Australian-Canadian Prostate Cancer Research Alliance



Abstracts

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Note: Submitting author, attending author









Gene Expression In Docetaxel Resistant PC3 Cells

<u>Manuel Altamirano-Dimas</u>, Melanie Lehman, Anne Heagert, Robert Bell, Junya Furakawa, Manju Sharma, Martin E Gleave and Michael E. Cox.

Department of Urologic Sciences, University of British Columbia, Vancouver Prostate Centre, Vancouver, Canada.

Prostate cancer is the most common cancer in Western men. Treatment for high risk localized disease includes androgen deprivation, prostatectomy, radiotherapy and adjuvant-neoadjuvant chemotherapy. For advanced disease there are no curative agents available. This situation has contributed to the development of multidisciplinary therapeutic procedures. Docetaxel is standard first line chemotherapy for men with castration resistant prostate cancer with a modest prolongation in patient survival and eventually the development of drug resistance. It is imperative to understand the molecular mechanisms of drug resistance to Docetaxel to revert this situation or develop new therapeutic approaches. Among the new drugs are carbazitaxel, arbiraterone acetate and OGX-011 which have shown a significant survival benefit.

We developed Docetaxel resistant PC3 cell lines by culturing in step-wise increased drug concentrations. Cells were continuously maintained in docetaxel, with treatments beginning at the initial IC50 of the respective parent cell lines. Media containing docetaxel was changed every 2-3 days. As cells displayed resistance to treatments of docetaxel the concentration was subsequently increased with final treatment doses of 10 nM. Resistance was judged based on decreased cell death and increased proliferation of cells. Age-matched parent cells whit no treatment were also maintained in culture.

RNA was extracted from resistant and no-resistant PC3 cells for the production of microarrays using Agilent G4112A Whole Genome 60mer Oligo microarray chip kits. Analysis was focused on 28,000 Stanford SOURCE annotated genes, normalized from triplicate experiments using the Bioconductor package LIMMA. Setting a threshold of > or < 2 fold difference in expression we found 1,111 genes differentially expressed (DE), 59% up-regulated and 41% down-regulated. The Gene Set Enrichment Analysis (GSEA) program selected 38enriched genes representing genes in the groups for cancer, stem cells, signal transduction, stress, apoptosis, Neuroendocrine and hypoxia. Further analysis of PC3 resistant microarrays shows groups in cytokines, ABC transporters and metabolism of xenobiotics.









GenomeLite: A Standalone Genome Browser

Jiyuan An 1,2, John Lai 1,2, Melanie L Lehman 3 and Colleen C Nelson 1,2,3

- 1. Australian Prostate Cancer Research Centre Queensland. Brisbane, Australia
- 2. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
- 3. Department of Urologic Sciences, University of British Columbia, Vancouver Prostate Centre, Vancouver, Canada.

Massive amounts of genetic data have been produced since the emergence of microarray and next generation sequencing platforms. Therefore, a genetic map, which visually shows expression at a given chromosomal locus becomes a necessary tool in systems biology research at the candidate gene level. Currently, the web based UCSC genome browser is the most widely used tool to show genetic maps. However, it suffers from network caused display delay for loading large custom track data, and limitations in showing high-throughput sequence data. We have developed a standalone, platform independent genome visualization tool (GenomeLite) where custom track data are stored in a database for quick retrieval, and extended wiggle data format is introduced to better visualize genomic sequencing data.









MiRDeep*: An Integrated Application Tool For MiRNA Identification From RNA Sequencing Data

Jiyuan An 1,2, John Lai 1,2, Melanie L Lehman 3 and Colleen C Nelson 1,2,3

- 1. Australian Prostate Cancer Research Centre Queensland. Brisbane, Australia
- 2. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
- 3. Department of Urologic Sciences, University of British Columbia, Vancouver Prostate Centre, Vancouver, Canada.

MiRDeep and its varieties are widely used to quantify known and novel miRNA from small RNA sequencing. This paper describes miRDeep*, our integrated miRNA identification tool, which is modeled off miRDeep, but the precision of detecting novel miRNAs is improved by introducing new strategies to identify precursor miRNAs. MiRDeep* has a user-friendly graphic interface and accepts raw data in FastQ and SAM/BAM format. Known and novel miRNA expression levels, as measured by the number of reads, are displayed in an interface which shows each RNAseq read relative to the pre-miRNA hairpin. The secondary pre-miRNA structure and read locations for each predicted miRNA are shown and kept in a separate figure file. miRDeep* is an integrated standalone application where sequence alignment, pre-miRNA secondary structure calculation, and graphical display are purely java-coded. This application tool can be executed using a normal personal computer with only 750Mb of memory. Using small RNAseq data that was generated in prostate cancer (LNCaP) cells, the precision of miRNA prediction was 81.01%-83.17% for miRDeep*, compared with 70.87%-78.47% for miRDeep and 19.76%-20.23% for miRanalyzer. miRDeep* also detected 35-45 novel miRNA compared with 45-74 for miRDeep and 848-1060 for miRanalyzer. Four of the novel miRNA predicted by miRDeep* were validated using stem-loop TaqMan PCR, as well as qualitative RT-PCR targeting the pre-miRNA.









ERG Expression And DNA Methylation Of Novel Biomarkers Correlates With Adverse Clinical Features Of Prostate Cancer And Is Associated With Poor Prognosis

Ken Kron 1,2, Liyang Liu 1,2, Dominique Trudel 3, Vaijayanti Pethe 1, John Trachtenberg 4, Neil Fleshner 4, Theodorus van der Kwast 2,3 and <u>Bharati Bapat 1,2</u>

- 1. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada
- 2. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada
- 3. Department of Pathology, University Health Network, University of Toronto, Toronto, ON, Canada
- 4. Department of Surgical Oncology, Division of Urology, University Health Network, University of Toronto, Toronto, ON, Canada

Purpose

Fusion of the TMPRSS2 gene with the ERG oncogene is found in approximately 50% of all prostate cancers, causing increased ERG expression leading to epigenetic aberrations. The aim of this study was to analyze the relationship between ERG expression, DNA methylation of three novel biomarkers, and clinicopathological features of prostate cancer.

Experimental Design

Immunohistochemistry for ERG protein was performed as a surrogate for TMPRSS2-ERG fusions. We analyzed methylation of CYP26A1, TBX15, and HOXD3 in 219 prostatectomy specimens using the quantitative MethyLight assay. DNA methylation was compared between ERG positive and negative cases and correlations of ERG and DNA methylation with clinicopathological features were analysed using chi-square, Spearman correlation, logistic regression, and Cox regression.

Results

ERG expression was highest in Gleason pattern 3 cancers (56.9%), lower in pattern 4 or 5 cancers (43.0%), and mostly absent in transition zone pattern 2 tumours (5.6%). TBX15 and HOXD3 methylation were significantly associated with pathological stage, Gleason score and Gleason pattern (p-values \leq 0.015). ERG expression and methylation of TBX15 and HOXD3 were significantly associated with greater stage and grade (p-values < 0.05). In a multivariate model for prediction of disease recurrence, ERG expression indicated favourable prognosis (p-value = 0.036) while high methylation of TBX15 was an indicator of poor prognosis (p-value = 0.05).

Conclusions

CYP26A1, TBX15, and HOXD3 are novel methylation markers of prostate cancer associated with ERG expression and clinicopathological variables, suggesting that incorporation of these markers may be useful in a pre and post-operative clinical setting.









A Randomized Phase II Study of OGX-427 Plus Prednisone vs. Prednisone Alone In Patients With Chemotherapy-Naive Metastatic Castration Resistant Prostate Cancer

Kim N. Chi, Sebastien Hotte, Susan Ellard, Joel Gingerich, Anthony Joshua, Evan Yu, Jenny Bazov, Martin E. Gleave

British Columbia Cancer Agency, Department of Urologic Sciences, University of British Columbia, Vancouver Prostate Centre, Vancouver, Canada.

