

**FLORIDA A&M UNIVERSITY
MINORITY PROSTATE CANCER TRAINING AND RESEARCH CENTER**

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**FACULTY PROSTATE CANCER DEVELOPMENT AWARDS PROGRAM
PROPOSAL GUIDELINES**

Project Title: Microarray Comparison of Prostate Tumor Gene Expression in African American Men and in Caucasian Men: A Feasibility Study
Name of PI: _____

Submission Deadline: June 6, 2003

Title Page

Directions: Save these forms as a Word or WordPerfect document. Highlight yellow area including brackets, then type in the requested information.

Project Title: Microarray Comparison of Prostate Tumor Gene Expression in African American Men and in Caucasian Men: A Feasibility Study

Investigator:

Co-investigators: None

Moffitt Cancer Center Collaborators: _____ and _____, Ph.D.

College/School: **Florida A & M University**

College/School Address: **College Of Pharmacy & Pharmaceutical Sciences, Tallahassee, Florida 32307**

Present Title at College/School: **Associate Professor**

Telephone: E-mail: _____

Grant Period: _____ from: **11/03/03**
to: **11/03/06**

Amount Requested: **\$40,000.00**

Brief Description of Project (Please single space):

Using standard molecular biology techniques, we propose to isolate, purify and label, with appropriate dyes in preparation for microarray analyses, total RNA from prostate tumor specimen obtained from African American Men and from Caucasian Men. We will focus on the analysis of androgen-regulated genes (ARG) to test the hypothesis that unique androgen receptor (AR) molecular signatures exists in prostate tumor specimen obtained from African American Men that are not present in prostate tumor specimen obtained from Caucasian men, due to AR polymorphisms. Polymorphisms in the androgen receptor (AR) found in African American Men may be potentially important in explaining the increased risk of prostate cancer in African American men. Dr. _____ will provide prostate tumor specimen to _____ from the Moffitt Tumor Specimen Bank. Due to the limited number of prostate tumor specimen samples on-hand for African American Men, we are proposing this pilot project as a feasibility study. However, it is anticipated that preliminary data obtained will allow for an initial "mining out of" unique AR molecular signatures. For the first time, as an outcome of these studies, it is expected that global gene expression profiles of prostate cancer in African American and Caucasian men will be obtained. The information learned from these global profiles will have far-reaching significant positive impact on the development of new pharmacogenomic therapies/strategies for effective prevention and successful management of prostate cancers, particularly in African American men.

Abstract Page

Project Title: **Comparison of Prostate Tumor Gene Expression in African American Men and in Caucasian Men: A Feasibility Study**

Investigator: **Ph.D.**

Moffitt Cancer Center Collaborators: **and Ph.D.**

College/School:

College OF Pharmacy & Pharmaceutical Sciences, Florida A & M University, Tallahassee, Florida 32307

Abstract (*Do not exceed the space provided; please single space.*):

African American men have the highest incident rate of prostate cancer of any ethnic group in the United States. Among racial groups, African American males are more likely to develop and to die from prostate cancer than Caucasian males. In spite of the widely appreciated magnitude of this problem, unfortunately, little progress has been made in understanding the root cause of prostate cancer health disparity observed in African American men. It is known that the CAG repeat polymorphism of the androgen receptor gene has been associated with increased risk for prostate cancer and the repeat length correlated with cancer stage and grade at presentation. Short CAG repeat length on the androgen receptor gene is associated with African American men and possibly with higher stage. Black men tend to have significantly shorter CAG repeats than Caucasian men. Hence racial variation in length of the androgen receptor gene CAG repeat may be potentially important in providing a hypothesis to explain the increased risk of prostate cancer in African American Men. To date, studies have not been done which compare variations in gene expression profiling of prostate tumors from African American men to that of Caucasian men. This study proposes a comparison of Prostate Tumor Gene Expression in African American Men to that of Caucasian men. The central hypothesis of this study is that unique androgen receptor (AR) molecular signatures exists in prostate tumor specimen obtained from African American Men that are not present in prostate tumor specimen obtained from Caucasian men, due to CAG repeat androgen receptor polymorphisms. Furthermore, we propose that AR mediated gene expression participates in prostate cancer in both African American males and Caucasian males, through regulation of cell survival and proliferation genes. However due to polymorphisms in the androgen receptor found in African American males, we propose that AR signaling can differentially influence disease outcome and resistance to chemotherapy in African American males. The PI, her Moffitt Center Collaborators and her Wayne State University consultants are well-prepared and have the competitive edge to carry out this pilot project study because of our collective experiences in molecular biology, genomics and prostate cancer research. This project is innovative in that it will use microarray data to define AR molecular signatures. This innovative approach is expected to yield the following outcomes. First, simple analysis of the cDNA array will yield the identity of up/down regulated genes; second Rosetta Resolver analysis of the cDNA array will yield gene clustering data and its significance; thirdly, Rosetta Resolver analysis of the cDNA array will pinpoint to the involvement pertinent biochemical pathways and gene otologies. Collectively these outcomes will establish how "aberrant" AR mediated gene expression plays a role in increased prostate cancer incidence and mortality rates in African American men. The outcomes of this research is expected to have significant positive effects on closing the gap in Prostate cancer health disparity because it will allow for the eventual development of new therapies/strategies for effective prevention and successful management of prostate cancers, particularly in African American men.

