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Award Number: W81XWH-08-2-0169

TITLE: HBCU Summer Undergraduate Training Program in Prostate  
Cancer Research

PRINCIPAL INVESTIGATOR: Shiv Srivastava

CONTRACTING ORGANIZATION: Henry M. Jackson Foundation  
Rockville, MD 20852

REPORT DATE: August 2009

TYPE OF REPORT: Annual Summary

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

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| REPORT DOCUMENTATION PAGE  |               |  | Form Approved<br>OMB No. 0704-0188         |  |
|--|---------------|--|--|--|
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| 1. REPORT DATE (DD-MM-YYYY)<br>01-08-2009  |               | 2. REPORT TYPE: Annual Summary           |  | 3. DATES COVERED (From - To)<br>17 July 2008 – 16 Jul 2009 |
| 4. TITLE AND SUBTITLE: HBCU Summer Undergraduate Training Program in Prostate Cancer Research  |               | 5a. CONTRACT NUMBER:<br>W81XWH-08-2-0169 |  |  |
|  |               | 5b. GRANT NUMBER: PC073564               |  |  |
|  |               | 5c. PROGRAM ELEMENT NUMBER:              |  |  |
| 6. AUTHOR(S) Shiv Srivastava, Ph.D.<br>Email: ssrivastava@cpdr.org   |               | 5d. PROJECT NUMBER                       |  |  |
|  |               | 5e. TASK NUMBER                          |  |  |
|  |               | 5f. WORK UNIT NUMBER                     |  |  |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br><br>Henry M. Jackson Foundation<br>Headquarters, 1401 Rockville Pike<br>CPDR, Rockville, MD 20852  |               | 8. PERFORMING ORGANIZATION REPORT        |  |  |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research<br>Materiel Command<br>Fort Detrick, MD 21702-5012   |               | 10. SPONSOR/MONITOR'S ACRONYM(S)         |  |  |
|  |               | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)   |  |  |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT<br><br>Approved for Public Release: Distribution unlimited   |               |  |  |  |
| 13. SUPPLEMENTARY NOTES:<br>N/A  |               |  |  |  |
| 14. ABSTRACT: In first year of the award, four meritorious students were selected for HBCU Summer Undergraduate Training Program in Prostate Cancer Research by USU-CPDR and UDC selection committee. The students were assigned to the faculty members of USU, focusing on specific research projects in basic science and database research. The ongoing projects in prostate cancer research provide them with knowledge, expert guidance and tools to successfully complete the assignments. Student and mentors met every morning to discuss the plan for the experiments, and evenings to summarize the experimental results and interpretations. Students participated in weekly seminars presented by the USU-CPDR faculty, researchers and guest speakers. This provided them exposure to the state-of-the-art prostate cancer, cell and molecular biology research. Students prepared and presented their research goals and objectives and experimental and progress in biweekly presentations. At the end of the training, each student made PowerPoint presentations of the completed project and conclusions in a seminar and submitted the entire project to the supervisors. Each student was given a certificate of completion of achievement. The students presented their experimental results at the Institutional and National meetings focusing the HBCU training and research. Overall, it was a very rewarding experience for the students as well as mentors. |               |  |  |  |
| 15. SUBJECT TERMS HBCU-Prostate Cancer Training, Center for Prostate Disease Research (CPDR), University of District of Columbia (UDC), DoD-PCR, Uniformed Services University (USU), Department of Surgery, Walter Reed Army Medical Center (WRAMC), Basic Science Research Program (BSRP).   |               |  |  |  |
| 16. SECURITY CLASSIFICATION OF: U  |               |  | 17. LIMITATION OF ABSTRACT<br><br>UU       | 18. NUMBER OF PAGES<br><br>25                              |
| a. REPORT U  | b. ABSTRACT U | c. THIS PAGE U                           |  |  |
|  |               |  | 19a. NAME OF RESPONSIBLE PERSON<br>USAMRMC |  |
|  |               |  | 19b. TELEPHONE NUMBER (include area code)  |  |

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## HBCU Summer Undergraduate Training Program in Prostate Cancer Research

**INTRODUCTION:** The goals of this training grant is to develop collaborations between Uniformed Services University/Center for Prostate Disease Research (USU/CPDR) and University of District of Columbia (UDC) that will provide a meaningful experience for UDC undergraduate students in prostate cancer research. Scientific breakthroughs of CPDR continue to unravel new gene defects that have potential as new biomarkers and/or therapeutic targets in improving the diagnosis and management of prostate cancer (CaP). Recently, CPDR researchers identified alterations of the ETS related gene (ERG), as a first high frequency, potentially causal oncogene alteration in CaP. CPDR programs have also been contributing to high impact research focusing on CaP in African Americans, who are at highest risk of this disease. **Summer Professional development training Program** is to provide an opportunity for motivated high school and undergraduate college students to gain exposure to the field of CaP biomedical research. The CPDR has provided this training to four to six students per year totaling 59 students who have gained a valuable experience through this program. Several of these students have embarked on educational paths leading to medical or graduate school. Ten plus CPDR faculty members with outstanding credentials in basic or clinical prostate cancer research contribute to these activities. **The objectives** of this training program are to: (1) recruit and motivate highly qualified undergraduate trainees from UDC; (2) provide them with a stimulating and intellectual environment that promotes state-of –the art training and education in CaP research; (3) motivate young researchers, who may contribute to CaP research centers at HBCUs.

Each student admitted to the program will be assigned to one of the faculty members of CPDR, will set up goals and will carry out a specific research assignment that is designated to yield new data and findings within the allotted time (12 weeks). Through regular lab meetings, seminars and personal discussions the students will interact with other fellow students, faculty members and staff. At the end of the summer experience, each student will present their research findings as PowerPoint presentations. The results will be included in a manuscript if warranted. Each student will be given a certificate of completion of achievement.

**BODY: Task 1. Selection Process:**

USU/CPDR summer internship program announcements for 2008 were made at the UDC to invite the applications from undergraduate students who were interested in pursuing an advanced education in medicine or medical research (Attachment # 1). The completed applications consisting of essay of interest, transcripts and letters of recommendation were considered for selection process. Four meritorious students were (GPA ranging from 3.2 to 3.8) selected by USU-CPDR and UDC the selection committee composed of the faculty advisors for the summer Undergraduate Training Program, PI and the Co-PI based on their interest in research, transcripts, letters of recommendation. The following are the successful applicants:



Francisco Saenz



Emmanuel Woode



Chiedozie J Ayika



Fiteh Yelekal

These applicants were assigned to faculty members of CPDR and other departments in USU to set-up goals to carry out a specific research projects.

**Task 2: Assignment of Mentors and Projects:**

The projects assigned to the individual students are well structured and well rounded programs that provide them with knowledge, expert guidance and tools to successfully complete the assignments. The knowledge and the technical experience they gain by performing experiments, seminars, discussions with scientists and data presentations during the training period will enhance their understanding of prostate cancer research and motivate them to pursue careers in basic science or clinical prostate cancer research.

