Estrogens, Obesity, Inflammation, and Breast Cancer—What Is the Link?

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Abstract

It gives me great pleasure to contribute to this special issue of Seminars which honors the career of Bruce Carr. As it happens, Bruce was my first Fellow upon my arrival at the Green Center for Reproductive Biology Sciences at UT Southwestern Medical Center in 1977. At that time, the Center was filled with luminaries of Reproductive Endocrinology, such as John Porter, Jack Johnston, Norman Gant, and of course the Director, Paul MacDonald, so to be given the responsibility of mentoring a new Fellow was a daunting responsibility. However, Bruce quickly rolled up his sleeves and plunged straight in, and we forged a relationship which led to some 36 manuscripts in 4 years. The first of these was entitled "The Role of Serum Lipoproteins in Steroidogenesis by the Human Fetal Adrenal Cortex," published in the *Journal of Clinical Endocrinology and Metabolism*, volume 49, pages 146–148, in 1979, and the authors were Simpson ER, Carr BR, Parker CR Jr, Milewich L, Porter JC, and MacDonald PC. Bruce quickly moved up the ranks of the Obstetrics/Gynecology Department to become full Professor and we went our separate ways professionally, but we remain close friends to this day. This special issue is indeed a worthy tribute to an outstanding career and especially to Bruce's role as editor-in-chief of Seminars which he has guided through the rapid evolution of the specialty, always maintaining a strong research focus and thus carrying on the rich tradition of the Green Center and the Obstetrics/Gynecology Department.

Keywords
► estrogens
► obesity
► inflammation
► breast cancer

Our work on breast cancer concerns the role of local aromatase expression in the breast as the source of estrogen driving breast cancer development in the postmenopausal woman.[1,2] Following the menopausal transition, the ovaries cease to make estrogens, which then becomes the responsibility of extragonadal sites such as breast, bone, and brain. Work from our laboratory and others indicates that estrogen produced in these sites acts locally rather than systemically in a paracrine and intracrine fashion.[3,4] Thus, in the case of the breast, our goal has been to define the mechanisms whereby aromatase expression is regulated within the breast, with the translational goal of developing new breast-specific inhibitors of aromatase expression as improved endocrine therapy for breast cancer treatment and prevention.

Aromatase in the Breast, Aging, and Breast Cancer

Within the breast, aromatase expression occurs in the adipose mesenchymal cells and increases fourfold in the presence of a tumor.[3] This occurs in conjunction with differential promoter usage such that the gonadal proximal promoter PII dominates relative to the promoter I.4.[5–7] We and others have shown that this is because inflammatory factors such as prostaglandin E_2 produced by the tumorous epithelium

activate aromatase expression in the surrounding breast adipose stromal cells via the EP2 receptor, which results in stimulation of adenylyl cyclase, and the EP1 receptor, which stimulates DAG and IP3 formation.[8,9] It is also an absolute requirement for a monomeric orphan member of the nuclear receptor family to bind to a nuclear receptor half site downstream of the CREs on the aromatase promoter PII, namely, LRH-1.[10,11] In addition, we have shown that a powerful coactivator of LRH-1 is PGC1α[12] and that the expression of PGC1α is stimulated 10-fold by the cyclic AMP pathway. Hence, these stimulatory pathways work in concert to facilitate tumor-driven aromatase expression within the breast mesenchyma, providing an important example of epithelial–mesenchymal interactions working to facilitate tumor development (►Fig. 1).

Our recent major interest is to understand the link between obesity and breast cancer. There is now substantial epidemiological evidence to support the conclusion that obesity is linked to the increased risk of several forms of cancer such as colon, endometrial, and breast cancer.[13,14] Given the obesity problem worldwide, the potential significance of this conclusion is that tens of millions more women may develop breast cancer in their senior years than was previously believed to be the case. The problem is compounded by the fact that breast cancer risk increases with aging. In postmenopausal life, this appears to be due primarily to an increased capacity of adipose tissue to synthesize estrogens as a function of age[15,16]; however, the mechanism of this increase is not entirely understood at this time.[17,18] While it is facile to say that obesity may be reversed or prevented by healthy diet and exercise, most individuals who are obese find it difficult to achieve permanent loss of weight by these methods; hence, other therapeutic interventions are required to stave off a global epidemic of breast cancer arising from the obesity pandemic.

