Establishment and characterization of Enzalutamide resistant prostate CDX models

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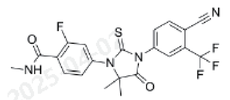
Abstract

Prostate cancer (PCa) is common and mostly androgen dependent cancer occurred in men and ranked fifth of the causes of death over the world, reduction the serum level of androgen become the priority therapy method for treating PCa patients. Although the first generation of anti-androgen receptor (AR) new drugs such as flutamide, nilutamide, and bicalutamide were launched few years ago and lighted new hopes for treatment of androgen dependent PCa, drug resistance was developed quickly and push the second generation of anti-AR drugs such as enzalutamide, apalutamide, and darolutamide to the market by their high specific binding to AR and better drug efficacy. Despite new anti-AR drugs exerted better efficacy, drug resistance still hampered the treatment of PCa patients, new challenge was burst for anti-PCa drugs through AR pathway. In this study, we developed an enzalutamide resistant C4-2B cell line in vitro by escalated the enzalutamide concentration gradually. The phenotype and genotype of C4-2B cell line were checked while enzalutamide concentration was increased. After 6 months of induction and identification, C4-2B enzalutamide resistant cell line was established and showed 140 times of drug-resistant index compare to that of parental cell. The in vivo drug-resistant efficacy was also confirmed in C4-2B enzalutamide resistant (C4-2B Enz-R) CDX models using 30 mpk enzalutamide through PO administration for 28 days (TGI=8.37%, T/C=93.22%) compared to the enzalutamide efficacies on LNCAP and 22RV1 CDX models. Western blot for androgen receptor expression level in tumor tissues and ELISA assay for prostate specific androgen (PSA) level in serum are highly correlated to the tumor volume of C4-2B Enz-R CDX models. The well characterized novel C4-2B Enz-R cell line and CDX model were appropriate for new drug discovery of anti-PCa drugs through AR pathway in vitro and in vivo.



Enzalutamide Chemical Structure: Enzalutamide (MDV3100), an androgen receptor (AR) antagonist, inhibited AR with an IC50 value of 36 nM in LNCaP prostate cells. Enzalutamide is an autophagy activator.

Methodology

Induction method of in vitro drug-resistant strains

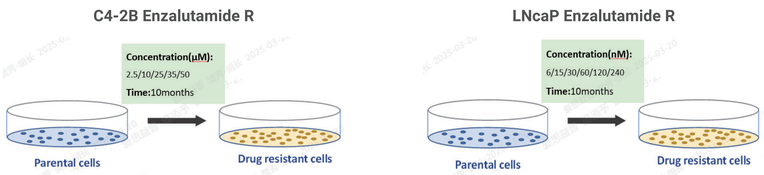


Figure 1: Schematic diagram of constructing drug-resistant strains.

Long term addition of drugs to stimulate C4-2B and LNCaP cells, continuous passage and culture, simulating the conditions experienced by tumor cells during in vivo chemotherapy, gradually leading to significant drug resistance in the cells.

Results

1. IC50 testing

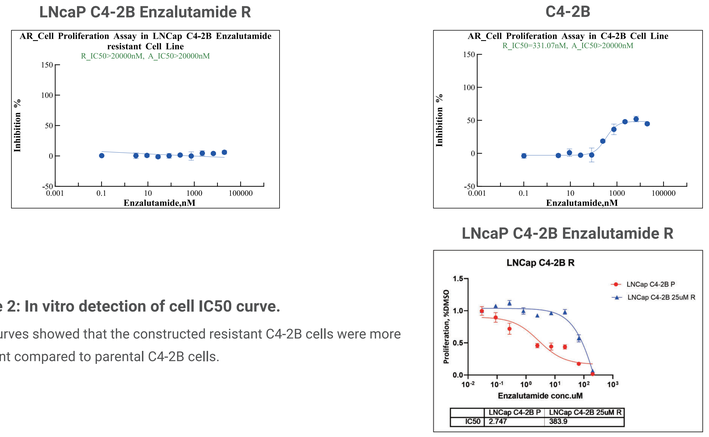


Figure 2: In vitro detection of cell IC50 curve.