**CPDR- A Multi-disciplinary Prostate Disease Focus: The USU-CPDR Basic Science Research Program (BSRP).** The 20,000 square foot state-of-the-art basic science laboratory facility is attracting the best and brightest to study the disease. Using biospecimens collected from volunteering military beneficiaries, the team has amassed a large bank of CaP specimens that are serving to unravel the defects of specific genes and biochemical pathways in CaP. The Basic Science Research Program of the CPDR is a multi-disciplinary CaP research endeavor, which continues to integrate collaborative efforts of basic and clinical sciences researchers. Multi-disciplinary team of cancer biologists, urologists, genitourinary pathologists, epidemiologists, bio-statisticians, medical/bio-informatitians and regulatory affairs specialists focuses on:

- Discovery and characterization CaP specific gene/protein alterations:.
- Evaluations of hormonal mechanisms with focus on androgen regulated transcriptome/ genome and *AR* expression and functions.
- Development of novel experimental models for better definition of CaP biology:
- Translational research defining diagnostic and prognostic bio-markers and novel therapeutic targets: For a comprehensive list of CPDR publications, please refer to our website, [www.cpdr.org](http://www.cpdr.org).

### Assignment of mentors, research projects to yield the new data:

Student: Mr. Francisco Saenz  
Mentor: Dr. Albert Dobi, Ph.D  
Research Project: Defining the role of NKX3.1 on the expression of TMPRSS2-ERG fusion Gene

Student: Mr. Emmanuel Woode  
Mentor: Dr. Gyorgy Petrovics, Ph.D  
Research Project: The Biological function of alternatively spliced ERG transcripts

Student: Mr. Chiedozie J Ayika  
Mentor: Dr. Meera Srivastava, Ph.D  
Research Project: Lack of p53 tumor suppressor effects in LNCaP prostate cancer cells

Student: Ms. Fiteh Yelekal  
Mentor: Dr. Bungo Furusato, MD  
Research Project: Increased levels of *SPARC* (osteonectin) in human prostate cancer tissues and its association with clinical metastasis

### Task 3: Training, Goals and Objectives:

Student: Mr. Francisco Saenz  
Objective: To define the function of NKX3.1 gene as a repressor of the TMPRSS2 promoter therefore limiting the expression of the TMPRSS2-ERG and subsequently the level of ERG.

Student: Mr. Emmanuel Woode  
Objective: To define the functions of cancer specific ERGs, in particular ERG8 one of the two truncated forms.

Student: Mr. Chiedozie J Ayika  
Objective: To study the correlation between the tumor suppressor activities of ANXA7 and p53 through the phosphorylation of FOXO3a.

Student: Ms. Fiteh Yelekal  
Objective: To study SPARC protein expression in a large cohort of patients by immunohistochemistry, whether the protein expression of SPARC can predict aggressive clinical behavior in retrospectively selected cohort of patients.

**Laboratory meetings:** Through laboratory meetings, seminars and personal discussions the students interacted with other fellow students, faculty members and staff.

- **Weekly meetings:** Students participated in department seminars presented by the USU-CPDR faculty and researchers as well as guest speakers to understand the research activities and the progress in the field of prostate cancer.
- **Biweekly seminar presentations:** Students presented their goals and objectives and experimental plan for the training period in the first presentation and progress in subsequent presentations.

At the end of the summer experience, each student prepared and presented their research findings as PowerPoint presentations.

- **Final seminar presentation:** Students presented the complete project report, and conclusions.
- **Report Submission:** Each student submitted the entire project as a hard copy and an electronic version to the supervisors.

**Task 4: Periodical meeting of Faculty advisors to monitor Student's progress.**

- **Meetings between student and mentor:** Student and mentors met every day morning to discuss the plan for the experiments, and evenings to summarize the experimental results and interpretations.
- Programmed interaction and oversight of the student interns by PI and Co-PI, faculty advisors, and mentors is presented in the following table:

| <b>TIMELINE</b> | <b>ITEM/ISSUE</b>  | <b>ACTION</b>   | <b>RESPONSIBLE AGENT</b> |
|-----------------|--|---|--------------------------|
| 90 Days*        | Student Interns  | Selection   | Faculty Advisors         |
| 60 Days*        | Mentors  | Appointment   | PI and Co-PI             |
| 30 Days*        | Research Projects With Goals & Objectives                        | Proposal  | Mentors & Students       |
| 14 Days*        | Research Projects  | Approval  | PI and Co-PI             |
| Day 1           | Research Internship  | Begins  | Mentors and Students     |
| Day 7           | Student Research Progress  | Weekly Review   | Mentors                  |
| Day 14          | Student Research Progress Student with Mentor                    | 2nd Week Review   | PI & Co-PI               |
| Day 30          | Student Research Progress Goals & Objectives Student with Mentor | 1st Monthly Review<br>Oral Presentation                   | Faculty Advisors         |
| Day 60          | Student Research Progress Goals & Objectives Student with Mentor | 2nd Monthly Review<br>Oral Presentation                   | Faculty Advisors         |
| Day 69          | Student Research Progress Goals & Objectives Student with Mentor | Semi Final Review   | PI and Co-PI             |
| Day 83          | Final Research Progress Report                                   | Review & Approval   | PI and Co-PI             |
| Day 90          | Submission of Presentation                                       | Research Report<br>Final Powerpoint and Oral Presentation | Student Interns          |

## **KEY RESEARCH ACCOMPLISHMENTS:**

All four students selected under HBCU Summer Undergraduate Training Program in Prostate Cancer Research have successfully completed their projects assigned to them.

Highlights of their project outline and experimental results were the following:

### **Francisco Sáenz**

- Knockdown of NKX3.1 changes the morphological features of VCaP cells
- NKX3.1 regulates ERG expression through TMPRSS2 gene promoter in prostate cancer.
- NKX3.1 regulates PSA, a marker expression.
- Knockdown of NKX3.1 did not alter the levels of PMEPA1

### **Emmanuel Woode**

- ERG8 was significantly silenced ectopically in HEK293 cells after treatment with ERG8 Si for 48 hours.
- No effect of ERG8 Si on ERG3.
- ERG8 specific siRNA is identified and can be used to down regulate ERG8 to study the biological functions associated with its expression in well differentiated prostate tumors.

### **Chiedozie J. Ayika**

- WT-ANXA7 induced tumor suppressor effects thereby demonstrating a capability to overcome PTEN-deficiency and constitutively activated status of PI3K-Akt survival cascade.
- DN-ANXA7 (which lacks phospholipid-binding properties) failed to match the WT-ANXA7-induced PCD and G1 cell growth arrest.
- p53 did not match tumor suppressor effects of WT- or even DN-ANXA7 that was presumably associated with the FOXO3A hyperphosphorylation and subsequent inhibition of the pro-apoptotic FOXO3A transcription.

### **Fiteh Yelekal**

- Higher *SPARC* expression in CaP is associated with poorly differentiated carcinoma and high Gleason Score.
- High *SPARC* expression in CaP mRNA levels is associated with an increased risk of PSA recurrence.
- Quantitative determination of *SPARC* expression in protein levels is associated with clinical metastasis status in this cohort.
- *SPARC* expression either in mRNA or protein level in primary CaP specimens may have prognostic utility especially in locally advanced CaP.



## REPORTABLE OUTCOMES:

During this period, the students have displayed tremendous of interest in the field of prostate cancer and have gained experience. The results obtained from their experiments were presented as posters in HBCU conferences at national level and were awarded scientific merit awards.

