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Aromatase, breast cancer and obesity: a complex interaction

Serdar E. Bulun, **Dong Chen**, **Irene Moy**, **David C Brooks**, and **Hong Zhao**
Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA

Abstract

Obesity has been associated with abnormally high expression of the enzyme aromatase in the breast, increased local estrogen production, and predisposition to breast hyperplasia and cancer. Increased adiposity in postmenopausal women may trigger signaling pathways that induce aromatase expression. In breast adipose fibroblasts, increased TNF production may induce the distal aromatase promoter, whereas increased local PGE_2 production may induce the proximal promoter region. We review here the mechanisms that control aromatase gene expression in breast adipose tissue, and the paracrine interactions between malignant breast epithelial cells and the surrounding adipose fibroblasts. Systematic characterization of these signaling pathways will facilitate the identification of potential drug targets to selectively reduce aromatase expression and excessive estrogen production, with therapeutic benefit.

Relationship between obesity and estrogen

Approximately two-thirds of adult US women have excess body fat: 36% are obese with a body mass index (BMI) of more than 30, whereas another one-third are overweight (BMI: 25–29) [1,2]. Overweight or obese postmenopausal women exhibit a threefold increased risk for developing breast cancer compared with normal-weight postmenopausal women [3–5]. Based on estimates by the American Cancer Society in 2002, more than 60 000 annual new cases of breast cancer are linked to obesity and increased adiposity in the US. That obesity is a risk factor for breast cancer has been recognized since the 1960s [6]; however, the molecular mechanisms that link obesity to breast cancer are unknown. It has been suggested that products of the subcutaneous adipose tissue in the breast and other peripheral sites may be responsible for the increased breast cancer risk associated with obesity. Studies have shown that estrogen, leptin or other adipose tissue-derived molecules may promote tumor development or growth [7–9]. In the case of estrogen, it might be possible that the hormone triggers development of malignancy and its growth via the induction of several key genes including progesterone receptor (PR), adenosine A1 receptor (ADORA1) and wingless-type MMTV integration-site family, member 11 (WNT11), in neighboring epithelial cells – all of which have been associated with tumor devel-opment or growth [10–16].

In the breast, benign or malignant epithelial cells lie in contact with endothelial cell-lined capillaries, undifferentiated adipose fibroblasts that are also known as preadipocytes, and lipid-filled mature adipocytes [17]. Aromatase, a member of the cytochrome P450 superfamily, is the enzyme responsible for key steps in the synthesis of estrogens [9]. Aromatase is expressed in a several tissues including undifferentiated adipose fibroblasts and breast tumors, but is not expressed in mature adipocytes [9,18]. A greatly increased mass of breast adipose tissue in obese women may locally increase estrogen production

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Corresponding author: Bulun, S.E. (s-bulun@northwestern.edu)..

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within the breast simply because of a higher number of aroma-tase-expressing fibroblasts [9]. In addition to this mass effect, aromatase expression per unit adipose tissue or cell may also increase with weight gain.

Estrogen, a product of the aromatase enzyme in adipose tissue, has long been suspected as the hormone responsible for increasing breast cancer risk in obese postmenopausal women. In fact, the most effective hormonal treatment of postmenopausal breast cancer has been the use of aroma-tase inhibitors that block aromatase activity in the breast and the periphery, thereby reducing the amount of local estrogen production – which in turn helps to suppress recurrence of the breast tumor tissue [19]. A key and unresolved question has been the relative contributions of breast adipose tissue, versus subcutaneous adipose tissue at other body sites, to the formation of estrogen that contributes to increased breast cancer risk and growth. Epidemiologic studies suggested that mildly increased venous blood estrogen levels might account for a portion of the link between obesity and breast cancer incidence [20]. However, the recently performed randomized Women's Health Initiative study, demonstrating a possible reduction in breast cancer risk in postmenopausal women that were administered estrogen-only hormone replacement, has challenged the role of mildly elevated circulating estrogen levels in breast cancer risk [15,16].