IC50 curves showed that the constructed resistant C4-2B cells were more resistant compared to parental C4-2B cells.

2. Cell proliferation of parents and drug-resistant strains

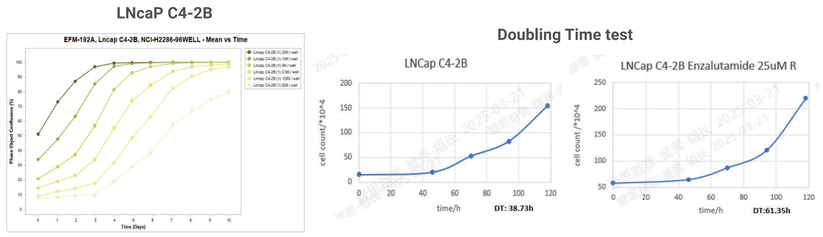


Figure 3: In vitro detection of cell fusion curve.

The fusion curve shows that the growth rate of the drug-resistant C4-2B cells constructed is slightly slower compared to the parental LNCaP C4-2B cells.

3. The CDX Model Targeting the AR

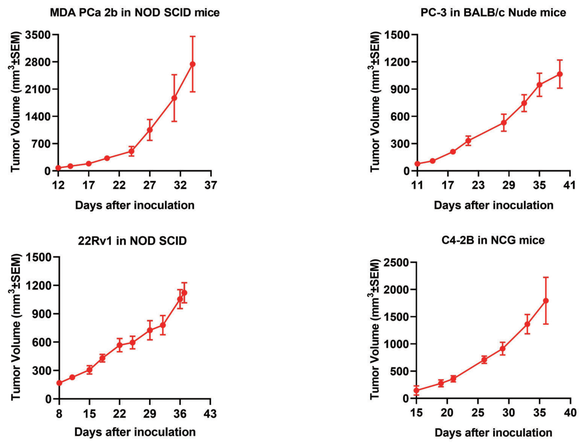


Figure 4: MDA Pca 2b, PC-3, 22RV1 and C4-2B tumor xenograft growth curves.

MDA Pca 2b, PC-3, 22RV1 and C4-2B exhibit good tumorigenic effects: fast tumorigenesis rate and uniformity of tumorigenesis.

4. In vivo efficacy evaluation of drug resistance models

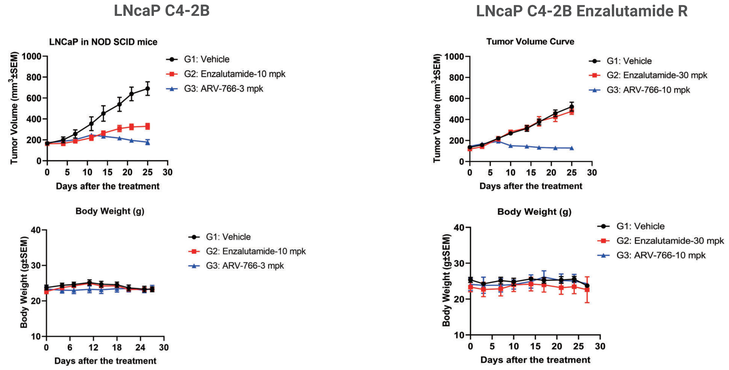


Figure 5: Changes in tumor volume and mouse weight in drug resistant models.

The pharmacological results of the in vivo drug resistance model showed that the tumor formation rate of the resistant strains was slower than that of the parents, and LNCaP C4-2B Enzalutamide R showed significant drug resistance. The weight maintenance of the mice in both models was good.