- Oral presentations in the presence of faculty and staff of CPDR and senior leaderships of USU (Department Chairman) and UDC (Dean, College of Arts and Sciences)

- **HBCU-UP National Research Conference, Atlanta, GA**

**Francisco Sáenz**, Rajesh Thangapazham, Deepak Kumar, Shiv Srivastava, and Albert Dobi. 2008. A Role for Conserved Regulatory Sequences in the Repression of TMPRSS2-ERG Fusion Gene, a Prevalent Oncogenic Alteration in Prostate Cancer.

**Emmanuel Woode**, Ying Hu, Gyorgy Petrovics, Deepak Kumar, and Shiv Srivastava. 2008. Evaluation of the variants of ERG oncogene in prostate cancer.

**Chiedozie J. Ayika**, Katerina Mezhevaya, Meera Srivastava, Yelizaveta Torosyan,, Deepak Kumar, and Shiv Srivastava. 2008. The Lack of p53 Tumor Suppressor Effects in LNCaP was Associated with FOXO3a Hyperphosphorylation.

**Yelekal Fiteh**, Deepak Kumar, Y. Fiteh, B. Furusato, C.A. DeRosa, Y. Chen, L. Ravindranath, C. Cook, J. Cullen, D.G. McLeod, G. Petrovics, I.A. and Sesterhenn, S Srivastava. 2008. Osteonectin (SPARC) Expression Correlates with PSA Recurrence after Radical Prostatectomy.

- **66<sup>th</sup> Annual Meeting of the BKX/NIS, Norfolk, VA**

**Yelekal Fiteh**, Deepak Kumar, Y. Fiteh, B. Furusato, C.A. DeRosa, Y. Chen, L. Ravindranath, C. Cook, J. Cullen, D.G. McLeod, G. Petrovics, I.A. and Sesterhenn, S Srivastava. Osteonectin (SPARC) Expression Correlates with PSA Recurrence after Radical Prostatectomy.

- **UDC Undergraduate Research Day- April 20, 2009**

**Yelekal Fiteh**, Deepak Kumar, Y. Fiteh, B. Furusato, C.A. DeRosa, Y. Chen, L. Ravindranath, C. Cook, J. Cullen, D.G. McLeod, G. Petrovics, I.A. and Sesterhenn, S Srivastava. Osteonectin (SPARC) Expression Correlates with PSA Recurrence after Radical Prostatectomy

**Emmanuel Woode**, Ying Hu, Gyorgy Petrovics, Deepak Kumar, and Shiv Srivastava. 2008. Evaluation of the variants of ERG oncogene in prostate cancer.

- **Awards:**

**Francisco Sáenz**, Rajesh Thangapazham, Deepak Kumar, Shiv Srivastava, and Albert Dobi. 2008. A Role for Conserved Regulatory Sequences in the Repression of TMPRSS2-ERG Fusion Gene, a Prevalent Oncogenic Alteration in Prostate Cancer. **HBCU-UP**

**National Research Conference, Atlanta, Georgia. (Francisco Sáenz, - 3<sup>rd</sup> Place-Biological Sciences (Oral)**

**Emmanuel Woode, Ying Hu, Gyorgy Petrovics, Deepak Kumar, and Shiv Srivastava**  
Evaluation of the Variants of ERG Oncogene in Prostate Cancer **UDC Undergraduate Research Day- April 20, 2009, (Emmanuel Woode : 3<sup>rd</sup> Place-Biology)**

**Yelekal Fiteh, Deepak Kumar, Y. Fiteh, B. Furusato, C.A. DeRosa, Y. Chen, L. Ravindranath, C. Cook, J. Cullen, D.G. McLeod, G. Petrovics, I.A. and Sesterhenn, S Srivastava.** Osteonectin (SPARC) Expression Correlates with PSA Recurrence after Radical Prostatectomy. **(Fiteh Yelekal 3<sup>rd</sup> Place-Biology)**

## **CONCLUSION:**

The students selected under HBCU Summer Undergraduate Training Program in Prostate Cancer Research program have successfully completed the projects assigned to them. They participated in daily meetings with the supervisors, received training in carrying-out the experiments, and recorded the data in electronic laboratory notebook on daily basis. Students participated in weekly seminars and made PowerPoint presentations every 2 weeks to show their progress. At the end of the 12 weeks, students made final presentations and submitted the complete report on the project (Attachments). This has been extremely rewarding experience for the students, mentors and for collaborating Institutions. The students have won numerous awards at national and local conferences serving towards HBCU training.

During the training period, the students learned the following:

- Basic skills in planning and execution of a research project focused on a defined question related to molecular and cell biology of prostate cancer or translational prostate cancer research.
- Presentation and development of a practical idea and its significance to prostate cancer
- Importance of prostate cancer research in high-risk population such as African Americans
- Preparation of high quality slide presentations on assigned topics
- Preparation of a final written report.
- Maintenance of electronic record of experiments
- Issues related to laboratory safety and laboratory hygiene
- Appreciated the power of scientific research and its application in decreasing suffering from a disease such as cancer
- Appreciated the dedication, perseverance and effort it takes to perform research of highest quality in the laboratory

## **REFERENCES:**

None

## **APPENDICES:**

### **Supporting Data:**

**Summer Research Opportunity Announcement:**

**Attachment#1**

**Mr. Francisco Saenz:** Highlights of research projects:

**Attachment#2**

**Mr. Emmanuel Woode:** Highlights of research projects:

**Attachment#3**

**Mr. Chiedozie J Ayika:** Highlights of research projects:

**Attachment#4**

**Ms. Fiteh Yelekal:** Highlights of research projects:

**Attachment#5**

#### **CPDR Web News Release:**

USU's Center for Prostate Disease Research,  
University of the District of Columbia  
Launch Successful Summer Internship Program:

**Attachment#6**

**USUHS News Release:** CPDR Receives Prostate Cancer  
Research Summer Internship Grant From DoD-PCRP:

**Attachment#7**



## SUMMER RESEARCH OPPORTUNITY

Summer research opportunity is available at the Center for Prostate Disease Research (CPDR) at Uniformed Services University of Health Sciences (USUHS) on a UDC-CPDR jointly funded grant from the Department of Defense. The UDC-CPDR Summer Program provides a 12-week summer research experience in prostate cancer research for undergraduate students majoring in science, technology, engineering and mathematics (STEM) disciplines. The goal of this program is to prepare a diverse, highly talented, educated, and skilled pool of scientists interested in Prostate Cancer Research. The students will be exposed to cutting edge research methods in prostate cancer. More information about CPDR can be found at <http://www.cpdr.org>

### Eligibility

1. The applicant must be a junior or senior at UDC when he/she returns to school in Fall 2008
2. Must be studying in STEM disciplines with an interest in Prostate Cancer Research.
3. Must have a cumulative GPA of 3.0 or above at the time of application.

### Stipend

The participants to this program will receive a stipend @ \$10/hr, 40h/week for 12 weeks.

### Start Date

June 1, 2008

### Application

Submit a letter of intent along with a short essay (1 page) on how this program will help you in achieving your career goals. The deadline for application is May15, 2008.

