian-suppressant treatment. In this review, we emphasize the most recent basic studies in detail and provide a short summary of recent clinical trials.

Key words: aromatase inhibitors/aromatase/cyclo-oxygenase-2/endometriosis/steroidogenic acute regulatory protein

Introduction

Endometriosis is a disease defined by the presence of endometrial tissue outside the uterus. Endometriotic implants contain all the regulatory proteins necessary to make estrogen de novo from cholesterol without the need for androgenic precursors. Therefore, a better understanding of the molecular mechanisms that regulate steroidogenic genes may permit us to develop more specific and effective treatment alternatives for endometriosis. In this review, we discuss the importance and function of endogenous steroidogenic genes in local estradiol (E₂) biosynthesis in endometriosis. We also discuss briefly the recent studies examining the use of an aromatase inhibitor together with ovarian suppressive therapy as a novel treatment of endometriosis.

Steroidogenic acute regulatory protein (StAR) facilitates the entry of cholesterol from the cytosol into the mitochondrion (Stocco et al., in press). This represents a major step for steroidogenesis. Then, six enzymes encoded by at least five specific genes catalyse the conversion of cholesterol to the biologically active estrogen, E₂. The aromatase enzyme catalyses the final and key step, i.e., the conversion of C₁₉ steroids to estrogens (Figure 1). Because only a single gene (CYP19) encodes aromatase in humans, targeted disruption of this gene or inhibition of its product effectively eliminates estrogen biosynthesis (Simpson et al., 2002).

Aromatase is expressed in some human cells including the ovarian granulosa cell, the placental syncytiotrophoblast, the testicular Leydig cell as well as various extravaginalular sites including the brain and skin fibroblasts (Simpson et al., 1994). The principal product of the ovarian granulosa cells during the follicular phase is E₂ (−17β). Additionally, aromatase is expressed in human adipose tissue. Whereas the highest levels of aromatase are in the ovarian granulosa cells in premenopausal women, the adipose tissue becomes the major aromatase-expressing body site after menopause (Grodin et al., 1973; Bulun and Simpson, 1994). Although aromatase level per adipose tissue fibroblast may be small, the sum of estrogen arising from all adipose tissue fibroblasts in the entire body makes a physiologic impact. The principal product of the ovary is the potent estrogen, E₂. In adipose tissue, estrogenically
weak estrone is produced from androstenedione of adrenal origin in relatively large quantities. However, at least half of this peripherally produced estrone is eventually converted to E2 in extraovarian tissues (MacDonald et al., 1979) (Figure 2).

The aromatase gene is transcribed from the telomere to the centromere, and the region encoding the aromatase protein spans 30 kb of its 3′-region (centromeric) and contains nine exons (II-X) (Shozu et al., 2003). The adenine-thymidine-guanine (ATG) translation start site is located in coding exon II. The upstream (telomeric) first exons associated with upstream promoters used alternatively in other human tissues were labelled as I.1, I.2a, I.4, I.7, I.f, I.6 and I.3. Nine coding exons are numbered from II to X. CRE, cAMP response element; NRHS, nuclear receptor half site.

Figure 2. The biologically active estrogen, estradiol (E2) is produced first from the ovary in reproductive-age women and next by conversion of circulating androstenedione (A) of adrenal and/or ovarian origins to estrone (E1) in peripheral tissues. E1 is further converted to E2 in normal or pathologic tissues.

