Role of activating transcription factor (ATF-2) in breast cancer: a possible cross talk with CYP2C19*17 allele

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Abstract

CYP2C19 plays a key role in the metabolism of estrogen. Promoter polymorphism of CYP2C19 (*17, -806C>T) causing ultra-rapid metabolizer phenotype for estrogen, may reduce the risk of breast cancer. Activating transcription factor (ATF-2) plays an important role in tumorigenesis. Estrogens influence the transcription of ATF-2 which in turn plays a role in the expression of several tumor suppressor and tumorigenic proteins. ATF-2 acts generally on tumor suppressor genes in mammary tissue but can also undergo estrogen mediated increased phosphorylation acting on tumorigenic genes, thus showing dual actions. We hypothesize that presence of CYP2C19*17 allele may enhance ATF-2 binding to its promoter region, hence may increase the expression of CYP2C19 causing more rapid metabolism of estrogens, protecting from the occurrence of breast cancer. Increased activity of CYP2C19 in the presence of CYP2C19*17 allele may alter the levels of phosphorylated ATF-2 by reducing the estrogen levels, leading to the increased transcription of tumorsuppressor genes. This will lead to decreased incidence of breast cancer in individuals carrying CYP2C19*17 allele.

Keywords: CYP2C19*17, ATF2, estrogen, Breast cancer, transcription

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Introduction

The role of life time estrogen exposure in the development of breast cancer is well accepted [1-2]. It is shown that estrogen acts through receptor dependent and independent mechanisms causing breast cancer [3]. Estrogens cause tumorigenesis by inducing cell proliferation, cell division and genotoxicity [4-6]. In breast cancer patients, estrogen levels in cancerous tissue has been reported to be higher compared to its urinary levels [7]. Thus, tissue specific estrogen levels have more predictive value in the causation of tumor. Here we propose a hypothesis on the role of altered levels of estrogens in relation to the activating transcription factor 2 (ATF-2) and CYP2C19*17 allele in tumorigenesis of breast cancer. We also attempt to explain the dual actions of ATF-2 in breast tissue.

CYP2C19 role in estrogen metabolism

It has been shown that metabolic genes which are selectively expressed in breast tissue such as CYP1B1 determine the tissue levels of estrogen [7]. CYP2C19 enzyme plays a role in the metabolism of estrogen. CYP2C19 is involved in catalyzing dehydrogenation of 17-β hydroxyl group and 16-α hydroxylation reaction during estrogen metabolism [8-9]. Promoter region polymorphism of CYP2C19, CYP2C19*17 (-806C>T and -3402C>T in strong linkage disequilibrium) causes increased activity of CYP2C19, resulting in ultra-rapid metabolism of estrogens, and might reduce the risk of breast cancer [10]. CYP2C19*17 mediated ultra-rapid metabolizer (UM) phenotype is apparently caused by an increased transcription of CYP2C19 as demonstrated by both...
in vitro and in vivo studies [11-12]. The promoter region of CYP2C19 surrounding -806C>T (*17 allele) has been proposed to bind to ATF-2, thus increasing the transcription of CYP2C19 [13]. Estrogen receptor-binding half site estrogen response element (ERE) in the CYP2C19 promoter has also been identified which interacts with estrogen receptor α down regulating CYP2C19 expression [14]. Thus the promoter region of CYP2C19 is viable to regulation by many factors. The altered expression of CYP2C19 in turn influences the estrogen levels by altering its metabolism. In the presence of CYP2C19*17 allele, increased metabolism of estrogens may alleviate their influence on the transcription of CYP2C19 and several other genes known to play a role in the tumorigenesis of breast cancer.

**Activating transcription factor 2 (ATF-2):**

Activating transcription factor 2 (ATF-2) is one of the key members of ATF-CREB (cAMP response element binding proteins) group of basic leucine zipper (bZIP) transcription factors. ATF-2 binds to cAMP response element (CRE) and exerts its action on the promoter regions of several genes, such as cyclin A, cyclin D, growth arrest and DNA damage inducible gene α (GADD45α) and maspin. It has been proposed that ATF-2 promotes survival of cells by regulating Bcl2 (B-cell lymphoma 2) expression in certain cell types such as chondrocytes [15].

