Adaptation of prostate cancer cell lines to tumor microenvironment culture conditions induces expression of androgen receptor splice variant 7 and contributes to drug resistance

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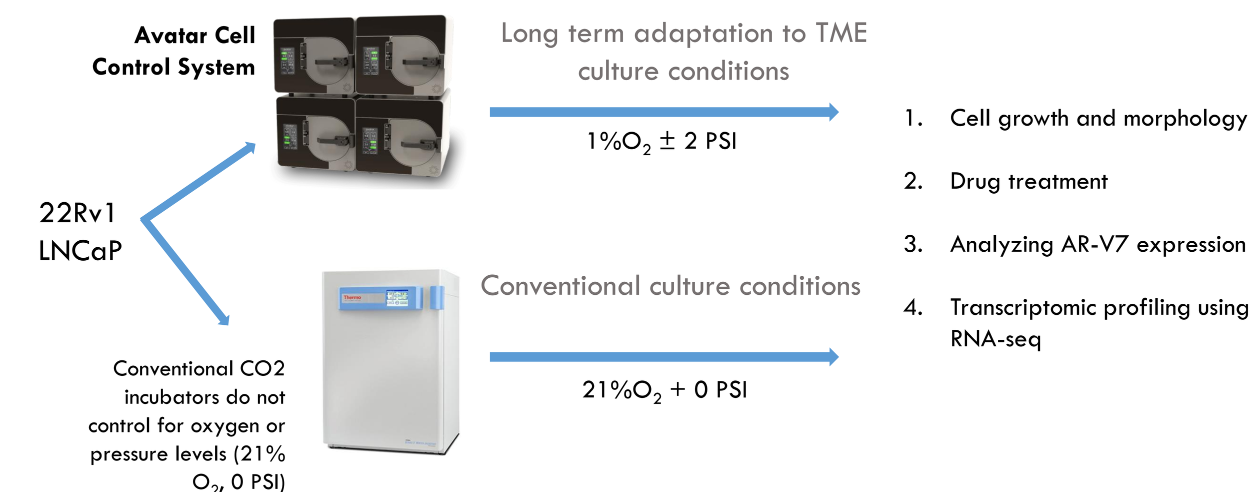
Culturing cells under conditions mimicking the tumor microenvironment increased expression of AR variant 7 and contributed to drug resistance

