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Title: Investigating the Expression, Role, and Targeting of Collagen Modifying Prolyl 4-Hydroxylase P4HA1 in Prostate Cancer Progression and Metastasis

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| 14. ABSTRACT Prostate cancer (PCa) is the most common malignancy and the second most common cause of cancer mortality of men in the United States. Complex molecular and signaling events converge leading to PCa initiation, unregulated growth, invasion, and metastasis. There is a growing concern that PSA screening leads to over-diagnosis resulting in excessive treatment of indolent PCa without significant clinical benefit. Thus identification and validation of novel diagnostic and prognostic molecular biomarkers of PCa as well as the oncogenic therapeutic targets are of critical importance for the early detection, management, and cure of PCa. Our recent studies utilizing gene expression profiling and next generation sequencing of prostate cancer tissues identified prolyl hydroxylase P4HA1, a key enzyme in collagen modification resulting in extracellular matrix modification in cancer as upregulated in primary PCa and castration resistant PCa. Being an enzyme, P4HA1 is a viable therapeutic target amenable to small molecule inhibition. | | | | | | |
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Title of the Grant: Investigating the Expression, Role, and Targeting of Collagen Modifying Prolyl 4-Hydroxylase P4HA1 in Prostate Cancer Progression and Metastasis

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Principal Investigator: Sooryanarayana Varambally

Annual Report: 09/30/2019- 9/29/2020

INTRODUCTION

Prostate cancer (PCa) is the most common malignancy and the second most common cause of cancer mortality of men in the United States. Complex molecular and signaling events converge leading to PCa initiation, unregulated growth, invasion, and metastasis. There is a growing concern that PSA screening leads to over-diagnosis resulting in excessive treatment of indolent PCa without significant clinical benefit. Thus identification and validation of novel diagnostic and prognostic molecular biomarkers of PCa as well as the oncogenic therapeutic targets are of critical importance for the early detection, management, and cure of PCa. Our recent studies utilizing gene expression profiling and next generation sequencing of prostate cancer tissues identified prolyl hydroxylase P4HA1, a key enzyme in collagen modification resulting in extracellular matrix modification in cancer as upregulated in primary PCa and castration resistant PCa. Being an enzyme, P4HA1 is a viable therapeutic target amenable to small molecule inhibition.

SPECIFIC AIMS:

Aim1. Evaluate the significance of P4HA1 expression in PCa.

Aim2. Investigate the functional role of P4HA1 in PCa metastasis.

Aim3. Targeting P4HA1 in PCa using specific small molecule inhibitor.

BODY

In order to identify novel biomarker and therapeutic targets for aggressive PCa, we analyzed high throughput data such as microarray and next generation transcriptome sequencing of PCa and identified prolyl 4-hydroxylase alpha subunit1 (P4HA1) as a gene overexpressed in PCa. During the first year of this project, we have obtained IACUC approvals, generated cell lines and also performed initial analysis of P4HA1, a collagen modifying enzyme inhibitor pythiDC in prostate cell lines.

Major Task 1: Evaluate the significance of P4HA1 expression in PCa.

Gene expression analysis: We have used multiple platforms and expression profiling studies to evaluate the expression P4HA1. Our data from both expression profiling and transcriptome sequencing suggests overexpression of P4HA1 (Fig. 1A and B) in prostate cancer compared to normal prostate tissues. Furthermore, a sub-class specific analysis using the TCGA prostate cancer datasets using the cancer data analysis web-portal UALCAN, that is developed by us show that various molecular sub-types of prostate cancers show higher expression of P4HA1 compared to normal prostate tissues. The molecular sub-types analyzed include the common gene fusions in prostate cancer, the ETS gene fusion positive PCAs, the FOXA1 and SPOP mutant prostate cancers.