Work from several laboratories over the past two dec-ades has suggested that breast adipose tissue fibroblasts are a crucial site for aromatase expression and estrogen production, and has linked them to the development of breast cancer [17,21,22]. By the same token, the malignant epithelial cells in an existing breast tumor are in direct contact with the surrounding adipose tissue, which is the major supplier of estrogen to cancer [23,24]. It is thus tempting to hypothesize that obesity may be associated with abnormally high expression of aromatase in breast adipose tissue fibroblasts, resulting in elevated levels of local estrogen in the breast and predisposition to breast hyperplasia and cancer. It is plausible that obesity may trigger signaling pathways that induce aromatase expres-sion. In fact, obesity is known to robustly increase adipose tissue levels of tumor necrosis factor (TNF), a known inducer of aromatase expression in adipose fibroblasts[25,26]. Obesity may also increase other local hormones such as prostaglandin E_2 (PGE_2) that are also known to induce aromatase gene expression in adipose tissue of the breast and elsewhere [26,27]. Aromatase in breast adipose tissue (versus adipose tissue at other body sites) might have a substantially higher impact on carcinogenesis be-cause of its proximity to breast epithelial cells. Therefore, the characterization of signaling pathways, which are activated in obese women and contribute to increased aromatase expression and activity and local estrogen ex-cess in the breast, will facilitate the identification of poten-tial drug targets for reducing the risk of breast cancer. Here we review the mechanisms that control aromatase expression in breast adipose tissue and the paracrine interactions between malignant breast epithelial cells and the surrounding adipose fibroblasts.

Mechanisms of aromatase overexpression in estrogen-responsive breast cancer

A single gene encodes aromatase, the key enzyme for estrogen biosynthesis, the inhibition of which effectively eliminates estrogen production. Today, aromatase inhibitors are the most effective endocrine treatments for estro-xsgen-responsive breast cancer [28]. Several head-to-head randomized clinical trials published since 2000 have dem-onstrated the superiority of aromatase inhibitors to the estradiol antagonist tamoxifen in the treatment of breast cancer [19,29–35]. Thus, inhibiting estrogen formation is therapeutically more efficacious than blocking its action. The ovaries, testes, adipose tissue, skin, the hypothalamus and placenta, all express aromatase, physiologically [36]. By contrast, breast adipose tissue bearing a tumor over-expresses aromatase, leading to local overproduction of

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estrogen that exerts paracrine and intracrine tumorigenic effects [36]. Although similar promoter regions of aroma-tase may be activated in normal and pathological tissues, the cellular and transcriptional regulatory mechanisms are extremely diverse and cell-specific [9,37]. This has led to a detailed investigation of the mechanisms underly-ing aromatase overexpression in each tissue in an effort to define specific molecular targets for developing new thera-peutics [9,38].

Alternatively used promoters, distributed over a 93 kb regulatory region upstream of a common coding region, control aromatase expression differentially in gonads, adi-pose tissue, bone, brain, skin, fetal liver and placenta [39]. Thus far, ten alternative promoters have been found in humans, including I.1, I.2 in placenta, I.4 in adipose tissue and skin, I.5 in fetal tissues, I.f in brain, I.7 in endothelial cells, I.6 in bone, I.3 in adipose tissue and PII in gonads (Figure 1) [9]. A distinct set of transcription factors reg-ulates each promoter in a signaling pathway- and tissue-specific manner.

Follicle-stimulating hormone (FSH) induces the most proximal promoter PII via a cAMP-dependent pathway involving the recruitment of steroidogenic factor-1 and β-catenin in human ovarian granulosa cells [41]. As will be detailed later, PGE_2 via cAMP coordinately induces theproximal cluster of promoters PII and I.3, which lie within an approximate 0.2 kb sequence, via recruitment of CCAAT/enhancer-binding protein-β (C/EBPβ), JunD and liver receptor homolog-1 (LRH-1), in human breast adipose fibroblasts [9,42]. By contrast, promoter I.4 that lies some 73 kb upstream of the coding region is induced by gluco-corticoids and the cytokines interleukin -6 or -11, and TNF. This leads to the activation of the c-Jun N-terminal kinase (JNK) activating kinase-1 (Jak1) and recruitment of signal transducer and activator of transcription-3 (STAT3), to-gether with glucocorticoid receptor (GR), in human adipose fibroblasts [9]. In cancer-free breast tissue, adipose fibro-blasts express low levels of aromatase via promoter I.4, whereas the promoter I.3/II region, which is occupied by transcriptional repressors, remains quiescent [43,44].

