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14. ABSTRACT:
Proteolysis of E-cadherin was rigorously studied a decade ago with specific attention focused on metalloproteinase activity that cleaved E-cadherin in the extracellular domain and generated an 80kDa (E-cad 80) fragment that was shown to disrupt epithelial cell-cell adhesion. This finding was of particular interest due to the fact that E-cad 80 was increased in the serum of cancer patients. Several of these studies demonstrated significant elevations in the serum of patients with gastric, hepatocellular, lung and breast cancer. Although several specific metalloproteinases were shown to cleave E-cadherin to the 80kDa species in vitro, these enzymes were not elevated in metastatic prostate cancer and were not tested under physiologic conditions. Through the use of cDNA microarray this laboratory identified a membrane bound, disentigrin metalloproteinase (ADAM-15) that is specifically upregulated at both the transcriptional and translational level in metastatic prostate cancer. Based on these observations, we hypothesize that the truncation and inactivation of E-cadherin is mediated by the ADAM-15 disentigrin in metastatic prostate cancer. The primary goal of this proposal is to demonstrate that ADAM-15 truncates E-cadherin in prostate epithelial cells, and that this activity promotes the malignant transformation of these cells.

15. SUBJECT TERMS
Disintegrin, metalloproteinase, prostate cancer, tumorigenesis

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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. Not only will the proposed studies address the functional role of ADAM-15 in the metastatic transformation of prostate epithelial cells; these results may also justify future studies pursuing ADAM-15 as a direct therapeutic target for metastatic prostate cancer.

BODY:

Over-expression of ADAM15 in minimally malignant prostate epithelial cells will determine if this over-expression cleaves E-cadherin and induces a malignant phenotype. The intent of aim 1 was to achieve stable high-level expression of ADAM15 in LNCaP cell line and determine if ADAM15 elevation induces E-cadherin cleavage as well as a malignant phenotype in this minimally malignant prostate cancer cell line. ADAM15 was tagged with GFP on its C-terminus and transfected into LNCaP cells. ADAM15-GFP over-expressing LNCaP cells were verified via western blotting and immunohistochemistry by our laboratory (Figure 1). The inactive precursor form of ADAM15 is a 110 kDa protein which is converted into the 90 kDa active form by the pro-protein convertase furin. We will use the stable LNCaP cell lines (LNCaP ADAM15-GFP) to perform cell motility, invasion and anchorage-independence assays to determine malignant potential of theses cell lines.

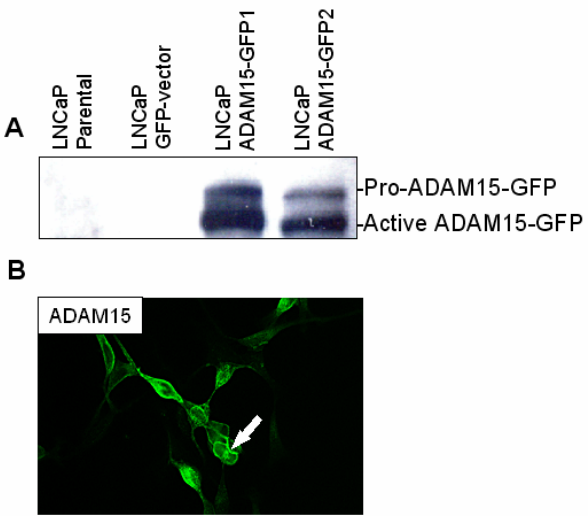

Figure 1. ADAM15-GFP LNCaP Cells. LNCaP cells were transfected with vector-GFP or ADAM15-GFP vector constructs. **(A)** Western blot showing the exogenous ADAM15-GFP in ADAM15-GFP transfected LNCaP cells. **(B)** Immunocytochemistry showing membranous staining (arrow) of ADAM15 in LNCaP cells.

