



# Testosterone and Dominant Negative Androgen Receptor Suppress Human Prostate Cancer Xenograft Proliferation in Athymic Nude Mice

David A. Parker<sup>1,2</sup>, O. Harris Ford<sup>2</sup>, Durairaj A. Jeyaraj<sup>2</sup>, Mark A. Titus<sup>2</sup>, Tal Kafri<sup>3</sup>, and James L. Mohler<sup>2,4,5,6</sup>

<sup>1</sup>Shaw University, Raleigh, NC, <sup>2</sup>UNC-School of Medicine Lineberger Comprehensive Cancer Center and <sup>3</sup>Gene Therapy Center Chapel Hill, NC, <sup>4</sup>Department of Urologic Oncology, Roswell Park Cancer Institute and <sup>5</sup>Department of Urology, <sup>6</sup>University at Buffalo School of Medicine and Biotechnology, Buffalo, NY



## ABSTRACT

Two cell cycle markers (Acinus and Ki67) are being investigated for a correlation between clinically assigned tumor aggressiveness and the proliferation status of newly diagnosed, clinically localized CaP as determined by immunohistochemistry. In addition, it is a possibility that dominant negative AR (DNAR) may be a potential treatment for CaP when matched with the aggressiveness of the tumor. Human CaP (CWR-R1) was injected into 90 castrated athymic nude mice. Two treatments were used (DNAR and LacZ) for the mice, androgen-stimulated and without T. The two potential markers were immunohistochemically stained to determine the effects of the treatments. Plenty of background staining occurred from aqueous fast-red substrate. No positive stain for Acinus protein. Amount of proliferating cells may have decreased in the DNAR treated groups.

## INTRODUCTION

Among all cancer deaths, CaP is the second leading cause of death among American men (1). Current methods for determining the aggressiveness of CaP tumors are not specific enough and there is a need for new, more efficient markers (2). Proliferation and apoptotic markers that may aid in the classification of the aggressiveness of CaP are Ki67 and Acinus..

Acinus is a protein that is cleaved by Caspase-3 and is responsible for chromatin condensation in the nucleus, which is an important step during the process of apoptosis (3). Ki67 is the second protein that will be examined. It has been used in several studies as a proliferation marker (4).

The aggressiveness index of clinically diagnosed patients is calculated by immunostaining for Acinus and Ki67 proteins. This index is then compared with corresponding clinical data to classify CaP aggressiveness.

One study created a DNAR by removing the first half of the N-terminal domain and fusing the mutant AR (AR122) to the Kruppel-associated box (KRAB) and to histone deacetylase 1 (HDAC1). The study suggests that dominant negative HDAC1-KRAB-AR122 suppresses transcription because the mutant AR competes with wild type AR for binding to the androgen response elements, leading to deactivation of the androgen response elements. Also, transcription is suppressed because of the activity of the HDAC1 and KRAB domain (5).

Thus, treatment with DNAR in human xenograft models should show an increase in Acinus protein however, only percentage of proliferation was observed. Since the percentage of Ki67 protein was lower in both of the DNAR treated groups when compared to the control, DNAR may only suppress proliferation rather than promote apoptosis.

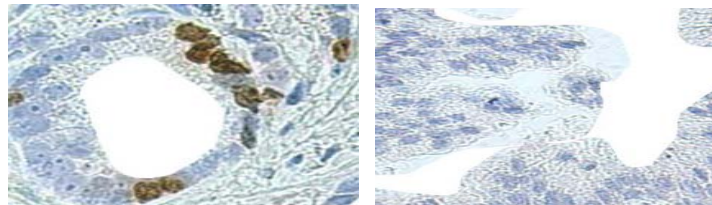
## REFERENCES

- Ilic D, O'Connor D, Green S, Wilt T. Screening for prostate cancer: a Cochrane systematic review. *Cancer Causes Control*. 2007 Apr;18(3):279-85.
- Costa VL, Henrique R, Jerónimo C. Epigenetic markers for molecular detection of prostate cancer. *Disease Markers*. 2007;23(1-2):31-41.
- Sahara S, Aoto M, Eguchi Y, Imamoto N, Yoneda Y, Tsujimoto Y. Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. *Nature*. 1999 Sep 9;401(6749):168-73.
- Brown DC, Gatter KC. Ki67 protein: the immaculate deception? *Histopathology*. 2002 Jan;40(1):2-11.
- Bramlett KS, Dits NF, Sui X, Jorge MC, Zhu X, Jenster G. Repression of androgen-regulated gene expression by dominant negative androgen receptors. *Mol Cell Endocrinol*. 2001 Oct 25;183(1-2):19-28.

