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14. ABSTRACT

The NCCU/BBRI-Duke/Urology Partnership in Prostate Cancer Research was developed to promote Collaborative Training, Research, and Community Dialogue and Outreach among scientists from NCCU and Duke. During the second funding period, we have first received full approval of our IACUC protocol and recently our IRB. We are now breeding the mouse model of prostate cancer deficient in βarrestin 2 (TRAMP-βarr2-/-) to further determine the role of βarr2 in prostate tumor development. We are also developing TRAMP-Alox5-/- mouse model to address the questions posed in project 3. The accrual of prostatic samples at Duke-VA has also started and processed samples will be made available to NCCU scientists for analysis as soon as significant numbers of cases and controls are collected. Three manuscripts directly related with the NCCU/BBRI-Duke/Urology Partnership have been submitted. One has been accepted and two are currently under revisions. The collaborative efforts have also led to the submission of 9 grant proposals with NCCU scientist as principal investigators. Unfortunately, the U54 application in partnership with UNC-Lineberger and Duke Comprehensive Cancer Centers entitled "NCCU-DCCC-LCCC Partnership In Cancer Research" was not funded. We are currently reviewing the proposal for a later submission.

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Introduction:

Prostate cancer is the most common type of cancer found in American men, other than skin cancer. Prostate cancer is the second leading cause of cancer death in men. The American Cancer Society estimated that there would be about 192,280 new cases of prostate cancer in the United States in 2009. About 27,360 men will die of this disease. While one man in six will get prostate cancer during his lifetime, only one man in 35 will die of this disease. African-American men are more likely to have prostate cancer and more likely to die of it than are white or Asian men. The reasons for this are still not known. National data also points to another important area of disparity for African Americans. According to the Nelson Diversity Surveys, African Americans, 12% of the US population, comprise less than 1% of the total tenured and non-tenured track investigators. Since it is well recognized that minorities are more likely to trust and cooperate with minority scientists in their community to address minority related health issues, building a diverse pool of scientists and clinical investigators is critical to reduce health disparities. The long-term goal of the NCCU/BBRI-Duke/Urology Partnership is to develop innovative approaches for prevention, detection, and treatment of prostate cancer, through research, training and collaboration between these two institutions. Community outreach through one of the leading Historically Black Colleges and Universities (HBCU) in this country will eventually help address the issue of health disparity in Durham and the surrounding area of North Carolina, where there is a large population of African Americans.

Body:

Task #1: Create the Prostate Cancer Disparity Research and Training Center (PCDRT) and develop plan for training.

a-d) As stated in last year progress report, these points have been accomplished.

e) The Fourth Annual Duke Prostate Center (DPC) Symposium "THE HOPE AND PROMISE OF PROSTATE CANCER THERAPY AND RESEARCH in 2008" and the Fourth Annual Duke Prostate Center (DPC) Symposium "LIVING WITH PROSTATE CANCER" were held in September 2008 at the Durham Marriott. Student, Postdoctoral Fellow, and scientist attended. The Fifth Annual Duke Prostate Center symposium is scheduled for Saturday, August 29, 2009. Students, Postdoctoral fellow, and scientists from NCCU are looking forward to participating in this event.

As stated in last year's progress report, our project entitled "Roles of Inflammation and Androgen Metabolism in Prostate Cancer Disparity" that was submitted in our EXPORT center grant has been funded. This grant provides each one of the co-PI's (Richardson, Chen and Grant) funding for

6 calendar months to support a postdoctoral fellow in their respective laboratory to increase research productivity.

Drs. Grant and Schildkraut USAMRMC training entitled: “Association of the UGT2B17 Gene Deletion Polymorphism and the Incidence of Ovarian Cancer and Ethnicity”. This project has allowed Dr. Grant to free more time from her teaching load to conduct research. She is currently writing a manuscript with the Duke team that focuses on ovarian cancer.

Our U54 3-way partnership proposal with Duke Comprehensive Cancer Center (DCCC) and UNC Lineberger Comprehensive Cancer Center (LCCC) entitled “NCCU-DCCC-LCCC Partnership in Cancer Research” received a priority score of **257**. Unfortunately, this is not a fundable score. We are currently revising the proposal for resubmission. A copy of the summary statement can be added to the progress report if needed.

Dr. Richardson’s RO1 grant entitled: Chemokine-mediated leukocyte functions has been funded for four more years.

Dr. Mukhopadhyay has submitted three grants as Principal Investigator:

1. CB2 Cannbinoid Receptor-mediated Regulation of Prostate Cancer Growth Submitted to NIGMS MBRS SCORE SC1 mechanism. (under review)

2. Targetting CB2 Cannbinoid Receptor in Prostate Cancer. RC1 NIH Recovery Act (under review)

3. RC2 NIA-NIH Recovery Act Grant Human Disease model in Zebrafish: Project 3: To generate a transgenic zebrafish model to study hypoxia-associated oxidative stress. (under review)

Dr. Chen has submitted five grants as Principal Investigator:

1. Investigator-initiated grant submitted to the Prevent Cancer Foundation in September 2008: “Chemopreventive mechanism of curcumin”. (not funded).

2. Investigator-initiated grant submitted to the ABMRF/the Foundation for Alcohol Research in September 2008: “Role of alcohol drinking in oral and prostate carcinogenesis”. (not funded).