### Submit your application to

#### **Dr. Deepak Kumar**

Co-PI and Director of the UDC-CPDR Summer Research Program

Department of Biological and Environmental Sciences

University of the District of Columbia

Building 44, Room 312

4200 Connecticut Avenue NW

Washington DC 20008

Telephone: (202) 274-5937

Fax: (202) 274-5776

Email: [dkumar@udc.edu](mailto:dkumar@udc.edu)

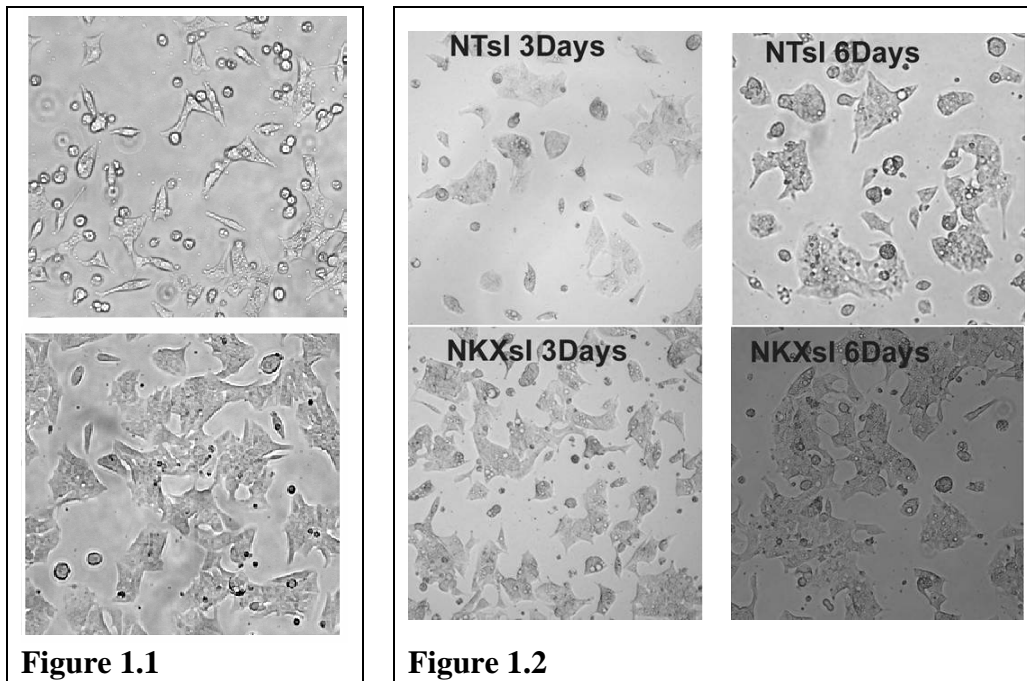
## Highlights of research projects assigned to the students (Provided by the students)

### Attachment #2

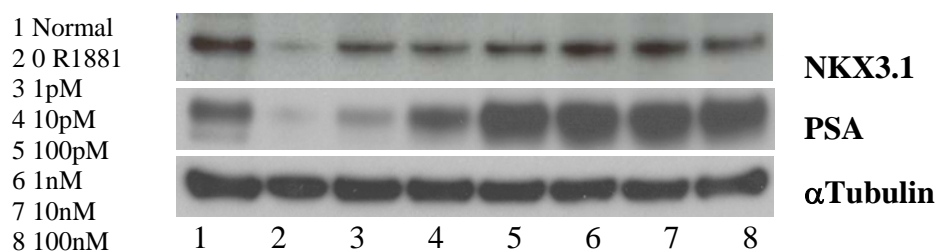
**Student:** Mr. Francisco Saenz  
**Mentor:** Dr. Albert Dobi, Ph.D  
**Research Project:** Defining the role of NKX3.1 on the expression of TMPRSS2-ERG fusion Gene

NKX3.1 gene is a transcription factor and is established as a tumor suppressor. The expression of NKX3.1 is found to be decreased in prostate cancers. NKX3.1 is normally expressed in prostate and several other organs and implicated in embryogenesis, organogenesis, and tissue maintenance. NKX3.1 is involved in the initiation of prostate development in male newborns and maintains differentiation of prostate epithelial cells in adults. Recent findings have suggested that in majority of prostate cancers, high levels of ETS-Related Gene (ERG) in prostate tumors were identified. Subsequent studies linked the cause of high levels of ERG to genomic rearrangement between TMPRSS2 promoter and ERG. TMPRSS2 is androgen-induced gene and studies and implicated prostate carcinogenesis. These findings suggest involvement of the TMPRSS2 promoter. **The main objective of this project is to define the function of NKX3.1 gene as a repressor of the TMPRSS2 promoter therefore limiting the expression of the TMPRSS2-ERG and subsequently the level of ERG.**

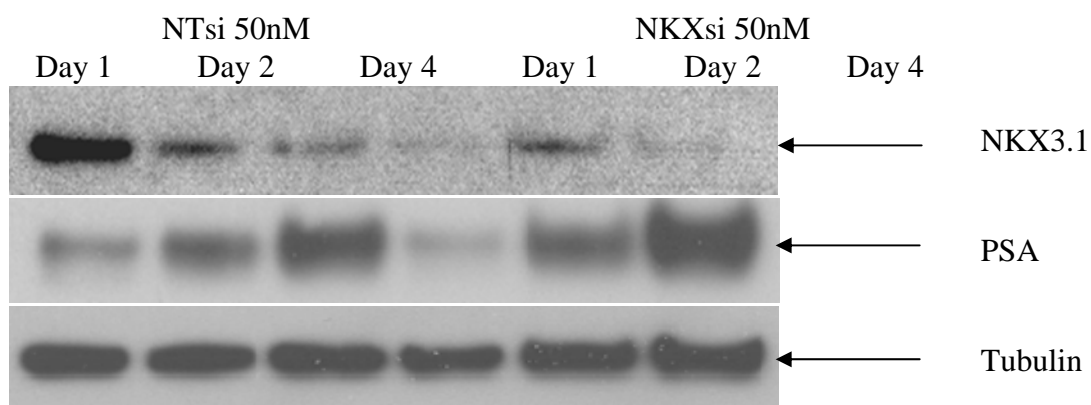
### Results:



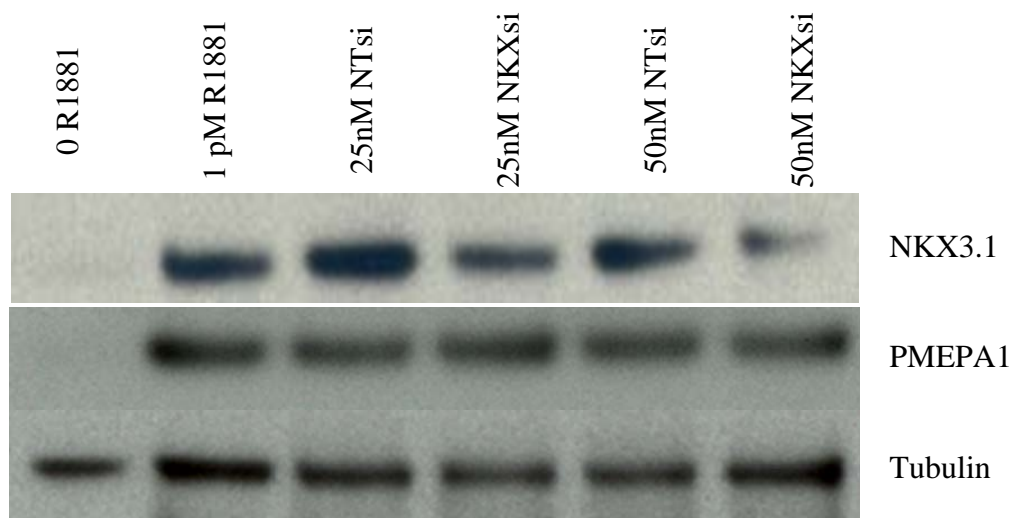
**Figure 1.1 shows VCaP cells grown under hormone-depleted environment (top) and induced growth by R1881 (bottom). Figure 1.2 shows VCaP cells transfected with NTsi/NKXsi at different time points.**



