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

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CONTRACTING ORGANIZATION: BAYLOR COLLEGE OF MEDICINE, Houston, TX

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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.

15. SUBJECT TERMS
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.

Unfortunately, COVID-19 has had a major negative impact on our research. Like most research institutions, we were shut down completely for many months and then proceeded with reopening 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.

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 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.

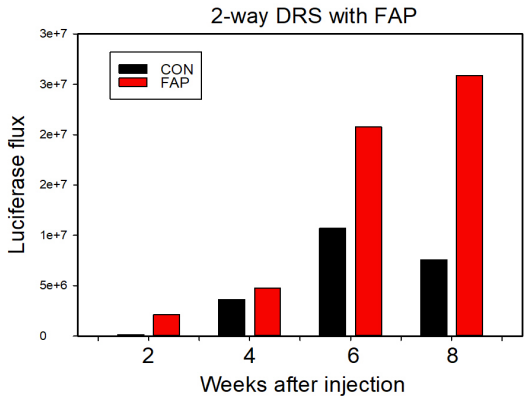

Figure 1. Enhanced tumor tumor by stromal FAP expression. LNCaP cells expressing luciferase were mixed with 19I prostate stromal cell expressing FAP or vector control cells (CON) and injected subcutaneously in SCID mice (2-way DRS).

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 markedly 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.

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. COVID19 related issues have prevented us from completing in vivo studies.

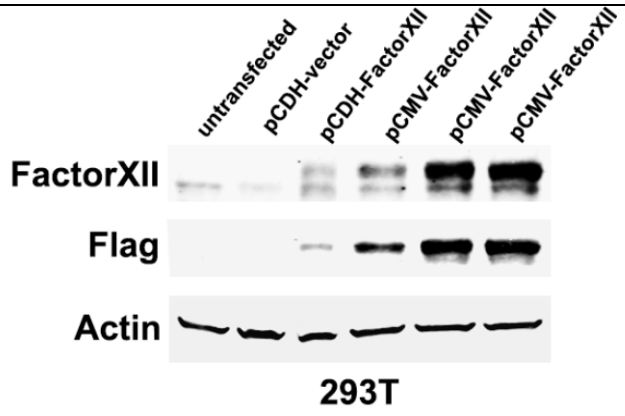

Figure 2. Expression of FX11 in 293T in two different expression vectors.

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.

