

AD _____

Award Number: DAMD17-02-1-0388

TITLE: X-Box Binding Protein-1 in Breast Cancer

PRINCIPAL INVESTIGATOR: Robert Clarke, Ph.D., D.Sc.

CONTRACTING ORGANIZATION: Georgetown University Lombardi Comprehensive
Cancer Center
Washington, DC 20007

REPORT DATE: August 2006

TYPE OF REPORT: Final Addendum

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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 Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 01-08-2006		2. REPORT TYPE Final Addendum		3. DATES COVERED 1 Aug 2002– 31Jul 2006	
4. TITLE AND SUBTITLE X-Box Binding Protein-1 in Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-02-1-0388	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Robert Clarke, Ph.D., D.Sc.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Georgetown University Lombardi Comprehensive Cancer Center Washington, DC 20007				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				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT The factors driving resistance to antiestrogens are unknown. Comparing the transcriptomes of antiestrogen responsive and resistant MCF-7 variants by serial analysis of gene expression, we have implicated several genes, including the human X-box binding protein-1 (XBP-1). XBP-1 is a cAMP response element (CRE) binding protein associated with estrogen receptor (ER) expression in gene expression profiles of human breast cancers. We hypothesize that overexpression of XBP-1 and/or activation of CRE contribute functionally to the ability of responsive cells to survive the metabolic stresses induced by exposure to antiestrogens. We also hypothesize that measuring expression of the XBP-1 protein will assist in better identifying antiestrogen resistant and/or responsive tumors. Aim 1: We will further study the likely functional role of XBP-1/CRE by overexpression through transfection into responsive cells, and inhibiting expression in resistant cells. Effects of these molecular manipulations on responsiveness to antiestrogens will be studied. We will; also identify signaling downstream of XBP1 that may explain how XBP1 modifies responsiveness to endocrine treatments in breast cancer cells. Aim 2: We will explore the prognostic and predictive significance of XBP-1 expression in a unique series of human breast cancer biopsies. Thus, we will begin to assess the extent to which XBP-1 is a candidate prognostic factor.					
15. SUBJECT TERMS Breast cancer, X-Box Binding Protein-1, antiestrogen resistance 1					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

X Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

X In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

X In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

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.



Oct 30, 2006

PI - Signature

Date

Table of Contents

Cover.....	1
SF 298.....	2
Foreward.....	3
Table of Contents.....	4
Introduction.....	5
Body.....	6
Key Research Accomplishments.....	6
Reportable Outcomes.....	11
Conclusions.....	11
References.....	12
Appendices	

INTRODUCTION

Antiestrogens are effective in premenopausal and postmenopausal patients, and in the chemopreventive, adjuvant and metastatic settings (1), probably through the induction of growth arrest/apoptosis (1). The triphenylethylene TAM, a partial agonist, is the most widely used antiestrogen. Long term TAM use reduces the incidence of contralateral breast cancer (antagonist) and primary breast cancer in high risk women (antagonist), maintains bone density (agonist) and increases the risk of endometrial carcinomas (agonist) (2). Newer antiestrogens include the “pure antagonist” ICI 182,780 (Faslodex; no agonist activity). In patients that had previously shown a response to TAM but recurred, Faslodex produces a response rate significantly higher than the response rate for crossover to another triphenylethylene (Toremifene) following TAM failure (3).

Antiestrogen Resistance. Most breast tumors that initially respond to TAM recur and require other endocrine or cytotoxic therapies (4). Despite over 10 million patient years of experience with TAM, the precise mechanisms that confer acquired resistance are unknown (1). Absence of ER expression is clearly important for *de novo* resistance (1). ER expression is *not* lost in most breast tumors that acquire antiestrogen resistance (5). Currently, there is little compelling evidence that expression of ER splice variants and mutant ER contribute significantly to antiestrogen resistance in patients (1;6). While the importance of wild type ER α is established as a mediator/predictor of antiestrogen responsiveness, that of ER β remains unclear. ER α may be the predominant species in most ER+ breast tumors (7;8), and is associated with a better prognosis (9). ER β is associated with a poorer prognosis, absence of PgR, and lymph node involvement (8;10). One small study reported higher ER β mRNA levels in resistant tumors (11). However, this association could not be separated from that between ER β and a more aggressive phenotype (8;10). Some studies report activities independent of ER function, which may initiate events that are necessary but not sufficient for antiestrogen-induced effects (1). Our research team has recently reviewed in detail the potential mechanisms of antiestrogen resistance in ER+ tumors (12).