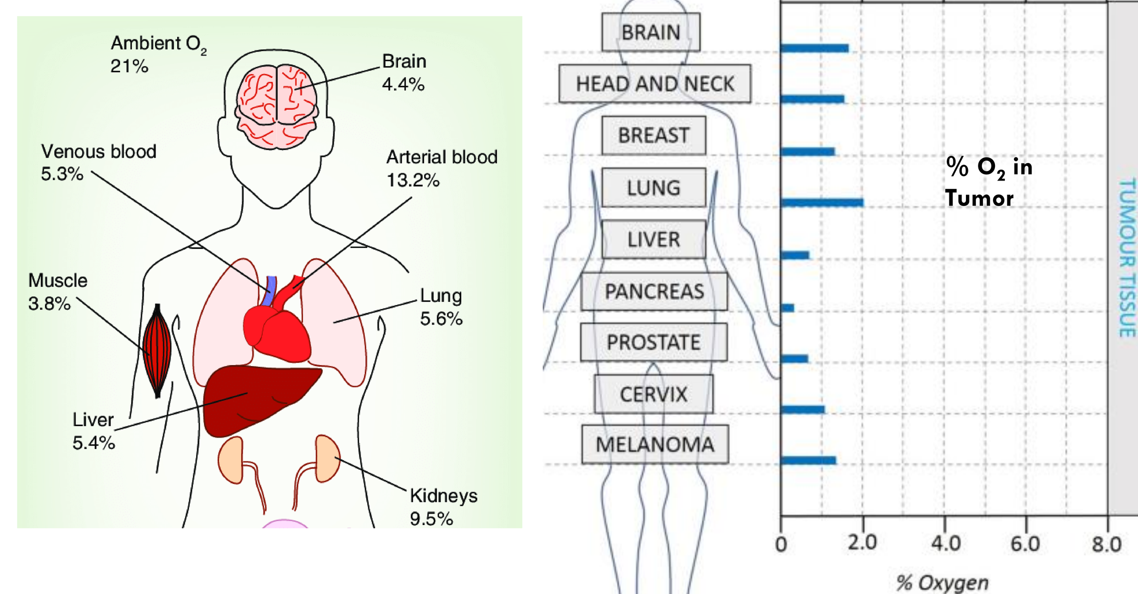
The tumor microenvironment (TME) has several characteristics that distinguish it from normal tissue, including elevated interstitial fluid pressures and hypoxia. Both pressurized and hypoxic culture conditions can influence gene and protein expression in tumor cells which can result in tumor metastasis and drug resistance. Enzalutamide is effective in treating metastatic castration-resistant prostate cancer (mCRPC), but majority of patients eventually develop resistance. One of the biomarkers for enzalutamide resistance is the expression of androgen receptor splice variant 7 (AR-V7), but the mechanism of AR-V7 upregulation in prostate cancer is still unclear. To investigate the influence of TME culture conditions on prostate cancer cell lines, 22Rv1 and LNCaP cells were incubated under pressurized and hypoxic culture conditions (2 PSI + 1%O2). We compared cell growth, gene expression profiles and drug response to enzalutamide treatment. 22Rv1 and LNCaP cells cultured under TME conditions exhibited increased resistance to enzalutamide treatment. Higher levels of AR-V7 expression were detected in both 22Rv1 and LNCaP grown in TME culture conditions. RNA-seq data identified 700+ differentially expressed genes in 22Rv1 and LNCaP cell lines adapted to TME culture conditions. GSEA analysis identified the upregulation of signaling pathways associated with hypoxia, glycolysis and epithelial-mesenchymal transition and the downregulation of xenobiotic metabolism and oxidative phosphorylation pathways in TME culture conditions. In summary, adaptation to TME culture conditions can promote the expression of AR-V7 in prostate cancer cell lines and can contribute to enzalutamide drug resistance. Adaptation of cell lines to TME culture conditions can provide meaningful insights into mechanisms of drug resistance while providing a novel approach for identifying drug targets and biomarkers of drug resistance.

Specific Aims

1. Identify the mechanism of prostate cancer cells resistance to enzalutamide treatment in the tumor microenvironment.
2. Generate a model for screening and evaluating chemotherapy drugs.

Materials and Methods





Growth rates of prostate cell lines acclimated to the TME culture conditions were similar to those grown in a standard CO_2 incubator