At this time, the cellular and molecular mechanisms underlying the increased risk of breast cancer associated with obesity and aging are incompletely understood. The AMP-activated protein kinase (AMPK) is now recognized to be a master regulator of energy homeostasis and the nexus for the convergence of endocrine signals including leptin, adiponectin, estradiol, androgens, and phytoestrogens.[19–21] AMPK activity is regulated primarily through phosphorylation at T172(α) by the upstream kinases LKB-1 and CaMKK; however, in most tissues LKB-1 appears to predominate. Furthermore, phosphorylation of the α-catalytic subunit of AMPK at S485 (α1) or S491 (α2) by PKA reduces its catalytic activity.[22] The possibility of a link between the LKB-1/AMPK pathway and aromatase expression in the breast arose from an unexpected source, namely, the rare condition of Peutz–Jeghers syndrome. Boys with this condition develop florid gynecomastia by the age of 6 or 7 years associated with the formation of Sertoli cell tumors. We studied such tumors some years ago and showed that they have very high rates of aromatase expression driven by promoter II thus explaining the gynecomastia in these boys.[23] The link with the LKB-1/AMPK pathway was revealed when it was shown that Peutz–Jeghers syndrome was due to mutations in the LKB-1 gene.[24]

Recently, a new family of CREB coactivators called CREB-regulated transcription coactivators (CRTCs; previously known as TORCs) has been shown to increase the expression of cyclic AMP responsive genes. When AMPK is active, CRTCs are sequestered in the cytoplasm due to phosphorylation by AMPK on S171 and binding to 14–3-3. In the absence of AMPK activity, CRTC2 is dephosphorylated and translocates to the nucleus where it associates with CREB and increases target
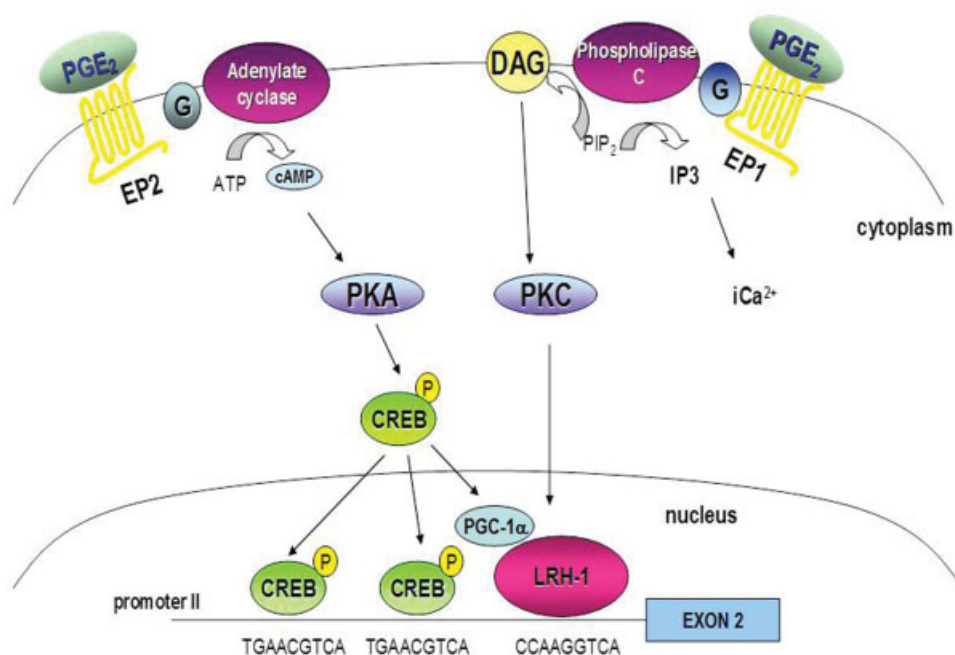

