0031-6997/05/5703-359–383$7.00
PHARMACOLOGICAL REVIEWS
Vol. 57, No. 3
50307/3052912
Pharmacol Rev 57:359–383, 2005
Printed in U.S.A

Regulation of Aromatase Expression in Estrogen-Responsive Breast and Uterine Disease: From Bench to Treatment

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This work was supported in part by the National Institutes of Health Grants CA67167, HD38691, HD46260, and HD40093 and grants from the Northwestern Memorial Foundation and the Friends of Prentice (to S.E.B.).

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Article, publication date, and citation information can be found at http://pharmrev.aspetjournals.org.
doi:10.1124/pr.57.3.6.

***Abstract*——A single gene encodes the key enzyme for estrogen biosynthesis termed aromatase, inhibition of which effectively eliminates estrogen production. Aromatase inhibitors successfully treat breast cancer and endometriosis, whereas their roles in endometrial cancer, uterine fibroids, and aromatase excess syndrome are less clear. Ovary, testis, adipose tissue, skin, hypothalamus, and placenta express aromatase normally, whereas breast and endometrial cancers, endometriosis, and uterine fibroids overexpress aromatase and produce local estrogen that exerts paracrine and intracrine effects. Tissue-specific promoters distributed over a 93-kilobase regulatory region upstream of a common coding region alternatively control aromatase expression. A distinct set of transcription factors regulates each promoter in a signaling pathway- and tissue-specific manner. Three mechanisms are responsible for aromatase overexpression in a pathologic tissue versus its normal counterpart. First, cellular composition is altered to increase aromatase-expressing cell types that use distinct promoters (breast cancer). Second, molecular alterations in stromal cells favor binding of transcriptional enhancers versus inhibitors to a normally quiescent aromatase promoter and initiate transcription (breast/endometrial cancer, endometriosis, and uterine fibroids). Third, heterozygous mutations, which cause the aromatase coding region to lie adjacent to constitutively active cryptic promoters that normally transcribe other genes, result in excessive estrogen formation owing to the overexpression of aromatase in many tissues.**

I. Introduction

A. The Aromatase Enzyme

The initial entry of cytosolic cholesterol into the mitochondrion, which is facilitated by steroidogenic acute regulatory protein (StAR[1]), represents a major step for steroidogenesis. Six enzymes encoded by at least five specific genes then catalyze the conversion of cholesterol to the biologically active estrogen estradiol. The aromatase enzyme catalyzes the final and key step, i.e., the conversion of C_{19} steroids to estrogens (Fig. 1).

The aromatase enzyme is localized in the endoplasmic reticulum of estrogen-producing cells (Sebastian and Bulun, 2001; Simpson et al., 2002). Aromatase enzyme complex is comprised of two polypeptides. The first of these is a specific cytochrome P450 (P450), namely aromatase cytochrome P450 (P450arom) (the product of the CYP19 gene) (Simpson et al., 2002). The second is a flavoprotein, NADPH-cytochrome P450 reductase and is ubiquitously distributed in most cells. Thus, cell-specific expression of P450arom determines the presence or absence of aromatase activity. For practical purposes, we will refer to "P450arom" as "aromatase" throughout this text. Since only a single gene (*CYP19*) encodes aromatase in mice and humans, targeted disruption of this gene or inhibition of its product effectively eliminates estrogen biosynthesis in these species (Simpson et al., 2002).

In the human, aromatase is expressed in a number of cells, including the ovarian granulosa cell, the placental syncytiotrophoblast, the testicular Leydig cell, and various extraglandular sites, including the brain and skin

[1] Abbreviations: StAR, steroidogenic acute regulatory protein; P450, cytochrome P450; P450arom, aromatase cytochrome P450; kb, kilobase(s); bp, base pair(s); FSH, follicle-stimulating hormone; ER, estrogen receptor; IL, interleukin; STAT, signal transducer and activator of transcription; PG, prostaglandin; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; C/EBP, CCAAT/enhancer-binding protein; PPAR, peroxisome proliferator-activated receptor; RT, reverse transcriptase; PCR, polymerase chain reaction; LRH-1, liver receptor homolog-1; SHP, short heterodimer partner; COX, cyclooxygenase; CRE, cAMP response element; ATF, activating transcription factor; CBP, cAMP response element binding protein-binding protein; HDAC1, histone deacetylase-1; SF-1, steroidogenic factor-1; COUP-TF, chicken ovalbumin upstream transcription factor; RACE, rapid amplification of cDNA ends; VEGF, vascular endothelial growth factor; GnRH, gonadotropin-releasing hormone; P450scc, cytochrome P450 side-chain cleavage; HSD, hydroxysteroid dehydrogenase; UTR, untranslated end; WT-1, Wilms' tumor-1; DAX-1, dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X gene 1.

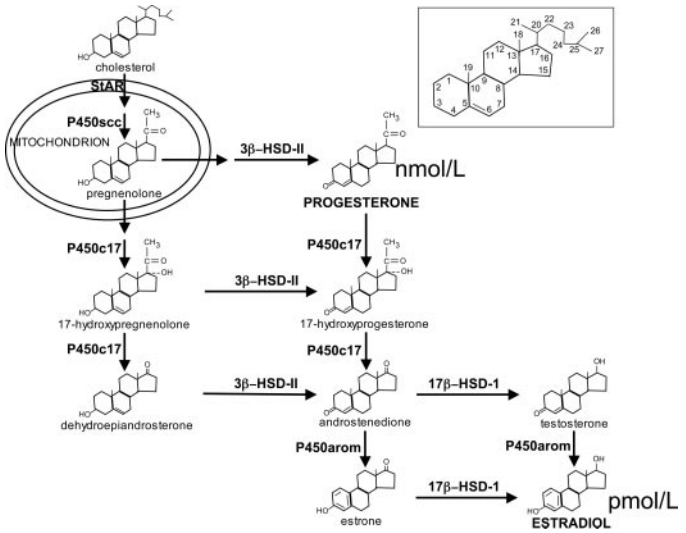


FIG. 1. Steroidogenic pathway. The biologically active steroids progesterone and estradiol are produced from cholesterol by a number of enzymes in the ovary. The key steps seem to be the entry of cholesterol into the mitochondrion facilitated by the StAR protein for progesterone production and the conversion of androstenedione to estrone catalyzed by aromatase (P450arom) for estrogen production. Please note that biologically active quantities of progesterone (nanomolar) are 100 to 1000 times higher than those of estradiol (picomolar). This underscores the fact that relatively very low quantities of aromatase enzyme and estradiol give rise to important biological functions.

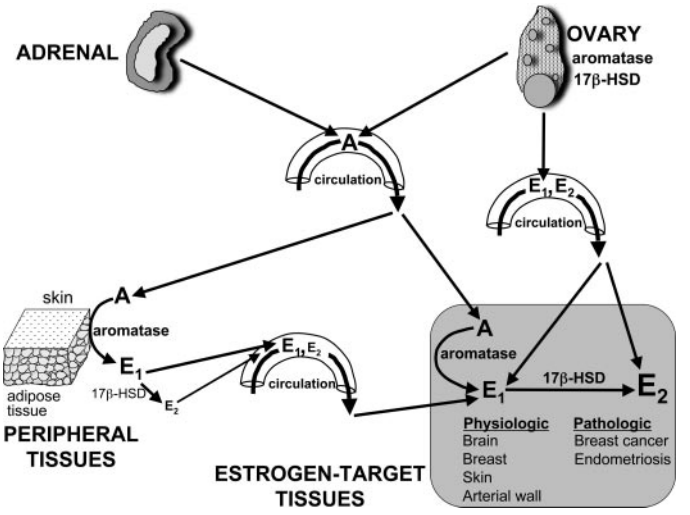


FIG. 2. Origins of estrogen in women. The biologically active estrogen estradiol (E_2) is produced in at least three major sites: 1) direct secretion from the ovary in reproductive-age women; 2) by conversion of circulating androstenedione (A) of adrenal and/or ovarian origins to estrone (E_1) in peripheral tissues; and 3) by conversion of A to E_1 in estrogen-target tissues. In the latter two instances, estrogenically weak E_1 is further converted to E_2 within the same tissue. The presence of the enzyme aromatase and 17β-HSD is critical for E_2 formation at these sites. E_2 formation by peripheral and local conversion is particularly important for postmenopausal women and for estrogen-dependent diseases such as breast cancer, endometriosis, and endometrial cancer.

fibroblasts (Simpson et al., 1994). The principal product of the ovarian granulosa cells during the follicular phase is estradiol (-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 (Fig. 2) (Grodin et al., 1973; Bulun and Simpson, 1994). Although aromatase level per adipose tissue fibroblast may be small, the sum of estrogen arising from billions of adipose tissue fibroblasts in the entire body makes a physiologic impact. The principal product of the ovary is the potent estrogen estradiol. 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 estradiol in extraovarian tissues (Fig. 2) (MacDonald et al., 1979).

B. The Aromatase (CYP19) Gene

The recent availability of the Human Genome Project on the Internet permitted us to reconstruct the entire aromatase gene by computer-assisted analysis (Sebastian and Bulun, 2001) (Fig. 3A). Two overlapping bacterial artificial chromosome clones contained the entire gene, which spans approximately 123 kb (Sebastian and Bulun, 2001) (Fig. 3A). The gene is transcribed from the telomere to the centromere, and the region encoding the aromatase protein spans 30 kb of the 3′-end and contains nine exons (II-X) (Shozu et al., 2003b). The ATG translation start site is located in coding exon II. The upstream (telomeric) 93 kb of the gene contains a number of promoters (Simpson et al., 1994; Sebastian and Bulun, 2001). The most proximal gonad-specific promoter II and two other proximal promoters, I.3 (expressed in adipose tissue and breast cancer) and I.6 (expressed in bone), are found within the 1-kb region upstream of the ATG translation start site in exon II, as expected (Fig. 3A). Promoter I.2, the minor placenta-specific promoter, is located approximately 13 kb upstream of the ATG site in exon II. The promoters specific for 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 ~33, 36, 43, 73, 78, and 93 kb, respectively, upstream of the first coding exon, the exon II (Fig. 3A) (Mahendroo et al., 1993; Sebastian and Bulun, 2001). 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 (Fig. 3B). 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 38 bp upstream of the ATG translation start site (Mahendroo et al., 1993). Consequently, the sequence encoding the open reading frame is identical in

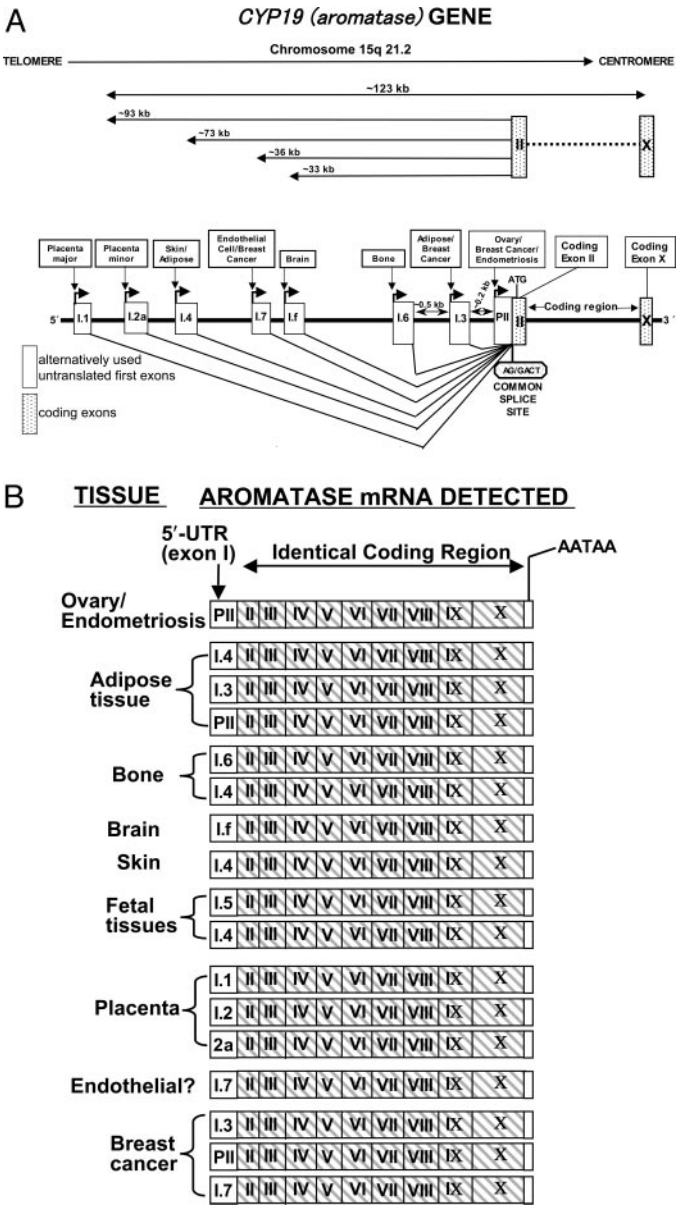


FIG. 3. A, aromatase (*CYP19*) gene. Expression of the aromatase gene is regulated by the tissue-specific activation of a number of promoters via alternative splicing. B, aromatase mRNA. Aromatase mRNA species contain promoter-specific 5′-UTRs. The coding region and encoded protein, however, are identical regardless of the promoter used.

each case. Thus, the expressed protein is the same regardless of the splicing pattern (Fig. 3B).

Aromatase expression in fish, birds, and lower mammals (rodents) is expressed in the brain and gonads via highly conserved promoters named I.f and II, respectively (Simpson et al., 1994). Aromatase is expressed in placenta and other extragonadal tissues such as the skin, adipose tissue, and bone of higher mammals via recruitment of additional promoters throughout the evolution. To our knowledge, the human is unique in that it has the largest number of tissues with aromatase expression and the highest levels of aromatase expression per tissue among all species.

The placental promoter I.1 is the most distally located promoter (93 kb), which gave rise to splicing of a 103-bp first exon onto the common splice junction immediately upstream of the ATG translational start site (Kamat et al., 1998; Jiang et al., 2000; Jiang and Mendelson, 2003) (Fig. 3A). The activity of promoter I.1 is the basis for 100 to 1000 times elevated levels of circulating estrogen in pregnant women (Kamat et al., 1998; Jiang et al., 2000; Jiang and Mendelson, 2003). Thus, recruitment of this promoter may have an evolutionary impact, since, of all species, humans are unique to acquire and maintain extraordinarily high levels of aromatase expression in placenta.

A transgene containing the human genomic region upstream of the placental exon I.1 was found to exhibit readily detectable promoter activity in the mouse placenta (Kamat et al., 2002). Thus, although mouse placental tissue does not express aromatase endogenously, it may contain the necessary transcriptional factors for human promoter I.1 expression. This suggests that the recruitment of aromatase expression in human placenta occurred via alterations in the mammalian genome throughout the evolution. One of the key mechanisms that permits the recruitment of such a large number of promoters seems to be the extremely promiscuous nature of the common splice acceptor site, since activation of each promoter gives rise splicing of an untranslated first exon onto this common junction immediately upstream of the translation start site in the coding region.

C. Physiologic Regulation of Aromatase Expression in Human Tissues

The primary site of aromatase expression in premenopausal women is the ovarian follicle, where FSH induces aromatase and thus estradiol 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) (Fig. 4). Men and postmenopausal also produce estrogen by aromatase that resides in extragonadal tissues such as adipose tissue and skin (Simpson et al., 1994) (Fig. 4). Estrogen produced in these extragonadal tissues are of paramount importance for the closure of bone plates and bone mineralization in both men and postmenopausal women, since the phenotype of men with defective genes of aromatase or estrogen receptor (ER)-α include severe osteoporosis and extremely tall stature with growth into adulthood (Bulun, 2000). A distal promoter (I.4) located 70 kb upstream of the coding region directs aromatase expression in adipose tissue and skin fibroblasts. Promoter I.4 in these tissues is regulated by combined action of a glucocorticoid and a member of the class I cytokine family [e.g., interleukin (IL)-6, IL-11, leukemia inhibitory factor, and oncostatin-M] (Fig. 4) (Zhao et al., 1995).

The alternative use of promoters comprises the basis for differential regulation of aromatase expression by

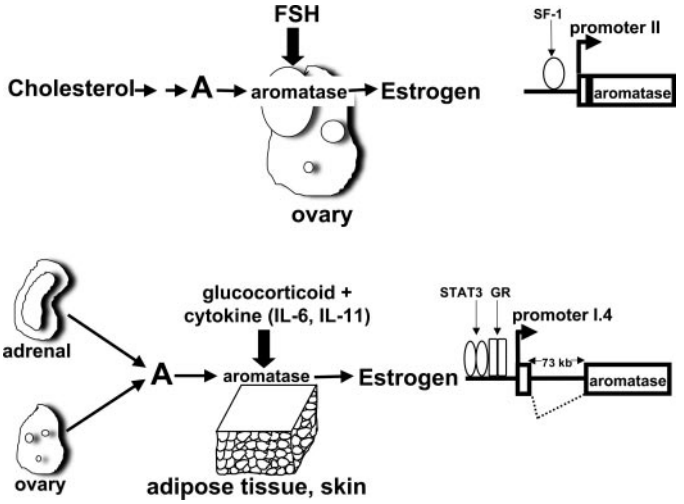


FIG. 4. Physiological regulation of aromatase expression. FSH induces aromatase expression via a cAMP-dependent pathway in ovarian granulosa cells via promoter II. SF-1 mediates this action of FSH. On the other hand, a combination of a glucocorticoid and a member of the class I cytokine family induces aromatase expression in skin and adipose tissue fibroblasts via promoter I.4 located 73 kb upstream of the coding region. Binding of STAT-3 and glucocorticoid receptor (GR) upstream of promoter I.4 mediates regulation of aromatase expression in these fibroblasts.

various hormones, growth factors, and cytokines in a tissue-specific manner. For example, extremely high baseline levels of the placental promoter I.1 activity are maintained constitutively in the syncytiotrophoblast and a consequence of decreasing levels of inhibitory transcription factors as cytotrophoblasts differentiate to a syncytiotrophoblast (Kamat et al., 1998; Jiang et al., 2000). On the other hand, extremely low baseline levels of promoter II in the ovary are stimulated strikingly by FSH via a cAMP-dependent pathway in the developing follicle (Simpson et al., 1994) (Fig. 4). Serum, cytokines, and growth factors are inhibitory to promoter II. In case of adipose and skin fibroblasts, promoter I.4 is used in vivo and activated coordinately by a glucocorticoid in the presence of a cytokine (IL-6, IL-11, leukemia inhibitory factor, and oncostatin M). Glucocorticoid receptors and the Janus kinase-1/signal transducer and activator of transcription (STAT)-3 pathway mediate this induction (Zhao et al., 1995).

Promoter use in cultured adipose tissue fibroblasts is a function of hormonal treatments. For example, in vitro studies showed that prostaglandin (PG) E_2 or cAMP analogs stimulate aromatase expression strikingly via proximally located promoters II and I.3, whereas treatment with a glucocorticoid plus a member of the class I cytokine family switches promoter use to I.4 (Zhao et al., 1995, 1996a).