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Other Support

Please list your other experiences relevant to this application:

Dr. [redacted] has the following skill sets (and associations) that will be of great benefit to her on this DOD subproject:

SDS-PAGE and western analysis, northern blot analysis, cDNA microarray analysis using Clontech's oxidative stress array (to examine the role manganese chloride plays in oxidative stress in Pheochromocytoma Cells). Moreover, Dr. [redacted] received an NIEHS K14 Minority Scientist Career Award to spend summer sabbaticals (1995-97) at Wayne State University's Environmental Health Sciences Center learning molecular biology and signal transduction protocols. As an Associate Member of the WSU EHS center Renee has use of all of its facility cores which includes: Gene Regulation and Gene Expression; Genomics and Bioinformatics, Signal Transduction, Cell Culture, and Flow Cytometry and Imaging Core Facilities. To date, Dr. [redacted] has confirmed RNA integrity of samples (prepared in her lab) on the Agilent bioanalyzer at Wayne State University. She has participated in an initial orientation on the running of the Agilent cDNA scanner and has had a short overview on the use of the Rosetta Resolver.

Please identify other grant applications that duplicate this application and certify that if a grant is awarded, there will be no duplication with other grants:

<u>Title</u>	<u>Sponsor</u>	<u>Amount</u>
NONE		

Budget Form

Please list the supplies/materials and the approximate dollar amount by category (below) needed to carry out the research project. Do not exceed the space provided.

Personnel:

Salary and fringe benefits for Dr. [redacted] \$10,770.

Dr. [redacted] will provide a minimum of 10% effort to this project; Funds are requested to cover 10 % of her annual salary including fringe for a total of \$10,770. Specifically she will isolate, purify and label, with appropriate dyes in preparation for microarray analyses, total RNA from prostate tumor specimen and their matched control specimen obtained African American men and from White men. Microarray chips will be taken for analysis to the cDNA array reader in the Genomics/Bioinformatics Facility Core Center at Wayne State University. Dr. [redacted] is an Associate Member of Wayne State University EHS center.

Dr. [redacted] has agreed to serve as consultant to [redacted] on this project **See attached letter.** Dr. [redacted] is director for the Institute of Environmental Health Sciences at Wayne State University and is also the center director for the NIEHS-funded Environmental Health Sciences (EHS) Center. The EHS center has five facility cores, one of which is the Genomics and Bioinformatics Facility Core. The Genomics and Bioinformatics Facility core at Wayne State University EHS center have expertise in the area of cDNA array analysis and Bioinformatics (use of rosetta resolver to interpret microarray data). The rationale for asking Dr. [redacted] to serve as consultant in this project rests on the fact that the costs of purchasing, scanning and analyzing agilent arrays is substantially less costly at Wayne State University compared to costs associated with use Moffitt's genomics center. Moreover, agilent gene chip technology were used in the NIEHS human genome project, which means a wealth of information is available for comparison to the results we obtain in this study.

Animals: No animals will be used in this project.

Drugs/Chemicals:

Dr. [redacted] has generously supplied LNCaP cells, DMSO and Synthetic Androgen R1881.

