AWARD NUMBER: W81XWH-14-1-0312

TITLE: The Role of NFIB in Prostate Cancer Progression

PRINCIPAL INVESTIGATOR: Magdalena Grabowska

CONTRACTING ORGANIZATION: Vanderbilt University
Nashville, TN 37240

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Nuclear factor I/B (NFIB) is a co-factor of androgen receptor (AR) and FOXA1. Previous studies in the laboratory have suggested a role for NFIB in controlling neuroendocrine differentiation (loss of AR expression, gain of neuroendocrine markers like synaptophysin) during prostate cancer progression. Knockdown of NFIB expression in LNCaP and C4-2B prostate cancer cell lines results in increased AR message and protein levels, but synaptophysin levels were not affected. 22RV1 cells, which are not reported to undergo neuroendocrine differentiation, expressed synaptophysin and AR at an increased level in response to NFIB over-expression. 22RV1-NFIB cells were also more proliferative than their 22RV1-vector controls in both serum-containing and serum-free media. These studies are defining a role for NFIB regulation of AR and synaptophysin in prostate cancer in vitro and suggest a role for NFIB during prostate cancer progression in vivo.

Prostate cancer, therapy acquired neuroendocrine prostate cancer, neuroendocrine prostate cancer, androgen receptor, NFIB
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1. INTRODUCTION:
Castrate resistant prostate cancer (CRPCa) maintains androgen receptor (AR) signaling but is no longer responsive to androgen deprivation therapy (ADT). This resistance is driven by multiple factors, including the expression of AR splice variants (AR-V) and the progression of neuroendocrine differentiation (NED) to therapy acquired neuroendocrine prostate cancer (NEPCa). However, new therapies continue to target the AR ligand binding domain, which is missing in AR-V, or altogether absent in therapy (t) induced NEPCa (tNEPCa). Our strategy, therefore, has focused on targeting AR via its co-factors, such as FOXA1 and the Nuclear Factor I family members (NFIA, NFIB, NFIC, and NFIX). We postulate that these transcription factors will have functions dependent on AR interaction, as well as independent functions. Our work and the work of others have demonstrated that neuroendocrine (NE) tumors can support castrate-resistant growth of prostate adenocarcinomas and that NED occurs frequently in CRPCa. Based on preliminary data, we believe that the transcription factor NFIB is a likely candidate for controlling AR expression and NED. It is possible, therefore, that patients who maintain high levels of NFIB will be more likely to undergo NED and fail ADT. The purpose of these studies has been two-fold. First, by analyzing clinical samples, we seek to determine whether NFIB expression in human PCa samples can predict biochemical recurrence. Secondly, by exploring the role of NFIB in driving NED in PCa cell lines in vitro and in vivo, we will define a mechanistic role for NFIB in driving NED. Successful completion of these studies may provide a novel biomarker of aggressive PCa, as well as define a role for NFIB in therapeutic failure.

2. KEYWORDS:
Prostate cancer, therapy acquired neuroendocrine prostate cancer, neuroendocrine prostate cancer, androgen receptor, NFIB

3. ACCOMPLISHMENTS:
- What were the major goals of the project?
The major goals of this project were to further my training in prostate cancer research, determine whether NFIB expression was a predictor of early biochemical recurrence in prostate cancer patients, as well as explore how NFIB could drive NED and support castrate resistant growth through neuroendocrine secretions. These goals were to be achieved through the following approved statement of work:

**Training-Specific Tasks:**

<table>
<thead>
<tr>
<th>Major Task 1: Training and educational development in prostate cancer research (only applicable to training award mechanisms)</th>
<th>Month</th>
<th>VUMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtask 1: Weekly meetings with mentors</td>
<td>1-24</td>
<td>Dr. Grabowska</td>
</tr>
<tr>
<td>Subtask 2: Present research at biweekly lab meetings and VUMC Prostate Cancer Center (VPCC) department group meetings</td>
<td>1-24</td>
<td>Dr. Grabowska</td>
</tr>
<tr>
<td>Subtask 3: Attend Translational Seminar Bridge Series and Urologic Oncology meetings</td>
<td>1-24</td>
<td>Dr. Grabowska</td>
</tr>
<tr>
<td>Subtask 4: Attend Society of Basic Urologic Research (SBUR), American Urologic Association (AUA), and IMPACT meetings.</td>
<td>1-24</td>
<td>Dr. Grabowska</td>
</tr>
</tbody>
</table>

*Milestone(s) Achieved: Presentation of project data at a national meeting, submission of manuscripts, preparation of K99/R00 grant application on basis of studies*
### Research-Specific Tasks:

**Specific Aim 1:** Examine if NFIB expression predicts aggressive disease by supporting failure of ADT via NED.

<table>
<thead>
<tr>
<th>Major Task 1: Determine whether NFIB correlates with aggressive disease, NED, and poor clinical outcome in human PCa patients.</th>
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<tbody>
<tr>
<td>Subtask 1: Obtain exempt IRB approval (Months 0-3)</td>
</tr>
<tr>
<td>Subtask 2: Patient review by Dr. Clark and request appropriate de-identified slides from Pathology</td>
</tr>
<tr>
<td>Subtask 3: Perform immunohistochemistry for NFIB and have it scored (Dr. Justin Cates, Pathology)</td>
</tr>
<tr>
<td>Subtask 4: With assistance from Dr. Clark, co-mentor, determine whether NFIB expression predicts NED and failure of therapy.</td>
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**Major Task 2:** Generate and characterize non-targeting shNT(control), shNFIB, and NFIB over-expressing cell lines

<table>
<thead>
<tr>
<th>Subtask 1: Transduce cell lines with non-targeting (shNT), shNFIB, PCH (vector) or NFIB constructs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory]</td>
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</table>

<table>
<thead>
<tr>
<th>Subtask 2: Examine cell lines generated in Aim 1b (subtask 1) for changes in AR, ARvs, NFIB, and markers of NED (SYN, CHGA, NSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory]</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subtask 3: Determine whether LNCaP and C4-2B cells generated in Aim 1b can no longer undergo NED in the absence of NFIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines used: LNCaP [AATC] and C4-2B [no longer commercially available, but established in laboratory]</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Subtask 4: Determine whether 22RV1 cells generated in Aim 1b gain the ability to undergo NED with NFIB over-expression as compared to EV cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines used: 22RV1 [AATC]</td>
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<tr>
<th>Subtask 5: If knockdown of NFIB in PC-3 or DU145 cells generated in Aim 1b restored AR expression, perform ChIP-seq to determine AR and NFIB binding sites/target genes</th>
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<tbody>
<tr>
<td>6-9 Dr. Grabowska</td>
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<tr>
<th>Subtask 6: Examine the consequence of NFIB loss in response to androgens (DHT) and anti-androgens (bicalutamide [Bic] and enzalutamide [Enz]) in cell lines generated in Aim 1b.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no</td>
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<tr>
<td>Specific Aim 2: Examine the role of NFIB during ADT response in vivo.</td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>Major Task 1: Perform xenograft experiments with the cell lines generated in Task 2</strong></td>
</tr>
<tr>
<td>Subtask 1: Generate twelve grafts per cell line established in Aim 1b. After three weeks, castrate half of the animals (proxy for ADT) for 2 weeks.</td>
</tr>
<tr>
<td>Cell lines used: LNCaP, 22RV1, PC-3, and DU145 [AATC]; C4-2B [no longer commercially available, but established in laboratory]</td>
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<tr>
<td>Mice: 12 mice per group, 5 cell lines, 2 groups [120 total]</td>
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<tr>
<td>Subtask 2: Stain grafts by immunohistochemistry (IHC) for NFIB, luminal markers (AR, CK8/18, PSA), basal markers (p63, CK14), and NED markers (SYN, CHGA, NSE)</td>
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<tr>
<td><strong>Major Task 2: Generate and characterize NE-10 shNFIB and non-targeting shNT cell lines</strong></td>
</tr>
<tr>
<td>Subtask 1: Utilizing lentiviral transduction, generate shNFIB and shNT NE-10 cell lines.</td>
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<tr>
<td>Cell lines: mouse neuroendocrine PCa, NE-10 [Matusik laboratory]</td>
</tr>
<tr>
<td>Subtask 2: Characterize the shNFIB and shNT NE-10 cell lines for global changes mediated by NFIB via RNA-seq</td>
</tr>
<tr>
<td>Cell lines: mouse neuroendocrine PCa, NE-10 [Matusik laboratory]</td>
</tr>
<tr>
<td><strong>Major Task 3: Establish dual xenografts with NE-10 and LNCaP cells</strong></td>
</tr>
<tr>
<td>Subtask 1: Athymic nude mice will host the following combinations of subcutaneous xenografts: NE-10 shNFIB + LNCaP shNT; NE-10 shNT + LNCaP shNT; NE-10 shNFIB + LNCaP shNFIB; NE-10 shNT + LNCaP shNFIB. Once tumors are established, animals will be castrated as a proxy for ADT</td>
</tr>
<tr>
<td>Cell lines: LNCaP [AATC], mouse neuroendocrine PCa, NE-10 [Matusik laboratory]</td>
</tr>
<tr>
<td>Mice: 12 mice per group, 4 groups, groups [48 mice total]</td>
</tr>
<tr>
<td>Subtask 2: Analyze tumors for tumor volume, mitotic index, invasion, and markers of NED (SYN, CHGA, NES)</td>
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</table>