D. Pathology Related to Aromatase Overexpression

Thus far, aromatase overexpression in four estrogen-responsive human diseases has been shown to be critical, since the use of aromatase inhibitors has been therapeutic in 1) breast cancer, 2) endometriosis, 3) endometrial cancer, 4)uterine fibroids, and 5) hypogonadism in aromatase excess syndrome. We will initially discuss the cellular and mechanisms responsible for aromatase overexpression in each disorder in detail. Then we will point out common mechanisms that mediate aromatase overexpression in these disorders.

Breast cancer, endometriosis, and uterine leiomyomata are highly responsive to estrogen for growth, as evidenced by high concentrations of estrogen receptors in these tissues (Bulun et al., 1997). Thus, increased estrogen formation as a consequence of aromatase expression in these tissues has primarily local (paracrine and intracrine) effects (O'Neill and Miller, 1987, 1988; James et al., 1990; Bulun et al., 1993, 1994a,b; Noble et al., 1996; Shozu et al., 2001). On the other hand, aromatase may be overexpressed in many human tissues ubiquitously in aromatase excess syndrome (Shozu et al., 2003b). This gives rise to increased circulating estrogen and both local and systemic (endocrine) effects of estrogen such as gynecomastia and hypogonadism in males and premature breast development and anovulation in females.

II. Aromatase and Breast Cancer

Paracrine interactions between malignant breast epithelial cells, proximal adipose fibroblasts, and vascular endothelial cells are responsible for estrogen biosynthesis and the lack of adipogenic differentiation in breast cancer tissue. It seems that malignant epithelial cells secrete factors that inhibit the differentiation of surrounding adipose fibroblasts to mature adipocytes and stimulate aromatase expression in these undifferentiated adipose fibroblasts (Meng et al., 2001). The in vivo presence of malignant epithelial cells also enhances aromatase expression in endothelial cells in breast tissue (Zhou et al., 2001). We developed a model in breast cancer, which reconciles the inhibition of adipogenic differentiation and estrogen biosynthesis in a positive feedback cycle (Fig. 5).

The desmoplastic reaction (formation of the dense fibroblast layer surrounding malignant epithelial cells) is essential for structural and biochemical support for tumor growth. In fact, the pathologists refer to 70% of breast carcinomas as "scirrhous"-type, indicating the rock-like consistency of these tumors (Haagensen, 1986). This consistency comes from the tightly packed undifferentiated adipose fibroblasts around malignant epithelial cells. Malignant epithelial cells achieve this by secreting large quantities of TNF and IL-11 that inhibit the differentiation of fibroblasts to mature adipocytes. Thus, large numbers of these estrogen-producing cells are maintained proximal to malignant cells (Meng et al., 2001; Zhou et al., 2001). At the same time, a separate set of factors secreted by malignant epithelial cells activates aromatase expression in surrounding adipose fibroblasts (Meng et al., 2001; Zhou et al., 2001) (Fig. 5).

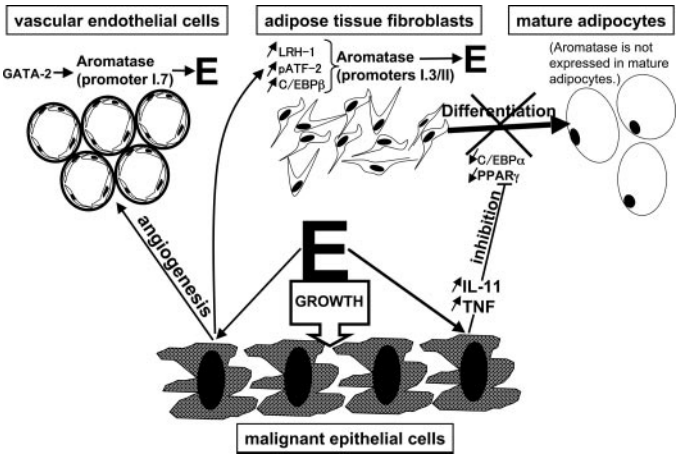


FIG. 5. Epithelial-stromal interactions in a breast tumor enhance estrogen formation, inhibition of adipogenic differentiation, and angiogenesis. Estradiol (E) up-regulates formation of antiadipogenic cytokines (TNF and IL-11) in epithelial cells. These cytokines inhibit adipogenic transcription factors C/EBPα and PPARγ to block adipogenesis and thus enhance accumulation of undifferentiated fibroblasts that express aromatase. Epithelial cells secrete PGE_2 and other factors that induce binding of the transcription factors LRH-1, phosphorylated ATF-2, and C/EBPβ to promoters I.3 and II to up-regulate aromatase expression in adipose fibroblasts. Since the aromatase promoter I.7 activity is also elevated in breast tumor tissue, we speculate that the transcription factor GATA-2 up-regulates this promoter in increased numbers of endothelial cells as a result of enhanced angiogenesis in breast cancer.

Malignant epithelial cells induce aromatase via activation of aberrant promoters in breast cancer tissue and adipose fibroblasts proximal to tumor (Fig. 6). The breast adipose tissue in disease-free women maintains

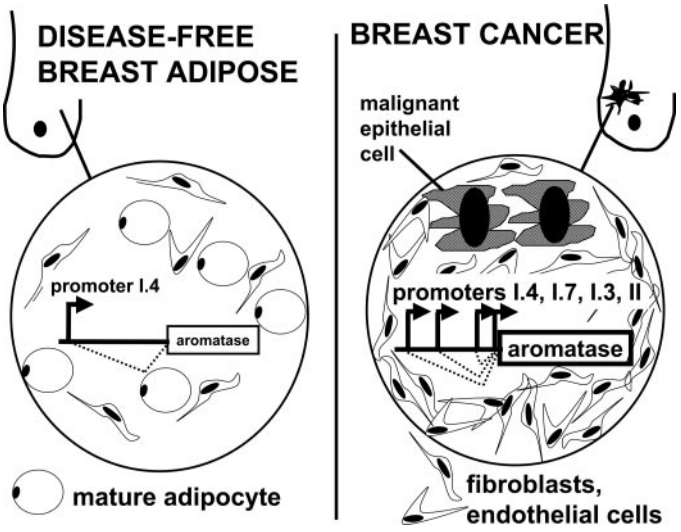


FIG. 6. Alternative promoter use for aromatase expression in normal and malignant breast tissues. The levels of total aromatase mRNA levels in breast cancer tissue are strikingly higher than normal breast tissue. The normal breast adipose tissue maintains low levels of aromatase expression primarily via promoter I.4. Promoters I.3 and II are used only minimally in normal breast adipose tissue. Promoter II and I.3 activities in the breast cancer, however, are strikingly increased. Additionally, the endothelial-type promoter I.7 is also up-regulated in breast cancer. Thus, it seems that the prototype estrogen-dependent malignancy breast cancer takes advantage of four promoters (II, I.3, I.7, and I.4) for aromatase expression. The sum of aromatase mRNA species arising from these four promoters markedly increase total aromatase mRNA levels in breast cancer compared with the normal breast.

low levels of aromatase expression primarily via promoter I.4 that lies 73 kb upstream of the common coding region (Mahendroo et al., 1993) (Fig. 6). The proximal promoters I.3 and II are used only minimally in normal breast adipose tissue (Mahendroo et al., 1993). Transcription via activity of promoters II and I.3 in the breast tumor fibroblasts and malignant epithelial cells, however, is strikingly increased (Harada et al., 1993; Agarwal et al., 1996; Utsumi et al., 1996; Zhou et al., 1996) (Fig. 6). Additionally, the endothelial-type promoter I.7 is also up-regulated in breast tumor tissue (Sebastian et al., 2002) (Fig. 6). Thus, it seems that the prototype estrogen-dependent malignancy breast cancer takes advantage of four promoters used in various cell types for aromatase expression (Fig. 6). The sum of aromatase mRNA species arising from these four promoters markedly increases total aromatase mRNA levels in breast cancer compared with the normal breast that uses almost exclusively promoter I.4 (Fig. 6).

A. Estrogen Formation in Breast Cancer

There are two sources of estrogen for breast cancer. First, estrogen that arises from extraovarian body sites such as subcutaneous adipose tissue and skin reaches breast cancer by way of circulation in an endocrine manner. Second, estrogen locally produced in breast cancer tissue makes an impact via paracrine or intracrine mechanisms.

1. Aromatase in Adipose Tissue and Skin: Endocrine Effect. The potential roles of extraovarian aromatase in human physiology and pathology were also recognized initially in the 1960s (MacDonald et al., 1968). These studies demonstrated that the conversion rate of plasma androstenedione to estrone in humans increased as a function of obesity and aging (Grodin et al., 1973; Hemsell et al., 1974) (Fig. 2). These studies also revealed the importance of extraovarian tissues (primarily adipose tissue and skin) as the origin of estrogen in postmenopausal women (Figs. 2 and 4). Extraovarian estrogen formation was shown to be correlated positively with excess body weight in both pre- and postmenopausal women and may be increased as much as 10-fold in morbidly obese postmenopausal women (Grodin et al., 1973; Hemsell et al., 1974). This elevation in association with both obesity and aging bears a striking relationship to the incidence of endometrial hyperplasia and cancer, which are more commonly observed in elderly obese women (MacDonald et al., 1978). It is now recognized that the continuous production of estrogen by the adipose tissue in these women is one of the risk factors of endometrial hyperplasia and cancer.

Evidence also suggests a role of estrogens produced by adipose tissue in the pathogenesis of the breast cancer. For example, breast cancer incidence correlates positively with the body fat content or serum estradiol levels in postmenopausal women, suggesting that estrogen collectively produced in all extraovarian sites reach the

breast tissue by circulation in an endocrine fashion and stimulate tumor growth (Huang et al., 1997; Hankinson et al., 1999). A role for adipose tissue estrogen biosynthesis in promoting the growth of breast cancer is implied because of the palliative effects of adrenalectomy in the past. Because estrone production by adipose tissue depends on plasma androstenedione secreted by the adrenal cortex as substrate, the role of adrenalectomy is explicable in terms of the denial of substrate precursor for adipose tissue estrogen biosynthesis. Today, reduction of estrogen biosynthesis in the adipose tissue is accomplished by the use of aromatase inhibitors in the treatment of breast cancer (Brodie et al., 1999a,b) (Fig. 7).

2. Local Aromatase in Breast Cancer Tissue: Paracrine/Intracrine Effect. Mechanisms giving rise to increased local concentrations of estrogen in breast cancer via aromatase overexpression within the tumor tissue have been demonstrated by a number investigators (Van Landeghem et al., 1985; Chetrite et al., 2000; Geisler et al., 2000). These studies showed strikingly increased local levels of estrone, estrone sulfate, and eastradiol in breast tumor tissue compared with circulating estrogen levels (Van Landeghem et al., 1985; Chetrite et al., 2000; Geisler et al., 2000).

A series of paracrine interactions between malignant breast epithelial cells and surrounding adipose stroma were uncovered and explained increased local estrogen levels in the breast bearing a cancer. For example, independent studies from at least six different laboratories indicated striking increases in aromatase enzyme activity and mRNA levels in breast fat adjacent to cancer compared with those in distal fat or disease-free breast adipose tissue (O'Neill et al., 1988; Bulun et al., 1993; Harada et al., 1993; Reed et al., 1993; Sasano et al., 1994; Utsumi et al., 1996; Zhou et al., 1996) (Fig. 7). We also found that an elevation in aromatase expression in adipose stroma surrounding malignant epithelial cells is regulated by complex cellular, molecular, and genomic mechanisms (Bulun et al., 1993; Agarwal et al., 1996; Zhou et al., 2001). Interestingly, the overall aromatase expression in breast adipose tissue in mastectomy specimens bearing a breast tumor was significantly higher than that in benign breast tissue removed for reduction mammoplasty (Agarwal et al., 1995, 1996, 1997).

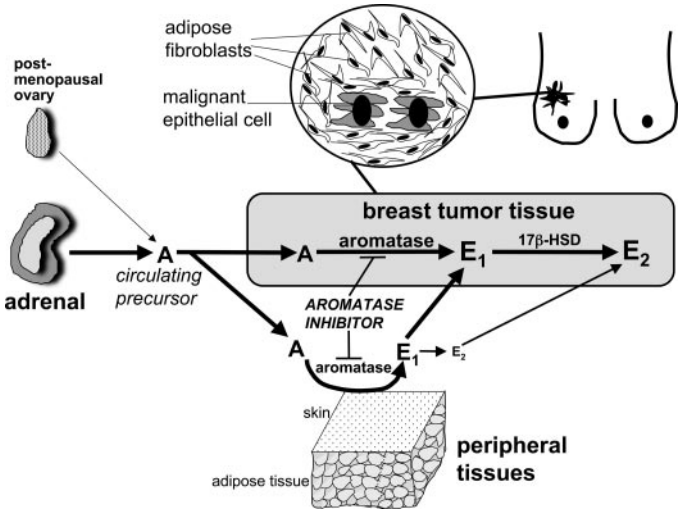


FIG. 7. Tissue sources of estrogen in postmenopausal breast cancer. This figure exemplifies the important pathologic roles of extraovarian (peripheral) and local estrogen biosynthesis in an estrogen-dependent disease in postmenopausal women. The estrogen precursor androstenedione (A) originates primarily from the adrenal in the postmenopausal woman. Aromatase expression and enzyme activity in extraovarian tissues such as fat increases with advancing age. The aromatase activity in skin and subcutaneous adipose fibroblasts gives rise to formation of systemically available estrone (E_1) and to a smaller extent estradiol (E_2). The conversion of circulating A to E_1 in undifferentiated breast adipose fibroblasts compacted around malignant epithelial cells and subsequent conversion of E_1 to E_2 in malignant epithelial cells provide high tissue concentrations of E_2 for tumor growth. The clinical relevance of these findings is exemplified by the successful use of aromatase inhibitors to treat breast cancer.

Estrogens can act both directly or indirectly on human breast cancer cells to promote proliferation. Breast cancer cells in culture elaborate a number of growth stimulants in response to estrogen, which can act in an autocrine and paracrine manner to stimulate their growth; however, there is also evidence that estrogens can directly induce proliferation of breast cancer cells. The pathologic significance of local aromatase activity in breast cancer was recognized based on the following in vitro data. MCF7 breast cancer cells, which were stably transfected to express an mouse mammary tumor virus-promoter-driven human aromatase cDNA and inoculated into oophorectomized nude mice, remained dependent on circulating androstenedione for their rapid growth (Yue et al., 1994). Further evidence for the importance of local aromatase expression in the breast tissue came from an in vivo mouse model demonstrating that aromatase overexpression in breast tissue is sufficient for maintaining hyperplasia in the absence of circulating estrogen and that aromatase inhibitors abrogated hyperplasia (Tekmal et al., 1999). These transgenic mice with mouse mammary tumor virus-promoter-driven local aromatase in breast tissue are more prone for breast cancer development (Kovacic et al., 2004).

B. Cellular Mechanisms That Regulate Aromatase Expression in Normal and Malignant Breast Tissue

1. Cellular Localization of Aromatase in Breast Cancer. Breast adipose tissue is primarily composed of mature lipid-containing cells and other stromal elements. This latter group of cells in the breast adipose tissue was characterized using immunohistochemical methods (Price et al., 1992). Ninety percent of these resident cells of adipose tissue are fibroblasts, i.e., the potential precursors of mature adipocytes, and another 7% represented endothelial cells. Most (80–90%) aromatase transcripts in adipose tissue was demonstrated to reside in fibroblasts compared with mature adipocytes (Price et

al., 1992). Moreover, aromatase enzyme activity was found to reside primarily in the fibroblast component of the adipose tissue in a previous study from the same laboratory (Ackerman et al., 1981).

Immunoreactive aromatase was localized to both the malignant epithelial cells and surrounding fibroblasts in breast tumor tissues (Santen et al., 1994; Sasano et al., 1996; Brodie et al., 1998). Different antibodies, however, showed variable affinity to malignant epithelial cells versus fibroblasts (Sasano et al., 2003). Immunoreactive aromatase was also observed in endothelial cells in normal breast tissue and breast tumors. Recently published data using RNA from laser-captured breast tumor cells showed aromatase mRNA in both stromal and malignant epithelial cells in tumor tissues from three patients (Sasano et al., 2003). Markedly high levels of aromatase enzyme activity have been consistently detected in breast adipose fibroblasts freshly isolated from breast tissue with or without cancer (Ackerman et al., 1981; Price et al., 1992). Aromatase enzyme activity in malignant breast epithelial cells, on the other hand, is either undetectable or extremely low (Pauley et al., 2000).

Adjacent adipose tissue including the dense fibroblast layer seems to account for most aromatase expression in breast tumors for the following reasons. First, the quantity of adjacent adipose tissue surrounding a clinically detectable breast tumor is comparatively very large; e.g., the volume of adipose tissue within a 1-inch radius of a 1- ml breast tumor is 129 ml. Second, the most intense aromatase immunostaining was observed in the adipose tissue fibroblasts located in and around the fibrous capsule (i.e., desmoplastic reaction) surrounding malignant cells (Sasano et al., 1994). Third, levels of aromatase expression and activity in fibroblasts isolated from breast adipose tissue or tumor are 10 to 15 times higher than those found in malignant epithelial cells or cell lines (Pauley et al., 2000).

2. Inhibition of Adipogenic Differentiation in Breast Cancer: Link to Aromatase Overexpression. Extraordinarily large quantities of TNF and IL-11 are produced and secreted by malignant breast epithelial cells (Meng et al., 2001) (Fig. 8). These two cytokines mediate one of the most commonly observed biological phenomena in breast tumors, the desmoplastic reaction. Desmoplastic reaction or accumulation of fibroblasts around malignant epithelial cells serves to maintain the strikingly hard consistency in many of these tumors (i.e., the traditional macroscopic description of malignant breast tumors as "scirrhous cancer") and increased local concentrations of estrogen via aromatase overexpression localized to these undifferentiated fibroblasts. The inhibition of differentiation of fibroblasts to mature adipocytes mediated by TNF and IL-11 is the key event responsible for desmoplastic reaction, because neither malignant cell-conditioned

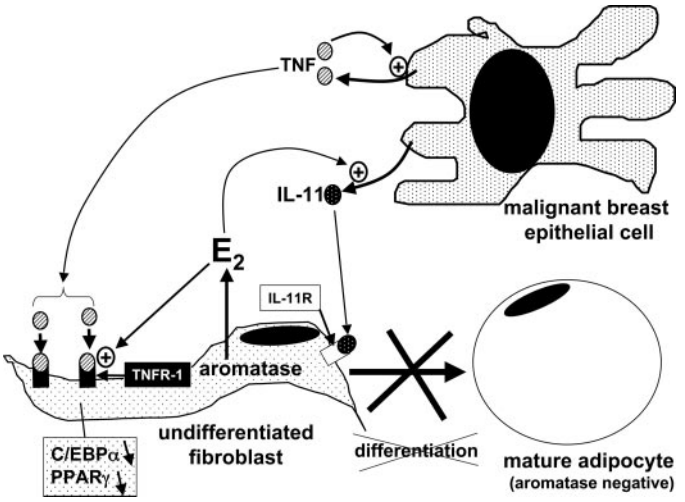


FIG. 8. Detail of epithelial-stromal interaction via estrogen and cytokines in breast cancer. Estradiol (E_2) increases secretion of antiadipogenic cytokines (IL-11) from malignant epithelial cells and up-regulates their antiadipogenic-type receptors (TNFR1) in undifferentiated fibroblasts. These redundant mechanisms give rise to accumulation of undifferentiated fibroblasts around malignant epithelial cells (desmoplastic reaction), which express aromatase and form E_2.

media nor these cytokines caused the proliferation of adipose tissue fibroblasts (Meng et al., 2001). Moreover, blocking both TNF and IL-11 in cancer cell-conditioned media using neutralizing antibodies is sufficient to reverse this antidifferentiative effect of cancer cells completely (Fig. 8) (Meng et al., 2001). In summary, desmoplastic reaction primarily occurs via the action of cytokines (TNF and IL-11) secreted by the malignant epithelial cells to inhibit the differentiation of adipose tissue fibroblasts to mature adipocytes. This tumor-induced block in adipocyte differentiation is mediated by the selective inhibition of expression of the essential adipogenic transcription factors C/EBPα and PPARγ (Meng et al., 2001) (Fig. 8).