Background

Heat Shock Protein 27 (Hsp27) is a stress-activated, multi-functional chaperone protein highly expressed in cancer that regulates many cell signaling and survival pathways implicated in cancer progression. In prostate cancer models, Hsp27 complexes with androgen receptor (AR) and enhances transactivation of AR-regulated genes. OGX-427 is a 2nd generation antisense that inhibits Hsp27 expression with in vitro and in vivo efficacy. Phase I studies have demonstrated tolerability and single agent activity.

Methods

Patients (pts) with CRPC, no/minimal symptoms and any prior treatment other than chemotherapy were randomized 1:1 to receive Prednisone 5 mg PO BID or P with OGX-427 600 mg IV x 3 loading doses followed by 1000 mg IV weekly. Primary endpoint was the proportion of pts progression free (PSAWG 2 criteria) at 12 weeks. A 2-stage MinMax design (H0 = 5%, HA >20%, α =0.1, β =0.1) will enrol 32 pts total per arm and provide 70% power to detect the difference at a 0.10 1-sided significance. Secondary endpoints include PSA decline, measurable disease response, and circulating tumour cell (CTC) enumeration.

Results

In the first 22 pts randomized (11 to OGX-427+P, 11 to P), baseline median age was 71 years (53-86), ECOG PS 0 or 1 in 64% and 36% of pts, median PSA 89 (6-606), metastases in bone/lymph nodes/liver or lung in 77%/64%/10%, 23% had prior treatment with P, and 91% had \geq 5 CTC/7.5 ml (median 18/7.5 ml). Thus far, 82% of pts randomized to OGX-427+P have had a PSA decline (55% with \geq 30% decline) and 18% a PSA increase; 40% of pts treated with P have had a PSA decline (20% with \geq 30% decline), 10% no change and 50% with PSA increase. CTC conversion from \geq 5 to <5/7.5 ml has occurred in 60% of pts randomized to OGX-427+P and 20% of pts treated with P alone. Grade 1-2 infusion reactions (e.g., chills, diarrhea, flushing) have occurred in 45% of pts receiving OGX-427+P and 1 pt developed hemolytic uremic syndrome after week 7 probably related to OGX.

Conclusions

Preliminary data provide clinical support for the role of Hsp27 in AR signalling and as a therapeutic target for prostate cancer. Enrolment on this study continues. Funded by a grant from the Terry Fox Research Institute.









Combination Therapy Using Lapatinib And MDV3100 Overcomes EGFR/Her2 Mediated Anti-androgen Resistance In Castration-Resistant Prostate Cancer

Jennifer Bishop

Vancouver Prostate Centre, Vancouver, Canada.

Therapeutic options for castration resistant prostate cancer (CRPC) are limited. However, new drugs, like MDV3100, aimed at targeting the androgen receptor (AR) that is reactivated even after castration, have been developed and shown promise in clinical trials. Unfortunately, despite the substantial antitumor activity of MDV3100 in some patients with CRPC, tumor recurrence does occur and suggests the adaptation of drug resistance in these anti-androgen treated patients. In this study, we investigated the mechanism of MDV3100 resistance in prostate cancer, and tried to improve therapeutic efficiency of MDV3100. We found that the expression of epidermal-growth factor receptor (EGFR) and HER2 were induced by MDV3100 treatment and increased in MDV3100-resistant tumors and cells, a phenotype that was dependent on the activity of the transcription factor YB-1. As a result, downstream targets of EGFR and HER2, ERK1/2 and Src were activated in MDV3100-resistant tumors and cells, resulting in activations of androgen receptor (AR) coactivator TIF-2 and AR itself. This data suggested that drug resistance was mediated by activation of EGFR/Her2. Therefore, we tested whether the dual EGFR/Her2 inhibitor, lapatinib, would have anti-tumor activity against MDV3100 resistant cells. MDV3100-resistant cells were sensitive to lapatinib, and activation of downstream targets of EGFR/Her2 were inhibited by lapatinib treatment. Most importantly, lapatinib exerted a synergistic therapeutic effect with MDV3100 in vitro and in vivo. Taken together, we have identified the activation of the EGFR/Her2 pathway as a mechanism driving anti-androgen resistance and have shown that combination therapy using lapatinib and MDV3100 may be a feasible and promising therapeutic option for castration-resistant prostate cancer.









ERG-like Signature For Prognostication Of Progression And Cancer Specific Mortality

<u>Tarek Bismar</u>

Pathology and Laboratory Medicine, Medicine Oncology, Biochemistry & Molecular Biology, University of Calgary

Purpose

ERG gene rearrangement has been proposed to define a distinct biological subtype of prostate cancer with potential therapeutic implications. However, previous studies assessing its prognostic value have yielded mixed results. Our purpose was to develop an *ERG-like signature* enriched in ERG-positive tumors that is prognostically robust.

Material and Methods

We used Singular Value Decomposition bioinformatics approach to analyze expression data previously generated from 46 prostate tumors to generate *ERG-like* signature with robust prognostic implication. The model was validated by immunohistochemistry and quantitative PCR using prostate cancer progression tissue microarray, and the prognostic significance of this signature was applied to several publicly available cohorts of prostate, breast and leukemia patients.

Results

We characterized and validated a 10-gene signature reflective of aggressive features of the *ERG* mediated transcriptome within each tumor. This signature was validated in relation to *ERG* fusion status and patient prognosis and was significantly associated with disease progression and cancer specific deaths in prostate cancer and across several tumor types. This signature, when coupled with Gleason score, identified prostate cancer patients at higher risk of cancer deaths more accurately than Gleason score alone or in combination with *ERG* status.

Conclusion

The *ERG*-like signature is more accurate than *ERG* status in stratifying patients with leukemia, breast and prostate cancers into different prognostic groups, and is reflective of aggressive features of the ERG-mediated transcription. This signature should be validated in conjunction with clinico-pathological parameters to confirm its ability to identify patients at highest risk for cancer progression and cancer death.









The Canadian Prostate Cancer Genome Network (CPC-GENE): A National Outcomes-Based DNA Sequencing Initiative

Paul C. Boutros 2,6, Michael Fraser 1,3, Emilie R. Lalonde 2,6, Alice Meng 3, Andrew Brown 2, Dominique Trudel 5, Taryne Chong 2, Amin Zia 2, Michelle Sam 2, Pablo H. Hennings-Yeomans 2, Fouad Yousif 2, Rob Denroche 2, Jeremy Johns 2, Lee Timms 2, Nicholas Buchner 2, Richard de Borja 2, Nicholas J. Harding 2, Michelle A. Chan-Seng-Yue 2, Jianxin Wang 2, Gaetano Zafarana 1,3, Maud HW Starmans 2, Melania Pintilie 1, Neil Fleshner 7, Stanislav Volik 4, Lakshmi Muthuswamy 2, Colin C. Collins 4, Thomas J. Hudson 2,6, Lincoln D. Stein 2, Timothy Beck 2, John D. McPherson 2, Theodorus van der Kwast 5, and Robert G. Bristow 1,3,6

- 1. Radiation Medicine Program, Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Ontario, Canada;
- 2. Ontario Institute for Cancer Research, Toronto, Ontario, Canada;
- 3. STTARR Innovation Program, Toronto, Ontario, Canada;
- 4. Vancouver Prostate Centre, Vancouver, British Columbia, Canada;
- 5. Department of Pathology, University Health Network, Toronto, Ontario, Canada;
- 6. Departments of Radiation Oncology and Medical Biophysics, University of Toronto, Toronto, Ontario, Canada'
- 7. Clinical Studies Resource Centre, Ontario Cancer Institute/Princess Margaret Hospital, Toronto, Ontario, Canada

Prostate cancer (CaP) is the most commonly diagnosed malignancy in Canadian men. In low or intermediate risk CaP localized to the prostate, treatments such as active surveillance, radical prostatectomy, image-guided external beam radiotherapy (IGRT) or brachytherapy are used. Current prognostic factors explain only a small proportion of the large variation in observed clinical outcome. Better predictors of patient outcome, response to therapy and prognosis are urgently required to individualize treatment, t optimize therapy, and minimize toxicities and other side effects.

