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

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Introduction & Objective

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.

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).

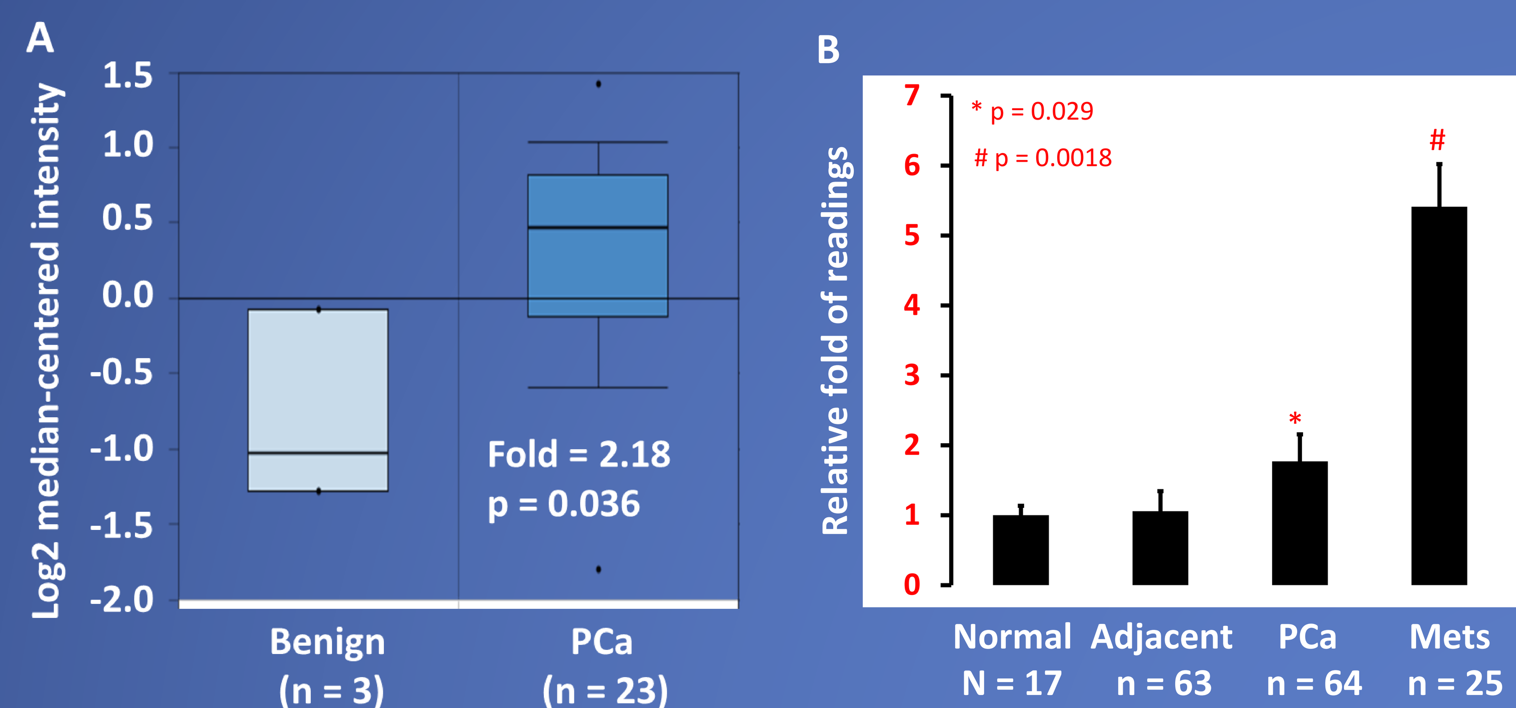

Fig 1. GAPDH expression in prostate cancers. Data mining for GAPDH expression was conducted using datasets from human prostate specimens, (A) GSE68882 and (B) GDS2545.