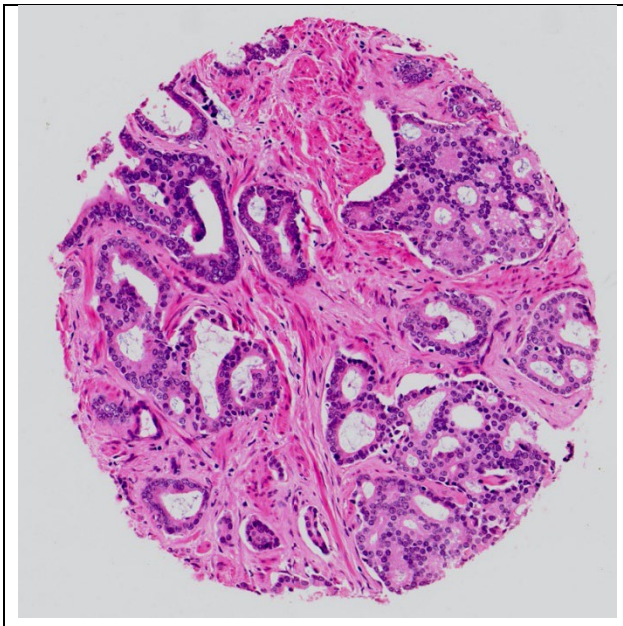

Figure 3A. H&E image of cancer core

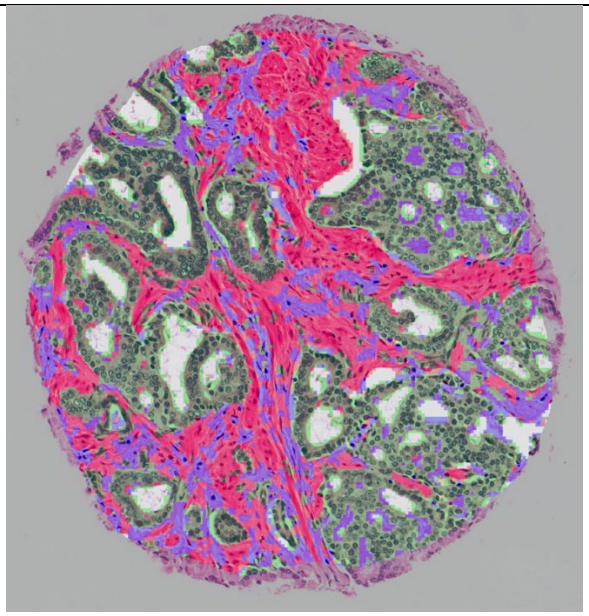

Figure 3B. Tissue segmented image of core shown in Fig 3A.

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

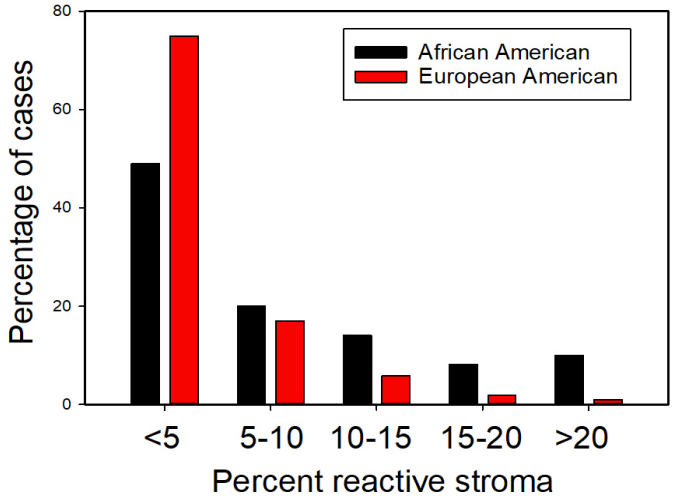

Figure 4: Reactive stroma in AA and EA prostate cancer in VA cohort

Percentage of total cases of AA or EA prostate cancer with the indicated percent reactive stroma by QRS analysis

Subtask 2: Validation studies of qRS.

To validate qRS Kaplan Meier analysis was used to determine survival in a large retrospective cohort of radical prostatectomy samples. Then qRS was validated in two additional, distinct cohorts that include international cases and tissue from both radical prostatectomy and biopsy specimens. In the developmental cohort (Baylor College of Medicine, n =482), patients whose tumor had qRS > 34% had increased risk of prostate cancer-specific death (HR 2.94; p=0.039). This result was replicated in two validation cohorts, where patients with qRS > 34% had increased risk of prostate cancer-specific death (MEDVAMC; n =332; HR 2.64; p =0.02) and also biochemical recurrence (Canary; n= 988; HR 1.51; p=0.001). By multivariate analysis, these associations were shown to hold independent predictive value when compared to currently used clinicopathologic factors including Gleason score and PSA. Thus qRS is a new, validated biomarker that predicts prostate cancer death and biochemical recurrence across three distinct cohorts. It measures host-response rather than tumor-based characteristics, and provides information not represented by standard prognostic measurements. These results have recently been published in *Human Pathology*[2]

We obtained the 132 case African American and 132 case European American TMAs from NYU from the DOD Prostate Cancer Biorepository Network along with associated de-identified data. These arrays have been scanned and confirm that AA PCa has a higher percentage of reactive stroma than EA PCa (p=.016, Mann-Whitney). Data is shown in Figure 5.

We have also 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.

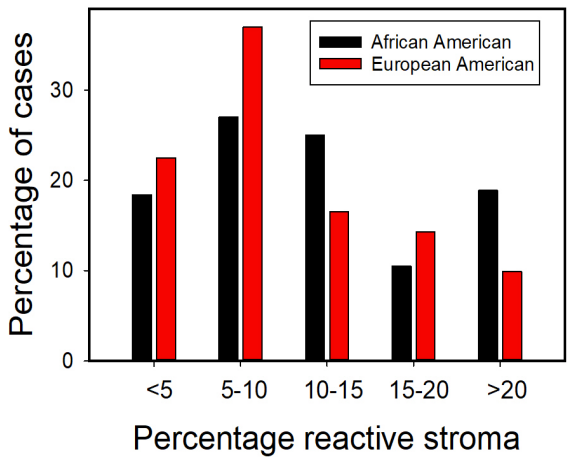

Figure 5: Reactive stroma in AA and EA prostate cancer in NYU cohort

Percentage of total cases of AA or EA prostate cancer with the indicated percent reactive stroma by QRS analysis

