AWARD NUMBER: W81XWH-16-1-0217

TITLE: Advancing Prostate Cancer Research by Providing Summer Research Opportunities for HBCU Students at the Cancer Center at UTHSCSA

PRINCIPAL INVESTIGATOR: Robin J. Leach, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas Health Science Center at San Antonio
San Antonio, TX 78229

REPORT DATE: August 2017

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

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  
August 2017

2. REPORT TYPE  
Annual

3. DATES COVERED  
15 Jul 2016 - 14 Jul 2017

4. TITLE AND SUBTITLE  
Advancing Prostate Cancer Research by Providing Summer Research Opportunities for HBCU Students at the Cancer Center at UTHSCSA

5a. CONTRACT NUMBER  
W81XWH-16-1-0217

5b. GRANT NUMBER  

5c. PROGRAM ELEMENT NUMBER  

5d. PROJECT NUMBER  

5e. TASK NUMBER  
E

5f. WORK UNIT NUMBER  

6. AUTHOR(S)  
Robin J. Leach, Ph.D.

E-Mail: leach@uthscsa.edu

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  
The University of Texas Health Science Center at San Antonio  
7703 Floyd Curl Drive  
San Antonio, TX 78229-3900

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)  
USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)  

12. DISTRIBUTION / AVAILABILITY STATEMENT  
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES  

14. ABSTRACT  
The DOD-funded summer research experience has provided a unique opportunity for students from a Historically Black University to work at a NCI-designated Cancer Center for 10 weeks. Their research is conducted in funded prostate cancer researchers’ laboratories. Before starting their research in the laboratory, the students are provided training in both laboratory methods as well as the biology of prostate cancer. During the summer, they are exposed to enrichment programs ranging from health disparity presentations to survivorship research. In addition, they obtain career guidance from the Associate Dean of the Graduate School who emphasizes the importance of this summer for exploring career opportunities. It is evident that the students contribute to the research in their laboratories by the high quality of their presentations at the end of summer poster session. Many of the students have previously considered a career in medicine, but for most of the students, this is their first exposure to a research-intensive environment. Some of the students are re-evaluating their long term career goals and exploring the possibility of either becoming a full time researcher or pursing a physician scientist degree. Thus, this summer experience is contributing to development of the next generation of prostate cancer researchers.

15. SUBJECT TERMS  
Prostate Cancer Research, Historically Black University

16. SECURITY CLASSIFICATION OF:
a. REPORT  
Unclassified

b. ABSTRACT  
Unclassified

c. THIS PAGE  
Unclassified

17. LIMITATION OF ABSTRACT  
Unclassified

18. NUMBER OF PAGES  
23

19a. NAME OF RESPONSIBLE PERSON  
USAMRMC

19b. TELEPHONE NUMBER (include area code)
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Introduction</td>
<td>4</td>
</tr>
<tr>
<td>2. Keywords</td>
<td>4</td>
</tr>
<tr>
<td>3. Accomplishments</td>
<td>4-5</td>
</tr>
<tr>
<td>4. Impact</td>
<td>5-6</td>
</tr>
<tr>
<td>5. Changes/Problems</td>
<td>6-7</td>
</tr>
<tr>
<td>6. Products</td>
<td>8-9</td>
</tr>
<tr>
<td>7. Participants &amp; Other Collaborating Organizations</td>
<td>9-13</td>
</tr>
<tr>
<td>8. Special Reporting Requirements</td>
<td>15</td>
</tr>
<tr>
<td>9. Appendices</td>
<td>15-23</td>
</tr>
</tbody>
</table>
1. **INTRODUCTION:**

This program provides students from a Historically Black College and University (HBCU), Huston-Tillotson University (HTU) in Austin, Texas, an opportunity to conduct prostate cancer research at a National Cancer Institute (NCI)- designated Cancer Center, UT Health San Antonio Cancer Center (UTHSA-CC; formerly known as the Cancer Therapy and Research Center at the University of Texas Health Science Center San Antonio). This is a distinct partnership between these two institutions since there are only nine HBCUs in the State of Texas and only four NCI-designated cancer centers. The students participate in a 10-week program and work directly with principal investigators who are funded to conduct prostate cancer research. The program begins with some didactic and laboratory training before the students are placed in a research laboratory. During the final week of the program, the students have an opportunity to present their research at a poster session where they present their findings to faculty and students.

2. **KEYWORDS:**

- Prostate Cancer
- Historically Black College and University
- NCI-Designated Cancer Center

3. **ACCOMPLISHMENTS:**

**What were the major goals of the project?**

The overall goal of this program is to develop opportunities to train minority students in southern Texas in prostate cancer research. This program provides undergraduate students with an opportunity to work at an NCI-designated cancer center and participate in state-of-the-art prostate cancer research in many focus areas including biomarkers, genetics, tumor biology, therapy and imaging. The prostate research mentors offer students research projects that encompass both basic biomedical research and translational research.

One goal of this program is to help encourage these students to learn about the graduate school experience and to equip them with research skills that will allow them to undertake careers in scientific research that will be focused on the field of prostate cancer. The program will last for 10 weeks; the first two weeks (reduced to one week in the second year of the program based on student feedback) includes course work (both didactic and laboratory based) that introduces the students to prostate cancer biology, as well as basic molecular techniques.

**What was accomplished under these goals?**

We were notified by the DOD that that we would be funded in spring 2016 and thus, we worked rapidly to identify students that could participate in the summer of 2016. The award actually was received in mid-June at which time we had already recruited students, confirmed mentors and selected the first year class of students. They began their research experience in early June and completed in mid-August. Below is a summary of the timeline and what has been accomplished under the award to date:

**Timeline:**

<table>
<thead>
<tr>
<th>Task</th>
<th>2016</th>
<th>2017</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruit students</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirm mentors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select Students</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internship program</td>
<td>2016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracking &amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Below is a summary of the timeline and what has been accomplished under the award to date:

- **Completed**
- **In Progress**
- **Pending**
The students participating in the first year of the program provided feedback in their final week at the cancer center on how their experience might have been improved. The one major concern was the limited amount of laboratory time to complete their project and so it was decided that for the subsequent year, we would reduce their didactic and laboratory training to a single week, allowing them more time in their prospective laboratories.

**What opportunities for training and professional development has the project provided?**

In addition to the two weeks of training described above, the students have weekly enrichment programs. These are conducted every Friday at noon and lunch is provided. The speakers for this program range from experts in Health Disparities to Cancer Survivorship. We also have a physician scientist discuss the dual M.D./Ph.D. degree with its advantages and disadvantages. The Associate Dean for the Graduate School, Dr. Nicquet Blake, also provides some career development information. Dr. Blake is one of the first presentations, so the students have an opportunity to meet with her one-on-one if they are so inclined. The students receive extensive laboratory training during the remaining weeks of the program. The new schedule for the one week of didactic training is provided below.

**How were the results disseminated to communities of interest?**

Nothing to Report.