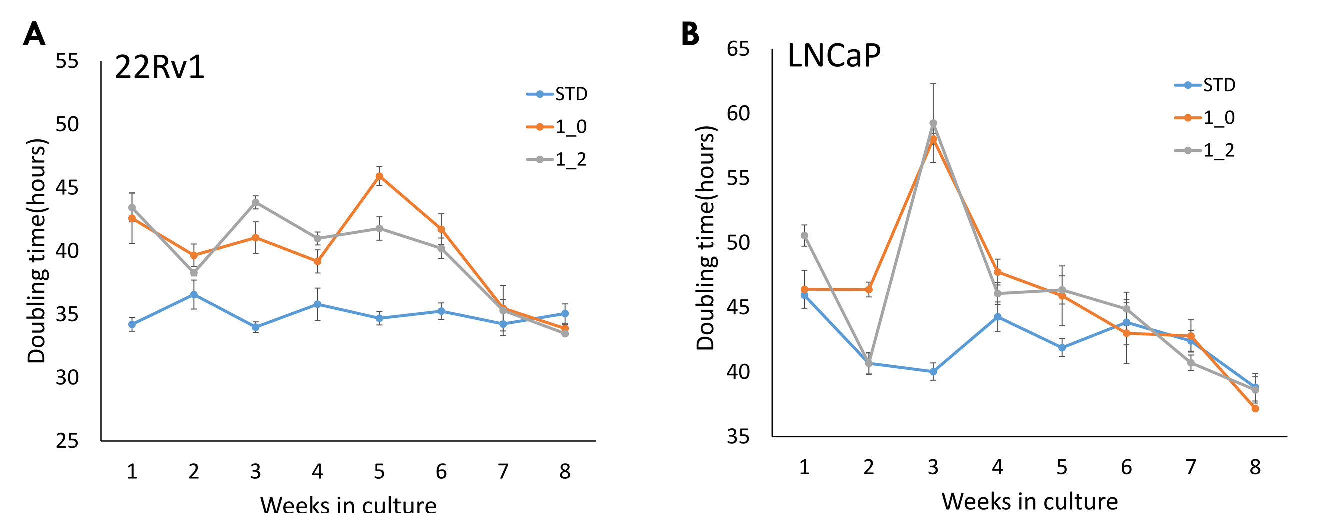


Figure 1. The doubling times of prostate cancer cell lines under TME and standard CO_2 incubator conditions. The doubling times of 22Rv1 (**A**) and LNCaP (**B**) under TME conditions had inhibited growth to start, however they recovered over time and resulted in growth rates similar to cells grown in a standard CO_2 incubator.