ATF-2 exhibit both tumor suppressor and oncogenic activities depending on cell, tissue and its availability [16, 17]. ATF-2 acts along with activator protein 1 (AP1) complex in tumorigenesis. Studies have shown that inhibition of ATF-2 by ATF-2 inhibitory peptides results in the suppression of tumorigenesis and metastasis in melanoma [18, 19]. Phosphorylation and overexpression of ATF-2 with altered subcellular localization and increased interaction with other API proteins, such as oncogenic JUN (JUN oncogene), is seen in various cancers [20]. ATF-2 expression has been proposed to have a diagnostic value in many cancers [21]. Thus the expression and phosphorylation of ATF-2 play a major role in different cancers. However loss of ATF-2 function has been associated with breast, lung cancers and neuroblastoma [22]. ATF-2 acts as tumor suppressor for breast cancer by activating the target genes such as Maspin and Gadd45α [23]. Further, ATF-2 binds to tumor suppressor genes breast cancer 1, early onset (BRCA1) and Maspin promoter regions to activate their transcription (23). Mice heterozygous (+/-) for ATF-2 developed more frequent breast cancer compared to homozygous (+/+), mice. In human breast cancer cells, ATF-2 mRNA was also shown to be lower compared to normal human mammary epithelial cells [24]. But there is also evidence for ATF-2 role in mediating tumor proliferation. Estradiol enhances cyclin D1 promoter transcription by activation of the p38 Mitogen activated protein kinase (MAP kinase) and phosphorylation of ATF-2, contributing to the breast cancer cell proliferation [25].

ATF-2 is ubiquitously present in all tissues and involved in multiple cellular responses to stresses namely hypoxia and DNA damage [17, 26]. Partial deregulation of ATF-2 is implicated in pathogenesis of cancer [22]. ATF-2 consists of basic structural region and leucine zipper domain that are essential for AP1 homodimerization and heterodimerization (27). ATF-2 exists as monomers in unstressed condition [28]. In response to stress or cytokines, ATF-2 is phosphorylated by either JNK or p38, which is required to egress ATF-2 intramolecular inhibition allowing its homodimerization or heterodimerization with other members of the AP1 family, such as JUN, CREB, and FOS (FBJ murine osteosarcoma viral oncogene homolog) [29]. The activity of ATF-2 also depends on post translational modification with heterodimeric components of AP1 network [30]. The DNA binding region of ATF-2 homodimers exhibits binding specificity to CRE sequences [31]. Nevertheless ATF-2 can interact with other promoter elements of interferon-γ, stress-response element depending on specific stimulus and cell type. Thus ATF-2 dimerization with different proteins significantly influences DNA binding specificity, affinity, and ultimately the transcription [32, 33].

**Hypothesis**

We propose that ATF-2 interacts with the promoter region of CYP2C19 in CYP2C19*17 allele carriers to increase its expression, and thereby increases the metabolism of estrogens. Thus presence of CYP2C19*17 allele might protect from the occurrence of breast cancer or increases the response to estrogen based therapy (e.g. tamoxifen treatment). Further, presence of CYP2C19*17 allele might increases ATF-2 recruitment altering its tissue levels impairing its action on other genes involved in tumorigenesis. Recently in silico analysis predicted alteration in the interaction of ATF-2 to the binding site in the presence of *17 allele in CYP2C19 promoter [13, 34]. Increased metabolism of estrogens in the presence of CYP2C19*17 allele might also diminish negative
influence of estrogens on the expression of CYP2C19, thus maintaining its higher activity. Higher levels of estrogens augments the activity of ATF-2 on genes like cyclin D1 by increasing transcription or phosphorylation of ATF-2, enhancing proliferation of breast cancer cells [25]. Where as in the presence of CYP2C19*17 allele, increased metabolism of estrogens along with enhanced recruitment of ATF-2 to CYP2C19 promoter region may decreases the levels of ATF-2 in mammary tissues resulting in shift of its control to tumor suppressor genes predominantly (Figure 1). Whereas estrogen mediated overexpression and phosphorylation of ATF-2 shifts its control to tumorigenic genes [25]. ATF-2 thus at higher levels of estrogen, could act on tumorigenic genes and at lower levels predominantly increases the transcription of tumor suppressor genes. Thus protective environment in the presence of CYP2C19*17 allele, is not only due to lower estrogen levels and but indirectly related to the altered regulation of tumor suppressor and tumorigenic genes by ATF-2. But in individuals with no CYP2C19*17 allele there is reduced metabolism of estrogens, thus higher estrogen levels might increase ATF-2 phosphorylation and may aggravate tumorigenesis (Figure 1). In addition higher estrogen levels in the absence of CYP2C19*17 allele may also further decrease the expression of CYP2C19 resulting

