

AD_____

Award Number: W81XWH-04-1-0244

TITLE: The Role of ADAM-15 Disintegrin in E-cadherin Proteolysis in Prostate Cancer Metastasis

PRINCIPAL INVESTIGATOR: Mark L. Day, Ph.D.

CONTRACTING ORGANIZATION: University of Michigan
Ann Arbor, MI 48109-1274

REPORT DATE: February 2008

TYPE OF REPORT: Final

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				<i>Form Approved</i> 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 01-02-2008		2. REPORT TYPE Final		3. DATES COVERED 20 JAN 2004 - 19 JAN 2008	
4. TITLE AND SUBTITLE The Role of ADAM-15 Disintegrin in E-cadherin Proteolysis in Prostate Cancer Metastasis				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-1-0244	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Mark L. Day, Ph.D. Email: mday@umich.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Michigan Ann Arbor, MI 48109-1274				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 The primary goal of this proposal was to demonstrate that the membrane disintegrin, ADAM-15, could cleave E-cadherin in prostate cancer cells, and that in this regard ADAM-15 would promote the metastatic growth of prostate cancer. The successful completion of this study has led to the confirmation of the functional role of ADAM15 in the cleavage of E-cadherin and metastatic growth of prostate cancer cells. These results justify continuing studies examining the metalloproteinase domain of ADAM15 as a direct therapeutic target for metastatic prostate cancer.					
15. SUBJECT TERMS Disintegrin, metalloproteinase, prostate cancer, tumorigenesis					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			USAMRMC
			UU	7	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
Introduction	5
Body	5
Key Research Accomplishments	6
Reportable Outcomes	6
Conclusion	NA
References	NA
Appendices	NA

REVISED REPORT W81XWH-04-1-0244:

In response to the comment by Dr. McGuire to reference specific figures in the publications has been provided (see below). In actuality all of the below mentioned tasks were accomplished and published or under review. The only difference was we change our experimental approach to knock down ADAM15 and reduced malignancy instead of over expressing ADAM15 and trying to increase malignancy.

We did not realize we needed to respond to the status of specific tasks as mentioned in the SOW. We provide the following responses to each task:

- **Task 1:** To determine if ADAM-15 activity can generate the 80kDa soluble form of Ecadherin in transfected cells.

- (Months 1-6). To over-express ADAM-15 in low expressing or non-expressing prostate epithelial cells and determine if E-cad 80 is released into the culture media.

Response: We have completed this task which is reported in a manuscript that was under review.

- (Months 6-18). To mutate the E-cadherin cleavage site and determine if ADAM-15 fails to truncate E-cadherin to the 80kDa species.

Response: We could never completely abrogate cleavage of E-cadherin by site mutation. This was likely due to the fact that other MMPs could cleave at near by sites or that the ADAM15 still bound to E-cadherin at a different location and could still cleave outside the mutated site.

- (Months 12-24). We will repress ADAM-15 expression by RNA interference in lines known to express ADAM-15 and determine if E-cadherin cleavage can be inhibited.

Response: This has been successfully completed and is reported in detail in the same manuscript that was under review for task 1a. However, what we realized following the successful knock down of ADAM15 in highly malignant prostate cancer lines (PC3) was a dramatic attenuation of the malignant characteristics of these cells.

Task 2: To determine if ADAM-15 activity promotes the malignant transformation of prostate epithelial cells to a metastatic phenotype.

Response: The approach of over-expressing ADAM15 in minimally malignant cells such as LNCaP had very undramatic results. We realized that all adenocarcinoma cell lines (prostate, breast, bladder, colon) over expressed ADAM15. In fact these were the same findings examining ADAM15 expression human cancer tissues as outlined in the neoplasia manuscript. We changed the focus then of the majority of the work to “knockdown” ADAM15 expression instead of over expressing the protein. The results were very dramatic as is outlined in the cancer research paper. Thus the knockdown strategy that was utilized in task 1c provided the model platform for all of task 2.

- Months (12-24). To determine if ADAM-15 expression results in increased cellular motility, ADAM-15 transfectants will be assayed by random motility through micro-spheres.

Response: We successfully over expressed ADAM15 in LNCaP cells and observed slight increases in motility; however these were not significant changes.

