rnaseqbrowser or http://sourceforge.net/ projects/rnaseqbrowser/

## 075

### Impeding the adaptive response of prostate cancer to androgen targeted therapies with relaxin receptor antagonist

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**Objective:** Androgen deprivation and androgen targeted therapies (ATT) are established treatments for prostate cancer (PCa). Although initially effective, ATT induces an adaptive response that leads to treatment resistance. Increased expression of relaxin-2 (RLN2) is an important alteration in the adaptive response. RLN2 has a well described role in PCa cell proliferation, adhesion and tumour growth. The objectives of this study were to develop cell models for studies of RLN2 signalling and to implement in vitro assays for evaluating the therapeutic properties of the unique RLN2 receptor (RXFP1) antagonist.
**Methods:** To validate the increased expression of RLN2 after the ATT we treated LNCaP cells with antiandrogens and used qPCR to determine its expression. We established doxycycline inducible RLN2 and RXFP1 knock-down (KD) and over-expression (OE) LNCaP models. We chemically synthesised RXFP1 antagonist and evaluated its therapeutic properties in cell proliferation and adhesion assays. To identify paracrine action of relaxin, we co-cultured inducible RLN2 KD and OE LNCaP models on bioengineered osteoblast matrices.
**Results:** We confirmed the increased expression of RLN2 after ATT in LNCaP cells. We established and validated inducible RLN2 and RXFP1 KD and OE LNCaP models. We showed that the KD of RLN2

and RXFP1 inhibited proliferation and adhesion of LNCaP cells. Our unique RXFP1 antagonist inhibited proliferation of LNCaP cells.
**Conclusions:** Our results show that the inhibition of relaxin signalling supressed proliferation and adhesion of PCa cells. Further confirmation of these findings in a preclinical model will be crucial step for translation of the RXFP1 antagonist into clinics.

## 076

### Characterisation of novel primary tumour derived epithelial prostate cancer cell lines

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Current translational and basic prostate cancer research is limited by the number of cell lines that truly reflect the spectrum of disease progression, with most commonly used cell lines being derived from metastatic lesions. There are essentially no prostate cancer cell lines derived from primary tumours or localised disease in wide use.
**Objective:** The objective of this work is to characterise novel primary tumour derived epithelial prostate cancer cell lines.
**Methods:** We have established a number of new prostate cancer cell lines derived from radical prostatectomy tissue taken from men with localised disease. Pathology of the tissue revealed benign and adenocarcinoma phenotypes with Gleason scores of 6–7 and patient PSA levels of 4–9 ng/mL. Cells were characterised for lineage markers, other prostate cancer relevant targets, STR profiling, RNAseq analysis and functional assays.
**Results:** RT-PCR and Western Blot analysis of lineage markers confirmed these cells are luminal in origin (cytokeratin/CK8 and 18 positive), with low to no AR expression. Most were also CK5, CK14

and p63 positive, which are basal markers, and CD133 negative suggesting a mixed lineage but not stem cell like. In initial anchorage independent growth assays, no growth/colony formation was observed, however, cells appeared to remain viable. This phenomenon may be attributed to their early tumour origins. Further characterisation of these lines is ongoing.
**Conclusions:** These results show potential for these cell lines as model for primary/localised disease. Development of contemporary cell lines, particularly from men with localised prostate cancer, will provide a repertoire of more clinically relevant research tools for the prostate cancer community.

## 077

### A multiple nucleotide length polymorphism (MNLP) mediates androgen regulation of IRX4 in prostate cancer

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**Objective:** Androgens and the androgen receptor (AR) play a crucial role in the initiation and progression of prostate cancer (PCa), regulating the expression of many PCa risk-associated genes. Iroquois Homeobox 4 (IRX4) has been recently identified with PCa risk and overexpressed in PCa. We observed a down-regulation of IRX4 expression in the cells undergoing epithelial to mesenchymal transition, suggesting its potential role in PCa progression and aim to delineate the androgen-mediated regulation of IRX4 in PCa.
**Methods:** In-silico analysis to identify the transcription factor (TF) binding sites regulating the IRX4 expression (Cistrome Finder database), followed by sequencing of these regions was performed. PCa cell lines (DuCaP and LNCaP) were treated with androgens for 48 h and IRX4 expression was determined by qRT-PCR.
**Results:** Binding of two crucial TFs – AR and ERG, was observed upstream of IRX4 in the VCaP cells while there was no AR binding in LNCaP cells at this locus, suggesting differential androgen responsiveness of IRX4. Moreover, IRX4 was up-regulated by androgens in DuCaP cells,