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TITLE: Tumor Microenvironment-Based Biomarkers in African American Prostate Cancer

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14. ABSTRACT

African American (AA) men have a higher incidence and significantly higher mortality rates from prostate cancer (PCa) than European American (EA) men. The central problem addressed in this proposal is to understand the biological basis for the more aggressive clinical behavior of PCa in AA men, to develop predictive tools to help manage PCa in AA men and identify novel therapeutic targets in PCa in AA men. We will test the hypothesis that AA PCa has both more extensive reactive stroma formation than in EA PCa and that there are qualitative differences in protein expression in the reactive stroma of AA PCa compared to EA PCa as well. Furthermore, we will determine if these differences in reactive stroma can explain, at least in part, the more aggressive clinical behavior of AA PCa. Our objective is to develop novel predictive tools that will be useful in treatment planning in AA men with PCa.

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NONE LISTED

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1. INTRODUCTION:

African American (AA) men have a higher incidence and significantly higher mortality rates from prostate cancer (PCa) than European American (EA) men. The central problem addressed in this proposal is to understand the biological basis for the more aggressive clinical behavior of PCa in AA men, to develop predictive tools to help manage PCa in AA men and identify novel therapeutic targets in PCa in AA men.

We have shown previously that formation of extensive reactive stroma in PCa is associated with biochemical recurrence and PCa specific death in primarily EA cohorts. In addition, we have shown that extensive reactive stroma is associated with specific gene expression changes and that these genes promote tumor progression in tissue recombination model systems. We will test the hypothesis that AA PCa has both more extensive reactive stroma formation than in EA PCa and that there are qualitative differences in protein expression in the reactive stroma of AA PCa compared to EA PCa as well. Furthermore, we will determine if these differences in reactive stroma can explain, at least in part, the more aggressive clinical behavior of AA PCa. Our objective is to develop novel predictive tools that will be useful in treatment planning in AA men with PCa.

It is important say a few words regarding the impact of COVID-19 on our research. Like most research institutions, we were shut down completely for many months. We have proceeded with reopening but in stages. Not only was time lost for experimentation but we also had to freeze all cell lines before we left the lab such that they had to be regrown (which takes considerable time) and thus we actually went backwards. Animal experiments were also shut down and mouse numbers were severely restrained. While Houston is emerging from a very bad COVID-19 surge in July, there does remain some uncertainty regarding the fall. However, we are now getting back on track and hopefully will be able to continue to accelerate over the coming year.

2. KEYWORDS: prostate cancer, African American, stroma

3. ACCOMPLISHMENTS:

A. Major Goals

Major Task 1: Obtain regulatory approvals (Months 1-4)

All regulatory approvals have been obtained and maintained

Major Task 2: Evaluate biology of reactive stroma in AA men using in vivo tissue recombination models (Months 4-36)

Subtask 1: Examine role of FAP in tumor progression.

We have initiated these experiments and have established 19I stromal cells with fibroblast activation protein (FAP) overexpression. These have been used to carry out a 2-way DRS experiment [1]with LNCAP cells expressing luciferase. As shown in Figure 1, FAP expression in 19I stromal cells increases tumor growth.

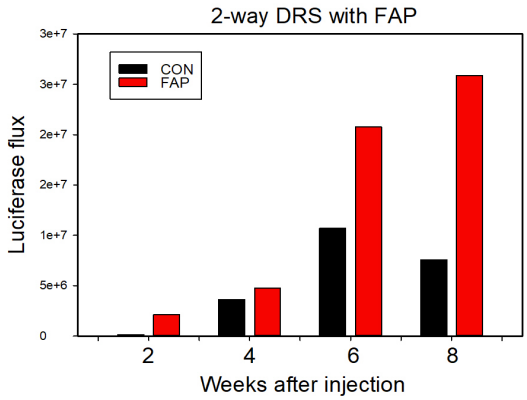

Figure 1. Enhanced tumor tumor by stromal FAP expression. LNCaP cells expressing luciferase were mixed with 19I prostate stromal cell expressing FAP or vector control cells (CON) and injected subcutaneously in SCID mice (2-way DRS).

Mice were imaged after luciferin injection using an IVIS imager to quantitate luciferase activity in tumors. Mean luciferase activity is shown.