Fig. 1 Diagram of the role of PGE2 in the regulation of aromatase expression in human breast adipose stromal cells.
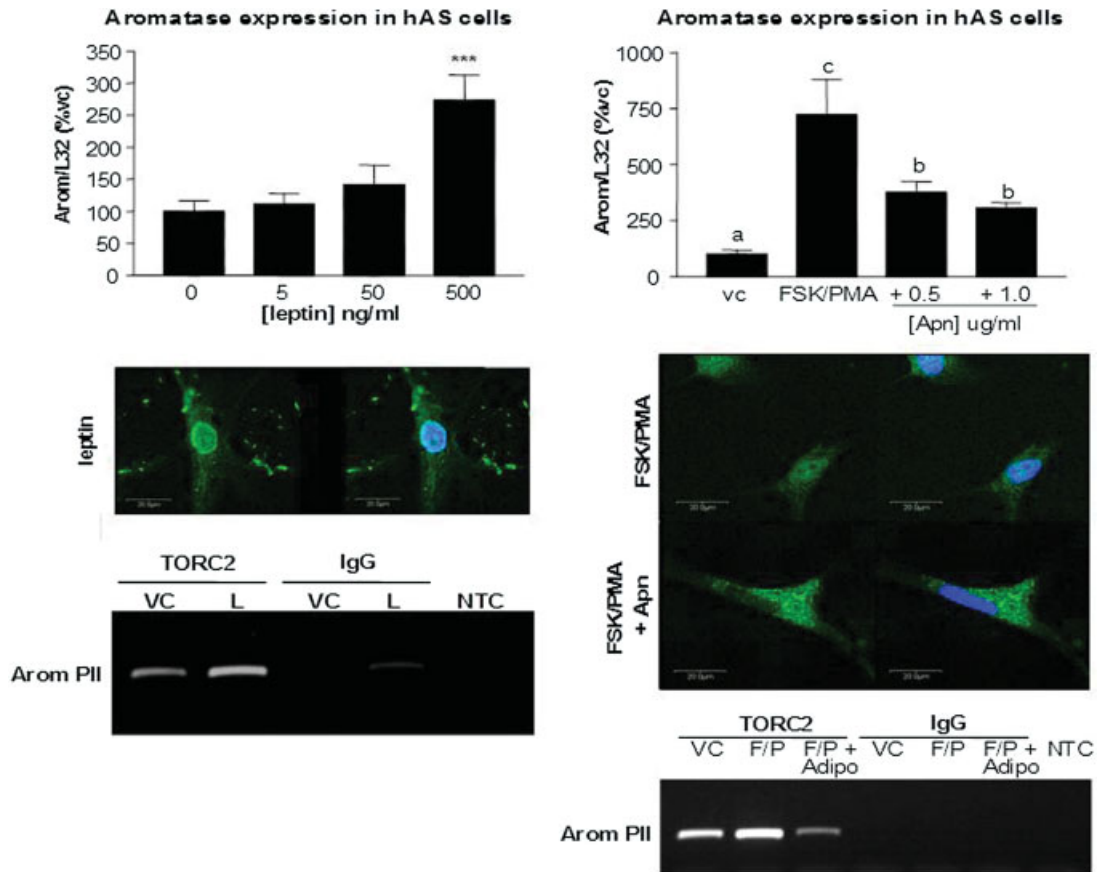
Fig. 2 Action of leptin to stimulate, and adiponectin to inhibit, aromatase expression in human breast adipose stromal cells.

gene expression.[25] We have shown that this is true in the case of aromatase in breast stromal cells. This provides a mechanism whereby the LKB-1/AMPK pathway can inhibit expression of aromatase in the breast. Furthermore, we have shown that leptin stimulates and adiponectin inhibits aromatase expression in breast mesenchymal cells via this pathway. In the case of leptin, this is associated with translocation of CRTC2 to the nucleus and binding to the aromatase promoter PII, whereas with adiponectin CRTC2 is retained in the cytoplasm and its binding to the promoter is decreased (►**Fig. 2**).[26] Consistent with this, we have shown that the CRTC2 mutant S171A remains in the nucleus and stimulates aromatase PII activity, whereas the S171D mutant remains in the cytoplasm and cannot stimulate PII activity. (S171 is the site of phosphorylation by AMPK.) We have also observed that Fsk/PMA treatment (to mimic PGE2) of these cells results in phosphorylation of $\alpha 1S485/\alpha 2S491$ residues in AMPK, which results in inhibition of AMPK activity. This is in turn associated with translocation of CRTC2 to the nucleus and binding to the PII promoter (►**Fig. 3**).