Acclimation of prostate cancer cells under TME culture conditions induced resistance to enzalutamide treatment

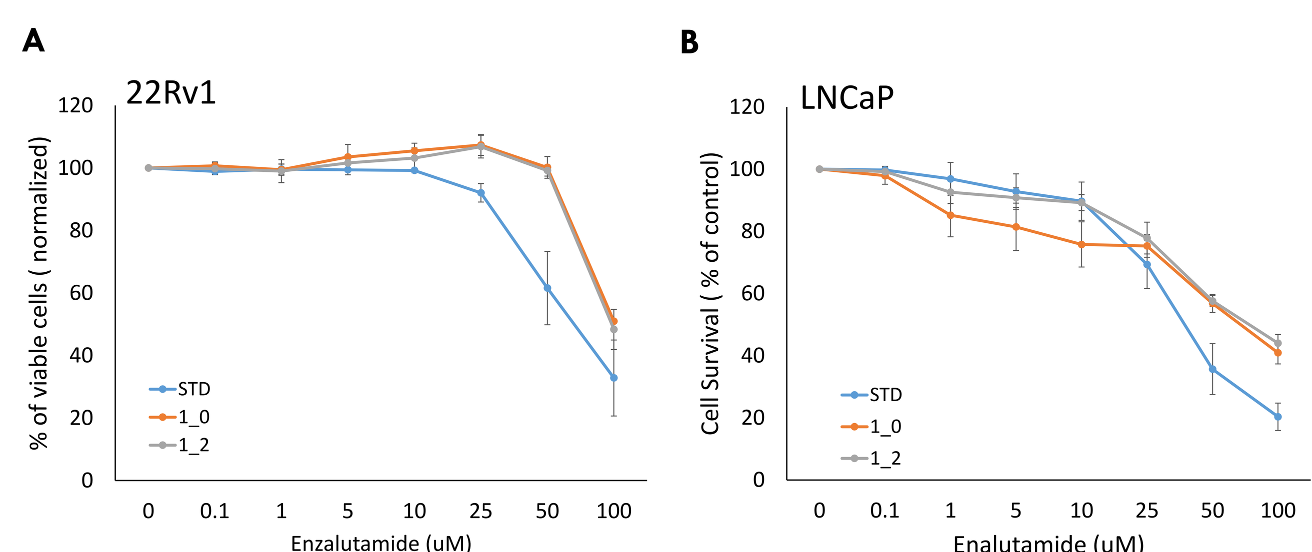


Figure 2. Adaptation of prostate cancer cells to the TME induced the resistance to enzalutamide treatment. Acclimation of 22Rv1 (**A**) and LNCaP (**B**) to TME (1% O_2 with or without 2 PSI) for 2 months induced the resistance to enzalutamide treatment compared to cells cultured in CO2 incubator.

