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13. ABSTRACT (Maximum 200 Words)

The purpose of this project is to investigate whether or not regulation of expression of the BRCA-1 gene in breast epithelial cells exposed to polycyclic aromatic hydrocarbons (PAHs) is mediated by the aryl hydrocarbon receptor (AhR). *The scope* of the project is to examine whether or not the AhR complexed with the AhR-nuclear transporter (ARNT) protein, binds to several xenobiotic responsive elements (XRE) strategically located at –539 bp (CCGTGGAA=Cyp1A1-like) and +20base pairs (bp) (GCGTG=XRE-1) from the transcription start site on exon-1A. Two additional XREs (GCGTG) have been localized at –107 bp in the intervening sequence upstream (XRE-2) and +218 bp (XRE-3) into exon-1B. Findings of the experiments conducted during the lest period include: 1) Completed testing of mutation contructs for XRE2 and investigated the effects of antagonists of the AhR and ERα on regulation of BRCA-1 transcription by estrogen and the dioxin-like compound TCDD in breast cancer MCF-7 cells. 2) Characterized the mechanism of interaction between the ERα and the AhR at the XRE-2 by chromatin immunoprecipitation assay (ChIP).

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BRCA-1, Benzo[a]pyrene, aromatic hydrocarbon receptor

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Introduction

The subject of the research is to investigate whether or not exposure to polycyclic aromatic hydrocarbons (PAHs) may be a risk factor in the onset of mammary neoplasia by altering transcription of the tumor suppressor gene, BRCA-1. The *purpose* of this project is to investigate whether or not changes in the expression of the BRCA-1 gene in breast epithelial cells induced by PAHs is mediated by the aryl hydrocarbon receptor (AhR). *The scope* of the project is to examine whether or not the AhR complexed with the AhR-nuclear transporter (ARNT) protein, binds to several xenobiotic responsive elements (XRE) strategically located at –539 bp (CCGTGGAA=Cyp1A1-like) and +20base pairs (bp) (GCGTG=XRE-1) from the transcription start site on exon-1A. Two additional XREs (GCGTG) have been localized at –107 bp in the intervening sequence upstream (XRE-2) and +218 bp (XRE-3) into exon-1B. Results presented in this progress report dealt with the role of the AhR in regulation of BRCA-1 transcription by the ERa and the cross-talk between the two receptors following stimulation of BRCA-1 transcription with estrogen.

Body

Synopsis

The tasks of the last year focused on completing testing of mutation contructs for XRE2 and investigated the effects of antagonists of the AhR and ERα on regulation of BRCA-1 transcription by estrogen and the dioxin-like compound TCDD in breast cancer MCF-7 cells. In addition, we wished to characterize the mechanism of interaction between the ERα and the AhR at the XRE-2 by chromatin immunoprecipitation assay (ChIP).

Mutation construct for XRE-2 and effects of the AhR-ligand TCDD

The results depicted in Figure 1 confirmed that estrogen stimulated transcription from the BRCA-1 promoter, whereas this effect was antagonized by the AhR-ligand TCDD. Mutation of the XRE-2 reduced basal activity and prevented the stimulation previously seen with estrogen (Figure 2). These results are very important because they indicate that 1) ligands of the AhR prevent estrogen stimulation of BRCA-1 transcription, and 2) the presence of the XRE-2 is required for estrogen activation of BRCA-1 transcription. The latter result establishes a role for the XRE-2 and the AhR in regulation of BRCA-1 transcription. This conclusion was corroborated by the results presented in Figure 3. These data suggested that cotreatment of MCF-7 cells transfecetd with the BRCA-1 promoter construct pGL3BRCA-1 with estrogen plus the AhR antagonist alpha-naphtoflavone (ANF) prevented estrogen stimulation of BRCA-1 transcription. Again, these results indicate that the AhR contributes to estrogen activation of BRCA-1 transcription. We then tested the effects of a pure antagonist (3'-methoxy-4'-nitroflavone, 3M4NF). The results presented in Figure 4 strongly confirm the role of the AhR in the estrogen upregulation of BRCA-1 transcription since cotreatment with 3M4NF abrogated the increase in promoter activity caused by estrogen.