Implicating XBP-1 in Antiestrogen Resistance. Initially, we explored differences in the transcriptomes of the MCF7/LCC1 (antiestrogen sensitive) and MCF7/LCC9 cells (antiestrogen resistant – resistant to both TAM and Faslodex) by serial analysis of gene expression (SAGE) as previously described (13), using the “SAGE” software (Dr. Kinzler, Johns Hopkins University). Most genes identified are not differentially expressed between MCF7/LCC1 and MCF7/LCC9 cells. Differentially expressed genes were selected by (a) the Tags compared represent ≤ 2 genes, (b) a Tag found in either the MCF7/LCC1 or MCF7/LCC9 SAGE library must represent 0.10% of the database, and (c) fold difference ~ 2 -fold. Evidence that a gene is expressed in breast cancers also was considered. No single criterion was considered an absolute requirement for selection. Among the genes we identified were cathepsin D, nucleophosmin (NPM), tumor necrosis factor (TNF) and XBP-1 (14).

To confirm the altered expression of XBP-1, we first performed Western analysis on proteins from MCF7/LCC1 and MCF7/LCC9 cells. We initially detected a ~ 5 -fold induction of XBP-1 protein in MCF7/LCC9 cells, comparable with the 4-fold induction in mRNA levels (14). Measuring protein levels and/or protein bound to responsive elements can be poor indicators of the functional activation of transcription factors. Since XBP-1 activates CREs, we measured directly CRE transcriptional activation using a CRE promoter-firefly luciferase reporter assay

(PathDetect *in vivo* signal transduction pathway *cis*-reporting system; Stratagene). Cells were transiently transfected with the appropriate plasmids using Qiagen's Superfect reagent. Normalization of transfection efficiency was made to a *Renilla* luciferase reporter driven by the constitutive cytomegalovirus promoter (Promega's Dual-luciferase reporter assay). The basal CRE activity is significantly increased in MCF7/LCC9 cells compared with MCF7/LCC1 cells (14).

Upregulation of CRE activation would be of limited use to cells if it could be inhibited by Faslodex-occupied ERs. Thus, we assessed the ability of Faslodex to affect CRE activation using the promoter-reporter assay. Faslodex treatments (10 nM) were administered for 48 hrs post-transfection. Faslodex treatment does not alter the transcriptional regulatory activities of the CRE promoter in either responsive MCF7/LCC1 or resistant MCF7/LCC9 cells (14). These data further imply a functional role for XBP-1 in acquired resistance to Faslodex. In responsive cells, the inability to induce CRE in the presence of Faslodex allows for the dominance of growth inhibitory signals leading to growth arrest/apoptosis. Resistant cells may survive growth inhibition/apoptosis by upregulating signaling through CREs. Since CRE-activation is required for MCF-7 cell proliferation (15), some breast cancer cells may survive antiestrogen treatment by upregulating factors that are not affected by ER-mediated signaling, e.g., XBP-1/CRE.

BODY

This is a final addendum report. In this report, we present primarily the work completed during the no-cost extension.

KEY RESEARCH ACCOMPLISHMENTS

We have continued to make good progress and have nearly completed most of the Tasks as proposed. We have made changes to Task 3 to change the direction in a more productive manner and obtained significant new data from gene expression microarray studies. These new studies provide powerful mechanistic insights not initially envisioned in the application. We are on target to complete most of the unfinished work and have requested a no-cost extension to help us do so.

