ADT/ANTI-AR THERAPY RESULTS IN GAPDH UPREGULATION IN PROSTATE CANCER

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Introduction

As the widespread use of potent anti-AR drugs in castration-resistant prostate cancer patients, neuroendocrine progression (t-NEPC or CRPC-NE) has emerged as a major clinical obstacle, accounting for more than 25-30% mortality of prostate cancers. Recent studies with patient-derived xenografts (PDX) revealed that t-NEPC model LTL-331R exerted a highly upregulated glycolytic activity, indicating a metabolic reprogramming in t-NEPC progression. Currently, targeting the altered glycolysis pathway in cancer cells has emerged as a potent cancer therapy. Especially, inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a critical glycolytic enzyme, achieved a profound anti-cancer outcome specifically in highly glycolytic cancers. Therefore, we investigated the role of GAPDH alteration in t-NEPC/CRPC-NE models and identified a novel GAPDH inhibitor Alternol as a potential therapy for NEPC patients.

Methods

GAPDH gene expression profiles in prostate cancers were analyzed using the published NCBI GDS datasets. GAPDH promoter-driven luciferase reporter assay and glucose consumption assay was conducted in LNCaP cells after Enzalutamide treatment. GAPDH protein expression was evaluated in tissue microarray sections, NEPC PDX tissue and xenograft tissues by immunohistochemistry. The novel small compound Alternol isolated from fungi fermentation was used in multiple prostate cancer cell lines for GAPDH activity assay. Cellular thermal shift assay (CETSA) was used to verify the interaction of Alternol with GAPDH protein in cells.

Results

Data mining for GAPDH expression showed that it is slightly higher in primary (1.7-2.18 folds) and significantly higher in metastatic prostate cancers (>5-folds) compared to normal or benign adjacent tissues (Fig 1). In addition, castration in mice caused a significant increase of GAPDH expression in prostate gland or subcutaneous xenograft tissues of prostate cancer (Fig 2). Consistently, GAPDH-LUC reporter activity was increased about 2-fold after androgen depravation, which was further enhanced (> 9-fold) by Enzalutamide in LNCaP cells. Interestingly, Enzalutamide also enhanced glucose consumption rate under androgen depravation condition (Fig 3). Molecular docking study confirmed our previous report that Alternol interacts with GAPDH at the catalytic active/NAD+ binding sites with a binding affinity at -10.1 kcal/mol (Fig 4A). This interaction was validated in CETSA assay in C4-2 and 22RV1 cells (Fig 4B & 4C). The functional consequence of Alternol-GAPDH interaction was evaluated using in vitro and in vivo GAPDH assays (Fig 5).

Conclusion

Androgen depravation plus anti-AR therapy resulted in GAPDH up-regulation in prostate cancer cells and tissues, suggesting a strong clinical relevance of GAPDH up-regulation in anti-AR treatment-induced NE progression of CRPC patients. Alternol interacts with GAPDH and potently suppressed GAPDH glycolytic activity *in vitro* (IC_{50} = 5.794 nM), which is 37.5-fold more potent than an existing GAPDH inhibitor Korningic Acid (IC_{50} = 217.6 nM). Alternol also lowered down the excessive GAPDH glycolytic activity in prostate cancer cells to the level close to benign cells without a total blockage, indicating a safe therapeutic feature.