Supplies: Funds totaling \$2,166 are requested for the purchase of supplies in the following categories listed below.

Cell Culture Supplies & Reagents. Funds totaling \$1,166 are budgeted for the purchase of supplies and reagents required for the culture of LNCaP cells including culture medium, fetal bovine serum and other media additives.

Molecular Biology supplies and reagents. Funds totaling \$1,000 are budgeted for the purchase of supplies and reagents required for the preparation of total RNA for microarray analysis (e.g. Triazol, DNase and RNeasy kits).

Other Supplies: Funds totaling \$24,314 will be needed for Agilent GeneChips (Hu1 and Hu2) and processing supplies and reagents.

In year 01 we will purchase 20 Agilent GeneChip Human Genome Hu1/Hu2 arrays (utilizing the Wayne State University discount price each array will cost approximately \$700). Supplies

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necessary to process the array at Wayne State University Genomics Facility is estimated at \$500 per array. The cost above is for performing the minimum number of replicate microarray experiments are described in this grant. Much of the costs associated with performing microarray experiments (e.g. labor costs) are defrayed by the Wayne State University EHS center because Dr. [redacted] is an associate member of the Environmental Health Science center at Wayne State University. The microarray experiments are the foundation of this pilot project study. Hence, in order to stay within the allotted budget of \$40,000, it will be necessary to use the genomics/bioinformatic facility at Wayne State University.

Funds in the amount of \$314 are requested to defray the costs associated with federal express shipping of samples (on dry ice) between FAMU and Moffitt and FAMU and Wayne State University.

Equipment: NONE

Operations (i.e., computer time, telephone, postage): NONE

Travel: Funds in the amount of \$1,750 are requested to defray the partial costs of travel of the principal investigator to the Moffitt Cancer Center for collaborative activities and to Wayne State University for Bioinformatics Workshop Training. The cost of \$750 to visit Moffitt will cover airfare (\$397), hotel (\$260), per diem (\$63), and taxi (\$30). The cost of \$1,000 visit Wayne State will cover airfare (\$450), hotel (\$457), per diem (\$63), and taxi (\$30).

TOTAL BUDGET REQUEST: \$ 40,000

Project Description

Provide a clear statement of the research that will be undertaken below. It must include: (1) an introduction; (2) a review of the relevant literature; (3) the objectives for the period of the proposed work; (4) significance of proposed work to eliminating prostate cancer disparity; (5) the research plan; (6) how the results will be disseminated; and (7) Potential benefit of the proposed project in enhancing your skills and experiences to maintain a competitive and successful program in prostate cancer research. 5-page limit

Racial variation in prostate cancer incidence in the United States is pronounced, with African American men having the highest rates. (Cancer Prevention & Early Detection: American Cancer Society) During 1992-1999, the average annual incidence rate for prostate cancer was 275.3 per 100,000 persons among African American males and 172.9 per 100,000 persons among Caucasian males. The average death rate per 100,000 persons was 75.1 for African American males and 32.9 for Caucasian males; hence prostate cancer mortality rate for that period was 2.3 fold higher in African American males than in Caucasian males (Cancer Prevention & Early Detection: American Cancer Society). The CAG repeat polymorphism of the androgen receptor gene has been associated with increased risk for prostate cancer and the repeat length correlated with cancer stage and grade at presentation. Short CAG repeat length on the androgen receptor gene is associated with African American men and possibly with higher stage (Bennett et al, 2003; DiTommaso et al 2002; Kittles et al , 2001) African American men tend to have significantly shorter CAG repeats than Caucasian men (Platz et al, 2000) Hence racial variation in length of the androgen receptor gene CAG repeat may be potentially important in providing a hypothesis to explain the increased risk of prostate cancer in African American men. To date, studies have not been done which compare variations in gene expression

