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Aromatase excess in cancers of breast, endometrium and ovary☆

Serdar E. Bulun*, Dong Chen, Meiling Lu, Hong Zhao, Youhong Cheng, Masashi Demura, Bertan Yilmaz, Regina Martin, Hiroki Utsunomiya, Steven Thung, Emily Su, Erica Marsh, Amy Hakim, Ping Yin, Hiroshi Ishikawa, Sanober Amin, Gonca Imir, Bilgin Gurates, Erkut Attar, Scott Reierstad, Joy Innes, Zhihong Lin

Robert H. Lurie Comprehensive Cancer Center and Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Northwestern University, Chicago, IL 60611, United States

Abstract

Pathogenesis and growth of three common women's cancers (breast, endometrium and ovary) are linked to estrogen. A single gene encodes the key enzyme for estrogen biosynthesis named aromatase, inhibition of which effectively eliminates estrogen production in the entire body. Aromatase inhibitors successfully treat breast cancer, whereas their roles in endometrial and ovarian cancers are less clear. Ovary, testis, adipose tissue, skin, hypothalamus and placenta express aromatase normally, whereas breast, endometrial and ovarian cancers overexpress aromatase and produce local estrogen exerting paracrine and intracrine effects. Tissue-specific promoters distributed over a 93-kb 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. In cancers of breast, endometrium and ovary, aromatase expression is primarly regulated by increased activity of the proximally located promoter I.3/II region. Promoters I.3 and II lie 215 bp from each other and are coordinately stimulated by PGE_2 via a cAMP-PKA-dependent pathway. In breast adipose fibroblasts exposed to PGE_2 secreted by malignant epithelial cells, PKC is also activated, and this potentiates cAMP-PKA-dependent induction of aromatase. Thus, inflammatory substances such as PGE_2 may play important roles in inducing local production of estrogen that promotes tumor growth.

Keywords: Aromatase; Aromatase inhibitor; Breast cancer; Endometrial cancer; Endometriosis; Uterine leiomyomata; Fibroids; Aromatase overexpression; Gain-of-function mutations; Aromatase excess syndrome; Gynecomastia; Estrogen; Estrogen biosynthesis; Endometrium; Uterus

1. Physiological regulation of aromatase expression in human tissues

1.1. The aromatase enzyme

The aromatase enzyme is localized in the endoplasmic reticulum of estrogen-producing cells [1,2]. Aromatase enzyme complex is comprised of two polypeptides. The first of these is a specific cytochrome P450, namely aromatase cytochrome P450 (the product of the *CYP19* gene) [1]. The second is a flavoprotein, NADPH-cytochrome P450 reductase and is ubiquitously distributed in most cells. Thus, cell-specific expression of aromatase P450 (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 [1].

In the human, aromatase is expressed in a number of cells including the ovarian granulosa cell, the placental syncytiotrophoblast, the testicular Leydig cell, as well as various extraglandular sites including the brain and skin fibroblasts [3]. The principal product of the ovarian granulosa cells during the follicular phase is estradiol. Additionally, aromatase is expressed in human adipose tissue. Whereas the highest

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* Corresponding author at: Department of Obstetric and Gynecology, Northwestern University, 303 E. Superior Street, Suite 4-123, Chicago, IL 60611, United States. Tel.: +1 312 503 0520; fax: +1 312 503 0095.
E-mail address: s-bulun@northwestern.edu (S.E. Bulun).

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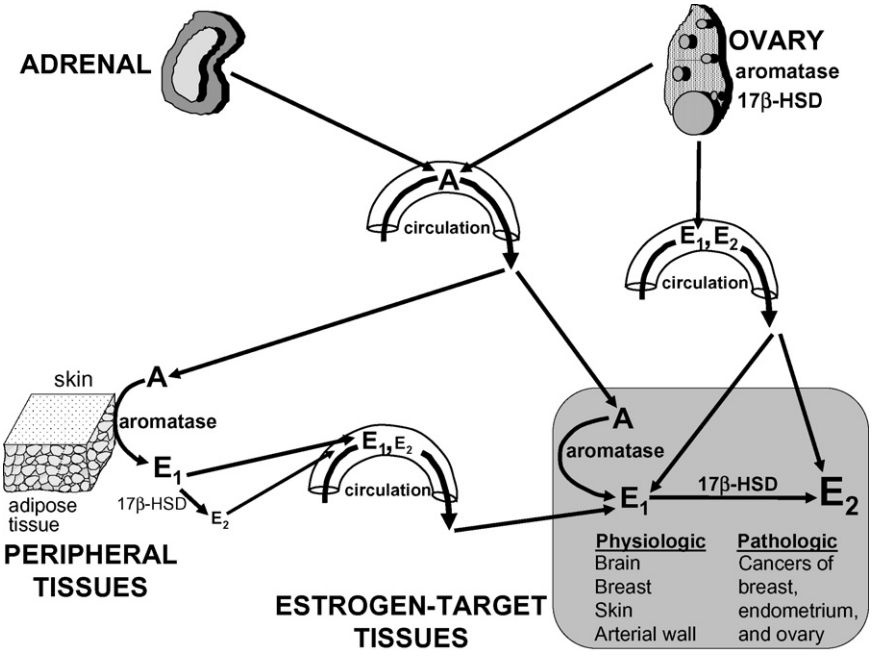

Fig. 1. Tissue sites of estrogen production in women. The biologically active estrogen, estradiol (E_2) is produced at least in three major sites: (1) direct secretion from the ovary in reproductive-age women; (2) by conversion of circulating androstenedione (A) of adrenal and/or ovarian origins to estrone (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-hydroxysteroid dehydrogenase (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.

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. 1) [4,5]. 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. 1) [6].

1.2. The CYP19 (aromatase) gene

The aromatase 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 9 exons (II-X) [7]. The ATG translation start site is located in coding exon II. The upstream (telomeric) 93 kb of the gene contains a number of promoters [2,3]. 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 to be located within the 1-kb region upstream of the ATG translation start site in exon II, as expected (Fig. 2). 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. 2) [2,8]. 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 [8]. 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. 2). 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 [8]. Consequently, the sequence encoding the open reading frame is identical in each case. Thus, the expressed protein is the same regardless of the splicing pattern (Fig. 2).

1.3. Normal hormonal pathways that regulate aromatase expression

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 [3]. Ovarian aromatase expression is mediated primarily by FSH receptors, cAMP production and activation