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Award Number: DAMD17-02-1-0389

TITLE: CRE Activation in Antiestrogen Resistance

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REPORT DATE: May 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20031216 049

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE May 2003	3. REPORT TYPE AND DATES COVERED Annual Summary (1 May 2002 - 30 Apr 2003)
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4. TITLE AND SUBTITLE CRE Activation in Antiestrogen Resistance	5. FUNDING NUMBERS DAMD17-02-1-0389
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012
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10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited
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12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words) The over-expression of the human X-base binding protein (h-XBP-1) and hence elevated levels of CRE transcription activator contributes to the antiestrogen resistance. Preliminary data using Western blot analysis indicates that E2-independent but responsive breast cancer cells that are responsive to antiestrogen showed elevated hXBP-1 protein level whereas, the cell line that is resistant to antiestrogen showed a lower level of hXBP-1 protein expression. Furthermore, a promoter-reporter assay measuring luciferase activity as an indicator of CRE activation showed that cells that are antiestrogen resistant had a four-fold increase in CRE activation when compared to the sensitive cells. HXBP-1 is transcription factor that belongs to the ATF/CRE family of transcription factors and can bind to and activate the CREs (cyclic AMP response elements). It is based on this relationship that the amount of hXBP-1 protein levels could be determined by measuring the level of CRE containing genes. In our experiments by over expressing hXBP-1 using transfection we well convert sensitive cells to resistant cell. On the other hand, cell resistant to antiestrogen will become sensitive by using an antisense oligonucleotide approach. Anchorage dependent as well as anchorage independent growth responses of the respective transformed cells to antiestrogen will also be measured.

14. SUBJECT TERMS HXBP-1-Human X-box binding protein; CRE- cyclic AMP response elements; E2-Estrogen
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15. NUMBER OF PAGES 10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified
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18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified
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20. LIMITATION OF ABSTRACT Unlimited
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FOREWORD

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

07.02.03



PI - Signature

Date

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INTRODUCTION

Human X box bonding protein (hXBP-1) has been associated with antiestrogen resistance in our lab. We believe that by the up regulation of hXBP-1 due to the over-expression of this gene, that there is some contribution to antiestrogen resistance. This resistance to antiestrogens may occur by growth induction or by apoptosis blockage. Once we have up regulated hXBP-1 it is expected that there must be increased CRE transcriptional activation, this will be measured by promoter- reporter assays. The purpose of these experiments is based on the fact that most patients with initial responsive breast tumors to antiestrogens acquire antiestrogen resistance (1). Although several possible mechanisms such as mutations in the estrogen receptors (ER) and loss of the ER expression have been associated with antiestrogen resistance other possible mechanisms that can be considered to contribute to antiestrogen resistance may also include Tamoxifen stimulated resistance mechanisms (1). Tamoxifen (TAM) or ICI 46, 477 is the most widely used antiestrogen for breast cancer treatment. Faslodex (ICI 182, 780) is the other antiestrogen therapy used for breast cancer treatment (2). It is effective in patients who have acquired resistance to TAM treatment. In our experiments the two cell lines MCF7 and T 47D are being used to determine resistance to these antiestrogens when hXBP-1 is overexpressed.

BODY

1. MCF7 cells sensitive to antistrogen were transfected with a pcDNA 3.1 vector (Invitrogen) containing a hXBP-1 insert for hXBP-1 over-expression. The empty vector was also transfected into MCF-7 cells to create a negative control.
2. Clone resistant to G 418 were selected to determine clones positive for the pcDNA 3.1/ hXBP-1 insert. Clones were expanded using 6 well plates.
3. hXBP-1 protein was extracted from these clones using Trizol reagent (Invitrogen). The amounts of proteins expressed in these cells were determined by Western blot analysis.
4. HXBP-1 activity was determined by measuring CRE transcriptional activation using a dual luciferase promoter- reporter assay system (Promega).

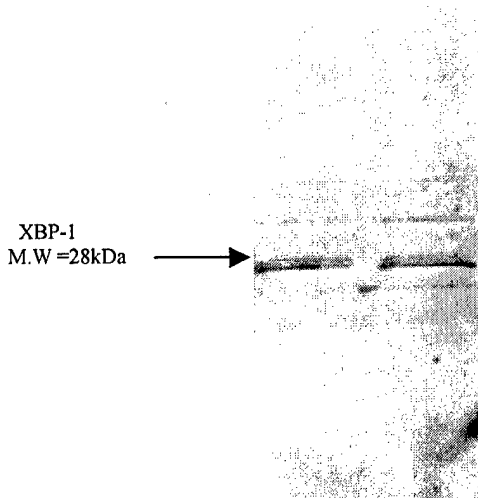


Fig. 1. Western blot to show two clones harboring hxbp-1 insert.

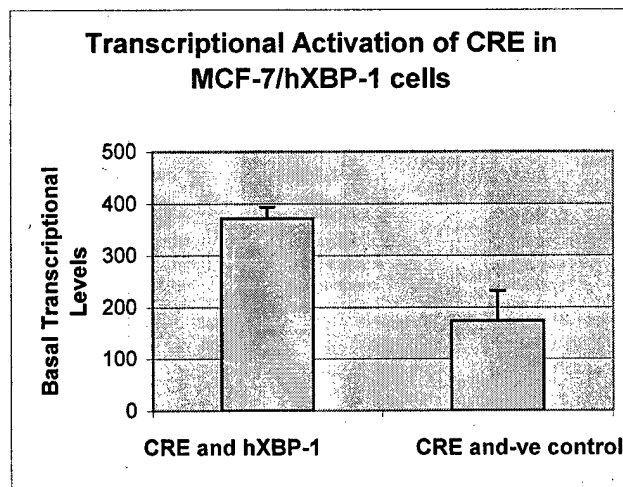


Fig.2. Transcriptional activation of hXBP-1 and CRE in MCF-7 cells. Negative control is empty pcDNA3.1 vector

KEY RESEARCH ACCOMPLISHMENTS

Task 1. To determine if sensitive cells can become resistant to antiestrogens Tamoxifen and Faslodex by the over-expression of hXBP-1 in breast cancer cells (Months 1-12):

- Transfected hXBP-1 into MCF/LCC1 (sensitive cells) with the vector containing the hXBP-1 insert.
- Cells were plated in media containing G418 to select resistant colonies (Months 1-6).
- Measured hXBP-1 expression levels in selected clones (Months 6-10).
- Confirmed hXBP-1 activity by measuring CRE transcriptional activity (Months 10-12).
- Currently T47D cells transfected with the pCDNA3.1 vector containing the hXBP-1 insert as well as the empty vector used as the negative control are now been grown in selective media for the selection of positive clones.

Course taken during the academic year 2001-2002: TBIO/PBIO
595 Tumor Endocrinology (3 credits)

REPORTABLE OUTCOMES

None at this current time.

CONCLUSION

- Over-expression of hXBP-1 was accomplished by the transfection of MCF-7 cells.

- Elevated levels of CRE in MCF7 cells transfected with the hXBP-1 indicate up regulation of hXBP-1.

- The MCF-7/hXBP-1 cells are now available for further characterization to explain the effects of XBP-1 expression on hormone responsiveness.

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