Chromatin immunoprecipitation assay

The implications of these results are significant because they establish a role for ligands of the AhR and ERα. In detail, our results suggest that the effects of estrogen on BRCA-1 transcription will depend on whether or not ligands of the AhR are present in the nuclear environment. To test this hypothesis we performed a series of experiments using the chromatin immunoprecipitation assay. This assay through a combination of immunoprecipitation with specific antibodies and PCR amplification allows examination of the occupancy of segments of promoter DNA by specific factors. In detail, we wished to examine the effects of TCDD on recruitment of the ERa at the XRE-2. The results of Figure 4 indicated that the treatment with TCDD stimulated the recruitment of the ERα as documented by the appearance of a band following PCR amplification of the DNA segment comprised by the oligonucleotides Ch-XRE-F and Ch-XRE-R (Figure 5). These results suggest that the presence of the AhR ligand TCDD facilitates the recruitment of the ERα and the XRE-2. In contrast, cotreatment with TCDD plus

estrogen causes a loss of the ERα from this site and the coincident loss of transcription activity.

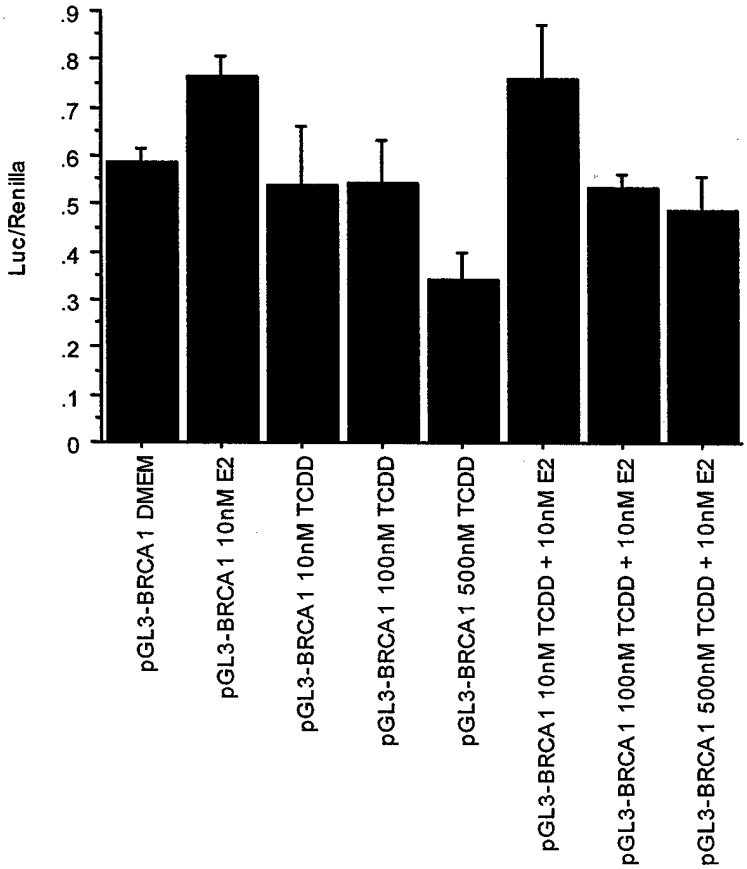


Figure 1. Cotreatment with TCDD represses estrogen-induced BRCA-1 transcription in breast cancer MCF-7 cells.

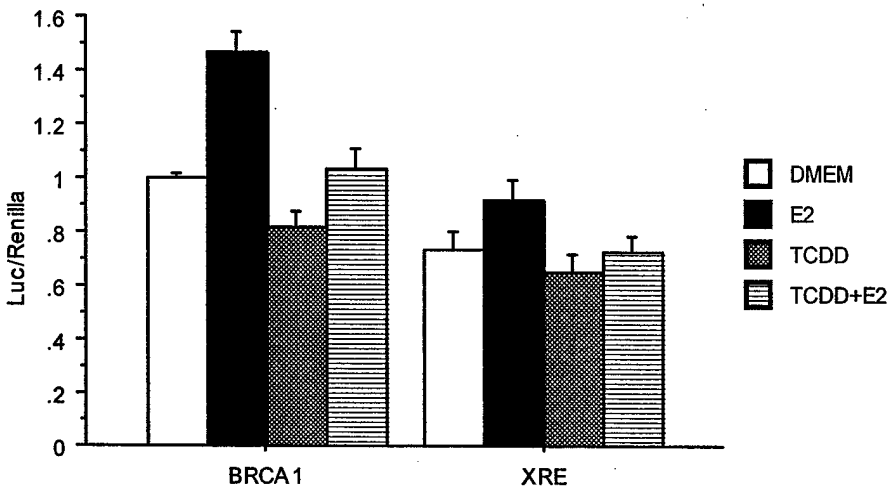


Figure 2. Mutation of XRE-2 prevents estrogen activation of BRCA-1 transcription.

