

AD _____

AWARD NUMBER: DAMD17-02-1-0408

TITLE: Anti-Estrogen Regulations of Macrophage Products that Influence Breast Cancer Cell Proliferation and Susceptibility to Apoptosis

PRINCIPAL INVESTIGATOR: Theodore Bremner, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Washington, DC 20060

REPORT DATE: August 2006

TYPE OF REPORT: Annual

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 (DD-MM-YYYY) 01-08-2006		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 5 Jul 2005 – 4 Jul 2006	
4. TITLE AND SUBTITLE Anti-Estrogen Regulations of Macrophage Products that Influence Breast Cancer Cell Proliferation and Susceptibility to Apoptosis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER DAMD17-02-1-0408	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Theodore Bremner, Ph.D. E-Mail: tbremner@howard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Howard University Washington, DC 20060				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					
14. ABSTRACT No abstract provided.					
15. SUBJECT TERMS Tamoxifen, Faslodex™, metastasis, apoptosis, MCF-7, MDA-MD-231, macrophages					
16. SECURITY CLASSIFICATION OF:				17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 8
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U	19a. NAME OF RESPONSIBLE PERSON USAMRMC		
				19b. TELEPHONE NUMBER (include area code)	

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	6

INTRODUCTION

In June 2006, I applied for a final no-cost extension to complete Task 2c of the project which required more time than previously anticipated. Progress was slow owing to difficulty in recruiting a qualified laboratory technician for a single year. During the spring and summer (2006), I trained an undergraduate student, Ms. Giselle Burnett, who is now assisting me with cell culture, western blotting and RT-PCR. The project will be completed in the current year (06/07).

BODY

STATEMENT OF WORK

Tamoxifen and tumor-associated macrophages

Task 1. Determine the effect of *in vitro* co-culture on gene expression in BC cells and THP-1- macrophages (Months 1 - 18):

- (a) Recruitment of postdoctoral fellow (Months 1 - 2)
- (b) Grow cells, set up co-cultures of BC and THP-1 macrophages (Months 1 - 7)
- (c) Isolate mRNA for gene expression array analysis, Months 4 - 12
- (d) Standardize and calibrate gene expression arrays for proliferation-related gene expression in BC cells co-cultured with THP-1 macrophages (Months 11 - 18)

Task 2. Studies on effects of anti-inflammatory and macrophage-modulating compounds on macrophage and BC gene expression (Months 16 - 36):

- (a) Co-culture of cells for studies on the effects of anti-inflammatory agents (Months 16 - 30)
- (b) Isolation of mRNA for gene RT-PCR, and gene expression arrays for Task 2 (Months 17 - 30)
- (c) **Western blotting, ELISA for cytokines, RT-PCR (Months 24 - 36)**

To date we have observed that when macrophages and breast cancer cells are co-cultured there is reciprocal regulation of gene expression in both cell types. Our data suggest that of the many cytokine and other genes whose levels of expression are altered when breast cancer cells and macrophages are grown together, *HIF-1 α* and *MIF* (macrophage migration inhibitory factor) showed significant changes that may influence macrophage function in relation to tumor progression. We have analyzed *HIF-1 α* gene expression by gene arrays and RT-PCR, and confirmed that HIF-1 is constitutively expressed in THP-1 monocytes and THP-1-derived macrophages. Furthermore, we have shown by TransAMTM ELISA, a transcription factor binding assay that measures the functionality of the HIF-1 heterodimer by its ability to bind a hypoxia response element (HRE) consensus sequence (5'-RCGTG-3'), that functional HIF-1 is expressed in THP-1 monocytes, and moderately up-regulated by 50 μ M cobaltous chloride, a hypoxia mimic (Fig. 1). HIF-1 α is also expressed in MCF-7, both under normoxia and hypoxia. Recent reports in the literature support a critical role for HIF-1 in breast cancer progression, because CXCR4, a chemokine receptor associated with metastasis [1], is a direct transcriptional target of HIF-1 [2]. CXCR4, a G-protein-coupled receptor, is activated upon binding to its ligand SDF-1/CXCL12 and stimulates several signal transduction pathways that regulate motility, chemotaxis, survival and proliferation of many cell types [3,]. Paradoxically, in our experiments, HIF-1 α expression is extinguished in THP-1 macrophages when the two cells are co-cultured (Fig. 2, A, B). MCF-7 cells, however, continue to express HIF-1 α (Fig. 2, C, D). It was recently reported that the human MIF gene contains a HRE in its 5' UTR and is a transcriptional target of HIF-1 [4]. Therefore, MIF expression may be driven by hypoxia through HIF-1. MIF has been implicated in paclitaxel resistance of prostate cancer due to modulation of the multidrug resistance (MDR-1) gene [5].

