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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, CA

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| 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, which generate nucleotides to support cell proliferation. Here, we aim to understand a role of PSPH expression and its regulation in prostate cancer (PCa) and its relation to PCa disparity. We discovered that MDAPCa2b cells, which were derived from African American (AA) PCa, and AA carcinoma associated fibroblasts (CAFs) express much higher levels of PSPH protein compared to benign associated fibroblasts, normal prostate epithelial cells and PCa cell lines from European American PCa. Knock-down of PSPH expression in MDA-PCa2b cells significantly inhibits the growth, colony formation and tumor growth. Analysis of publicly available PCa RNA seq data bases revealed that PSPH overexpression and amplification is associated with alterations of metabolism and immunity. The results suggest that PSPH may be a new target for treatment of PCa and AA CAFs may promote the proliferation of PCa. | | | | | | |
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1. Introduction

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.

2. Keywords

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

3. Accomplishments

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.

In order to obtain more appropriate controls, normal-associated fibroblasts (NAFs) were also developed from the tumor-free prostate of cystoprostatectomy specimens, including **14 EA NAFs and 2 AA NAFs lines**

In addition, additional **5 AA CAFs (out of 21 AA PCa patients)** and **18 EA CAFs (out of 37 EA PCa patients)** were developed during the past year. All the CAFs were validated for the origin of CAFs derived from the near stroma of prostate cancer through pathological examination.

RNA sequencing of gene expression profile of these AA and EA CAFs lines, as well as NAFs lines are in progress.

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

We have continued our effects on evaluating the tumor initiation and growth of xenografts of nontumorigenic BPH-1 cells using two different lines of patient-derived AA CAFs and two lines of EA CAFs. Each of the four 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 Oncolmaging Core using a T9.4 Brunker small animal. More pairs of CAF/BPH-1 recombination is also in progress.

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.

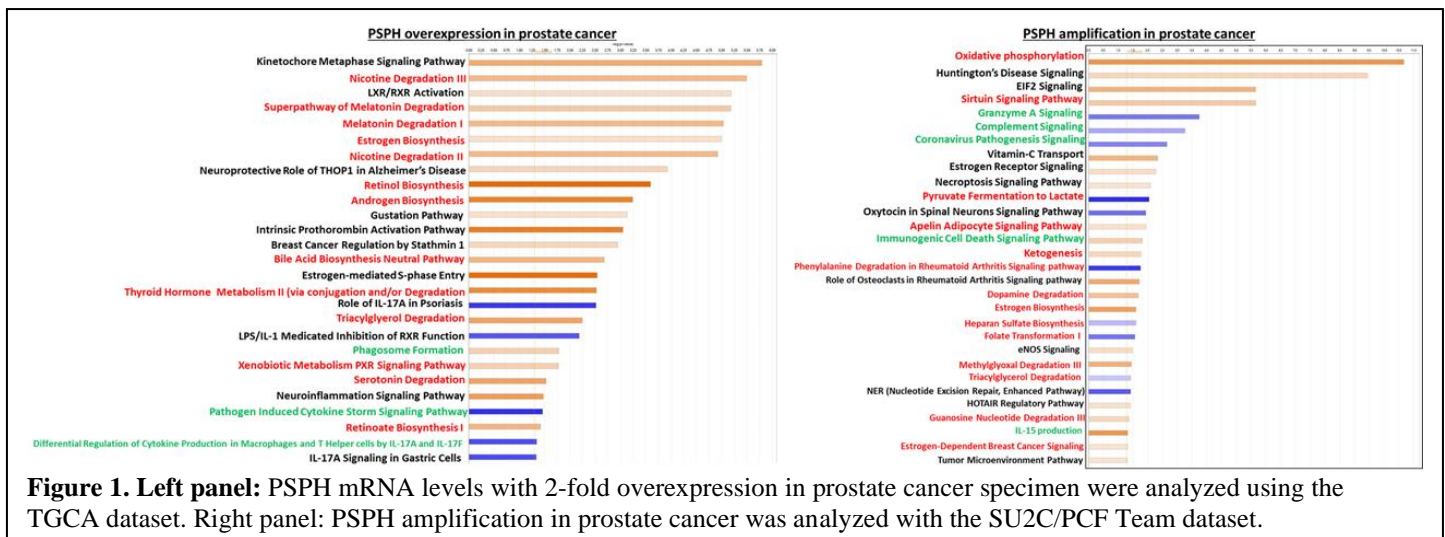
Specific Aim 3: determine the combined effects of docetaxel and TGF- beta inhibitor on the growth of MDA-PCa 2b/CAFs recombination