To determine if ADAM15 knockdown reduces the cleavage of E-cadherin as well as the metastatic phenotype of highly aggressive prostate cancer cells. The results from aim 1 may indicate the ADAM15 promotes a malignant phenotype; however this does not confirm that ADAM15 is directly inducing this phenotype. Thus, the intent of this aim is to confirm that ADAM15 is specifically inducing the malignant phenotype seen by using reverse genetics. To accomplish this task, we will utilize small interfering (si)-RNA-mediated knockdown of ADAM15 using a short hairpin (sh)-RNA construct. We have permanently reduced ADAM15 expression in PC3 cells using ADAM15 siRNA oligos in a lentiviral system. To directly assess the contribution of ADAM15 to prostate tumorigenesis, we examined the ability of ADAM15 knock down cells (shA15PC-3^{luc}) cells to grow as subcutaneous tumor in male SCID mice. Following injection of shA15PC-3^{luc} cells and vector control (vecPC-3^{luc}) cells into both flanks of 5 mice per cell line, we could demonstrate a dramatic reduction in tumor growth starting at 3 weeks using the bioluminescence (**figure 2**). This experiment demonstrates the utility of our luciferase-based PC-3 tumor system and the ability to monitor tumor growth and progression in live animals.

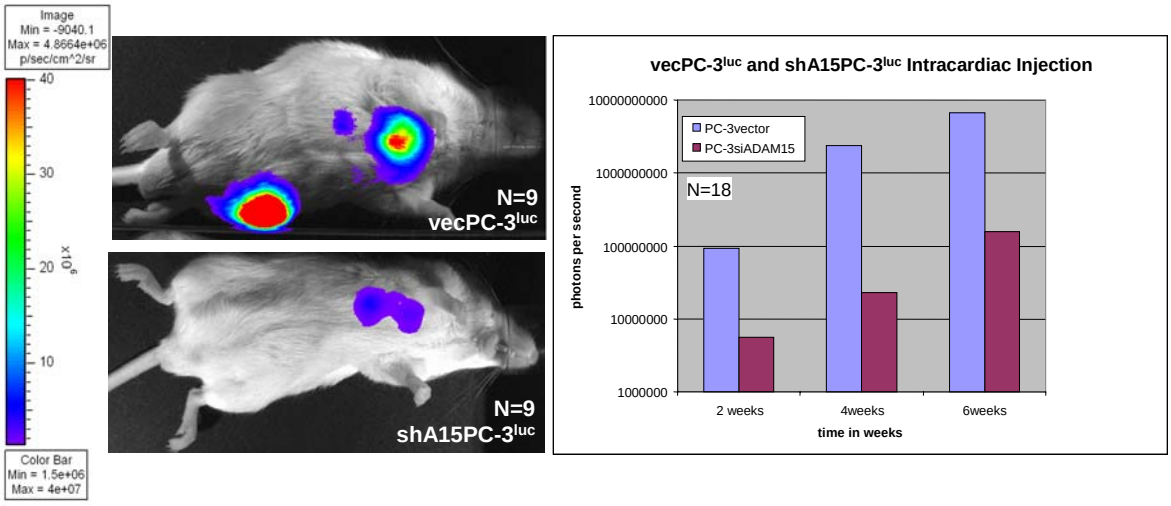

Figure 1. Loss of ADAM15 inhibits tumor metastasis in intracardiac injection model. shA15PC-3^{luc} and vecPC-3^{luc} cells were injected in to the left ventricle of 9 SCID mice per cell line. Metastatic growth was monitored by bioluminescence and plotted as photons per second. Statistical evaluation of these animal groups (n=9) was analyzed at different time points. For example, at week six, the animals were sacrificed and necropsy was performed on half of each study group. The student's t-test of the natural log of week 6 values was shown to have a statistical significance of p=0.004. Histological evaluations are still in progress.

ACCOMPLISHMENTS:

1. We have confirmed function of ADAM15 by demonstrating dramatic tumor reduction in vivo of ADAM15 knockdown PC-3 tumors.
2. We have confirmed that ADAM15 knockdown reduces interactions with vascular endothelial cells and trans-endothelial migration.
3. We have also confirmed function of ADAM15 by demonstrating dramatic reduction in the metastatic spread of human prostate cancer cells.

REPORTABLE OUTCOMES:

We have created several cell lines that express ADAM15-GFP and have successfully knocked down ADAM15 expression in PC-3 cells. Initial in vivo results indicate that ADAM15 does indeed play a tumor promoting 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 submitted two manuscripts describing the function of ADAM15 in human prostate cancer progression and metastasis.

CONCLUSIONS:

In summary this study to date has yielded two important categories of information:

1. Necessary reagents for the remainder of the study are being generated and versified.
2. The clinical data examining the expression of ADAM15 in prostate cancer has been published and 2 more functional papers have been submitted.