**Milestone(s) Achieved:** Characterize the role of NFIB during NED in vivo, publication of manuscripts

**What was accomplished under these goals?**
THIS SECTION CONTAINS UNPUBLISHED DATA

Training Tasks

Major activity/task 1: Training and educational development in prostate cancer research

Objective: The objective of this first task is to expand my training and education in prostate cancer research through meetings with my mentors (Drs. Matusik and Clark), departmental meetings, and attending national conferences.

Results/achievements: During the reporting period, I met with Dr. Matusik (primary mentor) at least once a week and with Dr. Clark (clinical co-mentor) monthly. I attended the monthly Translational Bridge Seminar Series and the bi-monthly Urologic Oncology Conference. I also attended annual meeting of the SBUR in Dallas, TX. In addition to the outlined tasks, I was also invited and attended the American Association for Cancer Research (AACR) Translational Cancer Research for Basic Scientists Workshop.

Research Tasks

Specific Aim 1

Major activity/task 1: Determine whether NFIB correlates with aggressive disease, NED, and poor clinical outcome in human PCa patients.

Objective: The objective of this first task of the research tasks is to examine human prostate cancer samples and determine whether NFIB expression predicts poor clinical outcome and if NFIB is associated with NED.

Results/achievements: This aim is still in progress and will be completed after the termination of the DOD Postdoctoral Fellowship. Thus far, I have generated the data collection sheet (See Appendices) and have received Institutional Review Board (IRB) approval. I have also developed a database within the RedCap systems to store the data collection sheets behind a password protected firewall and without identifiable information. I have been slowly navigating the excellent Vanderbilt University Medical Center (VUMC) resources, such as the biostatistics clinic to determine the optimal study design and patient number, as well the Vanderbilt Institute for Clinical and Translational Research (VICTR) for funding. Our initial study design, which proposed to look at prostate cancer tumors (1 case/slide) proved cost and effort-prohibitive, and we have transitioned into generating a tissue microarray so that we can evaluate multiple patients per slide. By working with the Vanderbilt Biostatistics clinic, we have determined that the minimal requirement for the microarray will be 69 patients in each arm of a matched case-control study. This number of patients falls well within the resources of the Vanderbilt Urologic Outcomes Database.

Also, during the tissue microarray design stage, I worked with the VUMC Translational Pathology Shared Resource Core to obtain an estimate for the cost of generating the microarray, which I hoped to fund through the VCTR resource. However, because the prostate cancer samples of interest are heavily sought after and each sample must be reviewed by a pathologist, we have opted not to duplicate effort with other investigators at Vanderbilt. As such, we are working with Dr. Pietenpol, the Director of the Vanderbilt-Ingram Cancer Center, who is currently generating a tissue microarray that will satisfy the requirements for our study. The tissue microarray has been designed as a nested case control study with clinical data derived from the Urologic Outcomes database from which Dr. Clark and I were working. In the cohort, half of the patients have had a biochemical recurrence, while the other half has not. Patients were matched on multiple factors, including stage, grade, and surgical margin status. Each patient has two cores represented in the tissue microarray. We are currently waiting for the completion of a tissue microarray to finish our studies.

Major activity/task 2: Generate and characterize non-targeting shNT(control), shNFIB, and NFIB over-expressing cell lines.

Objective: The objective of this aim was to generate NFIB knockdown cell lines (LNCaP, C4-2B, PC3, and DU145) and determine the consequences of NFIB loss. For LNCaP and C4-2B cells, we hypothesized that loss of NFIB would prevent NED (loss of AR, gain of synaptophysin), alter the response to DHT and sensitivity to bicalutamide, an anti-androgen. Similarly, 22RV1 cells, which do not undergo NED in response to ADT, were
used to determine if NFIB over-expression was sufficient to induce NED. PC-3 and DU145 cells are AR negative, and were used to determine if loss of NFIB was sufficient to re-express AR protein expression.

Results/achievements:

Cell line generation:
Five prostate cancer cell lines were used in the studies. LNCaP and its sub-line C4-2B both express AR and can undergo NED\(^1\). These cells were used to determine whether NFIB is required to undergo NED. Like C4-2B cells, 22RV1 cells express both AR and AR-V\(^2\), and are castrate resistant, but they do not undergo NED in charcoal stripped serum conditions\(^1\). These cells were utilized to determine if NFIB alone is sufficient to induce NED. Finally, PC-3 and DU145 cells lack AR, are classified as androgen independent, and do not undergo NED in charcoal stripped serum\(^1\). These cells were used to determine whether ablation of NFIB was sufficient to induce re-expression of AR.