KEY RESEARCH ACCOMPLISHMENTS

We have observed that:

- THP-1 monocytes and THP-1-derived macrophages produce functional HIF-1 (as opposed to HIF-1 α mRNA) under normoxia. Therefore, HIF-1 α is not targeted for proteasomal degradation under normoxia in these cells. The implications of this finding for tumor-associated macrophages are being explored.
- Our Western blot analysis of MIF shows that two forms of MIF are present in macrophages and MCF-7 cells, suggesting that MIF is either proteolytically processed or otherwise covalently modified in MCF-7. When MCF-7 is co-cultured with THP-1-derived macrophages, both forms are present in the MCF-7 cells (Fig. 3). Such variation has not been reported in the literature. Also, MIF protein is down-regulated in LPS-activated THP-1 macrophages.

REPORTABLE OUTCOMES

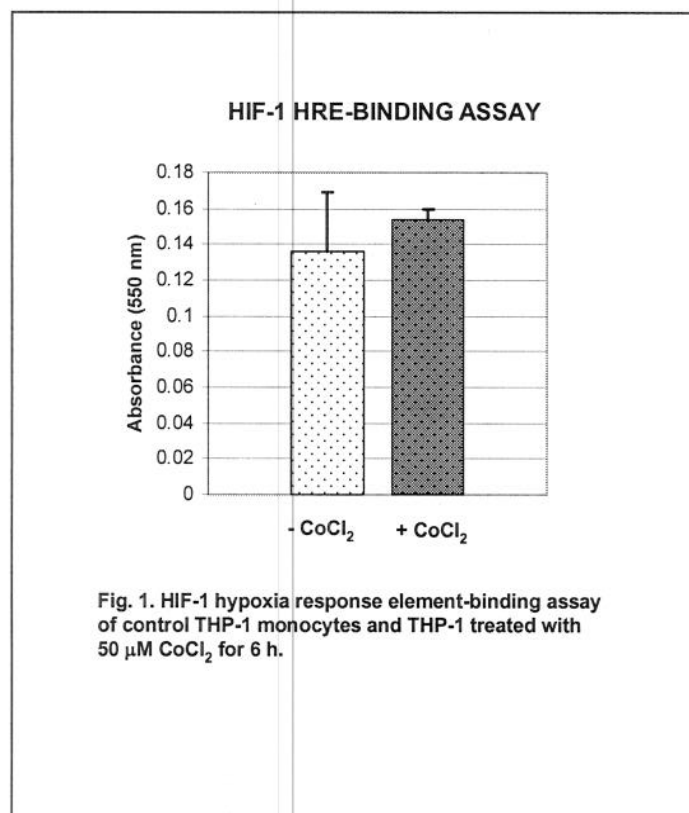
None

CONCLUSIONS

Both HIF-1 and MIF play important roles in the pathogenesis of breast cancer. Their activity and function in tumor-associated macrophages may drive tumor progression. While these proteins may provide growth and survival advantages to TAMs, they may also confer vulnerabilities, such as susceptibility to endogenous or exogenous agents. An exhaustive search for putative susceptibilities in the context of the tumor microenvironment may lead to the identification of new therapeutic targets.

REFERENCES

1. Muller, A., Homey, B., Soto, H. *et al.* (2001). Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410, 50-56.
2. Phillips, R. J., Mestas, J., Gharaee-Kermani, M., Burdick, M. D. *et al.* (2005). Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/ mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1 α . *J. Biol. Chem.* 280, 22473-22481.
3. Kucia, M., Reca, R., Miekus, K., Wanzeck, J. *et al.* (2005). Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. *Stem Cells* 23, 879-894.
4. Baugh, J. A. *et al.* (2006). Dual regulation of macrophage migration inhibitory factor (MIF) expression in hypoxia by CREB and HIF-1. *Biochem. Biophys. Res. Commun.* 347, 895-903.
5. Yu, D. S. Lin, J. C., Hsieh, D. S., Chang, S. Y., Lee, C. F. (2006). Modulation of MDR-1 gene by MIF and GSTp1 with drug resistance generation in hormone independent prostate cancer. *Arch. Androl.* 52, 283-291