3. R21 grant submitted to NIH in October 2008: “Identification of protein targets of curcumin for oral cancer prevention”. (**priority score: 198**; currently under revision)

4. R21 grant submitted to NIH in October 2008: “Role of Cdx2 in Barrett’s esophagus”. (unscored)

5. Investigator-initiated grant submitted to Takeda Pharmaceutical Inc. in November 2008: "Role of Nrf2/Keap1 pathway in esophageal development and resistance". (funded)

Task #2: Develop a core facility for the Collection of clinical samples and data.

a) Ms. Kelly Anderson, our research associate, left the program to go back to school. A new research associate, Ms. Kathryn Newman, has been recruited to assist the Duke team in the collection of clinical samples. She started on May 26th.

b) Because of the logistics concerning the accrual and distribution of samples with the Duke-VA, Ms. Newman is required to be on site 100% of the time. Thus, she was hired as a Duke employee. According to the original plan the Research Associate was supposed to be at Duke 50% of the time and, as a consequence, 50% salary support was requested in the original proposal. A revised budget reflecting this need was submitted and was approved.

c) Our IRB approval is now complete.

d) Dr. Grant and Dr. Freedland are currently training Ms. Newman in the collection, proper handling, and processing of tissue samples in the laboratory of Dr. Freedland at Duke.

Task #3: Develop 5 pilot studies focusing in the molecular, genetic socio-cultural aspects of prostate cancer incidence and disparities.

Below is the progress of each pilot project along with the significance and future directions.

Pilot Project #1: The UGT2B Gene Polymorphisms and its Association with Prostate Cancer Disparity

Investigator: Delores Grant, Ph.D. NCCU/BBRI
Collaborators: Cathrine Hoyo, Ph.D., Stephen Freedland, M.D., Joellen Schildkraut, Ph.D., Philip Febbo, M.D., Duke/Urology

A. Specific Aims

1. Determine whether there is an association between the *UGT2B17* deletion and *UGT2B15*^{D85Y} genotype in genomic DNA samples and

prostate cancer risk using a case control study in African and Caucasian population.
2. Compare expression of the *UGT2B17* gene in RNA samples from the prostate cancer cases and controls and determine whether these also vary by race;
3. Quantify serum glucuronides of testosterone and testosterone metabolites among controls to determine association with 0, 1, or 2 copies of *UGT2B17* and;
4. Compare expression levels of the *UGT2B17* gene in prostate cancer tissue and normal margins utilizing tissue microdissection and immunohistochemistry.

B. Studies and Results

In the second year of our study, we continue preparing IRB requests for the Veterans Administration Medical Center (VAMC) in Durham, the site where subjects will be accrued, North Carolina Central University (NCCU), the site of analysis, and the United States Army Medical Research and Material Command's (USAMRMC) Office of Research Protection (ORP), Human Research Protection Office (HRPO). We received initial approval to begin the accrual at the VAMC on May 8, 2009 and analysis at NCCU on March 13, 2009. Ms. Kelly Anderson, the research assistant, that was recruited and trained in year one, has been replaced by Ms. Kathryn Newman as of May 26, 2009 and is currently being trained in collecting peripheral blood, administering the 10-page questionnaire, entering data into an electronic data base, and preparing accrual reports. Other training will include assisting in amending Duke and the VA Institutional Review Board (IRB).

To accomplish training in population-based research, Dr. Freedland has included Dr. Grant in data analysis, manuscript review and publication activities in his group that are tangential to this project and documented in the publication section.

C. Significance

Successfully addressing the Aims outlined above will enhance our knowledge of prostate cancer by identifying a combination of biomarkers that could be used in conjunction with the PSA to improve our prediction of aggressive vs. non-aggressive prostate cancer at the early stage. Questionnaire data together with specimens collected using this funding will provide a resource for NCCU to explore additional hypotheses.

D. Plans

During the next and final funding cycle we will continue the accrual process approved on May 8, 2009. Drs. Freedland, Hoyo, and Grant will continue to train and co-mentor Ms. Tiffany Anderson, an NCCU Master of Science graduate student. As soon as feasible, we will be began laboratory and statistical analyses on data collected. If findings are promising, we will apply for additional funding to investigate this hypothesis in more samples.

E. Project-Generated Resources

Samples and questionnaire data will be generated and kept as a common resource for NCCU faculty. No other resources were generated.

Pilot Project #2: Role of β-arrestins in prostate cancer development and its contribution to Prostate Cancer Disparity

Investigator: M. Ricardo Richardson, PhD. NCCU/BBRI
Collaborators: Judd Moul, M.D., Duke/Urology

A. Specific Aims

1. To determine whether the level of expression of βarr-1 and/or βarr-2 are elevated in prostatic tissues from African American Men (AAM) relative to Caucasians American men (CAM).
2. To develop the TRAMP mouse model of prostate cancer in mice deficient in either βarr-1 or βarr-**2.**

B. Studies and Results

1. As reported last year, we have shown that expression of βarr-1, but not βarr-**2**, is markedly increased in prostatic cell lines from both AAM (E006AA) and CAM (PC3) compared to control prostate cell lines (RWPE1 and RWPE2). Because of the delay in the IRB approval, we have not been able to confirm these data in human samples from prostatic and control samples from both African American and Caucasian American men. Our goal is to determine whether overexpression of βarr-1 is associated with tumor aggressiveness. We hope to accomplish this goal this year.