For these studies, a Myc tagged- NFIB over-expression construct (NFIB-Myc, RC501590) and short hairpin RNA constructs targeting NFIB (shNFIB-GFP, TG311191) were purchased from Origene (Rockville, MD). The following cell lines have been generated through transfections with lipofectamine 2000 and selection with media supplemented with antibiotic (puromycin for knockdown cell lines and neomycin for over-expressing cell line):

1. 22RV1: 22RV1-Vector, 22RV1-NFIB
2. LNCaP: LNCaP-shScramble (control), LNCaP-shNFIB #1, LNCaP-shNFIB #2, LNCaP-shNFIB #3, LNCaP-shNFIB #4
3. C4-2B: C4-2B-shScramble (control), C4-2B-shNFIB #1, C4-2B-shNFIB #2, C4-2B-shNFIB #3, C4-2B-shNFIB #4
4. PC3: PC3-shScramble (control), PC3-shNFIB #1, PC3-shNFIB #2, PC3-shNFIB #3, PC3-shNFIB #4
5. DU145: DU145-shScramble (control), DU145-shNFIB #1, DU145-shNFIB #2, DU145-shNFIB #3, DU145-shNFIB #4

Figure 1: NFIB is not sufficient to prevent NED in LNCaP cells. Expression of AR, PSA (AR target gene), and neuronal markers (SYP, ENO2) in response to transient knockdown of NFIB under serum-free conditions. NT: non-targeting.

Figure 2: NFIB is frequently associated with AR and FOXA1 binding sites. A. Venn diagram of Chromatin immunoprecipitation then sequencing (ChIP-Seq) overlapping sites for AR, FOXA1, and NFIB. B. Breakdown of transcription factors associated with AR/FOXA1 peaks. C. Breakdown of transcription factors associated with NFIB peaks. D. Differentially regulated genes bound by transcription factor sets. E. Percentage of differentially regulated genes bound by transcription factor sets.
While cell lines were being established and characterized, preliminary studies were conducted using transient transfections of a previously published short-interfering RNA (siRNA) for NFIB. NFIB is not sufficient to prevent NED:

LNCaP cells, which can undergo NED in response to serum withdrawal, were transiently transfected with non-targeting siRNA or NFIB targeting siRNA (siNT, siNFIB) and placed in phenol-red free, serum-free media for three days (Figure 1). Cells were then collected, RNA was extracted, and quantitative real time PCR (qRT-PCR) was performed for markers of NED. NED is characterized by the loss of AR and AR-target gene expression (prostate specific antigen [PSA] with a concomitant gain of neuronal marker expression (synaptophysin [SYP], neuronal specific enolase [ENO2]). We hypothesized that loss of NFIB would prevent LNCaP cells from undergoing NED, mainly cells would maintain expression of AR and lose expression of SYP and ENO2. Analysis of transiently transfected LNCaP cells revealed that while loss of NFIB did increase AR and PSA expression (Figure 1A, B), it was not sufficient to prevent expression of SYP or ENO2, suggesting that while NFIB can regulate the expression of AR and AR-target genes, its downregulation is not sufficient to prevent the expression of genes associated with NED.

Figure 3: NFIB represses AR-target gene expression. Expression of PSA (A), TMPRSS2 (B), FKBP5 (C), and NKPX3-1 (D) in response to DHT and NFIB knockdown. NT: non-targeting. * P< 0.05, ** P < 0.01

NFIB modulates AR and AR-target gene expression in response to DHT

Transient transfection experiments in LNCaP cells also explored the role of NFIB in controlling AR/FOXA1/NFIB target genes. Our previous studies have demonstrated that while NFIB plays a largely repressive role in AR-target gene expression under serum-containing conditions. More recent chromatin immunoprecipitation then DNA sequencing (ChIP-Seq) studies has explored how frequently NFIB binding sites are associated AR/FOXA1 binding sites (Figure 2A, B, C). I observed that AR/FOXA1/NFIB binding sites are largely associated with genes whose expression increases following DHT exposure (Figure 2D, E). However, when NFIB is knocked down, the gene expression of these genes (TMPRSS2, FKBP5) increases even further, suggesting that the role of NFIB in this complex may serve as a brake to limit transcription (Figure 3A, B, C, and D). Interestingly, NFIB can also repress AR and AR-V7 message (Figure 4), suggesting that NFIB regulates AR expression under both serum-free and serum-containing conditions.

