

## **The role of the microenvironment in therapeutic response to anti-prostate cancer treatment.**

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### **Background:**

Cancers do not exist in isolation - surrounding any tumour are supportive cells, which create the microenvironment in which cancer cells reside. Cancer and microenvironment cells interact and communicate with each other, physically and via paracrine signalling. In the prostate cancer (PCa), androgen receptor (AR) signalling in the surrounding fibroblasts is strikingly distinct from that within cancer cells, and has specific functions to produce, maintain, and modulate the extracellular matrix (ECM) which surrounds and interacts with cancer cells. The supportive cells of metastatic sites differ from those in the primary site and produce different types of cellular microenvironments. Since the advent of second generation anti-androgen therapy there has been an increase in the presence of liver metastasis. This project investigates how AR and anti-androgen therapy affect the liver microenvironment and the subsequent effects on cancer.

### **Methods:**

Hepa1 liver cells, 22RV1 and LNCaP PCa cell lines were used in *in vitro* experiments. 3D-microenvironment culture systems were created from liver cell, in which cells were allowed to deposit ECM, PCa cells were then grown in the artificial microenvironments and their proliferation measured. PCa proliferation was also measured in response to conditioned media (CM) from liver cells treated with or without androgens. 1mm<sup>3</sup> pieces of primary liver samples were grown atop collagen sponges in explant culture and treated with 10nM DHT, 1uM Enzalutamide, or vehicle control. Tissue samples were analysed via histological techniques to examine ECM components. C57BL/6J transgenic mice crossed with Pten<sup>loxP/loxP</sup>;Pb-Cre4 were treated with 50 mg/kg Enzalutamide for three days, before harvesting. RNA and protein from cell lines and tissue were analysed via RT-qPCR and immunoblot.

### **Results:**

The liver expresses AR, and AR-signalling is active and can be manipulated via anti-androgens. Hepa1 proliferation was inhibited by androgen which could be partially reversed with increasing doses of enzalutamide. Analysis of liver with a mutated/non-functional AR compared to control liver, identified over 2000 genes differentially regulated. Of these, a number were involved in paracrine signalling and ECM. Androgen regulated was confirmed via RT-qPCR of DHT-treated Hepa cells and liver tissue explants. The CM from liver cells treated with DHT inhibited LNCaP and 22RV1 cell growth, whilst CM from liver cells treated with DHT and enzalutamide could stimulate more PCa growth. Livers from enzalutamide treated mice showed increased collagen fibres compared to control mice, as visualised by picro-direct-red staining. In 3D-ECM microenvironments, those created from liver cells treated with enzalutamide, were more supportive for PCa proliferation.

### **Conclusions:**

How the liver microenvironment responds to anti-hormonal therapy may make it more hospitable for cancer cell growth.

**Conflict of Interest:**

No conflict of interest to declare.

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