Prostate cancer is the most prevalent malignancy among men in the United States, resulting in over 35,000 deaths annually (1). It originates primarily from the luminal epithelial cells of the prostate gland, which arise from basal cells during prostate development and differentiation and are characterized by the expression of the androgen receptor (AR) and other specific markers. While androgen signaling is essential for the normal function and survival of luminal cells, it is also pivotal in the development and progression of prostate cancer (6).

Androgen deprivation therapy (ADT) has long been a cornerstone in the treatment of advanced prostate cancer. By reducing levels of androgens, ADT aims to inhibit androgen receptor signaling and therefore reduce the growth and proliferation of prostate cancer cells. However, despite the initial efficacy of ADT, most patients eventually develop resistance, known as Enzalutamide Resistance (ENZ-R) (3). The development of ENZ-R is marked by adaptive cellular mechanisms, including cellular plasticity and the emergence of neuroendocrine prostate cancer (CRPC-NEPC) and double-negative prostate cancer (CRPC-DNPC) phenotypes. While genomic alterations have been extensively investigated in ENZ-R, the role of epigenetic mechanisms remains less understood. Recent studies, including those from our laboratory, have pointed to increased expression and activity of DNA methyltransferases (DNMTs) such as DNMT1, DNMT3a, and DNMT3b, and the polycomb repressive complex 2 (PRC2) component EZH2 during prostate cancer progression (2,4). However, the direct role of DNMTs in ENZ-R has not been thoroughly evaluated.

In this study, we explore the potential of DNMT and EZH2 inhibitors to sensitize prostate cancer cells to enzalutamide. 5-Aza-2'-deoxycytidine (5-AZA-dC) is a broad-range DNMT inhibitor that incorporates into DNA and covalently traps DNMTs, thereby inhibiting DNA methylation and reactivation of silenced genes. GSK126 is a selective EZH2 inhibitor that impedes the histone methyltransferase activity of EZH2, reducing the tri-methylation of histone H3 on lysine 27 (H3K27me3), which is associated with active gene repression. Our results show that targeting these epigenetic regulators may help overcome ENZ-R resistance in Prostate Cancer.

**Introduction**

**Methods**

1. **Cell Culture:** LNCaP and LNCaP-ENZ-R cell lines were cultured under standard conditions with the LNCaP-ENZ-R receiving consistent treatment with 5µM ENZ.
2. **Western Blot Analysis:** Protein expression levels of DNMT and other prostate cancer markers were determined using Western blotting techniques.
3. **PCR Analysis:** DNMT gene expression was quantified through polymerase chain reaction (PCR) assays.
4. **Cell Growth and Proliferation:** The Incucyte live-cell analysis system was utilized to monitor and measure cell growth and proliferation rates.
5. ** Colony Formation Assay:** The ability of single cells to grow and form colonies was assessed using colony formation assays.

**Results**

**Fig 2. Enzalutamide Resistance is associated with increased DNMT Expression**

- **Fig 3 Effect of DNMT inhibition on cell growth and proliferation:** 3a: Cell Growth in LNCaP-ENZ-R Cells treated with varying concentrations of ENZ (5 μM and 10 μM), 5-AZA (5 μM and 10 μM), and their combinations were monitored 96 hours using the Incucyte live-cell analysis system. 3b: The growth and proliferation of LNCaP cells treated with varying concentrations of ENZ (5 μM and 10 μM), 5-AZA (5 μM and 10 μM), and their combinations were monitored 96 hours using the Incucyte live-cell analysis system. Cell Density: 3k cells per well, 200µL media.

**Fig 4 Inhibition of DNMT's decreased clonogenic activity of ENZ-Resistant PCa cells**

- **Fig 4 Effects of DNMT inhibition on Colony Formation:** A: Representative images of colonies formed by LNCaP cells following treatment with 5 µM ENZ, 5 µM 5-AZA, and their combination. Colonies were stained and quantified after a incubation period. The bar graph below depicts the number of colonies formed under each treatment condition; B: Representative images of colonies formed by LNCaP-ENZ-R cells following treatment with 5 µM ENZ alone or in combination with 5 µM 5-AZA. The bar graph below indicates a significant reduction in colony formation in LNCaP-ENZ-R cells treated with the combination of ENZ and 5-AZA. Statistical significance is indicated (* p < 0.05, ** p < 0.001).

**Fig 5 Enzyme-Resistant Prostate Cancer (ENR) Cells:** The proliferation of LNCaP cells treated with 5 µM ENZ, 5 µM 5-AZA, 5 µM GSK126, and their combinations were monitored over 96 hours using the Incucyte live-cell analysis system.

**Conclusions:**

Our data demonstrated that the DNA/Histone methylation pathways are deregulated in ENR prostate cancer cells, and that targeting DNA methyltransferases and EZH2 sensitized the prostate cancer cells enzalutamide (current treatment).

These studies suggest DNMT and EZH2 activities as a potential therapeutic vulnerability that can be exploited for limiting cellular plasticity, tumor progression, and therapy resistance in prostate cancer. Because DNMT and EZH2 inhibitors are currently approved for other malignancies, addition of these inhibitors to current treatment regimens could be readily explored in PCa.

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**References**