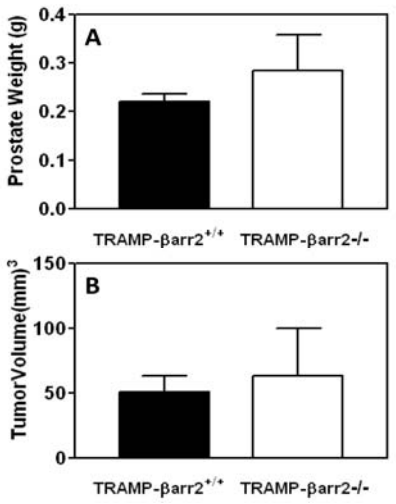

Fig 2-1. Tumor growth in TRAMP mice deficient in βarr-2 (TRAMP-βarr2$^{-/-}$) and control animals (TRAMP-βarr2$^{+/+}$). Prostate tumors (30 weeks) were isolated, weight and measured with a Thorpe caliper. Tumor volume was calculated using the formula: volume = (d_1 x d_2 x d_3) x 0.5236, where the d_n represents the three orthogonal diameter measurements. Tumor weight (A) and tumor volume (B).

2. We have obtained the final approval of our ACURO protocol and have bred the Transgenic Adenocarcinoma (TRAMP)-beta-arrestin 2 deficient mouse model (TRAMP-βarr2$^{-/-}$) with βarr2$^{-/-}$ knockout. We have followed the TRAMP-βarr2$^{-/-}$ along with control for the development of prostate tumor that is usually occur between 25 to 40 weeks in control mice. Up to 50 weeks, no significant difference in tumor volume or weight has been observed between the two groups. The survival rate between the two strains also overlaps suggesting that βarr2

plays no significant role in prostate tumor development and growth. Since the animal numbers were low, (5 TRAMP-βarr2$^{-/-}$ versus 5 TRAMP), our goal is to repeat the experiment with larger number of animals (20 versus 20) to repeat the experiments.

C. Significance

This project is specifically designed to address one significant issue in health disparity of prostate cancer, why African American men have a higher risk of developing prostate cancer and poorer clinical outcome than Caucasian American men.

D. Plans

1. To analyze prostatic tissues from human subjects and determine whether βarr1$^{-/-}$, not βarr2$^{-/-}$, promotes prostate tumor development and metastasis.

2. To generate a TRAMP-βarr1$^{-/-}$ mouse model to test the hypothesis. We have obtained the βarr1$^{-/+}$ mice from Dr. Lefkowitz laboratory at Duke. We will start breeding as soon as we can confirm the data using tissue samples.

Pilot Project #3: Role of 5-Lipoxygenase in Clinical Outcome of African American and Caucasian Prostate Cancer Patients

Investigator: Xiaoxin Chen, M.D., Ph.D., NCCU/BBRI
Collaborator: Leon Sun, M.D., Duke/Urology

A. Specific Aims

1. To determine whether expression and regulation of *Alox5* and *blt1* by promoter methylation and polymorphism may contribute to prostate cancer disparity between African American and Caucasian men.

B. Studies and Results

1. We have performed immunohistochemical staining of 5-lipoxygenase (5-Lox) on paraffin sections of 150 cases of African American prostate cancer and 150 cases of Caucasian American prostate cancer. Our research pathologist and postdoctoral fellow, Dr. Rong Qin, left our group. It took the new postdoctoral fellow, Bo Hu, some time to become familiar with the imaging system. We expect to start analysis of these samples in the next 3 months.

2. We have not yet reached a solid conclusion regarding the potential role of promoter methylation in regulating expression of these two genes. We examined the effect of 5-aza-2'-deoxycytidine (a DNA demethylating agent) on the expression of 5-Lox and BLT1 in two human prostate cancer cell lines (LNCaP, PC3) and two human normal prostate epithelial

cell lines (PrEC1 and PrEC2). Both RT-PCR and Western blotting failed to show dramatic up-regulation of these genes.

3. We examined expression of 5-Lox in the prostate tissues of wild-type mice and TRAMP mice. Significant overexpression of 5-Lox was observed in the mouse prostate tumor as compared with wild-type tissue. We have started to cross TRAMP mice with *Alox5* knockout mice to produce *Alox5*$^{-/-}$ TRAMP mice. The purpose is to determine whether knockout of *Alox5* may reduce tumorigenesis in TRAMP mice.

Initially we had problem in crossing TRAMP mice with Alox5 knockout mice. After trying several ways, we have obtained TRAMP/Alox5$^{-/-}$ mice (15 male and 5 female). All these mice and TRAMP/Alox5$^{+/-}$ mice are currently used for breeding more TRAMP/Alox5$^{-/-}$ mice. Since we can only use males for prostate cancer study, we expect another 3 months to generate enough animals for an animal experiment to compare TRAMP mice with TRAMP/Alox5$^{-/-}$ mice in prostate cancer development.

C. Significance

This project is specifically designed to address one significant issue in health disparity of prostate cancer, why African American men have a higher risk of developing prostate cancer and poorer clinical outcome than Caucasian American men.

D. Plans

1. To analyze the tissue samples which were stained for 5-Lox with an imaging system.
2. To improve our current methods of methylation analysis of the promoter regions of 5-Lox and BLT1 genes, and perform analysis of DNA samples from human prostate cancer tissues.
3. To breed male TRAMP/*Alox5*$^{-/-}$ mice and start an animal experiment to compare prostate cancer development in TRAMP and TRAMP/*Alox5*$^{-/-}$ mice.

Pilot Project #4: Anandamide-mediated Regulation of Prostate Cancer Cell Proliferation and Angiogenesis in African Americans

Investigator: Somnath Mukhopadhyay, PhD, NCCU/BBRI
Collaborator: Judd Moul, MD, Duke/Urology

A. Specific Aims

1) To define the role of CB1 and CB2 cannabinoid receptors in endocannabinoid methanandamide-mediated cell proliferation and androgen receptor expression in EA006AA African American prostate cancer cells.