**What do you plan to do during the next reporting period to accomplish the goals?**

We are well underway for the second year of the program. The students will be finishing up this week with the second year of summer training (completed in August 2017), and we will be working with our alumni to identify opportunities for them to present their research in other venues. It will also be important for the students as they apply for graduate and medical school that they receive letters of recommendation from their mentors in the program.

**4. IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

Participation in a 10-week research program is not likely to contribute significantly to new discoveries in prostate cancer; however, the goal of the program is to contribute to the development of the next generation of prostate cancer researchers. Many of the students have previously considered a career in medicine, but for most of the students, this is their first exposure to a research-intensive environment. After their summer experience, some of the students are re-evaluating their long-term career goals and exploring the possibility of either becoming a full time researcher or pursing a physician scientist degree. Thus, this summer experience is contributing to the development of the next generation of prostate cancer researchers, which will have a profound long term impact on the field.

**What was the impact on other disciplines?**

Training future scientists will have a broad impact on numerous disciplines, since discoveries in one field can often translate into another field.
What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Through this program, bright young minority students are encouraged to pursue a career in research, and this has many positive implications for society. Many students from Huston-Tillotson University had never considered a career in research because they were unaware of the numerous research opportunities, both in academics and industry. Thus, this experience may change their career trajectory, and as they interact with their family and peers, it may increase the number of future minority scientists. This is particularly important in prostate cancer where health disparities are so prevalent.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

During the first summer program, we followed the proposed schedule for two weeks of classroom training followed by 8 weeks in the laboratory. However, the students give a poster presentation during that last week, so the actual laboratory time is close to 7 weeks. During the final days of the program, the students had an opportunity to provide feedback on their summer experience. They felt it would have been more productive for them to reduce the original 2 weeks of training to a single week, thus providing more time for their actual bench work.

Shown below is the program provided in the first summer and how it was reduced for the second summer:

**Summer 2016: Introductory Course for Summer Students enrolled in the HBCU DOD Prostate Program**

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Tuesday 5/31</th>
<th>Wednesday 6/1</th>
<th>Thursday 6/2</th>
<th>Friday 6/3</th>
<th>Monday 6/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-11 a.m. Lecture</td>
<td>Orientation Room MED 238A</td>
<td>Overview of Prostate Cancer &amp; Prostate Biology Room MED 238A</td>
<td>Nucleic Acids Concentration and Integrity Room MED 5.573C</td>
<td>Methods Overview – Nucleic Acids Room MED 238A</td>
<td>Critical Thinking Journal Article Room 238A</td>
</tr>
<tr>
<td>12-1 p.m.</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Enrichment Program</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>1-4 p.m. Lab (on most days)</td>
<td>DNA &amp; RNA isolation Room MED 5.573C</td>
<td>2 pm Prostate Cancer – Clinical Perspective Room 238A</td>
<td>Reverse Transcription and QPCR Room MED 5.573C</td>
<td>SNP Genotyping Room 5.573C</td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>Tuesday 6/7</td>
<td>Wednesday 6/8</td>
<td>Thursday 6/9</td>
<td>Friday 6/10</td>
<td>Monday 6/13</td>
</tr>
<tr>
<td>9-11 a.m. Lecture</td>
<td>Methods Overview – Proteins (DOK) Room STRF-2.264.00</td>
<td>Protein Isolation Room STRF 270.1 9 a.m.-12 p.m.</td>
<td>Human Genetics &amp; Precision Medicine Room STRF-2.210.00</td>
<td>Critical Thinking Journal Article Room STRF-2.264.00</td>
<td>Critical Thinking Journal Article Room STRF-2.264.00</td>
</tr>
<tr>
<td>Time</td>
<td>Activity</td>
<td>Location</td>
<td>Time</td>
<td>Activity</td>
<td>Location</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>12-1 p.m.</td>
<td>Lunch</td>
<td>Room STRF 270.1</td>
<td>11-12 p.m.</td>
<td>Immunohistology prep</td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td>1-4 p.m.</td>
<td>12-1 p.m. Lunch</td>
<td>Lunch Room</td>
<td>11-12 p.m.</td>
<td>Immunohistology prep</td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td>Lab</td>
<td>Tissue Culture Techniques Room STRF 270.1</td>
<td>SDS-PAGE and</td>
<td>Lunch</td>
<td>Enrichment Program</td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue Culture Techniques Room STRF 270.1</td>
<td>Western Blotting</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Immunohistology</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Western Blotting</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Immunohistology</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Western Blotting</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Immunohistology</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Western Blotting</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Immunohistology</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
<tr>
<td></td>
<td>Room STRF 270.1 (1-5 p.m.)</td>
<td>Western Blotting</td>
<td></td>
<td></td>
<td>Room STRF 270.1</td>
</tr>
</tbody>
</table>

**Summer 2017: Introductory Course for Summer Students enrolled in the HBCU DOD Prostate Program**

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Monday, 6/5</th>
<th>Tuesday, 6/6</th>
<th>Wednesday, 6/7</th>
<th>Thursday, 6/8</th>
<th>Friday, 6/9</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-11 am</td>
<td>Orientation</td>
<td>Tissue Culture</td>
<td>Reverse Transcription</td>
<td>Protein Isolation</td>
<td>Western Blotting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overview; DNA &amp;</td>
<td>and QPCR Room MED 5.573C</td>
<td>Room MED 5.573C</td>
<td>Room MED 5.573C</td>
</tr>
<tr>
<td>Room MED 5.573C</td>
<td></td>
<td>RNA Isolation</td>
<td>Room MED 5.573C</td>
<td></td>
<td>Room MED 5.573C</td>
</tr>
<tr>
<td>12-1 pm</td>
<td>Lunch on your own</td>
<td>Medical and</td>
<td>Precision Medicine and Human Genetics</td>
<td>SDS-PAGE and Transfer</td>
<td>2-3 pm Clinical</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bioinformatics</td>
<td>Room MED 238A</td>
<td>Room MED 5.573C</td>
<td>Perspective for</td>
</tr>
<tr>
<td>Room MED 238A</td>
<td></td>
<td>Room MED 5.573C</td>
<td></td>
<td></td>
<td>Prostate Cancer</td>
</tr>
<tr>
<td>1-4 pm</td>
<td>Online Training</td>
<td>3-5 pm Western</td>
<td>3-5 pm Western</td>
<td>2-3 pm Clinical</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blotting continued</td>
<td>Blotting continued</td>
<td>Perspective for</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Room MED 5.573C</td>
<td>Room MED 238A</td>
<td>Prostate Cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Room MED 5.573C</td>
<td>Room MED 5.573C</td>
<td></td>
<td>Room MED 5.573C</td>
</tr>
</tbody>
</table>

**Actual or anticipated problems or delays and actions or plans to resolve them**

There was a delay in sponsoring two students to attend a national meeting. We did have one student, Anna Barbara O’James attend the Annual BKX Conference (Louisiana, March 15-18, 2017) and we anticipate supporting 3 more in the upcoming year. We are encouraging the students to attend the American Association for Cancer Research (AACR) meeting in Chicago in April 2018. The abstracts for this meeting are due December 1st. If they are unable to attend that meeting because of schedule conflicts, additional meeting opportunities will be identified and the students will receive up to $1500 from the grant toward their travel cost. Huston Tillotson University has additional funds that they plan to use if the student’s expenses exceed the allotted amount.