Bulleted List of Research Accomplishments (this reporting period)

- Completed analyses of microarray data and identified genes implicated in driving XBP-1's effects on cell cycle and apoptosis
- Selected second round of candidate genes for validation
- Validated differential expression of candidate genes from the second round of analysis by quantitative real-time PCR (qPCR)
- Generated new signal transduction pathway for XBP-1 based on validated genes from first and second round of microarray validation studies

Nota Bene: Please note that we changed the emphasis for Task 3 in the SOW in the prior report.

RESEARCH ACCOMPLISHMENTS

TASK 1: Overexpress XBP-1 in antiestrogen sensitive cells

This aim has been essentially completed. As previously reported, a small preliminary experiment was inconclusive with respect to XBP-1 conferring full estrogen-independence *in vivo* in MCF7/XBP-1 cells. As also indicated in the prior report, we chose to use the remaining resources to explore the cellular signaling data we had begun to generate last year (see Task 3; below).

TASK 2: Inhibit XBP-1 expression in antiestrogen resistant cells

We had some difficulty with the reproducibility of the siRNA. While our initial studies were promising (some were reported in an earlier report), we could not get readily interpretable data. We could not exclude some “off-target” effects, and so we chose not to pursue further these studies. Since we were quite successful with the other aims, included additional studies in Task 3, and the amount of work proposed here represented a relatively modest proportion of the overall proposal, we do not consider this problematic.

TASK 3: Explore the molecular events that confer XBP-1’s ability to affect endocrine responsiveness (modified Task for Statement of Work)

TASK 3: Timing of acquired increase in XBP-1 expression and CRE activation (original task)

In the prior report, we presented the results of some preliminary gene expression microarray studies comparing the MCF7/XBP-1 and control cells. Our primary focus for this final period of investigation was to continue working on this data set. Thus, we completed analysis of the data by performing a second run of analysis to finalize the gene list related to apoptosis and cell proliferation, and performed qPCR validation studies.

The experimental design for the microarray studies was provided in the prior report. We first reran the analysis to obtain a more robust assessment of differentially expressed genes, this time we included a more detailed *in silico* search of promoters of those genes selected from Bin 1 (we looked only in Bin 1). These searches were done only for those differentially expressed genes associated with apoptosis and cell cycle distribution, since these are the functions of primary interest to this research project. Table 1 shows the final list of differentially expressed genes that also contain at least one XBP-1 responsive element (CRE with ACGT core sequence) in their upstream regions; Fig 1 shows the results of the dimension reduction analysis and Bin 1 from within which these genes were identified. Previously, we had validated only three genes/proteins, Bcl2 (protein), ER α (protein), and aromatase (mRNA).

To study further the data on aromatase transcription, we established a new collaboration with Dr. Angela Brodie at the University of Maryland. Dr. Brodie is a world expert in the study of aromatase and she tested the cell lines for increased aromatase activity. Despite the significant

induction of aromatase mRNA as confirmed by quantitative real-time PCR (MCF7/XBP-1 cells; 8-fold; $p < 0.05$), Dr. Brodie was not able to detect increased enzyme activity. We then looked for protein by Western but we could not detect increased protein by Western. It is not clear if the increased transcription in the absence of translation or activation is unique to MCF-7 cells but it is interesting that we did not detect increased aromatase transcription in the T47D/XBP-1 cells. The aromatase gene has several promoters that contain XBP-1 responsive elements; promoters 1.3, 1.4, 1.7, and II are used in breast tissues and each contain at least one CRE-ACGT. Thus, the potential remains for XBP-1 to affect aromatase expression in other breast cancer cells or other estrogen-responsive tissues.