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Figure 3. Expression of the aromatase gene is regulated by the tissue-specific activation of many promoters via alternative splicing. Prostaglandin E2 (PGE2) induces the aromatase (CYP19) expression via cAMP. This gives rise to binding of steroidalogenic factor 1 (SF1) upstream of promoter II that is the regulatory region responsible for aromatase expression. We hypothesize that SF1 interacts with many other enhancer-type transcription factors including CCAAT–enhancer-binding protein-α (C/EBPα) and other possible additional yet unidentified enhancers (Enh) and co-activators (Co-Act-1 and Co-Act-2). First exons associated with upstream promoters used alternatively in other human tissues were labelled as I.1, I.2a, I.4, I.7, I.f, I.6 and I.3. Nine coding exons are numbered from II to X. CRE, cAMP response element; NRHS, nuclear receptor half site.

the brain (I.f), endothelial cells (I.7), fetal tissues (I.5), adipose tissue (I.4) and placenta (2a and I.1) are localized in tandem order at approximately 33, 36, 43, 73, 78 and 93 kb, respectively, upstream of the first-coding exon, exon II (Mahendroo et al., 1993; Sebastian and Bulun, 2001) (Figure 3). In addition to promoter II-specific sequences, transcripts containing two other unique sequences, untranslated exons I.3 and I.4, are present in adipose tissue and in adipose-tissue fibroblasts maintained in culture (Mahendroo et al., 1993). Transcription initiated by use of each promoter gives rise to a transcript with a unique 5′-untranslated end that contains the
sequence encoded in the first exon immediately downstream of this particular promoter. Therefore, the 5'-untranslated region of aromatase mRNA is promoter specific and may be viewed as a signature of the particular promoter used. It should be emphasized again that all of these 5'-ends are spliced onto a common junction immediately upstream of the ATG translation start site (Mahendroo et al., 1993). Consequently, the sequence encoding the open reading frame is identical in each case. Thus, the expressed protein is the same regardless of the splicing pattern.

Aromatase in premenopausal women is expressed primarily in the ovarian follicle, where FSH induces aromatase and consequently E2 production in a cyclic fashion (Simpson et al., 1994). Ovarian aromatase expression is mediated primarily by FSH receptors, cAMP production and activation of the proximal promoter II (Simpson et al., 1994). Men and postmenopausal women also produce estrogen by aromatase that resides in extraglandal tissues, such as adipose tissue and skin (Simpson et al., 1994). Estrogen produced in these extraglandal tissues is of paramount importance for the closure of bone plates and bone mineralization in both men and postmenopausal women, because the phenotype of men with defective genes of aromatase or estrogen receptor-α includes severe osteoporosis and extremely tall stature with growth into adulthood (Bulun, 2000). A distal promoter (I4) located 73 kb upstream of the coding region directs aromatase expression in adipose tissue and skin fibroblasts. Promoter I4 in these tissues is regulated by the combined action of a glucocorticoid and a member of the class I cytokine family [e.g. interleukin-6 (IL-6), IL-11, leukaemia inhibitory factor, oncostatin-M] (Zhao et al., 1995).

Aromatase is expressed in the stromal cells of endometriosis. Its expression is regulated primarily by promoter II. The second messenger in this signalling pathway is cAMP. The most potent ligand that induces aromatase via cAMP and promoter II in endometriotic stromal cells is prostaglandin E2 (PGE2) (Noble et al., 1997) (Figure 3).

Endometriosis and estrogen production

Endometriosis is the most common cause of chronic pelvic pain. It affects 6–10% of US women of reproductive age (approximately 3.6–6 million) (Giudice and Kao, 2004). Endometriosis is a systemic disorder that is characterized by the presence of endometrium-like tissue in ectopic sites outside the uterus, primarily on pelvic peritoneum and ovaries and linked to chronic pelvic pain, pain during sex and infertility (Giudice and Kao, 2004). In the United States, endometriosis is the third most common gynecologic disorder that requires hospitalization and a leading cause of hysterectomy. One report indicates that 9% of women are initially resistant to existing treatments, and 50% of the responders have recurrence of pain at the end of a 6-month follow-up (Vercellini et al., 1997). Aromatase inhibitors in combination with an oral contraceptive or progestin were found to be effective in the patients who were resistant to these existing medical and surgical treatments (Ailawadi et al., 2004; Amsterdam et al., in press). In a placebo-controlled prospective randomized study, an aromatase inhibitor plus GnRH analogue regimen showed a strikingly superior response compared with a GnRH analogue only regimen with respect to recurrence of pain after 24 months of the therapy (Soysal et al., 2004). Therefore, the novel treatment with aromatase inhibitors may have significant clinical advantages over the currently used treatments with respect to pain relief and recurrence of pain in patients with endometriosis.