**Changes that had a significant impact on expenditures**

Part of the travel funds for year one will be utilized in year 2.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to Report.
6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  
  Nothing to report.

- **Journal publications.**
  
  Nothing to report.

- **Books or other non-periodical, one-time publications.**
  
  Nothing to report.

- **Other publications, conference papers, and presentations.**

<table>
<thead>
<tr>
<th>Trainee (bold) and co-authors</th>
<th>Title of Presentation</th>
<th>Venue at UT Health San Antonio</th>
<th>Additional presentations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Johnson Bobmanuel,</strong> Xiang Gu, Hakim Bouamar, Lu-Zhe Sun</td>
<td>Immunohistochemistry (IHC) Assay on Mouse Mammary Tissue</td>
<td>2016 Summer Research Poster Presentation</td>
<td></td>
</tr>
<tr>
<td><strong>Anna Edem-Etuk,</strong> Suleman S. Hussain, Addanki P. Kumar</td>
<td>RPS6KB1 inhibition sensitizes prostate cancer cells to chemotherapy</td>
<td>2016 Summer Research Poster Presentation</td>
<td>Huston-Tillotson University Preview Day</td>
</tr>
<tr>
<td><strong>Darrion Jemerson,</strong> Alexandra Sharp, Suphada Lertphinyowong, Sarah Bulin, Ph.D., and David A. Morilak, Ph.D.</td>
<td>Cognitive Impairment Associated with Androgen Deprivation Therapy</td>
<td>2017 Summer Research Poster Presentation</td>
<td>Huston-Tillotson STEM meeting in September 2017</td>
</tr>
<tr>
<td><strong>Bomaonye Sokari,</strong> Saketh Amasa, Ratnesh Srivastava and A. Pratap Kumar</td>
<td>AMPK activates androgen receptor (AR)-signaling</td>
<td>2017 Summer Research Poster Presentation</td>
<td>Huston-Tillotson STEM meeting in September 2017</td>
</tr>
<tr>
<td><strong>Nangah Tabukum,</strong> Yanming Wu, Kexin Xu, PhD.</td>
<td>Plasmid Construction- A way to study the role of ATAD2</td>
<td>2017 Summer Research Poster Presentation</td>
<td></td>
</tr>
</tbody>
</table>
### 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

<table>
<thead>
<tr>
<th>Name</th>
<th>Project Role</th>
<th>Researcher Identifier</th>
<th>Nearest person month worked</th>
<th>Contribution to Project</th>
<th>Funding Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robin J. Leach, Ph.D.</td>
<td>PI</td>
<td>Professor, UT Health San Antonio</td>
<td>1</td>
<td>Oversaw the summer program. Involved in recruitment, selection and training of the students. Prepares progress report. Oversees accounting</td>
<td>National Cancer Institute and Owens Foundation</td>
</tr>
<tr>
<td>Amanda Masino, Ph.D.</td>
<td>Co-Investigator</td>
<td>Associate Professor, Huston-Tillotson University</td>
<td>.648</td>
<td>Faculty mentor for students at home institution. Helps with the recruitment and selection of students and provides mentoring throughout the year.</td>
<td>National Science Foundation, DOD, Office of Naval Research</td>
</tr>
<tr>
<td>Denise O'Keefe,</td>
<td>Co- Associate</td>
<td>0.5</td>
<td>Mentor students</td>
<td>DOD, NCI</td>
<td></td>
</tr>
</tbody>
</table>

- **Website(s) or other Internet site(s)**
  - Nothing to report.
- **Technologies or techniques**
  - Nothing to report.
- **Inventions, patent applications, and/or licenses**
  - Nothing to report.
- **Other Products**
  - Nothing to report.
<table>
<thead>
<tr>
<th>Ph.D. Investigator</th>
<th>Professor</th>
<th>and assist with didactic training</th>
<th>NCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teresa Johnson-Pais, Ph.D.</td>
<td>Co-Investigator</td>
<td>Associate Professor</td>
<td>1</td>
</tr>
<tr>
<td>Eva Garcia-Rodriguez</td>
<td>Coordinator</td>
<td>Research Coordinator - Senior</td>
<td>1.8</td>
</tr>
<tr>
<td>Students</td>
<td>See information provide in Table in Section</td>
<td>Undergraduates at Huston Tillotson University</td>
<td>2.0</td>
</tr>
</tbody>
</table>

### Participating Students

<table>
<thead>
<tr>
<th>Trainee</th>
<th>Research Mentor</th>
<th>Project Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson Bobmanuel</td>
<td>LuZhe Sun, Ph.D.</td>
<td><strong>Role of mammary stem/progenitor cell in tumorigenesis:</strong> Student will assist with immunohistochemical staining, solution making, and other routine lab chores, and help with data analysis and management, and literature review. Follow-Up: Preparing for the GRE and start submitting applications to graduate school before the end of this year. Proposing to apply to UT Health San Antonio along with other institutions.</td>
</tr>
<tr>
<td>Michael Esuruoso</td>
<td>Dean Bacich, Ph.D.</td>
<td><strong>The effect of metformin and folate on prostate cancer growth and gene regulation:</strong> Student will perform tissue culture of prostate cancer cell lines, and measure growth responses of these cells in response to altered folate and metformin. Students will learn and perform real time PCR for various folate regulating enzymes. They will isolate RNA from these cells, perform reverse</td>
</tr>
</tbody>
</table>
transcriptions and determine if these the transcription of these enzymes are altered in the above treatment of the cells. Students will isolate protein from cells and perform Western Analyses. Students will measure folate content of their treated cells via a bacterial folate assay.

**Follow-Up:** Preparing to apply for medical school.

<table>
<thead>
<tr>
<th>Anna Edem-Etuk</th>
<th>A. Pratap Kumar, Ph.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Targeted approaches for prostate cancer management:</strong> Student will assist with literature review, identifying molecules associated with radiation sensitization/resistance.</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-Up:</strong> Submitted applications to various graduate schools (Medical Sciences and/or Public Health)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A.B. O’James, A</th>
<th>Ron Rodriguez, M.D., Ph.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The student will assist with cell culture and help carry out a set of experiments that will be looking at the effect of folate and common supplements people take on cell growth. In particular, the experiment will entail growing human prostate cancer cells in media containing different levels of folate, supplemented with different amounts of omega 3 fatty acid (DHA), and checking on cell growth/death by using a cell viability assay.</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-Up:</strong> Preparing for the MCAT, currently a senior at Huston Tillotson University</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Darrion Jemerson</th>
<th>David Morilak, Ph.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darrion Jemerson worked on a pre-clinical project using rats to study the mechanisms and a potential treatment for cognitive impairment induced by androgen deprivation therapy for prostate cancer. He worked with a graduate student and a Research Assistant in the lab, comparing castrated male rats as a model of androgen deprivation therapy and intact controls. Within these groups, half of each were treated with a novel antidepressant drug, vortioxetine, which has beneficial effects on cognitive impairment in depression. The drug was given in the diet, and controls received standard diet. They tested the performance of the rats on the Novel Object Location test, a measure of visuospatial cognitive function, one of the more consistent deficits seen in prostate cancer patients treated with ADT, which is mediated in the hippocampus. And they measured change in the electrical response evoked in the medial prefrontal cortex by stimulating in the hippocampus, a measure of the integrity of this important pathway that is</td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Advisor</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Bomaonye Sokari</td>
<td>A. Pratap Kumar, Ph.D.</td>
</tr>
<tr>
<td>Nangah Tabukum</td>
<td>Kexin Xu, Ph.D.</td>
</tr>
<tr>
<td>Tiarra Walker</td>
<td>Denise O'Keefe, Ph.D.</td>
</tr>
</tbody>
</table>
an in vitro model, as future therapies may be able to be designed targeting these changes.