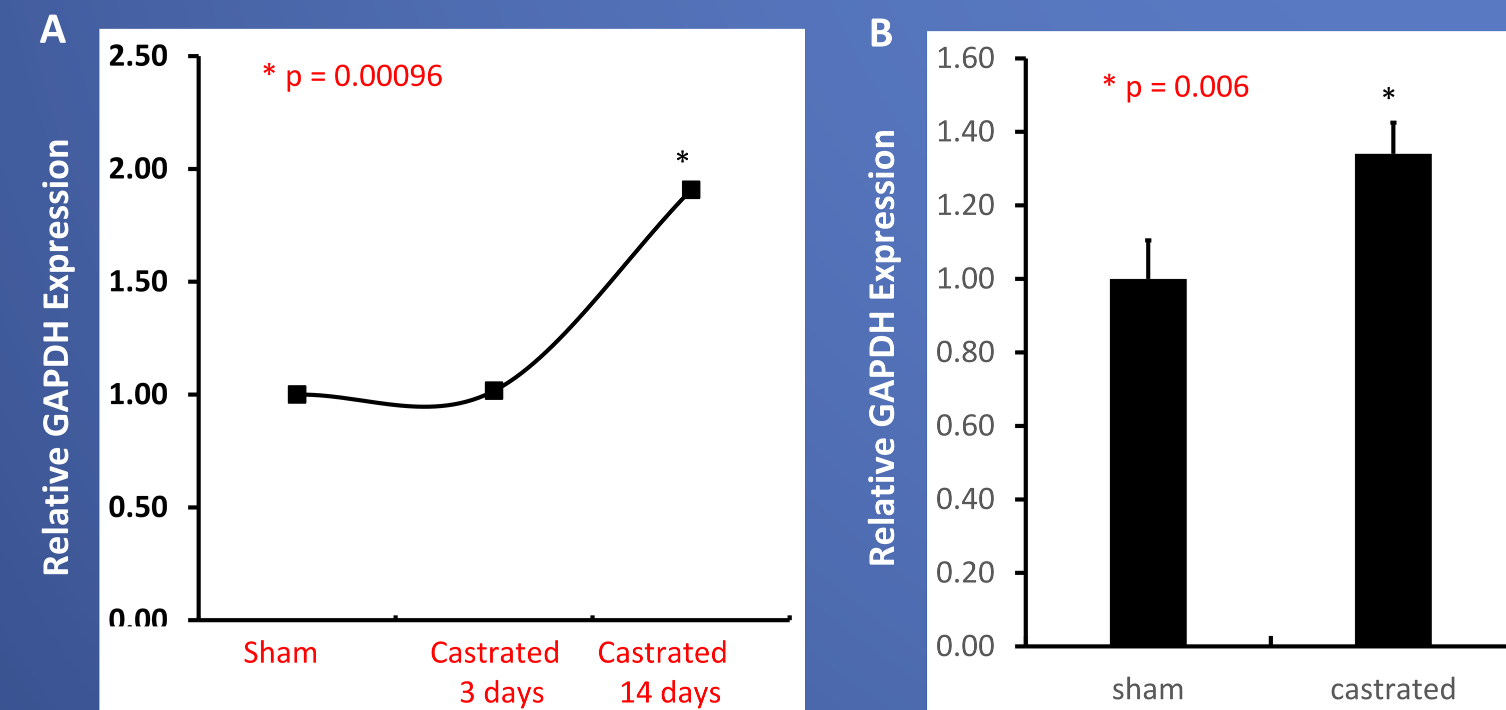

Fig 2. GAPDH expression after castration. Data mining for GAPDH expression was conducted using datasets from (A) mouse prostate tissue after 3-14 days of castration (GDS2562) and (B) LuCaP35 s.c. xenografts in SCID mice after 4-weeks castration (GDS4120). Epithelium-specific gene KRT18 was used as an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

