AWARD NUMBER: **W81XWH-19-1-0726**

TITLE: Carcinoma-Associated Fibroblasts from African American Prostate Cancer Promote Aggressive Tumors: Implications for Developing Novel Therapy

PRINCIPAL INVESTIGATOR: Dr. Xiaolin Zi

CONTRACTING ORGANIZATION: The Regents of the University of California, Irvine

REPORT DATE: October 2021

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	Annual	09/30/2020 to 09/29/2021

4. TITLE AND SUBTITLE

Carcinoma-Associated Fibroblasts from African American Prostate Cancer Promote Aggressive Tumors: Implications for Developing Novel Therapy

5a. CONTRACT NUMBER
W81XWH-19-1-0726

5b. GRANT NUMBER
PC181016

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Xiaolin Zi, Michael Lilly

E-Mail:xzi@uci.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Irvine
101 The City Dr S
Street2: Bldg 55, Rm 260
City*: Orange
State*: CA: California

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT : Metabolic reprogramming is one of the key characteristics of cancer and tumor microenvironment for fueling the rapid and self-sufficient growth of cancer cells. L-3-phosphoserine phosphatase (PSPH) is one of the five rate-limiting enzymes in the biosynthesis of serine from glucose. We are the first to show that PSPH is overexpressed in carcinoma associated fibroblasts (CAFs) and cancer tissues of African American (AA) prostate cancer (PCa) compared to those of European American (EA) PCa and distant carcinoma associated fibroblasts (dCAFs). High PSPH mRNA levels predict poor survival of prostate cancer. Knocking down the expression of PSPH in PCa DU145 cell line exhibits slow growth and overexpression of PSPH in benign associated prostate fibroblasts promote cell growth. In addition, we have developed primary cultures of 143 EA CAFs and matched BAFs and 53 AA CAFs and matched BAFs. Preliminary studies show that AA CAFs can transform tumorigenic growth and promote metastasis of benign prostate epithelial cells BPH-1 by co-inoculation in sub-Renal capsule xenograft experiment. The results suggest that PSPH may be a new target for treatment of PCa and AA CAFs may promote the aggressiveness of PCa.

15. SUBJECT TERMS
PSPH, prostate cancer disparity, TGF-beta

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	7	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Metabolic changes have been recognized as a hallmark of cancer, which include dysregulation of serine metabolism. In general, extracellular serine alone that enters cells via amino acid transporters is sufficient to meet the needs of tumor cell proliferation. However, some tumor cells can increase de novo serine synthesis through glycolysis intermediates even when sufficient extracellular serine is supplied. Actively synthesized serine is utilized for promoting nucleotide synthesis, redox homeostasis, amino acid transport, and folic acid metabolism, thereby enhancing tumor cell proliferation.

Kinseth et al. examined the differences in gene expression between AA and EA PCa by matching for age and pathological stage or Gleason scores as well as tumor-cell content and stroma-cell content. Striking differences in gene expression were observed in the stroma of AA patients relative to EA: 1016 genes with significant differences between the expression of EA and AA patients were observed. The vast majority (82%) of significant differences were downregulated. In this study, components of extracellular matrix (ECM), mediators of cellular immunity, mediators of the epithelial-to-mesenchymal transition (EMT) and L-3-phosphoserine phosphatase (PSPH) were the top differentially expressed genes in stroma of AA compared to EA men. PSPH was previously described as a gene expression biomarker in tumor tissues for identifying prostate, breast, endometrial and colorectal cancers specifically in AAs. However, our study was the first to show overexpression of PSPH gene in the cancer stroma of AA patients compared to EAs.

Therefore, in this study, we aim to understand the biological functions of PSPH and its regulatory mechanisms in the stroma of AA *versus* EA PCa using primary cultures of carcinoma associated fibroblasts (CAFs) and tissue recombination model in the sub-renal capsule of SCID mice.