Figure 4: NFIB controls AR and AR-V7 expression. Transient knockdown of NFIB in LNCaP cells under serum containing conditions results in increase AR and AR-V7 message. * P < 0.05
In preliminary studies utilizing the stable knockdown cell lines, this observation holds true, and knockdown of NFIB in LNCaP cells results in increased expression of AR (Figure 5A). However, the situation becomes more complex in the castrate resistant cell lines. In C4-2B cells, knockdown of NFIB has a variable response, with a loss of AR-V expression across all knockdown clones, but full length AR expression is variable (Figure 5A). In 22Rv1 cells, which are castrate resistant but are not reported to undergo NED, over-expression of NFIB appears to increase full length AR and AR-V expression. Interestingly, AR expression increases under serum-free conditions, as does SYP expression in 22Rv1 (Figure 5B). This observation suggests that NFIB over-expression in 22Rv1 can induce both AR and SYP under serum-free conditions. This finding is quite unexpected, and subsequent studies are evaluating whether these changes in AR and SYP are occurring in the same or whether NFIB over-expression has generated a heterogeneous population. It should be noted that while knockdown of NFIB is sufficient to induce an increase in AR gene expression in PC-3 and DU145 cells it is not sufficient to re-express AR protein (data not shown), suggesting that the loss of AR expression in androgen independent cells is a multi-

NFIB over-expression drives increased androgen-independent proliferation:

Proliferation studies utilizing our 22Rv1-NFIB and vector control cells have demonstrated that 22Rv1-NFIB cells are more proliferative than their vector control counterparts under serum-containing and serum-free conditions (Figure 6A, B). Notably, NFIB over-expression in cells resulted in a significant increase in growth rate in the presence of DHT (Figure 6C), likely due to the increased levels of AR. Expression of NFIB, however, did not sensitize the cells to anti-androgen (Bicalutamide, Figure 6D).

Figure 5: NFIB modulates AR expression. A. Western blot characterization of LNCaP and C4-2B NFIB-knockdown cells for AR expression. B. Western blot analysis of 22Rv1 cells over-expressing NFIB in response to one week of serum-free media.

Figure 6: NFIB supports increased proliferation of 22Rv1 cells. A. Proliferation assays comparing NFIB and vector control cell proliferation in serum (A), serum-free (B), DHT-supplemented (C), and bicalutamide (anti-androgen)-supplemented media (D). ** P<0.01, *** P < 0.001
Specific Aim 2
Outside of the scope of the reporting period.

- **What opportunities for training and professional development has the project provided?**
  Training and professional development has been described in Training Tasks above.

- **How were the results disseminated to communities of interest?**
  Some of the results have been reported in a manuscript (under revision for Endocrinology). The large data set generated by the ChIP-Seq analysis has been deposited into NCBI so that other investigators may interrogate the data set.

- **What do you plan to do during the next reporting period to accomplish the goals?**
  This award is being relinquished due to the awarding of a NCI K99/R00.

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     This project will have an impact on PCa research by further characterizing the role of NFIB in controlling AR expression and regulating NED. It also has the potential to define a novel biomarker for aggressive PCa.
   
   - **What was the impact on other disciplines?**
     Nothing to report.
   
   - **What was the impact on technology transfer?**
     Nothing to report.
   
   - **What was the impact on society beyond science and technology?**
     Nothing to report.

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
     Generation and characterization of cell lines took longer than anticipated and preliminary experiments were performed using transient transfections of previously published siRNA constructs targeting NFIB.
   
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     Nothing to report.
   
   - **Changes that had a significant impact on expenditures**
     Nothing to report.
   
   - **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**
     A manuscript including some of the preliminary studies using transient transfections is under review at *Endocrinology*. 

- **Journal publications.**
  Manuscript submitted and undergoing reversion².

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

- **Other publications, conference papers, and presentations.**
  Poster presentation at the annual meeting of the SBUR⁵.

- **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.

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7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

<table>
<thead>
<tr>
<th>Name:</th>
<th>Magdalena Grabowska</th>
</tr>
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<tbody>
<tr>
<td>Project Role:</td>
<td>PI</td>
</tr>
<tr>
<td>Researcher Identifier (ORCID ID):</td>
<td>0000-0003-1550-4925</td>
</tr>
<tr>
<td>Nearest person month worked:</td>
<td>12</td>
</tr>
<tr>
<td>Contribution to Project:</td>
<td>Dr. Grabowska has performed all experiments reported.</td>
</tr>
<tr>
<td>Funding Support:</td>
<td>Nothing to report.</td>
</tr>
</tbody>
</table>

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

Changes for PI:

Magdalena M. Grabowska: New support

1K99 CA197315-01 Grabowska (PI) 09/01/15 - 08/31/17

Pathway to Independence Award (K99/R00)
Nuclear Factor I/B Action in Castrate Resistant Prostate Cancer
This application explores how nuclear factor I/B (NFIB) interacts with androgen receptor and androgen receptor splice variants to drive lethal castrate resistant prostate cancer, as well as how NFIB supports cytokine secretions that promote castrate-resistance.
Role: Principal Investigator

Changes for senior key personnel:

Robert J. Matusik: Support changed from current to completed

5R01 DK055748-14 Matusik (PI) 04/06/09 - 03/31/14
NIH/NIDDK
Control of Prostate-Specific Gene Expression
Our hypothesis is that by identifying the transcription factors (TFs) that control prostate-specific gene expression, we are also identifying TF that play a critical role in prostate development.

Role: Principal Investigator

4R01 CA076142-14  Matusik (PI)  08/11/09 - 06/30/14
NIH/NCI (no cost extension)

Transgenic Mouse Models for Prostate Cancer
The overall goal of our grant has been to characterize how the NF-κB and Pten pathway co-operate in the progression of androgen dependent prostate cancer to castrate resistant prostate cancer. This work will use both cell culture and transgenic animal models to test the role of these pathways during tumor progression.

Role: Principal Investigator

5P20 DK097782-02  Hayward (PI)  09/30/12 - 07/31/14
NIH/NIDDK

Obesity, Inflammation and BPH
This planning center will develop preliminary data for subsequent proposals. The center is focused upon the integrating research in benign urologic disease into the broader clinical context. In particular links between the urologic research group the obesity institute and diabetes centers will be expanded. Research will focus on the role of inflammation subsequent to obesity regulated by various means in benign prostatic growth using in vivo and clinical population based models.

Role: Co-Investigator

5R01 DK067049-08  Hayward (PI)  08/01/10 - 05/31/14
NIH/NIDDK

Paracrine Regulation of BPH Pathogenesis
This grant examines the role of inflammation and subsequent modifications to paracrine signaling in establishing a proliferative microenvironment in the prostate leading to benign enlargement. Methods to alleviate the response to a pro-inflammatory environment are explored.

Role: Co-Investigator

5R01 DK087962-04  Fowke (PI)  07/01/10 - 06/30/14
NIH/NIDDK

Biomarkers of Obesity, Prostate Tissue Inflammation, and BPH Progression
This is a prospective epidemiologic study on the relationships between obesity biomarkers and inflammation in BPH. It also investigates the mechanisms linking obesity on prostate hyperplasia utilizing two mouse models of obesity. This multidisciplinary project will advance our understanding of obesity as a risk factor for BPH and lead to interventions to delay BPH progression by targeting obesity and inflammation pathways.

Role: Co-Investigator

5U54 CA163072-02  Moses (PI)  09/23/11 - 08/31/16
NIH/NCI

MMC, VICC & TSU: Partners in Eliminating Cancer Disparities
Molecular Mechanisms of SKP2 Targeting on Prostate Cancer Progression
We have previously shown in vitro and in vivo that the acute inactivation of Pten leads to the aberrant upregulation of p19Arf, p53, p21, and that S-phase kinase-associated protein 2 (Skp2) is an important downstream target of PI3K-AKT pathway. Activation of Skp2 promotes cancer progression and Skp2 deficiency significantly inhibits tumor development in mouse models. We hypothesize that an inhibition of Skp2 activity suppresses castration resistant prostate cancer (CRPC). We propose to test this hypothesis and to
study the molecular interaction of Skp2 with Pten-p53 pathway in cells and how Skp2 inactivation impacts prostate cancer progression in mouse models.
Role: Co-Investigator

Peter E. Clark: Support recently awarded
3P30 CA068485-19S1 Pitenpol (PI) 09/01/14 - 08/31/16
NIH/NCI
Cancer Center Support Grant Supplement
This supplement is a NCI directed tissue procurement program for next generation sequencing of advanced prostate and bladder cancer.
Role: Co-Investigator

Peter E. Clark: Support changed from current to completed
5P20 DK097782-02 Hayward (PI) 09/30/12 - 07/31/14
NIH/NIDDK
Obesity, Inflammation and BPH
This planning center will develop preliminary data for subsequent proposals. The center is focused upon the integrating research in benign urologic disease into the broader clinical context. In particular links between the urologic research group the obesity institute and diabetes centers will be expanded. Research will focus on the role of inflammation subsequent to obesity regulated by various means in benign prostatic growth using in vivo and clinical population based models.
Role: Co-Investigator

8. SPECIAL REPORTING REQUIREMENTS

- What other organizations were involved as partners?
  Nothing to report.
COLLABORATIVE AWARDS:
Not applicable.