Estrogen per se seems to potentiate this antiadipogenic action via indirect mechanisms. For example, treatment of T47D breast cancer cells with estradiol increased the mRNA levels of IL-11 by 3-fold (Crichton et al., 1996). Moreover, the cellular actions of TNF are mediated by two distinct receptors: TNF receptor type 1 (TNFR1) (also known as p60 in humans) and TNFR2 (p80). TNFR1 but not TNFR2 was found to be responsible for the inhibition of adipocyte differentiation using mutants of TNF specific for the stimulation of either receptor type (Hube and Hauner, 2000). We recently demonstrated that TNFR1 is responsible for inhibition of adipocyte differentiation in breast cancer (Deb et al., 2004). Interestingly, estradiol enhances this antiadipogenic effect by inducing TNFR1 levels in adipose fibroblasts (Deb et al., 2004) (Fig. 8).

Thus, large amounts of antiadipogenic cytokines (e.g., TNF and IL-11) secreted by malignant epithelial cells serve to maintain increased numbers of the aromatase-

expressing cell type, i.e., undifferentiated adipose fibroblast, in breast tumor tissue. This is further enhanced by stimulatory effects of estrogen on IL-11 production in cancer cells and on the TNF receptor type that mediates adipogenic inhibition (Fig. 8).

C. Molecular Mechanisms Responsible for Elevated Aromatase Expression in Breast Cancer

Alternative promoter use is a major mechanism that mediates increased aromatase expression in breast cancer. The normal breast adipose tissue maintains low levels of aromatase expression primarily via promoter I.4 that lies 73 kb upstream of the common coding region (Fig. 6). The proximally located promoters I.3 and II are used only minimally in normal breast adipose tissue. Promoter II and I.3 activities in the breast cancer, however, are strikingly increased (Harada et al., 1993; Agarwal et al., 1996; Utsumi et al., 1996; Zhou et al., 1996). Additionally, the endothelial-type promoter I.7 is also up-regulated in breast cancer (Sebastian et al., 2002). Thus, it seems that the prototype estrogen-dependent malignancy breast cancer takes advantage of four promoters (II, I.3, I.7, and I.4) for aromatase expression (Fig. 6). The sum of aromatase mRNA species arising from these four promoters markedly increases total aromatase mRNA levels in breast cancer compared with the normal breast that uses almost exclusively promoter I.4 (Fig. 6).

1. Up-Regulation of Promoters I.3 and II. Using an in vivo approach, we and two other groups demonstrated by quantitative exon-specific RT-PCR that the use of the proximal promoters II/I.3 are strikingly up-regulated in adipose tissue adjacent to breast cancer and in breast cancer tissue per se (Agarwal et al., 1996; Utsumi et al., 1996; Zhou et al., 1996). As noted earlier, promoters II and I.3 are located within 215 bp from each other and are coordinately induced by cAMP-dependent or -independent mechanisms in adipose fibroblasts in breast tumors. These promoters possibly share common regulatory DNA motifs.

Increased promoter I.3/II activity is, in part, the basis for increased aromatase expression in peri- and intratumoral adipose tissue fibroblasts (Fig. 6) (Agarwal et al., 1996). Over the past several years, we and others sought to elucidate the mechanisms underlying this cancer-induced increase in promoters I.3/II activity in adipose tissue fibroblasts.

There are two experimental models, which are not mutually exclusive, for the regulation of promoters I.3 and II in breast adipose fibroblasts in tumor tissue. In the first model, PGE_2 induces aromatase via promoters I.3 and II employing both cAMP/protein kinase A- and protein kinase C-dependent pathways (Zhao et al., 1996a). In this model, PGE_2 stimulates binding activity of an orphan nuclear receptor, LRH-1, to a nuclear receptor half-site (−136/−124 bp) upstream of promoter II (Clyne et al., 2002; Karuppu et al., 2002). Treatment with PGE_2 strikingly increased both LRH-1 expression and its binding activity to the aromatase promoter II in cultured adipose fibroblasts. LRH-1 overexpression significantly increased aromatase promoter II activity and aromatase enzyme activity in cultured adipose fibroblasts (Fig. 9). From an in vivo perspective, LRH-1 was up-regulated in undifferentiated fibroblasts in breast tumor tissue compared with those in disease-free breast tissue (C. Clyne, personal communication). It was suggested that a corepressor of LRH-1, short heterodimer partner (SHP), may inhibit aromatase expression in fibroblasts of normal breast tissue (Kovacic et al., 2004) (Fig. 9). The recently reported increases in cyclooxygenase (COX)-2 expression and beneficial effects of nonsteroidal anti-inflammatory in breast cancer support this model (Brueggemeier et al., 1999; Richards et al., 2002). In vivo evidence for increased PGE_2 formation in breast cancer, however, is still lacking at this time.

In an alternative experimental model, we found that conditioned medium from malignant breast epithelial cells (MCF7 or T47D) markedly induces aromatase expression in adipose tissue fibroblasts via promoters I.3 and II (Zhou et al., 2001) (Fig. 9). We hypothesize that a hormonal cocktail secreted from malignant epithelial cells induces aromatase in undifferentiated adipose fibroblasts via redundant pathways. For example, the addition of a COX-2 inhibitor or an adenylyl cyclase inhibitor or mutation of the LRH-1 binding site of the aromatase promoter II does not reverse induction of aromatase expression by malignant epithelial cell-conditioned medium. We isolated two *cis*-acting elements

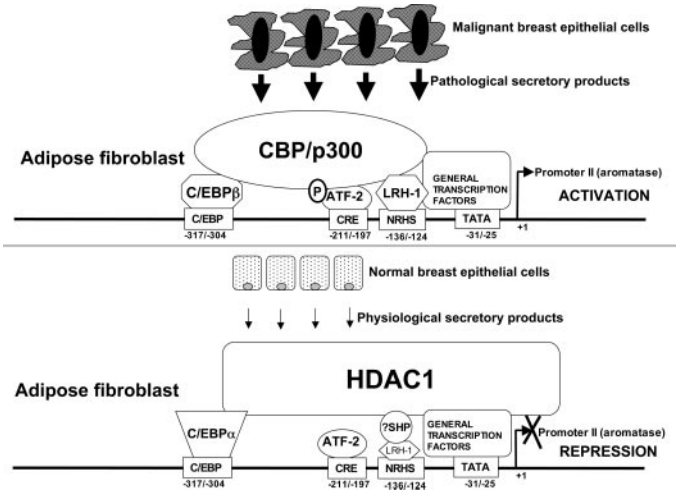


FIG. 9. Differential binding of stimulatory or inhibitory multimeric complexes to the aromatase promoter II regulatory region in malignant versus benign breast tissue. Conditioned media from malignant epithelial cells give rise to activation of aromatase promoter II in adipose fibroblasts. This is accompanied by binding of LRH-1, phosphorylated ATF-2, C/EBPβ, and a large cointegrator named CREB-binding protein or its homolog p300 (p300/CBP) to the promoter II regulatory region. Upon treatment of adipose fibroblasts with medium from normal breast epithelial cells, this promoter remains quiescent, and the same *cis*-acting elements are occupied by factors that are inhibitory (C/EBPα), not phosphorylated (ATF-2) or interacting with corepressors (SHP) and inhibitory cointegrators (HDAC1).

that stimulated promoter I.3/II use in response to cancer cell-conditioned medium (Zhou et al., 2001). Two critical elements were determined as a C/EBP site (−317/−304) and a CRE (−211/−197), since mutation of either element abolished conditioned medium-induced promoter activity (Fig. 9). Malignant epithelial cell-conditioned medium strikingly induced the expression of C/EBPβ, which binds to the C/EBP site in promoter I.3/II region and increases its activity (Zhou et al., 2001) (Fig. 9). In contrast, promoter I.3 and II are inhibited by transcription factors C/EBPα and δ that bind to the same site in adipose fibroblasts treated with benign epithelial cell conditioned medium.

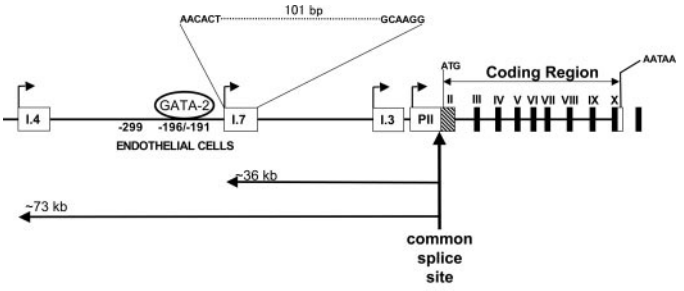
Malignant epithelial cell-conditioned medium also induced phosphorylation of ATF-2 and its binding to the CRE (Fig. 9). This CRE is occupied by nonphosphorylated ATF-2 in fibroblasts treated with benign epithelial cell-conditioned medium associated with inactivation of promoters I.3 and II. Moreover, chromatin immunoprecipitation PCR showed that the activator transcriptional complex in a malignant environment contains C/EBPβ, phosphorylated ATF-2, and p300/cAMP response element-binding protein-binding protein (CBP), whereas the inactivator complex (benign environment) contained nonphosphorylated ATF-2 and histone deacetylase-1 (HDAC1) (Fig. 9). Although this second model reflects in vivo conditions, the individual factors in malignant cell-conditioned medium, which contribute to up-regulation of aromatase, are not currently known. Cytokines in malignant cell-conditioned medium probably do not account for this up-regulation, since the TNF and IL-6 group of cytokines selectively induce only promoter I.4, which is not up-regulated either in vivo or in vitro in breast tumors (Zhao et al., 1995, 1996b; Agarwal et al., 1996; Zhou et al., 2001).

A unified model for promoter II/I.3 activation in breast cancer therefore predicts that malignant epithelial cells secrete a number of factors including PGE_2 (Fig. 9). These factors induce a number of signaling pathways in a redundant fashion to activate the transcription of the aromatase gene via promoter I.3/II in adipose fibroblasts. PGE_2 possibly arising from malignant epithelial cells is a candidate factor for activation of promoters I.3 and II in breast cancer. This, however, has not been demonstrated in vivo. Neither PGE_2 nor its downstream regulators cAMP or LRH-1-binding site in promoter II were found to be essential for activation of promoters II in adipose fibroblasts treated with malignant cell-conditioned medium (Zhou et al., 2001). In disease-free breast tissue, the incorporation of a number of transcriptional repressors into the multimeric complex that occupies promoter I.3/II region is associated with inhibition of transcription. Malignant epithelial cell conditioned medium, on the other hand, gives rise to replacement of this inhibitory complex by an activator transcriptional complex composed of distinct factors such as phosphorylated ATF-2, C/EBPβ, p300/CBP, and possibly LRH-1 (our unpublished observations) (Fig. 9).

In summary, the proximal promoters II and I.3 clustered within a 215-bp region are coordinately regulated. They remain quiescent in fibroblasts of normal breast tissue via redundant binding of multiple transcriptional inhibitors (Fig. 9). In a malignant breast environment, however, these promoter regions are occupied by multiple transcriptional enhancers as a result of activation of multiple signaling pathways in a fail-safe fashion to increase aromatase expression in breast fibroblasts (Fig. 9).

2. Regulation of Promoters II and I.3 in MCF7 Cells. Chen et al. (2002) studied the regulation of a number of gene reporter constructs of the promoter II/I.3 region. They found that estrogen receptor-related α-1 and CREB1 up-regulate and v-erbA-related factor-2, chicken ovalbumin upstream transcription factor (COUP-TF) inhibitor, retinoic acid receptor γ, Snail, and Slug proteins down-regulate this promoter region (Chen et al., 2002). The in vivo relevance of these findings will become clearer in the future once the relative significance of aromatase enzyme activity and estrogen biosynthesis is demonstrated in malignant epithelial cells.

3. Up-Regulation of Promoter I.7 in Breast Cancer. Studies summarized above employed exon-specific RT-PCR analysis of 5′-untranslated ends of aromatase mRNA in breast cancer tissues. This limited strategy permitted only the detection of promoters previously identified from healthy tissues (Agarwal et al., 1996; Utsumi et al., 1996; Zhou et al., 1996). Discovery-driven approaches designed to identify novel promoter regions in breast cancer or adjacent adipose tissues, however, have not been published until recently. To identify novel promoter regions in cancer tissues and proximal fat, we employed the 5′-rapid amplification of cDNA ends (RACE) procedure using total RNA isolated from breast cancer and proximal adipose tissue samples. We cloned a novel 101-bp untranslated first exon (I.7) that comprises the 5′-end of 29 to 54% of aromatase mRNA isolated from breast cancer tissues (Sebastian et al., 2002) (Fig. 10). The levels of aromatase mRNA with exon I.7 were significantly increased in breast cancer tissues and adipose tissue adjacent to tumors (Fig. 10). We identified a promoter immediately upstream of exon I.7 and mapped this to about 36 kb upstream of the ATG translation start site of the aromatase gene (Sebastian et al., 2002) (Fig. 10). Promoter I.7 is a TATA-less promoter containing *cis*-regulatory elements found in megakaryocytic and endothelial type promoters (Fig. 10). Maximal promoter activity could be demonstrated in human microvascular endothelial cells (Fig. 10). Binding of the transcription factor GATA-2 to a specific GATA *cis*-regulatory element in this promoter was critical for its regulation in endothelial cells (Sebastian et al., 2002). In conclusion, promoter I.7 is a GATA-2-regulated endothelial-type promoter of the human aro-



DISTRIBUTION OF P450arom PROMOTERS IN BREAST CANCER SAMPLES (n=5):

Number of 5′-RACE Clones sequenced	I.7	PII	I.3	I.4	Total
Breast Cancer 1	4	3	4	3	14
Normal Breast				10	10

5′-RACE/ Colony hybridization	Percentage of I.7-specific clones
Breast Cancer 2	39%
Breast Cancer 3	42%
Breast Cancer 4	42%
Breast Cancer 5	54%

FIG. 10. Promoter I.7. A novel first exon (5′-UTR) of aromatase mRNA species was originally cloned from human breast cancer tissues. This 5′-UTR was mapped to 36 kb upstream of the translation start site. We determined the distribution of I.7-specific mRNA in 5′-RACE libraries in breast cancer specimens. This suggested that I.7 that is a TATA-less promoter directed 29 to 54% of aromatase mRNA species in breast cancer. The −299/−35 region confers maximum basal activity in endothelial cells. This regulatory region contains at least three critical endothelial-type motifs, including *Ets*, GATA, and E47. The binding of GATA-2 protein to the −196/−191-bp site is important for baseline promoter activity in microendothelial cells.

matase gene and may increase estrogen biosynthesis in vascular endothelial cells of breast cancer. The activity of this promoter may also be important for intracrine and paracrine effects of estrogen on blood vessel physiology (Fig. 10).

D. Summary of Regulation of Aromatase Expression in Breast Cancer

Several alternative cellular and molecular mechanisms serve to maintain excessive levels of aromatase activity in breast stroma proximal to malignant epithelial cells. First, malignant epithelial cell-derived factors induce aromatase overexpression via the transcription factors LRH-1, C/EBPβ, and phosphorylated ATF-2 (Fig. 5). These factors are incorporated into a multimeric transcriptional complex that occupies the aromatase promoter I.3/II region in adipose tissue fibroblasts adjacent to epithelial cells. Second, aromatase is overexpressed in vascular endothelial cells of tumor tissue via binding of GATA-2 and other endothelial-type transcription factors to promoter I.7. These factors may also mediate angiogenesis in tumor tissue. Moreover, estrogen is known to induce the angiogenic factor VEGF in cancer cells (Nakamura et al., 1996, 1999; Ruohola et al., 1999; Shekhar et al., 2000) (Fig. 5). Third, we demonstrated recently that antiadipogenic cytokines IL-11 and TNF secreted by malignant epithelial cells block the differentiation of the aromatase-expressing cells (fibroblasts) to mature adipocytes that do not express aromatase (Fig. 5). These cytokines thereby secreted abundantly by malignant epithelial cells serve to maintain a dense layer of aromatase-expressing fibroblasts proximal to malignant epithelial cells to provide structural and hormonal support. Fourth, the expression of IL-11 in malignant epithelial cells and antiadipogenic-type TNF receptors in adjacent adipose tissue fibroblasts are up-regulated by estrogen produced as a consequence of elevated aromatase activity in breast tumors. This positive feedback involving complex epithelial-stromal interactions favor higher numbers of undifferentiated fibroblasts, angiogenesis, and increased local estrogen concentrations in breast tumors (Fig. 5). These four mechanisms interact to maintain high levels of estrogen production in a breast tumor.

E. Aromatase Inhibitors in the Treatment of Breast Cancer

Today, aromatase inhibitors are the most effective endocrine treatment of estrogen-responsive breast cancer (Santen, 2002) (Fig. 7). Six recent head-to-head randomized clinical trials published since 2000 demonstrated the superiority of aromatase inhibitors to tamoxifen in the treatment of breast cancer (Bonneterre et al., 2000; Mouridsen et al., 2001; Baum et al., 2002, 2003; Goss et al., 2003; Milla-Santos et al., 2003; Paridaens et al., 2003; Buzdar et al., 2004). Long-term side effect profiles of these agents will determine whether aromatase inhibitors will replace tamoxifen or other selective estrogen receptor modulators in the long run.

There are two intriguing implications of these results. First, it is pharmacologically more efficacious to block estrogen formation rather than its action at least by currently approved estrogen antagonists or selective estrogen receptor modulators. Second, the local effect of aromatase inhibitors at the target tissue level to block local estrogen formation possibly represents the most critical mechanism for the superior therapeutic potential of aromatase inhibitors (Fig. 7).