Funding

DoD PCRP190026

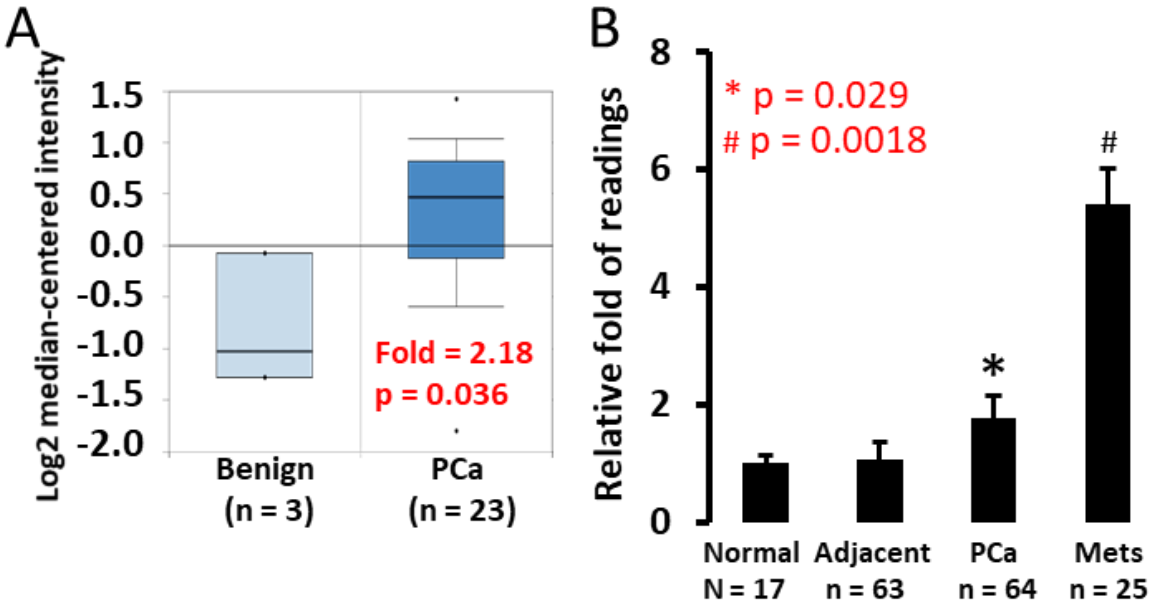

Fig 1. GAPDH expression in prostate cancers. Data mining for GAPDH expression was conducted using datasets from human prostate specimens (A, GSE6888236 and (B, GDS254537).

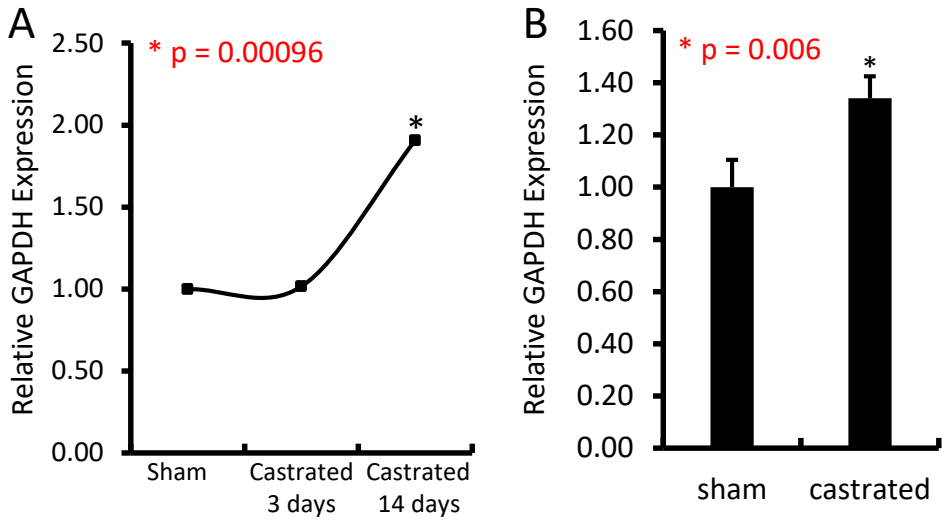

Fig 2. GAPDH expression after castration. Data mining for GAPDH expression was conducted using datasets from (A) mouse prostate tissue after 3-14 days of castration (GDS2562) and (B) LuCaP35 s.c. xenografts in SCID mice after 4-weeks castration (GDS4120). Epithelium-specific gene KRT18 was used for data normalization.

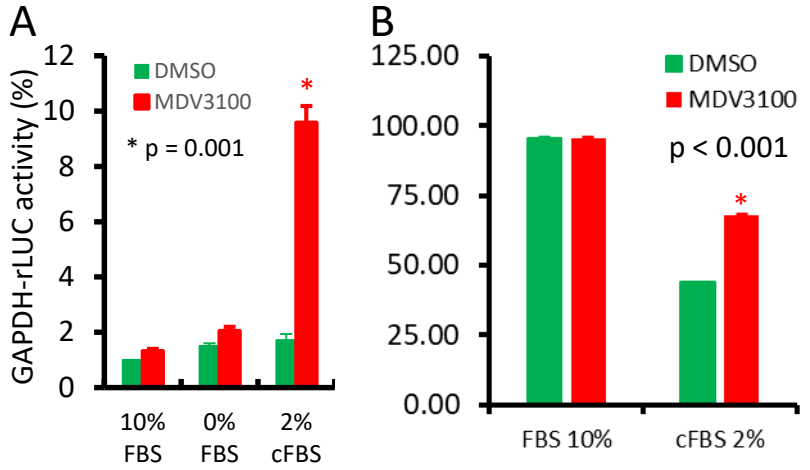

Fig 3. Enzalutamide enhances GAPDH expression and activity. **A** LNCaP cells transfected with GAPDH-rLUC constructs (Addgene#82479) were treated with DMSO or MDV3100 (Enzalutamide, 10 μM) for 24 h. Reporter activities were normalized with protein concentrations in corresponding samples. **B** Glucose levels in cell culture media were measured with a pre-assembled kit from Signa (catalog #GAGO20) in LNCaP cells after 24-h treatment as indicated.

