Hypoxia and pressure induce prostate androgen receptor variant expression and contribute to development of castration-resistant metastatic prostate cancer

Yunmin Li PhD, Bruce Adams PhD, Tianna Chow MSc, Luke Cassereau PhD, Bryan Downie PhD, & James Lim PhD

Xcell Biosciences Inc., 455 Mission Bay Blvd S, San Francisco, CA 94158

#P333

james@xcellbio.com

Culturing cells under conditions mimicking the tumor microenvironment increases expression of AR variants, including LNCaP, AVATAR, and 22Rv1.

**BACKGROUND**

The tumor microenvironment has several characteristics that distinguish it from normal tissue, including exposure to minimal oxygen and high interstitial fluid pressure. Both hypoxia and pressure can influence gene and protein expression of various cell types within a tumor. These changes in expression may serve as biomarkers, for patient stratification, or for treatment. For example, castration-resistant prostate cancer that is high levels of the AR mRNA variant 7 (ARV7) has been associated with resistance to enzalutamide and abiraterone.

We want to better understand the influence of how aspects of the microenvironment, such as hypoxia and pressure, influence androgen receptor (AR) signaling and metastatic properties of prostate cancer cells.

**METHODS**

In this report, we used a specialized cell culture system, AVATAR, to investigate the effects of hypoxia and pressure on prostate cancer cell lines, with the goal of analyzing androgen signaling in physiologically relevant culturing environments. We used a range of conditions to cover both oxygen concentration (1.5%-20%) and hydrostatic pressure levels (0 to 50 mmHg / 1.25 to 5 psi). We identified both common and unique gene signatures across these different cell lines, with the hypoxia conditions activating the HIF-1 pathway common across the cell lines studied, whereas pressure resulted in more restricted signatures. AR signaling was further studied in the cell lines 22Rv1 and LNCaP that were cultured under hypoxia and/or pressure and compared to standard cell culture conditions.

**RESULTS**

Hypoxia (1.5%) and/or pressure (2 PSI) induced higher expression of the mitotic variant ARV7 and to protein product in 22Rv1 cells. In LNCaP cells, AR-V7 protein product was not detectable under ambient (~20% O2) culture, but was detected under pressure and/or hypoxia. Expression of the normal AR protein did not change at the level of mRNA or protein in either 22Rv1 and LNCaP cells. Epithelial-mesenchymal transition regulators SLUG and ZEB1 were up-regulated under hypoxia, independent of pressure. Epithelial cell marker EPCAM and CAM was down-regulated by pressure or hypoxia, whereas the mesenchymal cell marker vimentin was up-regulated by the combination. Cell line sensitivity to therapies and their metastatic potential could also be affected by the microenvironment. For example, LNCaP cells were sensitive to the AR antagonist bicalutamide when cultured under standard culture conditions, whereas, when cultured under hypoxia and pressure culture, LNCaP cells were resistant to treatment.

**CONCLUSIONS**

Hypoxia and pressure up-regulated the expression of AR variants (especially AR-V7), and altered the expression of genes associated with mesenchymal transition, which contribute to castration-resistant metastatic prostate cancer development. These conditions can also influence how the cells respond to therapy. Physiologically-relevant conditions are essential factors for understanding the biology of prostate cancer as well as for drug development.

**Study objective:** Characterize the impact of the tumor microenvironment on prostate cancer cell lines, by altering oxygen and pressure levels during culture.