<i>Symbol</i>	<i>Gene Name</i>	<i>CRE^a</i>	<i>Fold Change (Affymetrix)^b</i>	<i>p value</i>	<i>Fold Change (qPCR)^b</i>	<i>p value</i>
<u>APBB2</u>	myloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	tc <u>ACGT</u> ga -1092 to -1081	-1.678	0.001	-1.33	0.001
BECN1	beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)	tc <u>ACGT</u> gag -290 to -282	1.641	0.042	1.01	0.752
<u>CRK</u>	v-crk sarcoma virus CT10 oncogene homolog (avian)	tc <u>ACGT</u> gg -1366 to -1354	-1.585	0.048	-1.96	0.003
CSPG6	chondroitin sulfate proteoglycan 6 (bamacan)	cg <u>ACGT</u> gg -677 to -665 tc <u>ACGT</u> gg -379 to -367	-2.122	0.017	1.039	0.87
<u>IL24</u>	interleukin 24	tg <u>ACGT</u> gg -1402 to -1390	-2.668	0.015	-9.7	<0.001
<u>MYC</u>	v-myc myelocytomatosis viral oncogene homolog (avian)	at <u>ACGT</u> gg -794 to -787	1.601	0.031	1.2	0.05
<u>PHLDA2</u>	pleckstrin homology-like domain, family A, member 2	ag <u>ACGT</u> tg -1095 to -1083	-1.808	0.001	-3.34	0.004
<u>S100A6</u>	S100 calcium binding protein A6 (calcyclin)	ac <u>ACGT</u> gg -709 to -697 tc <u>ACGT</u> gt -248 to -241	2.586	0.013	2.26	<0.001
TFDP1	transcription factor Dp-1	ag <u>ACGT</u> gt -1384 to -1377	1.626	0.025	1.22	0.366
TOP1	topoisomerase (DNA) I	tg <u>ACGT</u> cg, -51 to -39	-2.026	0.038	-1.09	0.512
<u>XRCC6</u>	X-ray repair complementing defective repair in Chinese hamster cells 6 (Ku autoantigen, 70 kDa)	cg <u>ACGT</u> gt -42 to -30 tg <u>ACGT</u> tag -97 to -90	1.602	0.010	1.6	0.016

Table 1: Genes selected for validation by quantitative real time PCR (qPCR). Gene symbols are HUGO annotations; those bolded and underlined were significant by Affymetrix and confirmed by qPCR.

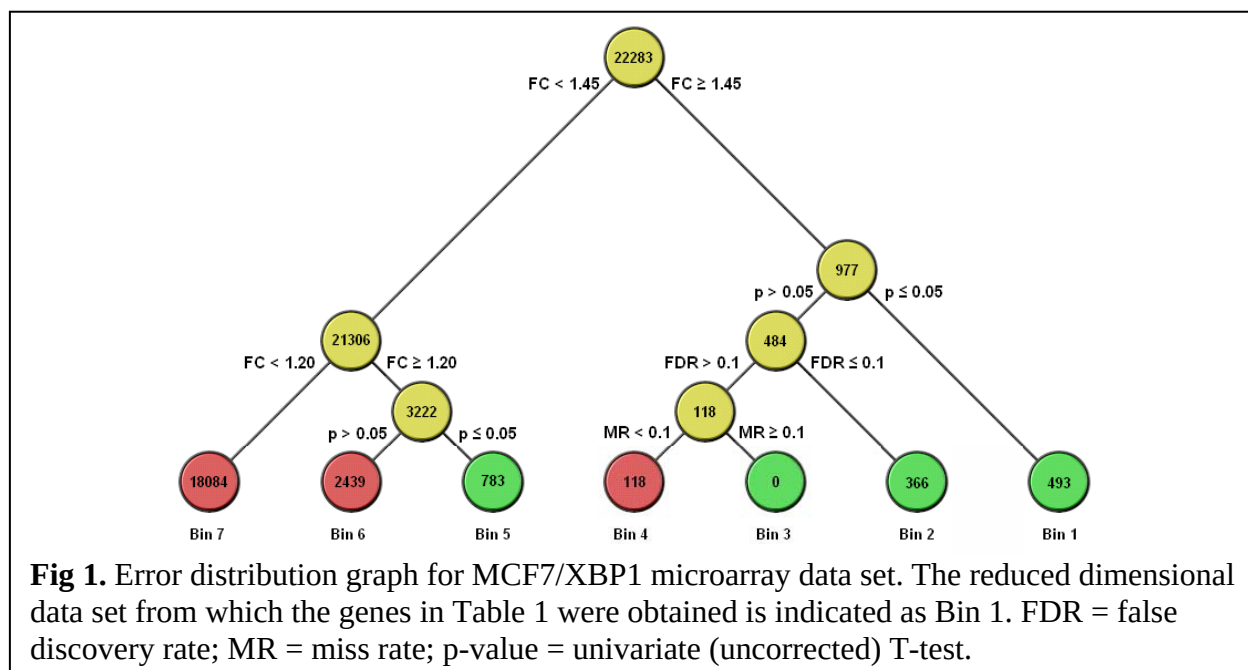
^aPutative XBP-1 binding site(s) in the promoter as predicted by MatInspector. The core **ACGT**