2) Characterization of anandamide-mediated regulation of matrix metalloprotease (MMP) activity in E006AA prostate cancer cells.

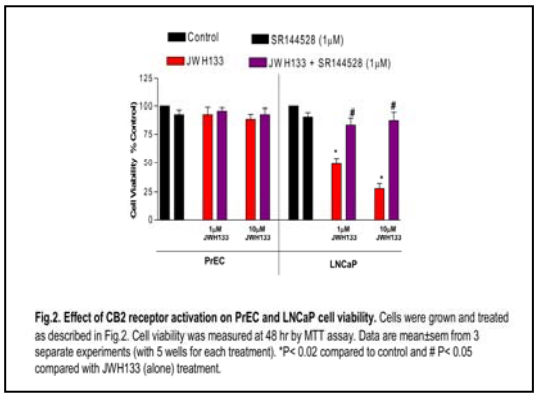

Fig.2. Effect of CB2 receptor activation on PrEC and LNCaP cell viability. Cells were grown and treated as described in Fig.2. Cell viability was measured at 48 hr by MTT assay. Data are mean±sem from 3 separate experiments (with 5 wells for each treatment). *P< 0.02 compared to control and # P< 0.05 compared with JWH133 (alone) treatment.

B. Studies and Results

In the previous progress report, it was indicated that in African American prostate cancer cell line (E006AA) the level of CB2 receptor (CB2R) expression is higher than normal prostate epithelial cells (PrEC). The data have shown that CB2 receptor activation (using CB2 receptor specific agonist JWH 133) produced significant decrease in cell proliferation and cell migration in a dose-dependent manner. It was also demonstrated that CB2R antagonist (SR144528) inhibited JWH133-induced decrease in E006AA cell migration and proliferation.

In the past year our research focused on the molecular signaling mechanism of CB2 receptor-mediated regulation of cell migration. We looked into CB2 receptor-mediated regulation of focal adhesion kinase (FAK) pathway in relation to cell migration. Since we were also interested to determine the differential effect (if any) of CB2 receptor activation between African-American prostate cancer cells vs. non -African-American prostate cancer cells, we first looked into the effect of CB2 receptor activation on FAK activation in non-African-American LNCaP prostate cancer cells. The findings of these experiments are described below:

1. First we found that under *in vitro* cell culture condition without any treatment (Control) LNCaP cells showed significant FAK phosphorylation at Tyr397 (Fig.1A).
2. Treatment with CB2 receptor agonist JWH133 produced a significant dephosphorylation at as early as 5 min of treatment and produced more robust dephosphorylation when tested at 15 min (Fig. 1A).
3. When cells were pre-treated with CB2 receptor antagonist SR1445282, FAK dephosphorylation at Tyr 397 was inhibited.

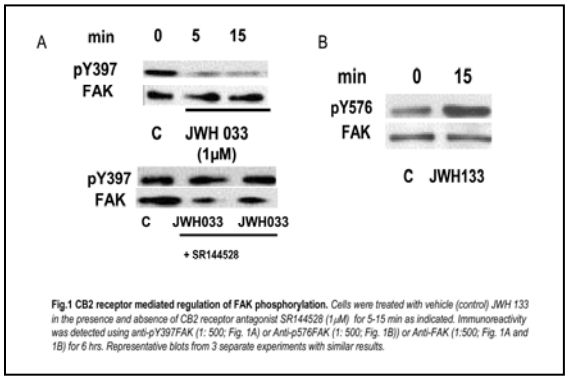

Fig.1 CB2 receptor mediated regulation of FAK phosphorylation. *Cells were treated with vehicle (control) JWH 133 in the presence and absence of CB2 receptor antagonist SR144528 (1μM) for 5-15 min as indicated. Immunoreactivity was detected using anti-pY397FAK (1: 500; Fig. 1A) or Anti-p576FAK (1: 500; Fig. 1B)) or Anti-FAK (1:500; Fig. 1A and 1B) for 6 hrs. Representative blots from 3 separate experiments with similar results.*

4. In contrast to dephosphorylation at Tyr397, JWH 133 treatment produced significant phosphorylation at Tyr 576 at 15 min compared to untreated controls.

This result clearly showed that activation of CB2 receptor produced differential effects on Tyr phosphorylation of FAK in a site-specific manner.

Besides looking into FAK phosphorylation, we have also looked into the effect of CB2 receptor activation on non malignant prostate epithelial cells and non-African-American prostate (LNCaP) cancer cells. As shown Fig.2, results from this set of experiments showed that CB2 receptor activation produced significant decrease in LNCaP cell viability but did not produced any significant change in non-malignant PrEc cell viability. These findings suggest that activation of CB2 receptor reduce cell viability of malignant cells with no effect on normal cells.

C. Significance

This project is addressing a health disparity of prostate cancer. Specifically, this project focuses on the effect of CB2 receptor activation on cell proliferation, viability and cell motility of non-African-American prostate cancer cells and African-American prostate cancer cells. The results from this study will be helpful to a) determine the differential effect of CB2 receptor activation in the regulation of prostate cancer in non African-American prostate cancer cells and African-American prostate cancer cells; b) validate CB2 receptor as a novel drug target for the regulation of prostate cancer. Thus, the outcome of this project may identify novel target for therapeutic intervention against prostate cancer.

D. Plans

1. To determine the effect of exogenous agonist mediated CB2 receptor activation as well as endogenous cannabinoid mediated CB2 receptor activation on FAK activity in African-American E006AA prostate cancer cells compared to non-African-American prostate cancer cells.