PSPH, prostate cancer disparity, TGF-beta, carcinoma associated fibroblasts

Aim 1: determine the differential ability of AA CAFs vs. EA CAFs to transform BPH-1 cells and the normal mouse prostate into tumorigenic products

Major Task 1: Establishment of primary CAFs and distant CAFs culture from prostatectomy specimens of both AA and EA prostate cancer patients.

. So far, patient-derived CAFs and matched benign associated fibroblasts (BAFs) from the contralateral tumor-free portion of the prostate have been derived from fresh radical prostatectomy (RP) surgical specimens of 53 AAs and 143 EAs: Gleason scores ranging from 3+3 to 5+5 and age ranging from 43 to 73. Tumor-adjacent stroma and distant stroma were identified by cryostat frozen sections of the tumor-bearing tissue. Five normal-associated fibroblasts were also developed from the tumor-free prostate of cystoprostatectomy specimens.

The stroma cells are grown in medium, which supports fibroblast growth and eliminates epithelial cells over successive passages. All lines have been serially passaged 5 times to eliminate non-fibroblast cells. To confirm that our cells are fibroblasts, we have performed RNAseq on ten EA CAFs and ten AA CAFs using an Illumina HiSeq 4000 system and analyzed the data by matching of deduplicated reads to the Ensembl genome database. Three stromal markers (VIM, COL17A1, CALD1) are significantly higher than two epithelial markers (CK 8 and 18) for the 8 primary CAF cultures (**Fig. 1**). Furthermore, to enhance the rigor and validate the ancestry of our CAFs, we have used LASER principal component analysis of single-nucleotide polymorphisms (SNPs) from the obtained RNA Seq data. A large SNP profile for quantitatively assessing the genetic ancestries of all AA and EA cells was conducted through our collaboration with Dr. Rick Kittles at the City of

Hope including all proposed CAFs and commercial lines (**Fig. 2**). The South Carolina AA population is dominated by the ethnically characterized Gullah group with little European gene admixture.