Acclimation of prostate cancer cells under TME culture conditions induced AR-V7 expression

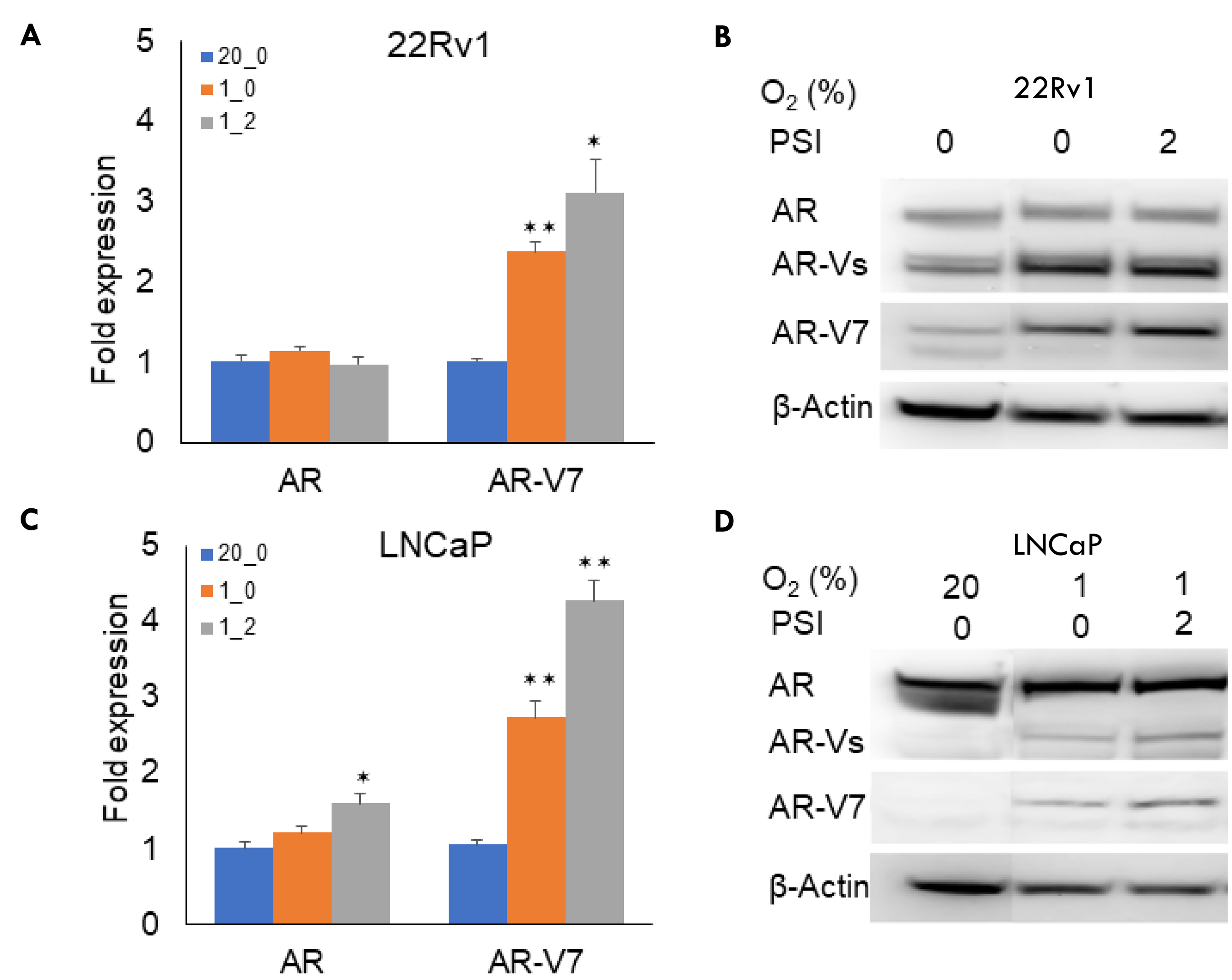


Figure 3. Adaptation of prostate cancer cells to the TME induced the expression of androgen receptor variants expression, especially AR-V7. qPCR (**A**) and western blot (**B**) measured the expression of AR and AR-V7 in TME adapted 22Rv1 cells. qPCR (**C**) and western blot (**D**) measured the expression of AR and AR-V7 in TME adapted LNCaP cells. Results shown are representative of three independent experiments. *P < 0.05; **P < 0.01.

Tumor microenvironment (TME) culture conditions altered gene expression profiles of 22Rv1 and LNCaP cells