- Months (12-24). We will determine if ADAM-15 promotes an invasive phenotype using a modified Boyden chamber invasion assay on individual ADAM-15 clones and vector-only controls.

Response: We successfully over expressed ADAM15 in LNCaP cells and observed slight increases in invasion through Boyden chambers, but as with the motility assays these were not significant changes and were thus not publishable.

- Months (24-36). We will determine if ADAM-15 expression promotes the anchorage-independent growth of prostate cancer cells in soft agar.

Response: We successfully over-expressed ADAM15 in LNCaP cells and observed increased growth of these cells in soft agar, but as with the invasion assays these were not significant changes and were thus not publishable.

- Months (12-36). To determine if ADAM-15 clones exhibit *in vivo* tumor growth by subcutaneous injection into nude mice.

Response: We successfully over-expressed ADAM15 in LNCaP cells and observed increased growth of these cells following subcutaneous injection. These results were complemented by the dramatic attenuation of *in vivo* growth and metastasis as shown in figures 1 and 6 of the cancer research manuscript.

INTRODUCTION:

A key determinant in the metastatic progression of prostate cancer is the dissociation of cancer cells from the primary tumor that may result from inadequate cell adhesion. In tumors of epithelial origin, the disruption of cellular adhesion appears to arise, in part, through alterations of the E-cadherin cell-adhesion system. In our original proposal we hypothesized that the disintegrin metalloproteinase, ADAM 15, is closely associated with the metastatic progression of prostate cancer and could possibly cleave E-cadherin into proteolytic fragments. Examination of both cDNA and tumor micro arrays demonstrated increased expression of ADAM-15 in metastatic prostate cancer. It was also important to note that the chromosomal location for ADAM-15, on 1q21, is a region of specific high-level amplification in prostate cancer metastasis. Taken together, this information provided a compelling rationale for the proposed studies and supports our central hypothesis: ADAM-15 specifically targets the extracellular domain of E-cadherin and disrupts the adhesive integrity of epithelium during the metastatic progression of prostate cancer. The results of this 3 year DOD Idea Award established that ADAM15 plays a central role in prostate cancer progression and metastasis, but also has identified both E-cadherin and N-cadherin as proteolytic substrates. These results (outlined below) justify the continuing studies ADAM15 both mechanistically and as a direct therapeutic target for metastatic prostate cancer.

BODY:

Using human tumor and cDNA microarray technology, we have recently demonstrated that the ADAM15 disintegrin is significantly overexpressed during the metastatic progression of human prostate cancer. In the current study, we utilized lentiviral-based shRNA technology to downregulate ADAM15 in the metastatic prostate cancer cell line, PC-3. ADAM15 downregulation dramatically attenuated many of the malignant characteristics of PC-3 cells *in vitro* and prevented the subcutaneous growth of PC-3 cells in SCID mice. By inhibiting the expression of ADAM15 in PC-3 cells (figure 1 cancer research manuscript) we demonstrated decreased cell migration and adhesion to specific extracellular matrix (ECM) proteins in figure 2 of the *cancer research* manuscript. This was accompanied by a reduction in the cleavage of N-cadherin by ADAM15 at the cell surface. FACS analysis revealed reduced cell surface expression of the metastasis-associated proteins α_v integrin and CD44 (figure 4 cancer research paper). Furthermore, MMP9 secretion and activity were abrogated in response to ADAM15 reduction. In an *in vitro* model of vascular invasion, loss of ADAM15 reduced PC-3 adhesion to, and migration through vascular endothelial cell monolayers (figure 5). Using a SCID mouse model of human prostate cancer metastasis, we found that the loss

of ADAM15 significantly attenuated the metastatic spread of PC-3 cells to bone figure 6. Taken together, these data strongly support a functional role for ADAM15 in prostate tumor cell interaction with vascular endothelium and the metastatic progression of human prostate cancer. Results of this study were published this year in Cancer Research (see appendix A)