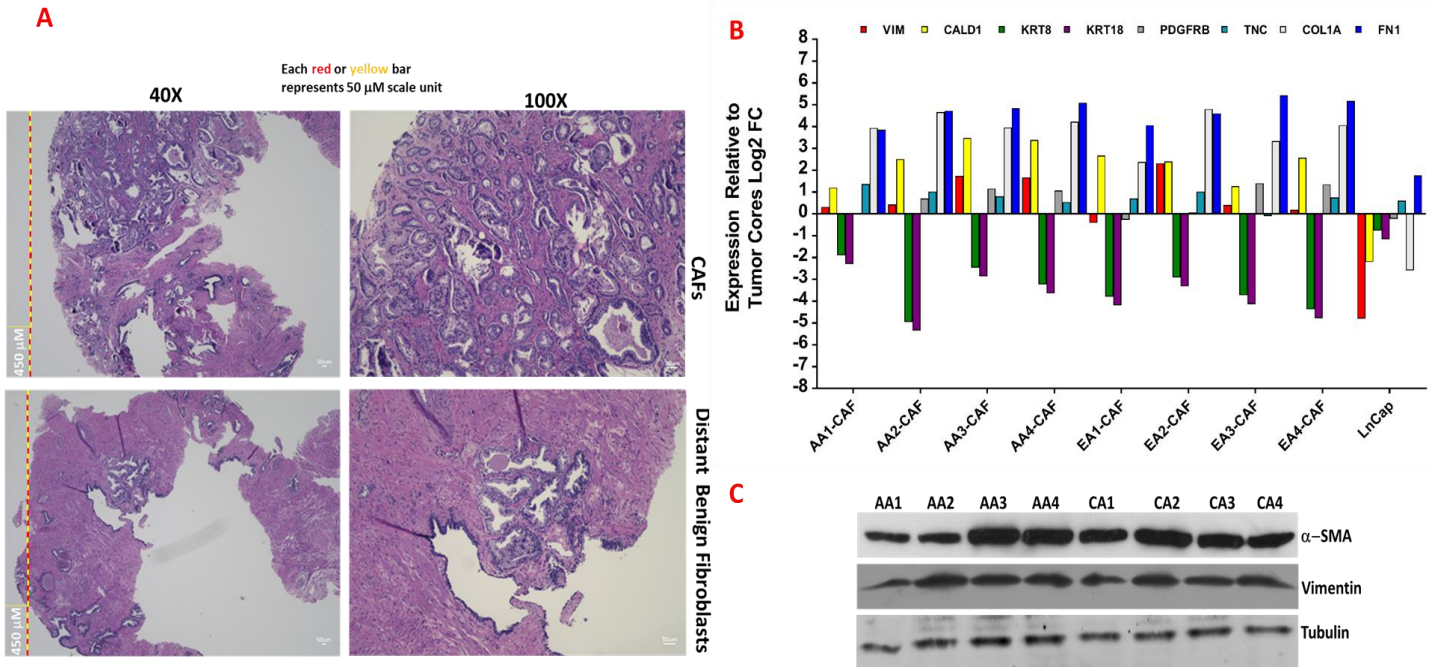


Fig. 1. A, H &E staining and histological evaluation of prostate cancer and stroma components**. B,** RNA seq analysis of expression of epithelial and stroma markers in AA and CA CAFs**. C.** Western blotting analysis of stroma markers in AA and CA CAFs**.**

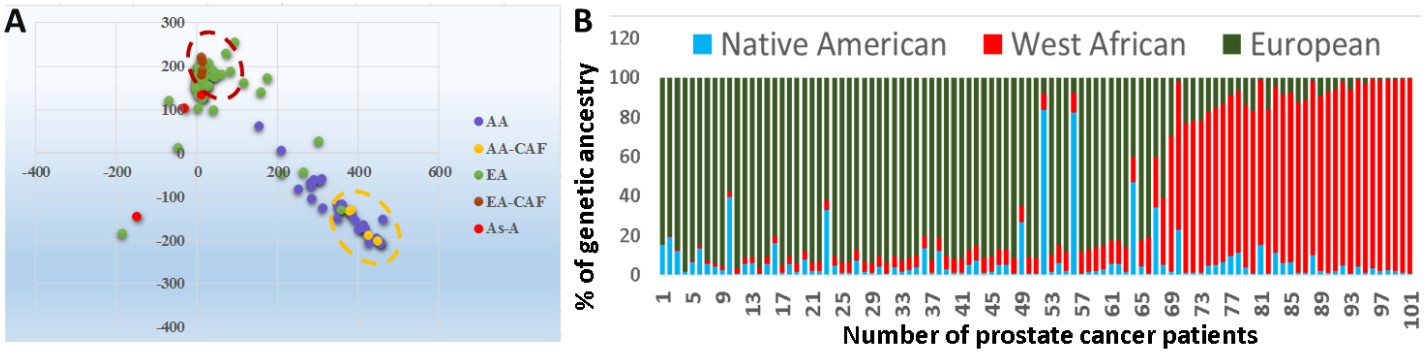
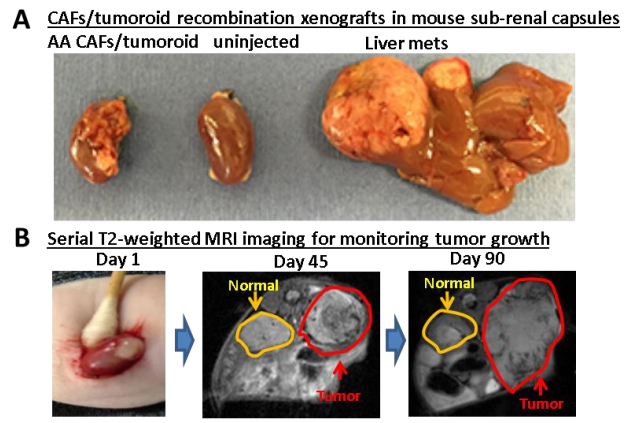