Endometriosis is an estrogen-dependent disease. The clinical significance of estrogen biosynthesis in endometriosis is exemplified by the clinical observations that estrogen is essential for growth of endometriosis. We and others demonstrated abundant aromatase expression and local estrogen production in endometriotic tissue (Kitawaki et al., 1997; Zeitoun et al., 1999; Bulun et al., 2001; Fang et al., 2002; Gurates et al., 2002; Yang et al., 2002). The subsequent introduction of aromatase inhibitors into the treatment of endometriosis successfully underscored the presence of aromatase in endometriotic tissue (Takayama et al., 1998; Ailawadi et al., 2004). These recent results were suggestive that aromatase inhibitors might treat endometriosis more effectively than GnRH analogues via suppression of local estrogen formation in endometriotic tissue.

Inflammation and growth in endometriosis

There are two pathologic processes, namely inflammation and growth, which are responsible for chronic pelvic pain and infertility and endometriosis. Estrogen, growth factors and metalloproteinases enhance the growth and invasion of endometriotic tissue, whereas PGs and cytokines mediate pain, inflammation and infertility (Bruner et al., 1997; Ryan and Taylor, 1997). Research work from our laboratory and other investigators over the past 10 years uncovered a molecular link between inflammation and estrogen production in endometriosis (Bulun et al., 2001). This is mediated by a positive feedback cycle that favours expression of the key steroidogenic genes, most notably StAR and aromatase, expression of cyclo-oxygenase-2 (COX-2) and continuous local production of E2 and PGE2 in endometriotic tissue (Noble et al., 1997; Tsai et al., 2001; Sun et al., 2003) (Figure 4). We find that the aberrantly expressed transcription factor steroidogenic factor 1 (SF1) mediates PGE2–cAMP-dependent co-activation of multiple steroidogenic genes, in particular STAR and aromatase, in endometriosis (our unpublished observations) (Zeitoun et al., 1999).

Investigation of intact tissues and cultured stromal cells in human endometriosis research

Many molecular abnormalities were demonstrated in vivo in intact tissues of surgically removed endometriosis (Figure 5). The prototype abnormality was the presence of significant levels of StAR and aromatase activity and expression of protein and mRNA in the stromal cell component of endometriosis, whereas StAR or aromatase expression was either absent or barely detectable in the eutopic endometrium of disease-free women (Noble et al., 1996, 1997; Tsai et al., 2001; Gurates et al., 2002; Sun et al., 2003). The eutopic endometrium of women with endometriosis contains low but significant levels of aromatase mRNA and enzyme activity and represents an intermediate state of this disease. It appears that upon retrograde menstruation and implantation of this inherently abnormal tissue on pelvic peritoneal surfaces, aromatase expression and enzyme activity are amplified by up to 400 times (Noble et al., 1996, 1997). COX-2 expression that is important for PGE2 synthesis is increased markedly in both eutopic endometrium and endometriotic tissue of women with endometriosis (Ota et al., 2001; Wu et al., 2005).
Figure 4. Two basic pathologic processes, namely growth and inflammation, are responsible for chronic pelvic pain and infertility, which are the primary devastating symptoms of endometriosis. Estrogen enhances the growth and invasion of endometriotic tissue, whereas prostanoids (PGs) and cytokines mediate pain, inflammation and infertility. Estradiol (E2) is produced locally in endometriotic tissue. The precursor, androstenedione (A) of ovarian, adrenal or local origin becomes converted to estrone (E1) that is in turn reduced to E2 in endometriotic implants. Endometriotic tissue is capable of synthesizing A from cholesterol via the activity of steroidogenic acute regulatory protein (StAR) and other steroidogenic enzymes also present in this tissue. E2 directly induces cyclo-oxygenase-2 (COX-2), which gives rise to elevated concentrations of prostaglandin E2 (PGE2), in turn, is the most potent known stimulator of StAR and aromatase in endometriotic stromal cells. This establishes a positive feedback loop in favour of continuous estrogen and PG formation in endometriosis. Disruption of this feedback loop using an aromatase inhibitor is an effective treatment of endometriosis.