Update: Current Junior at Huston-Tillotson University

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Robin J. Leach, Ph.D. Updates:

Elucidating the Effects of Obesity on Bladder Cancer Progression - completed

CTRC at UTHSCSA: Genomics Shared Resource; reduced from 1.2 cal months to 0.96 cal months

Texas Cancer Diagnostics Pipeline Consortium - completed

The Cancer Bioinformatics Initiative: A UTSA/UTHSCSA Partnership; reduced from 2.16 cal months to 0.6 cal months – no cost extension ending in August 2017.

NCI, R01, Improving prostate biopsy efficiency: The finasteride challenge test - completed

The San Antonio Center of Biomarkers of Risk for prostate cancer (SABOR); renewed, but new effort is 0.12 cal months

Cancer Therapy & Research Center – CURE Supplement; new award
NIH/NCI
$221,814
8/1/16-7/31/19
0 cal months (part of parent grant)
Funded through the Diversity Training Program of the National Cancer Institute aims to increase the number of underrepresented populations engaged in basic, translational and population-based biomedical cancer research by providing a rich training environment for both promising high school and undergraduate students who are currently students in the San Antonio area of South Texas.

Improving the detection of prostate cancer in men with low PSA; new award
UTHSCSA CTRC
$60,000
7/15/16-8/31/17
1.2 cal months
The goal of this current study is to evaluate 30 men with abnormal urinary biomarkers and normal PSA with imaging and offer them prostate biopsies.

MUSC Transdisciplinary Collaborative Center in Precision Medicine & Minority Men’s Health, U54MD010706; new award
Medical University of South Carolina/NCI
$ 354,745
7/08/16-3/31/21
0.6 cal months
The overarching goal of the Medical University of South Carolina (MUSC) Transdisciplinary Collaborative Center (TCC) in Precision Medicine for Minority Men's Health is to determine the most effective ways to integrate, interpret, and apply biological, social, psychological, and clinical determinants of disease risks and outcomes into more precise medical strategies to prevent, diagnose, and treat chronic health conditions and diseases.

Detecting prostate cancer in men with low PSA; new award
The William & Ella Owens Medical Research Foundation
$100,000
4/1/2017-3/31/2018
0.24 cal months
To improve the utility of PSA for the early detection of prostate cancer, we propose to develop a new assay that will detect the presence of this variant in serum from men who are being screened for prostate cancer.

The University of Texas Adult Clinical Center (UTACC) U01AR071150; new award
UT Medical Branch at Galveston
University of Texas Adult Clinical Center, NIH Molecular Transducers of Physical Activity Consortium (MoTrPAC)
$39,396
12/07/2016-11/30/2022
0.12 cal months
The purpose of the MoTrPAC is to catalyze the identification and mapping of molecular and cellular transducers underlying the physiological effects of physical activity.

Denise O’Keefe, Ph.D. Updates:

Oncogenic LINE-1 Retroelements Sustain Prostate Tumor Cells and Promote Metastatic Progression - completed

Folate and PSMA interact to regulate DNA methylation in the prostate; reduced from 4.8 cal months to 0.36 cal months, no cost extension – NCI – R01

An Interventional Study to Reduce Folate Levels in Men on Active Surveillance for Prostate Cancer; new award
UTHSCSA CTRC
$39,556
8/15/2016-8/14/2017
0.12 cal months

Novel Regulation and Oncogenic Mechanisms of Fatty-Acid Synthase (FASN) in Aggressive Prostate Cancer, W81XWH-17-1-0244; new award
DOD
$858,752
7/15/2017-7/14/2020
3.6 cal months
Our overall hypothesis is that dietary folate has a novel regulatory role in FASN expression and function through modulation of AMPK action and/or epigenetic modulation that is mediated by PSMA.

Teresa Johnson-Pais, Ph.D. Updates:
Texas Cancer Diagnostics Pipeline Consortium – completed

Prostate SPORE Pilot: Biobanking and Pathology; new award
UTHSCSA CTRC
$46,401
12/15/2016-12/14/2017
0.12 cal months

Recruiting and Retaining Underrepresented Students R25GM095480; new award
NIH
$374,215
5/1/17-4/30/2018
Here we propose a series of systematic interventions designed to sharpen critical thinking skills and develop grant and manuscript writing as well as presentation skills. Successful integration of these strategies will prepare IMSD scholars for the most competitive positions in the biomedical workforce.

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not applicable
QUAD CHARTS: Not applicable

9. APPENDICES:

Posters of all students included.
Immunohistochemistry (IHC) Assay on Mouse Mammary Tissue

Johnson Bobmanuel, Xiang Gu, Hakim Bouamar, Lu-Zhe Sun
The University of Texas Health Science Center at San Antonio

Introduction of IHC

- Immunohistochemistry (IHC) = Immunology + histology + chemistry.

- The main purpose of IHC is to use antibodies to detect protein expression on tissue samples.

- In order to achieve this goal, I will be using the IHC assay to detect the protein expression on mammary stem cells / tissues from mouse mammary gland.

- A mammary gland comprises of ducts with working units made of two layers, an inner layer called the Luminal cells and an outer layer called the [2] myoepithelial cells which is confined to a basement membrane called basal cell.

- It is evident today that scientists have discovered that “the very rare population of stem/progenitor cells within the tumor is present in various tissues [1] and some of these stem cells give rise to tumors.” Previous studies have shown that this population of stem cells are resistant to therapy because each time we try to kill a tumor, the tumor shrinks and grows back after a while.

- Recent discoveries in stem cell biology have shown that these “tumors contain self-renewal property [1] that stimulates tumorigenesis” and most often the stem cell initiating tumor are resistant to therapy.

antibody is K8 and the host is rabbit, therefore the secondary antibody will be biotin goat anti-rabbit.

- **Fixation:** Its function is to preserve the shape and localization of the protein. Some epitopes may not be altered over fixation. The tissue used for this experiment was fixed in Carnoy’s fixative solution overnight.

- **Sectioning:** Tissue section is recommended to be cut at 3-5um and placed in water bath of about 37 degree Celsius and allowed to dry for about two hours before commencing IHC.