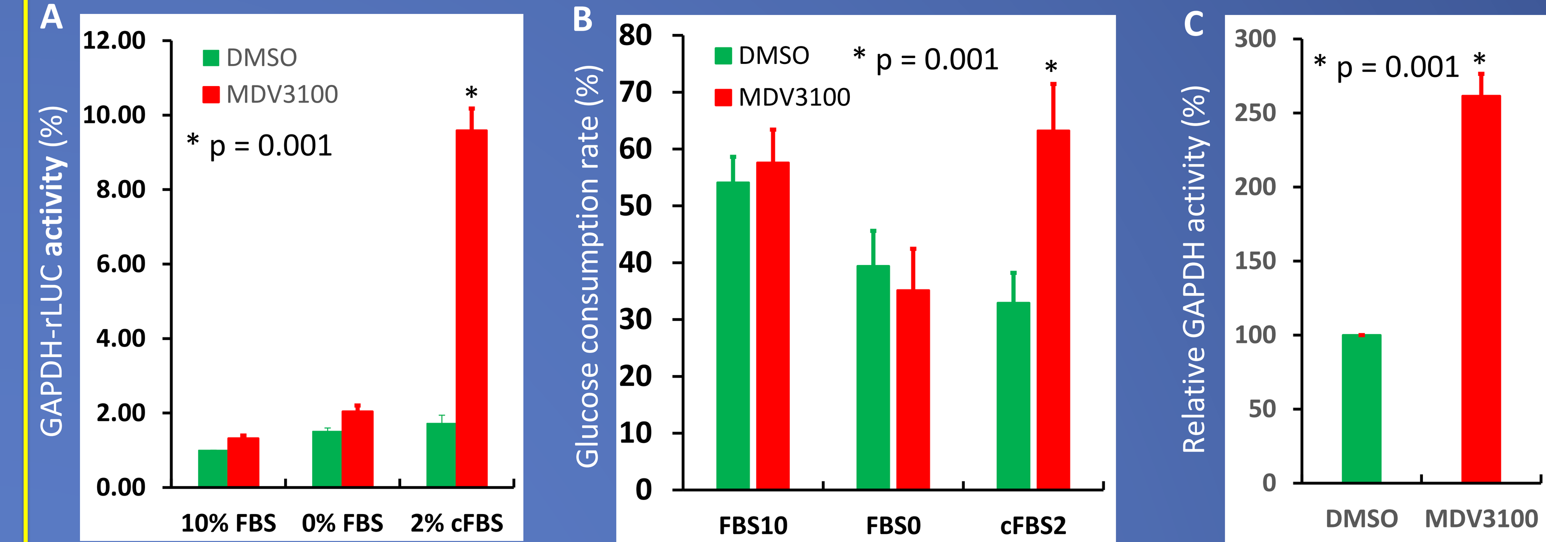

Fig 3. GAPDH expression and activity were enhanced after ADT and MDV3100 treatment. A LNCaP cells transfected with GAPDH-rLUC constructs (Addgene#82479) were treated with DMSO or MDV3100 (10 μM) for 24 h under different serum condition as indicated. GAPDH-rLUC activities were normalized with protein concentrations in corresponding samples. B LNCaP cells were treated with DMSO or MDV3100 (10 μM) for 24-h under different FBS condition as indicated. Glucose levels in cell culture media were measured with a pre-assembled kit from Signa (catalog #GAGO20). C GAPDH activity was assessed using purified GAPDH enzyme (5.0 μg/assay) from rabbit muscle (BioVision catalog #K680). The substrate G3P (0.5 mM) is converted to 1,3-bisphosphate glycerate, yielding NADH captured by a developer chemical to form a color product with a maximal absorption at 450 nm. MDV3100 was used at 20 μM. The statistical analysis was carried out using Student t-test comparing MDV3100 to DMSO in the corresponding group.

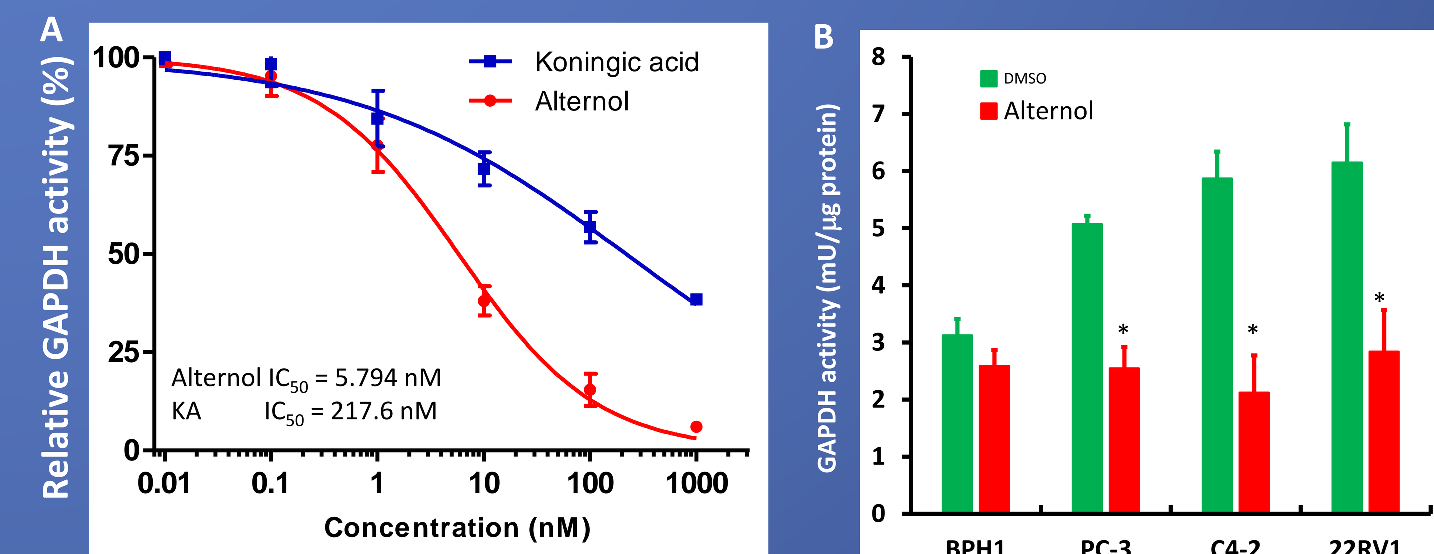

Fig 5. Alternol suppresses GAPDH activity. A GAPDH in vitro activity assay was described in Fig 3E. B Cells were treated with DMSO or Alternol (10 □M) for 4 h and cellular proteins were extracted for GAPDH activity assay using the BioVision kit. The asterisk indicates a significant difference compared to DMSO (p < 0.05, Student t-test).