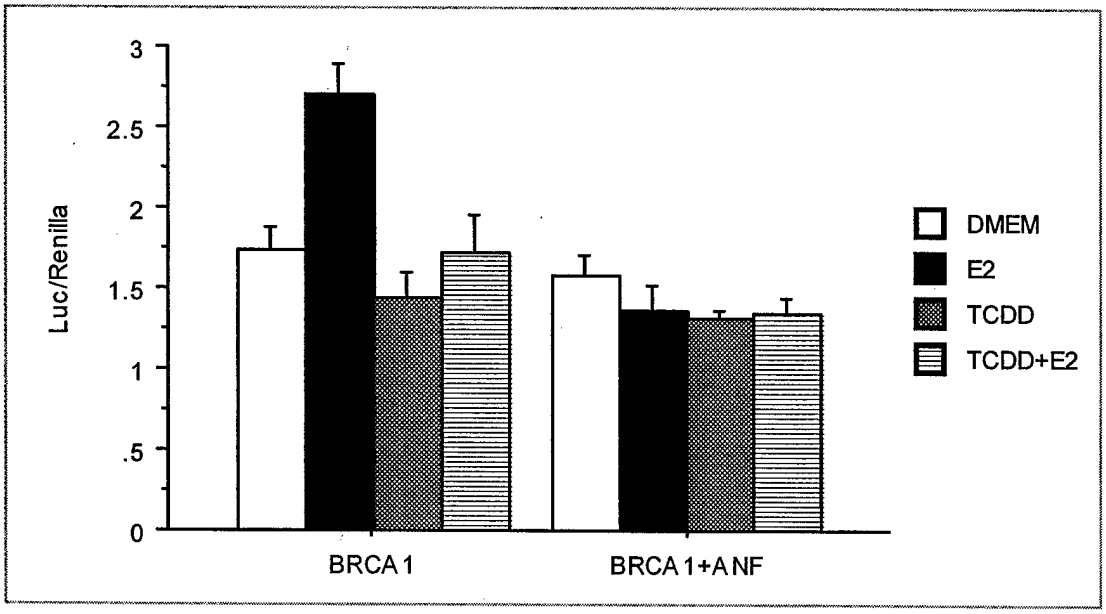


Figure 3. The AhR antagonist ANF prevents estrogen stimulation of BRCA-1 transcription.

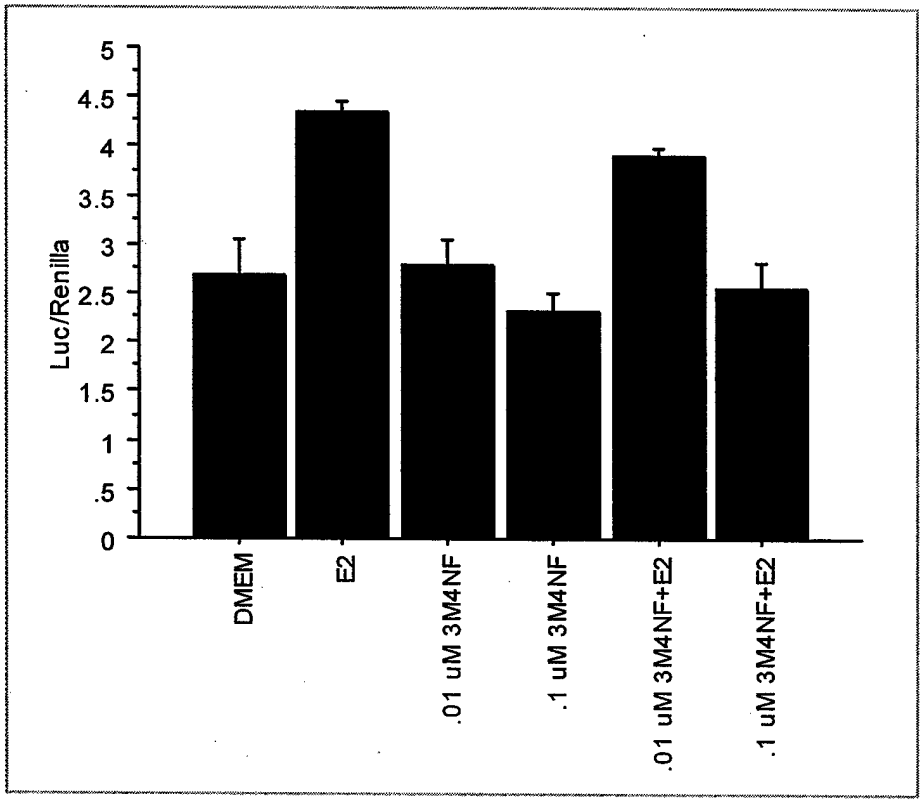
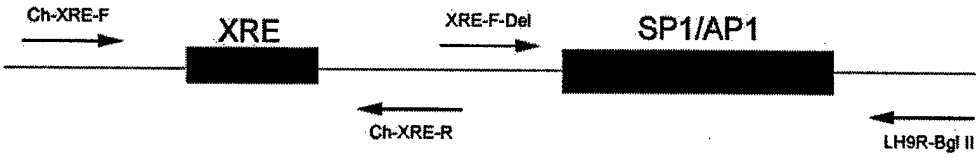