Figure 1. A possible cross talk of ATF2 with CYP2C19*17 in breast cancer. Dotted arrows in presence of CYP2C19*17

1. CYP2C19 metabolizes estrogens and is expressed in mammary tissues. Presence of *17 allele in the promoter region of CYP2C19 recruits ATF-2, and increases CYP2C19 activity, which further increases the metabolism of estrogens.

2. Estrogens modulate the transcription process of ATF-2 and its phosphorylation [24], which further may influence its metabolism by altering the activity of CYP2C19.

3. ATF-2 play a role in estrogen mediated tumor proliferation [25]. When there are lower levels of estrogen in the presence of CYP2C19*17 allele, there is less phosphorylation of ATF-2 levels and altered action on tumorigenic genes and vice versa.

4. ATF-2 increases the expression of both tumorigenic and tumor suppressor genes depending on the presence of estrogen in the mammary tissues. In the absence of CYP2C19*17 allele, estrogen levels will be high, which increases phosphorylation of ATF-2. This affects ATF-2 control on tumor suppressor genes and vice versa.
Higher levels of estrogen augments the activity of ATF-2 on genes like cyclin D1 by increasing transcription or phosphorylation of ATF-2 enhancing proliferation of breast cancer cells [25]. Where as in the presence of CYP2C19*17 allele, increased metabolism of estrogen along with higher recruitment of ATF-2 to CYP2C19 promoter region may decreases the levels of ATF-2 in mammary tissues resulting in shift of its influence to tumor suppressor genes predominantly. (Figure 1) Thus, explaining dual actions of ATF-2 as, being tumor suppressor normally [23] while estrogen mediated overexpression and phosphorylation leads to its action on tumorigenic genes [25]. ATF-2 thus at higher levels of estrogen, could act on tumorigenic genes and at lower levels predominantly increases the transcription of tumor suppressor genes. The protective environment in the presence of CYP2C19*17 allele, is not only due to decrease in the estrogen levels and its effects but indirectly also due to shifting of ATF-2 to tumor suppression. But in individuals with no CYP2C19*17 allele there is reduced metabolism of estrogens, thus high estrogens could increase ATF-2 phosphorylation and thus could be a risk factor or may aggravate tumorigenesis (Figure 1). In addition higher estrogen levels in the absence of CYP2C19*17 allele may also further decrease the expression of CYP2C19 resulting in more aggravated levels of estrogens.

CYP2C19*17 has also been shown to be in strong linkage disequilibrium with CYP2C8*1 and CYP2C9*1 wild alleles [35]. Furthermore, another study has shown that each copy of CYP2C8/9 *1/*4/*1/*1 allele is associated with significantly lower risk of nodal involvement in breast cancer patients [36]. However, CYP2C8*4 has shown to lower metabolic activity of the enzyme [37]. Thus the protective effect of CYP2C19*17 may also be contributed by CYP2C8*1 and CYP2C9*1 in breast cancer. These enzymes collectively have influence on the metabolism of estrogens (increased estrogen metabolism and decreasing its influence on mammary tissues) thus affecting breast cancer occurrence.

**Conclusion:**

ATF-2 is a possible drug target whose activity can be manipulated for therapeutic benefit. ATF-2 manipulation for therapy may be more beneficial in subjects carrying CYP2C19*17 allele with lower levels of estrogen. Thus, the turnover of ATF-2, estrogen and their interaction with CYP2C19 may be playing an important role in tumorigenesis of breast cancer.

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**Conflicts of interest:**

The authors declare no conflicts of interest

**References**