QUAD CHARTS:
Not applicable.

9. **APPENDICES:**
   Appendix 1: References
   5. Grabowska, M. M. *et al.* in *Society of Basic Urologic Research* (Dallas, TX, 2014).
RRP - Case Manager Form

De-identified Study ID:_______

Race: ____White   ____Black   ____Asian   ____Hispanic   ____Indian (American)   ____Other   ____DNA

Pre-OP Info

PSA:_____ Men's Wellness Class:____Y____N____DNA  Salvage Case after Radiation:____Y____N
Hormones: Lupron/Casodex:____Y____N
5-ARI: Avodart/Proscar/Propecia:____Y____N
Classified as castrate resistant?_____ Y ____N
T Clinical Stage 2010:______TX____T0____T1a____T1b____T1c____T2a____T2b____T2c____T3a____T3b____T4
____DNA
N Clinical Stage 2010:______NX____N0____N1
M Clinical Stage 2010:______MX____M0____M1____M1a____M1b____M1c
Metastatic locations:____
Group:____I____IIA____IIB____III____IV

Gleason Primary @BX:_________
Gleason Secondary @BX:_________
Total Gleason Score:_________
SHIM:____Y_____N,______(1-25)
AUA SI:____Y_____N,______(0-35)

OP Info

Pre-Op PCV:_________ Post-Op PCV:_________
BMI:________,____DNA

Post-OP Info

RRP - Case Manager Form - PATHOLOGY

Tumor %:_____________ Post-Op Tumor Grade:Gleason Primary:_________
Prostate Volume:_____________  Gleason Secondary:_________
Gleason Tertiary:_________
Gleason Total:_________
Margin Status:____Positive ____Negative

Apex _____Y_____N
Base _____Y_____N
Lateral _____Y_____N
Anterior _____Y_____N
Posterior: ____Y  ____N

Bladder Neck: ____Y  ____N

Other: ____Y  ____N

T Stage 2010: _____TX _____T0 _____T2a _____T2b _____T2c _____T3a _____T3b _____T4 _____DNA

AJCC stage 2010: _____I  _____IIA  _____IIB  _____III  _____IV  _____DNA

Seminal Vesicle Invasion: ____Y  ____N

Nodol Involvement

_____Not Sampled

_____Not Lymph Tissue

_____Sampled as Group  Total # Positive: __________  Total # Sampled: __________

Right Side  Total Right # Positive: __________  Total Right # Sampled: __________

Left Side  Total Left # Positive: __________  Total Left # Sampled: __________

Distant Metastasis: _____PCS(pre-clinical stage) _____M1 _____M1a _____M1b _____M1c _____DNA

Bladder Neck Invasion: _____Microscopic  _____Gross  _____None  _____DNA

Extracapsular Extension: ____Y  ____N  _____DNA
De-identified Study ID: ______

Race: ___White ___Black ___Asian ___Hispanic ___Indian (American) ___Other ___DNA

Pre-OP Info
PSA: ___ Men's Wellness Class: ____Y____N____DNA
Salvage Case after Radiation: ____Y____N
Hormones: Lupron/Casodex: ____Y____N
5-ARI: Avodart/Proscar/Propecia: ____Y____N
Additional Therapies Prior to Recurrence: ______________
__________________________________________

At Time of Initial Prostate Cancer Diagnosis (if available)
T Clinical Stage 2010: ___TX____T0____T1a____T1b____T1c____T2a____T2b____T2c____T3a____T3b____T4
N Clinical Stage 2010: ___NX____N0____N1
M Clinical Stage 2010: ___MX____M0____M1____M1a____M1b____M1c
Group: ___I____IIA____IIB____III____IV
Gleason Primary @BX: __________
Gleason Secondary @BX: __________
Total Gleason Score: __________

OP Info
Procedure: ____Bone Resection ____Channel TURP
## De-identified outcomes data