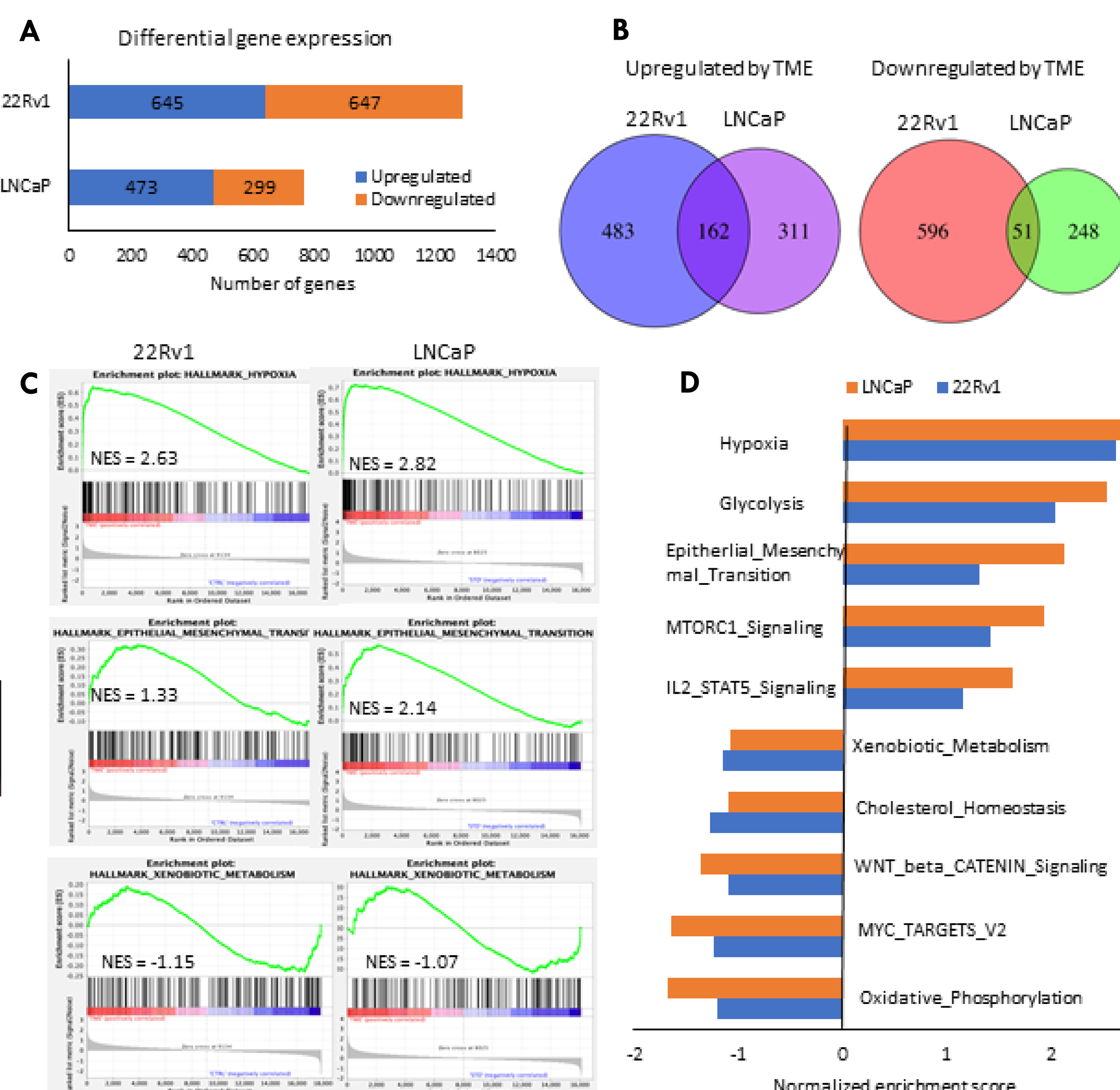


Figure 4. Transcriptomic analysis of the prostate cancer cell 22Rv1 and LNCaP under tumor microenvironment conditions. (**A**) Number of genes significantly up- or down-regulated in tumor microenvironment conditions in 22Rv1 and LNCaP cells. (**B**) Venn diagrams of overlapped up and downregulated genes in 22Rv1 and LNCaP cells grown in TME conditions. (**C**) Gene set enrichment analysis (GSEA) plots for the hallmark signatures of hypoxia, epithelial-mesenchymal transition and xenobiotic metabolism in 22Rv1 and LNCaP cells. (**D**) GSEA signatures enrichment scores for significantly enriched pathways in 22Rv1 and LNCaP cells under TME conditions

Upregulation of KDM3A expression in TME acclimated prostate cancer cells contribute to increased AR-V7 expression