2. To elucidate the effect of exogenous agonist mediated CB2 receptor activation as well as endogenous cannabinoid mediated CB2 receptor activation on MMP activity in African-American E006AA prostate cancer cells compared to non-African-American prostate cancer cells.

3. To identify the signaling pathways that connects CB2 receptor activation with FAK and MMP regulation in relation to cell proliferation, cell viability and cell motility in African-American E006AA prostate cancer cells compared to non-African-American prostate cancer cells.

Pilot Project #5: Feasibility of Endurance Exercise Training on Cardiovascular Risk Factors Following Radical Prostatectomy among Men with Localized Prostate Cancer: A Community-Based Intervention

Investigators: Dave Tulis Ph.D., NCCU/BBRI

Collaborators: Catherine Hoyo, Ph.D., Lee Jones, Ph.D., Stephen Freeland, M.D., Duke/Urology

A. Specific Aims

1. To determine the effects of home-based endurance exercise training on exercise capacity following radical prostatectomy among with men with localized prostate cancer.
2. To assess the changes in other markers of CVD (i.e., lipid profile, blood pressure, fasting insulin, C-reactive protein, and weight status).
3. To explore the potential differential effects of exercise training between white and black American prostate cancer patients on specific aims 1 and 2.

B) Studies and Results

Dr. David Tulis the NCCU co-PI in this pilot project, left NCCU last September and accepted a faculty position at East Carolina University. Thus, since we do not have a faculty Dr Tulis expertise in the BBRI, this project has been discontinued. As shown in the last progress report, a questionnaire was developed. The data generated thus far has led to the submission of the manuscript entitled "Exercise is Associated with a Reduced Risk of Prostate Cancer in a Cohort of Veterans Undergoing Prostate Needle Biopsy" that has been accepted in the journal Urology. Below is the abstract:

Purpose: Molecular and epidemiologic evidence suggests a potential association between exercise and prostate cancer risk reduction. We sought to further characterize this relationship by examining the association between exercise and cancer risk among men undergoing prostate needle biopsy.
Materials and Methods: 342 men undergoing a prostate biopsy at the Veterans Affairs Hospital in Durham, NC were asked to complete a survey regarding current exercise behavior. Participants were asked average frequency of mild, moderate, and strenuous intensity exercise in a typical week, as well as average duration. Total current exercise was calculated in terms of metabolic equivalent task (MET) hours per week. The primary outcomes were prostate biopsy result and biopsy Gleason sum.
Results: 190 (56%) subjects provided written consent, had prostate biopsy pathology results available, and completed the exercise survey. After adjusting for age, race, body mass index, PSA, and digital rectal exam findings, men who reported being at least moderately active ($\geq$9 MET hrs/wk) were significantly less likely to have cancer on biopsy (OR=0.41, CI=0.20-0.85, p=0.02). Furthermore, among men with cancer, not being sedentary (i.e. $\geq$3 MET hrs/wk) was associated with a trend toward lower risk of high-grade disease, Gleason $\geq$7 (OR=0.48, CI=0.18-1.29, p=0.15).
Conclusions: Men who reported exercising more had a lower risk of having prostate cancer, and among men with cancer, they tended to have lower grade disease. Further investigation is required to confirm these findings in a larger sample size and to better characterize the molecular mechanisms through which exercise may affect the risk of developing prostate cancer, especially high-grade disease.

Task #4: Training Determine the effects of home-based endurance exercise training on exercise capacity following radical prostatectomy among with men with localized prostate cancer.

This task has suffered two setbacks. First, Dr. Oates the Postdoctoral Fellow at NCCU working with the researchers at Duke left the program to accept a faculty position at Tennessee State University (TSU). In addition, Dr. David Tulis (NCCU PI) who was working on the exercise project with Dr. Lee Jones (Duke PI), has recently accepted a position at East Carolina State University (ECU). As indicated early, the funds for this project were rebugeted to hire the Research Associate 100% at Duke.

Key Research Accomplishments: N/A

Reportable Outcomes:

Antonelli, J., Jones, LW, Banez, LL, Thomas, J-A, Anderson, K, Taylor, LA, Gerber, L, Crowe, N, Anderson, T, Hoyo, C, Grant, DJ, Freedland, SJ. 2009. Exercise is Associated with a Reduced Risk of Prostate Cancer in a Cohort of Veterans Undergoing Prostate Needle Biopsy. J. Urology. *(Accepted)*

Thomas, J-A, Antonelli, J, Banez, LL, Hoyo, C, Grant, DJ, Demark-Wahnefried, W, Platz, EA, Anderson, K, Taylor, LA, Gerber, L, Anderson, T, Crowe, N, Freedland, SJ. 2009. Androgenetic Alopecia and Prostate Cancer Risk in an Equal Access Multiethnic Case Control Series of Veterans. Cancer Epidemiology Biomarkers and Prevention. *(Submitted)*

Johnson, I., Saha, A., Schaller, M., Pizzo, S. and Mukhopadhyay, S. (2009) Activation of CB2 Cannabinoid Receptor regulates prostate cancer cell proliferation and motility. Cancer Research 2009 (*Submitted*)

Conclusion:

Our progress for the second year of the funding period of the NCCU-Duke Collaborative Center in Prostate Cancer has somewhat limited due to the delay in obtaining IRB and ACURO approval. Our collaborators at Duke have done a remarkable job throughout the process and NCCU scientists have learned a lot. We are awaiting the collection and processing of the tissues samples to confirm the data obtained in vitro and decide whether or not the aims of pilot #2 need to be amended. Another drawback this year is the departure of Ms Kelly Anderson, our research associate from the program after one year of intense training. She has been replaced with Ms. Kathryn Newman. Due budget shortages and State restriction in faculty hiring, we have not been able to replace Dr. David Tulis and Dr. Veronica Oates, the co-investigators in project #5. As a consequence, this project has been discontinued and the data collected have been accepted for publication. A total of four manuscripts directly related to the project has been submitted or accepted for publication. In addition, with the assistance of the duke collaborators, NCCU scientists have submitted nine grants.