E2 is produced from cholesterol through six serial enzymatic conversions in two ovarian cell types that co-operate in a paracrine fashion. The two rate-limiting steps include the entry of cholesterol into the mitochondrion facilitated by StAR and the conversion of androstenedione to estrone by aromatase (Figure 1). We and others recently showed that StAR, aromatase and all other necessary steroidogenic enzymes are expressed in vivo in endometriosis enabling this tissue to synthesize E2 from cholesterol de novo (Bulun et al., 2001; Tsai et al., 2001) (and our unpublished data). Additionally, PGE2 induces expression of the steroidogenic genes StAR, P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase (3β-HSD) type II, 17-hydroxylase/17–20-lyase and aromatase (P450arom) in endometriotic stromal cells. The PGE2-dependent inductions of StAR and aromatase mRNA levels were the highest in comparison with other steroidogenic genes (our unpublished observations). In contrast, normal endometrium does not biosynthesize E2. Both endometriosis and normal endometrium contain the enzyme 17β-HSD-1 that catalyses the final step that is the conversion of estrone to E2. What separates normal endometrium from endometriosis, however, is the in vivo lack of StAR and aromatase.

The following definitions have been used in our past publications. The terms, ‘endometriotic tissue’, ‘endometriotic cell’ and ‘endometriosis’, refer to the pathologic ectopic endometrium-like tissues isolated from the pelvic peritoneum or ovaries during surgery. The term, ‘endometrium’, refers to eutopic endometrial tissue that is the mucosal layer of the uterine cavity. ‘Normal endometrium’ and ‘normal endometrial cells and tissues’ refer to the eutopic endometrium from disease-free women. The term, ‘endometrium from an endometriosis patient’, refers to eutopic endometrial tissue or cells from patients with endometriosis. ‘Endometriotic stromal cells’ are isolated from the walls of cystic endometriosis lesions (endometriomas) in the ovaries (Ryan et al., 1994). These cells in primary culture have been characterized extensively in Robert Taylor’s and our laboratories (Ryan et al., 1994; Noble et al., 1997). Endometriotic stromal cells display the differentiation markers observed in eutopic endometrial stromal cells in culture. For example, progestin-treated endometriotic stromal cells express prolactin mRNA (readily detectable by northern analysis), albeit in significantly lower levels compared with eutopic endometrial cells, whereas control ovarian granulosa cells do not show detectable prolactin mRNA.

The key molecular abnormalities described in this article were first observed in vivo as differential expression of steroidogenic genes in endometrial and endometriotic tissues. The molecular mechanisms responsible for these abnormalities were then determined in primary endometriotic and endometrial stromal cells in culture. We found that most of these molecular mechanisms were altered expression and/or DNA-binding activity of transcription factors that regulate steroidogenic genes. Then, these in vitro-demonstrated molecular mechanisms were verified in human tissues to demonstrate the in vivo roles of these transcription factors in human endometriosis.