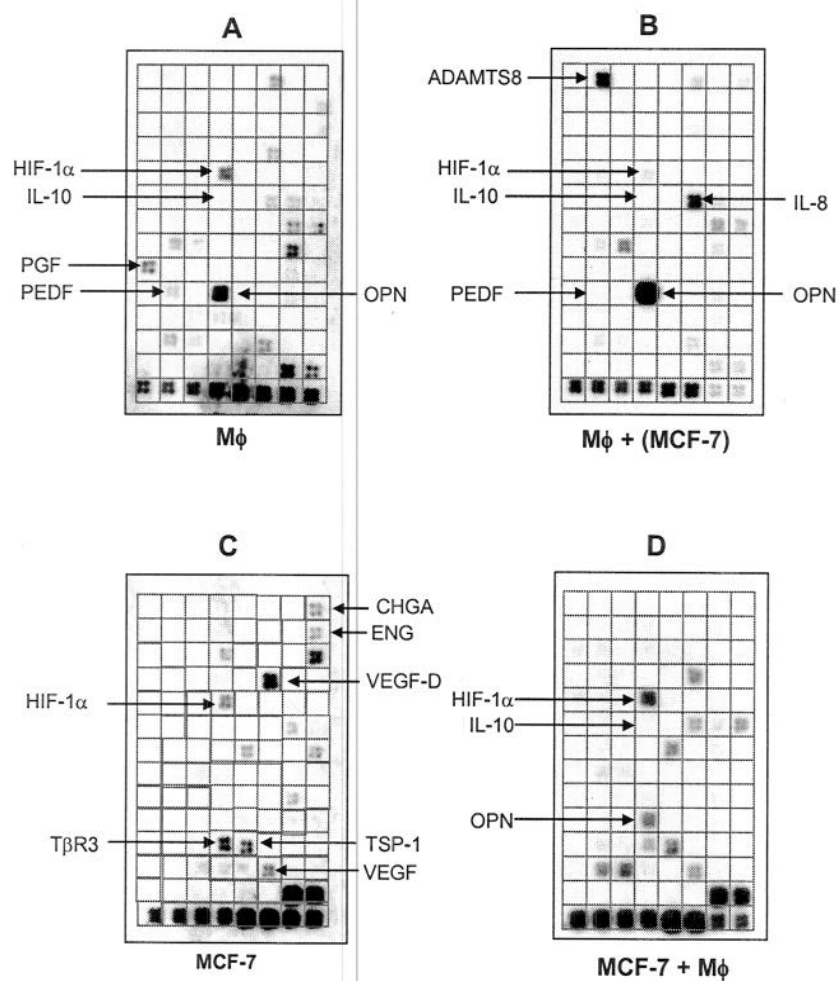


Fig. 2. Angiogenesis related gene expression in A, THP-1-derived macro-phages; B, macrophages co-cultured with MCF-7; C, MCF-7 cells; and D, MCF-7 co-cultured with macrophages.

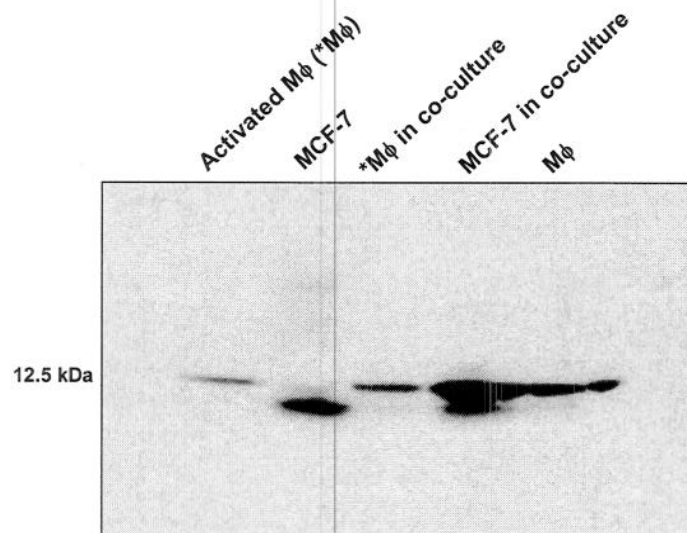


Fig. 1. Western blot of MIF in THP-1-derived macrophages and MCF-7 breast cancer cells. LPS-activated macrophages, MCF-7 cells, activated macrophages co-cultured with MCF-7, MCF-7 cells co-cultured with activated macrophages, and resting macrophages were probed for MIF protein. For each sample, 50 μ g lysate protein was resolved in 4-20% SDS-PAGE gradient gels, electroblotted on to nitrocellulose membrane and probed with rabbit polyclonal anti-human MIF IgG (Santa Cruz Biotechnology; FL-115, sc-20121). Secondary antibody was horse radish peroxidase-conjugated goat anti-rabbit IgG.