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profiling of prostate tumors from the African American population to the gene expression profiling of prostate tumors from the Caucasian population. Hence, the central hypothesis of this proposed study is that unique AR molecular signature(s) exists in prostate tumors in African American men that are not present in prostate tumors in Caucasian men. Furthermore, we propose that AR mediated gene expression participates in prostate cancer in both African American men and Caucasian men, through regulation of cell survival and proliferation genes; and that polymorphisms in the androgen receptor cause "aberrant" AR signaling which can differentially influence prostate cancer disease outcome and resistance to chemotherapy in African American men. We will investigate the role of AR mediated gene expression in prostate cancer in African American males by gene expression profiling using cDNA microarray to "mine out" AR molecular signatures. Due to the limited number of prostate tumor specimen samples on-hand for African American men, we are proposing this project as a pilot study to obtain very preliminary data (N=4) that will allow for an initial comparison of prostate tumor gene expression profiling in African American men and Caucasian men. To test this hypothesis, the objectives for the period of proposed work are:

1. **To define an AR-associated molecular signature in a prostate cancer cell line.** The human prostate cancer cell line LNCaP (androgen dependent, low Stat3 activity), will be used to identify expression profiles of potential AR-regulated genes in prostate cancer using Agilent microarray technology. We will modulate AR-associated gene expression levels using various concentrations of the synthetic androgen, R1881 in order to restrict the field of candidate AR-associated genes. These studies will provide a preliminary AR-associated molecular signature in the LNCaP prostate cancer cell line. (Timeline: months 1-4 of year one).

2. **To identify the AR-associated molecular signature in primary prostate cancer in African American men.**

The AR molecular signatures defined from the LNCaP cell line model will be used to identify AR-associated molecular signature in tumor specimen from African American men and Caucasian men who have undergone radical prostatectomy. Gene expression profiles in tumor specimens will be compared directly to control non-tumor tissues in the same patients. These studies will critically test our hypothesis and assess the AR molecular signature as an explanation for the increased risk of prostate cancer in African American men. (Timeline: months 2 – 9 of year one)

3. **To identify additional sources for procurement of African American prostate tumor specimens.**

In order prepare for a scaled up version of the pilot project experiments described in this grant proposal, we will need to procure additional African American prostate tumor specimens. Our goal is to increase the number of specimen from n =8 to n =50. (Timeline: months 1-12 of Year One).

Significance of proposed work to eliminating prostate cancer disparity.

Collectively the expected outcomes of this study are (1) the identity of up/down regulated genes from the global gene expression profile of Prostate cancer in African American and Caucasian; defined AR molecular signatures (2) Use of Rosetta Resolver analysis of the cDNA array will yield gene clustering data and its significance; (3) Rosetta Resolver analysis of the cDNA array will point to pertinent biochemical and gene otologies. Collectively these outcomes will establish that how AR mediated gene expression plays a role in increased prostate cancer incidence and mortality rates in African American men. The knowledge learned from this pilot project has

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far-reaching ramifications and will lay the ground work for closing the gap in Prostate cancer health disparity by pin-pointing molecular targets for the development of new pharmacogenomics therapies/strategies for effective prevention and successful management of prostate cancers, particularly in African American men. Currently, there is no simple way to determine whether people will respond well, badly or not at all to a medication; therefore pharmaceutical companies are limited to developing drugs using a "one size fits all" system. Pharmacogenomics now allows for the development of drugs to which the "average" patient will respond. But in the future, as the genes involved in drug response are identified, tailor made drugs may one day be an option for ablating health disparities in populations who show substantially higher incidence and mortality rates in disease states such as cancer.

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BACKGROUND

Metastatic carcinoma of the prostate is the second leading cause of cancer mortality among American men, and is especially prevalent among African Americans. Clinical diagnosis of prostate cancer is rare before age 40, but increases steadily thereafter (Carter, 2001). Thus in the United States, it is estimated that 1 in 55 men between the ages of 40 and 59 will develop the disease. Thus, as longevity increase and the baby boom population ages, the impact of prostate cancer upon our society will be increasingly felt.

It is well known that prostatic epithelial cells and early carcinoma cells proliferate in response to androgen, by virtue of their abundant expression of AR. However, it has also been known for many years that treatment of prostate cancer patients with synthetic estrogens, such as diethylstilbestrol, often results in the regression of hormonally responsive tumors. While it was originally thought that these effects were secondary to suppression of the pituitary-testicular axis, with consequent lowering of circulating androgen levels, it has more recently been appreciated that estrogenic hormones and chemicals exert growth-altering effects directly on prostate cells. Current evidence suggests that prostate epithelial cells express at least one form of estrogen receptor (ER). For example, one recent study reported that cultured normal prostate epithelial cells and the androgen-responsive prostate carcinoma cell line, LNCaP, each express only ERB, and exhibit estrogen-inducible expression of pS2 and progesterone receptor (PR) (Lau, LaSpina, Lonmg, Ho, 2000). These results suggest that the LNCaP cell line is a reasonable model for studying androgen- and estrogen-dependent effects on human prostate cell growth and function.