sequence is indicated; numbers indicate the position of the sequence relative to the transcription start site

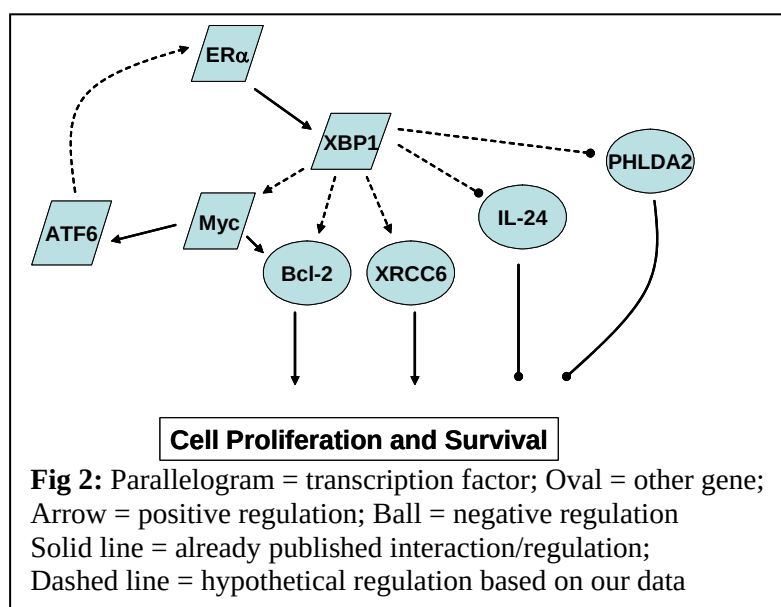
^bFold Change in MCF7/XBP-1 cells relative to MCF7/c (control cells)

XBP-1 mediated antiestrogen resistance is characterized by increased cell cycle progression and reduced levels of apoptosis. As a transcription factor, it is likely that XBP-1 controls the resistant phenotype by regulating the transcription of cell cycle- and apoptosis-associated genes. In our gene expression microarray studies, we found several genes annotated with these functions, that contain ACGT CREs in their 5' sequences, and that are likely to be significantly altered by XBP-1 overexpression (based on simple univariate analysis). For example, IL24 is strongly downregulated by XBP-1 overexpression (-2.68 fold by microarray, and -9.7 fold by qPCR). Also known as melanoma differentiation-associated gene 7 (mda-7), IL24 is an established inducer of apoptosis in breast cancer cell lines (16). While this gene has not been previously associated with estrogen or antiestrogen responsiveness, downregulation of proapoptotic molecules like IL24/mda-7 may play a functional role in endocrine resistance. Similarly, PHLDA2 (also known as IPL or TSSC3) is significantly downregulated in MCF7/XBP-1 cells (-1.8 fold by microarray, and -3.3 fold by qPCR). PHLDA2 is one of the only apoptosis-associated genes known to be imprinted. Moreover, PHLDA2 is located on chromosome 11p15, a region thought to harbor tumor suppressor activities and that also is altered in several cancers including breast cancer (17). In the mouse, where expression of maternal PHLDA2 is highest in extra-embryonic structures, knockout of this gene leads to overgrowth of the placenta (18), suggesting that dysregulation of PHLDA2 can promote hyperproliferation.