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

Experimental results

[1.PSPH overexpression or amplification in prostate cancer is associated with alterations in metabolism and immunity.](#) We have previously reported that PSPH is overexpressed in AA prostate cancer and the tumor microenvironment. To further understand the significance of PSPH over-expression in prostate cancer, we performed a systematic transcriptome analysis of PSPH overexpression and amplification in prostate cancer primary tumor samples in the TGCA dataset (TCGA, Firehose Legacy) and metastatic tumor samples in the SU2C/PCF Team dataset (SU2C/PCF Dream Team, PNAS 2019), respectively. After associated pathway enrichment of differentially expressed genes (DEGs) was detected by Gene Set Enrichment Analysis (GSEA), gene control network was constructed by ingenuity pathway analysis (IPA) software. Figure 1 shows that



PSPH overexpression and amplification are mainly associated with alterations in metabolism and immunity. Notably, in primary prostate cancer tumors, PSPH overexpression is associated with increased androgen and estrogen biosynthesis, suggesting a role in androgen receptor signaling. In metastatic prostate cancer tumors, PSPH amplification is associated with oxidative phosphorylation, tumor microenvironment, estrogen biosynthesis, folate transformation, and immunogenic cell death signaling pathway. The effect of PSPH expression on genomic expression profiles in prostate cancer cell lines and stromal cells are also being examined.

2. [PSPH knock-down in androgen sensitive prostate cancer cells LNCaP and MDA-PCa 2b inhibits cell proliferation.](#) We next examined the effect of PSPH suppression by stably short hairpin (sh)RNA transfection on the growth of androgen sensitive LNCaP and MDA-PCa 2b cells. Figure 2 shows that LNCaP and MDA-

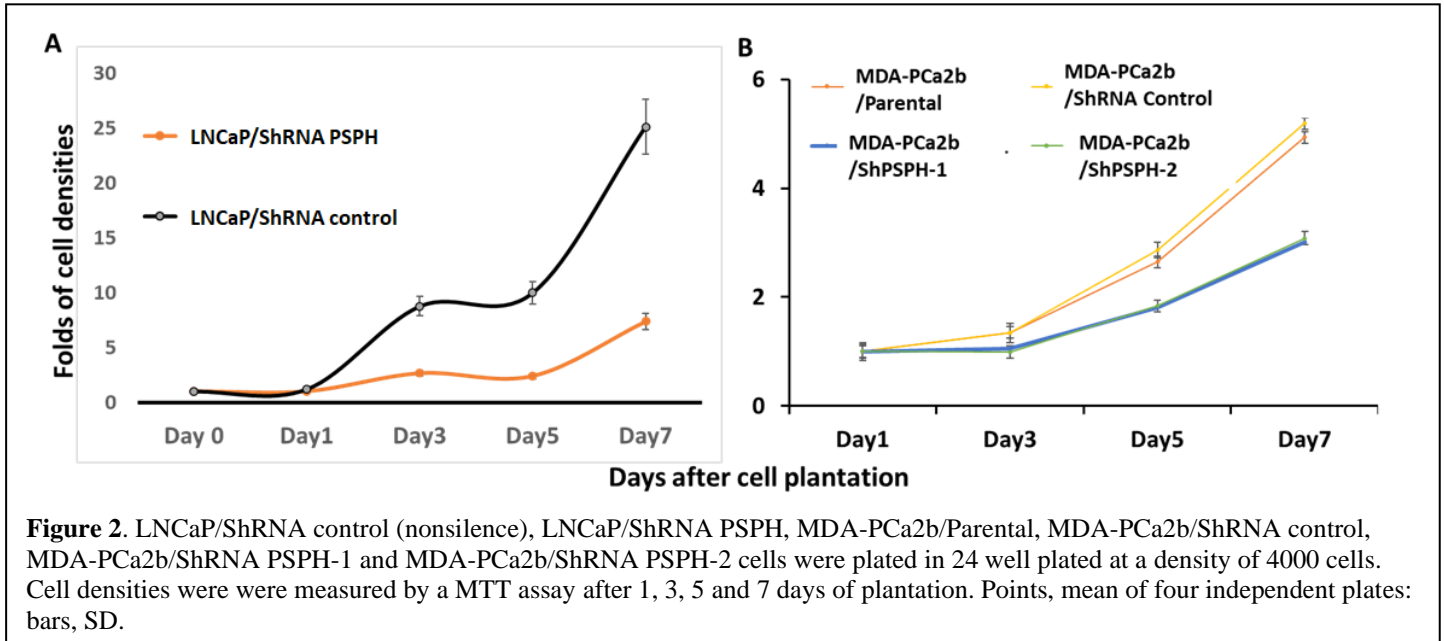


Figure 2. LNCaP/ShRNA control (nonsilence), LNCaP/ShRNA PSPH, MDA-PCa2b/Parental, MDA-PCa2b/ShRNA control, MDA-PCa2b/ShRNA PSPH-1 and MDA-PCa2b/ShRNA PSPH-2 cells were plated in 24 well plated at a density of 4000 cells. Cell densities were were measured by a MTT assay after 1, 3, 5 and 7 days of plantation. Points, mean of four independent plates: bars, SD.