Targeting aromatase in breast cancer as a therapeutic strategy was first conceptualized in the 1960s (Santen, 2002). Aminoglutethimide was the first aromatase inhibitor tested for this purpose. Although the first-generation aromatase inhibitor aminoglutethimide was as efficacious as tamoxifen in the treatment of breast cancer, its adverse side effects precluded widespread use (Santen, 2002). Tamoxifen was introduced in the 1970s and became the gold standard for hormonal treatment of breast cancer (Santen, 2002). Second-generation aromatase inhibitors were tested in Europe in the 1980s and were found to be as efficacious as tamoxifen (Santen, 2002). Finally, the third-generation aromatase inhibitors were approved in the United States to treat postmenopausal breast cancer in the 1990s and proven to be superior to tamoxifen (Lu et al., 1998; Bonneterre et al., 2000; Dixon et al., 2000; Mouridsen et al., 2001; Baum et al., 2002, 2003; Goss et al., 2003; Milla-Santos et al., 2003; Paridaens et al., 2003; Buzdar et al., 2004). These new inhibitors have a benign side-effect profile

and suppress estrogen production in extraovarian tissues and within the breast cancer tissue itself. This effectively blocks estrogenic action, reduces recurrences, and prolongs disease-free survival in postmenopausal women with breast cancer (Baum et al., 2002, 2003). Aromatase inhibitors are also effective in the treatment of breast cancer that becomes resistant to treatment with tamoxifen (Goss et al., 2003).

In these studies, tumors that express ER were more responsive to aromatase inhibitors compared with the tumors with an unknown receptor status (Baum et al., 2002, 2003). Future studies are required to determine whether aromatase inhibitors might be beneficial in ER-negative tumors via ER-independent mechanisms.

III. Aromatase and Endometriosis

Endometriosis is an estrogen-dependent disease that affects 6 to 10% of American women of reproductive age (approximately 4–6 million) and is the most common cause of chronic pelvic pain (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 is 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 is a leading cause of hysterectomy. Only 50% of women with endometriosis achieve pain relief in response to existing hormonal treatments or conservative surgery (Vercellini et al., 1997). Thus, there is a clear need to develop novel and effective therapies for endometriosis.

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 in the treatment of endometriosis successfully underscored the presence of aromatase in endometriotic tissue (Takayama et al., 1998; Ailawadi et al., 2004). These recent results suggested that aromatase inhibitors might treat endometriosis more effectively than GnRH analogs via suppression of local estrogen formation in endometriotic tissue. Thus, as in breast cancer, aromatase is a critical therapeutic target in endometriosis.

A. Mechanisms of Growth and Inflammation in Endometriosis

Two basic pathologic processes, growth and inflammation, are responsible for chronic pelvic pain and infertility, which are the primary devastating symptoms of endometriosis. Estrogen, growth factors, and metalloproteinases enhance the growth and invasion of endometriotic tissue, whereas prostaglandins 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 favors expression of key steroidogenic genes, most notably StAR and aromatase, expression of COX-2, and continuous local production of estradiol and PGE_2 in endometriotic tissue (Noble et al., 1997; Tsai et al., 2001; Sun et al., 2003) (Fig. 11). We find that the aberrantly expressed transcription factor steroidogenic factor-1 (SF-1) mediates PGE_2-cAMP-dependent coactivation of multiple steroidogenic genes, most notably StAR and aromatase, in endometriosis (our unpublished observations) (Zeitoun et al., 1999).

B. Definitions of Experimental Models and Abnormal Tissues in Women with Endometriosis

We and others demonstrated a number of molecular abnormalities in endometriosis (Fig. 12). The prototype

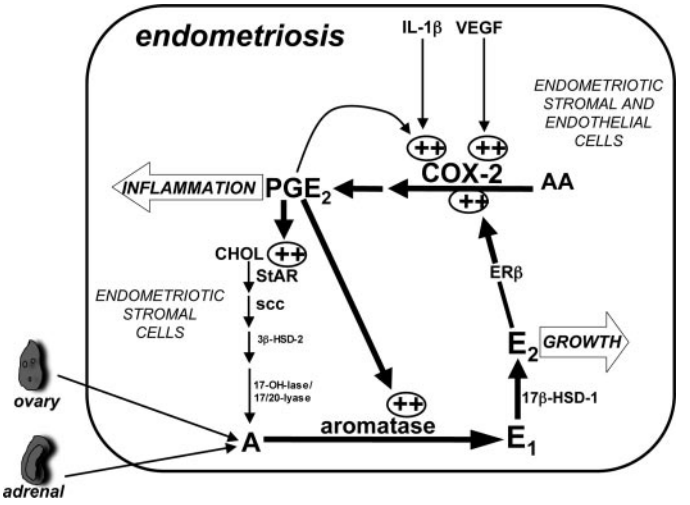


FIG. 11. A positive-feedback cycle for estrogen and prostaglandin formation. Two basic pathologic processes, growth and inflammation, are responsible for chronic pelvic pain and infertility, which are the primary devastating symptoms of endometriosis. Estrogen enhances the growth and invasion of endometriotic tissue, whereas prostaglandins and cytokines mediate pain, inflammation, and infertility. Estradiol is produced locally in the endometriotic tissue in both ovulatory and postmenopausal women. The precursor androstenedione (A) of ovarian, adrenal, or local origin becomes converted to estrone (E_1), which is in turn reduced to estradiol (E_2) in the peripheral tissues and endometriotic implants. Endometriotic tissue is capable of synthesizing androstenedione from cholesterol via the activity of StAR and other steroidogenic enzymes (SCC, 3β-HSD-2, and 17-hydroxylase-17-20-lyase) present in endometriosis. We also demonstrated significant levels of 17β-HSD-1 (reductase) expression in endometriosis, which catalyzes the conversion of estrone to estradiol. Estradiol directly induces COX-2, which gives rise to elevated concentrations of PGE_2 in endometriosis. IL-1β, VEGF, and PGE_2 itself are also potent inducers of COX-2. PGE_2 in turn is the most potent known stimulator of StAR and aromatase in endometriotic stromal cells. This establishes a positive-feedback loop in favor of continuous estrogen and prostaglandin formation in endometriosis.

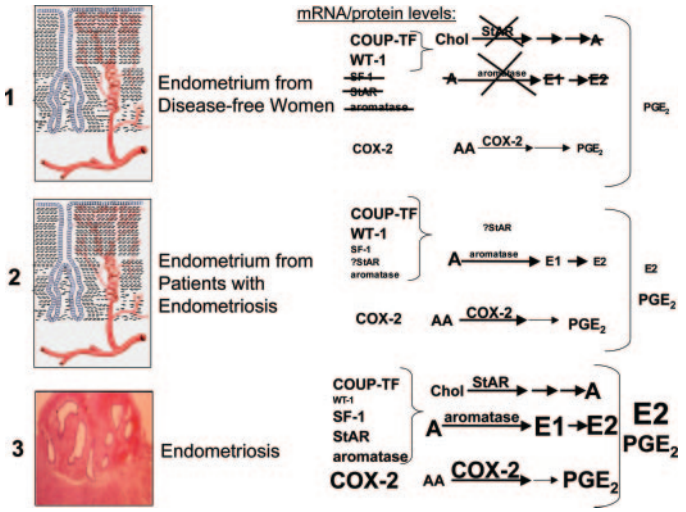


FIG. 12. Molecular abnormalities in endometriosis. In the endometrium of disease-free women, StAR and aromatase are not expressed because the stimulatory transcription factor SF-1 is absent, and inhibitory factors COUP-TF and WT-1 are present. Relatively low levels of COX-2 are detected that give rise to some PGE_2 production. In the endometrium of patients with endometriosis, very small quantities of SF-1 and aromatase are detectable and give rise to low levels of estrogen production, whereas COX-2 expression and PGE_2 formation are markedly elevated. In the ectopic lesions (endometriotic tissue), elevated levels of SF-1, StAR, and aromatase are the basis of significant androstenedione (A), estrone (E_1), and estradiol (E_2) formation. High COX-2 expression enhances formation of large amounts of PGE_2. AA, arachidonic acid.

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; Noble et al., 1997; Tsai et al., 2001; Gurates et al., 2002; Sun et al., 2003) (Fig. 12). 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 seems 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, which is important for PGE_2 synthesis, is increased markedly in both eutopic endometrium and endometriotic tissue of women with endometriosis (Ota et al., 2001; Wu et al., 2004) (Fig. 12).

We define herein the terminology to be used in reference to the tissues and cells that are used as model systems. 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 lines 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 gene expression in endometrial and endometriotic tissues (Fig. 12). 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. Finally, we went back to human tissues and verified these novel molecular mechanisms by demonstrating altered levels of transcription factors in vivo.

C. Estrogen Formation in Endometriosis

The biologically active estrogen estradiol is produced from cholesterol through six serial enzymatic conversions in two ovarian cell types that cooperate 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 (Figs. 1 and 11). We and others recently showed that StAR, aromatase, and all other steroidogenic enzymes are expressed in vivo in endometriosis, enabling this tissue to synthesize estradiol from cholesterol de novo (Bulun et al., 2001; Tsai et al., 2001; our unpublished data). Additionally, PGE_2 induces expression of the steroidogenic genes StAR, side-chain cleavage (P450scc), 3β-hydroxysteroid dehydrogenase (HSD) type 2, 17-hydroxylase/17-20-lyase (P450c17), and aromatase (P450arom) in endometriotic stromal cells (Figs. 11 and 12). The PGE_2-dependent inductions of StAR and aromatase mRNA levels were the highest compared with other steroidogenic genes (our unpublished observations). In contrast, normal endometrium does not biosynthesize estradiol. Both endometriosis and normal endometrium contain the enzyme 17β-HSD-1 that catalyzes the final step, which is the conversion of estrone to estradiol (Fig. 11). What separates normal endometrium from endometriosis, however, is the in vivo lack of StAR and aromatase (Fig. 12). Physiologically significant levels of these gene products are not detected in normal endometrial tissue or PGE_2-stimulated endometrial stromal cells (our unpublished observations).

Until recently, we focused our investigation 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, implies that estrogen is synthesized de novo from cholesterol and that endometriotic aromatase is not solely dependent for substrate on adrenal or ovarian secretion. These new findings revised our view of the pathogenesis of estrogen biosynthesis in endometriosis (Figs. 11 and 12).

D. Cellular Mechanisms That Regulate Aromatase Expression in Eutopic Endometrium and Endometriosis

Both StAR and aromatase are expressed primarily in the stromal cells of endometriosis (Fig. 13). This has been supported by a number of 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; our unpublished observations). Abundant StAR activity and aromatase enzyme activity are present in stromal cells isolated and cultured from ovarian endometriotic cysts (endometriomas) (Noble et al., 1997; Zeitoun et al., 1999; Tsai et al., 2001; Sun et al., 2003) (Fig. 13). StAR and aromatase mRNAs are present only in the 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 analog stimulates StAR mRNA and protein levels and aromatase mRNA in endometriotic stromal cells in a time- and concentration-dependent fashion (Tsai et al., 2001) (Fig. 13). PGE_2 stimulates the production of progesterone, 17-hyroxyprogesterone, androstenedione, and estradiol primarily via induction of StAR and aromatase in endometriotic cells (Noble et al., 1997; Tsai et al., 2001) (Fig. 13).

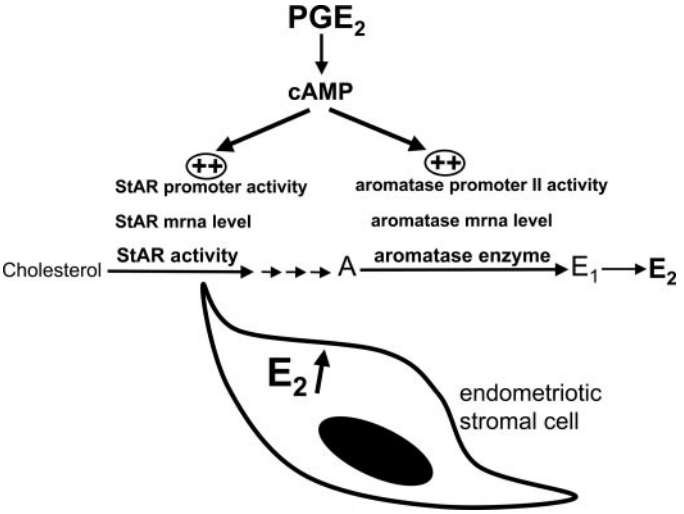


FIG. 13. Effects of PGE_2 on StAR and aromatase expression in endometriotic stromal cells.

StAR or aromatase expression or steroid hormone production is virtually undetectable in eutopic endometrial stromal cells from disease-free women (Fig. 12). High number of cycles of PCR may amplify aromatase mRNA in endometrial biopsies, but we could not detect aromatase enzyme activity in cultured endometrial stromal cells of disease-free women (Noble et al., 1997) (Fig. 12). Aromatase activity or mRNA could not be induced by PGE_2 or cAMP analogs in stromal cells from disease-free women (Noble et al., 1997).

On the other hand, 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) (Fig. 12). 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, prostaglandins, cytokines, and decreased production of molecules important for differentiation and implantation (Lessey et al., 1994; Tseng et al., 1996; Kao et al., 2003; Wu et al., 2004).

E. Prostaglandin E_2 Biosynthesis and Action in Endometriosis

1. Prostaglandin E_2 Biosynthesis in Endometrial Disease. PGE_2 plays a key role in the estrogen biosynthetic pathway in endometriotic tissue because it is a potent stimulator of 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 cyclooxygenase catalyzes the conversion of arachidonic acid to PGG_2, which is then converted to PGE_2 by the enzyme PGE_2 synthase. Two distinct genes, referred to as COX-1 and COX-2, encode the cyclooxygenase enzyme. COX-2 is the inducible gene that is regulated by a whole host of factors (Figs. 14 and 15). 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).

The first line of evidence for a positive-feedback link between the productions of PGE_2 and estradiol comes from breast tissue. 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 estradiol in breast cancer cells and by

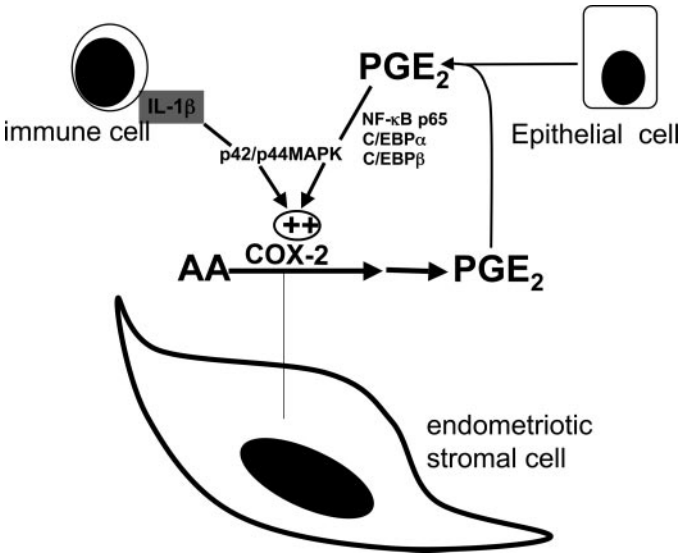


FIG. 14. Effects of PGE_2 and IL-1β on COX-2 expression in endometrial and endometriotic stromal cells. PGE_2 or IL-1β induces COX-2 expression in endometrial and endometriotic stromal cells primarily via a p42/p44 mitogen-activated protein kinase-dependent pathway.

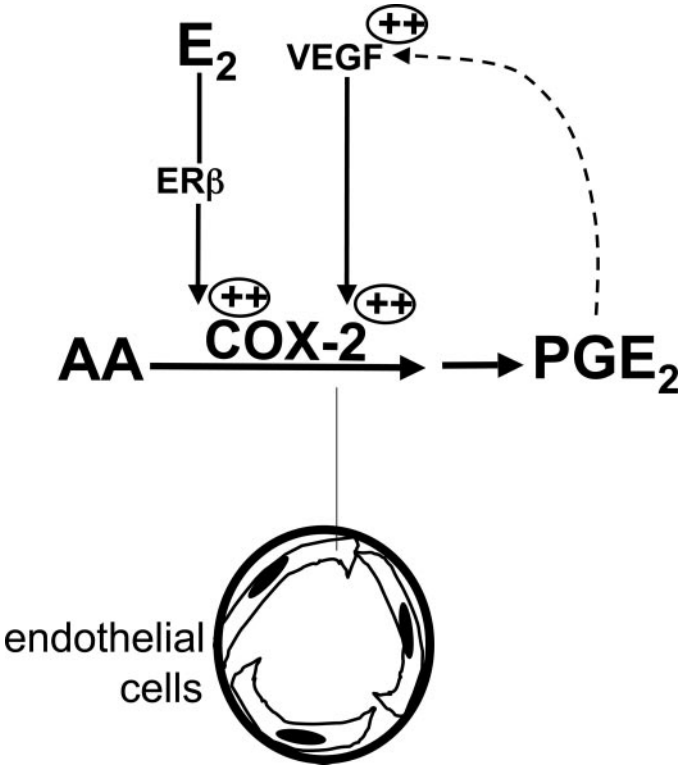


FIG. 15. Effects of estradiol and VEGF on COX-2 expression in uterine endothelial cells. Estradiol (E_2) or VEGF induces COX-2 expression in uterine microendothelial cells rapidly over minutes. These endothelial cells contain ER-β but not ER-α.

IL-1β in a variety of fibroblasts (Miller et al., 2000; Frasor et al., 2003).

Most importantly, COX-2 is strikingly up-regulated in stromal cells of endometriosis, whereas its expression is also significantly higher in endometrium of patients with endometriosis compared with endometrium of disease-free patients (Ota et al., 2001; Wu et al., 2004). We and others and demonstrated that IL-1β and PGE_2 themselves induce COX-2 in endometriotic and endometrial stromal cells, whereas VEGF and PGE_2 rapidly induced COX-2 in uterine endothelial cells (Tamura et al., 2002a,b,c, 2003, 2004; Wu et al., 2004) (Figs. 14 and 15). Thus, a large number of pathways in endometriosis induce COX-2 to increase PGE_2 formation in this tissue (Figs. 14 and 15). The regulation of PGE_2 synthase in endometriosis is not known.

2. Prostaglandin E_2 Action on Endometriotic and Endometrial Stromal Cells. 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 (our unpublished observations). Use of receptor-selective agonists, however, showed that only the EP_2 receptor is responsible for PGE_2-mediated StAR and aromatase expression in endometriotic stromal cells (Zeitoun and Bulun, 1999). Stimulation of the EP_2 receptor rapidly increases intracellular cAMP (Zhao et al., 1996a; Noble et al., 1997) (Fig. 13). Treatment with PGE_2 or a cAMP analog gives rise to comparable increases in StAR and aromatase activity, protein, and mRNA levels (Noble et al., 1997) (Fig. 13). On the other hand, neither PGE_2 (despite the presence of EP_2 receptors) nor cAMP analogs can induce StAR or P450arom in normal eutopic endometrial stromal cells (Noble et al., 1997). Thus, the block for PGE_2-dependent steroidogenesis in eutopic endometrial stromal cells is mediated by inhibitory mechanisms downstream of cAMP.

F. Molecular Mechanisms Responsible for Increased Expression of Steroidogenic Genes in Endometriosis

1. Up-Regulation of Promoter II for Increased Aromatase Expression. 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 ends (UTRs) but an identical coding region (Sebastian and Bulun, 2001; Sebastian et al., 2002) (Fig. 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 activation of promoter II (Fig. 16).