The <u>C</u>anadian <u>Prostate Cancer Genome <u>Network</u> (CPC-GENE) is an outcomes-based initiative that will sequence specimens from at least 300 localized CaP patients who underwent surgery or radiotherapy. Specimens are derived from pre-treatment biopsies, radical prostatectomies, and paired bloods and are directly linked to clinical outcome in which patients have > 6.5 years median follow-up after treatment. Patients either responded (~70%) or did not respond to local therapy (~30%). In a sub-set of patients, the intra- and inter-prostatic heterogeneity of intermediate risk prostate cancer is being addressed using matched frozen and FFPE-derived biopsy and prostatectomy samples. Our unique approach allows **DNA-based studies to be directly linked to clinical outcomes** and glean information regarding the genetic signature of patients with solely localized cancers versus patients who have both local tumors and occult metastatic disease.</u>

Following retrospective consenting, initial WGS of DNA from pre-treatment frozen biopsies (>70% cellularity) was performed using sequencing libraries constructed from as little as 100 ng of DNA, to generate coverage depths of 50x for tumour samples and 30x for reference samples. Following alignment (Novoalign) and variant calling (GATK), WGS data were compared with genotyping results for ~330,000 loci generated using the Affymetrix OncoScan platform. To date, single nucleotide variants detected using arrays have been validated >98% of the time by WGS data (n=20), confirming that the use of a low-input library does not hinder mutation detection. Sequencing does not exhibit significant genome-wide coverage biases. CNV calls were similarly compared and validated between the genotyping arrays and the next-generation sequencing data. Finally, targeted sequencing of DNA from frozen biopsies revealed non-synonymous mutations in *p53*, *PDGFRA*, *NRAS*, and *BRAF*, suggesting that biopsies can be utilized to develop sequencing-based genetic signatures.







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We are currently analysing the remainder of our radiotherapy and radical prostatectomy cohorts by WGS and OncoScan. These data will be complemented by RNAseq and methylome analyses. Genomic data will be correlated with clinical outcome using biostatistical and machine-learning techniques to generate prognostic and predictive signatures for intermediate risk prostate cancer. Taken together, these studies of prostate cancer could help to generate predictors of treatment outcome and patient prognosis, enabling personalized medicine.

(Supported by Prostate Cancer Canada, the Ontario Institute for Cancer Research, the PMH Prostate Cancer Research Program/Campbell Family Research Institute and the Canadian Institutes for Health Research).









The Multifarious Roles Of Hedgehog In Promoting Prostate Cancer Progression To A Therapy Resistant State

Menggian Chen, Na Li, Ralph Buttyan The Vancouver Prostate Centre, Vancouver BC, Canada

Introduction

Hedgehog (Hh) is a primal signaling pathway that regulates cell fate, tissue morphogenesis and function during embryonic development. Canonical Hh is a reciprocal paracrine-driven process in which Hedgehog ligands (hedgehogs [hhs]), secreted from developing epithelial cells, bind to receptors on adjacent mesenchymal/stromal cells that, in the end, activate Gli-mediated transcription. In turn, the hh-stimulated mesenchymal/stromal cells secrete (undefined) substance(s) that promote further organization, growth and differentiation of the developing epithelium. In adults, key mutations in genes that regulate Hh signaling can confer autocrine-like Hh activity that is strongly associated with certain malignancies. These mutations are rarely found in prostate cancer (PCa) yet there are ample published reports of active elements of Hh signaling in prostate tumors. Here, we present evidence that aspects of Hh signaling may be critically involved in the progression of PCa to the therapy-(hormone-) resistant state by two separate actions in the tumor microenvironment.

Methods

Human prostate cancer (LNCaP, VCaP, and androgen independent [AI] variants of LNCaP) and primary human prostate stromal cells (PrSCs) were used. Expressions of Hh signaling regulators, SHH, Smo, Gli1, Gli2 and Gli3 were upregulated by infection with lentiviruses. Knockdown of Smo, Gli1 and Gli2 were done with siRNAs. Expressions of all these and other androgen-dependent proteins (AR, PSA, KLK2 and PGC) were measured by qPCR and/or Western blotting. ChIP assays were used to identify the presence of Gli2 at AR genomic binding sites in AI LNCaP cells. Canonical Hh signaling was induced in cells by a Smo agonist (SAG) and was suppressed by a Smo inhibitor (KAA-cyclopamine). Testosterone (T) biosynthesis in DHEA-fed PrSCs was measured by an ELISA assay.

Results 1) Hh and effects on PCa cells: Chronic androgen deprivation induces Gli2 overexpression in androgen growth-dependent PCa cells that supports expression of androgen-regulated genes without altering the expression of the androgen receptor (AR). Treatments with Hh antagonist or Smo-/Gli2-knockdowns suppressed the expression of AR dependent genes and blocked AI growth. Likewise, exogenous overexpression of Gli2 in androgen-dependent PCa cells enabled AI growth. Co-immunoprecipitation studies identified an interaction between Gli2 and AR proteins in PCa cells mediated by a domain in the C-terminus of Gli that interacts with the N-terminal domain of AR. Gli2 was bound to promoter/enhancer regions of androgen-dependent genes in AI cells. **2)** Androgen deprived PCa cells also upregulate Sonic Hedgehog expression and release it in a paracrine-active form. Treatment of primary PrSCs with an Hh agonist induced the expression of a repertoire of steroid biosynthetic genes and significantly increased the output of T from DHEA from these cells.

Conclusions

Collectively our studies suggest that chronic androgen deprivation increases Gli2 activity in prostate cancer cells that hypersensitizes endogenous AR to the androgen-poor environment and enables AI growth. Likewise, chronic androgen deprivation induces hh expressions and release from PCa cells in a paracrine active form. PrSCs treated with a Hh agonist activate expressions of steroidogenic genes that enables them to abundantly produce and secrete T from DHEA. These outcomes suggest that regional Hh activities induced by androgen deprivation promotes PCa progression to a hormone-resistant state through at least two different mechanisms, one involving the tumor cell and one involving adjacent stromal cells.

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Hsp27 Is An Essential Effector Of EGF-induced Epithelial To Mesenchymal Transition In Prostate Cancer

Thomas Cordonnier

The Vancouver Prostate Centre, Vancouver BC, Canada

Prostate Cancer (PCa) causes a worldwide threat on the health of men. To date, no effective therapy allows the abrogation of prostate cancer's progression to castration resistant prostate cancer (CRPC) and lethal invasive metastatic forms. Recent evidence suggests that acquisition of androgen independence may be due to up-regulation of growth factors/receptor signaling pathway, notably the epidermal growth factor (EGF) and is related receptor (EGF-R). Both, EGF and EGF-R are up-regulated in PCa during progression to CRPC. Carcinoma progresses to metastasis through a process called epithelial-to-mesenchymal transition (EMT). Malignant cells discard epithelial restraints and acquire invasive abilities that facilitate their dissemination to permissive micro-environments. In PCa, elevated expression of heat shock protein 27 (Hsp27) occurs and is linked with CRPC progression. As Hsp27 and EGF are up-regulated in CRPC, often associated with metastasis and poor prognosis, we suggest that increased Hsp27 enhances EGF-induced EMT and thereby metastasis progression. Our results showed that the molecular chaperone Hsp27 drives EMT in PCa cells by decreasing epithelial cell markers, upregulating mesenchymal markers and enhancing cell migration and MMP-9 activity. Hsp27 was also required for the EGF-induced EMT in PCa cells via modulation of several signaling pathways including p38, Pi3K/AKT and MEK/Erk. Loss of Hsp27 decreased EGF dependent p38, Akt, Erk phosphorylation. In addition, our experiments also highlight that the GSK3ß phosphorylation and also β-catenin phosphorylation and its nuclear translocation induced by EGF are abrogate in stable knockdown prostate cancer cells. These results indicate that Hsp27 play an essential role in EGF-induced EMT via direct or indirect β -catenin modulation. In vivo, targeting Hsp27 using the phase II trial antisense oligonucleotide OGX-427 significantly reduced tumor cell metastasis in a PCa murine model. This study reveals Hsp27 as a crucial effector of EGF-dependent and independent EMT and suggests that targeting Hsp27 is a viable treatment strategy for metastatic PCa.









