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

Principal Investigator: **Sooryanarayana Varambally**

Contracting Organization: **University of Alabama at Birmingham**

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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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Principal Investigator: Sooryanarayana Varambally
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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

We have shown earlier that the P4HA1 expression in multiple prostate cancer datasets and identified its overexpression. The gene expression of P4HA1 is increased in prostate cancer samples compared to benign prostate cells.

Furthermore, we have performed immunohistochemical analysis to detect and quantify P4HA1 protein expression in prostate cancer tissues. We used prostate cancer tissue microarray (TMA) for this. The staining showed increased P4HA1 protein in prostate cancer tissues in both Caucasian and African American prostate cancer tissues. The staining intensity has been quantified biostatistical analysis is being performed. During the current funding period, we have performed multiple in vitro and in vivo experiments to test the effect of P4HA1 overexpression

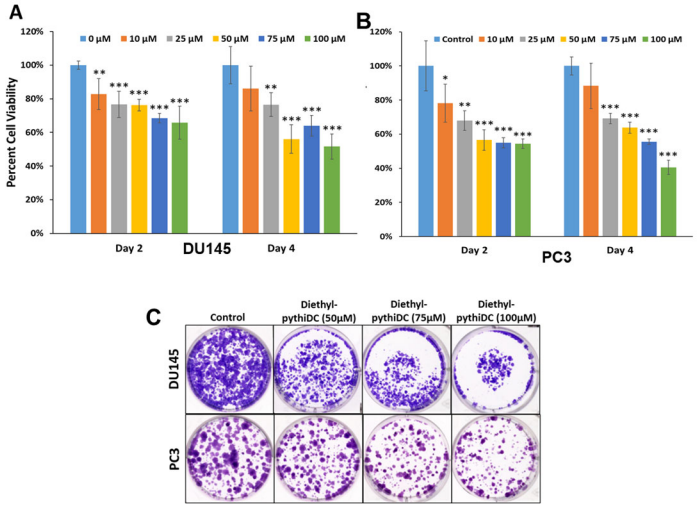

Figure 1: PythiDC inhibit the proliferation of A) DU145 and B) PC3 PCa cells. Colony assay for DU145 and PC3 cells. Cells were treated with PythiDC and stained with crystal violet.

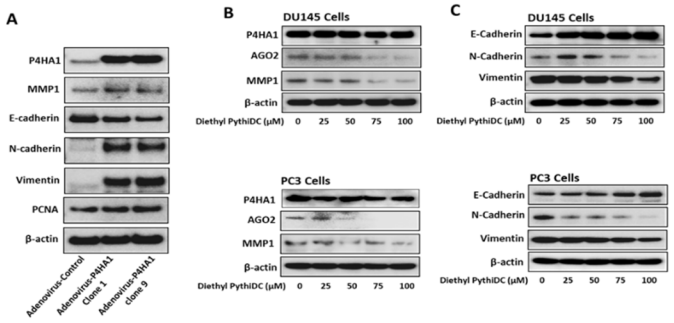

Figure 2: Modulation of P4HA1 or blocking with PythiDC treatment results in multiple molecular changes including EMT changes in prostate cancer cells. A. PrEC cells were infected with P4HA1 or control vector. Expression of P4HA1, MMP1, N-cadherin, vimentin, E-cadherin, and PCNA were determined by western blot analysis. **B**. DU145 and PC3 cells were treated with P4HA1 inhibitor diethyl-phythiDC (25-100 µM) for 48 hrs and protein lysates were prepared for western blot analysis. Expression of P4HA1, AGO2, and MMP1 were measured using specific antibodies. **C.** Expression of N-cadherin, Vimentin and E-cadherin in DU145 and PC3 cells after treatment. Equal loading of protein was confirmed by stripping the membrane and re-probing it for β-actin.

Treatment of prostate cancer cells DU145 and PC3 with P4HA1 inhibitor PythiDc resulted in reduced prostate cancer cell proliferation (**Fig. 1 A, B**) and colony formation (**Fig. 1 C**).

At the molecular level, overexpression of P4HA1 in normal prostate epithelial cells PrEC, it lead to increase in expression of MMP1 and PCNA, upregulates mesenchymal markers N-Cadherin and Vimentin and downregulates epithelial marker E-cadherin (**Fig. 2A**). Treating prostate cancer cells with P4HA1 PythiDC resulted in downregulation of AGO2 and MMP1 expression (**Fig. 2B**) and modulation of epithelial mesenchymal Transition (EMT) markers (**Fig. 2C**).