Distinct cellular and molecular mechanisms are respon-sible for aromatase expression in breast cancer versus disease-free breast tissue [9]. First, cellular composition is altered in breast cancer such that aromatase-expressing undifferentiated adipose fibroblasts accumulate around malignant epithelial cells. Second, molecular alterations in adipose stromal cells favor binding of transcriptional enhancers versus inhibitors to the normally quiescent aromatase promoter I.3/II region. Work from several labo-ratories has suggested that these two mechanisms support local aromatase overexpression in breast cancer and ac-count for the majority of aromatase expression in breast cancer [24,45,46]. A third mechanism has been described and involves increased activity of distal promoter regions such as I.4 and I.7, and this also contributes to aromatase overexpression in breast tumors (Figure 1) [47,48]. Finally, heterozygous mutations, which cause the aromatase cod-ing region to lie adjacent to constitutively active cryptic promoters that normally transcribe other genes, may ac-count for aromatase overexpression in breast and other tissues and excessive estrogen formation (Figure 1) [49]. The clinical significance of this mechanism in cancer de-velopment is not yet known.

Estrogen formation in breast cancer

There are two sources of estrogen in postmenopausal breast cancer. First, estrogen may arise from aromatase activity in extraovarian body sites, such as subcutaneous adipose tissue and skin, and may reach breast cancer in an endocrine manner. Breast cancer incidence correlates pos-itively with body fat content and serum estrogen levels in postmenopausal women, suggesting that this endocrine mechanism stimulates tumor development [50,51]. Sec-ond, an increase in the local concentrations of estrogen may result from aromatase

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overexpression within the tumor tissue; this mechanism has been demonstrated by several laboratories [52–54]. These groups demonstrated significantly elevated levels of estrone, estrone sulfate and estradiol in breast tumor tissue compared with circulating levels [52–54]. Several groups consistently found increased aromatase enzyme activity and mRNA levels in breast fat adjacent to the cancer tissue compared with distal fat or disease-free breast adipose tissue [17,21,45,46,55–57]. In vivo evidence from a transgenic mouse model revealed that aromatase overexpression in breast tissue was sufficient for maintaining hyperplasia in the absence of circulating estrogen and that aromatase inhibitors abrogated hyper-plasia [58]. These mice developed mammary tumors more rapidly than their wild-type littermates [58].

The biologically active estrogen is estradiol. Aromatase catalyzes the conversion of androstenedione to estrone or testosterone to estradiol. Because the major circulating precursor steroid in postmenopausal women is androstene-dione, reductive enzymes are required for estradiol forma-tion. The enzymes 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) and aldo–keto reductase family 1, member C3 (AKR1C3), which can convert estrone to estradiol and an-drostenedione to testosterone, respectively, are present in various cell types of malignant and benign breast tissues and complement aromatase activity for the formation of estradiol from circulating androstenedione (Figure 2)[58,59].

Cellular localization of aromatase in breast cancer

Approximately 90% of aromatase enzyme activity and mRNA in breast adipose tissue is found in undifferentiated fibroblasts rather than mature adipocytes [60] (Figure 2). Immunoreactive aromatase has been localized to both malignant epithelial cells and surrounding fibroblasts in breast tumor tissues [61–63]. The biologic relevance of immunoreactive aromatase detected by different antibo-dies, however, remained debatable [64]. Markedly high levels of aromatase enzyme activity and gene expression via activation of the promoter I.3/II have been consistently detected in breast adipose tissue or fibroblasts, freshly isolated from breast tissue, with or without cancer[24,60,65]. Aromatase enzyme activity in primary malig-nant breast epithelial cells or cell lines, by contrast, was either undetectable or extremely low [66] (Figure 2).