Pathophysiology of estrogen biosynthesis in endometriosis

E2 is produced from cholesterol through six serial enzymatic conversions in two ovarian cell types that co-operate in a paracrine fashion. The two rate-limiting steps include the entry of cholesterol into the mitochondrion facilitated by StAR and the conversion of androstenedione to estrone by aromatase (Figure 1). We and others recently showed that StAR, aromatase and all other necessary steroidogenic enzymes are expressed in vivo in endometriosis enabling this tissue to synthesize E2 from cholesterol de novo (Bulun et al., 2001; Tsai et al., 2001) (and our unpublished data). Additionally, PGE2 induces expression of the steroidogenic genes StAR, P450 side-chain cleavage, 3β-hydroxysteroid dehydrogenase (3β-HSD) type II, 17-hydroxylase/17–20-lyase and aromatase (P450arom) in endometriotic stromal cells. The PGE2-dependent inductions of StAR and aromatase mRNA levels were the highest in comparison with other steroidogenic genes (our unpublished observations). In contrast, normal endometrium does not biosynthesize E2. Both endometriosis and normal endometrium contain the enzyme 17β-HSD-1 that catalyses the final step that is the conversion of estrone to E2. What separates normal endometrium from endometriosis, however, is the in vivo lack of StAR and aromatase.

Our initial focus was on aromatase expression in endometriosis and hypothesized that the substrate androstenedione for this enzyme originated primarily from the adrenal and/or ovary and...
Arrive at the target tissue (i.e. endometriosis) via circulation. The recent demonstration of StAR and a complete set of steroidogenic enzymes including aromatase within the endometriotic stromal cell, however, imply that estrogen is synthesized de novo from cholesterol and that endometriotic aromatase is not solely dependant for substrate on adrenal or ovarian secretion. In view of these new findings, we revised our hypothesis regarding the source of substrate for the aromatase enzyme.

**Molecular and cellular mechanisms that regulate aromatase expression in endometriosis**

The stromal cells of endometriosis have both StAR and aromatase. This has been supported by many in vivo and in vitro observations. We and others demonstrated immunoreactive aromatase in stromal cells of extraovarian and ovarian endometriotic tissue (Kitawaki et al., 1997) (and our unpublished observations). Abundant StAR activity and aromatase enzyme activity are present in stromal cells isolated and cultured from ovarian and endometriotic endometriomas (Noble et al., 1997; Zeitoun et al., 1999; Tsai et al., 2001; Sun et al., 2003). StAR and aromatase mRNA species are present only in stromal but not epithelial cell compartment of endometriosis (Noble et al., 1997; Tsai et al., 2001; Sun et al., 2003).

PGE$_2$ or a cAMP analogue stimulates StAR mRNA and protein levels and aromatase mRNA in endometriotic stromal cells in a time- and concentration-dependent fashion (Tsai et al., 2001). PGE$_2$ stimulates the production of progesterone, 17-hydroxyprogesterone, androstenedione and E$_2$ primarily via induction of StAR and aromatase in stromal cells isolated from endometriosis (Noble et al., 1997; Tsai et al., 2001). StAR or aromatase expression or steroid hormone production is virtually undetectable in eutopic endometrial stromal cells from disease-free women. High number of cycles of PCR may amplify aromatase mRNA in endometriotic stromal cells, but we could not detect aromatase enzyme activity in cultured endometrial stromal cells from disease-free women (Noble et al., 1997). Aromatase activity or mRNA could not be induced by PGE$_2$ or cAMP analogues in stromal cells from disease-free women (Noble et al., 1997). Small but significant levels of aromatase enzyme activity and mRNA were detected in eutopic endometrial tissue and stromal cells of women with endometriosis (Noble et al., 1996, 1997). This is consistent with the observations of many other investigators that the eutopic endometrial tissue of women with endometriosis exhibits multiple molecular abnormalities that may be linked to aberrant production of estrogen, PGs, cytokines and decreased production of molecules important for decidual transformation of stromal cells (Lessey et al., 1994; Tseng et al., 1996; Kao et al., 2003; Wu et al., 2005).