2. Transcriptional Mechanisms Responsible for Increased Expression of the Aromatase Gene in Endometriosis. We uncovered a number of 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., SF-1) in biopsied endometriotic tissues (in vivo) and cultured endometriotic stromal cells (in vitro) (Figs. 12 and 16).

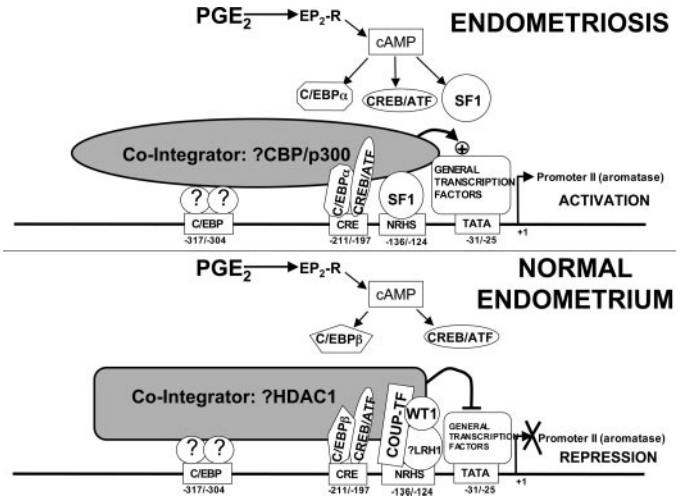


FIG. 16. Differential binding of stimulatory or inhibitory transcription factors to aromatase promoter II in endometriotic versus normal endometrial stromal cells. Both endometriotic and normal endometrial stromal cells contain intact PGE_2 receptors that give rise to intracellular cAMP formation. Treatment of normal endometrial cells with a cAMP analog does not induce aromatase expression, whereas this strikingly stimulates aromatase expression in endometriotic cells. Differential aromatase expression in these two cell types depends on binding activity of transcription factors downstream of cAMP. The key stimulator of aromatase (via promoter II) in endometriotic cells is the aberrantly expressed SF-1. C/EBPα is also stimulatory in this cell type. In endometrial stromal cells, however, inhibitory factors comprise the multimeric complex that occupies this promoter. In particular, COUP-TF and the corepressor WT-1 and C/EBPβ are inhibitory. The possible role of WT-1 is the repression of potential transactivating effects of LRH-1, which is a surrogate of SF-1 and found in large amounts in this cell type. It is also possible that the stimulatory complex contains the cointegrator CBP/p300, whereas the inhibitory complex is associated with HDAC1.

A flip side of this enhancer-mediated mechanism serves as a second mechanism to suppress steroidogenic gene expression in normal eutopic endometrial stromal cells exposed to PGE_2 or cAMP analogs (Fig. 16). This involves the redundant presence and steroidogenic promoter-binding activity of multiple transcriptional inhibitors (COUP-TF) and corepressors of SF-1 [e.g., Wilms' tumor-1 (WT-1)] serving as fail-safe mechanisms (Figs. 12 and 16).

The key regulatory elements that regulate aromatase in endometriosis are within the 500-bp region that lies upstream of promoter II (Fig. 16). We found a dose-dependent direct competition between the orphan nuclear receptors SF-1 (enhancer) and COUP-TF (inhibitor) for occupancy of a nuclear receptor half-site (−136/−124 bp) to which SF-1 bound with a higher affinity (Zeitoun et al., 1999). COUP-TF is expressed in both eutopic endometrial and endometriotic cells, whereas SF-1 is expressed in endometriotic but not normal eutopic endometrial cells (Figs. 12 and 16). COUP-TF is part of the transcriptional system that inhibits aromatase in eutopic endometrial stromal cells; in contrast, aberrantly expressed SF-1 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 favoring aromatase expression (Zeitoun et al., 1999) (Fig. 16).

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 C/EPBα stimulated whereas C/EBPβ inhibited P450arom promoter in endometriotic and eutopic endometrial cells (Yang et al., 2002) (Fig. 16). Unexpectedly, C/EBPα or β was found to bind to the −211/−197 CRE but not the nuclear factor-IL-6 sites in the aromatase promoter II (Fig. 16). 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 (Figs. 16).

Dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X gene 1 (DAX-1), a corepressor of SF-1, inhibits SF-1-dependent expression of aromatase in endometriotic and endometrial cells (Gurates et al., 2003). Moreover, we demonstrated for the first time that WT-1, another coregulator of SF-1, also acts as a corepressor and inhibits SF-1-dependent activity of the aromatase promoter II in endometriotic/endometrial stromal cells (Fig. 16) (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) (Figs. 12 and 16). Thus, WT-1 seems to be a physiologically significant corepressor for the inhibition of steroidogenesis in eutopic endometrium (Fig. 16).

On the other hand, p300/CBP, which is a coactivator of SF-1, enhances promoter II activity in endometriotic and endometrial stromal cells (our unpublished observations; Fig. 16). These findings together suggest that SF-1 acts as a master switch to activate the promoters of aromatase and other key steroidogenic genes. Corepressors or coactivators of SF-1 in endometrial versus endometriotic cells may further modify SF-1-dependent transcriptional activity.

G. Activation of Multiple Steroidogenic Promoters in Endometriosis

As indicated above, 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, SF-1, 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 (Fig. 17). Additionally, similar transcription factors (SF-1, C/EBPs, and CREB) mediate PGE_2-dependent regulation of both promoters (Christenson and Strauss, 2001; Manna et al., 2002, 2003; Yang et al., 2002) (Fig. 17). These promoters remain quiescent under the influence of inhibitory tran-

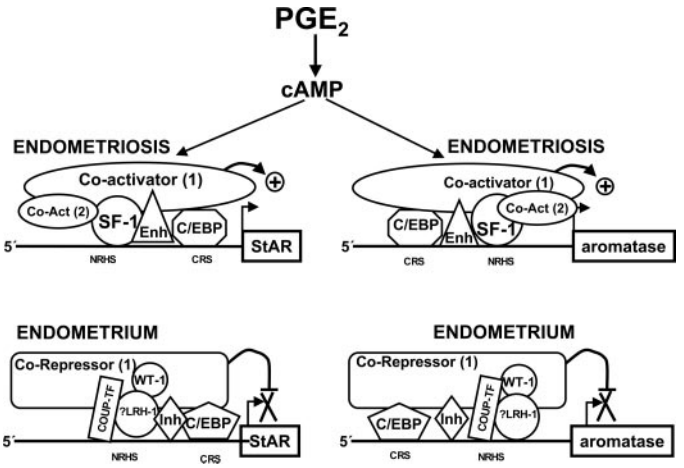


FIG. 17. Regulation of multiple steroidogenic promoters coordinately by common *cis*-acting elements and transcription factors in endometriosis. The *cis*-acting elements in StAR and aromatase promoters and transcription factors that bind to these promoters are very similar. Both multimeric complexes that occupy StAR and aromatase promoters in PGE_2-treated endometriotic stromal cells contain common factors such as SF-1 and a C/EBP subtype. On the other hand, normal endometrial stromal cells from disease-free patients do not express StAR or aromatase mRNA after cAMP analog or PGE_2 stimulation. Multimeric complexes that occupy these promoters are significantly different than those in endometriotic cells and do not contain SF-1. Coact (2), second hypothetical coactivator; Enh, hypothetical enhancer; lnh, hypothetical transcription factor.

scription factors (e.g., COUP-TF) and corepressors (e.g., WT-1) in normal endometrial stromal cells (Fig. 17). Our unpublished observations demonstrated that there are no differences between endometrium and endometriosis with respect to LRH-1 levels. SF-1, however, is readily detectable in endometriosis, whereas it is absent in endometrium. These data strongly suggest that SF-1 expression in endometriosis is primarily responsible for PGE_2-mediated estrogen formation, in particular via induction of StAR and aromatase promoters (Fig. 17).

H. Summary of Regulation of Promoter II in Endometriotic Stromal Cells and Breast Adipose Fibroblasts

Both endometriotic stromal cells and adipose fibroblasts in breast tumors overexpress aromatase via promoter II using apparently similar transcriptional mechanisms. There are, however, several cell-specific differences. First, PGE_2 or a cAMP analog coordinately stimulates two very closely located promoters, II and I.3, in adipose fibroblasts but only promoter II in endometriosis (Figs. 6 and 13). Second, the nuclear receptor half-site in promoter II is occupied by LRH-1 in adipose fibroblasts that do not express SF-1 and by SF-1 in endometriotic stromal cells that express both SF-1 and LRH-1 (Figs. 9 and 16). Third, C/EBPβ stimulates promoter II in breast tumor adipose fibroblasts and inhibits it in endometriotic stromal cells (Figs. 9 and 16). These tissue-specific differences may be explained in part by differential binding of transcription factors and coregulators to a key promoter in each cell type.

I. Aromatase Inhibitors in the Treatment of Endometriosis

Aromatase expression and local estrogen biosynthesis 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; Razzi et al., 2004; Shippen and West, 2004; Soysal et al., 2004).

We introduced aromatase inhibitors into the treatment of endometriosis in 1998 by a case report of postmenopausal endometriosis (Takayama et al., 1998) (Fig. 18). This patient had her uterus and both ovaries surgically removed and was therefore postmenopausal but continued to have a 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 much frequently encountered form of premenopausal endometriosis, some form of ovarian suppression needs to be added to aromatase inhibition; otherwise, estrogen depletion in the hypothalamus causes FSH secretion and ovarian stimulation (Fig. 18). Thus, an aromatase inhibitor was administered together with a

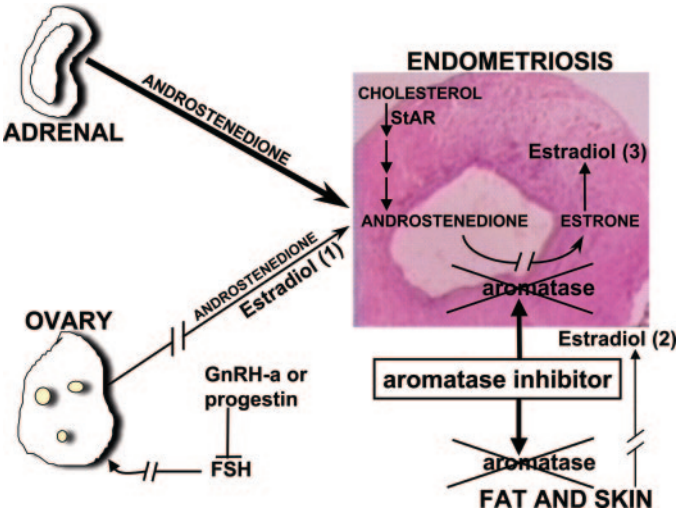


FIG. 18. Sources of estrogen and treatment options in a woman with endometriosis. There are three potential sources of estrogen for endometriosis. 1) Estradiol secreted from the ovary may be decreased or eliminated by FSH suppressants (e.g., GnRH analogs or progestins). 2) Estradiol that arises from skin or fat may be suppressed by aromatase inhibitors. 3) Local estradiol biosynthesis is also effectively suppressed by an aromatase inhibitor. Since aromatase inhibitors in currently prescribed doses cause ovarian stimulation and follicle formation via increasing gonadotropins, aromatase inhibitors are administered in combination with a progestin or a GnRH analog (GnRHa) in premenopausal women. There is no need to add an ovarian suppressing agent to the aromatase inhibitor treatment in a postmenopausal woman.

GnRH agonist, a progestin, 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) (Fig. 18). 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 (Ailawadi et al., 2004). One randomized study ($n = 80$) that combined an aromatase inhibitor with a GnRH agonist showed recurrence of pain in only 10% of patients in the GnRH-agonist-plus-aromatase-inhibitor arm versus 55% in the GnRH-agonist-only arm within 24 months from the completion of the treatment (Soysal et al., 2004) (Fig. 18). The combination of an aromatase inhibitor with an oral progestin or an oral contraceptive gave rise to similar results, and we predict that many patients and physicians will prefer these simpler oral regimens (Amsterdam et al., 2003; Ailawadi et al., 2004; Shippen and West, 2004).

These reports suggest the following. 1) Aromatase inhibitors effectively treat pelvic pain associated with endometriosis, which is resistant to existing therapeutic modalities. 2) An aromatase inhibitor is the medical treatment of choice for persistent postmenopausal endometriosis. 3) Use of aromatase inhibitors in premenopausal women with endometriosis requires ovarian suppression via the addition of a GnRH analog, progestin, or combination oral contraceptive. 4) Side-effect profiles were quite favorable and did not include bone loss in most of these regimens. Thus, aromatase inhibitors represent one of the most promising new treatments of pain associated with endometriosis.

IV. Aromatase and Endometrial Cancer

The role of aromatase expression and the therapeutic use of aromatase inhibitors have been well defined for breast cancer endometriosis. Aromatase is also expressed in endometrial cancer tissue, and aromatase inhibitors have been used to treat endometrial cancer (Bulun et al., 1994a, 2001; Rose et al., 2000; Burnett et al., 2004). The pathologic significance of local estrogen biosynthesis via aromatase expression in endometrial cancer tissue or the therapeutic value of aromatase inhibitors in its management, however, is not clear at the moment.

Endometrial cancer is the most common gynecological malignancy found in women (Inoue et al., 1994). These tumors have high concentrations of estrogen receptors, their growth is clearly enhanced by estrogen, and unopposed estrogen exposure (in the absence of progesterone) predisposes women to development of endometrial cancer (Inoue et al., 1994). Although there is no consistent evidence of increased concentrations of circulating estrogen in women with endometrial cancer, the local concentration of estradiol in endometrial cancer tissues was reported to be higher than that in blood and in the endometrium of cancer-free women (Naitoh et al., 1984; Vermeulen-Meiners et al., 1986; Nagasako et al., 1988; Potischman et al., 1996; Sherman et al., 1997; Berstein et al., 2003). It is therefore conceivable that endometrial cancer itself synthesizes estradiol in situ, which then contributes to growth and possibly carcinogenesis.

A conversion study demonstrated significant conversion of androstenedione to estrone in endometrial cancer tissue (Yamamoto et al., 1993). Aromatase protein and mRNA were detected in endometrial cancer using immunohistochemistry and RT-PCR, whereas aromatase expression was low or undetectable in endometrial hyperplasia (a precursor lesion of endometrial cancer) (Bulun et al., 1994a; Watanabe et al., 1995). These observations suggest that intratumoral aromatase may play a role in the pathology of endometrial cancer. Immunoreactive aromatase was found in malignant epithelial, endometrial stromal, and myometrial cells. Aromatase in stromal but not epithelial cells correlated positively with advanced surgical stage and poor survival (M. Shozu, personal communication).

Currently available third-generation aromatase inhibitors may be used for endocrine treatment of endometrial cancer (Berstein et al., 2002). Treatment of endometrial cancer tissues in vitro with aromatase inhibitors demonstrated that in situ depletion of estrogen results in decreased cell proliferation of tumor cells (Sasano et al., 1999). Treatment of women with endometrial cancer with aromatase inhibitors blocked estrogen production in vivo in tumor tissue (Yamamoto et al., 1990). Safety data from the clinical trials of postmenopausal women with breast cancer indicated a preventive role of aromatase inhibitors in that an aromatase inhibitor reduced the risk of endometrial cancer (Duffy and Greenwood, 2003).

On the other hand, the therapeutic efficacy of aromatase inhibitors in advanced endometrial cancer is not clear. The Gynecologic Oncology Group performed a phase II trial of anastrozole in advanced, recurrent, or persistent endometrial cancer (Rose et al., 2000). Twenty-three patients were entered, all with grade 2 or 3 cancers. Two partial responses were noted (Rose et al., 2000). In a recent report, two cases of reproductive-aged women with grade 1 endometrial cancer who were treated with medroxyprogesterone acetate and anastrozole daily for 3 and 6 months subsequently reverted to normal endometrium. A progestin combined with the elimination of production of estrogen may be an effective therapy in well differentiated endometrial cancer in the obese premenopausal woman (Burnett et al., 2004). Thus, there are some early encouraging results. It is, however, too early to predict whether aromatase inhibitors will be used widely in the treatment of endometrial cancer.

V. Aromatase and Uterine Fibroids (Leiomyomata)

Uterine fibroids (leiomyomata), benign tumors of myometrial origin, are the most common neoplasms of the uterus. Symptomatic leiomyomata are diagnosed in 30% of women over 30 years of age and represent the leading cause of hysterectomy (Andersen and Barbieri, 1995). Each leiomyoma is composed of monoclonal proliferation of a transformed myocyte derived from myometrium. The pathogenesis of this transformation remains unknown, although cytogenetic studies have implicated the involvement of several genes that may function as tumor suppressor genes in some leiomyomata, such as high mobility group protein I-C on chromosome 12q15 (Hu and Surti, 1991; Sreekantaiah et al., 1994; Ozisik et al., 1995; Hennig et al., 1997; Van de Ven, 1998; van der Heijden et al., 1998; Gattas et al., 1999; Schoenmakers et al., 1999). On the other hand, it is widely accepted that the growth of uterine leiomyomata is up-regulated by sex steroids, namely estrogen and progesterone secreted from the ovary (Stewart, 2001). Leiomyoma develops only after the commencement of menstrual cycles and becomes symptomatic usually in the 30s or 40s. At the time of menopause, leiomyomata in a portion of patients start to regress. Thus, the ovary is thought to be the major source of sex steroids for leiomyoma growth.

In addition to endocrine estrogen from the ovary, we and others reported the possible contribution of in situ estrogen to leiomyoma growth, namely estrogen synthesized in leiomyoma cells. Leiomyomata per se express aromatase at strikingly higher levels than the surrounding myometrium and can synthesize estrogen (Folkerd et al., 1984; Yamamoto et al., 1985; Bulun et al., 1994b; Sumitani et al., 2000). In fact, the tissue concentrations of estrogen are elevated in leiomyoma nodules compared with levels in surrounding myometrium (Otubu et al., 1982; Pasqualini et al., 1990). Moreover, it was shown in vitro that estrogen synthesized in leiomyoma smooth muscle cells in culture is sufficient to promote proliferation in an intracrine fashion, since the stimulation of aromatase activity increased cellular proliferation, whereas the addition of an aromatase inhibitor inhibited proliferation (Sumitani et al., 2000). A recent report provided limited but promising evidence that uterine leiomyomata shrink in response treatment with an aromatase inhibitor (Shozu et al., 2003a).

A. Molecular Basis for Estrogen Dependence of Uterine Leiomyomata for Growth

A large body of experimental and clinical evidence shows that estrogen stimulates the growth of uterine leiomyomata (Li and McLachlan, 2001; De Leo et al., 2002; Walker, 2002). Estrogen may directly increase proliferation of leiomyoma cells or indirectly increase growth by enhancing progesterone action in human leiomyomata (Andersen and Barbieri, 1995; Rein et al., 1995; Palomba et al., 2002; Walker, 2002). In an in vivo rat model, uterine leiomyomata were shown to be dependent on estrogen for growth (Houston et al., 2001; Walker, 2002). In this model, progestins were inhibitory for estrogen-dependent growth (Hodges et al., 2002). In human leiomyoma cells, however, both estradiol and progesterone enhanced proliferation in vitro. Estrogen induces leiomyoma growth possibly by altering the expression of a large number of genes (Li and McLachlan, 2001; Walker, 2002). These include but are not limited to estradiol-dependent increases in epidermal growth factor and epidermal growth factor receptor expression in leiomyoma cells (Li and McLachlan, 2001). Additionally, human ER-α polymorphisms have recently been reported to be associated with uterine leiomyomata. Recently, a negative cross-talk was uncovered between ER and PPAR signaling pathways (Houston et al., 2003). These data indicate a strong relationship between estrogen and pathogenesis of leiomyomata.