- **Antigen retrieval:** This is a very crucial step which allows the site of the protein to be recognized by the antibody by unfolding. It does this by undoing the inter and intra-protein bridges that have been made by the fixative solution which unmasks and expose the epitope to the antibody.

- **Blocking:** This will help to neutralize the unspecific binding by mimicking the secondary antibody. The primary antibody will recognize the antigen from the goat serum instead of the unspecific proteins from the tissue.

How it works

![Diagram](image)

Figure 1. IHC staining results. A: Negative control. B: K8 Antibody stains the luminal layer, C: SMA Antibody stains the basal layer, D: K14 Antibody stains the basal layer.

Results

Conclusion

The above result was obtained after many attempts of a 2-day IHC protocol. The proteins were well expressed on the mouse mammary gland but the background stain was relatively high. The IHC protocol is crucial and tricky, it is a powerful tool and can be carried out at a much more lower cost. However, the pitfall for IHC is that we can stain just one protein at a time. To overcome this problem in the future, I would make use of the Immunofluorescence assay which is more sensitive and more than one antibody can be used at a time.

References


Methods

When doing IHC, there are certain parameters to put into consideration. They include;

- **Antibody selection (primary and secondary):** When choosing the antibody, one needs to look-up information about the host of the primary antibody from the manufacturer or datasheet which will further determine the choice of secondary antibody. It should not be randomly picked but carefully selected. For instance, the primary
The effect of metformin and folate on prostate cancer growth

Michael A. Esuruoso¹, Kyle S. Johnson², Wasim H. Chowdhury M.S.², Denise S. O’Keefe Ph.D.², Dean J. Baech Ph.D.²
¹Huston-Tillotson University, Austin, TX
²Department of Urology, The University of Texas Health Science Center at San Antonio, TX

INTRODUCTION

Prostate cancer is the most common form of cancer in American men and the second most common cause of cancer death (1). Metformin, an FDA approved oral drug mostly utilized in the treatment of type 2 diabetes to improve blood sugar levels in diabetic patients (2), recent studies have demonstrated that the use of metformin results in decreased incidence and improved outcomes for prostate cancer (3). There are ongoing clinical studies examining the effect of metformin in patients with advanced prostate cancer. However, the exact mechanisms by which metformin exerts its anti-tumor effects are not fully understood, with some reports indicating that it may be through its ability to activate AMPK and inhibition of mTOR (3). However, others have revealed that its anti-tumor effects are nucleotide metabolism and regulation of folate (4). In this experiment, we are looking at the effect of metformin and different forms of folate on prostate cancer growth and folate regulation.

RESULTS

We initially grew the cells in regular RPMI media, which contains 2.5 mM folate as its folate source, in the presence of 0, 1, and 5 mM Metformin for 3 days. We then examined the expression of folate transporters (FOLH1, PCTF), and reduced folate carrier (RFC), 3 molecules that are involved in cellular folate uptake.

Materials and Methods

Measurement of LNCaP Cell growth

In order to measure the growth of LNCaP cells, LNCaP cells were cultured in RPMI media. A 96-well assay was performed by plating cell numbers in 24 well plates with various concentrations of metformin (0, 2.5, 5.0, and 7.5 mM) and 0.5% fetal bovine serum (FBS) and 0.5% SMITH for a period of 7 days. During the duration of the experiment, triplicate samples from all different treatments were collected daily at each 24 hours from where the optical density was measured. The amount of absorbance measured via absorbance was measured in the M.Luc LNCaP cells in a graph based on the time points of the experiment. The graph was used to measure the growth response of the cells in response to altered folate and metformin.

RNA Extraction and Reverse Transcripase

Plated cells from all different concentration treatments were then harvested using TRIzol to extract RNA, which was subsequently quantified and utilized for cDNA synthesis via reverse transcription. Real time Polymerase chain reaction (PCR) was done for FOLH1, PCTF and RFC which are all folate regulating genes as well as GUSB which was the control gene in a normal housekeeping gene from all cDNA samples that were made from the different treatments.

Cells in the 2.5 mM SMITH were unable to be harvested for RNA extraction due to the majority of cells ceasing viability. The other cells still viable at the other concentrations on the 24 well plates were used to extract RNA and make cDNA for qPCR analysis at 7 days.

Conclusion

Based on the results obtained, it appears that Metformin treatment of LNCaP cells is correlated with a change in expression of genes involved with folate metabolism. Although Metformin treatment alone resulted in a decreased expression of FOLH1, PCTF, and RFC, when Metformin treatment was paired with varying levels of folate acid and 5SMITH an increase in expression of FOLH1 was observed, which could be explained by the different time points of 3 versus 7 days, but also indicating a need for further experiments. The lack of prediction of lincRNA in the LNCaP treated with high levels of 5SMITH indicate that a large proportion of cells died, which was not expected. Interestingly after the initial 48 hour of cell death, the cells that had survived in the higher concentrations began to grow at the same rate as the cells that were at lower concentrations of 5SMITH. Further experiments are necessary to determine that exact biochemical mechanisms occurring.

REFERENCES


Acknowledgements

This work is supported by the U.S. Army Medical Research Acquisition Activity W81XWH-16-1-0127.
RPS6KB1 inhibition sensitizes prostate cancer cells to chemotherapy?

Anna Edem-Etuk, Suleman S. Hussain, and Addanki P. Kumar

Departments of Urology, Pharmacology, Cancer Therapy and Research Center, The University of Texas Health Science Center at San Antonio; South Texas Veterans Health Care System

Introduction

- Prostate cancer (PCA) is the second leading cause of cancer-related death in U.S. men.\(^1\)
- Castration resistant prostate cancer (CRPC) has a mean survival of only 19 months.\(^2\)
- Docetaxel (Dox) & Enzalutamide (ENZ) are currently used for CRPC treatment.\(^3\)
- However, Dox and ENZ cause a minimal increase in survival (~5 months) and patients develop resistance.\(^4\)
- Hence, there is need to find novel CRPC treatments.
- PI3K/mTOR pathway is deregulated in 40% localized PCA patients and almost all CRPC patients.\(^5\)
- RPS6KB1 is downstream effector of mTOR pathway, which controls protein translation, cell proliferation, cell survival.\(^6\)
- Role of RPS6KB1 in PCA is not well studied.
- We tested if downregulation of RPS6KB1 sensitizes PCA cells to Dox and ENZ.

Results

1. **Fig. 1**: (A) Validation of RPS6KB1 knockdown at protein and RNA level in PC-3 (NTC, C1, C2) and LNCaP (NTC, 5M, 10M) PCA cells. (B) Phase-contrast images of PC-3 and LNCaP cell lines; stably silenced for RPS6KB1. (C) Anchorage-independent growth was measured in PC-3 (NTC, C1, C2) cells using soft agar assay. (D) Surviving fraction of PC-3 (NTC, C1, C2) and LNCaP (NTC, 5M, 10M) cells using colony formation assay.