Mobile Advice & Testing Service (MATS): Introduction Of A Novel, Nurse-led Prostate Cancer Education And Testing Service

<u>Crowe H</u>, Bugeja P, Wootten A, Murphy D, Challacombe B, Costello A.

Australian Prostate Cancer Research Centre, Epworth, Melbourne, Australia, Peter MacCallum Cancer Institute, East Melbourne, Australia, Guy's Hospital, London, England

Introduction & Objectives

In Australia, as in other countries, there is confusion in the community about the need for prostate cancer (CaP) testing. Divided opinions amongst health professionals about the benefits or harm of CaP treatment add to this. As a consequence, many men in the recommended age group for CaP testing do not have access to information about their personal risk of developing CaP, in order to make a choice of whether or not to undergo testing. We established a workplace Mobile Advice & Testing Service to give men easy access to this information. We piloted the service and evaluated the effectiveness, feasibility and acceptance of the program.

Materials & Methods

We reviewed existing guidelines for CaP testing and developed a testing protocol for the MATS. We created an education program for the workplace sessions about CaP testing and treatments, and associated risks and benefits. We modified an existing Prostate Cancer Knowledge Questionnaire (PCKQ) and developed a Quality Assurance (QA) questionnaire to assess the usefulness of the service. These were piloted and revised as required.

Predominantly male workplaces were contacted, invited to participate, and site visits scheduled. The first visit was an education session delivered by a male urologist, with time for questions and answers. Individual appointments with urology nurses were scheduled, with the opportunity for CaP testing at that time. PCKQs were completed prior to the education session and repeated at the completion of the consultation with the nurses, with the QA questionnaire. Pre- and post – PCKQs responses were compared. Demographic and general health related data were also collected. All testing results were reviewed by a urologist, and results sent to both participant and their General Practitioner (GP) with recommendations about future testing.

Results

101 men attended the sessions at 3 worksites, 67 being in the target population (40 – 70 years). Comparison of pre- and post-PCKQs demonstrated improved CaP knowledge following the sessions.

PROSTATE CANCER KNOWLEDGE	None	Little	Moderate	High
Pre-PCKQ	11%	52.5%	31.5%	4%
Post-PCKQ	3%	31%	56%	10%

44% reported they do not regularly attend a GP. Of those in the recommended age for testing, 56% had not been tested. Of those who had been tested 86% reported they had a Prostate Specific Antigen (PSA), and only 53% a digital rectal examination (DRE). 7 of 101 attending the nurse consultations had an abnormal PSA and/or DRE and were recommended for urologist review and further investigation. The MATS experience was rated Highly Satisfactory – Satisfactory by all participants

Conclusions

This nurse led MATS provides an alternative, convenient forum for men to obtain information about prostate cancer and to undergo testing if they wish to do so. It provides a model of care that could be adopted in many settings.









Leave No Base Unturned: Understanding Human Development And Disease Through An Integrated Analysis Of Gene Regulatory Architecture

Marcel E. Dinger

University of Queensland Diamantina Institute, R-Wing, Princess Alexandra Hospital, Woolloongabba, QLD 4102.

Approximately 98% of the human genome comprises noncoding DNA, the function of which is largely unknown. Intriguingly, more than 85% of polymorphisms identified to be associated with disease in genome-wide studies (GWAS) occur within noncoding regions, suggesting that understanding the role of these regions of the genome will be important for understanding and potentially treating disease. Noncoding DNA can transact function through various mechanisms, including acting as a template for noncoding regulatory RNAs, target sites for regulatory protein or RNA interactions, or as a substrate for other epigenetic modifications, such as histone modifications or chemical modifications to the DNA itself. It is apparent that perturbation of any of these regulatory processes can affect normal cell behaviour and result in disease. Importantly, it is becoming increasingly clear that perturbations in the regulatory processes of the cell are commonly the first events that occur in advance of the onset of disease. However, there is very little known about which parts of the genome are responsible for these regulatory functions. Therefore, the understanding of the regulatory architecture of key processes involved in disease onset provide an excellent opportunity for determining disease susceptibility and identifying therapeutic targets for early intervention. By specifically targeting regions of the genome associated with disease as identified by GWAS, we aim to apply a combination of methods including DNase I hypersensitivity profiling, RNA sequencing, methylome sequencing and chromatinimmunoprecipitation sequencing, to unravel the roles of every nucleotide in these regions. This agnostic approach provides a means to connect data from GWAS with function and potentially mechanism. We are currently using this approach to identify new molecular mechanisms that underlie the pathobiology of melanoma and endometriosis. The approach could similarly be employed to understand other diseases where extensive GWAS data is available, such as prostate cancer.









MRI-based Radiation Therapy Treatment Planning

Jason Dowling *, Jonathan Lambert 2,3 , Shakes Chandra 1, Joel Parker 2, Jidi Sun 2,3, Peter Stanwell 2,3, Jurgen Fripp 1, Olivier Salvado 1, Peter B. Greer 2,3

- 1. Australian e-Health Research Centre, CSIRO ICT Centre, Australia;
- 2. Calvary Mater Newcastle Hospital, Australia;
- 3. University of Newcastle, Australia; *Jason.dowling@csiro.au

External beam radiation therapy (EBRT) is a major clinical treatment which is indicated in 60% of prostate cancer patients [1]. EBRT uses high energy x-ray beams combined from multiple directions to deposit energy (dose) within the patient tumour region (the prostate) in order to destroy cancer cells. Prostate radiation therapy dose planning currently requires computed tomography (CT) scans as they contain electron density information needed for patient dose calculations. However magnetic resonance images (MRI) have significant advantages over CT. Prostate borders on MRI are smaller than CT on the same patient, thus the treatment volume for MR-based planning is smaller compared to CT. The prostate is more accurately defined on MRI due to the greater contrast resolution of MRI over CT, resulting in reduced inter-observer variability. Extra treatment margins which may be added to account for delineation uncertainties in CT are reduced. Therefore the use of MRI leads to a reduction in extra-prostatic tissue (i.e. normal surrounding tissue) being irradiated and reduced morbidity.

A collaborative project between the Calvary Mater Newcastle Hospital Department of Radiation Oncology and the CSIRO Biomedical Imaging Group is working towards the development of MRI-alone prostate radiation therapy planning [2]. To date, a number of research issues have been addressed, including the development of DICOM-RT interfaces between commercial clinical treatment planning systems and research software platforms; methods to automatically segment organs of interest from MRI; and tools to automatically assign electron density information to MR scans for radiotherapy dose calculations for treatment planning.

Dynamic 3D multi-atlas methods have been developed to automatically identify the boundaries of the prostate and other pelvic organs from MRI scans [3]. Using the same clinical dataset and manual contours from 50 clinical scans [4], a median Dice similarity coefficient (DSC) of 0.86 has been achieved with an average surface error between manual and automatic contours of 2.00mm. This result is very close to the median inter-observer (n=3) DSC of 0.87 from the same dataset. The goal of atlas based segmentation is to segment any scan, however the training database can be too small to cover the range of morphology observed in the population. Therefore a further improvement to the selection process has been to automatically detect scans which are unlikely to find an adequate match in the database, and provide a warning that these may not be segmented correctly. Statistical shape models have also been investigated to improve the accuracy of prostate segmentation [5].

In terms of dose, the agreement between the MRI based pseudo-CTs and planning CTs for 26 patients has been quantified by differences in the point dose at isocentre and distance to agreement in corresponding voxels. Dose differences have been found to be less than 2%. Chi values indicate that the planning CT and pseudo-CT dose distributions are equivalent. No significant differences (p>0.9) were found between CT and pseudo-CT Hounsfield Units for organs of interest [6].

The work to date has provided the necessary tools for MRI-alone treatment planning and adaptive MRI-based prostate radiation therapy. Validation of these tools is currently occurring at the Calvary Mater Newcastle Hospital.