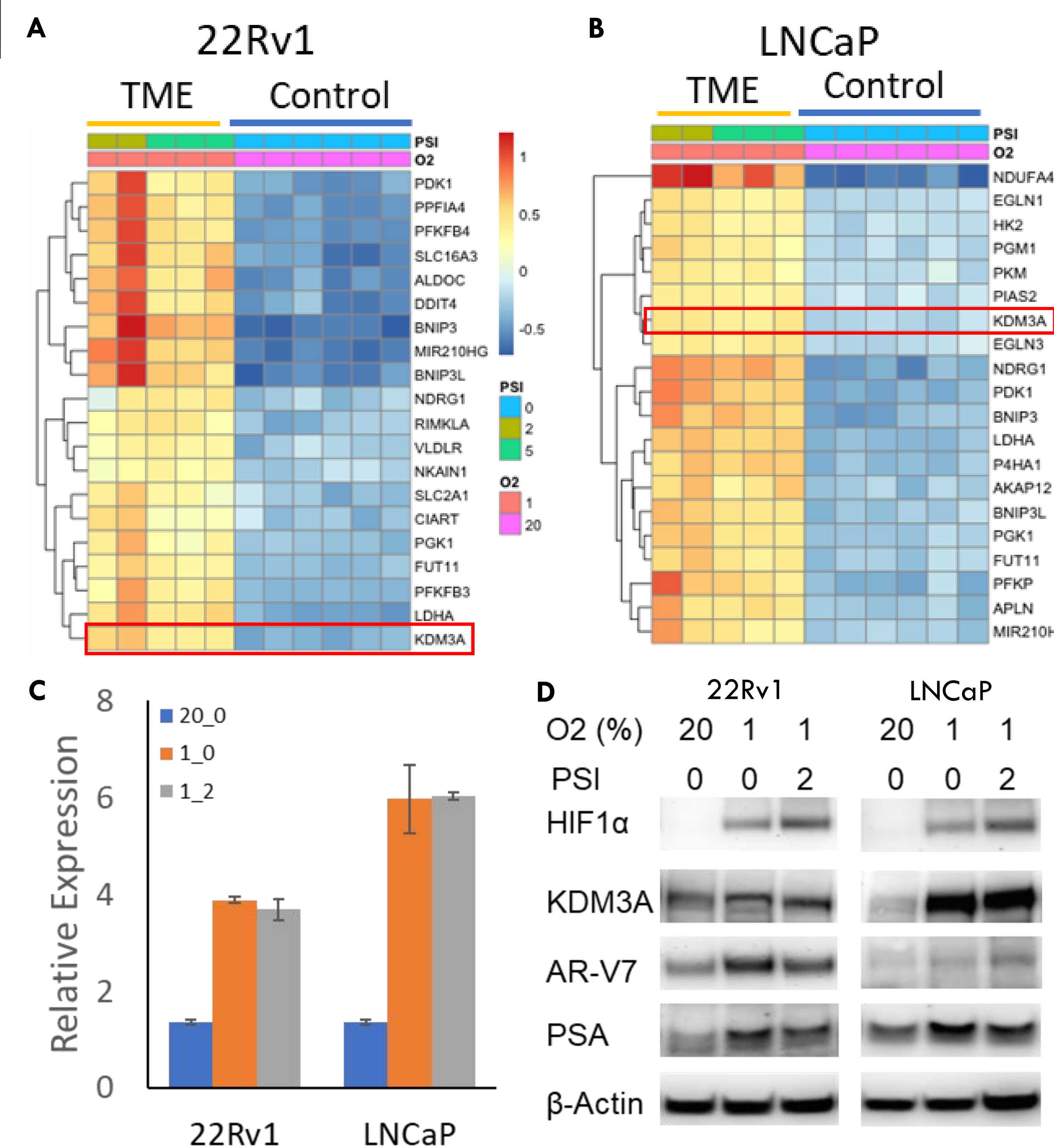


Figure 5. TME upregulated the expression of histone demethylase KDM3A in 22Rv1 and LNCaP cells. Heatmap of the top 20 differentially expressed genes in 22Rv1 (**A**) and LNCaP (**B**). qPCR results confirmed that the expression of KDM3A in 22Rv1 and LNCaP cells was upregulated in TME conditions (**C**). Western blot results showed that increased levels of HIF-1α and KDM3A proteins in TME conditions (**D**). KDM3A promoted alternative splicing of AR-V7, resulting in higher levels of AR-V7 in TME conditions and induced PSA protein expression. β-Actin was used as loading control.

Prostate cancer cell lines cultured under TME culture conditions show increased expression of Vimentin