5.Transcriptome sequencing analysis of drug-resistant strains

C4-2B Enzalutamide R

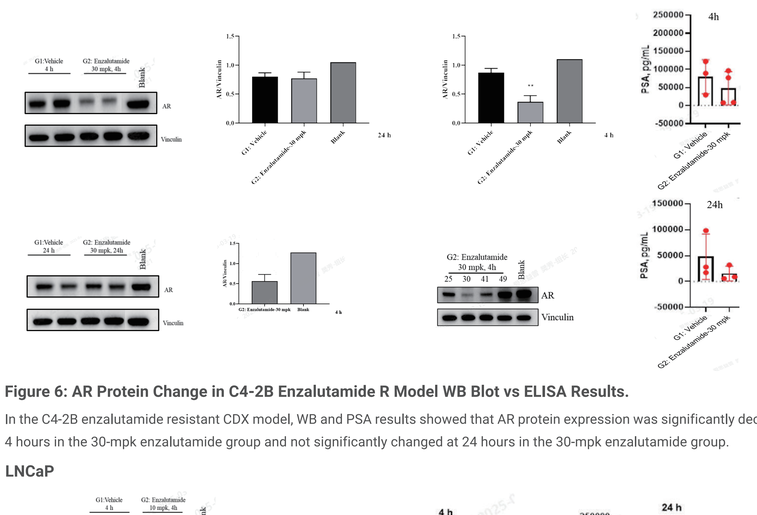


Figure 6: AR Protein Change in C4-2B Enzalutamide R Model WB Blot vs ELISA Results.

In the C4-2B enzalutamide resistant CDX model, WB and PSA results showed that AR protein expression was significantly decreased at 4 hours in the 30-mpk enzalutamide group and not significantly changed at 24 hours in the 30-mpk enzalutamide group.

LNCaP

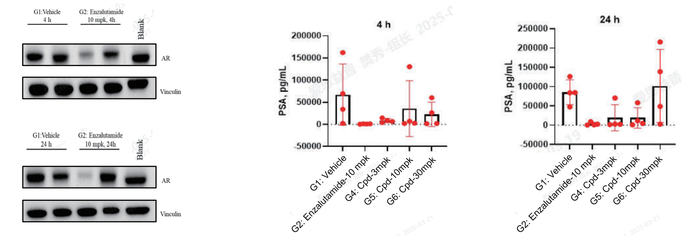


Figure 7: AR Protein Change in LNAaP Model WB Blot vs ELISA Results.

In the LNCap CDX model, WB and PSA results showed that AR protein expression was significantly decreased in the 10-mpk enzalutamide group at 4 h and 24 h.

Discussion

According to the results of Western blot, AR protein in C4-2B Enz R was significantly degraded in the group of Enzalutamide after 4 hours of administration, with significant statistical differences, degradation of AR protein was not evident in the groups after 24 days of dosing. Although enzalutamide is only an AR antagonist, it has been shown in the literature to decrease AR expression in C4-2B, C4-2B Enz R, and LNCaP cells [1, 2] . However, the degradation of AR in tumor tissue became less pronounced after 24 h of dosing likely due to resistance of C4-2B enzalutamide R cells. By detecting the concentration of human PSA in plasma, it can be found that there is a higher concentration of human PSA in the plasma of mice inoculated with tumors, and the production of PSA can be reduced to some extent after the addition of drugs.

Summary

The in vivo drug resistance model can be used to evaluate the efficacy and toxicity of new drugs. By testing the anti-tumor activity of new drugs in drug resistance models, their effects on drug-resistant tumors can be determined, guiding drug development and optimization. Using C4-2B Enzalutamide Rresistant cell and LNCaP, 22RV1 cell lines to screen for new compounds or combination therapies that may overcome or reverse drug resistance can help identify potential therapeutic strategies and improve drug efficacy.

References

1. Liadi YM, Campbell T, Hwang BJ, Elliott B, Odero-Marah V. High Mobility Group AT-hook 2: A Biomarker Associated with Resistance to Enzalutamide in Prostate Cancer Cells. Cancers (Basel). 2024 Jul 24;16(15):2631.

2. Erb HHH, Oster MA, Gelbrich N, Cammann C, Thomas C, Mustea A, Stope MB. Enzalutamide-induced Proteolytic Degradation of the Androgen Receptor in Prostate Cancer Cells Is Mediated Only to a Limited Extent by the Proteasome System. Anticancer Res. 2021 Jul;41(7):3271-3279.