**Figure 1.3 . Increased expression of NKX3.1 protein increase with increase in concentration of R1881.**

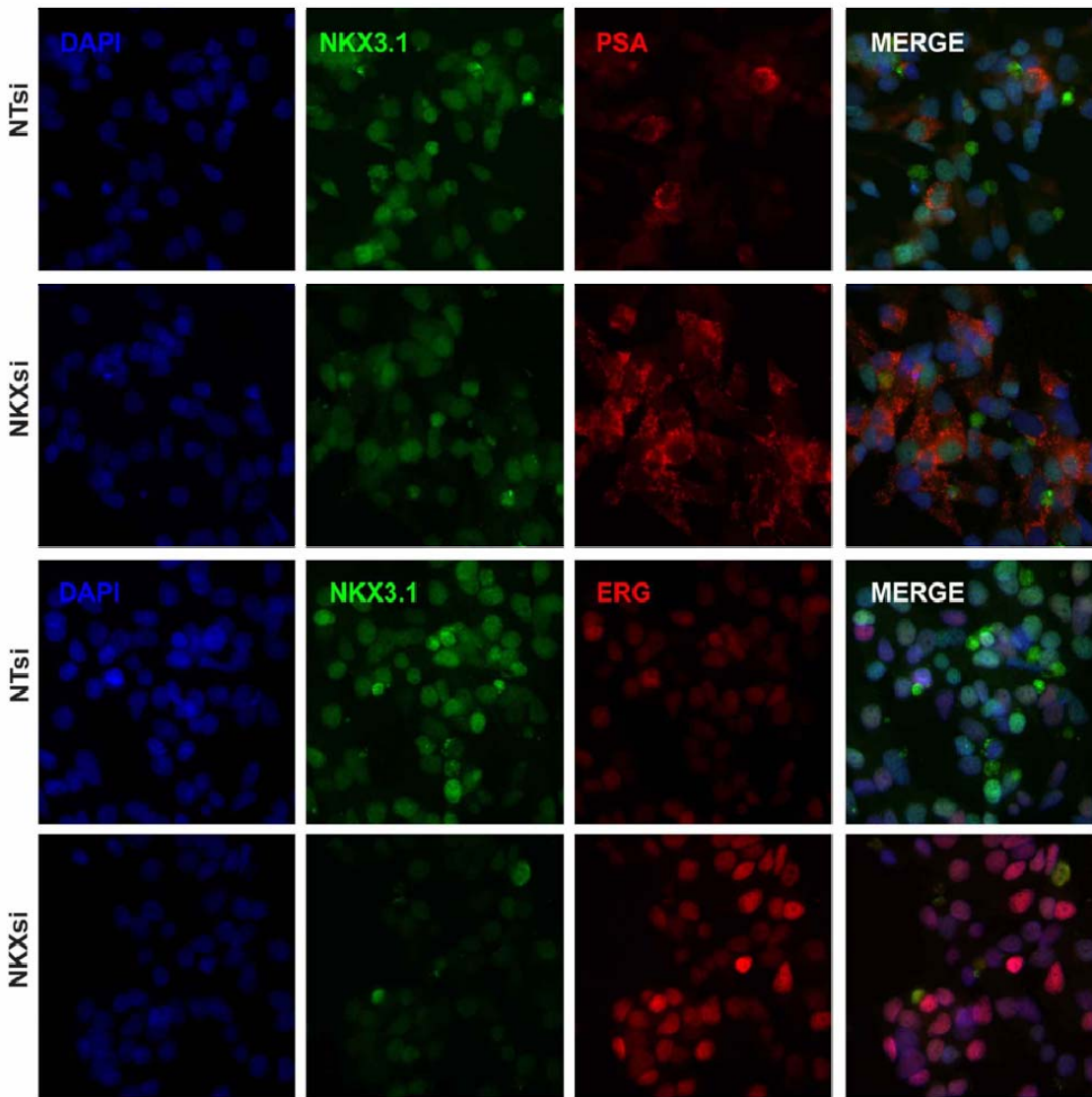


**Figure 2.1. Knock-down of NKX 3.1 expression by siRNA induces the levels of PSA**



**Figure 2.2. PMEPA1 levels are not affected by the silenced NKX samples.**





**Figure 3. Expression of of PSA and ERG in NKX3.1siRNA treated VCaP cells: Elevated levels of PSA (red) and ERG (red) in response to NKX knock-down suggest the NKX3.1 mediated regulation.**

**Conclusions:**

- Knockdown of NKX3.1 changes the morphological features of VCaP cells
- NKX3.1 regulates ERG expression through TMPRSS2 gene promoter in prostate cancer.
- NKX3.1 regulates PSA, a marker expression.
- Knockdown of NKX3.1 did not alter the levels of PMEPA1

## Attachment# 2

**Student:** Mr. Emmanuel Woode  
**Mentor:** Dr. Gyorgy Petrovics, Ph.D  
**Research Project:** The Biological function of alternatively spliced ERG transcripts

In TMPRSS2-ERG fusion positive prostate cancers, five tumor specific isoforms of ERG were detected (Hu et al. Clinical Cancer Research 2008). Three of the five were identified as full length prototypical gene product (type I) while the remaining two were identified to be truncated (type II) and without the DNA binding domain. Quantitative PCR results showed that that the truncated forms were relatively abundant than the full length product. **We hypothesized that the truncated isoforms (TMPRSS2-ERG8) may function as dominant negative forms.** Therefore, we focused on studying the functional characteristics of cancer specific ERGs, in particular ERG8 one of the two truncated forms.

### Results:

Identification of ERG8 siRNA specific to study the biological functions:

A.

B.

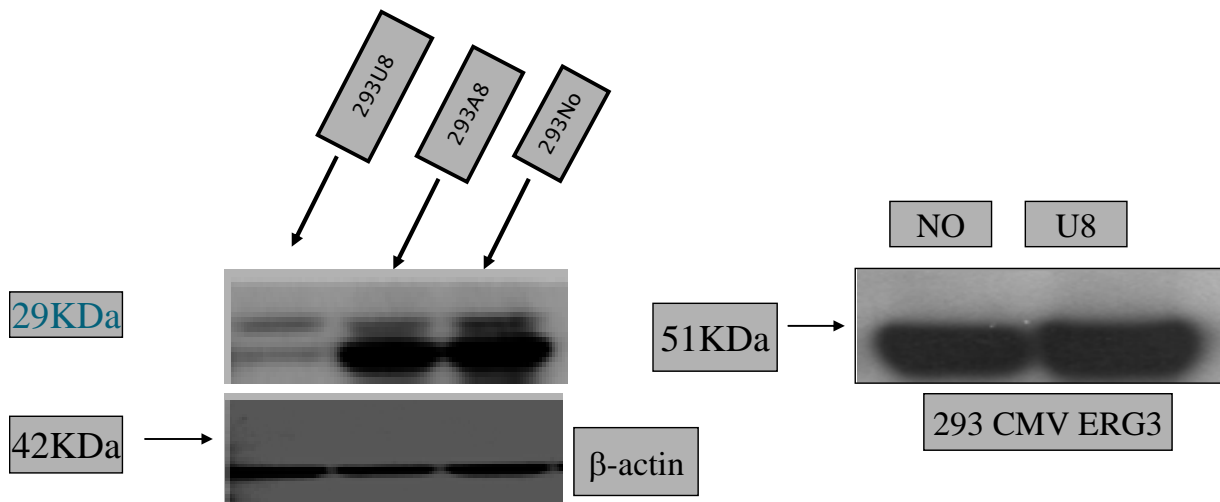


Fig 1A and 1B: Western Blot result for ectopic ERG8 silencing but not ERG3 by antisense to ERG8 (U8).