**Production and action of PGE$_2$ in endometriosis**

PGE$_2$ plays a key role in the estrogen biosynthetic pathway in endometriotic tissue, because it is a potent stimulator of the key steroidogenic genes (Noble et al., 1997; Zeitoun et al., 1999; Tsai et al., 2001; Sun et al., 2003). The most significantly induced steroidogenic genes are StAR and aromatase (Noble et al., 1997; Zeitoun et al., 1999; Tsai et al., 2001; Sun et al., 2003). Thus, PGE$_2$ production in endometriosis is extremely important. The enzyme cyclo-oxygenase catalyses the conversion of arachidonic acid to PGH$_2$, which is then converted to PGE$_2$ by the enzyme PGE$_2$ synthase. Two distinct genes, called COX-1 and COX-2, encode the cyclo-oxygenase enzyme. COX-2 is the inducible gene that is regulated by many factors. PGE$_2$ synthase is also encoded by multiple genes (Murakami et al., 2003). The inducible membrane-bound PGE$_2$ synthase was found in many human tissues including the benign and malignant endometrium and breast (Jabbour et al., 2001; Karuppu et al., 2002; Ni et al., 2002; Sun et al., 2004).

An in vivo association was reported between aromatase and COX-2 expression in breast-cancer tissues. PGE$_2$ synthesized in breast-cancer epithelial cells was proposed to stimulate P450arom expression in breast fibroblasts (Brueggemeier et al., 1999; Richards et al., 2002). Moreover, PGE$_2$ synthase was shown to be up-regulated by E$_2$ in breast-cancer cells and by IL-1β in a variety of fibroblasts (Miller et al., 2000; Frasor et al., 2003).

COX-2 is up-regulated significantly in stromal cells of endometriosis. Its expression is also significantly higher in endometrium of patients with endometriosis in comparison with endometrium of disease-free patients (Ota et al., 2001; Wu et al., 2005). We and others demonstrated that IL-1β and PGE$_2$ itself induce COX-2 in endometriotic and endometrial stromal cells (Tamura et al., 2002a,b,c). Additionally, vascular endothelial growth factor (VEGF) and E$_2$ rapidly induced COX-2 in uterine endothelial cells (Tamura et al., 2003, 2004; Wu et al., 2005). Thus, many pathways in endometriosis induce COX-2 to increase PGE$_2$ formation in this tissue. The regulation of PGE$_2$ synthase in endometriosis is not known (Figure 4).

Both endometriotic and eutopic endometrial stromal cells express similar mRNA and protein levels of the known PGE$_2$ receptor subtypes including EP$_1$, EP$_2$, EP$_3$ and EP$_4$. Use of receptor-selective agonists, however, showed that EP$_2$ receptor is primarily responsible for PGE$_2$-mediated aromatase expression in endometriotic stromal cells (Zeitoun and Bulun, 1999). Stimulation of the EP$_2$ receptor rapidly increases intracellular cAMP (Zhao et al., 1996; Noble et al., 1997). Treatment with PGE$_2$ or a cAMP analogue gives rise to comparable increases in StAR and aromatase activity, protein and mRNA levels (Noble et al., 1997). Neither PGE$_2$ (despite the presence of EP$_2$ receptors) nor cAMP analogues can induce StAR or P450arom in normal eutopic endometrial stromal cells (Noble et al., 1997). Therefore, the inhibition for PGE$_2$-dependent steroidogenesis in eutopic endometrial stromal cells is mediated by molecular mechanisms downstream of cAMP.

**Mechanisms responsible for increased expression of steroidogenic genes in endometriosis**

Transcription of the aromatase gene in human tissues is regulated by at least 10 distinct promoters each giving rise to aromatase mRNA species with variable 5'-untranslated sequences but an identical coding region (Harada et al., 1990, 1993; Sebastian and Bulun, 2001; Sebastian et al., 2002) (Figure 3). Extraovarian endometriotic tissue and ovarian endometrioma-derived cells use almost exclusively promoter II, which is the PGE$_2$/cAMP-responsive proximal promoter, for aromatase expression in vivo (Noble et al., 1996, 1997; Zeitoun et al., 1999). Thus, aberrant aromatase expression in endometriosis is primarily mediated by the activation of promoter II (Figure 3).
We uncovered many molecular abnormalities that are responsible for PGE$_2$-cAMP-dependent aromatase expression in endometriosis. One critical mechanism is mediated by aberrantly expressed key transcriptional enhancers (e.g. SF1) in biopsied endometriotic tissues (in vivo) and cultured endometriotic stromal cells (in vitro).

