Hypotheses in the Life Sciences Volume 2 Issue 1 pp 15-19

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 a 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 tumour 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 tumorsupressor 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 AP1 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 *Mapsin* 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 *AFT-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* might increase 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

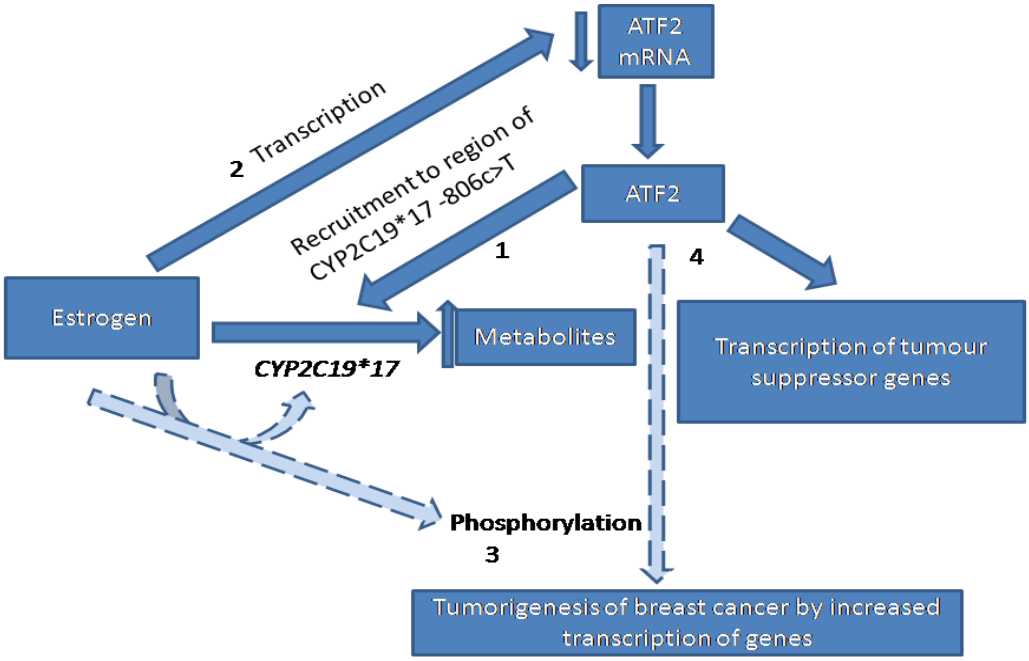

Figure 1. A possible cross talk of ATF2 with CYP2C19*17 in breast cancer. Dotted arrows in presence of CYP2C19*17

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.

Conflicts of interest:

The authors declare no conflicts of interest

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