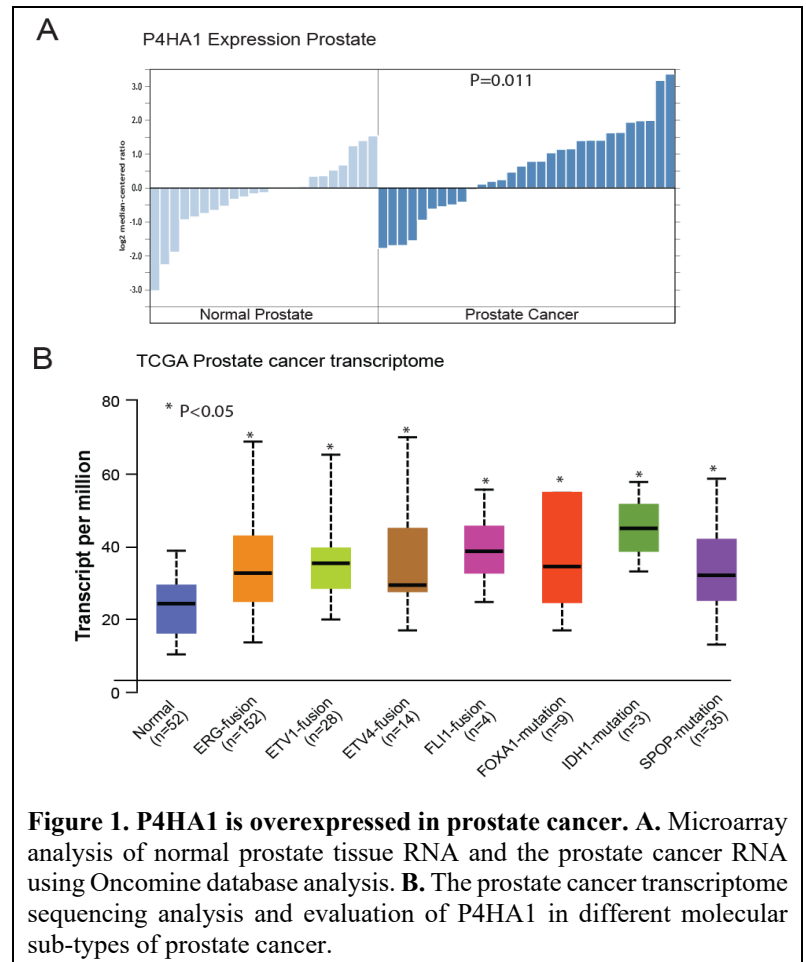
Further analysis of genes involved in collagen metabolism showed that apart from proline collagen modifying enzymes, the enzymes involved in collagen lysyl oxidases (*LOX*) and procollagen-lysine, 2-oxoglutarate 5-dioxygenase (*PLOD*)

Next, in order to evaluate the P4HA1 protein expression, we have constructed now multiple tissue microarrays containing PCa tissue sample from different Gleason grades.

The tissue microarrays also contain different racial groups including African American PCa tissues. Our collaborator Dr. George Netto, an expert GU pathologist leads the TMA construction effort. In the second year of funding effort. We will also perform Fluorescence *In Situ* Hybridization (FISH) to evaluate P4HA1 locus amplification.

Major Task 2: Investigate the functional role of P4HA1 in PCa metastasis.

Here, in order to test the mechanism of action of P4HA1 in PCa cell, we will modulate the P4HA1 expression. We have generated the stable knockdown of P4HA1 in PCa cells and also the construct for overexpression of P4HA1 in normal prostate cells. We have earlier shown the authenticity of both the knockdown constructs and overexpression constructs using prostate cancer cell lines and normal prostate cell PrEC respectively.