Acknowledgements

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Gleason Grading Agreement Between Urologic Pathologists In An Active Surveillance Clinical Trial Highlights Problematic Patterns For Differentiating Grade 3 From Grade 4

Michael Bonham MD/PhD 1, Sarah Hawley MS 2, Lawrence True MD 3, Jesse McKenney MD 2, Dean Troyer MD 4, <u>Ladan Fazli</u> MD 5, Marlo Nicolas MD 6, Lakshmi Kunju MD 7, Jeffry Simko MD/PhD1 and the PASS Clinical Trial Team; 1 University of California, San Francisco, San Francisco, CA, United States; 2 Canary Foundation, Palo Alto, CA, United States; 3 University of Washington, Seattle, WA, United States; 4 Eastern Virginia Medical School, Norfolk, VA, United States; 5 University of British Columbia, Vancouver, British Columbia, Canada; 6University of Texas Health Science

Background

Active Surveillance is becoming a more accepted management strategy for patients with prostate cancers that have a low risk of disease progression (1-3). Many such treatment strategies involve close monitoring of these patients with serial exams, serum PSA levels and various prostate biopsy protocols. While there are various protocols for active surveillance management, most rely heavily on the Gleason grade obtained from serial prostate biopsy for determining when to recommend conventional treatment, with scores of 6 or lower deemed acceptable for continuing on with an active surveillance strategy. This requirement places a heavy emphasis on the discrimination between Gleason patterns 3 and 4 to distinguish low risk Gleason score 6 cases from those of higher Gleason score. Our multiinstitutional prospective active surveillance study of patients with previously untreated clinically localized prostate cancer at diagnosis (PASS; The Prostate Active Surveillance Study) involves annual prostate biopsy with the study outcome of disease progression defined as upgrading to Gleason score 7 or higher(4). Study patients so upgraded are generally counseled to end active surveillance and receive therapy with curative intent. Therefore, we instituted a rapid biopsy review program that study pathologists could submit biopsy images to when consensus grading was sought on borderline/ difficult Here, we present a summary of Gleason grades assigned by the seven genitourinary cases. pathologists participating in this trial (study pathologists) for such borderline cases submitted over an 18 month period.

Methods

For tumors with features borderline between Gleason patterns 3 and 4, a Rapid Biopsy Review Program was created to provide consensus grading of biopsies obtained in the PASS trial. Digital images from borderline areas of 39 cases (out of a total of ~600 biopsies performed) were distributed to the 7 study pathologists with instructions to provide a score within 24 hours of receipt. For each case, at least two images at 100X and 200X magnification from areas of borderline grade were circulated by electronic mail as JPEG images. Results of these grade assignments are shown here. Cases that lacked a clear consensus on Gleason score 6 versus 7 were examined for the presence of irregular poorly formed glands, areas of questionable glandular fusion or other confounding features.

Conclusion

We evaluated the variability in Gleason grade assignments for prostate biopsies obtained from a series of men on an active surveillance clinical protocol. Study pathologists showed significant differences of opinion over the presence of Gleason pattern 4 in small tumor foci. There were 10 cases without consensus over Gleason score 6 verses Gleason score \geq 7, areas of questionable glandular fusion and/or small poorly formed glands were observed in all cases. This study suggests the extent of Gleason grading variability is a problem in this setting. Solutions include the development of universally accepted histological criteria for grading and the discovery of novel molecular criteria to help guide management of active surveillance patients, if the presence of any Gleason pattern 4 cancer glands or higher is used to trigger a move to curative therapy in these patients.









Developing Novel Models For Studying The Role Of EMT In Prostate Cancer Invasion And Metastasis

Stylianou, Nataly 1; Kashyap, Abhishek 2; Gunter, Jennifer 1; Nelson, Colleen 1 and Hollier, Brett 1,2.

- 1. Australian Prostate Cancer Research Centre-QLD;
- 2. Tissue Repair and Regeneration Program; Institute of Health and Biomedical Innovation, Brisbane, Queensland

The metastatic spread of cancer cells from the primary site to critical organs is the major cause of death in patients with prostate cancer (PCa). The epithelial-to-mesenchymal transition (EMT) is an embryonic program that cancer cells can reactivate to acquire a metastatic phenotype. While current cellular models for studying EMT have proved useful, they do not accurately recapitulate the temporal changes that occur during EMT and are not ideal for *in vivo* studies. This project aims to develop novel conditional PCa cell lines, whereby the induction and reversion of EMT (MET) can be tightly regulated in a temporal manner. The PCa cell models developed may be particularly useful in defining the temporal gene expression changes that mediate EMT (and MET). These cell models may also provide a novel conditional PCa xenograft for investigating the role of EMT in tumour progression wherein EMT can be initiated and reversed *in vivo*. Taken together, it is hoped that these models will provide us with an increased understanding of the EMT program and its relevance to PCa progression that can then be used to design better EMT targeted therapies for improving patient outcomes.









Modafinil For Fatigue Associated With Docetaxel-based Chemotherapy: A **Randomized Controlled Trial**

Elizabeth Hovey 1, Tal Rapke, Paul De Souza, Gavin Marx, Phillip Parente, Andrew Hill, Anthony Bonaventura, Tony Michele, Paul Craft, Ehtesham Abdi, Andrew Lloyd

1. Department of Medical Oncology, Prince of Wales Hospital, New South Wales

Background

Chemotherapy-induced fatigue is a common complaint for patients with cancer. We investigated whether modafinil, a psychostimulant, could reduce fatigue in patients on chemotherapy.

Methods

A multicenter, randomized, double-blind, placebo-controlled, parallel group study was conducted in patients with metastatic prostate or breast cancer suffering significant chemotherapy-related fatigue whilst undergoing docetaxel-based chemotherapy. Patients were enrolled at the start of their 3rdor subsequent cycles of docetaxel which was continued for up to four further cycles (defined here as 'treatment periods'). Patients were randomized 2:1 to receive modafinil 200mg daily or placebo for 15 days during each treatment period. Fatigue was evaluated by the MD Anderson Symptom Inventory (MDASI). The primary endpoint was MDASI area under the curve (AUC) during the first 7 days of study medication for the first two treatment periods (possible range 0-70). Other validated tools were used to record disturbances in sleep, mood and functional status.

Results

Eighty-three patients (65 with prostate cancer) were randomized and received at least one dose of study medication.

	Least squ			
Treatment period	Placebo (N=28)	Modafinil (N=55)	Difference	Р
1 & 2*	39.6	35.9	-3.7 [-8.9,1.4]	0.15
1	39.4	38.0	-1.4 [-7.0,4.2]	0.62
2	40.1	33.7	-6.4 [-12.2,-0.6]	0.03

MDASI AUC₃₋₁₀ scores for the first two treatment periods

* Primary endpoint. 95% confidence intervals in brackets

The number of grade 3 or 4 adverse events (AEs) was 16/55 (29.1%) in the modafinil group and 5/28 (17.9%) in the placebo group. The toxicity profile was largely consistent with docetaxel-based chemotherapy and with previously reported AEs associated with modafinil use in the community; 11 AEs were possibly related to docetaxel; 1 to modafinil and 9 to neither treatment.

Conclusions

Managing chemotherapy-related fatigue remains a major challenge. Despite not reaching the primary endpoint, there was a consistent trend towards improvement of chemotherapy-related fatigue in the modafinil arm. Further studies are needed to better understand the clinical implications of these findings. Funding sanofi-aventis; Study ID NCT00917748.









Identification And Characterisation Of Androgen Regulated Gene Expression Profiles In Prostate Cancer Cells

Varinder Jeet 1, Melanie Lehman 2, Colleen Nelson 1, 2

- 1. Australian Prostate Cancer Research Centre-Queensland, Princess Alexandra Hospital, Queensland University of Technology
- 2. Vancouver Prostate Centre, University of British Columbia

Prostate cancer (PCa) is the most common form of cancer in Australian men and remains the second leading cause of cancer related deaths after lung cancer. Androgenic action through the Androgen Receptor (AR) plays a pivotal role in the growth, differentiation and regulation of prostate gland in addition to prostate carcinogenesis. Since the discovery by Hughes and Huggins that growth of prostate cancer cells is dependent on androgens, androgen deprivation therapy (ADT) has been the mainstay of non-organ confined PCa. However, due to the characteristic heterogeneity of this disease and lack of proper understanding of the molecular mechanisms underlying progression of PCa, the tumour reappears after ADT and becomes castration resistant.