Fig. 2 A, Analysis of SNPs using RNA Seq. reads with LASER server. Principle component analysis of RNA Seq of study group (4 EA & 4 AA) against a larger-sized reference group (1000 Genomes Project) to estimate genotype, in this case race. The dashed ovals include the locus of PC coordinates of authentic EA (upper) and AA (lower) individuals (the placement of some symbols is on the edge of their coordinates to avoid overlap. The plot represents similar data from our lab for which ethnicity is known. **B,** Percentages of genetic ancestry for patient-derived CAFs.

Major Task 2: Sub-Renal capsule recombination xenograft assay.

Our hypothesis is that the CAFs from prostate tumors of AA patients promote more aggressive disease than those from EA patients. To begin testing this hypothesis, we compared the tumor initiation and growth of xenografts of nontumorigenic BPH-1 cells using two different lines of patient-derived AA CAFs and one line of EA CAFs. Each of the three lineages was implanted under the kidney capsule of 5 adult male severe combined immunodeficiency (SCID) mice each. The initiation and growth of the xenografts was followed by in vivo magnetic resonance imaging (MRI) imaging at the UCI OncoImaging Core using a T9.4 Brunker small animal. As summarized in Table 1, 8 of the 10 mice inoculated with AA CAF-BPH-1 combinations led to large xenografts (**Fig. 3**). Furthermore, one inoculation of each AA patient-derived AA CAF line led to large liver metastases (**Fig. 3**) which were confirmed upon necropsy. None of the 5 mice inoculated with EA CAF-BPH-1

combinations developed resolvable tumors and no metastases were found upon necropsy. More pairs of CAF/BPH-1 recombination is in progress.



C

Table 1: Tumorigenicity of BPH-1 cells with EA or AA CAFs (sub kidney capsule; SCID, males; 100K BPH-1 cells + 250K CAFs)

RACE	Number of Mice inoculated	Take-rate	Metastasis
EA CAF	5	0	0
AA CAF #1	5	4 mice (80%)	Liver x 1 (20%)
AA CAF #2	5	4 mice (80%)	Liver x 1 (20%)

Fig. 3. A, representative photographs of AA CAFs/BPH1 cell recombination in mouse sub-renal capsules and liver metastases. **B,** Day 1 shows sub-renal capsule implantation of LNCaP/LEF1 cells underneath the renal capsule. Day 45 and 90 show normal kidney (yellow) and tumor growth (red) by serial MRI imaging. **C.** Table 1.

Major Task 3: Prostate orthotopic xenograft experiment

Pairs of AA and EA CAFs have been injected into the prostate of the NOD/SCID mice. Experiments are in progress to monitor tumor growth in the prostate.

Specific Aim 2: determine the impact of the enzymatic activity or expression of PSPH and high serine production in in vivo tumor growth.

Major Task 4: Whether PSPH expression and activity affect the CAFs mediated tumorigenic transformation or tumor growth?

CAFs with stable overexpression of PSPH and stable suppression of PSPH, and MDA-PCa 2b with stable suppression have been made and further characterization for growth and activities is in progress. After characterization, tissue recombination experiments will be performed.

Specific Aim 3: determine the combined effects of docetaxel and TGF- beta inhibitor on the growth of MDA-PCa 2b/CAFs recombination

Major Task 5: Evaluation of in vivo anticancer efficacy of docetaxel in combination with TGF-beta inhibitor.