Following its confinement within the prostate gland, the adenocarcinoma may spread via the lymphatic or blood supply (Carter, 2001). Local extension tends to be into and through the prostatic capsule, bladder base, and seminal vesicles (Carter, 2001). Prostate cancers frequently metastasize to the bone, lung, liver or epidural space. (Carroll, 2001). The spread to the bone is believed to result from bi-directional interaction of prostatic epithelial cell and bone stroma, which results in a favorable microenvironment for growth.

The possibility for successful treatment of prostate cancer depends on its stage. Thus, early-stage prostate cancer, still confined to the prostate, can be successfully treated by surgical removal of the gland. (Carter, 2001) If the disease has spread beyond the boundaries of the prostate, it may be managed, but not cured, by androgen ablation therapy (i.e. estrogen therapy). Thus three- distinct cellular phenotypes for prostate cancer cells have been postulated (Carter, 2001) to exist, consisting of androgen-dependent cancer cells, which require androgen stimulation for maintenance and growth, and are thus, similar to normal

prostate cells (Lau, LaSpina, Lonmg, Ho, 2000); Androgen-sensitive cancer cells that do not die if no androgen is present, although their growth rates are decreased in the absence of androgen and (Tessier, and Matsumura , 2001); androgen-independent cells that neither die nor slow their growth in the absence of androgen (Carrol, Lee, Fuks, Kantoff, 2001). Of these phenotypes, only the androgen-dependent and androgen-sensitive cancer cells may be managed by androgen ablation therapy. With treatment, patients having such cancers may survive for many years. Ultimately, however, many androgen-responsive cancers progress to an androgen-independent state, at which point they become , for all practical purposes, untreatable (Carrol, Lee, Fuks, Kantoff, 2001). Hence, the androgen-responsive period represents a critical window during the progression of prostate cancer, which largely determines whether there is to be hope for the management of the disease.

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African Americans tend to present with more advanced disease and have poorer prognosis than Caucasian Men. It is therefore critical to gain a thorough understanding of underlying cause of the higher incidence and Prostate Cancer mortality rate seen in African American men.

The underlying cause for the Prostate Cancer disparity in African American men is not known. Some studies have attributed this disparity to social, economic, educational, hereditary and dietary differences among racial groups (Carrol, Lee, Fuks, and Kantoff, 2001). Migrant studies have supported environmental, social or dietary components to prostate cancer development. For example, among foreign-born Asian Americans, the risk of developing prostate cancer increased with years of residence in North America and with saturated fat intake (Whittemore , Kolonel, Wu, John, Gallagher, Howe, Burch, Hankin, Dreon, West, 1995).

Other studies support the prevailing thought that Androgen receptor polymorphisms increases a man's risk of prostate cancer (Sartor, Zheng, and Eastham, 1999), while other investigators argue that small differences in the number of CAG repeats in both black and Caucasian patients do not appear to be a strong indicator of risk (Chen, Lamharzi, Weiss, Etzioni, Dightman, Barnett, DiTommaso, Goodman, 2002) or aggressive disease but that this size polymorphism may be one of many genetic and environmental risk factors involved in prostate cancer. This investigator favors the idea that androgen polymorphism CAG repeat may be potentially important in providing a hypothesis to explain the increased risk of prostate cancer in African American men.

Hence, the research plan below will be undertaken to test the hypothesis that androgen polymorphism CAG repeats cause significant differences in global gene expression in African American men compared to Caucasian men. In large measure, the major intent of this study is to allow the tumor specimen to indicate what the gene expression profile of Prostate Cancer look like in African American and Caucasin men. This global profile will also indicate to us what genes, biochemical pathways are likely to be most important in influences this disease state in African American men. This approach is made possible by the availability of powerful new technologies that permit the global evaluation of a cell's phenotype, at the cellular and molecular levels.