| Record ID (auto-numbered and de-identified) | ________________________________ |
| Race                                      | ________________________________ |
| Age (at prostatectomy)                    | ________________________________ |
| Body Mass Index (BMI)                     | ________________________________ |
| PSA (at diagnosis)                        | ________________________________ |
| Salvage case after radiation?             | ________________________________ |
| Did patient receive neoadjuvant androgen deprivation therapy (ADT)? | ○ Yes  ○ No |
| Type of neoadjuvant therapy               | ________________________________ |
| Is patient considered castrate resistant at time of prostatectomy? | ________________________________ |
| T 2010 Clinical Stage                     | ○ TX  ○ T0  ○ T1a  ○ T1b  ○ T1c  ○ T2a  ○ T2b  ○ T2c  ○ T3a  ○ T3b  ○ T4 |
| N 2010 Clinical Stage                     | ○ NX  ○ N0  ○ N1 |
| M 2010 Clinical Stage                     | ○ MX  ○ M0  ○ M1  ○ M1a  ○ M1b  ○ M1c |
| Location of metastatic deposits           | ________________________________ |
| Gleason primary @ BX                       | ________________________________ |
| Gleason secondary @ BX                     | ________________________________ |
| Total Gleason                              | ________________________________ |
| Preop PCV                                  | ________________________________ |
| Post-op PCV                                | ________________________________ |
### Pathology

<table>
<thead>
<tr>
<th>Tumor Percent</th>
<th>____________________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate volume</td>
<td>____________________________</td>
</tr>
</tbody>
</table>

**Post-op tumor grade: Gleason primary**

| ____________________________ |

**Post-op tumor grade: Gleason secondary**

| ____________________________ |

**Post-op tumor grade: Gleason tertiary**

| ____________________________ |

**Margin Status**

- **positive**
- **negative**

**Margin Status: Apex**

- **Yes**
- **No**

**Margin Status: Base**

- **Yes**
- **No**

**Margin Status: Lateral**

- **Yes**
- **No**

**Margin Status: Anterior**

- **Yes**
- **No**

**Margin Status: Posterior**

- **Yes**
- **No**

**Margin Status: Bladder neck**

- **Yes**
- **No**

**Margin Status: Other**

- **Yes**
- **No**

**Pathology T 2010 Clinical Stage**

- **TX**
- **T0**
- **T1a**
- **T1b**
- **T1c**
- **T2a**
- **T2b**
- **T2c**
- **T3a**
- **T3b**
- **T4**

**AJCC stage 2010**

- **I**
- **IIA**
- **IIB**
- **III**
- **IV**

**Seminal vesicle invasion**

- **Yes**
- **No**

**Nodal involvement**

- **Not Sampled**
- **Not Lymph Tissue**
- **Sampled as Group**

**Nodal involvement: Sampled, right side**

| ____________________________ |

**Nodal involvement: Postive, right side**

| ____________________________ |

**Nodal involvement: Sampled, left side**

| ____________________________ |
Nodal involvement: Positive, left side

Total positive nodes

Total nodes sampled

Distant Metastasis

- MX
- PCS
- M1
- M1a
- M1b
- M1c
- DNA

Bladder neck invasion

- Microscopic
- Gross
- None
- DNA

Extracapsular invasion

- Yes
- No
- DNA

Recurrence

Did patient recur?

- Yes
- No

Time to biochemical recurrence (months)

Therapies subsequent to biochemical failure

Castrate Resistant Prostate Cancer

Did patient undergo bone resection or TURP to relieve compression or obstruction following failure of ADT?

- Yes
- No

Did patient undergo prostatectomy at VUMC/Has clinical data been previously entered?

- Yes
- No

Race

Age (at prostatectomy)

PSA (at diagnosis)

Salvage case after radiation?

- Yes
- No

Androgen deprivation therapy?

- Yes
- No

Therapy after failure of ADT
At primary diagnosis: clinical stage
- TX
- T0
- T1a
- T1b
- T1c
- T2a
- T2b
- T2c
- T3a
- T3b
- T4
- DNA

At primary diagnosis: N 2010 Clinical Stage
- NX
- N0
- N1

At primary diagnosis: M 2010 Clinical Stage
- MX
- M0
- M1
- M1a
- M1b
- M1c

At primary diagnosis: Group
- I
- IIA
- IIB
- III
- IV

At primary diagnosis: Gleason primary

At primary diagnosis: Gleason secondary

At primary diagnosis: Gleason sum

Surgical procedure
- Channel TURP
- Bone Resection

**Histological analysis**

NFIB staining: tumor nuclear intensity

NFIB staining: tumor nuclear distribution

NFIB staining: tumor sum

NFIB staining: normal adjacent nuclear intensity

NFIB staining: normal adjacent nuclear distribution

NFIB staining: NAT sum

NFIB staining: CRPC nuclear intensity

NFIB staining: CRPC nuclear distribution

NFIB staining of CRPC sample (sum)
Androgen receptor status

Neuroendocrine differentiation status

Is this prostate cancer classified as neuroendocrine PCa or small cell PCa?

○ Yes
○ No

NFIB staining: NEPCa nuclear intensity

NFIB staining: NEPCa nuclear distribution

NFIB status in NEPCa (sum)