Figure 4. the pure antagonist 3M4NF antagonizes BRCA-1 transcription induced by estrogen.

A



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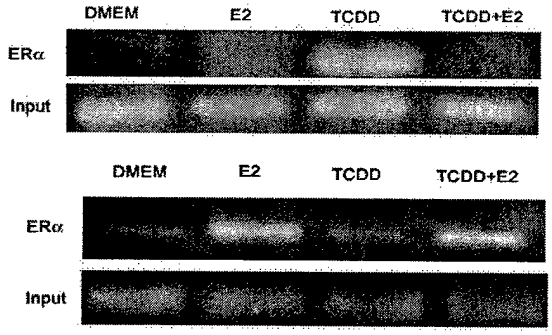

Figure 5. Chromatin immunoprecipitation assay of ERα at the XRE-2. Bands represent PCR amplification products following crosslinking and immunoprecipitation with an antibody for the ERα. The PCR products were generated using oligonucleotides flanking the A) XRE-2 or B) an Sp-1/AP-1 site known to be targeted by the ERα (positive control).

Key research Accomplishments

- Completed analysis of mutation contruct for XRE2 and the role of antagonists of the AhR in regulation of estrogen stimulation of BRCA-1 transcription.
- Obtained new evidence by ChIP assay of cross-talk between AhR ligands and estrogen in recruitment of the ERα at the BRCA-1 promoter.

Reportable Outcomes

1. Brandon D. Jeffy. Dissertation for a Ph.D. in Cancer Biology, The University of Arizona, December 2004.

2. Jennifer Ku. Data acquired through the execution of this project is being used for the preparation of a Ph.D. dissertation for the Cancer Biology. The support of the US Army Medical Research and Materiel Command has been acknowledged in the Acknowledgment section of these reports:
    1. Abstracts presented at the 94th Meetings of the AACR, 842:4230Breast Cancer and Environmental Mutagens Conference, Environmental Mutagen Society, Research Triangle Park, NC, September 22-25, 2001.
    2. Posters presented at the Arizona Cancer and Southwest Environmental Health Sciences Centers, The University of Arizona, Tucson, AZ.
    3. Preclinical Models of Breast Cancer Research Conference. 24th Congress of the International Association for Breast Cancer Research, UC Davis, Sacramento.

Conclusions

Summary

Based on the data obtained through the completion of the experiments outlined in the Body section of this report, we can conclude that exposure to ligands of the aromatic hydrocarbon receptors regulates transcription of the BRCA-1 gene, likely through XREs comprised in the BRCA-1 promoter. The mechanism being proposed is that through binding to AhR-binding domains, the AhR regulates the expression of BRCA-1. In detail, we propose that the XRE-2 selected as a prototype of other XREs found in the BRCA-1 gene, is required for estrogen activation of BRCA-1 transcription.

Importance and Implications

The findings of this Report confirm the original assumption that AhR-ligands may contribute to basal and estrogen regulation of BRCA-1 expression. These conclusions have been confirmed with the results of the transfections with mutation constructs for XRE-2. Results obtained with ChIP assay presented with this report have clarified that the ERα is recruited at the XRE-2 may contribute to formation of a transcription complex with the AhR.

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