The converse experiment in which FAP is knocked down in 19I cells has proven more difficult. FAP knockdown slows growth of 19I cells, suggesting that FAP promotes growth of both stromal and cancer cells. The 19I stromal cells are intrinsically slow growing so it has been difficult to establish clonal cell lines with FAP knockdown. Our new strategy is to start with a larger number of 19I cells and infect with lentivirus expressing 19I siRNA. We will then use these cells (and vector controls) after a few passages for in vivo experiments after confirming 19I knockdown in siRNA infected cells.

Subtask 2: Evaluate role of FXII in tumor progression.

We have cloned a Flag-tagged FXII gene into pCDH and pCMV lentivirus expression vectors. As shown in Figure 2, both express FXII protein at robust levels, with pCMV showing superior expression. We have recently begun establishing 19I-FXII cell lines after recovering the 19I cell line, which had been frozen down during the COVID-19 pandemic closure.

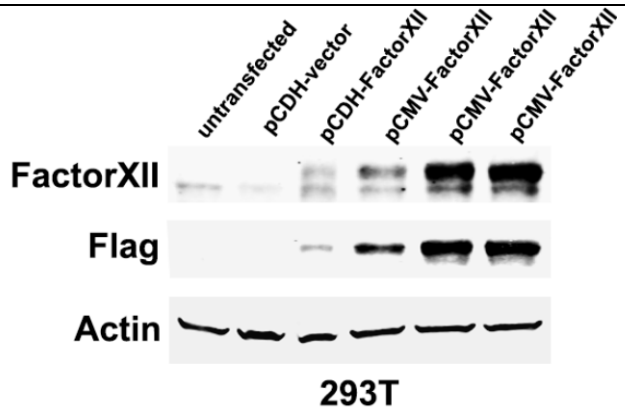

Figure 2. Expression of FX11 in 293T in two different expression vectors.

Flag-tagged FXII gene in pCDH or pCMV expression vector was transfected into 293T cells and Western blots performed with anti-FXII antibody, anti-Flag and anti-actin. Robust FXII was seen with both constructs with pCMV having higher than pCDH.

Major Task 3: Reactive stroma and stromal markers of disease progression in AA PCa (Months 4-36)

Subtask 1: Quantitative Reactive Stroma grading in a population of AA patients to select those who need adjuvant treatments above therapy standard of care.

We have scanned the 256 case tissue microarray (TMA) of African American prostate cancers and a similar EA TMA of 206 cases from the Michael E DeBakey VA Medical Center. An example of image segmentation of one of the cores is shown in Figure 2.

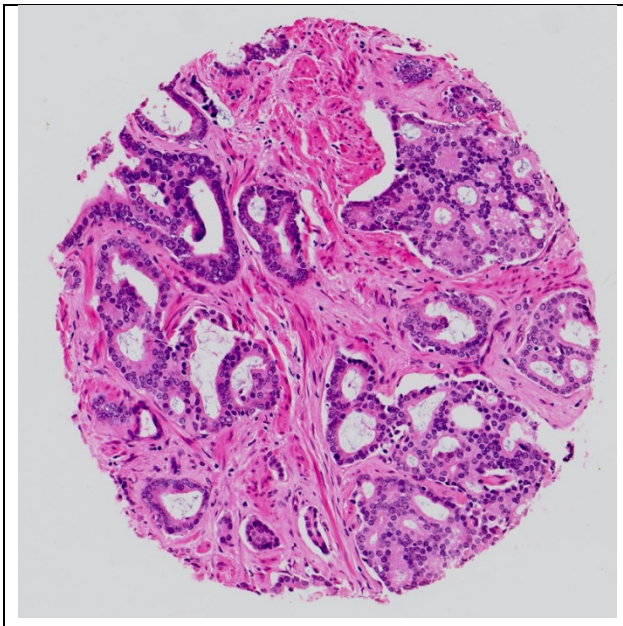

Figure 3A. H&E image of cancer core

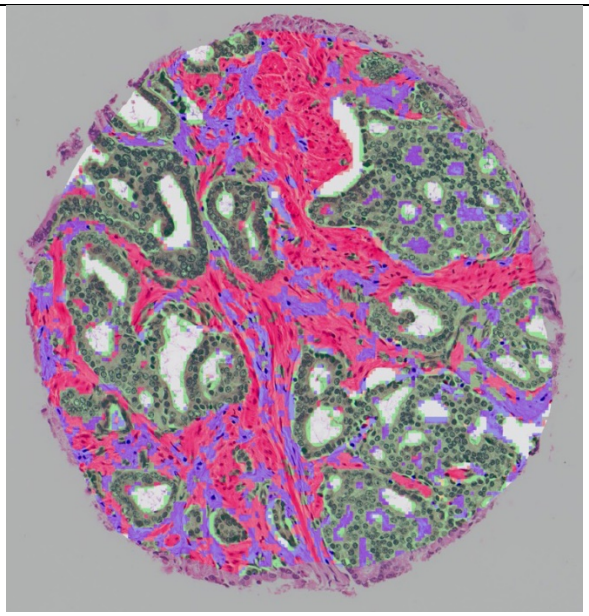

Figure 3B. Tissue segmented image of core shown in Fig 3A.