The dense layer of undifferentiated adipose fibroblasts (desmoplastic reaction) surrounding malignant epithelial cells is essential for the structural and biochemical support of tumor growth [67]. Malignant epithelial cells secrete large quantities of the antiadipogenic cytokines TNF and IL-11, which inhibit the differentiation of fibroblasts to mature adipocytes primarily via suppression of the adipogenic tran-scription factors C/EBPα and peroxisome proliferator-acti-vated receptor-γ (PPARγ) (Figure 2). Thus, large numbers of estrogen-producing adipose fibroblasts are maintained proximal to malignant cells [43,68] (Figure 2).

Signaling pathways that regulate promoter region I.3/II in breast tumor fibroblasts

It has been demonstrated that the use of the proximal promoter I.3/II region accounts for the majority of in vivo aromatase expression in adipose tissue that is located adjacent to breast cancer, and in breast cancer tissue[24,45,46]. In vitro, treatment of undifferentiated adipose fibroblasts with cell-conditioned media derived from ma-lignant breast epithelia was found to induce aromatase expression [43]. This effect could be mimicked by PGE_2, which is present in high concentrations in media that have been conditioned with malignant epithelial cells, but not in media conditioned with benign breast epithelial cells [43]. Different actions of PGE_2 are mediated via specific G-protein-coupled cell-surface receptors, named E-prosta-noid (EP) receptors, subdivided into four types termed EP_1 through EP_4 [69]. PGE_2

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induction of aromatase occurs through the EP_1 and EP_2 receptor subtypes, which are linked to protein kinase C (PKC) and A (PKA) pathways[70,71]. In fact, this PGE_2 effect could be reproduced using a combination of dibutyryl (Bt_2) cAMP and phorbol diace-tate (PDA), which also stimulate PKA and PKC, respec-tively [27]. In summary, malignant breast epithelial cells secrete significant quantities of PGE_2 that activates PKA and PKC pathways, which in turn induce aromatase ex-pression via the proximal I.3/II promoters in adjacent adipose fibroblasts (Figure 3).

To investigate potential mechanisms for blocking aro-matase activity and estrogen production in breast adipose fibroblasts surrounding malignant epithelial cells, the downstream effectors of the PGE_2–cAMP–PKA/PKC path-way have been systemically investigated [27]. These efforts have helped to define the signaling components that con-nect the transcription factors bound to the tumorigenic promoter I.3/II region of the aromatase gene. Studies on adipose fibroblasts pointed to the involvement of twospecific terminal MAP kinases: JNK and p38 (Figure 3) [27]. Detailed dissection of this pathway indicated that PGE_2 treatment of breast adipose fibroblasts activated both PKA and PKC and their downstream effectors, JNK and p38, which collectively were necessary for estro-gen production mediated via the I.3/II promoter region of the aromatase gene [27]. These studies have highlighted to role of these terminal MAP kinases in activating aroma-tase gene expression and have pointed to the possibility of using JNK and p38 as potential new drug targets for breast tissue-specific ablation of aromatase expression.

Transcriptional complexes that regulate the I.3/II promoter region in breast tumor fibroblasts

Promoters I.3 and II are located approximately 0.2 kb apart and share several cis-acting elements necessary for promot-er activation, including a nuclear receptor half-site, C/ EBPβ-binding sites, AP-1 binding sites, and cAMP-response elements (CREs) (Figure 3) [43,44]. A large number of transcription factors, which may potentially regulate this complex region, have been reported. Very few of these, however, were demonstrated to have a functional role in human adipose fibroblasts. In this review we focus on transcriptional enhancers that were characterized using in situ techniques such as chromatin immunoprecipitation PCR, or were shown to regulate aromatase mRNA or en-zyme activity using siRNA-based knockdown techniques in primary human adipose fibroblasts. These factors are limit-ed to C/EBPβ, CREB-binding protein (CBP), liver receptor homolog 1 (LRH-1), JunB and JunD [42,44,72–79].