In contrast to the suppression of tumor suppressor-like activities, we observe upregulation of proliferative and/or prosurvival genes by XBP-1: c-Myc, XRCC6, and the calcium-binding protein S100A6 (calcyclin). Increased S100A6 expression has been demonstrated in several different cancerous versus normal tissues (19), and in 32% of breast tumors (20). In pulmonary fibroblasts, S100A6 antisense oligonucleotides inhibit cell proliferation, (21). In WI-38



embryonic lung cells, any delay in the upregulation of S100A6 transcripts in late G1 is accompanied by a delay in S phase entry (22). The molecular mechanism(s) by which S100A6 regulates proliferation is unclear, but its calcium binding function may be important. XRCC6 (also known as the Ku70 autoantigen) is a DNA repair molecule essential to the end-joining process (reviewed in (23)). Two single nucleotide polymorphisms (SNPs) in the XRCC6 gene are significantly associated with breast cancer risk (24), and XRCC6 overexpression has recently been implicated in acquired cisplatin resistance in ovarian cancer cells (25). Finally, c-Myc is a well-established pro-survival molecule in human breast cancer and mouse mammary tumorigenesis (26). Capable of modulating both cell growth and cell death, cMyc is overexpressed in 70% of breast tumors, and amplified at least 3-fold in 16% of breast tumors (27). Moreover, inducing c-Myc expression in MCF-7 cells can confer resistance to Faslodex (28). To our knowledge, this is the first report that XBP-1 can regulate c-Myc expression in breast cancer cells. Whether the regulation of cMyc signaling by XBP-1 contributes to the resistance phenotype in T47D cells is currently under investigation.



The signaling network predicted by these interactions is shown in Fig 2. We include ATF6 even though it is not differentially expressed because it can be activated by Myc and induce ER; both ER and Myc are upregulated in the MCF7/XBP-1 cells. XBP-1 and ER can interact to increase the transcriptional activation of ER (activation would not be detected by the micorarrays); ER also is known to regulate Myc and Bcl2, so these may be affected directly by XBP-1 (through the CREs) and/or through XBP-1 increasing transcriptional

activation of ER. In a recent collaborative study with colleagues in the U.K., we then showed how increased ER expression and phosphorylation is sufficient to confer antiestrogen resistance. Establishing the functional relevance of other nodes in this pathway and confirming the regulatory interactions will be used as the basis for a future application to NIH or another funding agency.

TASK 4: Explore XBP-1 expression in clinical samples

We have completed and published the first study on the clinical samples - a reprint will be forwarded to the DOD BCRP. We will continue with these studies in additional clinical material as it becomes available.

REPORTABLE OUTCOMES

We have now published some of our data and presented other data at meetings and in abstracts. Another paper has been submitted and this will be forwarded to the DOD BCRP once it has been accepted for publication. Publications in the past 12 months are listed below.

