but down-regulated in LNCaP cells. Sequencing of the AR binding region identified a MNLP (rs38668493) where a 47 bp sequence is replaced by a novel 21 bp sequence. The DuCaP cells have an intact AR binding site (47 bp/47 bp) whereas the LNCaP cells have deleted AR binding site (21 bp/21 bp) which may explain this discrepancy.
Conclusion: The unique MNLP genotype upstream of IRX4 appears to regulate AR binding, and directs the androgen-mediated regulation of IRX4. Functional studies using reporter gene assay for the MNLP genotype may provide further insights for the regulation of IRX4 expression in PCa.

078
Modulation of protease expression in prostate cancer cells after androgen deprivation

Thomas Kryza, Gregor Tevz, Stephen McPherson, Melanie Lehman, Carson Stephens, Brett Hollier, Colleen C Nelson and Judith Clements
Australian Prostate Cancer Research Centre – Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, Brisbane, Australia

Objective: Understanding mechanisms associated with the emergence of castration resistant prostate cancer cells (CRPC) after androgen deprivation therapy (ADT) is essential to create new therapeutic agents to counteract this aggressive form of prostate cancer (PCa). Because proteases are involved in almost all cancer associated mechanisms such as cell proliferation, invasion and metastasis, we are interested in their modulation in PCa after ADT and their involvement in CRPC.
Method: First, we screened for protease expression in hormone sensitive prostate cancer cells (LNCaP) after ADT and anti-androgen treatments using a custom PCa microarray. Secondly, using RT-qPCR and western-blots, we confirmed the deregulation of several proteases which are repressed by androgen but induced after ADT. Finally, we analysed their expression in C4-2B cells, which are LNCaP derived CRPC, and in Oncomine databases.
Results: We identified several proteases overexpressed in LNCaP cells after ADT which are already proposed as involved in the aggressiveness of PCa or other cancers: BMP1, TLL1 and TLL2, three proteases of the astacin family; MMP16, a membrane bound MMP; and KLK14, a protease of the same family as PSA. We also showed that several of these proteases are over-expressed in C4-2B cells compared to LNCaP cells and in Oncomine databases.
Conclusion: Androgen deprivation and androgen targeted therapies in PCa is conducive to the deregulation of protease gene expression with the overexpression of several proteases known to be associated with aggressiveness of PCa or other cancers. In vitro and in vivo confirmation of their role in the androgen deprived environment as seen with ADT will determine their potential utilisation as therapeutic targets for CRPC.

079
Extracellular vesicles mediate paracrine signalling in androgen deprived prostate cancer

Carolina Soekmadji[1], Jayde Ruelcke[2], Chenwei Wang[1], James D Riches[3], Stephen McPherson[1], Pamela J Russell[1], Michelle M Hill[2], Guido W Jenster[4] and Colleen C Nelson[1]
[1]Australian Prostate Cancer Research Centre – Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Translational Research Institute, Brisbane, Australia
[2]University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia
[3]Central Analytical Research Facility, Institute for Future Environments, Queensland University of Technology, Brisbane, Australia
[4]Erasmus MC, Josephine Nefkens Institute, Rotterdam, The Netherlands

The androgen receptor (AR) is the main therapeutic target for advanced prostate cancer (PCa). Current treatments have focused on inhibiting the transcriptional activity of the AR, however androgens can also induce non-genomic effects by facilitating the initiation of kinase signaling cascades in PCa. Cells, including PCa, secrete extracellular vesicles (EV), which are able to mediate communication between cells and can also contribute towards these processes.
Objective: We investigated the effect of androgens and androgen deprivation in regulating EV secretion and the role of EV in mediating paracrine signalling in AR+ PCa cells.
Methods: PCa cells were grown in charcoal stripped serum and treated with androgen dihydrotestosterone (DHT) or antagonist MDV3100 (Enzalutamide). EV was isolated from conditioned medium using differential ultracentrifugation method, analysed by electron microscopy (morphology), qNANO (size) and protein content (LCMS/MS), followed by bioinformatic (R) and pathway analysis (Ingenuity). Data was compared with microarray transcriptome wide analysis to investigate the contribution of AR. Functional analysis by treating androgen deprived LNCaPs with CD9-enriched EV and the effect of knock down CD9 on cellular growth was observed using life imaging system Incucyte.
Results: We found that androgen deprivation increases the secretion of EV in AR+ PCa cells. However, DHT increases the secretion of CD9-enriched EV, even though androgen treatment does not alter the mRNA level of CD9. The CD9-enriched EVs were able to increase the growth rate of androgen-deprived LNCaPs, while siCD9 reduced cell growth. Androgen manipulations minimally influenced the EV content, suggesting that androgens alter the EV pathway(s) discrete from genomic AR action to mediate cellular proliferation in LNCaPs.

080
Polysaccharopeptide enhanced the anti-cancer effect of gamma-tocotrienol through activation of AMPK

Patrick Ling and Ji Liu
Australian Prostate Cancer Research Centre – Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Australia

Objective: Prostate cancer (PCa) frequently relapses after hormone ablation therapy. Unfortunately, once progressed to the castration resistant stage, the disease is regarded as incurable as prostate tumours are highly resistant to conventional chemotherapy. Therefore, an effective treatment strategy is urgently needed for improving the treatment outcome of the patients.
Method: We recently reported that the two natural compounds polysaccharopeptide (PSP) and Gamma-tocotrienols (γ-T3) possessed potent anti-cancer activities through targeting of CSCs. In the present study, using both prostate cancer cell line and xenograft models, we seek to investigate the therapeutic potential of combining γ-T3 and PSP in the treatment of prostate cancer.