### Conclusions:

- ERG8 and ERG3 expression vectors were made and tested in HEK 293 cells for the expression of ERG8 (28kD) and ERG3 (53kD).
- ERG8 was significantly silenced ectopically in HEK293 cells after treatment with ERG8 Si for 48 hours (Figure 1A).
- No effect of ERG8 Si on ERG3 (Figure 1B).
- ERG8 specific siRNA is identified and can be used to down regulate ERG8 to study the biological functions associated with its expression in well differentiated prostate tumors

### Attachment# 3

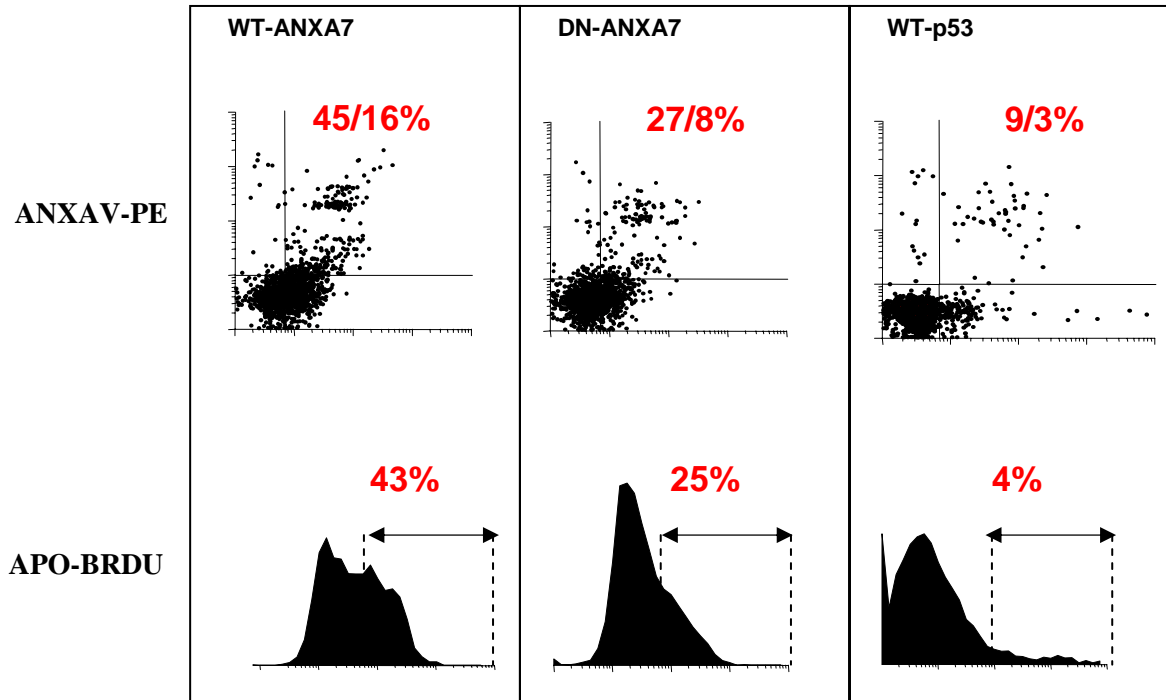
**Student:** Mr. Chiedozie J Ayika

**Mentor:** Dr. Meera Srivastava, Ph.D

**Research Project:** Lack of p53 tumor suppressor effects in LNCaP cells was associated with FOXO3a hyper-phosphorylation

Annexin 7 (ANXA7) is a member of the annexin family of calcium-dependent phospholipid-binding proteins. ANXA7(+/-) mice have a cancer-prone phenotype and ANXA7 acts as a tumor suppressor gene in human cancers. In particular, loss of heterozygosity and reduction of ANXA7 protein expression was associated with the aggressive metastatic and hormone-refractory prostate cancer. In earlier studies, apoptosis detection assays and cell cycling showed that p53 failed to match programmed cell death (PCD), and cell growth arrest that were induced by Annexin A7 (ANXA7) in androgen-responsive prostate cancer cells (LNCaP). Cell cycle regulator p53 is known to phosphorylate FOXO3a that can affect its nuclear localization and suppress FOXO3a transcription, thereby preventing the FOXO3a-induced cell cycle arrest and apoptosis. **Our working hypothesis is that ANXA7 and p53 may phosphorylate FOXO3a in a reciprocal way to regulate PCD in androgen-responsive prostate cancer cells, LNCaP. Therefore, we proposed to study the correlation between the tumor suppressor activity of p53 and ANXA7 through the phosphorylation of FOXO3a.**

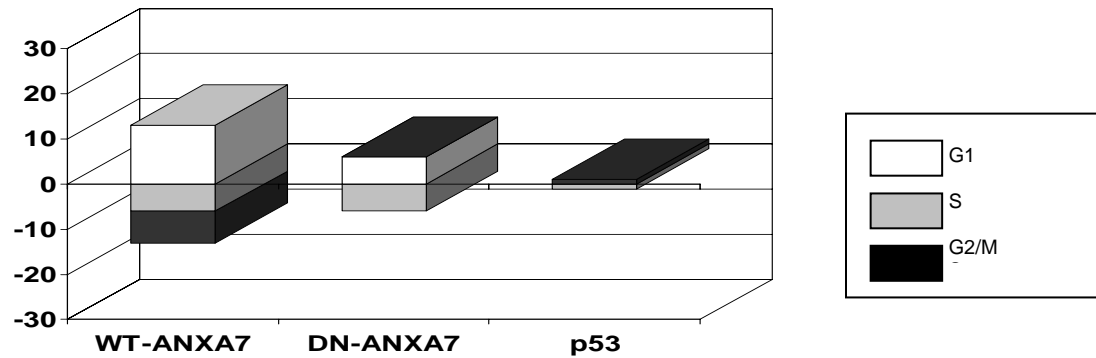
#### Results:



**Figure 1. PCD Responses Androgen-Sensitive Prostate Cancer Cells (LNCaP) Transfected With WT/DN-ANXA7 OR p53**

AnnexinV-PE assay showed double PCD rates for WT-ANXA7 whereas DN-ANXA7 failed to reach the same levels of late apoptosis and phosphatidylserine exposure on dying LNCaP cells ( $p < 0.001$  for both comparisons). Based on APO-BRDU assay, WT-ANXA7 caused a 2-fold PCD increase compared to DN-ANXA7 ( $p < 0.001$ ) inducing DNA fragmentation almost in a half of LNCaP cell population. In the meantime, p53 caused only a slight increase in early apoptosis with phosphatidylserine exposure. PCD responses to WT/DN-ANXA7 and p53 (18hr) were assessed by Annexin V-PE and APO-BRDU (Panel A and B, respectively) as described in Panel