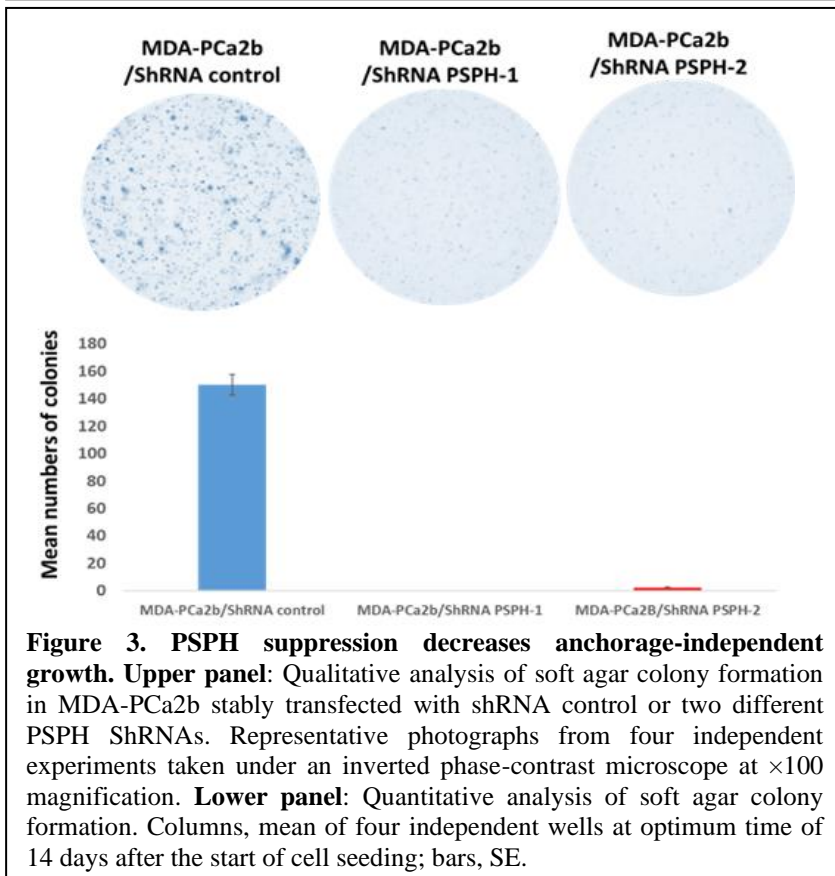
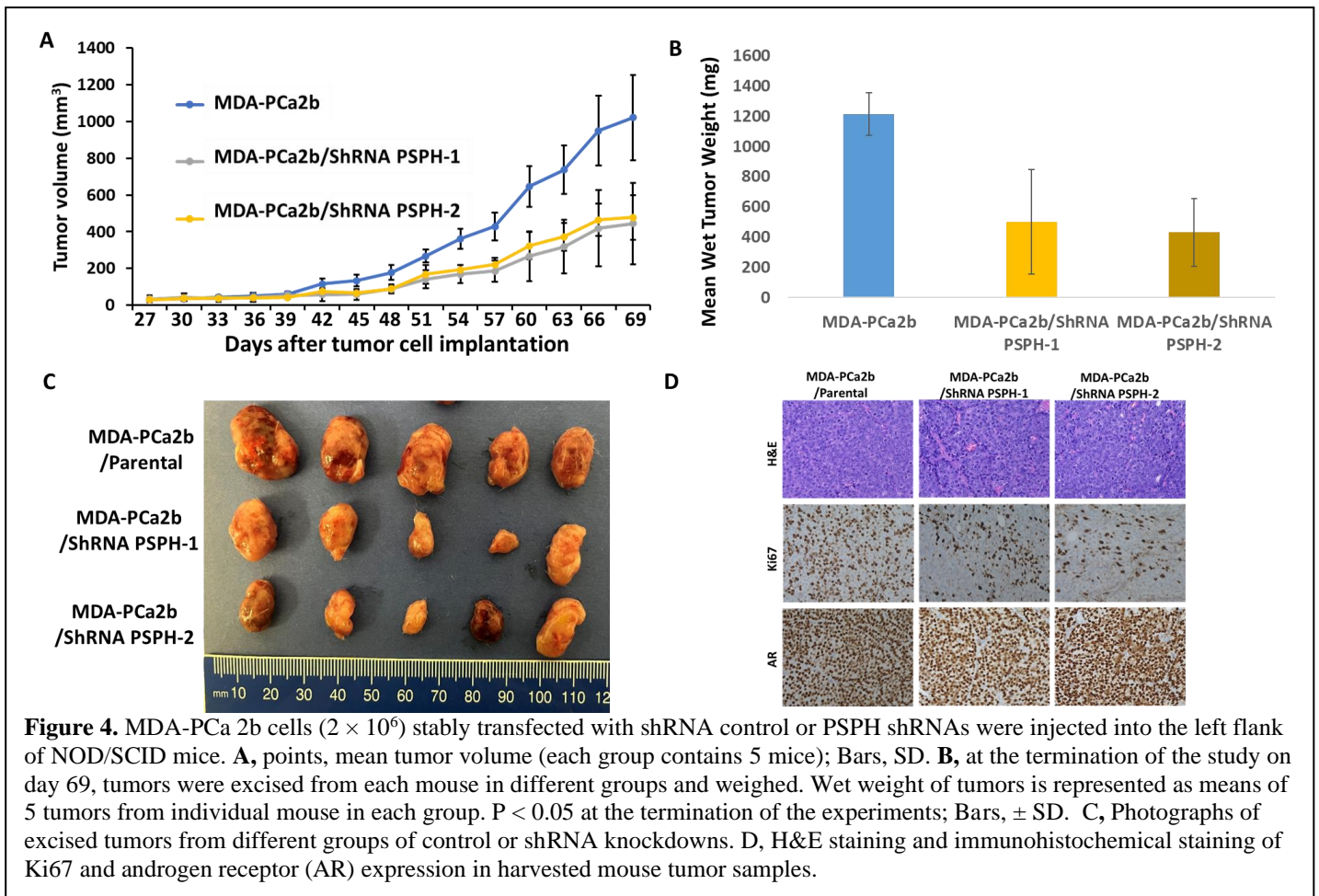