1. Zhu, Y., Singh, B., Hewitt, S., Liu, A., Gomez, B., Wang, A. & Clarke, R. "Expression patterns among proteins associated with endocrine responsiveness in breast cancer: interferon regulatory factor-1, human X-box binding protein-1, nuclear factor kappa B, nucleophosmin, estrogen receptor-alpha, and progesterone receptor." *Int J Oncol*, 28: 67-76, 2006.
2. Kuske, B., Naughton, C., Moore, K., MacLeod, K.G., Miller, W.R., Clarke, R. Langdon, S.P. & Cameron, D.A. "Endocrine therapy resistance can be associated with high estrogen receptor alpha (ER α) expression and reduced ER α phosphorylation in breast cancer models." *Endocr Related Cancer*, in press.
3. Gomez, B.P., Riggins, R.B., Klimach, U., Zhu, Y., Zwart, A., Wang, M., Wang, A. & Clarke, R. "Human X-Box Binding Protein-1 confers both estrogen-independence and antiestrogen resistance in breast cancer." submitted.
4. Gomez, B.P., Riggins, R.B., Zhu, Y., Zwart, A., Wang, A & Clarke, R. "Human X box binding protein-1 (XBP-1) induces antiestrogen resistance via deregulation of cell cycle progression and the intrinsic apoptotic pathway." *AACR Annual General Meeting*, 2006 (presented as a podium presentation).

CONCLUSIONS

Our data confirm a potentially important role for XBP-1 in breast cancer. We have successfully overexpressed XBP-1 in MCF-7 cells, shown that XBP-1 binds to ER α , and induces estrogen-independence and antiestrogen resistance. We have optimized the use of tissue microarrays and demonstrated the detectable presence of XBP-1 protein in breast tumors. We have performed gene expression microarrays on XBP-1 transfected and control cells and identified several key leads to explain how XBP-1 may function. We have confirmed the differential expression of most of the selected genes and constructed a signaling pathway to explain how XBP-1 may act to affect breast cancer cell proliferation and cell survival.

References

- (1) Clarke R, Leonessa F, Welch JN, Skaar TC. Cellular and molecular pharmacology of antiestrogen action and resistance. *Pharmacol Rev* 2001;53:25-71.
- (2) Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst* 1998;90:1371-88.
- (3) Vogel CL, Shemano I, Schoenfelder J, Gams RA, Green MR. Multicenter phase II efficacy trial of toremifene in tamoxifen- refractory patients with advanced breast cancer. *J Clin Oncol* 1993;11:345-50.
- (4) EBCTCG. Early Breast Cancer Trialists' Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomized trials. *Lancet* 1998;351:1451-67.
- (5) Johnston SRD, Saccanti-Jotti G, Smith IE, Newby J, Dowsett M. Change in oestrogen receptor expression and function in tamoxifen-resistant breast cancer. *Endocr Related Cancer* 1995;2:105-10.
- (6) Karnik PS, Kulkarni S, Liu XP, Budd GT, Bukowski RM. Estrogen receptor mutations in tamoxifen-resistant breast cancer. *Cancer Res* 1994 Jan 15;54(2):349-53.
- (7) Leygue E, Dotzlaw H, Watson PH, Murphy LC. Altered estrogen receptor α and β messenger RNA expression during human breast tumorigenesis. *Cancer Res* 1998;58:3197-201.
- (8) Speirs V, Parkes AT, Kerin MJ, Walton DS, Carelton PJ, Fox JN, et al. Coexpression of estrogen receptor α and β : poor prognostic factors in human breast cancer. *Cancer Res* 1999;59:525-8.
- (9) Fitzgibbons PL, Page DL, Weaver D, Thor AD, Allred DC, Clark GM, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. *Arch Pathol Lab Med* 2000;124(7):966-78.
- (10) Dotzlaw H, Leygue E, Watson PH, Murphy LC. Estrogen receptor- β messenger RNA expression in human breast tumor biopsies: relationship to steroid receptor status and regulation by progestins. *Cancer Res* 1999;59:529-32.
- (11) Speirs V, Malone C, Walton DS, Kerin MJ, Atkin SL. Increased expression of estrogen receptor beta mRNA in tamoxifen- resistant breast cancer patients. *Cancer Res* 1999 Nov 1;59(21):5421-4.
- (12) Clarke R, Liu MC, Bouker KB, Gu Z, Lee RY, Zhu Y, et al. Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene* 2003 Oct 20;22(47):7316-39.