- Establish orthotopic xenograft models of AA CAFs and MDA-PCa2 cells.
- Randomizing into different treatment groups.
- MRI monitoring tumor growth
- Histology analysis of xenograft tumors.
- Evaluating the effect of the TGF beta inhibitor and /or docetaxel on collagen disposition and expression of biomarkers
- Statistical analysis

AA CAFs secreted more active TGFβ1 but less chemokines than EA CAFs. We assayed the secretion of 31 different mediators including cytokines, chemokines or growth factors for 19 AA and 32 EA patient-derived CAFs by applying serum-free conditioned medium (CM) of passage 5 cells to the bead-based platform of Magpix. Among them, eotaxin/CCL11, IP-10/CXCL-10, and MCP-1/CCL2 were significantly reduced whereas *TGF-β1 levels were significantly higher in CM of AA vs. EA CAFs* (**Fig 4A to D**). These results suggest that TGF-beta signaling may play an important in cancer promoting effects in the stroma. The data provide a rational to test the combined effect of docetaxel and TGF beta inhibitor in AA CAFs/MDA-PCa recombination xenograft models.

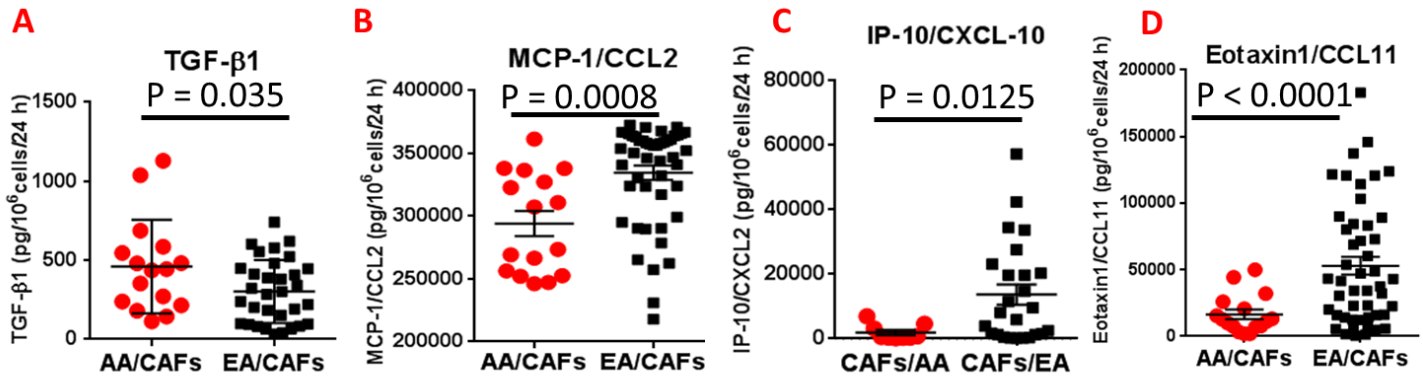


Fig. 4. Cytokines/chemokines were differentially secreted in CM of AA vs. EA CAFs

4. Impact

4.1. We have developed unique resources of CAFs and BAFs from many different AA and EA PCa patients. The ethnic identification was determined by large SNP analysis. These resources provide an important tool for dissecting the stroma regulatory features of PCa of different races.

4.2. We found that AA CAFs secreted more TGF beta leading to immunosuppression in tumor microenvironment, which may play a role in promoting the aggressiveness of AA prostate cancer.

5. Changes/Problems

The subaward PI Dr. Yang Liu at Chapman University has resigned from her position at Chapman University due to her health issues and family reasons. She also has withdrawn from the collaboration.

The proposed experiments using nanoparticles of docetaxel and TGF-beta inhibitor are no longer feasible due to changes in personnel. Therefore, we have proposed an alternative by directly testing the combined effect of docetaxel and TGF beta inhibitor in AA CAFs/ MDA-PCa2 cell recombination models. The alternative still addresses Aim 3 and tests the original hypothesis, which is within the scope of the science in the original proposal. A modified SOW was made. I have contacted Ms. Charlotte Ballard for requesting the approval of the modification.

6. Products

6.1. Patient-derived CAFs and BAFs.
6.2. PSPH expression lentivirus and plasmid constructs

7. Participants & Other Collaborating Organizations

7.1. Medical University of South Carolina
7.2. City of Hope

8. Special Reporting Requirements: N/A

9. Appendices: None