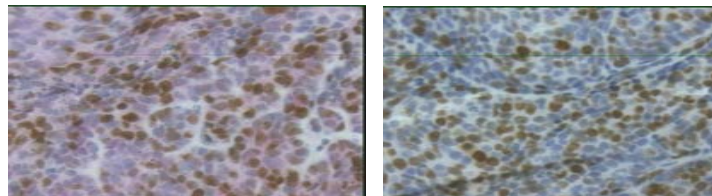
## METHODS

- Athymic nude mice were tattooed #1-90
- All mice were castrated, 45 mice received T pellet implant (12.5 mg).
- Mice were subdivided into 4: 22 mice (group 1) were injected with CWR-R1 (DNAR) with T; 22 mice (group 2) were injected with CWR-R1 (DNAR) without T. 22 mice (group 3) were injected with CWR-R1 (LacZ) with T; 22 mice (group 4) were injected with CWR-R1 (LacZ). Each of the four groups were inoculated unilateral with 1.25x10<sup>6</sup>/100 µL matragel.
- Tumors were harvested at 1.3 cm<sup>3</sup>.
- 1/2 of each tumor was prepared in formalin fix for paraffin embedding (4% formalin/PBS).
- 1/2 of each tumor was frozen.
- Immunohistochemical staining (M.O.M. kit) for visualization of the androgen receptor.
- Immunohistochemical staining (M.O.M. kit) was used to visualize the amount of Acinus and Ki67 proteins (dual stain).

## ACINUS IMMUNOSTAINING DIAGNOSTIC BIOPSY

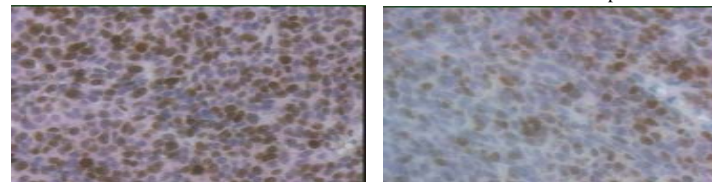


## RESULTS (Pilot Study)



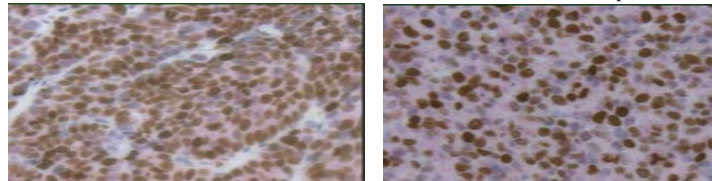
Control - 91: 45% prolif.

Control - 95: 47% prolif.



DNAR- 36: 34% prolif.

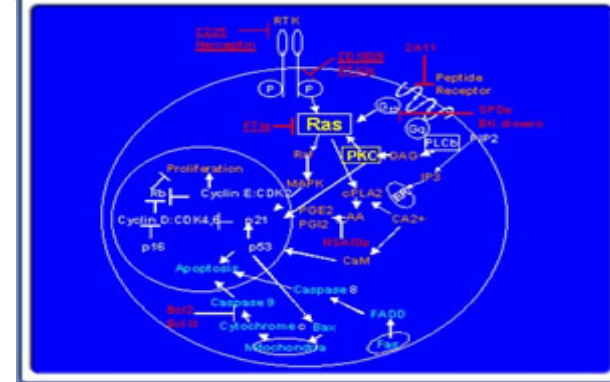
DNAR+T -13: 40% prolif.



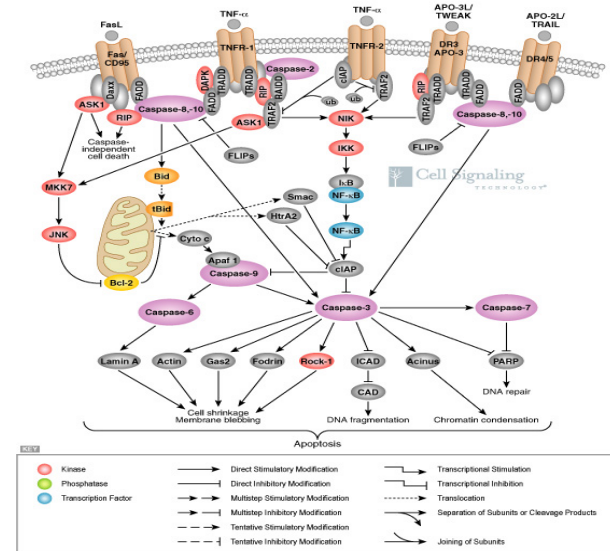
LacZ- 83: 82% prolif.

LacZ+T- 59: 53% prolif.

## Ki67 PATHWAY



## ACINUS PATHWAY



## CONCLUSIONS

There were no positive stains for Acinus. Nonspecific background staining occurred as a result of the aqueous fast-red substrate. However, there appeared to be less proliferation in both of the DNAR treated groups.

## Acknowledgements

Dr. P.A. Godley, Division of Hematology/Oncology UNC School of Medicine  
Dr. G. Sancar, Dept of Biochemistry and Biophysics UNC School of Medicine

D.P. was supported by the Shaw University-University of North Carolina at Chapel Hill Undergraduate Program in Prostate Cancer Research and Training (SUUPRT), funded by the Department of Defense Prostate Cancer Research Program CDMRP (PC061634).