2. **Fig. 2**: (A) LNCaP (NTC, 5M, 10M) and PC-3 (NTC, C1, C2) were treated with different doses of Dox for 72h and MTT assay was performed to measure cell viability. (B) LNCaP (NTC, 5M, 10M) and PC-3 (NTC, C1, C2) were treated with different doses of ENZ for 72h and MTT assay was performed to measure cell viability.

Conclusions

- RPS6KB1 does not affect cell morphology, but decreases both anchorage independent and dependent cell growth.
- RPS6KB1 inhibition sensitizes PC-3 and LNCaP cells to Dox treatment.
- RPS6KB1 inhibition does not affect ENZ sensitivity in PC-3 & LNCaP cells.
- PCA patients have higher expression of RPS6KB1.

References


Acknowledgements

Supported by U.S. Army Medical Research Acquisition Activity W81XWH-16-1-0217 and NCCAM (R01 AT007448) and VA-MERIT Award (101 BX 006766, APK).
Introduction

- 47-53% of prostate cancer patients who undergo Androgen Deprivation Therapy (ADT) exhibit significant cognitive impairment in areas such as memory, visuo-spatial ability, attention, and executive function.
- The medial prefrontal cortex (mPFC) is important to many of these cognitive processes. MRI studies indicate by proactivity in the mPFC, reduced gray matter volume, and reduced functional connectivity of the mPFC at rest in ADT patients compared to those who did not undergo this treatment.
- Also, clinical studies show that ADT produces the most prominent impairment in visuospatial cognition such as spatial recognition and memory. These domains are mediated by the hippocampus (Hpc).
- Vortioxetine, a novel multimodal antidepressant with characteristics similar to an SSRI and has a specific efficacy for the cognitive impairment seen in depression.

Methods

Animals and Drug Dose:
- Sprague Dawley male rats (intact & physically castrated) weighed 225-240g upon arrival.
- Rats were singly housed and received either the control or vortioxetine diet.
- Vortioxetine diet (0.6 g/kg corresponding to a dose of approx. 28 mg/kg/day) was administered for 17 days prior to testing. This included 10 days of free-feeding followed by seven days of food restriction (14 g/day).

AST (Attentional Set-Shifting Test):
- Rats must learn to associate a cheerful reward with positive stimuli: texture of the digging medium or the odor on the rim of the pot.
- 6 consecutive correct trials are required before moving on to the next phase.
- In the extra-dimensional set-shifting task, the positive stimuli becomes negative, requiring a shift in cognitive set, which is dependent on the mPFC.

NOL (Novel Object Recognition Test):
- Evaluates rodent’s ability to recognize that one of two familiar objects in the environment has changed location.
- Evaluate the interaction time with the object in the novel location versus the total interaction time with both objects.
- Discrimination Ratio (DR) = (Tn-TF)/(Tn+Tf).
- This task requires visuospatial recognition and memory, which are mediated by the Hpc.

Evoked Field Potential Recordings:
- Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and body temperature was maintained at 37°C for duration of procedure.
- A stainless steel stimulating electrode was placed in the right medial dorsal thalamicus (from bregma, DV: -5.4; AP: -2.5, ML: 1.0 mm) while a second stainless steel recording electrode was placed in the right mPFC (DV: -3.5, AP: +3.0, ML: 1.0 mm).
- Local field potentials were recorded in the mPFC after stimulation in the MDT.
- Data represented as a current-response curve by stimulating the MDT with 30 pulses (100-600 µA in 100 µA increments, 260-µ pulse width, 0.1 Hz).

Results

![Figure 1. Castrated male rats treated with control chow exhibited significantly higher number of trials to criterion on the set-shifting task than intact male rats treated with control chow (P<0.0001). Chronic vortioxetine treatment reversed the deficit in set-shifting performance in castrated male rats (P<0.0001). All data are presented as mean ± SEM, n=10-12 groups.](image1)

![Figure 2. Castrated male rats treated with control chow exhibited an attenuated electrophysiological response in the mPFC evoked by stimulating the excitatory afferent input from the MDT compared to intact rats treated with control diet. Chronic dietary vortioxetine normalized the evoked response of castrated male rats to a level comparable to that seen in intact control rats. All data are presented as mean ± SEM, n=4-6 per group.](image2)

![Figure 3. Castrated male rats treated with control chow exhibited an impairment in visuospatial memory as indicated by a lower discrimination ratio than intact male rats treated with control chow. Chronic dietary vortioxetine treatment reversed the deficit in castrated male rats. All data are presented as mean ± SEM, n=4-6 per group.](image3)

Conclusion

- Androgen deprivation by physical castration in rats produced a cognitive deficit in both AST and NOL, which models the human cognitive impairment seen after ADT.
- Also, vortioxetine treatment reversed the deficit in cognitive performance in castrated male rats, suggesting vortioxetine may be effective in treating cognitive impairment in ADT patients.
- Finally, androgen deprivation caused a decrease in evoked afferent response in the mPFC, while chronic vortioxetine treatment increased evoked response in the mPFC after stimulating in the MDT.

Future Directions

- Investigate changes in functional plasticity by stimulating excitatory afferents from the ventral Hpc and recording evoked field potentials in the mPFC.
- Use Golgi stain technique to assess the structural changes in the mPFC.
- Characterize the differences in gene expression, focusing on signaling pathways, transmitters, and modulators that are regulated by androgen that are important to cognition.
- Clinical trials are on-going to test the effects of vortioxetine on cognitive impairment in prostate cancer patients who undergo ADT.

Acknowledgements

This work is supported by pilot funds from the UT-HSA Cancer Therapy & Research Center and the U.S. Army Medical Research Acquisition Activity W81XWH-16-1-0217.
Common Supplements can augment the efficacy of Valproic Acid in treating Prostate Cancer


1Department of Urology, The University of Texas Health Science Center at San Antonio
2Huston-Tillotson University, Austin, TX
3James Buchanan Brady Urological Institute and Department of Urology, The Johns Hopkins School of Medicine
4Department of Medicine, The University of Texas Health Science Center at San Antonio

Abstract

Rising prostate specific antigen (PSA) levels in prostate cancer (CaP) patients treated with surgery or radiation is known as biochemical recurrence (BCR). BCR indicates disease relapse, which is often treated with androgen deprivation therapy (ADT). ADT has multiple morbidities which may compromise quality of life and there is controversy regarding early or late use of ADT for BCR. There is lack of a clear overall survival advantage with the use of immediate ADT, and alternative treatment options that could eliminate or delay ADT would be beneficial. Castrate-resistant prostate cancer (CRPC) is the lethal phenotype and therefore the target of current therapy. There have been numerous studies demonstrating the utility of histone deacetylase inhibitors (HDACi) in treating CRPC. We have studied Valproic acid, an HDACi, in treating CaP as well as bladder cancer in animal models. Docosahexaenoic acid (DHA) or omega-3 Fatty Acid (FA), is a primary structural component of the human brain, skin, semen, testicles and retina. Fish oil is a great source of DHA, and is a popular nutritional supplement. Even though there is controversy concerning the benefits of DHA, there are over two decades of research that show beneficial effects of DHA on bone health, as well as a chemosensitizing agent in treating cancer. Similar efficacy of cell kill by VPA can be achieved with half the dose when combined with DHA. This potentially can reduce the side effects associated with VPA treatment. Chronic treatment with DHA, VPA, and as well as the combination results in epigenetic changes in the treated cells. Folate, which provides the methyl group in DNA methylation, also enhanced cell kill when combined with DHA & VPA.