Moreover, we have shown that the ratio of estradiol to testosterone also regulates the LKB1/AMPK pathway in adipose tissue.[19] In mouse adipose tissue and in 3T3L1 cells, testosterone or dihydrotestosterone inhibits, and estradiol stimulates, LKB1 expression, and ERα binds to the LKB1 promoter in the presence of estradiol.[19] Thus, we predict that when the circulating ratio of testosterone to estradiol is

high, as in the postmenopausal years, then aromatase expression in the breast is stimulated.

Obesity, Inflammation, and Breast Cancer

With obesity now recognized to be an inflammatory condition, research activity has turned to the role of inflammatory
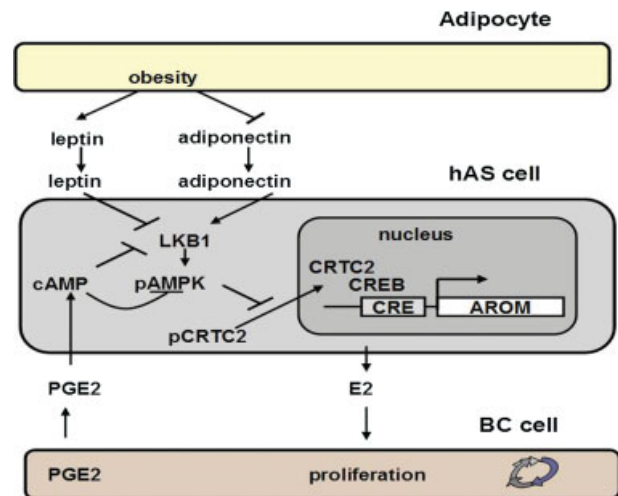

Fig. 3 Diagram of the role of obesity to stimulate breast cancer proliferation.

mediators as drivers of aromatase expression in the breast and breast cancer risk. PGE2 is such a mediator and as indicated above is a major driver of aromatase expression in human breast adipose stromal cells. We have previously shown that inflammatory cytokines also drive aromatase expression in these cells, notably IL-6 and TNFα. Recent work from Dannenberg's group[27] has shown that the lipid-laden adipocytes in the breasts of obese women are frequently surrounded by macrophages, indicative of an inflammatory condition. Moreover, they showed that aromatase expression in the breasts of these women was higher than in nonobese women, and correlated with increased levels of breast COX2 (the first enzyme on the pathway to prostaglandins including PGE2) and also PGE2 itself. In recent work, we have shown that PGE2 acts in a similar fashion to leptin to inhibit the LKB1/AMPK pathway, presumably by activation of PKA as indicated above. Thus, a second mechanism whereby PGE2 stimulates aromatase expression is provided.[26]

AMPK—The Master Regulator

Thus, we see that AMPK plays a central role in the mechanisms whereby inflammation and obesity regulate aromatase expression in the breast, namely, as an inhibitory factor. These results immediately provide a new and commanding explanation for the link between obesity, aging, and breast cancer risk. First, obesity, whether premenopausal or age-related, results in a decrease in circulating adiponectin and increase in leptin. Second, postmenopausally when the ovaries cease to make estrogen, the ratio of testosterone to epitestosterone increases. Third, obesity results in the formation of inflammatory mediators, notably PGE2. Each of these would in turn result in a decrease in activity of the LKB1/AMPK pathway in breast adipose, resulting in increased expression of aromatase. The resulting increase in estrogen formation in the breast would lead to increased breast cancer proliferation (►Fig. 3).

Hence, factors that stimulate AMPK have the potential to be a new generation of breast cancer therapeutics. Moreover, since promoter II drives aromatase expression in the breast and promoter I.4 drives expression in bone, and AMPK is inhibitory solely of promoter II–driven aromatase expression but not promoter I.4–driven expression, such factors should be breast-specific. Thus, they would not inhibit aromatase in other sites where estrogens have important roles, such as bone, and thus should not give rise to the contraindications, such as arthralgia and bone loss, which cause many women to abandon endocrine therapy for breast cancer. One such factor is the antidiabetic drug metformin, which acts by stimulating AMPK and which we have shown is inhibitory of PGE2-stimulated aromatase expression in adipose stromal cells.[28] Several studies have indicated that metformin is protective against breast cancer and inhibits the growth of breast cancer cells in culture. However, the action of metformin to stimulate AMPK is unclear and is indirect, so there is currently much interest to develop specific agonists of AMPK.

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