An opposite counterpart of this pathologic enhancer-mediated mechanism serves as a physiologic mechanism to suppress steroidogenic gene expression in normal eutopic endometrial stromal cells exposed to PGE$_2$ or cAMP analogues. This involves the redundant presence and steroidogenic promoter-binding activity of multiple transcriptional inhibitors [e.g. chicken ovalbumin upstream promoter transcription factor (COUP-TF)] and corepressors of SF1 [e.g. Wilms’ tumour-1 (WT-1)] serving as fail-safe mechanisms to inhibit aromatase.

The key regulatory elements that regulate aromatase in endometriosis are within the 500-bp region that lies upstream of promoter II. We found a dose-dependent direct competition between the orphan nuclear receptors SF1 (enhancer) and COUP-TF (inhibitor) for occupancy of a nuclear receptor half site (NRHS) (−136/−124 bp), to which SF1 bound with a higher affinity (Zeitoun et al., 1999). COUP-TF is expressed in both eutopic endometrial and endometriotic cells, whereas SF1 is expressed in endometriotic but not normal eutopic endometrial cells (Figure 5). COUP-TF is part of the transcriptional system that inhibits aromatase in eutopic endometrial stromal cells; in contrast, aberrantly expressed SF1 in endometriotic stromal cells overrides this inhibition by competing for the same regulatory element. Thus, the mechanism here is differential expression of an enhancer in endometriosis but not in eutopic endometrium favouring aromatase expression (Zeitoun et al., 1999).

Additional redundant mechanisms serve to inhibit aromatase in normal endometrium and stimulate it in endometriosis (Yang et al., 2002). For example, we demonstrated that overexpression of CCAAT–enhancer-binding protein-α (C/EBPα) stimulated, whereas C/EBPβ inhibited P450arom promoter in endometriotic and eutopic endometrial cells (Yang et al., 2002). Unexpectedly, C/EBPα or -β was found to bind to the −211/−197 cAMP response element (CRE) but not the nuclear factor IL-6 sites in the aromatase promoter II. Moreover, C/EBPβ was selectively down-regulated in vivo in endometriosis but not in eutopic endometrium indicating that differential down-regulation of a transcriptional inhibitor in endometriosis is an additional mechanism for aromatase expression in this pathologic tissue.

Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX-1), a co-repressor of SF1 inhibits SF1-dependent expression of aromatase in endometriotic and endometrial cells (Gurates et al., 2003). Moreover, we demonstrated for the first time that WT-1, another co-regulator of SF1, also acts as a co-repressor and inhibits SF1-dependent activity of the aromatase promoter II in endometriotic/endometrial stromal cells (Gurates et al., 2002, 2003). Intriguingly, WT-1 but not DAX-1 is selectively down-regulated in vivo in endometriotic stromal cells (Gurates et al., 2002). Thus, WT-1 seems to be a physiologically significant co-repressor for the inhibition of steroidogenesis in eutopic endometrium.

On the other hand, p300/CREB (CRE binding protein (CREB)), which is a co-activator of SF1, enhances promoter-II activity in endometriotic and endometrial stromal cells (our unpublished observations). These findings together are suggestive that SF1 acts as a master switch to activate the promoters of aromatase and other key steroidogenic genes. Co-repressors or co-activators of SF1 in endometrial versus endometriotic cells may further modify SF1-dependent transcriptional activity.

A PGE$_2$–cAMP-dependent signalling system activates multiple steroidogenic genes

Endometriotic tissue or stromal cells in primary culture express the complete cascade of steroidogenic genes and thus can potentially make estrogen from cholesterol. In contrast to normal endometrium, SF1, StAR and aromatase are specifically and distinctly up-regulated in endometriotic tissues and cultured cells. The regulatory elements in StAR and aromatase promoters are very similar. Additionally, similar transcription factors (SF1, C/EBPs and CREB) mediate PGE$_2$–dependent regulation of both promoters (Christenson and Strauss, 2001; Manna et al., 2002; Yang et al., 2002; Manna et al., 2003). These promoters remain quiescent under the influence of inhibitory transcription factors (e.g. COUP-TF) and co-repressors (e.g. WT-1) in normal endometrial stromal cells. These data are strongly suggestive that SF1 expression in endometriosis is primarily responsible for PGE$_2$-mediated estrogen formation, in particular via induction of StAR and aromatase promoters.