Analysis of the percentage reactive stroma in the African American and European American prostate cancers by QRS analysis in these arrays confirms our hypothesis that AA PCa has significantly higher reactive stroma than EA PCa. The mean percent reactive stroma was significantly higher in AA versus EA PCa , specifically 8.6 +/- 0.6 Vs 3.7 +/- 0.3, $p<.001$ (mean +/-SEM, Mann-Whitney). Breaking the data into groups by percent reactive stroma indicates that AA PCa is significantly skewed toward higher reactive stroma (Figure 4). Overall, 32% of AA PCa have 10% or more reactive stroma while only 8.7% of EA PCas have >10% reactive stroma

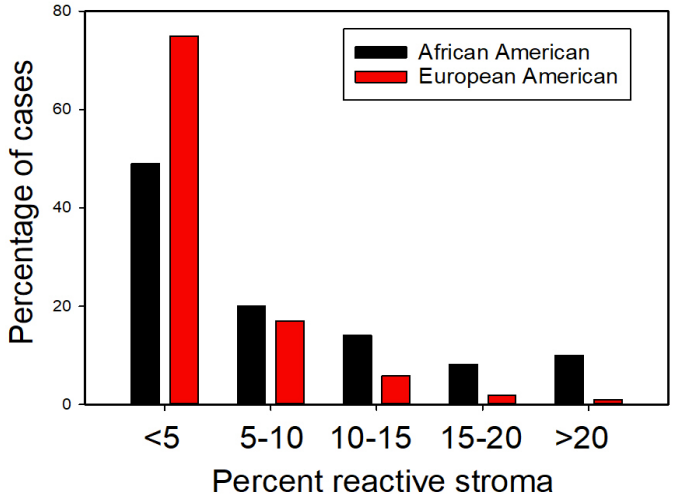

Figure 4: Reactive stroma in AA and EA prostate cancer

Percentage of total cases of AA oEA prostate cancer with the indicate percent reactive stroma by QRS analysis

Subtask 2: Validation studies of qRS.

We have obtained the 132 case African American and 132 case European American TMA's from NYU from the DOD Prostate Cancer Biorepository Network along with associated de-identified data. We have also obtained and scanned the AA and EA TMAs from Washington University from the DOD Prostate Cancer Biorepository Network along with associated de-identified data.. These TMAs have been scanned and QRS analysis is complete. Data analysis is in progress

Subtask 3: Analysis of novel stromal biomarkers found in AA populations with PCa.

We are in the process of validating antibodies COMP, FAP and CXCL14 for immunohistochemistry. To date, the COMP and CXCL14 antibodies have been validated.

B. Training and Professional Development

Nothing to report

C. Dissemination to communities of interest

Nothing to report

D. Plans for coming year

We plan to proceed with the outlined Statement of Work. We have not had any major technical issues that constitute a major impediment to our planned experiments. However, the rate of progress will in part be determined by the course of the COVID-19 pandemic in Houston. We are cautiously optimistic that the worst is over.

4. IMPACT

The data presented here and the preliminary data presented in our application have proven our underlying hypothesis that AA PCa has more abundant reactive stroma than EA PCa. In the coming year our goal is to further validate this finding, understand its clinical significance and explore the underlying biology of reactive stroma in AA PCa.

5. CHANGES/PROBLEMS

See above regarding FAP knockdown cells. COVID-19 has significantly impacted our progress in the past year as described above but we are getting back to the laboratory.

6. PRODUCTS

We have developed a FAP overexpressing 19I stromal cell line. Other 19I cell lines are under development.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Participants

Name: Michael Ittmann MD PhD
Project Role: Principal investigator
Nearest person month worked: 1.2 calendar months
Contribution to Project: Overall coordination and data analysis
Funding Support: The following changes in funding support have occurred since this proposal was activated:

Merit Review (Ittmann) 4/1/2019-3/31/2023 3.0 calendar
Dept of Veterans Affairs
A Novel Oncogenic Axis in African American Prostate Cancer
The goal of this project is to characterize the role of RGS12 in African American prostate cancer. No overlap.