Results

The following table shows the effects of VPA and DHA on LNCaP cells treated for 72 hours:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Cell Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>No VPA</td>
<td>100%</td>
</tr>
<tr>
<td>0.3 mM VPA</td>
<td>90%</td>
</tr>
<tr>
<td>0.6 mM VPA</td>
<td>80%</td>
</tr>
<tr>
<td>0.3 mM DHA</td>
<td>50%</td>
</tr>
<tr>
<td>0.6 mM DHA</td>
<td>25%</td>
</tr>
<tr>
<td>VPA + DHA</td>
<td>5%</td>
</tr>
</tbody>
</table>

Figure 1: Demographics of the participants in a Randomized, Controlled Phase II Study of Vatproic Acid in Patients with Non-Metastatic Biochemical Progression of CaP.

Acknowledgements

U.S. Army Medical Research Acquisition Activity Grant W81XWH-15-1-0211

Conclusion

- Combining DHA with VPA can reduce the dose of VPA required for cell kill.
- The combination of a common dietary supplement such as DHA has the potential to reduce the side effects of VPA, as a reduced dose could be used to treat patients.
- DHA, VPA, and the combination treatment resulted in changes in methylation in the central elements of genes involved in tumorigenesis, tumor progression, differentiation, metabolism, signal transduction.
- The addition of Folic Acid, another common dietary supplement further enhances the cell kill by VPA.
- Patients need to be advised on effects of dietary supplements, as they can positively (as seen here) or even potentially negatively affect the efficacy of certain drugs.

Figure 2: VPA treatment resulted in the increase in PSA doubling time in Prostate cancer patients with biochemical recurrence. The treatment also resulted in a loss in quality of life.

Figure 3: LNCaP cells were plated in 96 well plates and treated with DHA, VPA, and a combination (25 μM DHA + 0.3 mM VPA) for 72 hours. The addition of 25 μM DHA was able to reduce the requirement of VPA by almost half the dose.

Figure 4: NuNu mice with sub-cue LNCaP xenografts were fed a defined diet containing DHA or Sunflower oil, and provided with 0.2% VPA in drinking water. The rate of tumor growth was reduced to 20.3 mm3/day in the combination group as compared to the VPA (34.0 mm3/day), DHA (41.8 mm3/day), and no treatment (97.5 mm3/day).

Figure 5: LNCap-M.LUC cells were plated in three 24 well plates, in increasing concentrations of folinic acid (0 nM, 50 nM, 2.3 μM), and treated with DHA, VPA and a combination (25 μM DHA + 0.3 mM VPA). The combination, in the presence of high folinic acid, had the largest cell kill compared to the cells treated with the other drug conditions.

Figure 6: LNCap-M.Luc cells were grown in RPMI1640 (2.3 μM Folic Acid) or in RPMI containing 50 nM Folic Acid for 4 weeks before repeating the experiment described in figure 5. The effect of high folinic acid on the increased cell kill is diminished when the cells are grown in physiological levels of folinic acid before conducting the experiment.

Changes in Methylation upon chronic treatment with DHA, VPA or a combination:

LNCAp cells were grown in RPMI-1640 with 10% FBS containing 10 nM DHA, 0.3 mM VPA, or a combination of the two drugs for 110 days. The genomic DNA was isolated from these cells and changes in methylation were analyzed using the Illumina MethylationEpiKit.

Some of the genes that are differentially methylated in both the DHA and VPA treatment but further enhanced in the combination are: MAPK10, PDGFD, FAM110B, FMRN2, GPM6A, UNC93B, ADCY8, LTBPI.

Some of the genes that are Differentially methylated at a much higher rate in the combination treatment are: MAPK10, STAT3, FHT, PDE1A, CFT, LIM1, STAT3, RABGGT8, MIR21, FPR3.
Does AMPK activates AR in human prostate cell line?

Bomaonye Sokari, Saketh Amasa, Ratnesh Srivastava and A. Pratap Kumar.
The University of Texas Health Science Center at San Antonio

Introduction

- Prostate cancer is the second most leading cause of cancer related deaths amongst American men.
- AMPK is a stress sensor that is expressed in all the cells. It consists of three subunits (alpha-catalytic, beta and gamma- regulatory subunits).
- AMPK is activated in PCA due to hypoxia and metabolic stress found in tumor cells. Activation of AMPK restores cellular energy balance by promoting ATP generating processes which helps in cell survival.
- The role of AMPK in context dependent and its role in PCA is not clear.
- Objective is to test whether AMPK promotes or inhibits PCA cell growth.

Results

- AMPK knockdown was confirmed in LNCaP cells at mRNA and Protein level.
- Similar level of knock-down shows differential biological outcome. In clonogenic assay, CL1 shows fewer number of colonies with no significant effect on CL2.
- Stress causing agents including androgen deprivation may help knock-down cells to proliferate more.
- AMPK knock-down makes cells more sensitive to AR antagonist, Enzalutamide and chemotherapeutic agent Doxetaxel.
- Sensitivity to the drugs depends on the amount of AMPK present in the cells.

Conclusion

- AMPK knockdown was confirmed in LNCaP cells at mRNA and Protein level.
- Similar level of knock-down shows differential biological outcome. In clonogenic assay, CL1 shows fewer number of colonies with no significant effect on CL2.
- Stress causing agents including androgen deprivation may help knock-down cells to proliferate more.
- AMPK knock-down makes cells more sensitive to AR antagonist, Enzalutamide and chemotherapeutic agent Doxetaxel.
- Sensitivity to the drugs depends on the amount of AMPK present in the cells.

References


Acknowledgements

This work is supported by the U.S Army Medical Research Acquisition Activity W81XWH-16-1-0217. Supported in part by CPRIT (RP130166APK).
Plasmid Construction- A way to study the role of ATAD2
Nangah Tabukum, Yanning Wu, Kexin Xu, PhD.
The University of Texas Health Science Center at San Antonio

Introduction
- Androgen receptor (AR) plays a pivotal role in prostate cancer, primarily by regulating different gene programs elicited by androgens, which is important for cancer cell proliferation, survival, and differentiation.
- ATAD2, a new member of the AAA+ ATPase family proteins, as a novel AR coactivator, ATAD2 interacts directly with AR and enhances its transcriptional activity, and is required for androgen-simulated expression of a specific subgroup of genes.
- Although ATAD2 is hardly detected in normal human prostate tissue, high levels of ATAD2 are found in hormone-independent prostate cancer cell lines, xenograft tumor, and a subset of prostate cancers with high Gleason scores. These findings suggest that ATAD2 plays an important role in prostate cancer by mediating specific AR functions in cancer cell survival and proliferation (1).