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

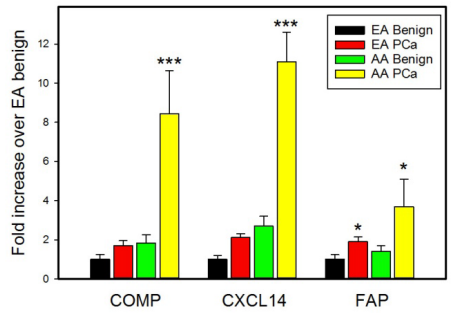

Figure 6. Q-RT-PCR of stromal gene in AA and EA PCa and benign prostate tissues.
Expression is normalized to β-actin and shown as mean +/- SEM with mean level in EA benign tissue for each gene considered as 1.0 to facilitate comparison. Statistically significant increases in PCa versus benign tissue from the same race is indicated by asterisks *p<.05; ***p<.001

We have identified three genes associated with reactive stroma that are markedly upregulated in AA PCa: COMP, FAP and CXCL14 as shown by Q-RT-PCR (Figure 6). Antibodies for these three proteins have been validated for IHC. Analysis of tissue microarrays is in progress.

B. Training and Professional Development

Nothing to report

C. Dissemination to communities of interest

The following manuscript has been published:

1. Ruder S, Gao Y, Ding Y, Bu P, Miles B, De Marzo A, Wheeler T, McKenney JK, Auman H, Fazli L, Simko J, Coll AH, Troyer DA, Carroll PR, Gleave M, Platz E, Trock B, Han M, Sayeeduddin M, True LD, Rowley D, Lin DW, Nelson PS, Thompson IM, Feng Z, Wei W, Brooks JD, Ittmann M, Lee M, Ayala G: Development and Validation of a Quantitative Reactive Stroma Biomarker (qRS) for Prostate Cancer Prognosis, Hum Pathol 2022, 122:84-91

D. Plans for coming year

We will continue preparing data we have obtained for future publications.

4. IMPACT

The data presented here have proven our underlying hypothesis that AA PCa has more abundant reactive stroma than EA PCa and that more broadly reactive stroma enhances tumor progression.

5. CHANGES/PROBLEMS

COVID-19 has significantly impacted our progress.

6. PRODUCTS

We have developed an FAP overexpressing 19I stromal cell line and Factor XII expression vectors.

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.

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 overlap.

2U54MD007597-31 (Kwabi-Addo) 08/01/19-07/31/24 0.6 calendar
NIH (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 (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.

DAMD W81XWH-20-1-0926 (Yen) 9/30/2020-9/29/2023 0.6 calendar
DOD Prostate Cancer Program (salary support only)
AZI1 RNA-Driven Gene Fusion in Prostate Cancer
The goal of this proposal is to evaluate the role AZI1 in prostate cancer. No overlap.

U01CA257328-01 (Haiman) 05/01/2021 – 04/30/2026 0.6 calendar
NCI to Ittmann lab
Multiethnic GWAS and TWAS to Inform Risk Prediction for Prostate Cancer
The goal of this project is to determine genetic risk factors for prostate cancer. We will be supplying specimens for analysis. 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.

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
2. Ruder S, Gao Y, Ding Y, Bu P, Miles B, De Marzo A, Wheeler T, McKenney JK, Auman H, Fazli L, Simko J, Coll AH, Troyer DA, Carroll PR, Gleave M, Platz E, Trock B, Han M, Sayeeduddin M, True LD, Rowley D, Lin DW, Nelson PS, Thompson IM, Feng Z, Wei W, Brooks JD, Ittmann M, Lee M, Ayala G: Development and Validation of a Quantitative Reactive Stroma Biomarker (qRS) for Prostate Cancer Prognosis, Hum Pathol 2022, 122:84-91

9. APPENDICES: None