Figure 3. PSPH suppression decreases anchorage-independent growth. **Upper panel:** Qualitative analysis of soft agar colony formation in MDA-PCa2b stably transfected with shRNA control or two different PSPH ShRNAs. Representative photographs from four independent experiments taken under an inverted phase-contrast microscope at $\times 100$ magnification. **Lower panel:** Quantitative analysis of soft agar colony formation. Columns, mean of four independent wells at optimum time of 14 days after the start of cell seeding; bars, SE.

PCa2b cell with PSPH suppression exhibited much slower growth rates compared to those transfected with control nonsilence RNAs or parent MDA-PCa2b cells. These results suggest that PSPH plays a role in proliferation of prostate cancer cells.

3. [The effect of PSPH knock-down on colony formation and cell invasion of MDA-PCa 2b cells.](#) Figure 3 shows that PSPH knockdown by stable PSPH shRNA transfections inhibited the anchorage-independent growth of MDA-PCa 2b cells by more than 90% compared to shRNA control transfection (Figure 3; Student's t test, $P < 0.01$). In addition, we have performed the transwell cell invasion assay. PSPH knock down didn't have any significant effects on the capacity of the invasiveness of MDA-PCa 2b. These results suggest that PSPH play a more important role in cell proliferation. Further studies are also in progress to evaluate the expression of PSPH in stroma on proliferation of MDA-PCa 2b using co-culture system in soft agar.

4. PSPH knock-down results in reduced tumorigenicity of MDA-PCa 2b cells in NOD/SCID mice on colony and inhibits cell proliferation in in vivo in tumor tissues. Figure 4A shows that the PSPH knockdowns in MDA-PCa



2b cells resulted in a significant decrease in the growth rate of tumors compared to control (ANOVA, $P < 0.01$). The wet tumor weights were 1211.8 ± 141.6 in the control group and 499.5 ± 346.0 mg and 430.0 ± 225.8 mg in the PSPH shRNA knockdown group (Figure 4B; $n = 5$, mean \pm SD; $P_s < 0.05$, Student's t test). PSPH knockdown attenuated tumor growth by approximately 65 to 59%.

Immunohistochemical analysis shows that tumor sections from PSPH knockdown tumor tissues exhibited a marked decrease in Ki-67, whereas there is no obvious alterations in numbers of AR positive cells in tumor tissues between shRNA knockdowns and control. Further experiments are in progress to understand the mechanisms of PSPH's action on tumor cell proliferation.

4. Impact

4.1. We have developed unique resources of CAFs and dCAF from many different AA and EA PCa patients and NAFs from the tumor-free prostate of cystoprostatectomy specimens. 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 discovered that PSPH over-expression or amplification is associated with alterations of metabolism and immunity in prostate cancer tumor tissues.

4.3. PSPH suppression results in anti-proliferative effects on anchorage-dependent and-in dependent growth and in vivo tumor growth, which was accompanied by significantly decreased number of Ki-67 positive cells.

5. Changes/Problems

None.

6. Products

- 6.1. Patient-derived CAFs, distant CAFs and CAFs.
- 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