B. Regulation of Estrogen Biosynthesis in Leiomyoma Smooth Muscle Cells

We and others demonstrated local estrogen biosynthesis in intact leiomyoma tissue explants as well as cultured leiomyoma smooth muscle cells (Bulun et al., 1994b). The incubation of these cells with labeled androstenedione gives rise to significant conversion of this precursor steroid to estrone. Estrone is weakly estrogenic and needs to be converted to estradiol to exert full biologic activity. Estrone is further reduced to the biologically active estrogen estradiol by 17β-hydroxysteroid dehydrogenase activity in leiomyoma tissue (Sumitani et al., 2000). This is verified by significant stimulation of proliferation of leiomyoma smooth muscle cells by androstenedione to the same extent as estradiol (Sumitani et al., 2000). The addition of an aromatase inhibitor blocked the stimulatory effect of androstenedione (Sumitani et al., 2000) (Fig. 19). These results indicate that androstenedione is locally converted to biologically significant quantities of estrone and further to estradiol by leiomyoma smooth muscle cells (Fig. 19). Moreover, aromatase seems to be the key enzyme in this process, since the inhibition of aromatase activity blocked proliferation. Aromatase activity was significantly stimulated by a cAMP analog, PGE_2, or a combination of a glucocorticoid and a cytokine (IL-1β) (Bulun et al., 1994b; Shozu et al., 2002) (Fig. 19).

C. Aromatase Expression in Uterine Leiomyoma Tissues

Aromatase mRNA was detectable by quantitative RT-PCR in 91% of 35 leiomyomata from 26 women (Bulun et al., 1994b; Sumitani et al., 2000). Aromatase mRNA was also detectable in 75% of normal-seeming myometrial tissues ($n = 24$) biopsied 2 cm from a leiomyoma. Aromatase mRNA was not detectable in myometrial tissues from disease-free uteri ($n = 8$). To provide a perspective, leiomyoma aromatase mRNA levels were similar to those ordi-

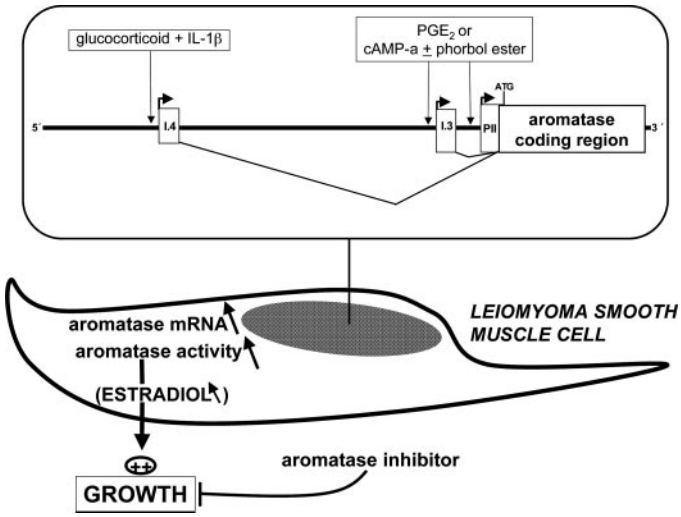


FIG. 19. Aromatase expression in uterine fibroids (leiomyomata). Leiomyoma-derived smooth muscle cells express aromatase and synthesize estrogen. Inhibition of aromatase activity leads to inhibition of growth under both in vitro and in vivo circumstances. Alternative promoters stimulated by a distinct set of hormones direct aromatase expression in leiomyoma smooth muscle cells. In vivo analyses of leiomyoma tissues also reveal the utilization of these promoters. cAMP-a, cAMP analog.

narily found in breast adipose tissue and were 1.5- to 25-fold higher than those in adjacent myometrial tissues.

There was no correlation between aromatase mRNA levels and leiomyoma size, uterine weight, or patient age, although there was a positive trend between advancing age and aromatase mRNA levels. For example, the highest mRNA levels were detected in patients above age 45, and leiomyoma tissue from a 65-year-old patient contained moderately high aromatase mRNA levels (Bulun et al., 1994b; Sumitani et al., 2000). These data suggest that local aromatase expression may be important for the availability of estrogen to leiomyoma tissue despite severely decreased circulating estrogen levels in perimenopausal or postmenopausal women.

D. Regulation of Aromatase Expression in Leiomyoma Tissues and Smooth Muscle Cells

We and others used leiomyoma tissue explants and smooth muscle cells in monolayer culture as model systems to study the regulation of aromatase mRNA levels (Bulun et al., 1994b; Shozu et al., 2002). Myometrial smooth muscle cells isolated from normal uteri did not contain aromatase activity or aromatase mRNA (Bulun et al., 1994b; Sumitani et al., 2000; Shozu et al., 2001). In our hands, a cAMP analog gave rise to maximum induction, whereas glucocorticoids alone or together with serum moderately increased aromatase mRNA levels (Bulun et al., 1994b). The alterations in aromatase enzyme activity induced by hormonal treatments were accompanied by comparable changes in the levels of aromatase mRNA (Bulun et al., 1994b) (Fig. 19).

Our published and unpublished observations on 14 samples from 13 patients indicated that cAMP-responsive promoters I.3 and II (86%) are primarily used for aromatase expression, whereas promoter I.4 (14%) plays a less important role (Bulun et al., 1994b) (Fig. 19). A Japanese group of investigators recently reported that promoter I.4-specific aromatase mRNA species were predominantly present in most leiomyoma tissues ($n = 6$) (Shozu et al., 2002). A glucocorticoid plus IL-1β stimulated aromatase enzyme activity and mRNA levels maximally in cells isolated from these specimens (Shozu et al., 2002) (Fig. 19). These investigators also demonstrated that glucocorticoids and IL-1β regulated aromatase expression via promoter I.4 (Fig. 19). The discrepancy between their results and ours may be attributed to race-dependent differences, since our samples were obtained from African American, white, and Hispanic patients, whereas all patients in the Japanese study were of Asian origin (Bulun et al., 1994b; Shozu et al., 2002). Studies are underway to understand the basis of these different findings from two different laboratories.

E. Treatment of Uterine Leiomyomata with Aromatase Inhibitors

A preliminary but promising piece of clinical evidence for the significance of local aromatase expression was published recently (Shozu et al., 2003a). These authors described the shrinkage of a large leiomyoma in a 53-year-old menopausal woman using an aromatase inhibitor (Shozu et al., 2003a). Because the patient did not have ovarian function, the therapeutic effect was postulated to be mediated by blocking local aromatase activity in the leiomyoma (Fig. 19). This case report provides limited but promising evidence for a role of aromatase in the biology of uterine leiomyomata and the future use of aromatase inhibitors to treat these tumors. To our knowledge, several pilot clinical trials are in progress and should provide a better insight to this question.

VI. Aromatase Excess Syndrome

A. Background and Clinical Manifestations

Wilson and McPhaul and their colleagues described a genetically inherited form of estrogen excess in henny-feathered Sebright roosters (George and Wilson, 1980; McPhaul et al., 1988; Matsumine et al., 1990). In birds, aromatase is normally expressed in the ovaries and brain but not in skin fibroblasts (McPhaul et al., 1988; Simpson et al., 2002). In Sebright chickens with the autosomal dominant henny-feathered trait, aromatase is overexpressed in skin fibroblasts, leading to inappropriate estrogen formation in skin and a female pattern of feather development in roosters (George and Wilson, 1980; Matsumine et al., 1990). Furthermore, the 5′-untranslated region of aromatase mRNA in skin fibroblasts suggested that a unique promoter regulated the expression of aromatase (Matsumine et al., 1990). We published two distinct rearrangements in chromosome 15, which may be responsible for the human counterpart of this disorder, i.e., aromatase excess syndrome (Shozu et al., 2003b) (Fig. 20).

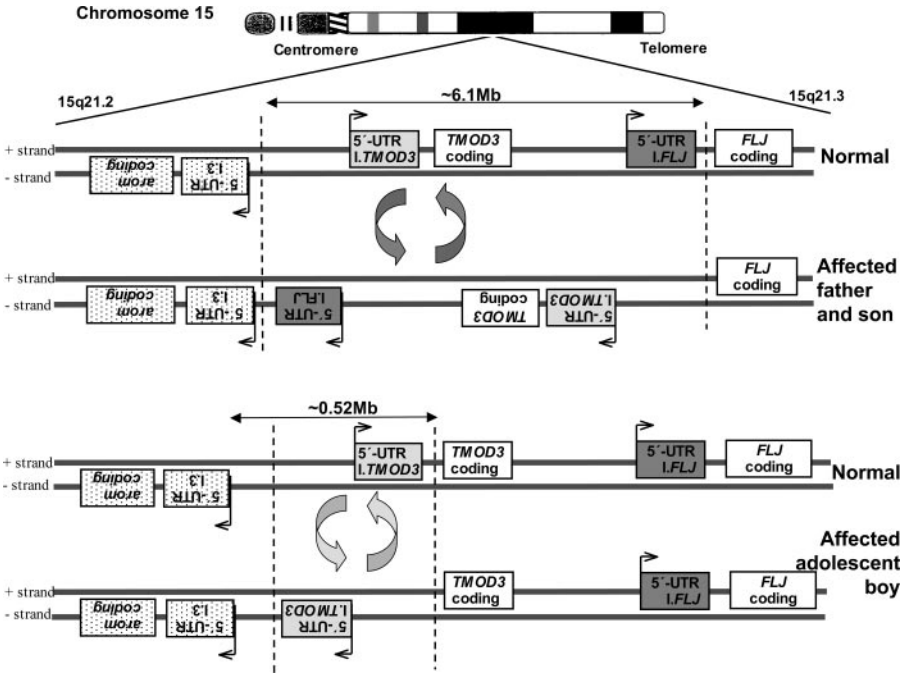


FIG. 20. Mechanism of two inversion mutations that give rise to aromatase excess syndrome. Two genes (FLJ and TMOD3) that lie telomeric to the aromatase gene on chromosome 15 have a similar structure to that of the aromatase gene in that their coding regions are separated from the first untranslated exons (I.FLJ and I.TMOD3) by long intronic sequences. Proposed inversion mutations and estimated breakpoints (dotted lines) are indicated. These heterozygous mutations give rise to the formation of cryptic promoters that transcribe aromatase ubiquitously in many tissues.

Boys affected by this syndrome present with growth of breast tissue (gynecomastia), whereas girls present with premature breast development (thelarche). Both sexes exhibit a premature growth spurt, early fusion of epiphyses, and decreased adult height. Adult men are undervirilized, whereas adult women experience irregular uterine bleeding. Increased conversion of steroid precursors to estrogens in extraglandular tissues is responsible for excessive estrogen formation. MacDonald and his colleagues described originally a feminized, prepubertal adopted boy in whom large amounts of estrone and estradiol were produced by extraglandular aromatization of plasma androstenedione (Hemsell et al., 1977). Five families were subsequently described in which several members had estrogen excess as a result of increased extraglandular aromatization inherited in an autosomal dominant fashion (Berkovitz et al., 1985; Leiberman and Zachmann, 1992; Stratakis et al., 1998; Martin et al., 2003; Shozu et al., 2003b).

B. Genetic Basis for Familial Aromatase Excess Syndrome

We evaluated for genetic abnormalities a 36-year-old man, his 7-year-old son, and an unrelated 17-year-old boy with severe gynecomastia of prepubertal onset and hypogonadotropic hypogonadism caused by elevated estrogen levels (Shozu et al., 2003b). Aromatase activity and mRNA levels in fat and skin and conversion of circulating androstenedione to estrone were severely elevated. Treatment with an aromatase inhibitor decreased serum estrogen levels and normalized gonadotropin and testosterone levels. The 5′-untranslated regions of aromatase mRNA contained the same sequence (FLJ) in the father and son and another sequence (TMOD3) in the unrelated boy; neither sequence was found in control subjects nor had they been previously described (Fig. 20). These 5′-untranslated regions normally make up the first exons of two ubiquitously expressed genes clustered in chromosome 15q21.2 to 3 in the following order (from telomere to centromere): *FLJ*, *TMOD3*, and aromatase. The aromatase gene is normally transcribed in the direction opposite to that of *TMOD3* and *FLJ*. Two distinct heterozygous inversions reversed the direction of the *TMOD3* or *FLJ* promoter in the patients (Shozu et al., 2003b) (Fig. 20).

We found that overproduction of estrogen arose from gain-of-function mutations in chromosome 15, giving rise to the formation of cryptic promoters that regulate the aromatase gene. These constitutively active promoters normally serve to transcribe two ubiquitously expressed genes—*FLJ* and *TMOD3*—that encode products homologous to muscle proteins in many human tissues. The functions of *FLJ* and *TMOD3* are not known (Cox and Zoghbi, 2000; Nagase et al., 2000) (Fig. 20). An identical mutation involving the *FLJ* promoter was transmitted from a father to his son in an autosomal-dominant manner (Fig. 20). The cryptic promoter in a third unrelated patient was distinct and arose from the *TMOD3* gene (Fig. 20). The relatives of this third patient were not affected. Therefore, this rearrangement seemed to be the result of a new mutation.

These mutations are somewhat analogous to transgenic mice, in which a gene product fused to a constitutively active promoter is expressed in multiple tissues after random insertion. The tissue selectivity (or nonselectivity) and level of activity of this aberrant promoter determines the tissue distribution and levels of mRNA encoded by this gene. In human peripheral tissues and gonads, basal levels of aromatase mRNA is extremely low. Only FSH induces aromatase expression physiologically to very high levels in the ovaries of premenopausal women during cycle days 5 to 14. Thus, in general, low basal expression of aromatase is tightly controlled in the peripheral tissues because comparatively low levels of aromatase expression and formation of estrogen (measured in picomoles per liter in the serum) are sufficient for many estrogenic effects. The mutations described in aromatase excess syndrome represent a major deviation from this physiology and are caused by constitutively active cryptic promoters that take control over aromatase expression.

C. Discussion on Gain-of-Function Mutations That Affect the Aromatase Gene

The upstream region of the aromatase gene may represent a "hot spot" for mutations. Occasional mutations, such as those that we described, caused extremely high aromatase activity and striking clinical consequences. More common rearrangements, however, may go clinically unrecognized and cause subtle degrees of estrogen excess, which may increase the risks of estrogen-dependent disease, such as cancers of the breast and endometrium, endometriosis, and uterine fibroids.

Adult men with this syndrome had hypogonadotropic hypogonadism. During treatment with an aromatase inhibitor, their estrogen levels declined and testosterone, luteinizing hormone, and follicle-stimulating hormone levels rose to normal. This response suggests a crucial role of estrogen in the suppression of both gonadotropins in men. Despite low testosterone levels in these patients, luteinizing hormone remained suppressed, possibly owing to the high levels of circulating estrogen. These mutations may have given rise to the overexpression of aromatase in the brain and thus to increased local estrogen production, which might also have contributed to the suppression of gonadotropins.

Aromatase inhibitors may potentially be used to prevent or halt the progression of gynecomastia in young boys (Plourde et al., 2004). The potential objective of long-term treatment of adult men with an aromatase inhibitor is to restore gonadal function, but this approach is not clearly justified for several reasons (Shozu et al., 2003b). First, men with this condition are not at risk for osteoporosis. Second, although men with estrogen excess may be subfertile, infertility is not a uniform feature of the syndrome. Third, the consequences of long-term exposure to high levels of estrogen in men are not known. We suggest that these men should periodically be evaluated for breast and prostate disease, given the potentially deleterious effects of estrogen on these tissues (Shozu et al., 2003b; Plourde et al., 2004).

There are no published reports regarding the use of the new-generation aromatase inhibitors in women with aromatase excess syndrome. It is conceivable that these women are at increased risk for developing breast cancer, endometrial cancer, macromastia, endometriosis, and uterine fibroids. Thus, aromatase inhibitors may potentially be used for prevention or treatment. Answers to these questions require clinical trials in carefully selected populations.

VII. Conclusions Regarding Aromatase Overexpression in Estrogen-Dependent Human Disease

Several principles emerge from the data summarized in this review. First, human aromatase encoded by a single gene acts as a master switch in physiological and pathological systems to turn on estrogen formation or serves as a therapeutic target to turn off estrogen biosynthesis. By the same token, estradiol acts as a master switch in many biological systems to regulate hundreds to thousands of diverse genes at one time point, which makes it extremely difficult to therapeutically target any of these downstream genes. Second, biologically active quantities of aromatase mRNA and protein and their product estrogen are relatively small. For example, circulating or tissue estradiol is measured in picomole quantities that are 100 to 1000 orders of magnitude smaller than those used for testosterone measured in nanomolar quantities. Thus, comparatively small levels of aromatase expression have physiologically or pathologically critical consequences. Third, local aromatase expression and estrogen biosynthesis seems to be a far more critical therapeutic target compared with circulating estrogen. Fourth, complex and redundant epigenetic mechanisms are responsible for aromatase expression in the estrogen-dependent pathologic tissues. These range from accumulation of aromatase-expressing cell types to aberrant expression of transcriptional enhancers and permit the use of additional promoters in pathological tissues, which are normally silenced in disease-free tissue counterparts. Most notably, the two proximal promoters, I.3 and II, which are located within 215 bp from each other and coordinately regulated by PGE_2 or protein kinase A- and C-dependent pathways, are aberrantly activated in breast cancer, endometriosis, endometrial cancer, and uterine fibroids. Finally, gain-of-function-type germ cell mutations give rise to cryptic promoters that overexpress the aromatase gene ubiquitously in many tissues. We speculate that the promiscuous nature of the splice acceptor site immediately upstream of the coding region is in part responsible for regulation of the aromatase gene by multiple alternatively used physiological or pathological promoters.

REFERENCES

Ackerman GE, Smith ME, Mendelson CR, MacDonald PC, and Simpson ER (1981) Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture. *J Clin Endocrinol Metab* **53:**412–417.

Agarwal VR, Ashanullah CI, Simpson ER, and Bulun SE (1997) Alternatively spliced transcripts of the aromatase cytochrome P450 (CYP19) gene in adipose tissue of women. *J Clin Endocrinol Metab* **82:**70–74.

Agarwal VR, Bulun SE, Leitch M, Rohrich R, and Simpson ER (1996) Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients. *J Clin Endocrinol Metab* **81:**3843–3849.

Agarwal VR, Bulun SE, and Simpson ER (1995) Quantitative detection of alternatively spliced transcripts of the aromatase cytochrome P450 (CYP19) gene in aromatase-expressing human cells by competitive RT-PCR. *Mol Cell Probes* **9:**453–464.

Ailawadi R, Jobanputra S, Kataria M, Gurates B, and Bulun S (2004) Treatment of endometriosis and chronic pelvic pain with letrozole and norethindrone acetate: a pilot study. *Fertil Steril* **81:**290–296.