Incubation of breast adipose fibroblasts with malignant epithelial cell-conditioned medium or with PGE_2 was shown to lead to recruitment of a stimulatory transcrip-tional complex, comprising C/EBPβ, CBP, JunB, JunD and LRH-1, to the aromatase promoter I.3/II region [44,72–79] (Figure 3). Thus, in undifferentiated adipose fibroblasts in breast tumors, the proximally clustered aromatase promo-ters I.3/II are coordinately regulated by PKA- and PKC-dependent mechanisms (Figure 3). As mentioned above, these promoters are usually quiescent in fibroblasts of normal breast tissue due to the binding of a transcriptional inhibitory complex. In a malignant breast environment, however, the promoter region I.3/II is occupied by several transcriptional enhancers as a result of the activation of multiple signaling pathways that ultimately increase aro-matase expression in breast fibroblasts [43,44] (Figure 3).

The effect of PGE_2 on the aromatase promoters is fairly complex. PGE_2 has been shown both to activate the proxi-mal aromatase promoters I.3/II and to suppress the distal promoter I.4 activity, in a reciprocal fashion. The net result, however, is a robust increase in total aromatase mRNA levels and enzyme activity in adipose fibroblasts, indicating that PGE_2

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has a dominant stimulatory effect on the promoter I.3/II region [42]. Because JNK is a crucial MAP kinase that mediates PGE_2-dependent aromatase expression in adipose fibroblasts, the roles of its target transcription factors c-Jun, JunB and JunD have recently been examined [42]. Among these, JunD was found to be the crucial transcription factor that mediated PGE_2 induc-tion of aromatase, and this involved binding to a cAMP response element (CRE) at the promoter I.3/II region [42]. JunB supported this induction primarily via maintaining steady-state JunD levels. In this context, PGE_2 treatment simultaneously suppressed the distal promoter I.4 via the recruitment of c-Jun and JunD which, in this case, para-doxically acted as transcriptional silencers [42]. This line of evidence suggests that targeting JunD may potentially achieve selective ablation of local aromatase expression, and hence estrogen formation in breast cancer, via inhibi-tion of the tumorigenic aromatase promoters I.3/II (Figure 3).

Targeting the aromatase promoter I.3/II region as a therapeutic strategy

Inhibiting the aromatase promoter I.3/II region, which is overactive in adipose fibroblasts associated with breast cancer, is an attractive therapeutic option because it avoids the side effects observed with the currently available nonselective aromatase inhibitors, which cause total estro-gen deprivation. The crucial question is whether ablating this promoter region would lower breast tissue estrogen levels sufficiently to produce a therapeutic effect. This question may be answered via the use of in vivo models in the future. Promoter I.3/II is induced by the common PKA/PKC pathway and may potentially be targeted using several strategies such as the use of PGE_2 receptor block-ers, kinase inhibitors, or by knockdown technology such as RNA interference. PGE_2 is only one of the hormones that stimulate this pathway, and two of its receptors, EP_1 and EP_2, may mediate its activity for aromatase induction, introducing redundancy into the system. Thus, suppres-sion of its production or action via targeting its synthetic enzymes or receptors may not be sufficient to achieve a therapeutic effect. The terminal MAP kinases which lie downstream of PKA/PKC, namely p38 and JNK, are other potential targets. JNK inhibition appears to provide near-complete ablation of aromatase activity in adipose fibro-blasts [27]. Several transcriptional enhancers that bind to the promoter I.3/II region were proposed as potential possible therapeutic targets. Very few of these factors, however, were clearly demonstrated to have functional roles. For example, knockdown of LRH-1 or JunD de-creased aromatase expression in human adipose fibro-blasts [42]. JunD knockdown almost completely abolished aromatase activity in primary adipose fibro-blasts, suggesting that targeting of JNK or JunD may produce the most profound aromatase inhibition in breast adipose tissue associated with cancer [27,42]. It should be noted that both JNK and JunD are widely expressed signaling effectors. Thus, their inhibition may cause sys-temic side effects, which may prohibit their widespread use in the treatment of breast cancer. It would be preferable to target the PKA/PKC-dependent transcriptional complex that is assembled at the aromatase promoter I.3/II in breast adipose fibroblasts as a more specific target.