Despite the fact that most prostate tumours are castrate-resistant, AR function and expression are almost invariably maintained leading to the AR dependent growth of castrate resistant cells. Therefore, investigation of the molecular mechanisms by which AR regulates transcription of target genes that are responsible for such action may open new gateways for the development of novel therapeutic targets and diagnostic markers. In an attempt to unmask these genetic progenitors and to expand the scope of current microarray technology, we have created a unique custom-made prostate cancer microarray with 180K probes. These probes target annotated exons, differential 3'untranslated regions (UTRs), expressed sequence tags (ESTs) mined from public databases, and transcripts identified from Next Generation Sequencing (NGS) studies in our model systems.

In this study, we studied the expression profiles of various androgen-regulated genes in LNCaP cells and identified several candidate genes for prostate cancer diagnosis and therapy. We analysed these genes by using web based UCSC genome browser and Ingenuity Analysis programmes and identified several novel and already described androgen regulated genes in addition to the unique androgen binding sites with RIP-CHIP analysis. Of particular interest was the expression of genes involved in glucose and ion transport (SLC26A3, SLC16A6, SGK1, STEAP4). These genes were specifically upregulated by androgens as compared to other hormones and were repressed by anti-androgen treatment. We have so far validated the microarray findings by real-time PCR in three prostate cancer cell lines (LNCaP, 22RV1 and LAPC4) and are in the process of targeting the selected genes functionally to study their effects on different pathways. These preliminary results suggest that these genes and the pathways they control may serve as the new therapeutic modalities for the treatment and diagnosis of castration resistant prostate cancer.









Porphysomes: Intrinsically Multifunctional Nanovesicles For Focal Laser Therapy To Treat Prostate Tumor

Cheng S. Jin 1,2,4, Jonathan F. Lovell 2,4, Robert G. Bristow 3,4, and Gang Zheng* 1,2,3,4

- 1. Department of Pharmaceutical Sciences, University of Toronto, Ontario, M5S 3M2
- 2. Institute for Biomaterials and Bioengineering, University of Toronto, Ontario, M5G1L7
- 3. Department of Medical Biophysics, University of Toronto, Toronto, Ontario, M5G 2M9
- 4. Ontario Cancer Institute, University Health Network, Ontario M5G 1L7

Introduction

We have recently discovered porphysomes, the first all-organic nanoparticles with intrinsically multimodal biophotonic properties. These are bilayer nanovesicles that self assemble from a single porphyrin-lipid conjugate. The very high porphyrin packing density (83,000 porphyrin per particle) results in structure-dependent 'super'-quenching, which, in turn, gives unique photothermal and photoacoustic properties that are unprecedented for organic nanoparticles. In this study, we investigated porphysomes as photothermal therapy (PTT) agents to eradicate tumors in both subcutaneous xenograft and orthotopic prostate tumor model.

Method

The studies were preformed first on subcutaneous tumor model. After i.v. injection of porphysomes, biodistribution was conducted at different time to define treatment time point. PTT was performed on tumors with laser transdermally delivered (671nm, 1.4 W/cm², 60s) at 24 hours post-administration. An infrared thermal camera was used to monitor the real-time temperature increase of tumor during the treatment. The PTT efficacy was evaluated by monitoring the tumor size and mice survivals, comparing with two control groups (mice treated with either porphysomes or laser alone). In the preliminary study using PC3 orthotopic prostate tumor model, ⁶⁴Cu-chelated porphysomes served as PET probes, and were used for biodistribution study. PTT was performed using transdermally delivered laser, and the efficacy was evaluated by monitoring the orothotopic tumor growth by Magnetic resonance imaging (MRI) and Bioluminescence imaging (BLI).

Results

Porphysomes can passively accumulate in tumors and induced tumor temperature increase from 30 °C to 62 °C rapidly upon the laser irradiation. After laser treatment, an eschar formed and healed within 2 weeks. All mice survived after 150 days without tumor recurrence (Figure 1). However, in both control groups, tumors continued growing and reached the defined end point within 3 weeks. In orthotopic prostate tumor study, ⁶⁴Cu-chelated porphysomes passively accumulated in tumor by 6.5 %ID/g at 24 hours post-injection. PTT induced rapid temperature increase in treated tumor area. Both MRI and BLI showed that the tumor growth was significantly suppressed following PTT, comparing to control groups (Figure 2).

Conclusion

The intrinsically multifunctional porphysomes dissipated the absorbed energy as heat efficiently, thus were potent PTT agents for imaging-guided cancer therapy. The promising PTT results on orthotopic prostate tumor model indicated the potential of developing porphysome technology toward the future clinical translations. Studies are ongoing now, investigating the theranostic applications of porphysomes to treat prostate tumor by focal PTT.







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Figure 1: Subcutaneous mice xenograft: the tumor change and survival of mice in the treatment group and control groups after photothermal therapy.



Figure 2: Orthotopic mice prostate tumor model: bioluminescence signal of PC3 tumor was significantly reduced after the PTT treatment.

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PIM Kinase Inhibitors Synergize With The EGFR-TKI Gefitinib To Inhibit Prostate **Cancer Cell Proliferation And Clonogenic Cell Survival**

Allan Siu 1, John Van 1, Julia Hanchard 1, Carl Virtanen 2 and Jan Jongstra 1

- 1. Genetics and Development Division, Toronto Western Research Institute, University Health Network, Toronto, Canada and Department of Immunology, University of Toronto, Toronto, Canada and
- 2. Microarray Centre, University Health Network, Toronto, Canada.

The PIM family of oncogenic serine/threonine kinases regulates tumour cell proliferation, survival and drug resistance. To identify proliferative signaling pathways that are regulated by PIM kinases we analyzed gene expression differences in DU-145 and PC3 prostate cancer derived cells induced by treatment with the recently developed highly selective PIM kinase inhibitor M-110. This identified 97 genes the expression of which is affected by M-110 in both cell lines. We then focused on the M-110 induced up regulation of the *MIG6* gene that encodes a negative regulator of EGFR signaling. Here we show that M-110 and the structurally unrelated PIM kinase inhibitor SGI-1776 up regulate MIG6 in DU-145 and PC3 cells. Knockdown of PIM-1 but not of PIM-2 or PIM-3 also up regulates MIG6 expression, which identifies MIG6 as a PIM-1 regulated gene. In agreement with the role of MIG6 protein as a negative regulator of EGFR signaling we found that M-110 treatment inhibits EGF induced EGFR activation and the activation of the downstream ERK MAPkinase pathway. The biological significance of these findings are demonstrated by the fact that co-treatment of DU-145 or PC3 cells with the EGFR tyrosine kinase inhibitor Gefitinib and M-110 or SGI-1776 has synergistic inhibitory effects on cell proliferation and clonogenicity. These experiments define a novel biological function of PIM-1 as a positive co-regulator of EGFR signaling and suggest that PIM inhibitors may be used in combination therapies to increase the efficacy of EGFR tyrosine kinase inhibitors.









Characterization Of New Epithelial Prostate Cell Lines

Jovanovic L. 1, Lawrence M. 2, Nelson C. 1 and Clements J. 1, 2

- 1. Australian Prostate Cancer Research Centre Queensland, Institute for Health and Biomedical Innovation, Queensland University of Technology
- 2. Institute for Health and Biomedical Innovation, Hormone Dependent Cancer Program, Queensland University of Technology

Most commonly utilised prostate cancer cell lines - LNCaP, PC3 and DU145, have been derived from metastatic deposits and are representative of advanced disease. Despite several attempts to establish human prostate cancer cell lines from primary sites or from localized disease, most of emerging cell lines are either derived from the existing ones, or represent new metastatic or normal prostate epithelial cell lines.

We used a series of stage-specific differentiation markers and their expression profiles to characterise a series of HPV16 E6 and E7 immortalized prostate cell lines derived from men with localized prostate carcinoma. Panels of cytokeratin, neuroendocrine and stem-cell markers have been used for molecular profiling. AR, PSA and kallikrein locus gene expression patterns were also tested. We believe that some of these cell lines can be relevant tools for prostate stem cell and prostate cancer research.