Amsterdam L, Gentry W, Rubin S, Jobanputra S, and Bulun SE (2003) Treatment of endometriosis-related pelvic pain with a combination of an aromatase inhibitor (anastrozle) plus a combination oral contraceptive: a novel approach (Abstract). *Proceedings of the 85th Annual Endocrine Society Meeting* **1:**360.

Andersen J and Barbieri RL (1995) Abnormal gene expression in uterine leiomyomas. *J Soc Gynecol Investig* **2:**663–672.

Baum M, Buzdar A, Cuzick J, Forbes J, Houghton J, Howell A, and Sahmoud T (2003) Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early-stage breast cancer: results of the ATAC (arimidex, tamoxifen alone or in combination) trial efficacy and safety update analyses. *Cancer* **98:**1802–10.

Baum M, Budzar AU, Cuzick J, Forbes J, Houghton JH, Klijn JG, and Sahmoud T (2002) Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial. *Lancet* **359:**2131–2139.

Berkovitz GD, Guerami A, Brown TR, MacDonald PC, and Migeon CJ (1985) Familial gynecomastia with increased extraglandular aromatization of plasma C 19-steroids. *J Clin Investig* **75:**1763–1769.

Berstein L, Maximov S, Gershfeld E, Meshkova I, Gamajunova V, Tsyrlina E, Larionov A, Kovalevskij A, and Vasilyev D (2002) Neoadjuvant therapy of endometrial cancer with the aromatase inhibitor letrozole: endocrine and clinical effects. *Eur J Obstet Gynecol Reprod Biol* **105:**161–165.

Berstein L, Tchernobrovkina A, Gamajunova V, Kovalevskij A, Vasilyev D, Chepik O, Turkevitch E, Tsyrlina E, Maximov S, Ashrafian L, et al. (2003) Tumor estrogen content and clinico-morphological and endocrine features of endometrial cancer. *J Cancer Res Clin Oncol* **129:**245–249.

Bonneterre J, Thurlimann B, Robertson JF, Krzakowski M, Mauriac L, Koralewski P, Vergote I, Webster A, Steinberg M, and von Euler M (2000) Anastrozole versus tamoxifen as first-line therapy for advanced breast cancer in 668 postmenopausal women: results of the Tamoxifen or Arimidex Randomized Group Efficacy and Tolerability study. *J Clin Oncol* **18:**3748–3757.

Brodie A, Long B, and Lu Q (1998) Aromatase expression in the human breast. *Breast Cancer Res Treat* **49:**S85–S91.

Brodie A, Lu Q, Liu Y, and Long B (1999a) Aromatase inhibitors and their antitumor effects in model systems. *Endocr Relat Cancer* **6:**205–210.

Brodie A, Lu Q, and Long B (1999b) Aromatase and its inhibitors. *J Steroid Biochem Mol Biol* **69:**205–10.

Brueggemeier RW, Quinn AL, Parrett ML, Joarder FS, Harris RE, and Robertson FM (1999) Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens. *Cancer Lett* **140:**27–35.

Bruner KL, Matrisian LM, Rodgers WH, Gorstein F, and Osteen KG (1997) Suppression of matrix metalloproteinases inhibits establishment of ectopic lesions by human endometrium in nude mice. *J Clin Investig* **99:**2851–2857.

Bulun S (2000) Aromatase deficiency and estrogen resistance: from molecular genetics to clinic. *Semin Reprod Med* **18:**31–39.

Bulun SE, Economos K, Miller D, and Simpson ER (1994a) CYP19 (aromatase cytochrome P450) gene expression in human malignant endometrial tumors. *J Clin Endocrinol Metab* **79:**1831–1834.

Bulun SE, Noble LS, Takayama K, Michael MD, Agarwal V, Fisher C, Zhao Y, Hinshelwood MM, Ito Y, and Simpson ER (1997) Endocrine disorders associated with inappropriately high aromatase expression. *J Steroid Biochem Mol Biol* **61:**133–139.

Bulun SE, Price TM, Mahendroo MS, Aitken J, and Simpson ER (1993) A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. *J Clin Endocrinol Metab* **77:**1622–1628.

Bulun SE and Simpson ER (1994) Competitive RT-PCR analysis indicates levels of aromatase cytochrome P450 transcripts in adipose tissue of buttocks, thighs and abdomen of women increase with advancing age. *J Clin Endocrinol Metab* **78:**428–432.

Bulun SE, Simpson ER, and Word RA (1994b) Expression of the CYP19 gene and its product aromatase cytochrome P450 in human leiomyoma tissues and cells in culture. *J Clin Endocrinol Metab* **78:**736–743.

Bulun SE, Yang S, Fang Z, Gurates B, Tamura M, Zhou J, and Sebastian S (2001) Role of aromatase in endometrial disease. *J Steroid Biochem Mol Biol* **79:**19–25.

Burnett A, Bahador A, and Amezcua C (2004) Anastrozole, an aromatase inhibitor and medroxyprogesterone acetate therapy in premenopausal obese women with endometrial cancer: a report of two cases successfully treated without hysterectomy. *Gynecol Oncol* **94:**832–834.

Buzdar AU, Vergote I, and Sainsbury R (2004) The impact of hormone receptor status on the clinical efficacy of the new-generation aromatase inhibitors: a review of data from first-line metastatic disease trials in postmenopausal women. *Breast J* **10:**211–217.

Chen S, Itoh T, Wu K, Zhou D, and Yang C (2002) Transcriptional regulation of aromatase expression in human breast tissue. *J Steroid Biochem Mol Biol* **83:**93–99.

Chetrite G, Cortes-Prieto J, Philippe J, Wright F, and Pasqualini J (2000) Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal and in cancerous, human breast tissues. *J Steroid Biochem Mol Biol* **72:**23–7.

Christenson L and Strauss JI (2001) Steroidogenic acute regulatory protein—an update on its regulation and mechanism of action. *Arch Med Res* **32:**576–586.

Clyne CD, Speed CJ, Zhou J, and Simpson ER (2002) Liver receptor homologue-1 (LRH-1) regulates expression of aromatase in preadipocytes. *J Biol Chem* **277:**20591–20597.

Cox PR and Zoghbi HY (2000) Sequencing, expression analysis and mapping of three unique human tropomodulin genes and their mouse orthologs. *Genomics* **63:**97–107.

Crichton MB, Nichols JE, Zhao Y, Bulun SE, and Simpson ER (1996) Expression of transcripts of interleukin-6 and related cytokines by human breast tumors, breast cancer cells and adipose stromal cells. *Mol Cell Endocrinol* **118:**215–220.

De Leo V, Morgante G, La Marca A, Musacchio MC, Sorace M, Cavicchioli C, and Petraglia F (2002) A benefit-risk assessment of medical treatment for uterine leiomyomas. *Drug Safety* **25:**759–779.

Deb S, Amin S, Imir AG, Yilmaz MB, Suzuki T, Sasano H, and Bulun SE (2004) Estrogen regulates expression of tumor necrosis factor receptors in breast adipose fibroblasts. *J Clin Endocrinol Metab* **89:**4018–4024.

Dixon J, Renshaw L, Bellamy C, Stuart M, Hoctin-Boes G, and Miller W (2000) The effects of neoadjuvant anastrozole (Arimidex) on tumor volume in postmenopausal women with breast cancer: a randomized, double-blind, single-center study. *Clin Cancer Res* **6:**2229–35.

Duffy S and Greenwood M (2003) The endometrial cancer data from the ATAC (arimidex, tamoxifen, alone or in combination) trial indicates a protective effect on anastrozole (arimidex) upon the endometrium. *Breast Cancer Res Treat* 82 **(Suppl 1):**S29.

Fang ZJ, Yang S, Gurates G, Tamura M, Simpson ER, Evans D, and Bulun SE (2002) Genetic or enzymatic disruption of aromatase inhibits the growth of ectopic uterine tissue. *J Clin Endocrinol Metab* 87:3460–3466.

Folkerd EJ, Newton CJ, Davidson K, Anderson MC, and James VH (1984) Aromatase activity in uterine leiomyomata. *J Steroid Biochem Mol Biol* **20:**1195–1200.

Frasor J, Danes JM, Komm B, Chang KC, Lyttle CR, and Katzenellenbogen BS (2003) Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. *Endocrinology* **144:**4562–4574.

Gattas GJ, Quade BJ, Nowak RA, and Morton CC (1999) HMGIC expression in human adult and fetal tissues and in uterine leiomyomata. *Genes Chromosomes Cancer* **25:**316–322.

Geisler J, Berntsen H, and Lonning P (2000) A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol and estrone sulphate levels in breast cancer tissue. *J Steroid Biochem Mol Biol* **72:**259–264.

George FW and Wilson JD (1980) Pathogenesis of the henny feathering trait in the Sebright bantam chicken. *J Clin Investig* **66:**57–65.

Giudice L and Kao L (2004) Endometriosis. *Lancet* **364:**1789–1799.

Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, et al. (2003) A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N Engl J Med* **349:**1793–1802.

Grodin JM, Siiteri PK, and MacDonald PC (1973) Source of estrogen production in postmenopausal women. *J Clin Endocrinol Metab* **36:**207–214.

Gurates B, Amsterdam A, Tamura M, Yang S, Zhou J, Fang Z, Amin S, Sebastian S, and Bulun S (2003) WT1 and DAX-1 regulate SF-1-mediated human P450arom gene expression in gonadal cells. *Mol Cell Endocrinol* **208:**61–75.

Gurates B, Sebastian S, Yang S, Zhou J, Tamura M, Fang Z, Suzuki T, Sasano H, and Bulun SE (2002) WT1 and DAX-1 inhibit aromatase P450 expression in human endometrial and endometriotic stromal cells. *J Clin Endocrinol Metab* **87:**4369–4377.

Haagensen CD (1986) *Diseases of the Breast*. W.B. Saunders Company, Philadelphia.

Hankinson S, Willett W, Manson J, Colditz G, Hunter D, Spiegelman D, Barbieri R, and Spiezer F (1999) Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst* **90:**1292–1299.

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 carcinogenesis. *Proc Natl Acad Sci USA* **90:**11312–11316.

Hemsell D, Edman C, Marks J, Siiteri P, and MacDonald P (1977) Massive extraglandular aromatization of plasma androstenedione resulting in feminization of a prepubertal boy. *J Clin Investig* **60:**455–464.

Hemsell DL, Grodin JM, Brenner PF, Siiteri PK, and MacDonald PC (1974) Plasma precursors of estrogen. II. Correlation of the extent of conversion of plasma androstenedione to estrone with age. *J Clin Endocrinol Metab* **38:**476–479.

Hennig Y, Rogalla P, Wanschura S, Frey G, Deichert U, Bartnitzke S, and Bullerdiek J (1997) HMGIC expressed in a uterine leiomyoma with a deletion of the long arm of chromosome 7 along with a 12q14–15 rearrangement but not in tumors showing del(7) as the sole cytogenetic abnormality. *Cancer Genet Cytogenet* **96:**129–133.

Hodges LC, Houston KD, Hunter DS, Fuchs-Young R, Zhang Z, Wineker RC, and Walker CL (2002) Transdominant suppression of estrogen receptor signaling by progesterone receptor ligands in uterine leiomyoma cells. *Mol Cell Endocrinol* **196:**11–20.

Houston KD, Copland JA, Broaddus RR, Gottardis MM, Fischer SM, and Walker CL (2003) Inhibition of proliferation and estrogen receptor signaling by peroxisome proliferator-activated receptor gamma ligands in uterine leiomyoma. *Cancer Res* **63:**1221–1227.

Houston KD, Hunter DS, Hodges LC, and Walker CL (2001) Uterine leiomyomas: mechanisms of tumorigenesis. *Toxicol Pathol* **29:**100–104.

Hu J and Surti U (1991) Subgroups of uterine leiomyomas based on cytogenetic analysis. *Hum Pathol* **22:**1009–1016.

Huang Z, Hankinson SE, Colditz GA, Stampfer MJ, Hunter DJ, Manson JE, Hen-

nekens CH, Rosner B, Speizer FE, and Willett WC (1997) Dual effects of weight and weight gain on breast cancer risk. *J Am Med Assoc* **278:**1407–1411.
Hube F and Hauner H (2000) The two tumor necrosis factor receptors mediate opposite effects on differentiation and glucose metabolism in human adipocytes in primary culture. *Endocrinology* **141:**2582–2588.
Inoue M, Okayama A, Fujita M, Enomoto T, Tanizawa O, and Ueshima H (1994) A case-control study on risks factors for uterine endometrial cancer in Japan. *Jpn J Cancer Res* **85:**346–350.
Jabbour H, Milne S, Williams A, Anderson R, and Boddy S (2001) Expression of COX-2 and PGE synthase and synthesis of PGE(2) endometrial adenocarcinoma: a possible autocrine/papacrine regulation of neoplastic cell function via EP2/EP4 receptors. *Br J Cancer* **85:**1023–1031.
James VHT, Reed MJ, Lai LC, Ghilchik MW, Tait GH, Newton CJ, and Coldham NG (1990) Regulation of estrogen concentrations in human breast tissues. *Ann NY Acad Sci* **595:**227–235.
Jiang B, Kamat A, and Mendelson C (2000) Hypoxia prevents induction of aromatase expression in human trophoblast cells in culture: potential inhibitory role of the hypoxia-inducible transcription factor Mash-2 (mammalian achaete-scute homologous protein-2). *Mol Endocrinol* **14:**1661–1773.
Jiang B and Mendelson C (2003) USF1 and USF2 mediate inhibition of human trophoblast differentiation and CYP19 gene expression by Mash-2 and hypoxia. *Mol Cell Biol* **23:**6117–6128.
Kamat A, Alcorn J, Kunczt C, and Mendelson C (1998) Characterization of the regulatory regions of the human aromatase (P450arom) gene involved in placenta-specific expression. *Mol Endocrinol* **12:**1764–1777.
Kamat A, Hishelwood M, Murray B, and Mendelson C (2002) Mechanisms in tissue-specific regulation of estrogen biosynthesis humans. *Trends Endocrinol Metab* **13:**122–128.
Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, and Giudice LC (2003) Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology* **144:**2870–2881.
Karuppu D, Kalus A, Simpson E, and Clyne C (2002) Aromatase and prostaglandin inter-relationships in breast adipose tissue: significance for breast cancer development. *Breast Cancer Res Treat* **76:**103–109.
Kitawaki J, Noguchi T, Amatsu T, Maeda K, Tsukamoto K, Yamamoto T, Fushiki S, Osawa Y, and Honjo H (1997) Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. *Biol Reprod* **57:**514–519.
Kovacic A, Speed C, Simpson E, and Clyne C (2004) Inhibition of aromatase transcription via promoter II by short heterodimer partner in human preadipocytes. *Mol Endocrinol* **18:**252–259.
Leiberman E and Zachmann M (1992) Familial adrenal feminization probably due to increased steroid aromatization. *Hormone Res* **37:**96–102.
Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, and Strom BL (1994) Aberrant integrin expression in the endometrium of women with endometriosis. *J Clin Endocrinol Metab* **79:**643–649.
Li S and McLachlan J (2001) Estrogen-associated genes in uterine leiomyoma. *Ann NY Acad Sci* **948:**112–120.
Lu Q, Yue W, Wang J, Liu Y, Long B, and Brodie A (1998) The effects of aromatase inhibitors and antiestrogens in the nude mouse model. *Breast Cancer Res Treat* **50:**63–71.
MacDonald PC, Edman CD, Hemsell DL, Porter JC, and Siiteri PK (1978) Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. *Am J Obstet Gynecol* **130:**448–455.
MacDonald PC, Grodin JM, and Siiteri PK (1968) The utilization of plasma androstenedione for estrone production in women, in *Excerpta Medica Int. Congr. Series No. 184* (Gual C and Ebling FJG eds) pp 770–776, Progress in Endocrinology, Proceedings of the Third International Congress of Endocrinology; 1968 Jun 30–Jul 5; Mexico. Excerpta Medica Foundation, Amsterdam, The Netherlands.
MacDonald PC, Madden JD, Brenner PF, Wilson JD, and Siiteri PK (1979) Origin of estrogen in normal men and in women with testicular feminization. *J Clin Endocrinol Metab* **49:**905–916.
Mahendroo MS, Mendelson CR, and Simpson ER (1993) Tissue-specific and hormonally-controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. *J Biol Chem* **268:**19463–19470.
Manna P, Dyson M, Eubank D, Clark B, Lalli E, Sassone-Corsi P, Zeleznik A, and Stocco D (2002) Regulation of steroidogenesis and the steroidogenic acute regulatory protein by a member of the cAMP response-element binding protein family. *Mol Endocrinol* **16:**184–199.
Manna P, Eubank D, Lalli E, Sassone-Corsi P, and Stocco D (2003) Transcriptional regulation of the mouse steroidogenic acute regulatory protein gene by the cAMP response-element binding protein and steroidogenic factor 1. *J Mol Endocrinol* **30:**381–397.
Martin R, Lin C, Nishi M, Billerbeck A, Latronico A, Russell DW, and Mendonca BB (2003) Familial hyperestrogenism in both sexes: clinical, hormonal and molecular studies of two siblings. *J Clin Endocrinol Metab* **88:**3027–3034.
Matsumine H, Wilson JD, and McPhaul MJ (1990) Sebright and Campine chickens express aromatase P-450 messenger RNA inappropriately in extraglandular tissues and in skin fibroblasts. *Mol Endocrinol* **4:**905–911.
McPhaul MJ, Noble JF, Simpson ER, Mendelson CR, and Wilson JD (1988) The expression of a functional cDNA encoding the chicken cytochrome P-450 arom (aromatase) that catalyzes the formation of estrogen from androgen. *J Biol Chem* **263:**16358–16363.
Meng L, Zhou J, Hironobu S, Suzuki T, Zeitoun K, and Bulun S (2001) TNFalpha and IL-11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively downregulating C/EBPalpha and PPARgamma: mechanism of desmoplastic reaction. *Cancer Res* **61:**2250–2255.
Milla-Santos A, Milla L, Portella J, Rallo L, Pons M, Rodes E, Casanovas J, and Puig-Gali M (2003) Anastrozole versus tamoxifen as first-line therapy in postmenopausal patients with hormone-dependent advanced breast cancer: a prospective, randomized, phase III study. *Am J Clin Oncol* **26:**317–322.
Miller LJ, Kurtzman SH, Anderson K, Wang Y, Stankus M, Renna M, Lindquist R, Barrows G, and Kreutzer D (2000) Interleukin-1 family expression in human breast cancer: interleukin-1 receptor antagonist. *Cancer Investig* **18:**293–302.
Mouridsen H, Gershanovich M, Sun Y, Perez-Carrion R, Boni C, Monnier A, Apffelstaedt J, Smith R, Sleeboom HP, Janicke F, et al. (2001) Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III study of the International Letrozole Breast Cancer Group. *J Clin Oncol* **19:**2596–606.
Murakami M, Nakashima K, Kamei D, Masuda S, Ishikawa Y, Ishii T, Ohmiya Y, Watanabe K, and Kudo I (2003) Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenasesa-1 and -2. *J Biol Chem* **278:**37937–37947.
Nagasako S, Asanuma N, and Nagata Y (1988) Plasma concentration of estrogens and androgens in postmenopausal women with or without endometrial cancer. *Nippon Sanka Fujinka Gakkai Zasshi* **40:**707–713.
Nagase T, Kikuno R, Hattori A, Hondo Y, Okamura K, and Ohara O (2000) Prediction of the coding sequences of unidentifed human genes. XIX. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. *DNA Res* **31:**347–355.
Naitoh K, Honjo H, Yamamoto T, Urabe M, Ogino Y, Yasumura T, and Nambara T (1984) Estrone sulfate and sulfatase activity in human breast cancer and endometrial cancer. *J Steroid Biochem* **33:**1049–1054.
Nakamura J, Lu Q, Aberdeen G, Albrecht E, and Brodie A (1999) The effect of estrogen on aromatase and vascular endothelial growth factor messenger ribonucleic acid in the normal nonhuman primate mammary gland. *J Clin Endocrinol Metab* **84:**1432–1437.
Nakamura J, Savinov A, Lu Q, and Brodie A (1996) Estrogen regulates vascular endothelial growth/permeability factor expression in 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors. *Endocrinology* **137:**5589–5596.
Ni H, Sun T, Ding N, Ma X, and Yang Z (2002) Differential expression of microsomal prostaglandin E synthase in implantation sites and decidual cells of mouse uterus. *Biol Reprod* **67:**351–358.
Noble LS, Simpson ER, Johns A, and Bulun SE (1996) Aromatase expression in endometriosis. *J Clin Endocrinol Metab* **81:**174–179.
Noble LS, Takayama K, Putman JM, Johns DA, Hinshelwood MM, Agarwal VR, Zhao Y, Carr BR, and Bulun SE (1997) Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. *J Clin Endocrinol Metab* **82:**600–606.
O'Neill JS, Elton RA, and Miller WR (1988) Aromatase activity in adipose tissue from breast quadrants: a link with tumor site. *Br Med J* **296:**741–743.
O'Neill JS and Miller WR (1987) Aromatase activity in breast adipose tissue from women with benign and malignant breast diseases. *Br J Cancer* **56:**601–604.
Ota H, Igarashi S, Sasaki M, and Tanaka T (2001) Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. *Hum Reprod* **16:**561–566.
Otubu JA, Buttram VC, Besch NF, and Besch PK (1982) Unconjugated steroids in leiomyomas and tumor-bearing myometrium. *Am J Obstet Gynecol* **143:**130–133.
Ozisik YY, Meloni AM, Altungoz O, Surti U, and Sandberg AA (1995) Translocation (6;10)(p21;q22) in uterine leiomyomas. *Cancer Genet Cytogenet* **79:**136–138.
Palomba S, Sena T, Morelli M, Noia R, Zullo F, and Mastrantonio P (2002) Effect of different doses of progestin on uterine leiomyomas in postmenopausal women. *Eur J Obstet Gynecol Reprod Biol* **102:**199–201.
Paridaens R, Dirix L, Lohrisch C, Beex L, Nooij M, Cameron D, Biganzoli L, Cufer T, Duchateau L, Hamilton A, et al. (2003) Mature results of a randomized phase II multicenter study of exemestane versus tamoxifen as first-line hormone therapy for postmenopausal women with metastatic breast cancer. *Ann Oncol* **14:**1391–1398.
Pasqualini JR, Cornier E, Grenier J, Vella C, Schatz B, and Netter A (1990) Effect of Decapeptyl, an agonistic analog of gonadotropin-releasing hormone on estrogens, estrogen sulfates and progesterone receptors in leiomyoma and myometrium. *Fertil Steril* **53:**1012–1017.
Pauley R, Santner S, Tait L, and Bright R (2000) Regulation of CYP19 aromatase transcription in breast stromal fibroblasts. *J Clin Endocrinol Metab* **85:**837–846.
Plourde P, Reiter E, Jou H, Desrochers P, Rubin S, Bercu B, Diamond FJ, and Backeljauw P (2004) Safety and efficacy of anastrozole for the treatment of pubertal gynecomastia: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab* **44:**28–33.
Potischman N, Hoover R, Brinton L, Siiteri P, Dorgan J, Swanson C, Berman M, Mortel R, Twiggs L, Barrett R, et al. (1996) Case-control study of endogenous steroid hormones and endometrial cancer. *J Natl Cancer Inst* **88:**1127–1135.
Price T, Aitken J, Head J, Mahendroo MS, Means GD, and Simpson ER (1992) Determination of aromatase cytochrome P450 messenger RNA in human breast tissues by competitive polymerase chain reaction (PCR) amplification. *J Clin Endocrinol Metab* **74:**1247–1252.
Razzi S, Fava A, Sartini A, De Simone S, Cobellis L, and Petraglia F (2004) Treatment of severe recurrent endometriosis with an aromatase inhibitor in a young ovariectomised woman. *BJOG* **111:**182–184.
Reed MJ, Topping L, Coldham NG, Purohit A, Ghilchik MW, and James VH (1993) Control of aromatase activity in breast cancer cells: the role of cytokines and growth factors. *J Steroid Biochem Mol Biol* **44:**589–596.
Rein MS, Barbieri RL, and Friedman AJ (1995) Progesterone: a critical role in the pathogenesis of uterine myomas. *Am J Obstet Gynecol* **172:**14–18.
Richards JA, Petrel TA, and Brueggemeier RW (2002) Signaling pathways regulating aromatase and cyclooxygenases in normal and malignant breast cells. *J Steroid Biochem* **80:**203–212.
Rose P, Brunetto V, VanLe L, Bell J, Walker J, and Lee R (2000) A phase II trial of anastrozole in advanced recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol* **78:**212–216.
Ruohola J, Valve E, Karkkainen M, and Joukov V (1999) Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. *Mol Cell Endocrinol* **149:**29–40.