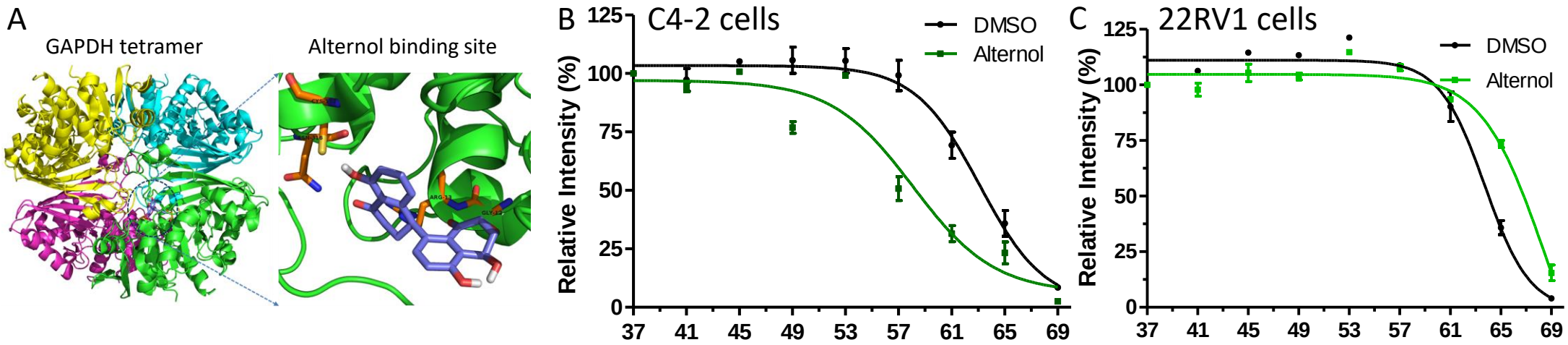

Fig 4. Alternol interacts with GAPDH. **A** in silicon docking analysis was conducted using crystal structure for human liver GAPDH protein derived from Research Collaboratory for Structural Bioinformatics Protein Data Bank (1ZNQ). **B&C** C4-2 and 22RV1 cells were treated with Alternol (10 μM) for 4 h, followed by CETSA assays.

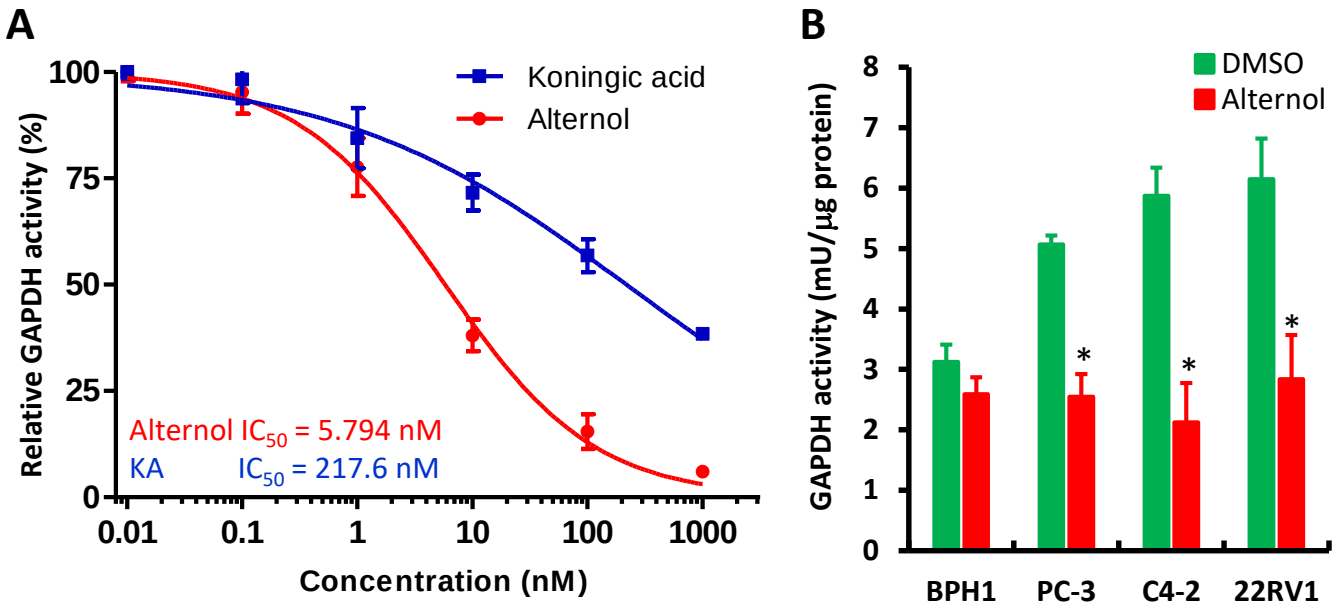

Fig 5. Alternol suppresses GAPDH activity. **A** GAPDH in vitro activity assay. **B** Cells were treated with DMSO or Alternol (10 μM) for 4 h and cellular proteins were extracted for GAPDH activity assay using the BioVision kit. The asterisk indicates a significant difference compared to DMSO ($p < 0.05$, Student t-test).