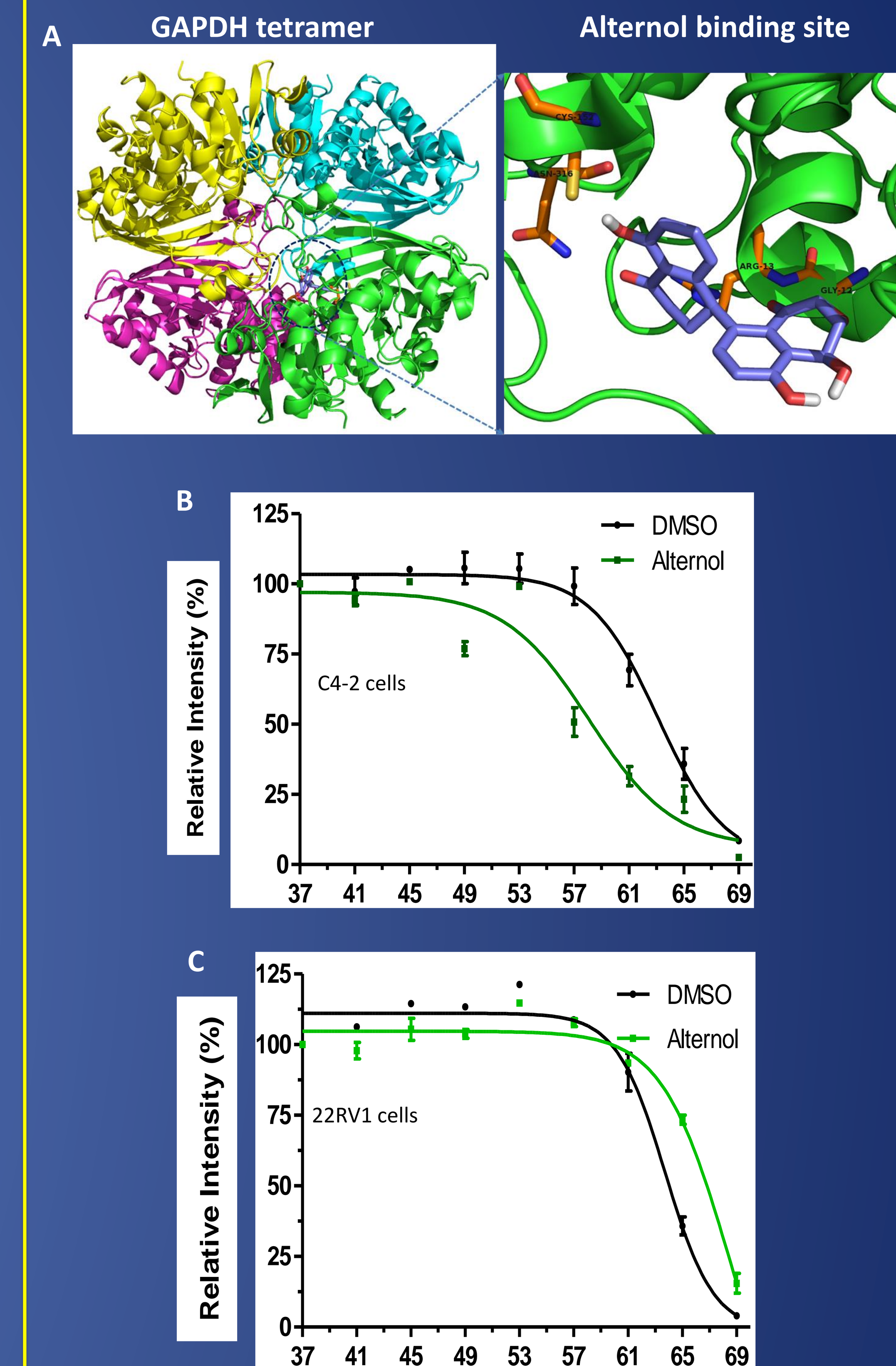

Fig 4. Alternol interacts with GAPDH. A Computer-aid in-silicon docking analysis was conducted using crystal structure for human liver GAPDH protein derived from Research Collaboratory for Structural Bioinformatics Protein Data Bank (1ZNQ)56. B&C C4-2 and 22RV1 cells were treated with Alternol (10 □M) for 4 h, followed by CETSA assays as described54

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 prostate cancer cells to the level close to benign cells without a total blockage, indicating a safe therapeutic feature.