Ryan I, Schriock ED, and Taylor R (1994) Isolation, characterization and comparison of human endometrial and endometriosis cells in vitro. *J Clin Endocrinol Metab* **78:**642–649.
Ryan IP and Taylor RN (1997) Endometriosis and infertility: new concepts. *Obstet Gynecol Surv* **52:**365–371.
Santen R (2002) To block estrogen's synthesis or action: that is the question. *J Clin Endocrinol Metab* **87:**3007–3012.
Santen RJ, Martel J, Hoagland M, Naftolin F, Roa L, Harada N, Hafer L, Zaino R, and Santner SJ (1994) Stromal spindle cells contain aromatase in human breast tumors. *J Clin Endocrinol Metab* **79:**627–632.
Sasano H, Edwards DP, Anderson TJ, Silverberg SG, Evans DB, Santen RJ, Ramage P, Simpson ER, Bhatnagar AS, and Miller WR (2003) Validation of new aromatase monoclonal antibodies for immunohistochemistry: progress report. *J Steroid Biochem Mol Biol* **86:**239–44.
Sasano H, Frost A, Saitoh R, Harada N, Poutanen M, Vihko R, Bulun S, Silverberg S, and Nagura H (1996) Aromatase and 17beta-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. *J Clin Endocrinol Metab* **81:**4042–4046.
Sasano H, Nagura H, Harada N, Goukon Y, and Kimura M (1994) Immunolocalization of aromatase and other steroidogenic enzymes in human breast disorders. *Hum Pathol* **25:**530–535.
Sasano H, Sato S, Ito K, Yajima A, Nakamura J, Yoshihama M, Ariga K, Anderson T, and Miller W (1999) Effects of aromatase inhibitors on the pathobiology of the human breast, endometrial and ovarian carcinoma. *Endocr Relat Cancer* **6:**197–204.
Schoenmakers EF, Huysmans C, and Van de Ven WJ (1999) Allelic knockout of novel splice variants of human recombination repair gene RAD51B in t(12;14) uterine leiomyomas. *Cancer Res* **59:**19–23.
Sebastian S and Bulun SE (2001) A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the human genome project. *J Clin Endocrinol Metab* **86:**4600–4602.
Sebastian S, Takayama K, Shozu M, and Bulun S (2002) Cloning and characterization of a novel endothelial promoter of the human CYP19 (aromatase P450) gene that is up-regulated in breast cancer tissue. *Mol Endocrinol* **16:**2243–2254.
Shekhar M, Werdell J, and Tait L (2000) Interaction with endothelial cells is a prerequisite for branching ductalalveolar morphogenesis and hyperplasia of preneoplastic human breast epithelial cells: regulation by estrogen. *Cancer Res* **60:**439–449.
Sherman M, Sturgeon S, Brinton L, Potischman N, Kurman R, Berman M, Mortel R, Twiggs L, Barrett R, and Wilbanks G (1997) Risk factors and hormone levels in patients with serous and endometrioid uterine carcinomas. *Mod Pathol* **10:**963–968.
Shippen E and West WJ (2004) Successful treatment of severe endometriosis in two premenopausal women with an aromatase inhibitor. *Fertil Steril* **81:**1395–1398.
Shozu M, Murakami K, Segawa T, Kasai T, and Inoue M (2003a) Successful treatment of a symptomatic uterine leiomyoma in a perimenopausal woman with a nonsteroidal aromatase inhibitor. *Fertil Steril* **79:**628–31.
Shozu M, Sebastian S, Takayama K, Hsu WT, Schultz RA, Neely K, Bryant M, and Bulun SE (2003b) Estrogen excess associated with novel gain-of-function mutations affecting the aromatase gene. *N Engl J Med* **348:**1855–65.
Shozu M, Sumitani H, Segawa T, Yang HJ, Murakami K, and Inoue M (2001) Inhibition of in situ expression of aromatase P450 in leiomyoma of the uterus by leuprorelin acetate. *J Clin Endocrinol Metab* **86:**5405–5411.
Shozu M, Sumitani H, Segawa T, Yang HJ, Murakami K, Kasai T, and Inoue M (2002) Overexpression of aromatase P450 in leiomyoma tissue is driven primarily through promoter I. 4 of the aromatase P450 gene (CYP19). *J Clin Endocrinol Metab* **87:**2540–8.
Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, Speed C, and Jones M (2002) Aromatase—a brief overview. *Annu Rev Physiol* **64:**93–127.
Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, et al. (1994) Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev* **15:**342–355.
Soysal S, Soysal M, Ozer S, Gul N, and Gezgin T (2004) The effects of post-surgical administration of goserelin plus anastrozole compared to goserelin alone in patients with severe endometriosis: a prospective randomized trial. *Hum Reprod* **19:**160–167.
Sreekantaiah C, Ladanyi M, Rodriguez E, and Chaganti RS (1994) Chromosomal aberrations in soft tissue tumors. Relevance to diagnosis, classification and molecular mechanisms. *Am J Pathol* **144:**1121–1134.
Stewart EA (2001) Uterine fibroids. *Lancet* **357:**293–298.
Stratakis CA, Vottero A, Brodie A, Kirschner LS, DeAtkine D, Lu Q, Yue W, Mitsiades CS, Flor AW, and Chrousos GP (1998) The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription. *J Clin Endocrinol Metab* **83:**1348–1357.
Sumitani H, Shozu M, Segawa T, Murakami K, Yang HJ, Shimada K, and Inoue M (2000) In situ estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. *Endocrinology* **141:**3852–3861.
Sun HS, Hsiao KY, Hsu CC, Wu MH, and Tsai SJ (2003) Transactivation of steroidogenic acute regulatory protein in human endometriotic stromal cells is mediated by the prostaglandin EP2 receptor. *Endocrinology* **144:**3934–3942.
Sun T, Li S, Diao H, Teng C, Wang H, and Yang Z (2004) Cyclooxygenases and prostagaldin E synthases in the endometrium of the rhesus monkey during the menstrual cycle. *Reproduction* **127:**465–473.
Takayama K, Zeitoun K, Gunby RT, Sasano H, Carr BR, and Bulun SE (1998) Treatment of severe postmenopausal endometriosis with an aromatase inhibitor. *Fertil Steril* **69:**709–713.
Tamura M, Deb S, Sebastian S, Kunihiro O, and Bulun S (2004) Estrogen up-regulates cyclooxygenase-2 via estrogen receptor in human uterine microvascular endothelial cells. *Fertil Steril* **81:**1351–1356.
Tamura M, Sebastian S, Tang S, Gurates B, Ferrer K, Sasano H, and Bulun SE (2002a) Up-regulation of cyclooxygenase-2 expression and prostaglandin synthesis in endometrial stromal cells by malignant endometrial epithelial cells: a paracrine mechanism mediated by PGE2 and nuclear factor-KB. *J Biol Chem* **277:**26208–26216.
Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, and Bulun SE (2002b) Interleukin-1beta elevates cyclooxygenase-2 protein level and enzyme activity via increasing its mRNA stability in human endometrial stromal cells: an effect mediated by extracellularly regulated kinase 1 and 2. *J Clin Encrinol Metab* **87:**3263–3273.
Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, and Bulun SE (2002c) Vascular endothelial growth factor upregulates cyclooxygenase-2 expression in human endothelial cells. *J Clin Endocrinol Metab* **87:**3504–3507.
Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, Okamura K, and Bulun S (2003) Induction of cyclooxygenase-2 in human endometrial stromal cells by malignant endometrial epithelial cells: evidence for the involvement of extracellularly regulated kinases and CCAAT/enhancer binding proteins. *J Mol Endocrinol* **31:**95–104.
Tekmal R, Kirma N, Gill K, and Fowler K (1999) Aromatase overexpression and breast hyperplasia, an in vivo model—continued overexpression of aromatase is sufficient to maintain hyperplasia without circulating estrogens and aromatase inhibitors abrogate these preneoplasatic changes in mammary glands. *Endocr Relat Cancer* **6:**307–314.
Tsai SJ, Wu MH, Lin CC, Sun HS, and Chan HM (2001) Regulation of steroidogenic acute regulatory protein expression and progesterone production in endometriotic stromal cells. *J Clin Endocrinol Metab* **86:**5765–5773.
Tseng JF, Ryan IP, Milam TD, Murai JT, Schriock ED, Landers DV, and Taylor RN (1996) Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metab* **81:**1118–1122.
Utsumi T, Harada N, Maruta M, and Takagi Y (1996) Presence of alternatively spliced transcripts of aromatase gene in human breast cancer. *J Clin Endocrinol Metab* **81:**2344–2349.
Van de Ven WJ (1998) Genetic basis of uterine leiomyoma: involvement of high mobility group protein genes. *Eur J Obstet Gynecol Reprod Biol* **81:**289–293.
van der Heijden O, Chiu HC, Park TC, Takahashi H, LiVolsi VA, Risinger JI, Barrett JC, Berchuck A, Evans AC, Behbakht K, et al. (1998) Allelotype analysis of uterine leiomyoma: localization of a potential tumor suppressor gene to a 4-cM region of chromosome 7q. *Mol Carcinog* **23:**243–247.
Van Landeghem AAJ, Poortman J, Nabuurs M, and Thijssen JHH (1985) Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. *Cancer Res* **45:**2907–2912.
Vercellini P, Cortesi I, and Crosignani P (1997) Progestins for symptomatic endometriosis: a critical analysis of the evidence. *Fertil Steril* **68:**393–401.
Vermeulen-Meiners C, Poortman J, Haspels A, and Thijssen J (1986) The endogenous concentration of estradiol and estrone in pathological human postmenopausal endometrium. *J Steroid Biochem* **24:**1073–1078.
Walker CL (2002) Role of hormonal and reproductive factors in the etiology and treatment of uterine leiomyoma. *Recent Prog Horm Res* **57:**277–94.
Watanabe K, Sasano H, Harada N, Ozaki M, Niikura H, Sato S, and Yajima A (1995) Aromatase in human endometrial carcinoma and hyperplasia. Immunohistochemical, in situ hybridization and biochemical studies. *Am Jpathol* **146:**491–500.
Wu M, Wang C, Lin C, Chen L, Chang W, and Tsai S (2004) Distinct regulation of cyclooxygenase-2 by interleukin-1β in normal and endometriotic stromal cells. *J Clin Endocrinol Metab* **90:**286–295.
Yamamoto T, Fukuoka M, Fujimoto Y, Kitawaki J, Nakakoshi M, Yoshihama M, and Okada H (1990) Inhibitory effect of a new androstenedione derivative, 14 alpha-hydroxy-4-androstene-3,6,17-trione (14 alpha-OHAT) on aromatase activity of human uterine tumors. *J Steroid Biochem* **36:**517–521.
Yamamoto T, Kitawaki J, Urabe M, Honjo H, Tamura T, Noguchi T, Okada H, Sasaki H, Tada A, Terashima Y, et al. (1993) Estrogen production of endometrium and endometrial cancer tissue; influence of aromatase on proliferation of endometrial cancer cells. *J Steroid Biochem Mol Biol* **44:**463–468.
Yamamoto T, Takamori K, and Okada H (1985) Effect of aminoglutethimide on androstenedione aromatase activity in human uterine leiomyoma. *Horm Metab Res* **17:**548–549.
Yang S, Fang Z, Takashi S, Hironobu S, Jianfeng Z, Gurates B, Tamura M, Ferrer K, and Bulun SE (2002) Regulationof aromatase P450 expression in endometriotic and endometrial stromal cells by CCAT/enhancer binding proteins: decreased C/EBPbeta in endometriosis is associated with overexpression of aromatase. *J Clin Endocrinol Metab* **87:**2336–2345.
Yue W, Zhou D, Chen S, and Brodie A (1994) A new nude mouse model for postmenopausal breast cancer using MCF-7 cells transfected with the human aromatase gene. *Cancer Res* **54:**5092–5095.
Zeitoun K, Takayama K, Michael MD, and Bulun SE (1999) Stimulation of aromatase P450 promoter (II) activity in endometriosis and its inhibition in endometrium are regulated by competitive binding of SF-1 and COUP-TF to the same cis-acting element. *Mol Endocrinol* **13:**239–253.
Zeitoun KM and Bulun SE (1999) Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. *Fertil Steril* **72:**961–969.
Zhao Y, Agarwal VR, Mendelson CR, and Simpson ER (1996a) 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:**5739–42.
Zhao Y, Nichols JE, Bulun SE, Mendelson CR, and Simpson ER (1995) Aromatase P450 gene expression in human adipose tissue: Role of a Jak/STAT pathway in regulation of the adipose-specific promoter. *J Biol Chem* **270:**16449–16457.
Zhao Y, Nichols JE, Valdez R, Mendelson CR, and Simpson ER (1996b) Tumor necrosis factor-alpha stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter I. 4. *Mol Endocrinol* **10:**1350–1357.
Zhou C, Zhou D, Esteban J, Murai J, Siiteri PK, Wilczynski S, and Chen S (1996) Aromatase gene expression and its exon I usage in human breast tumors. Detection of aromatase messenger RNA by reverse transcription-polymerase chain reaction. *J Steroid Biochem Mol Biol* **59:**163–171.
Zhou J, Gurates B, Yang S, Sebastian S, and Bulun SE (2001) Malignant breast epithelial cells stimulate aromatase expression via promoter II in human adipose fibroblasts: an epithelial-stromal interaction in breast tumors mediated by C/EBPbeta. *Cancer Res* **61:**2328–2334.