DNA microarray analysis is an exceedingly powerful technique that permits the evaluation of a cell's molecular phenotype. The technology has, historically, been very expensive, and therefore not widely available for use. The technology also requires specialized equipment, resources and expertise. This applicant is fortunate enough to be an associate member of an NIEHS funded Environmental Health Sciences (EHS) Center. As an associate member of this center, this applicant has the privilege to use the core facilities at WSU's EHS. Membership allows this applicant to order microarray chips at discounted prices and to use the array reader and Rosetta Resolver at significantly reduced price. This EHS center is located at Wayne State University

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(WSU) in Detroit, Michigan. One such facility is the Microarray and Bioinformatics Facility Core (MBFC). Dr. Raymond F. Novak, director of WSU's EHS center, has invested heavily into obtaining the expertise and resources that are necessary to support genomics-based research. The WSU Microarray and Bioinformatics Facility Core is capable of performing microarray analysis using a variety of platforms and is equipped with the following: an Agilent G2565 dual-laser microarray scanner, an Axon array microarray scanner and an ABI Prism 7000 RT-PCR for validation of microarray results. Bioinformatics resources for microarray data mining include a Sun V880 dual processor server on which resides the Rosetta Resolver microarray database and analysis system. This system allows investigators to remotely access manage and analyze their microarray data. In addition, the facility at WSU's EHS supports a fulltime bioinformatics director and staff who are available for consultation on all matters on experimental design and data interpretation. Letters of support from Drs Raymond Novak and Alan Dombkowski are included with this application.

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The Research Plan.

Objective 1. To define androgen receptor-associated molecular signature(s) in a prostate cancer cell line.

Rationale: The primary goal of this experiment is to identify the specific subset of AR regulated genes that define a AR molecular signature in LNCaP cell line. For this purpose we will use the synthetic androgen, R1887, to induce gene expression in the LNCaP cells. We predict that such genes represent elements of a "universal" AR molecular signature associated with human tumors and expect that there will be additional genes regulated by AR either directly or indirectly, which comprise a AR molecular signature specific to prostate cancer cells. These studies will provide a preliminary AR molecular signature in prostate cancer cells.

Rationale for Use of LNCaP cells as An Experimental Model Of Prostate Cancer.

Of the available prostate carcinoma cell lines that are readily grown and manipulated in culture, only LNCaP cell line, derived from a patient with lymph node metastasis, is androgen responsive. It should be noted that a particular feature of this cell line is that it expresses a mutated AR, which has been shown to be activated by an abnormally broad spectrum of agents, including estrogens and pregnenolone (Grigoryev, Long, Njar, Brodie, 2000). Like normal prostate epithelial cells, there is evidence that ERB is expressed in the LNCaP cell line (Lau, LaSpina, Lonmg, Ho, 2000;) although the precise level of its expression has not been established, especially at the protein level. Thus overall the LNCaP cell line appears to be a suitable model for use in defining AR molecular signatures because it is androgen responsive.

Experimental Strategy.

Cell Culture

LNCaP cells will be standard ATCC protocols.

Cell Viability Assay

The effect of the synthetic androgen, R1887, on the cell viability of LNCaP cells will be assessed using the promega MTS assay.

Exposure of LNCaP Cells to R1887

The results of the MTS Assay will help define a useful high and low concentration of R1887 to use for LNCaP cell exposure. Experimental samples will include the following groups.

Cultured LNCaP cells will be exposed to three different concentrations of R1887 for a period of 24 hours. Concentrations of R1887 will be determined from MTS cell viability assays. After 24 hours, cells will be lysed and total RNA from each replicate will be isolated using Triazol method.

Control (N=3)
 Low [R1887] (N=3)
 High [R1887] (N=3)

The following steps will be followed .

- 1.Total RNA Isolation
- 2.RNA integrity Using Agilent Bioanalyzer
- 3.Labelling of total RNA with appropriate dyes in preparation for micro array analysis
- 4.Scanning of the cDNA array
- 5.Use Rosetta Resolver to meaningfully interpret global gene expression in LNCaP prostate cell lines.