- (13) Velculescu VE, Zhang L, Vogelstein B, Kinzler KW. Serial analysis of gene expression. *Science* 1995 Oct 20;270(5235):484-7.
- (14) Gu Z, Lee RY, Skaar TC, Bouker KB, Welch JN, Lu J, et al. Association of interferon regulatory factor-1, nucleophosmin, nuclear factor-kappaB, and cyclic AMP response element binding with acquired resistance to faslodex (ICI 182,780). *Cancer Res* 2002 Jun 15;62(12):3428-37.
- (15) Lee YN, Park YG, Choi YH, Cho YS, Cho-Chung YS. CRE-transcription factor decoy oligonucleotide inhibition of MCF-7 breast cancer cells: cross-talk with p53 signaling pathway. *Biochemistry* 2000 Apr 25;39(16):4863-8.
- (16) Su ZZ, Madireddi MT, Lin JJ, Young CS, Kitada S, Reed JC, et al. The cancer growth suppressor gene mda-7 selectively induces apoptosis in human breast cancer cells and inhibits tumor growth in nude mice. *Proc Natl Acad Sci USA* 1998 Nov 24;95(24):14400-5.
- (17) Lee MP, Feinberg AP. Genomic imprinting of a human apoptosis gene homologue, TSSC3. *Cancer Res* 1998 Mar 1;58(5):1052-6.
- (18) Frank D, Fortino W, Clark L, Musalo R, Wang W, Saxena A, et al. Placental overgrowth in mice lacking the imprinted gene Ipl. *Proc Natl Acad Sci U S A* 2002 May 28;99(11):7490-5.
- (19) Ilg EC, Schafer BW, Heizmann CW. Expression pattern of S100 calcium-binding proteins in human tumors. *Int J Cancer* 1996 Nov 4;68(3):325-32.
- (20) Cross SS, Hamdy FC, Deloulme JC, Rehman I. Expression of S100 proteins in normal human tissues and common cancers using tissue microarrays: S100A6, S100A8, S100A9 and S100A11 are all overexpressed in common cancers. *Histopathology* 2005 Mar;46(3):256-69.
- (21) Breen EC, Tang K. Calcyclin (S100A6) regulates pulmonary fibroblast proliferation, morphology, and cytoskeletal organization in vitro. *J Cell Biochem* 2003 Mar 1;88(4):848-54.
- (22) Cosenza SC, Owen TA, Soprano DR, Soprano KJ. Evidence that the time of entry into S is determined by events occurring in early G1. *J Biol Chem* 1988 Sep 5;263(25):12751-8.
- (23) Smider V, Chu G. The end-joining reaction in V(D)J recombination. *Semin Immunol* 1997 Jun;9(3):189-97.
- (24) Fu YP, Yu JC, Cheng TC, Lou MA, Hsu GC, Wu CY, et al. Breast cancer risk associated with genotypic polymorphism of the nonhomologous end-joining genes: a multigenic study on cancer susceptibility. *Cancer Res* 2003 May 15;63(10):2440-6.

- (25) Sakamoto M, Kondo A, Kawasaki K, Goto T, Sakamoto H, Miyake K, et al. Analysis of gene expression profiles associated with cisplatin resistance in human ovarian cancer cell lines and tissues using cDNA microarray. *Hum Cell* 2001 Dec;14(4):305-15.
- (26) Jamerson MH, Johnson MD, Dickson RB. Of mice and Myc: c-Myc and mammary tumorigenesis. *J Mammary Gland Biol Neoplasia* 2004 Jan;9(1):27-37.
- (27) Deming SL, Nass SJ, Dickson RB, Trock BJ. C-myc amplification in breast cancer: a meta-analysis of its occurrence and prognostic relevance. *Br J Cancer* 2000 Dec;83(12):1688-95.
- (28) Venditti M, Iwasiow B, Orr FW, Shiu RP. C-myc gene expression alone is sufficient to confer resistance to antiestrogen in human breast cancer cells. *Int J Cancer* 2002 May 1;99(1):35-42.