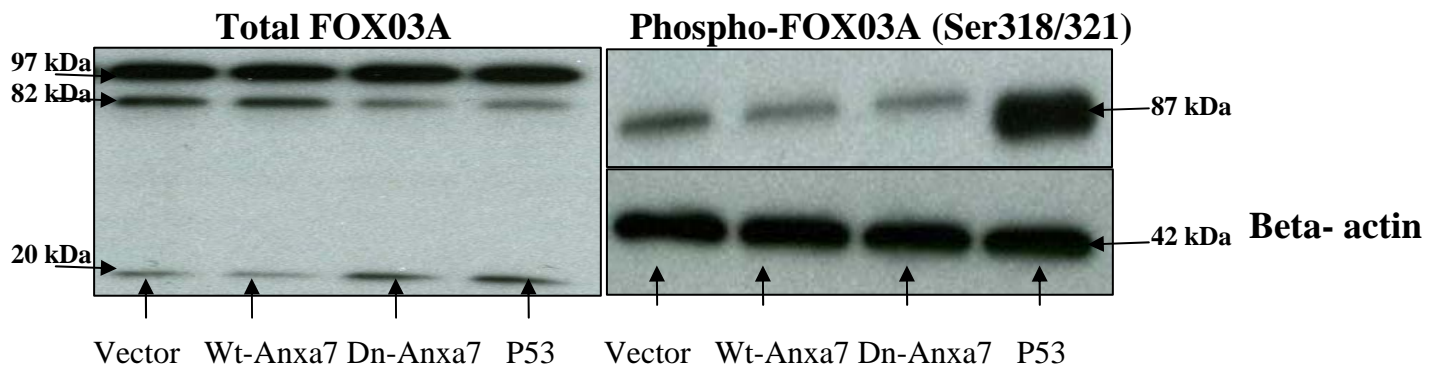
A: first numbers – phosphatidylserine exposure and second numbers – membrane permeabilization by Annexin V-PE; Panel B: DNA fragmentation by APO- BRDU.



**Figure 2. Cell Cycling In Androgen-Sensitive Prostate Cancer Cells (LNCaP) Transfected With WT/DN-ANXA7 OR p53.**

WT-ANXA7 caused a moderate G1 increase which was not matched by DN-ANXA7 ( $pZ < 0.001$ ). Unlike WT-ANXA7, p53 lacked essential cell cycling effects in LNCaP.

Graphs represented the differences (delta %) in cell numbers in different phases in response to WT/DN-ANXA7 and p53 (18hr); mean values from replicates (%) were presented after subtraction of control levels in corresponding vectors.



**Figure 3. Expression Of Total And Phosphorylated FOXO3A Protein In Androgen-Sensitive Prostate Cancer Cells (LNCaP) Transfected With WT/DN-ANXA7 OR p53**

### Conclusion:

- In a conventional model of androgen-sensitive prostate cancer (LNCaP), the phospholipid-binding WT-ANXA7 induced tumor suppressor effects thereby demonstrating a capability to overcome PTEN-deficiency and constitutively activated status of PI3K-Akt survival cascade. On the other hand, DN-ANXA7 (which lacks phospholipid-binding properties) failed to match the WT-ANXA7-induced PCD and G1 cell growth arrest.
- In contrast, a canonical cell cycle regulator p53 did not match tumor suppressor effects of WT- or even DN-ANXA7 that was presumably associated with the FOXO3A hyperphosphorylation and subsequent inhibition of the pro-apoptotic FOXO3A transcription.

## Attachment# 4

**Student:** Ms. Fiteh Yelekal

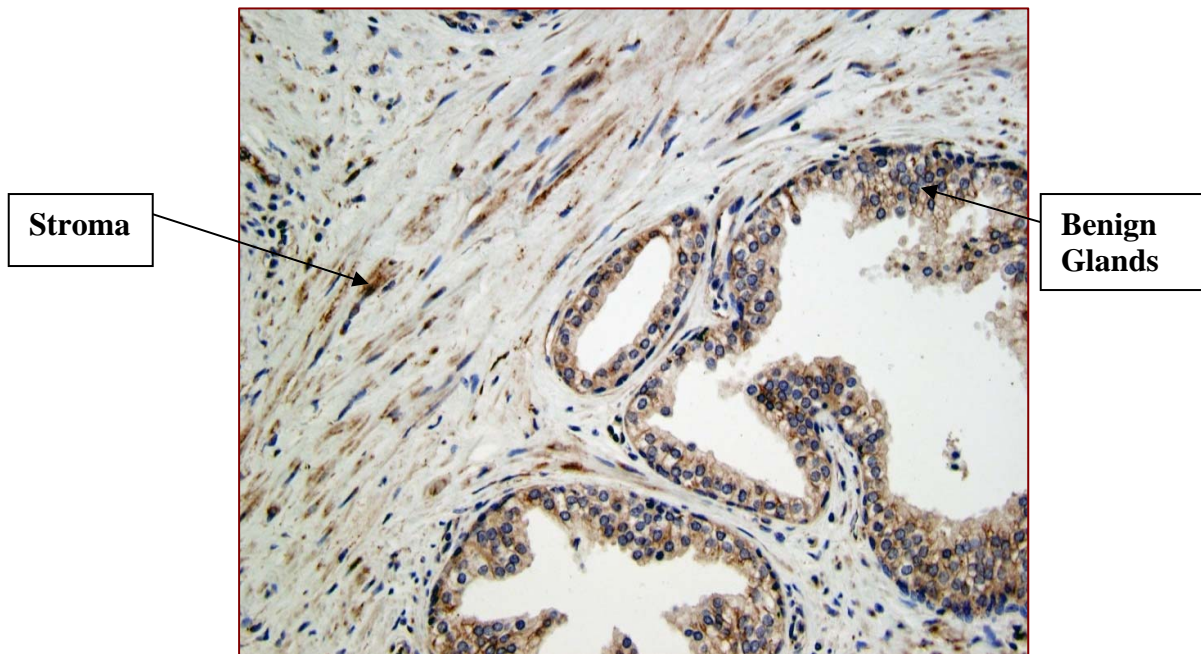
**Mentor:** Dr. Bungo Furusato, MD

**Research Project:** Increased levels of *SPARC* (osteonectin) in human prostate cancer tissues and its association with clinical metastasis

Comparative gene expression signatures of well/moderately differentiated and poorly differentiated prostate cancer (CaP) cells along with knowledge based gene function and transcriptional regulation analyses highlighted alterations of *SPARC*, and genes linked to it, in poorly differentiated CaP. *SPARC* is a secreted glycoprotein that supports the migration of CaP cells to bone and demonstrates increased expression in CaP metastatic foci (mCaP) as well as mCaP cell lines. Bone is by far (80-85%) the primary site of prostate cancer metastasis. *SPARC* also plays a major role in tissue remodeling, wound repair, cellular differentiation, morphogenesis, cell migration, and angiogenesis. **We hypothesized that quantitative determination of *SPARC* expression levels in prostate tumor cells may have potential to predict aggressive clinical behavior in newly diagnosed patients.** Our objective is to Study *SPARC* protein expression in a large cohort of patients by immunohistochemistry, whether the protein expression of *SPARC* can predict aggressive clinical behavior in retrospectively selected cohort of patients.