DOD Prostate Cancer Research Program Idea (Mitsiades) 10/1/2018-9/30/2021 0.6 calendar
DOD Prostate Cancer Program
Sensitization of castration resistant prostate cancer to chemotherapy via BRCA-1/BRCA-2 induced DNA replication stress
The goal of this proposal is to enhance the efficacy of chemotherapy in advanced prostate cancer by inducing DNA replication stress. Dr. Ittmann is providing pathology support. No overlap.

RO1CA227559 (Sreekumar/Palapattu) 05/01/2019-04/31/2024 0.12 calendar
NIH
Metabolic Rewiring Promotes AA PCa by Regulating Stromal-Epithelial Interaction
The goal of this proposal is to examine metabolism in African American prostate cancer Dr. Ittmann is providing pathology support. No overlap.

PC181023 (Lotan) 9/1/2019-8/31/2022 0.24 calendar
DOD Prostate Cancer Research Program HDA
Epigenomic Landscape of Primary Prostate Cancer in African American Men
The goal of this proposal is to examine the epigenetic alterations in African American prostate cancer. We will provide samples for a validation cohort. No onerlap

2U54MD007597-31 (Kwabi-Addo) 08/01/19-07/31/24 0.6 calendar
NIH $56,681 (BCM directs)
Epigenetic regulated genes in African American Prostate Cancer Patients
The goal is to understand the biological basis for the more aggressive clinical behavior of prostate cancer in African American men and to begin to develop predictive tools to help manage prostate cancer in African American men. No overlap.

W81XWH-19-1-0410 (Frigo) 7/15/2019-7/14/2022 0.6 calendar
DOD Prostate Cancer Program $7,964 (salary support only)
Therapeutic Targeting of the Glutamine Transporter SLC1A5 in Advanced Prostate Cancer
To rigorously evaluate SLC1A5's role in prostate cancer metabolism and test whether SLC1A5 represents a viable therapeutic target. No overlap.

Name: Gustavo Ayala MD
Project Role: Qualified Collaborator
Nearest person month worked: 1.2 calendar months
Contribution to Project: Coordinating of human tissue analysis efforts and data analysis
Funding Support: None

Name: MinJae Lee, PhD
Project Role: Biostatistician
Nearest person month worked: 1.2 calendar months
Contribution to Project: Dr. Lee is analyzing the tissue microarray data
Funding Support: None

Name: Jianghua Wang MD
Project Role: Co-investigator
Nearest person month worked: 6 calendar months
Contribution to Project: Dr. Wang has carried out all the biological experiments described in Major Task 1, above

Name: Yi Ding, Ph.D
Project Role: Co-investigator
Nearest person month worked: 3.0 calendar months
Contribution to Project: Dr. Ding is responsible for all technical aspects described in Major Task 3, above

Collaborating organizations

This proposal was funded as a collaboration between Dr. Ittmann and his group at Baylor College of Medicine (BCM) and Dr. Ayala and his group at University of Texas Health Science Center (UTHSC) School of Medicine. We are located across the street from each other in the Texas Medical Center in Houston, TCX

Organization Name: Baylor College of Medicine
Location of Organization: One Baylor Plaza, Houston, TX 77030
Partner's contribution to the project: The biological experiments are primarily carried out at BCM with some tissues supplied by BCM as well
Financial support: The grant independently funds efforts at BCM
Facilities: BCM has independent facilities
Collaboration: We collaborate as needed on a daily basis
Personnel exchanges: No exchange of personnel
Other: None

Organization Name: University of Texas Health Science Center (UTHSC) School of Medicine
Location of Organization: 6431 Fannin St Houston, TX 77030
Partner's contribution to the project: The tissue based analysis is being carried out primarily at UTHSC
Financial support: The grant independently funds efforts at UTHSC
Facilities: UTHSC has independent facilities
Collaboration: We collaborate as needed on a daily basis
Personnel exchanges: No exchange of personnel
Other: None

8. REFERENCES

1. Dakhova O, Rowley D, Ittmann M: Genes upregulated in prostate cancer reactive stroma promote prostate cancer progression in vivo, Clin Cancer Res 2014, 20:100-109

9. APPENDICES: None