Regulation of aromatase expression in human tissues

Serdar E. Bulun and Evan R. Simpson
Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Departments of Obstetrics/Gynecology and Biochemistry, University of Texas Southwestern Medical Center at Dallas, Texas, USA

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Summary

Extraglandular conversion of C₁₉ steroids to estrogens takes place primarily in the stromal cell compartments of adipose tissue and is catalyzed by aromatase cytochrome P450 (P450arom, the product of the CYP19 gene). CYP19 gene expression and aromatase activity in breast adipose stromal cells in culture are subject to complex hormonal regulation, which was recently found to be mediated in part by alternative use of tissue-specific promoters of the CYP19 gene. It has been proposed that increased local aromatase activity in breast adipose tissue may influence the growth of breast carcinomas. Using competitive RT-PCR, we quantified P450arom transcripts in breast adipose tissue from mastectomy specimens. In 10 out of 15 patients, the highest transcript levels were found in the quadrant where the tumor was located. We also found the highest proportions of adipose stromal cells vs. adipocytes in these quadrants. These findings suggest that regional differences in the relative proportions of the histologic components give rise to local elevated concentrations of estrogens. Although the initiating events are not known, once a neoplastic change has occurred, tumor growth may be promoted by these locally increased estrogen levels. We are currently investigating alternative promoter use for CYP19 gene transcription to explain this association. Our results underscore the importance of aromatase inhibitors as effective agents in treatment of hormone-responsive breast cancer, since aromatase inhibitors reduce local aromatase activity as well as blood estradiol levels.

Introduction

The conversion of C₁₉ steroids to estrogens is catalyzed by the enzyme complex named aromatase, which comprises a specific form of cytochrome P450 (P450arom, the product of the CYP19 gene) and a flavoprotein, NADPH-cytochrome P450 reductase, which is a ubiquitous component of most cells [1,2]. Aromatase expression occurs in a number of human tissues and cell types, including syncytiotrophoblast of placenta [3], hydatid moles [4], JEG-3 cells (a choriocarcinoma-derived cell line) [5], fetal hepatocytes [6], ovarian granulosa cells [7], and testicular Leydig cells [8]. Additionally, in other species, the activity was shown to be present in Sertoli [9], Leydig [10,11], and germ cells [12] in the male, and in several sites in the brain of both sexes [13]. In addition, aromatase activity and P450arom transcripts have been detected in both

Address for correspondence and offprints: Serdar E. Bulun, M.D., Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9051, USA; Tel: 214-648-3260; Fax: 214-648-8683
breast adipose tissue and breast tumor tissue [14-20]. Adipose tissue is the principal site of estrogen formation in postmenopausal women [21,22]. Estrogen production by adipose tissue increases as a function of aging and obesity. The increased estrogen production in elderly obese women is believed to play a role in the pathogenesis of endometrial cancer. Furthermore, estrogen produced by adipose tissue within the breast may act locally to promote the growth of breast carcinomas [16,23,24]. The usefulness of estrogen antagonists as well as inhibitors of aromatase in the management of breast cancer has long been recognized. The implication of adipose tissue estrogen biosynthesis in the maintenance of growth of breast cancer is apparent from the palliative effects of adrenalectomy. Since estrogen production by adipose tissue is dependent for substrate on circulating androstenedione produced by the adrenal cortex, the role of adrenalectomy is explicable in terms of the denial of substrate precursor for adipose tissue estrogen biosynthesis. It should be pointed out that the product of aromatase activity in adipose tissue, namely estrone, is less estrogenic than estradiol. Evidence from at least two laboratories indicates that 17β-hydroxysteroid dehydrogenase present in breast tumor tissue is capable of locally converting estrone into estradiol [25,26].

Fat distribution may be a contributing factor in the etiology of breast cancer. Increased waist-to-hip ratio in women was implicated as a risk factor for breast cancer [27]. However, conflicting results were published regarding this issue [28]. Our findings are in agreement with DeRidder et al [29], in that the adipose tissue of the buttocks contains much higher levels of P450arom transcripts than that of the abdomen or thighs [30]. Therefore, even if postmenopausal women with central obesity are at a higher risk, this should not be due to increased circulating estrogens produced in the adipose tissue.

Initially aromatase activity and subsequently P450arom transcripts have been detected both in breast adipose tissue and in tumor tissue [14,15,17-20]. It has been proposed that the growth of breast carcinomas may be influenced by local estrogen biosynthesis in surrounding adipose tissue [16]. We previously demonstrated that aromatase activity in adipose stromal cells in culture is regulated primarily by changes in the level of mRNA encoding P450arom [31]. More recently, we have investigated the expression of the CYP19 (P450arom) gene in breast adipose tissue as a function of proximity to a tumor and in breast cancer tissue per se, using a RT-PCR amplification procedure employing an internal standard to correct for variation in amplification from sample to sample.

**Regulation of aromatase expression in human adipose tissue**

We have utilized human adipose stromal cells in culture as a model system to study the regulation of aromatase activity in human fat, having determined that aromatase activity and P450arom mRNA levels are much higher in the stromal elements of adipose tissue, i.e. the potential adipose precursor cells, than in adipocytes themselves [14,17]. In these stromal cells, we have observed that aromatase expression is subject to complex and multifactorial regulation which is correlated with comparable changes in the levels of P450arom mRNA [31,32]. In cultured human adipose stromal cells, aromatase activity is stimulated by glucocorticoids [33] and by cyclic AMP analogs [34]. The stimulatory effects of cyclic AMP are potentiated by phorbol esters [35] which activate protein kinase C, and inhibited on the other hand by serum, as well as by a number of growth factors including EGF, TGFβ-1, TGFα, interleukin 1β, TNF, bFGF, and PDGF [35]. By contrast, the stimulatory action of glucocorticoids requires the presence of serum, but this action of serum can be mimicked in part by growth factors such as PDGF, as well as by phorbol esters. The actions of these various stimulatory and inhibitory factors on enzyme activity are paralleled by