Treatment of endometriosis with aromatase inhibitors

The observation that local estrogen biosynthesis takes place in endometriotic implants prompted us and other investigators to target aromatase in endometriosis using its third-generation inhibitors. Among these inhibitors, anastrozole and letrozole were used successfully to treat endometriosis in both postmenopausal and premenopausal women (Takayama et al., 1998; Amsterdam et al., 2003; Ailawadi et al., 2004; Raphael et al., 2004; Shippen and West, 2004; Soysal et al., 2004).

In 1998, we published the first article reporting the treatment of postmenopausal endometriosis with an aromatase inhibitor (Takayama et al., 1998). This postmenopausal woman had her uterus and both ovaries surgically removed and was therefore postmenopausal but continued to have a large persistent pelvic mass and severe pain (Takayama et al., 1998). An aromatase inhibitor effectively eradicated the mass and eliminated her pain (Takayama et al., 1998). This was followed by another case report confirming that an aromatase inhibitor is the medical treatment of choice in postmenopausal endometriosis that is a relatively rare condition (Razzi et al., 2004).

In the treatment of premenopausal endometriosis, some form of ovarian suppression needs to be added to the currently available doses of aromatase inhibitors. If the ovary is not suppressed concomitantly, estrogen depletion in the hypothalamus may cause FSH secretion and ovarian stimulation. Thus, an aromatase inhibitor was administered together with a GnRH agonist, a progesterin, progesterone or a combination oral contraceptive in four phase-II trials (Amsterdam et al., 2003; Ailawadi et al., 2004; Shippen and West, 2004; Soysal et al., 2004). All four studies showed a significant benefit of an aromatase inhibitor in reducing pelvic pain. One study showed laparoscopic evidence of eradicating visible pelvic endometriotic implants and significantly decreasing pain.
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underlying estrogenic control of proliferation and cell phenotype. Endocrinology 144,4562–4574.


Harada N, Yamada K, Saito K, Kibe N, Doihmae S and Takagi Y (1990) Struc-

Harada N, Utsumi T and Takagi Y (1993) Tissue-specific expression of the human aromatase cytochrome P450 gene by alternative use of multiple exons I and promoters, and switching of tissue-specific exons I in carcino-
genesis. Proc Natl Acad Sci USA 90,11312–11316.


based implantation failure and infertility. Endocrinology 144,2870–2881.

Karuppur D, Kalus A, Simpson ER and Clynce C (2002) Aromatase and progest-

chrome P450 protein and messenger ribonucleic acid in human endometri-


MacDonald PC, Madden JD, Brenner PF, Wilson JD and Siiteri PK (1979) Origin of estrogen in normal men and in women with testicular feminiza-

Mahendroo MS, Mendelson CR and Simpson ER (1993) Tissue-specific and hormonally-controlled alternative promoters regulate aromatase cyto-


Manna PR, Eubank DW, Lalli E, Sassone-Corsi P and Stocco DM (2003) Transcriptional regulation of the mouse steroidogenic acute regulatory protein gene by the CAMP response-element binding protein and ster-

cer Invest 18,293–302.


ocrinol Metab 82,600–606.

Ota H, Igarashi S, Sasaki M and Tanaka T (2001) Distribution of cyclooxygen-
ase-2 in eutopic and ectopic endometrium in endometriosis and adenomy-
mosis. Hum Reprod 16,561–566.


Zhao Y, Agarwal VR, Mendelson CR and Simpson ER (1996) Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 137,5379–5742.

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