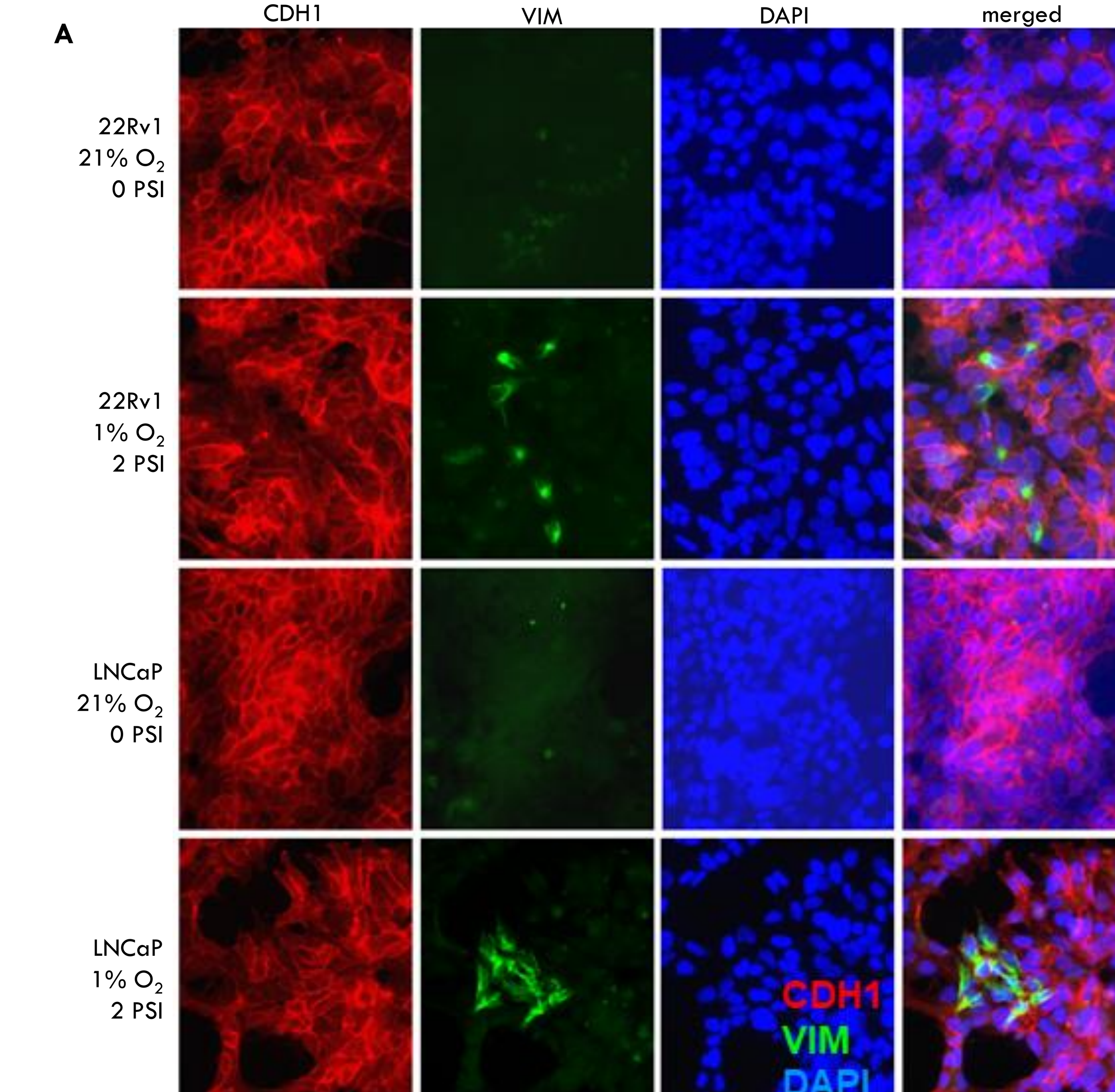


Figure 6. Tumor microenvironment induced epithelial-mesenchymal transition (EMT) in 22Rv1 and LNCaP cells. 22Rv1 and LNCaP cells were cultured under TME or standard CO2 incubator for 3 days, expression of epithelial cell marker E-cadherin (CDH1) and mesenchymal cell marker Vimentin (VIM) was analyzed by immunofluorescence staining (**A**), qPCR (**B**) and Western blot (**C**). The results showed mesenchymal cell marker VIM was upregulated in 22Rv1 and LNCaP cells under TME conditions.

Summary

1. Tumor microenvironment induced histone demethylase KDM3A expression in prostate cancer cells.
2. KDM3A promotes alternative splicing of AR variant 7 (AR-V7) in prostate cancer cells and contribute to enzalutamide resistance.
3. TME culture condition induced the epithelial-mesenchymal transition in prostate cancer cell lines.
4. Adaptation of cell lines to TME culture conditions can provide meaningful insights into mechanisms of drug resistance and deliver a novel approach to identify targets and biomarkers of drug resistance.

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