Reference:

1. "The Nelson *Diversity* Surveys" Nelson, *D. J.*: Norman, OK, 2002; http://*cheminfo.chem.ou.edu/faculty/djn/diversity*

Appendix:

1. Exercise and Prostate Cancer Risk in a Cohort of Veterans Undergoing Prostate Needle Biopsy.

Exercise and Prostate Cancer Risk in a Cohort of Veterans Undergoing Prostate Needle Biopsy

Jodi A. Antonelli[1,2], Lee W. Jones[3], Lionel L. Bañez[1,2], Jean-Alfred Thomas[1,2], Kelly Anderson[1,2], Loretta A. Taylor[1,2], Leah Gerber[1,2], Tiffany Anderson[2,4], Catherine Hoyo[5], Delores Grant[4], Stephen J. Freedland[1,2,6]

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Funding/Support: Department of Veterans Affairs, Department of Defense, and the AUA Foundation/Astellas Rising Star in Urology Award

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Abstract

Purpose: Epidemiologic and molecular evidence suggest potential associations between exercise and prostate cancer risk reduction. We sought to further characterize this relationship by examining exercise and cancer risk among men undergoing prostate needle biopsy.

Materials and Methods: A total of 190 men who underwent a prostate biopsy at the Durham Veterans Affairs Medical Center completed a questionnaire on current exercise behavior. Participants were asked average frequency of mild, moderate, and strenuous intensity exercise in a typical week, as well as average duration, as assessed by the Godin Leisure Time Exercise Questionnaire. Total current exercise was calculated in terms of metabolic equivalent task (MET) hours per week. Primary outcome measures were prostate biopsy result and Gleason sum.

Results: After adjusting for age, race, BMI, PSA, DRE, family history, previous prostate biopsy, and comorbidity score men who reported ≥9 MET hrs/wk of exercise were significantly less likely to have cancer on biopsy (OR=0.35, CI=0.17-0.75, p=0.007). Furthermore, among men with malignant biopsy results, reporting moderate exercise (3-8.9 MET hrs/wk) was associated with a lower risk of high-grade disease, Gleason ≥7 (OR=0.14, CI=0.02-0.94, p=0.04).

Conclusions: These results provide the first evidence for an association between exercise and prostate cancer risk as well as grade at diagnosis in men scheduled to undergo prostate biopsy. Specifically, moderate exercise was associated with a lower risk for prostate cancer and among men with cancer, lower grade disease. Further investigation using an objective measure of exercise in a larger sample size is required to confirm these findings.

Introduction

Exercise is a pleiotropic intervention that has been postulated to influence multiple molecular pathways implicated in prostate cancer tumor biology. Specifically, exercise has been shown to lower serum levels of metabolic and sex steroid hormones hypothesized to stimulate prostate cancer, including insulin-like growth factor-1 (IGF-1), insulin, leptin, and testosterone.[1-3] Chronic exercise is also a potent stimulator of endogenous antioxidant protection pathways thereby decreasing lipid peroxidation and reducing reactive oxygen species.[4] One possible mechanism responsible for the age-associated increase in prostate cancer incidence is oxidative damage from reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxide.[5] Previous studies have suggested that the immune system may have an impact on prostate cancer pathogenesis[6] and the literature supports that exercise may improve innate immune function and surveillance.[7]

By exerting the aforementioned biological actions, exercise has been postulated to confer a prostate cancer risk reduction. Numerous studies investigated the potential protective effect of exercise against prostate cancer risk.[8-13] While many studies found increased exercise was associated with reduced prostate cancer risk,[8-11] not all studies concurred.[12, 13] To date, no prior study has examined the association between exercise and prostate cancer among men undergoing prostate biopsy. We prospectively enrolled men scheduled to undergo prostate biopsy, assessed current exercise behavior using a validated questionnaire, and examined the association between current exercise, prostate cancer risk, and tumor grade. We hypothesized that those who exercised more would be less likely to have prostate cancer and among men with cancer, less likely to have high-grade disease.

Materials and Methods

Study population

After obtaining institutional review board approval, male patients of the Durham Veterans Affairs Medical Center (DVAMC) in Durham, NC scheduled to undergo a prostate needle biopsy between January 2007 and October 2008 were eligible to participate in this study. A total of 288 subjects, without a known diagnosis of prostate cancer, underwent a prostate biopsy and had pathological results available for review; 50 men declined participation in the study. Of the remaining 238 men who signed the informed consent form (83%), we excluded 22 due to incomplete exercise questionnaires and 17 due to missing data on height, weight, or documented pre-biopsy digital rectal exam (DRE), resulting in a final study population of 190 (66%).

Exercise was assessed using the leisure score index (LSI) of the Godin Leisure-Time Exercise Questionnaire[14] which was given to the participant by study personnel at an appointment on the morning of, and prior to the prostate biopsy. Participants were instructed to complete the questionnaire at home at their convenience and return it to study personnel in a provided, pre-addressed stamped envelop. The LSI contains three questions that assess the average frequency of mild, moderate, and strenuous intensity exercise during free time in a typical week. There is also a corresponding question for each of the three exercise intensities that asks the average duration in minutes for a typical session of activity within that exercise intensity. Participants were asked to respond based on current exercise behavior.