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The primary goal with the LNCaP cell line will be to identify a preliminary molecular signature of candidate AR-regulated genes that may predict their important roles in human prostate cancer. Candidate genes present in the AR molecular signature serve as one approach for identification of AR-regulated genes associated with primary prostate cancer in African American men and in Caucasian men. The focus of these studies is on global patterns of gene expression that begin to define a AR molecular signature, as opposed to identifying specific genes regulated by AR or assessing their casual roles in oncogenesis.

Objective #2. To identify AR associated molecular signature in primary prostate cancer in African American men.

Once we have defined an AR molecular signature in LNCaP cells, we will search for this gene expression profile in prostate tumor specimen in African American men and compare to their non-tumor matched control specimen. Likewise, we will search for this gene expression profile in prostate tumor specimen in Caucasian men and compare to their non-tumor matched control specimen. In addition, Dr. [redacted] will take the lead in correlating the expression of genes in the AR molecular signature with pathological stage, Gleason score, and PSA failure after prostatectomy as established prognostic factors. Our prediction is that AR molecular signatures in tumors from African American men will be uniquely different than the AR molecular signatures in Caucasian men. Another important question is whether the presence of an AR molecular signature in adjacent non-tumor tissues in African American men is different than that of Caucasian men.

Experimental Strategy

Experimental Samples:

African American Men		Caucasian Men	
Prostate Tumor specimen	N=4	Prostate Tumor specimen	N=4
Matched Control	N=4	Matched Control	N=4

Dr. [redacted] has 8 prostate tumor specimen from African American Men and 163 prostate tumor specimen from Caucasian men. The budget for this pilot project will allow us to analyzed four specimen from each experimental group. The following outline will be followed .

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1. Use Triazol, to isolate total RNA from each sample (Perform in Dr. [redacted] Lab)
2. Verify RNA integrity of each sample on the agilent bioanalyzer
3. Label each total RNA sample with appropriate dyes in preparation for micro array analysis (perform at Wayne State Environmental Health Sciences Center)
4. Hybridize labeled total RNA to micro array (at Wayne State)
5. Scan micro array (at Wayne State)
6. Use Rosetta Resolver to meaningfully interpret global gene expression in African American prostate tumor specimen and in their matched controls (at FAMU via WSU network)
7. Use Rosetta Resolver to meaningfully interpret global gene expression in Caucasian prostate tumor specimen and in their matched controls (at FAMU via WSU network)
8. Search for AR molecular signature in the gene expression profiles obtained.
(work with Drs. [redacted] and Dr. [redacted])

Objective #3. To identify additional sources for procurement of African American prostate tumor specimens.

To date, I have found out that a tumor bank or repository exists in Detroit, Michigan that has thousands of biological samples taken from prostate cancer patients, especially African American prostate cancer patients. I am told that tumor samples, blood samples, etc can be obtain for approximately \$75.00 per sample. If funded, I will verify this lead. Purchase of these samples would be cost prohibited in this pilot project; hence the purchase costs would have to be included in the budget of a future external grant application to the Department of Defense. My source of information comes from Dr. [redacted], an epidemiologist at Henry Ford Hospital in Detroit Michigan.

How the results will be disseminated

Results and data from this pilot project will be very preliminary due to number of samples analyzed (N=4), therefore dissemination will via scientific poster presentation at American Association of Cancer Research conference which will be held in Orlando, Florida in 2004. It is also highly likely that a manuscript in the form of a short communication will be published during year two of this grant. More importantly, preliminary data will be used to secure external funding for a scaled up version of this pilot project.

POTENTIAL BENEFIT OF THE PROPOSED PROJECT IN ENHANCING YOUR SKILLS AND EXPERIENCES TO MAINTAIN A COMPETITIVE AND SUCCESSFUL PROGRAM IN PROSTATE CANCER RESEARCH

