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Review

Transcriptional control of local estrogen formation by aromatase in the breast

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ABSTRACT

Aromatase is the critical enzyme that converts androgens to estrogens. It is frequently highly expressed in the tumour bearing breast of women diagnosed with estrogen receptor positive tumours, resulting in dramatically increased local estrogen production to drive tumour progression. Expression of aromatase is regulated primarily at the transcriptional level of its encoding gene *CYP19A1*, located on chromosome 15 of the human genome. A characteristic feature of *CYP19A1* expression is its use of alternative promoters to regulate transcription in a tissue-specific manner. In breast cancer, the increase in aromatase expression is mediated *via* higher expression of the distal adipose-specific promoter I.4 and a switch to the preferential use of proximal promoters I.3 and II. This results in a net increase of *CYP19A1* transcripts in tumour-bearing breast up to 3–4-fold higher than normal breast. Current aromatase inhibitors – whilst efficacious – exhibit significant side effects that reduce patient compliance. Understanding the transcription factors and signalling pathways that control aromatase expression will lead to opportunities to develop breast-specific inhibitors with an improved side-effects profile.

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Contents

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1. Introduction

Estrogens, the primary female sex hormone, are responsible for key physiological processes related to the growth, differentiation and physiology of the reproductive system, and also have significant effects on bone, liver, brain and cardiovascular systems [1]. They are also implicated in a number of pathologic disease states, including breast, ovarian, endometrial, colon and prostate cancers [2–4]. In premenopausal women, estrogen is produced in the ovary where it then circulates in the body in an endocrine fashion. However, upon cessation of ovarian function in post-menopausal women, extragonadal sites including the brain, bone, skin fibroblasts and adipose tissue produce estrogen which then acts in a local paracrine manner [5].

High systemic levels of estrogen is a major risk factor for developing breast cancer, with up to 70% of post-menopausal breast cancers classified as estrogen-receptor alpha positive (ER+), dependent on the hormone for continued growth and proliferative advantage [6,7]. Tumours may derive estrogen from circulation, but the primary source of estrogen in postmenopausal women is *via* local production by undifferentiated breast adipose fibroblasts (BAFs) surrounding malignant cells [8–10]. In fact, estrogen concentrations within the local breast tumour environment are up to ten times higher than circulating levels [11]. Increased activity of aromatase in breast cancer is one of the primary mechanisms leading to increased local estrogen production in breast cancer, and as such this enzyme is of major therapeutic interest. As the rate-limiting step in the conversion of androgens to estrogens, aromatase performs a critical function in maintaining estrogen excess in ER+ breast cancers. Regulated largely at the transcriptional level by its encoding gene *CYP19A1*, it is the target of aromatase inhibitors (AIs) which have come into mainstream use for ER+ breast cancer therapy [12,13]. An understanding of aromatase regulation, and reasons for the observed increase in activity in breast cancer, is critical for the development of breast-specific inhibitors which aim to minimise the side-effects seen with current AI therapies where estrogen production is inhibited throughout the body.

This review will outline the aromatase enzyme and its role in steroid hormone biosynthesis as well as lipid metabolism. The structure of the gene encoding aromatase, *CYP19A1*, and the key transcriptional promoters will be examined. Finally, research in the use of AIs for ER+ breast cancer therapy will be examined, discussing how our developing understanding of *CYP19A1* transcriptional regulation can be harnessed to design more specific and potent inhibitors.

2. The aromatase enzyme and its role in intracrine estrogen production

Aromatase catalyses the final and rate-limiting step of estrogen biosynthesis. It is localised in the endoplasmic reticulum of estrogen producing cells. In humans, aromatase is expressed in a number of tissues including ovarian granulosa cells [14], placental syncytiotrophoblast [15], bone [16], brain [17], skin fibroblasts [18] and adipose tissue mesenchymal cells [19]. The highest levels of aromatase are in the ovarian granulose cells in premenopausal women, however, adipose tissue becomes the major site of aromatase expression after menopause [20,21].

The aromatase enzyme complex is comprised of two polypeptides: aromatase cytochrome P450 (P450arom), and a flavoprotein, NADPH-cytochrome P450 reductase [22]. It is the presence of the first polypeptide, P450arom, in cells that governs the presence of estrogen. The biosynthesis of estrogen occurs through binding of the aromatase enzyme to C_{19} androgenic steroid substrates and catalysing the aromatase reaction leading to the formation of the phenolic A ring which is characteristic of biological estrogens [23,24].

Although the reproductive effects of estrogen in females have been well understood for decades, the recent development of knockout mouse models has identified hitherto unexpected functions of estrogen in both males and females. The aromatase knockout (ArKO) mouse contains targeted disruptions of the *Cyp19* gene, causing almost a complete abolition of estrogen production in homozygous animals [25]. Aberrations were seen in both male and female ArKO mice including; reproductive and bone phenotypes and accumulation of excess adipose tissue. In the female ArKO mouse, there were profound effects on the reproductive system including; underdeveloped external genitalia and uteri, and ovulation failure leading to infertility [26]. The ArKO male mice showed immature sexual behaviour and disrupted spermatogenesis, which combined resulted in reduced fertility [27]. Both sexes of the ArKO mouse had increased adiposity, demonstrating the importance of aromatase activity in lipid metabolism [28]. Skeletal abnormalities were also observed in the ArKO mouse, with a decrease in bone mass of both males and females [29].

Aromatase deficiency also occurs in humans with mutations in the gene encoding for aromatase. The inability of the placenta to convert androgens to estrogens in the aromatase deficient female foetus results in excess androgens *in utero* and thus these newborns have ambiguous genitalia [30]. At puberty, these individuals fail to develop breasts and initiate menstruation, have cystic ovaries and delayed bone age. However, most female patients are supplemented with estrogen which leads to regression of these symptoms [31–34]. In contrast, prepubertal development for aromatase deficient males are normal, however they do exhibit symptoms later on in life such as obesity and associated metabolic disorders, osteoporosis, failure of epiphysial fusion with resulting tall stature, impaired fertility and loss of libido, consistent with the phenotypes of ArKO mice [35–38].

Aromatase excess as a result of genetic alterations has also been clinically reported, whereby males presented with prepubertal gynecomastia [39] and females undergo premature onset of puberty, had an enlarged uterus and irregularities of the menstrual cycle [40,41]. Both males and females present with premature growth spurt, early fusion of growth plates and as a result decreased adult height. In adulthood, aromatase excess male patients are undervirilised and adult female patients experience irregular uterine bleeding. These phenotypes are the result of excessive conversion of androgens into estrogens and patients are effectively treated with aromatase inhibitors [42]. Combined clinical reports from aromatase excess and deficient patients demonstrate the importance of estrogen in numerous non-reproductive functions and hence the role of the aromatase enzyme.

3. The *CYP19A1* gene and its regulation in the breast

Aromatase is encoded by the *CYP19A1* gene, located on chromosome 15, band q21 of the human genome [43]. The full length of *CYP19A1* is 123 kb [44], of which the coding region accounts for approximately 30 kb [42]. The upstream 93 kb contains eight tissue-specific promoters that control the highly tissue-specific expression of *CYP19A1*, corresponding to sites of estrogen production (Fig. 1 and Table 1). Differential use of these promoters in different tissues gives rise to tissue specificity, and this may be detected through exon-specific RT-PCR [8]. Promoters are transcribed and spliced into a common junction immediately upstream of the ATG translational start site, resulting in the same aromatase protein being produced despite transcripts with differing 5′ untranslated regions [45].