Concluding remarks

Breast cancer is unique in that it develops and thrives in adipose tissue, which provides structural and paracrine support for the growing tumor. The undifferentiated adi-pose fibroblast is the primary cell type that is in contact with the malignant epithelial cell; these two cell typessynergize in favor of tumor growth. A key contribution of adipose fibroblasts to a breast tumor is estrogen produc-tion via aromatase expression [10]. In obese women, in-creased production of TNF may induce the distal aromatase promoter in increased numbers of adipose fibroblasts in the breast and other body sites. Indepen-dently, increased local PGE_2 production as a function of obesity and aging may also induce the proximal promoter region of the aromatase gene in breast adipose fibroblasts. In vivo, the proximal promoter

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region is the primary regulator of aromatase expression in breast adipose tissue proximal to malignant epithelial cells. Identification of the key effectors of this signaling pathway that connects its exogenous stimulator PGE_2 and the proximal aromatase promoter is crucial for developing novel and tissue-specific therapeutic strategies [27,42–44,80]. Targeting this path-way may reduce aromatase expression and excessive es-trogen production sufficiently to produce a clinical benefit. Selective inhibition of aromatase expression in the breast may offer a treatment modality with minimal systemic side effects, in contrast to whole-body estrogen depriva-tion that is associated with the currently available nonse-lective aromatase inhibitors.

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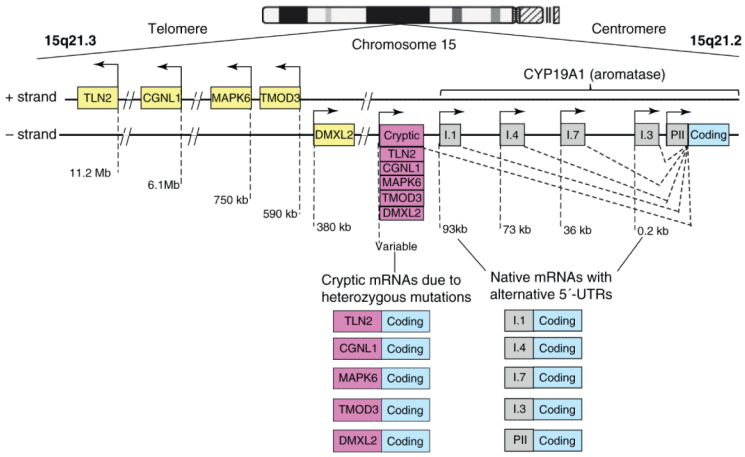

Figure 1.

Structure of the CYP19A1 gene. The human CYP19A1 (aromatase) gene is transcribed in the direction from the telomere towards the centromere of chromosome 15 and contains approximately 10 alternatively used native promoters that regulate its expression in a partially tissue-specific fashion. Activation of each promoter transcribes on mRNA species that contains a specific 5′-untranslated region (5′-UTR), which serves as the signature of that particular promoter. Five other genes (TLN2, CGNL1, MAPK6, TMOD3 and DMXL2) clustered in tandem order lie next to aromatase at its telomeric aspect. Heterozygous inversions or deletions change the direction of these genes' promoters and their 5′-UTRs and move them upstream of the aromatase gene. These cryptic promoters then inappropriately overexpress aromatase in multiple human tissues and cause estrogen excess. The most common manifestation is the feminine growth of breast tissue in young boys (prepubertal gynecomastia) (see [40] for further details).

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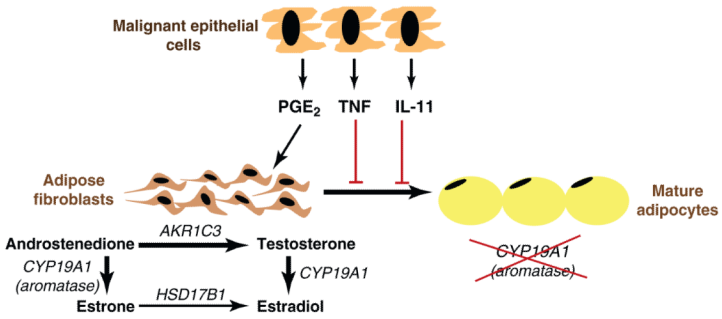

Figure 2.