I have an earned Ph.D degree in biochemistry and some experience in molecular biology. To date, most of my research work has been in the area of metal neurotoxicity and signal transduction. At this juncture in my career, I would like to use my very transferable molecular and biochemistry skills to extend my research endeavors to laboratory research in the area of prostate cancer health disparity. In order to be able to enhance my skills and experiences for establishing a competitive and successful laboratory program in prostate cancer research here at Florida A&M University - Pharmacy, I recognize the advantages/benefits of becoming involved with this Department of Defense Prostate Cancer Development program. Participation in this DOD development award program will allow me the opportunity to receive mentoring from and engage in thought provoking discussions with established investigators such as Dr. [redacted], Dr. [redacted] and Dr. [redacted]. Visit to their laboratory and quarterly participation in their lab group meetings will give a audience to sound out ideas and get feedback on my research

findings as this project develops. Already, Dr. [redacted] has provided helpful critique of my hypothesis and objectives and Dr. [redacted] has been available via telephone and emails to answer questions and help me stay focus as this proposal developed. To this end, I have crafted a pilot project for inclusion in this grant application entitled "Microarray Comparison of Prostate Tumor Gene Expression in African American Men and in Caucasian Men: A Feasibility Study. The opportunity to work with Drs [redacted] and Dr. [redacted] as collaborators on this grant will provide the needed mentoring vehicle that will ensure that I become equipped with the expertise that is necessary for accomplishment of this pilot project. In addition, participation in this program will provide opportunities to participate in Moffitt Cancer Center seminars and forums and which will provide avenue for coming up to speed in the field of prostate cancer research. Taken together, these enrichment activities with Moffitt scientists will cultivate in me a true competitive edge as I seek external funds when this pilot project is completed.

Plan for External Funding

Provide a detailed description of your plans to expand the current research for submission to a major external funding agency. Agencies and programs should be specified and the dates for proposal submission should be indicated.

The expected outcomes of this pilot project will yield:

- (1) the identity of up/down regulated genes from the global gene expression profile of Prostate cancer in African American men and Caucasian and defined AR molecular signatures
- (2) gene clustering data and its significance
- (3) Rosetta Resolver analysis of the cDNA array that points to pertinent biochemical and signal transduction pathways that may *preferentially* influence prostate cancer disease outcome and resistance to chemotherapy in African American men.

Plans to expand the current research for submission to major external funding agencies are as follows:

We will submit a full proposal to seek funds from the Department of Defense for a full-scale version of this pilot project. The objectives of the expanded study will include but are not limited to

- a. Initiate a scaled up version of the pilot project described in this DOD development proposal. Increase the number of tumor specimen examined from N=4 to N=50 for both African American Men and for Caucasian Men. Using standard molecular biology techniques, purify and label, separately, cellular RNA from prostate tumors obtained from African American males and from Caucasian males with appropriate dyes in preparation for the micro array analyses. Obtain a comparison microarray from the analysis of 50 replicates for each racial group tested, determine the alteration in global gene expression using Rosetta Resolver analysis and
- b. Identify and validate(by independent means real-time PCR and western blotting). AR molecular signatures observed in LNCap cell lines, and in prostate tumor specimen from African American men and Caucasian men.

Timeline for the Proposed Project

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(Timeline: months 1-4 of year one)

1. To define an AR-associated molecular signature in a prostate cancer cell line. The human prostate cancer cell line LNCaP (androgen dependent, low Stat3 activity), will be used to identify expression profiles of potential AR-regulated genes in prostate cancer using Agilent microarray technology. We will modulate AR-associated gene expression levels using various concentrations of the synthetic androgen, R1881 in order to restrict the field of candidate AR-associated genes. These studies will provide a preliminary AR-associated molecular signature in the LNCaP prostate cancer cell line.

(Timeline: months 2 – 9 of year one)

2. To identify the AR-associated molecular signature in primary prostate cancer in African American men.

The AR molecular signatures defined from the LNCaP cell line model will be used to identify AR-associated molecular signature in tumor specimen from African American men and Caucasian men who have undergone radical prostatectomy. Gene expression profiles in tumor specimens will be compared directly to control non-tumor tissues in the same patients. These studies will critically test our hypothesis and assess the AR molecular signature as an explanation for the increased risk of prostate cancer in African American men. (Timeline: months 2 – 9 of year one)

(Timeline: months 1-12 of Year One).

Task 3. To identify additional sources for procurement of African American prostate tumor specimens.

In order prepare for a scaled up version of the pilot project experiments described in this grant proposal, we will need to procure additional African American prostate tumor specimens. Our goal is to increase the number of specimen from $n=8$ to $n=50$. (Timeline: months 1-12 of Year One)

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