To calculate the metabolic equivalent task (MET) hours of total current exercise, the frequency of exercise sessions per week within each intensity category was multiplied by the average reported duration in hours, weighted by an estimate of the MET for that intensity,

summed across all intensities, and expressed as average total MET hrs/wk. The weighted values for each exercise intensity were as follows: mild (3 METs, e.g., easy walking, yoga), moderate (5 METs, e.g., brisk walking, tennis), and strenuous (9 METs, e.g., running, vigorous swimming). For example, for a subject who reported mild activity for one hour three times a week and moderate activity for thirty minutes twice a week, the following calculation would be used to compute total current MET hrs/wk of exercise, 3 x (1 x 3) + 5 x (0.5 x 2) = 14 MET hrs/wk.

Statistical analysis

A joint recommendation of the American College of Sports Medicine (ACSM) and the American Heart Association (AHA) published in 2007 stated that 7.5-12.5 MET hrs/wk is the minimal amount of activity recommended to achieve substantial health benefits over and above the routine activities of daily living.[15] The exercise categories for this study, chosen based upon this recommendation and previous work among women with breast cancer,[16] were <3 (sedentary), 3-8.9 (mildly active), 9-17.9 (moderately active), and ≥18 MET hrs/wk (highly active). This corresponds to walking at an average pace for <1, 1-2.9, 3-5.9, and ≥6 hrs/wk.

The association between exercise as a categorical variable and other continuous or categorical variables was assessed using the Kruskal-Wallis test or chi-square test, respectively. Age, prostate specific antigen (PSA), body mass index (BMI), number of previous prostate biopsies, and Charlson comorbidity score[17] were examined as continuous variables and race (black vs. non-black), family history (first-degree relative vs. negative), previous prostate biopsy (yes vs. no) and DRE finding (normal vs. suspicious for cancer) were examined as categorical variables. PSA and BMI were logarithmically transformed due to their non-normal distribution. Our primary outcomes were the comparisons between exercise and biopsy status (positive or

negative) among all participants and exercise and Gleason sum (<7 or ≥7) among men with cancer. To determine whether exercise independently predicted biopsy status or high-grade disease, we used a multivariate logistic regression analysis adjusting for age, race, serum PSA, BMI, DRE finding, family history, previous prostate biopsy, number of previous negative prostate biopsies, and Charlson comorbidity score[17]. In the analysis predicting high-grade disease, only men with a positive biopsy were included. Associations with a $p<0.05$ were considered statistically significant. All statistical analyses were performed using STATA version 10.0 (StataCorp, College Station, Texas).

Results

The clinical and demographic data for the 190 men included in this study are shown in Table 1. Of these men, prostate biopsy revealed cancer in 79 subjects (42%), 35 (44%) of whom had high-grade disease defined as a Gleason sum ≥7. A total of 111 (58%) subjects reported <3 MET hrs/wk of exercise and were considered sedentary, while 33 (17%) subjects reported ≥18 MET hrs/wk and were considered highly active.

Exercise was significantly associated with age (p=0.04) and comorbidity score (p=0.03) (Table 1), which was driven by the higher median age and comorbidity score in the 9-17.9 MET hrs/wk category. There were no significant associations between exercise and race, BMI, PSA, DRE, family history, and previous biopsy status. Increasing amounts of exercise were significantly associated with benign biopsy status (p=0.05) and low-grade disease (Gleason sum <7) (p=0.05) (Table 1).

In multivariate analysis, any level of exercise (≥3 MET hrs/wk) was associated with a trend toward a lower risk of having cancer on biopsy (OR=0.56, CI= 0.30-1.06, p=0.07). As the amount of exercise increased the risk reduction for having cancer on biopsy also increased (Table 2). Specifically men who reported 9-17.9 MET hrs/wk had a lower risk of having cancer (OR=0.44, p=0.14), though this did not reach statistical significance. However, men exercising ≥18 MET hrs/wk were at a significantly lower risk of having cancer on biopsy (OR=0.30, p=0.01) (Table 2). When we combined these two groups, there was a statistically significant reduction in cancer risk for men exercising ≥ 9 MET hrs/wk (OR=0.35, p=0.007) (Table 3).

Among men with cancer, on multivariate analysis, there was a trend toward a lower risk of high-grade disease among men who reported being active at any level (i.e. ≥3 MET hrs/wk) (OR=0.41, CI=0.13-1.28, p=0.13). When this was analyzed further, men who engaged in

moderate exercise (3-8.9 MET hrs/wk) had a significantly lower risk of high-grade disease on biopsy (OR=0.14, p=0.04), while exercising beyond 9 MET hrs/wk was not significantly linked with lower risk of high-grade disease (OR=0.52, p=0.38) (Table 4).

Discussion

Despite numerous epidemiological studies investigating the association between exercise and prostate cancer risk, the existence and/or nature of this association remains unclear.[18] We sought to further characterize this association by examining self-reported exercise in veterans scheduled to undergo prostate biopsy. We found that men exercising ≥9 MET hrs/wk were significantly less likely to have cancer on biopsy. For men with cancer, those reporting moderate exercise (3-8.9 MET hrs/wk) had lower grade disease. If confirmed in future studies, these findings suggest that a moderate amount of exercise may lead to a reduced risk of being diagnosed with prostate cancer and for men with cancer, may portend a lower risk of high-grade disease.