Stromal–epithelial interactions for estrogen production in breast cancer. Breast cancer grows in an environment of adipose tissue. Adipose fibroblasts differentiate to mature adipocytes in the breast. Both cell types lie in close proximity to benign or malignant epithelial cells. The products of malignant and benign cells determine the key biological features of this micro-environment. For example, malignant cells and adipose fibroblasts secrete PGE_2 that induces aromatase (CYP19A1) expression in undifferentiated adipose fibroblasts. Aromatase converts androstenedione to estrone, that is further converted to biologically active estradiol, by 17β- hydroxysteroid dehydrogenase type 1 (HSD17B1) in the same cell or in the malignant epithelial cell. Alternatively, the enzyme aldo–keto reductase family 1, member C3 (AKR1C3), which is expressed in myofibroblasts of breast tissue, converts androstenedione to testosterone that may be readily aromatized to estradiol. Thus, PGE_2 induces estradiol production directly or indirectly via regulating aromatase enzyme activity in adipose fibroblasts. Once an adipose fibroblast is differentiated into a mature adipocyte it loses its capacity to express aromatase and form estradiol. Malignant breast epithelial cells secrete antiadipogenic cytokines such as TNF and IL-11 to inhibit this differentiation. It appears that malignant epithelial cells maintain neighboring adipose cells in an undifferentiated state to maximize paracrine estradiol biosynthesis.

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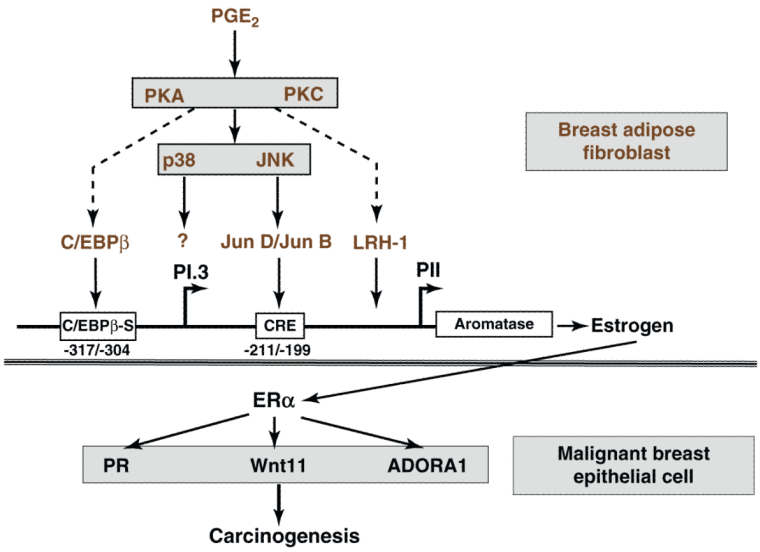

Figure 3.

Signaling pathways for estrogen production and action. PGE_2 that originates from malignant epithelial cells or adipose fibroblasts activates specific signaling mechanisms to induce aromatase expression in adipose fibroblasts. The best characterized and most potent components of the PGE_2-dependent signaling pathway in the adipose fibroblast are PKA/PKC and p38/JNK. Some of the key transcription factors downstream of this pathway include C/EBPβ, JunD and JunB, and LRH-1 which bind to the tumorigenic aromatase promoter region I.3/II (see text). The key cis-regulatory elements, C/EBPβ-S and CRE and the promoters II and I.3, cluster within a sequence of less than 300 bp. LRH-1 binds to a nuclear receptor half-site located more proximal to promoter II (PII). It is very likely that these key elements regulate the activity of both promoters coordinately. The details of this complex regulation are not well understood. Estrogen produced as a consequence of aromatase activity acts on estrogen receptor-α (ERα) in neighboring epithelial cells to enhance carcinogenesis by transactivating specific genes. PKA, protein kinase A; PKC, protein kinase C; C/EBP, CCAAT/enhancer binding protein; C/EBPβ-S, C/EBPβ site; CRE, cAMP response element; PR, progesterone receptor, Wnt, wingless-type MMTV integration-site family; ADORA, adenosine A1 receptor.