Physical And Psychosocial Benefits Of Physical Activity For Men With Prostate Cancer

Justin Keogh

Faculty of Health Sciences and Medicine, Bond University

Context

Prostate cancer patients, especially those on androgen deprivation therapy (ADT) experience many symptoms that make it difficult to maintain their independence and quality of life. As ADT act via reducing testosterone production, exercise may offset many of the ADT side-effects and that of the cancer itself.

Objectives

This study sought to conduct a systematic review of the literature on whether exercise could reduce symptoms and improve quality of life for prostate cancer patients.

Methods

Using relevant databases and keywords, 12 training studies were found meeting the inclusion and exclusion criteria.

Results

Grade A level evidence was observed for the benefits of exercise in improving muscular endurance, aerobic endurance and overall quality of life as well as reducing fatigue in prostate cancer patients. Grade B evidence also suggested that exercise may improve prostate cancer patients' muscle mass, muscular strength, functional performance (walking and sit to stand speed) as well as health-related, social and physical quality of life. These effects appeared greater for group- rather than home-based exercise; especially if these programs including resistance training.

Conclusion

It is recommended that most prostate cancer patients be encouraged to exercise regularly by their clinicians and significant others. Where possible, this exercise should be group-based and include some resistance training. Future research in this area should directly compare group- and home-based as well as resistance, aerobic and combined resistance and aerobic training to better elucidate the most effective forms of exercise for this population and what factors affect initiation and adherence to such programs.









Microscopic Assessment Of Fresh Prostate Tumour Specimens Yields Significantly Increased Rates Of Correctly Annotated Samples For Downstream Analysis

<u>Michael Kerger</u>*¥, Matthew K.H. Hong*, John Pedersen†, Timothy Nottle†, Andrew Ryan†, John Mills†, Justin S. Peters*, Daniel Moon*, Helen Crowe*, Anthony J. Costello*, Niall M. Corcoran*‡ and Christopher M. Hovens*

*Division of Urology, Department of Surgery, Royal Melbourne Hospital, University of Melbourne, Parkville and Australian Prostate Cancer Research Centre at Epworth Hospital, Richmond, ¥ The Carlo Vaccari Scholarship,

† TissuPath Pathology Services, Glen Waverley, and

‡Department of Urological Sciences and Vancouver Prostate Centre, University of British Columbia, Vancouver, BC Canada.

Aims

To assess if performing frozen sections of tissue biopsies from fresh radical prostatectomy specimens, prior to tissue banking, could improve the identification of the banked samples compared to standard fresh tumour banking procedures.

Methods

Tissue biopsies banked from 332 fresh prostatectomy specimens were assessed for accuracy of diagnosis, comparing two separate methods of tumour identification: one in which tumour was identified in the gross specimen by visual inspection (n=155) and one in which rapid frozen sectioning was applied (n=177). The associations with correct tumour annotation and clinicopathological variables, including age, pre-operative prostate specific antigen (PSA) levels, pathological Gleason score, pathological T stage, tumour volume and surgical margins, were examined using univariable and multivariable binary logistic regression models.

Results

For the gross visual inspection cohort the rate of correctly identifying and banking specimens containing prostate cancer was 69%. For the cohort assessed with rapid frozen sections, 94% of banked specimens actually had cancer. On multivariable analysis, we found that only frozen sectioning and tumour volume variables were independent predictors of correctly banked tumour specimens whilst all other routinely reported pathological variables had no influence on the success rates of fresh prostate tumour banking.

Conclusion

The success rate for correctly banking fresh prostate tumour specimens is directly related to the tumour volume. Frozen section scrutiny of prostate samples is recommended to prevent misclassification of the banked material.









Prostate Cancer: PTEN Genomic Deletion Is Associated With Poor Prognosis And **Reduced AR Signalling**

Joshua Ejdelman 1, Khalil Choucair 1, Fadi Brimo 2, Simone Chevalier 1 & Jacques Lapointe 1.

- 1. Department of Surgery, Division of Urology
- 2. Department of Pathology, McGill University and the Research Institute of the McGill University Health Centre (MUHC-RI), Montreal, Quebec, Canada

Prostate cancer (PCa), a leading cause of cancer death in Canadian men, displays a broad range of clinical outcome from relatively indolent to lethal metastatic disease. Several genomic alterations have been identified in PCa which may serve as predictors of disease progression. PTEN, residing at chromosome 10q23, is a negative regulator of the AKT survival pathway and a tumor suppressor frequently deleted in PCa. The androgen receptor (AR) signalling pathway is known to play an important role in PCa and its blockade constitutes a commonly used treatment modality. Using Fluorescence In Situ Hybridization (FISH) on 43 primary PCa specimens we found 18 cases of PTEN deletion. Kaplan-Meier analysis showed that loss of PTEN was associated with disease recurrence (P=0.03). Concurrently, immunohistochemical staining for AR found significantly lower levels of AR expression within those tumors deleted for PTEN (P<0.05). To validate these observations we looked at an existing copy number alteration (CNA) and gene expression profiling dataset of 64 PCa samples, 17 of which were PTEN deleted. We confirmed the predictive value of PTEN deletion in disease recurrence (P=0.03). PTEN deletion was also linked to the diminished expression of PTEN (P<0.01) and AR (P=0.02). Furthermore, gene set enrichment analysis (GSEA) revealed a diminished expression of genes downstream of AR signalling in PTEN deleted tumors. Altogether, our data suggest that PTEN deleted tumors expressing low levels of AR may represent a worse prognostic subset of PCa and serve as a challenge for therapeutic management.









Detecting Sense-antisense Transcripts Differentially Regulated By Androgens In Prostate Cancer Cells

Melanie L. Lehman 1,2; John Lai 1; Jiyuan An 1; Stephen C. Hendy 2; Colleen C. Nelson 1,2

- 1. Australian Prostate Cancer Research Centre–Queensland, Queensland University of Technology, Princess Alexandra Hospital, Level 1, Building 1, 199 Ipswich Road, Brisbane QLD 4102 Australia;
- 2. Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver BC V6H 3Z6 Canada

The human transcriptome is far more complex than previously thought with the potential for a single genomic locus to undergo overlapping, interleaved and antisense transcription in a cell and context dependent manner. We have used strand-specific Illumina RNA sequencing to detect loci where sense and antisense transcripts are expressed in an androgen-sensitive prostate cancer cell line (LNCaP) following treatment with a physiological androgen (DHT). We detected loci where both sense and antisense transcripts were up- or down-regulated by DHT, as well as loci where sense and antisense transcripts were oppositely regulated by DHT. Over half of the sense-antisense transcripts that were oppositely regulated by DHT mapped to a processed transcript in the NCBI RefSeg reference database. We further studied one instance of oppositely regulated sense-antisense transcription at the CTBP1 locus. CTBP1 protein-coding transcript levels were decreased by DHT while the antisense transcript levels (GenBank Accession: AK092548; non-coding transcript sequenced from prostate tissue) were increased by DHT. The pattern of expression was reversed with anti-androgen treatment (MDV3100 and bicalutamide). Knock-down of the CTBP1 antisense transcript via siRNA led to an increase in CTBP1 protein levels. This highlights the intimate relationship of sense-antisense transcription and how changes to the sense-antisense transcript ratio can be reflected at the protein level. The detection of these sense-antisense transcripts also highlights the importance of adopting strandspecific RNA sequencing protocols to accurately map the strand of transcripts. Incorrect strand assignment of sequencing reads will misrepresent the levels of protein-coding transcripts in downstream network analyses, as well as mask the detection of novel non-coding transcripts. Oppositely regulated sense-antisense pairs such as the one described at the CTBP1 locus suggests that genomic context should be a component in the analysis of regulatory networks.









Using Protein Biophysical Properties To Design Effective Drug Screening Assays For The Molecular Chaperones HspB1

Barbara Lelj Garolla Di Bard

Vancouver Prostate Centre, Vancouver, Canada.