Over the last several decades, numerous studies have examined the effect of physical activity, including recreational, occupational, and/or household activities and incidence of prostate cancer in a variety of cohorts from Finnish athletes to healthcare professionals.[8-13] More than half of these studies found an inverse relationship between exercise and prostate cancer risk, while other studies showed no association, and a few even found exercise increased prostate cancer risk.[18] The majority of these studies were case-control designs in which exercise was self-reported and cancer incidence was ascertained from medical records and/or state cancer registries.

Several biochemical mechanisms lend support for the link between exercise and prostate cancer risk reduction proposed by much of the epidemiological literature. Specifically, chronic exercise is a potent stimulator of endogenous antioxidant protection pathways,[4] promotes innate immune function and surveillance,[7] and leads to reductions in systemic inflammation and pro-inflammatory factors.[19] Additionally, aerobic exercise lowers serum levels of sex steroid

hormones and several metabolic substances hypothesized to stimulate prostate cancer, such as testosterone, IGF-1, insulin, and leptin.[1-3] The aforementioned pathways altered by exercise are hypothesized to be important in prostate cancer pathogenesis and thereby provide biochemical evidence to further support a potential link between exercise and prostate cancer.

While no single study is going to clarify the complex association between exercise and prostate cancer risk, we hoped to shed some light on the matter by studying this association in a novel population. The current study is unique because the cohort consisted of men recommended to undergo prostate biopsy based upon elevated PSA and/or suspicious DRE; a population for which exercise and prostate cancer risk has not been explored previously. Presumably, men without cancer on biopsy (i.e. the "control" group) had a benign explanation for their elevated PSA and/or abnormal DRE such as benign prostatic hyperplasia (BPH). Therefore our control group was likely enriched for men with BPH. The significance of this is that prior studies have suggested lack of physical activity may be a risk factor for BPH.[20, 21] Thus, it is possible our "control" population, by the mere fact it was enriched for BPH patients, had lower physical activity than more typical "healthy" control populations. Indeed, this is reflected by the fact that 50% of our "controls" reported being sedentary. Therefore, the fact that men with prostate cancer had even lower levels of exercise than a group with lower than typical physical activity, is noteworthy. Moreover, these data suggest that while both prostate cancer and BPH may be correlated with lack of physical activity, the association with prostate cancer may be stronger.

In addition to lower cancer risk, we also found that men who exercised between 3-8.9 MET hrs/wk were less likely to have high-grade tumors. While other studies have examined the relationship between exercise and advanced and/or metastatic cancer, only one has investigated exercise and tumor grade at diagnosis. In a sub-analysis of their data, Giovannucci et al. using a

prospective cohort study found a statistically significant inverse relationship between vigorous physical activity and Gleason grade at diagnosis for case subjects 65 years or older.[9] Thus, our data, albeit in a much smaller population (n=80 cases vs. 1800), found similar results in that moderate exercise appeared to be associated with lower grade disease. We recognize that defining "high-grade disease" as Gleason sum $\geq$ 4+3 would potentially be a more accurate description of high-grade disease as Gleason 3+4 and 4+3 are clinically distinct entities, however, due to only a small number of patients in this cohort having a Gleason sum $\geq$ 4+3 (n=17) we extended the definition to include Gleason sum $\geq$ 7.

The current results must be interpreted in light of certain limitations. This cohort of Veteran Affairs medical center patients represents a referral population and not a true screening population. Therefore one must exercise caution when generalizing these results outside of this specialized population. Furthermore, the number of men in this study was small and thus our results are subject to type I error and thus these results should be interpreted with caution. Also it is highly conceivable that increased exercise correlated with other healthy behaviors such as diet or other unmeasured confounders. While we saw no association between exercise and obesity measures, this does not negate the possibility of an association between exercise and diet or other factors. As such, we are unable to state whether exercise alone is responsible for the benefits seen in this study.

Another potential limitation of this study includes the self-reported nature of the exercise data. This limitation could have led to both an over and an underestimation of the amount of exercise subjects reported. Specifically, 58% of subjects reported participating in <3 MET hrs/wk which corresponds to walking at an average pace <1 hr/wk. Although this limitation is important to recognize, it also should be noted that the exercise survey has important strengths.

In addition to being a validated instrument to assess exercise behavior, the timing of the questionnaire supports a non-biased representation of exercise across all subjects in that participants completed the survey prior to knowing the result of their prostate biopsy; therefore, subjects were not influenced by their biopsy status when reporting their activities. Moreover, if exercise was misreported by some, this should not be differential by biopsy status and therefore this would tend to bias the results toward the null. Therefore, the reduced risk of prostate cancer associated with exercise in this study may actually be underestimated.

Conclusions

We found that engaging in exercise which meets the ACSM/AHA recommendations for adults, specifically ≥9 MET hrs/wk of exercise, was associated with a significant risk reduction in prostate cancer in a cohort of veterans undergoing prostate biopsy. Furthermore, among men with cancer on biopsy, engaging in moderate exercise, specifically 3-8.9 MET hrs/wk, was associated with lower grade disease (Gleason sum <7). Our results lend further support to the hypothesis that exercise may lower the risk of prostate cancer and for men with cancer may lower the risk for high-grade disease. These results are consistent with biochemical evidence which has shown exercise affects molecular pathways central to prostate cancer tumor biology. Studies utilizing objective measures of exercise in a larger sample size are needed to better determine the true association between exercise, prostate cancer risk, and tumor grade.

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