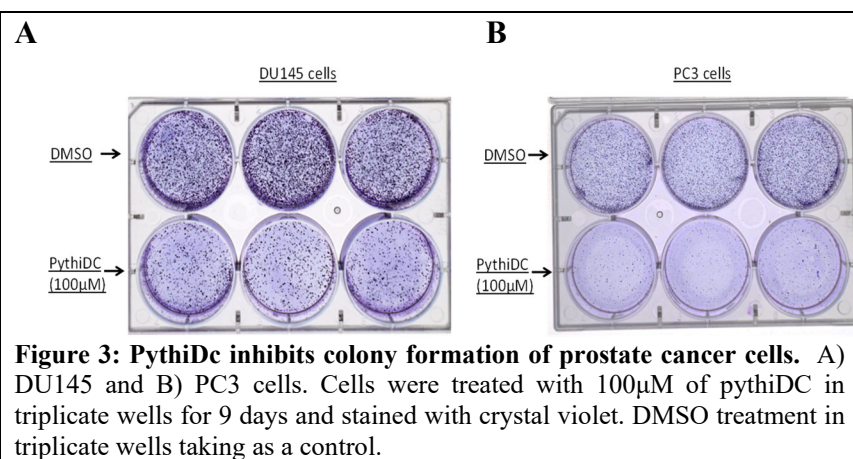
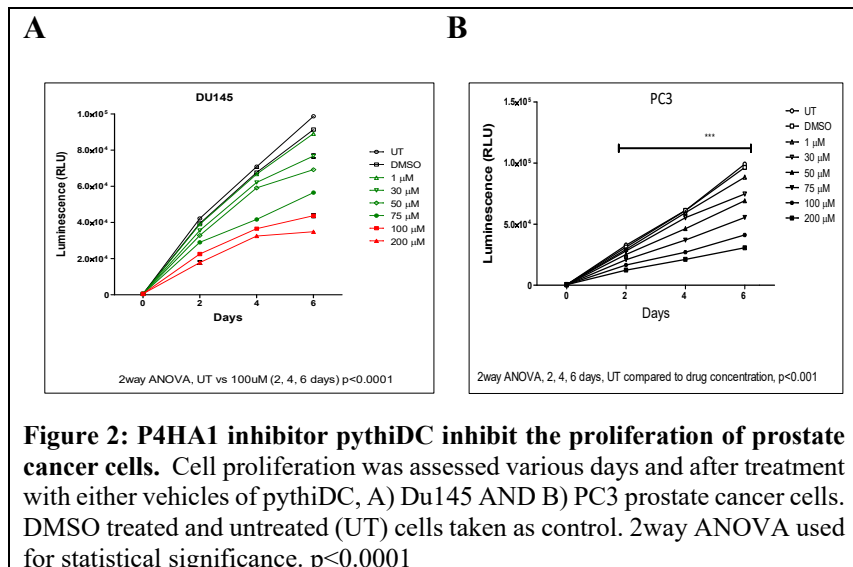


We will use the RNA from these P4HA1 modulated cells and perform RNA sequencing analysis and identify pathways and molecular networks alterations due to P4HA1 modifications. In the second year, we will initiate all the functional studies required to accomplish the tasks to accomplish the goals of this major task.

Major Task 3: Targeting P4HA1 in PCA using specific small molecule inhibitor.

This specific task is focusing on targeting P4HA1 with a long term goal of targeting aggressive prostate cancer expressing P4HA1. In order to test this, we used in our initial experiments pythiDC, a specific inhibitor of P4HA1 and performed cell proliferation assay. As shown in **Fig. 2A** and **B**, increased concentration of pythiDC reduced the proliferation of both DU145 and PC3 prostate cancer cells.

Next, we performed colony formation assay using DU145 and PC3 prostate cancer cells and evaluated the effect of 100uM pythiDC in colony formation (**Fig. 3 A and B**)



KEY RESEARCH ACCOMPLISHMENTS:

- Analyzed multiple prostate cancer datasets to evaluate the expression of P4HA1 and other enzymes related to collagen metabolism
- Constructed the tissue microarray containing PCa patient samples for immunohistochemical staining
- Generated stable prostate cancer cell line in which P4HA1 is modulated
- Performed *in vitro* P4HA1 inhibition assays using pythiDC. Obtained IACUC approval for animal experiments

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have initiated the proposed studies and finished analyzing the transcriptomic data showing the overexpression of P4HA1 in PCa. We also have PCa TMA constructed by our collaborator Dr. George Netto and ready for immunohistochemical studies.

PERSONNEL RECEIVING PAY FROM THIS GRANT

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