Next, we utilized mouse models to study the effectiveness of PythiDC in regulating prostate tumor growth using immunocompromised mice. We used PC3 and DU145 PCa cell lines. As

shown in photographs **(Fig 3A and B)**, there is a reduction in tumor size upon treating mice with P4HA1 inhibitor. Quantification of tumor weight **(Fig 3C and D)** after treatment with vehicle and diethyl-pythiDC at the time of termination of experiment showed statistically significant reduction in the tumor weight after treatment with PythiDC. Furthermore, pythiDC treatment modulates EMT markers in tumors of immunocompromised mice subcutaneously injected with PC3 and DU145 cells **(Fig 3E and F).**

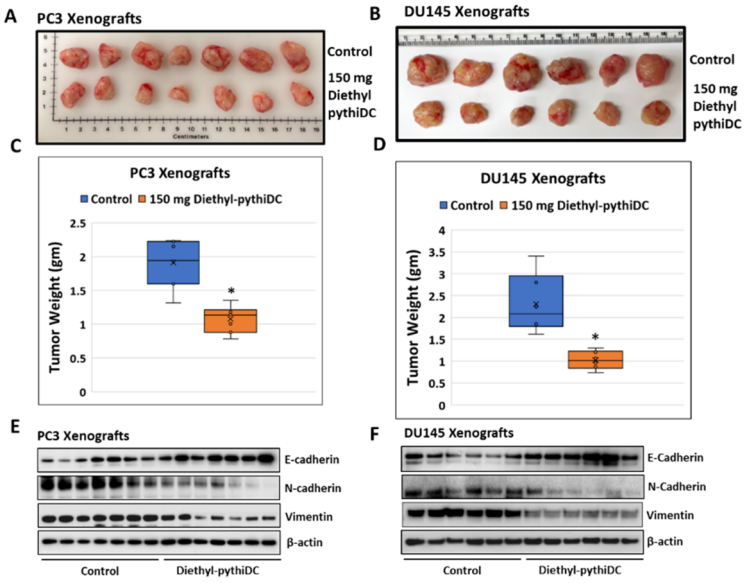

Figure 3: Diethyl-pythiDC treatment reduces prostate tumor growth in immunocompromised mice. NSG mice were subcutaneously injected with $2x10^6$ PC3 cells or $5x10^6$ DU145 cells to initiate tumor growth. When tumor volume reached 100 mm^3, the mice were randomly divided into two groups in both PC3 and DU145 injected cells. The mice were treated with vehicle (served as control) or 150 mg/kg. b.wt. pythiDC twice per week. **A &B .** Photographs of mouse tumors after treatment with vehicle and diethyl-pythiDC at the time of termination of experiment. **C&D.** Tumor weights at the termination of experiments are shown for vehicle- and diethyl-pythiDC-treated mice. Error bar indicates mean ± SD, $*P = 0.0003$ (PC3 xenografts) and $*P = 0.004$ (DU145 xenografts) . At the termination of the experiment the tumors were harvested from the mice treated with vehicle or diethyl-pythiDC. Protein lysates were prepared, and western blot analysis were performed. **E&F.** Showing expression of N-cadherin, Vimentin and E-cadherin.

KEY RESEARCH ACCOMPLISHMENTS:

- Analyzed multiple prostate cancer datasets to evaluate the expression of P4HA1 and other enzymes related to collagen metabolism
- Analyzed the effect of P4HA1 inhibitor PythiDC on cell proliferation, colony formation, and EMT markers
- Studied the effect of P4HA1 overexpression in PrEC cells on EMT markers, MMP1, and PCNA
- Peformed *In vivo* tumor xenograft studies and investigated the effect of P4HA1 inhibitor PythiDC
- Investigated the molecular changes upon treatment of tumors with PythiDC

REPORTABLE OUTCOMES

None

CONCLUSIONS

We have initiated the proposed studies and finished analyzing the genomic and transcriptomic data showing the overexpression of P4HA1 in PCa. We also have PCa TMA ready constructed by our collaborator Dr. George Netto. We finished standardizing the immunohistochemical staining of prostate TMA's using P4HA1 specific antibodies. The staining was evaluated by expert pathologist and is being evaluated by statistician to fund the correlation between P4HA1

staining intensity and prostate cancer progression. During the current funding period, we have performed *in vivo* tumor growth studies and evaluated the effect of P4HA1 inhibitors in PCa tumor growth. We will be evaluating the TMA immunostaining data and the PCa metastasis data in the coming period.

PERSONNEL RECEIVING PAY FROM THIS GRANT

Sooryanarayana Varambally, Ph.D.
George Netto, M.D.
Sejong Bae, Ph.D.
Selvarangan Ponnazhagan, Ph.D.
Darshan Shimoga Chandrashekar, Ph.D., Postdoctoral fellow
Farrukh Afaq, Ph.D., Research Associate
SanthoshKumar Karthikeyan, MS, Student.

Note: The beginning of COVID19 pandemic did effect to an extent some of the proposed experimental work and the timeline of the performance. We will continue and finish the remaining part of the project during this year and if allowed, will request no cost extension if need arises.