### Results:

Weak to moderate staining of *SPARC* in the benign glands and in stroma cells. The brown areas are *SPARC* positive areas. Stroma cells are *SPARC* positive only in the above area but not on the entire section of the slide



## Statistical data results:

### Association of SPARC level with metastasis

| SPARC level      | Metastasis status |      |     |     | P value |
|------------------|-------------------|------|-----|-----|---------|
|                  | No                |      | Yes |     |         |
|                  | N                 | %    | N   | %   |         |
| SPARC positive % |                   |      |     |     | 0.0076  |
| <25%             | 6                 | 100% | 0   | 0%  |         |
| 25%-50%          | 7                 | 50%  | 7   | 50% |         |
| 51-75%           | 10                | 59%  | 7   | 41% |         |
| >75%             | 4                 | 24%  | 13  | 76% |         |
| SPARC intensity  |                   |      |     |     | 0.0184  |
| Weak             | 16                | 73%  | 6   | 27% |         |
| Moderate         | 8                 | 33%  | 16  | 67% |         |
| Strong           | 3                 | 38%  | 5   | 62% |         |

Fisher exact tests were used to test the difference of % of metastasis among different SPARC levels. This table showed that patients with higher SPARC positive % have higher % of metastasis ( $P = 0.0076$ ), and patients with higher SPARC intensity have higher % of metastasis ( $P = 0.0184$ ).

### Conclusions:

- Higher *SPARC* expression in CaP is associated with poorly differentiated carcinoma and high Gleason Score.
- High *SPARC* expression in CaP mRNA levels is associated with an increased risk of PSA recurrence.
- Quantitative determination of *SPARC* expression in protein levels is associated with clinical metastasis status in this cohort.
- *SPARC* expression either in mRNA or protein level in primary CaP specimens may have prognostic utility especially in locally advanced CaP.





Center for Prostate Disease Research

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### > CPDR News

## USU's Center for Prostate Disease Research, University of the District of Columbia Launch Successful Summer Internship Program

September 16, 2008

**BETHESDA, Md.** — The Center for Prostate Disease Research (CPDR), Department of Surgery, Uniformed Services University (USU) and the University of the District of Columbia (UDC) were granted an award of \$198,000 by the Department of Defense, United States Army Medical Research and Materiel Command (USAMRMC) for training gifted students from the University of the District of Columbia (UCD) in prostate cancer research.

The program goal is to immerse young students from historically black colleges and universities in prostate cancer research within the framework of a structured summer training program. CPDR is credited for landmark discoveries in prostate cancer research such as, the increased prevalence of prostate cancer among African American men and the recent discovery of frequent Ets-related Gene (ERG) oncogene over expression at early stages of prostate cancer.

In 2008, four UDC students were selected on a rigorous competitive academic basis for the twelve-week program. Every student chosen to participate in the program demonstrated outstanding excellence in advanced prostate cancer research. On Aug. 20, 2008 the students presented highlights of their training and research at the CPDR Headquarters in Rockville, Md. In attendance were Rachel Petty, Ph.D., Dean of the College of Art and Sciences at UDC; COL David G. McLeod, MC, USA (Ret.), Director and Founder of CPDR and Professor of Surgery (USU); COL David Burris, MD, FACS, DMCC, Professor and Chair, Department of Surgery, USU; and the Principal Investigators of the program -Shiv Srivastava, Ph.D., Co-Director of CPDR and Professor of Surgery; and Deepak Kumar, Ph.D., Assistant Professor, UDC, and the Summer Program Coordinators Albert Dobi, Ph.D., Assistant Director and Taduru Sreenath, Ph.D., Senior Staff Scientist and the CPDR research faculty and staff.

**The names of the presenters and the titles of their concluding presentations are:**

**Fiteh Yelekal:** *"Increased levels of SPARC in prostate cancer is associated with metastasis"*

**Emmanuel Woode:** *"Biological function of ERG in prostate cancer"*

**Chiedozie Joseph Ayika:** *"A role for the tumor suppressor Annexin7 in prostate cancer"*

**Francisco R. Saenz:** *"Defining NKX3.1 as a negative regulator of ERG"*

The CPDR mission is fulfilled primarily through its three principal programs – the Clinical Research Center, the Basic Science Research Program and the National Multicenter Prostate Cancer Database– and through a robust education and training program that operates out of its Headquarters location, the Clinical Research Center, and the original laboratories at USUHS. CPDR is also committed to patient outreach, primarily through its affiliation with the WRAMC US TOO! organization and through a heavy schedule of health fairs in which it participates.



**Uniformed Services University**  
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4301 Jones Bridge Road  
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Release No.08-08-39

Aug. 11, 2008

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## News Release

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# CPDR Receives Prostate Cancer Research Summer Internship Grant From DoD-PCRP

**BETHESDA, Md.** — The Prostate Cancer Research Program (PCRP) Collaborative Undergraduate Historically Black College And University Student Summer Training Program was awarded by the Department of Defense, United States Army Medical Research and Materiel Command (USAMRMC) to the Uniformed Services University of the Health Sciences' (USU) Center for Prostate Disease Research (CPDR) and the University of the District of Columbia (UDC) collaborative team.

A successful collaborative effort between Dr. Shiv Srivastava, Department of Surgery, USU/CPDR and Dr. Deepak Kumar, UDC, provides a great opportunity for talented students to take part in the new Prostate Cancer Training Program that is conducted during their summer break. Historically Black Colleges and Universities (HBCU) have a diverse student body with a majority of students classified as minorities. Health Disparities such as the higher incidence of prostate disease among African American men that have been the focus of the CPDR research team are increasingly being addressed in various diseases including prostate cancer. So what better way to increase awareness of such trends and move towards a cure than to:

1. Recruit and encourage highly qualified undergraduate trainees by providing them with a stimulating and intellectual environment that promotes state-of-the-art training and education in prostate cancer research.
2. Motivate young researchers, who may contribute to prostate cancer research centers at HBCUs
3. Ensure that the new generation of biomedical scientists is properly trained to continue the fight against prostate cancer.

At the Uniformed Services University's Center for Prostate Disease Research (CPDR), the four selected summer students from UDC are paired with one of the faculty members of USU, and focus on specific research projects in basic science and database research. There are goals and objectives for both mentors and students. Students are actively involved in the program through progressive research, regular lab meetings, seminars and personal discussions with faculty members, staff and fellow students. Research findings are presented by the students, at the end of the summer experience aiming scientific publications. The National Conference Award provides a unique opportunity for gifted students in a leading institution of the prostate cancer field.



*Learning to Care for Those in Harm's Way*

Located on the grounds of Bethesda's National Naval Medical Center and across from the National Institutes of Health, USU is the nation's federal school of medicine and graduate school of nursing. The university educates health care professionals dedicated to career service in the Department of Defense and the U.S. Public Health Service. Students are active-duty uniformed officers in the Army, Navy, Air Force and Public Health Service, who are being educated to deal with wartime casualties, national disasters, emerging infectious diseases, and other public health emergencies. Of the university's more than 4,000 physician alumni, the vast majority serve on active duty and are supporting operations in Iraq, Afghanistan, and elsewhere, offering their leadership and expertise.

For more information, contact the Office of External Affairs at 301-295-1219.

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