Steps
1. Choose the vector and analyze its restriction sites
2. Preparation of interest DNA to be cloned
   - Amplify template DNA using PCR (M0405)
   - Target size = 4kb; confirm thru gel
3. Purification of the PCR products
   - Follow protocol QiAquick PCR Purification Kit Using a Microcentrifuge (4kb target size)
   - Confirm size, check for non-specific bands and concentration (185ng/μl)
4. Use Restriction Enzyme to digest cloning vector and PCR products
   - Tube #1 (vector): 5ul + 1.5ul NdeI + 5ul buffer 3.1 + 38.5 1×ED
   - Tube #2 (ATAD2): 5.4ul + 1.5ul NdeI + 5ul buffer 3.1 + 38.1 H2O
   - Leave in 37°C overnight
5. Dephosphorylate Vector
   - To reduce chance of self ligation
   - Add 3ul of Rsnp to Vector
   - Store in 37°C for 30 minutes
6. Ligation with T4 DNA ligase (M0202)
   - Vector 12.5ul
   - ATAD2 8ul
   - T4 DNA ligase buffer 2ul
   - Ligase 1ul
   - water -

Purpose
To construct a plasmid containing Flag-HA and ATAD2 in order to find out and further analyze the role it plays in cell regards to cell survival and proliferation in prostate cancer.

Flowchart
Construct Plasmid → Generate Virus ATAD2-Flag-HA → Infect target cells → coinmunprecipitate HA-tag and Flag→tag

Materials and Methods
Backbone- Flag-HA
Insert- ATAD2
Primers- ATAD2-F, ATAD2-R
Protocols
- Ligation Protocol with T4 DNA Ligase (M0202)
- PCR Using Q5 Hot Start High-Fidelity DNA Polymerase (M0492)
- QiAquick PCR Purification Kit using a Microcentrifuge
- Plasmid DNA Purification Using the QiAprep Spin Miniprep Kit and a Microcentrifuge
- Midi-Prep (Boyder Lab)
- Gene Subcloning Procedure

References

Acknowledgements
This work is supported by the U.S. Army Medical Research Acquisition Activity W81XWH-16-1-0217.
4 R08 CA178199 from NHT/NCI
RR140972 from CPRIT
PC160180 from DOD PCRP
Regulation of LIN7A by Methylation in Prostate Cancer Cell Lines
Tiarra Walker, Keith Ashcraft Ph.D., Desiree Wilson Ph.D., Robin Leach Ph.D., Denise O’Keefe, Ph.D.
The University of Texas Health Science Center at San Antonio

Introduction
Prostate Cancer (PCa) is the second most common cancer in American men. It is now estimated that there will be approximately 161,360 new cases of PCa and about 26,730 deaths from PCa this year alone. Around one in seven men develop PCa during their lifetime. PCa is more common in older men, with an average age of 65 at time of diagnosis. In efforts to reduce PCa mortality, Prostate Specific Antigens (PSA) screenings have been utilized to detect prostate cancer prior to symptomatic disease. However, this has caused a concern of over treatment in low grade cancers. Biomarkers have become an essential tool in PCa by helping to create a more personalized approach for patients, and identifying whether the disease will become more aggressive over time. A novel area of research for PCa biomarker is DNA hypermethylation. DNA methylation (a process of adding a methyl group to cytosines within the genome) is an epigenetic alteration which can impact gene expression. Regions that are differentially methylated in tumors with different outcomes can be useful as potential biomarkers for aggressive prostate cancer. This study aims to identify regions that are differentially methylated between PCa patients with good outcomes and those with poor outcome. Additionally, we plan to confirm the methylation-dependent regulation of genes associated with these DNA regions in PCa cell lines.

Abstract
Previous data was generated from a genome-wide methylation array comparing primary prostatectomy on a group of patients with a minimum of 5 year clinical outcome data. They were distinguished either as No Evidence of Disease recurrence (NED) or Metastasis (MET). Using this data we identified 3 gene regions that were hyper-methylated in METS, (CDCA7L, LIN7A, PDZD4). These genes were tested to see if they are regulated by methylation using PCa cell lines treated with decitabine (methyltransferase inhibitor) and measuring gene expression through qPCR. PCa and LNCaP prostate cancer cell lines were treated with decitabine and DNA and RNA were then isolated and analyzed to assess the conversion of bisulfite DNA for High Resolution Methylation (HRM) and cDNA for Reverse transcription. The LNCaP proved to have little to no change between the vehicle and treated for all three genes. There was a slight change in the genes CDCA7L and PDZD4 for PC3. LIN7A demonstrated a four fold increase after treatment, suggesting that LIN7A is regulated by methylation in these cells. HRM analysis showed no evidence of methylation in the CDCA7L region but the primers for LIN7A were inconclusive. Using MxPlex, it was found that methylation of this LIN7A region correlated significantly with expression in prostate tumors in the Texas Cancer Genome Atlas data (TCGA).

Materials and Methods
Decitabine Treatment: 10nM PC3 or LNCaP cells were harvested in T25s and treated with 5nM of decitabine for 7 days. Media and drug were refreshed daily. RNA and DNA were isolated for gene expression and methylation analysis.

Gene Expression: 1ug of isolated RNA was converted to cDNA using Applied Biosystems Reverse Transcription Kit. Gene expression was then performed using primers for CDCA7L, LIN7A, and PDZD4. U61941 was used as reference gene. qPCR was performed in a LightCycler 96 (Roche).

Bisulfite Conversion: Bisulfite conversion was used on the DNA isolated using the EZ DNA methylation kit to obtain bisulfite converted DNA (BS-DNA).

High Resolution Melting (HRM): BS-DNA was used to test methylation with BS Specific or methylation specific primers. Melt curves for vehicle and treated DNA were identified and compared to controls of 0% methylated DNA, 100% methylated, and a 1:1 combination of 0 and 100% Methylated DNA is expected to have a higher melt peak due to the higher GC content of the product after bisulfite conversion.

Results

![Figure 1. Primers Designed against regions from previous data from methylation array.](image1)

![Figure 2. Gene expression after 5nM decitabine treatment in PC3 and LNCaP.](image2)

![Figure 3. No Significant change in methylation of CDCA7L region as shown by High Resolution Melt with Bisulfite converted DNA.](image3)

![Figure 4. Mecpsx Data from the Texas Cancer Genome Atlas.](image4)

Conclusion
1. We found three gene regions hyper-methylated in the primary tumor of patients that later progressed to metastatic disease.
2. LIN7A showed a significant change in gene expression between PC3 vehicle and PC3 decitabine treatment.
3. LIN7A methylation correlates with expression in prostate tumors according to TCGA.
4. LIN7A methylation represents a potential biomarker as well as a potential pathway of intervention for treatment.
5. CDCA7L and PDZD4 also represent biomarkers but do not appear to be regulated by methylation in PCa cell lines.

References

Acknowledgements
This work is supported by the US Army Medical Research Acquisition Activity W81XWH-15-1-0217.