We have also examined the ability of ADAM15 to process the E-cadherin molecule into the soluble form (sE-cad). We have shown that sE-cad can be measured in the serum of prostate cancer patients, which we hypothesize is mediated by ADAM15. Utilizing two complementary models, overexpression and stable shRNA-mediated knockdown of ADAM15 in breast cancer cells; we demonstrated that ADAM15 cleaves E-cadherin in response to growth factor deprivation. We also demonstrate that the extracellular shedding of E-cadherin was abrogated by a metalloproteinase inhibitor and a catalytically inactive ADAM15 mutant. We report here the novel observation that the soluble E-cadherin fragment was found in complex with EGFR family members in prostate cancer cells in an ADAM15-dependent manner. These interactions appeared to induce receptor activation and signaling supporting both cell migration and proliferation. In this study, we provide evidence that ADAM15 catalyzes the cleavage of E-cadherin to generate a soluble fragment that in turn binds to and stimulates EGFR receptor signaling. This manuscript is currently under review.

ACCOMPLISHMENTS:

1. We have confirmed function of ADAM15 by demonstrating dramatic tumor reduction in vivo of ADAM15 knockdown PC-3 tumors.
2. We have also confirmed function of ADAM15 by demonstrating dramatic reduction in the metastatic spread of human prostate cancer cells.
3. We have confirmed that both N-cadherin and E-cadherin are substrates of ADAM15.

REPORTABLE OUTCOMES:

We have created several cell lines that express ADAM15-GFP and have successfully knocked down ADAM15 expression in PC-3 cells and LNCaP cells. Initial in vivo results indicate that ADAM15 does indeed play a tumor progression to metastasis role in prostate cancer. Abdo Najy who is a graduate student in my lab received a DOD predoctoral fellowship that will cover his salary and tuition through the remainder of this project. We have published a manuscript in the journal *Neoplasia*, which is the first comprehensive study of ADAM15 in prostate cancer. The DOD is cited as the funding source. We have published a manuscript in the journal *Cancer Research*, which is the first functional study of validating ADAM15 function in prostate cancer metastasis. (appendix A and B). We have one manuscript under review describing the function of ADAM15 in the cleavage of E-cadherin human prostate cancer.

BIBLIOGRAPHY:

1. Kuefer R, Day KC, Kleer C and Day M.L. The ADAM15 Disintegrin is Associated with Aggressive Prostate and Breast Cancer. *Neoplasia* . 8(4):319-29. 2006
2. Najy A., Day K.C., and Day M.L. ADAM15 supports prostate cancer metastasis by modulating tumor cell-endothelial cell interaction. *Cancer Research*. 15;68(4):1092-9. 2008.

MEETING ABSTRACTS:

1. Najy, A.J., Day, K.C., Sargent, E.E., Wright, C.W., and Day, M. L. The Disintegrin-tallopoteinase, ADAM15, Supports the Metastatic Progression of Prostate Cancer. The National Graduate Student Research Festival (NGSRF). October 2007. National Institute of Health Campus, Bethesda, MD

2. Najy, A.J., Day, K.C., Sargent, E.E., Wright, C.W., and Day, M. L. The Disintegrin-Metalloproteinase, ADAM15, Supports the Metastatic Progression of Prostate Cancer. The Department of Defense (DoD) Prostate Cancer Research Program (PCRP) IMPaCT: Innovative Minds in Prostate Cancer Today. September 2007. Atlanta, GA.
3. Najy, A.J., Day, K.C., Sargent, E.E., Wright, C.W., and Day, M. L. ADAM15 Plays a Critical Role in Prostate Cancer Metastasis by Modulating Metastatic-associated Proteins. May 2007. Michigan Prostate Research Colloquium. Detroit, MI.
4. Najy, A.J., Day, K.C., Sargent, E.E., Wright, C.W., and Day, M. L. The Role of the Disintegrin-Metalloproteinase, ADAM15, in Prostate Cancer Progression. SBUR 2006 Annual Fall Meeting. November 2006. Phoenix, AZ. Travel Award Winner
5. Najy, A.J., Day, K.C., Sargent, E.E., Wright, C.W., Nör, J., and Day, M. L. ADAM15 Attenuates Tumor Development in Prostate Cancer. May 2006. Michigan Prostate Research Colloquium. Ann Arbor, MI.

PERSONNEL RECEIVING PAY FROM THE RESEARCH EFFORT:

Mark L. Day PhD PI
Kathleen C Day Senior research associate