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"Role of DNA Methyltransferases in Enzalutamide Resistant Prostate Cancer"

INTRODUCTION: Prostate cancer is the most common malignancy in men and results in over 35,000 deaths annually in the USA. Androgen deprivation therapy (ADT) has been an effective strategy for reducing cancer cell growth and proliferation. Enzalutamide (ENZA) is a next-generation androgen receptor inhibitor approved by the FDA for the treatment of metastatic castration-resistant prostate cancer (mCRPC). ENZ-R is marked by adaptive cellular mechanisms that includes both cellular plasticity and the emergence of CRPC- NEPC and CRPC-DNPC phenotypes. While genomic alterations have been extensively investigated in ENZ-R, little is known about the plausible epigenetic mechanisms in this process. Recent studies from our lab and others have pointed to increased expression and activity of DNMT's (DNMT1, DNMT3a and DNMT3b) during prostate cancer progression, however, direct role of DNMTs in ENZ-R has not been evaluated. In this study, we aimed to determine the role of DNMTs in ENZ-R prostate cancer.

METHODS: LNCaP cells were obtained from ATCC Inc. The cells were cultured in RPMI-1640 (Corning #10-040-CV). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS), Cat# 10437028-Gibco; 1% penicillin-streptomycin, Cat# 15070-063-Gibco; and were maintained at 37°C, 5% CO_2 in a humidified incubator. LNCaP-ENZR cells were generated by culturing LNCaP cells in progressive concentrations of ENZ-R for over 3 months and were maintained in media supplemented with 5uM Enzalutamide. Cell growth and proliferation was measured in the IncuCyte® Cell Count Proliferation Assay. Clonogenic activity was measured by colony formation, and cell migration was assessed by wound-healing assay. Protein levels were measured by Western Blot analysis, while gene expression was evaluated by real time PCR

RESULTS: We observed that treatment of PCa (LNCaP Cells) with 5Aza-dC (5uM and 10uM) resulted in significant ($p<0.01$) inhibition of cell proliferation in a time dependent manner. The inhibition of cell growth was more pronounced than treatment with ENZA (5uM and 10uM). Moreover, treatment with a combination of both 5Aza-dC and ENZA was more effective in limiting cell growth and proliferation. We uncovered that DNA methylation (DNMT1, DNMT3A, and DNMT3B) was upregulated in ENZ-R cells. Our results also show that 5Aza-dC treatment of established ENZ resistant PCa Cells (LNCaP-ENZR cells) also reduced growth and proliferation significantly ($p<0.01$). Combination treatment of the cells with 5Aza-dC and ENZA decreased clonogenic activity. Results of cell migration are still in progress.

CONCLUSIONS: Our data demonstrated that the DNA methylation pathway is deregulated in ENZ-R Prostate cancer cell lines, and that targeting DNA methyltransferases sensitized the prostate cancer cells enzalutamide (current treatment). These studies suggest DMNT activity as a potential therapeutic vulnerability that can be exploited for limiting cellular plasticity, tumor progression, and therapy resistance in prostate cancer. Because DNMT inhibitors are currently approved for other malignancies, addition of these inhibitors to current treatment regiments could be readily explored in PCa.