The heat shock protein B1 (HspB1) is an ATP-independent molecular chaperones that is overexpressed in castrate resistant prostate cancer (CRPC) and its expression correlate with poor prognosis. HspB1 is up regulated in the natural stress response to chemotherapy and infers increased survival to cancer cells. We believe that it is a novel therapeutic anticancer targets for small molecule inhibitors with preclinical and phase II clinical proof of concept using the antisense OGX-427.

HspB1 is an oligomeric chaperone with a temperature dependent self-association. It can inhibit protein precipitation in vitro but because it lacks an enzymatic activity have so far been considered an undruggable target. HspB1 biophysical properties have been the focus of several studies in the last decade and we are using this information to design effective drug screenings. We are employing a multi tactic approach by designing 3-4 assays for drug discovery based on various HspB1-protein interactions or HspB1 oligomerization properties.

Moreover we are focusing on finding suitable conditions for protein crystallization. If the structure of HspB1 can be elucidated, alongside the development of screening assays for on target inhibition, this will facilitate discovery of novel drugs that will be translated into clinical trials and effective therapies in prostate cancer patients.









Defining Optimal Coating Conditions For The LNCaP Prostate Cancer Cell Line

<u>Michelle S. Liberio</u> a,b, Martin Sadowski b, Carolina Soekmadji b, Rohan A. Davis a, Colleen Nelson b

- a. Eskitis Institute, Griffith University, Nathan, QLD 4111, Australia
- b. Australian Prostate Cancer Research Centre, Queensland University of Technology, Brisbane, Queensland, Australia.

Cell-surface adhesion is mediated through specialized membrane receptors called integrins. Besides mediating cellular adhesion, integrins also lead to initiation of a signaling cascade inside the cell, which directs cellular process such as cell survival, proliferation, differentiation, and migration. Extracellular matrix (ECM) proteins play an important role in the generation and maintenance of tissue architecture, are cell line specific and function through their specific interaction with integrins. Weak cell-surface adhesion of cell lines is a common problem of tissue culture research and presents technical limitations to the design of experiments. To overcome this problem, various surface coating protocols have been developed. However, due to methodological limitations, a comparative and precise real-time measurement of their impact on cell attachment and proliferation has not been conducted. The androgen-sensitive human prostate adenocarcinoma cell line, LNCaP, represents an early stage of the disease and is one of the most important model systems in prostate cancer research. Their characteristically weak attachment to the surface of tissue culture vessels and cover slips have impeded their manipulation and analysis since LNCaP cells can be easily dislodged through modest mechanical forces like fluid shear stress. To improve the adherence of LNCaP cells to the culture surface, we compared different coating reagents (poly-L-lysine, poly-L-ornithine, collagen IV, fibronectin, and laminin) and culturing conditions, e.g. cell density, and analysed their impact on cell proliferation, adhesion and morphology with a real-time cell analyser (RTCA). RTCA is a label free methodology that measures these cellular parameters based on impedance changes. We also performed viability assays and microscopy at different time points to validate the RTCA data. Our findings are a helpful tool for the selection of the ideal coating reagent and culture conditions for the LNCaP cell line with respect to their effect on proliferation rate, attachment, morphology and cellular cytoskeleton arrangement.









Gamma-Tocotrienol As An Effective Agent In Targeting Prostate Cancer Stem **Cell-like Population**

MingTat (Patrick) Ling 1, Sze-Ue Luk 2, Terence Kin-Wah Lee 3

- 1. Australian Prostate Cancer Research Centre Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology
- 2. Department of Anatomy The University of Hong Kong, Hong Kong, Hong Kong SAR
- 3. Department of Pathology, The University of Hong Kong, Hong Kong, Hong Kong SAR

Emerging evidence supports that prostate cancer originates from a rare sub-population of cells, namely prostate cancer stem cells (CSCs). Conventional therapies for prostate cancer are believed to mainly target the majority of differentiated tumor cells but spare CSCs, which may account for the subsequent disease relapse after treatment. Therefore, successful elimination of CSCs may be an effective strategy to achieve complete remission from this disease. Gamma-tocotrienols (γ -T3) is one of the vitamin-E constituents which have been shown to have anticancer effects against a wide-range of human cancers. Recently, we have reported that γ -T3 treatment not only inhibits prostate cancer cell invasion but also sensitizes the cells to docetaxel-induced apoptosis, suggesting that γ -T3 may be an effective therapeutic agent against advanced stage prostate cancer. Here, we demonstrate for the first time that y-T3 can down-regulate the expression of prostate CSC markers (CD133/CD44) in androgen independent (AI) prostate cancer cell lines (PC-3 & DU145), as evident from western blotting and flow cytometry analysis. Meanwhile, the spheroid formation ability of the prostate cancer cells was significantly hampered by y-T3 treatment. In addition, pre-treatment of PC-3 cells with y-T3 was found to suppress tumor initiation ability of the cells. More importantly, while CD133-enriched PC-3 cells were highly resistant to docetaxel treatment, these cells were as sensitive to y-T3 treatment as the CD133-depleted population. Our data suggest that γ -T3 may be an effective agent in targeting prostate CSCs, which may account for its anticancer and chemosensitizing effects reported in previous studies.









Chemopreventive Effect of PSP Through Targeting Of Prostate Cancer Stem Cell-like Population

MingTat (Patrick) Ling 1, Sze-Ue Luk 2, Terence Kin-Wah Lee 3,

- 1. Australian Prostate Cancer Research Centre Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology
- 2. Department of Anatomy The University of Hong Kong, Hong Kong, Hong Kong SAR
- 3. Department of Pathology, The University of Hong Kong, Hong Kong, Hong Kong SAR

Recent evidence suggested that prostate cancer stem/progenitor cells (CSC) are responsible for cancer initiation as well as disease progression. Unfortunately, conventional therapies are only effective in targeting the more differentiated cancer cells and spare the CSCs. Here, we report that PSP, an active component extracted from the mushroom Turkey tail (also known as *Coriolus versicolor*), is effective in targeting prostate CSCs. We found that treatment of the prostate cancer cell line PC-3 with PSP led to the down-regulation of CSC markers (CD133 and CD44) in a time and dose-dependent manner. Meanwhile, PSP treatment not only suppressed the ability of PC-3 cells to form prostaspheres under non-adherent culture conditions, but also inhibited their tumorigenicity *in vivo*, further proving that PSP can suppress prostate CSC properties. To investigate if the anti-CSC effect of PSP may lead to prostate cancer chemoprevention, transgenic mice (TgMAP) that spontaneously develop prostate tumors were orally fed with PSP for 20 weeks. Whereas 100% of the mice that fed with water only developed prostate tumors at the end of experiment, no tumors could be found in any of the mice fed with PSP, suggesting that PSP treatment can completely inhibit prostate tumor formation. Our results not only demonstrated the intriguing anti-CSC effect of PSP, but also revealed, for the first time, the surprising chemopreventive property of oral PSP consumption against prostate cancer.









Insulin Increases *de novo* Steroidogenesis In Prostate Cancer Cells

<u>Amy Lubik</u> 1, Jennifer Gunter 2*, Steve Hendy 1, Hans Adomat 1, Adrian Herington 2, Michael Pollak 3, Colleen Nelson 1, 2.

- 1. Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada.
- 2. Australian Prostate Cancer Research Centre Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.
- 3. Departments of Medicine and Oncology, Jewish General Hospital and McGill University, Montreal, QC, Canada.

Androgen-deprivation therapy (ADT) is used to treat recurrent and metastatic prostate cancer resulting in tumour regression. However, this response initially seen with ADT eventually gives way to regrowth of prostate tumour cells no longer reliant on testicular androgens, referred to as castrate resistant prostate cancer (CRPC), and is currently considered incurable. We have previously demonstrated that prostate tumour cells in the face of ADT synthesise their own androgens *de novo* reactivate the androgen receptor and promote CRPC. We aim to understand what promotes this transition to *de novo* androgen synthesis. ADT is associated with metabolic syndrome including the key persistence of high levels of circulating insulin (hyperinsulinaemia) which in turn is associated with more rapid progression to CRPC and increased cancer mortality. We hypothesise that insulin may also influence steroidogenesis in CRPC; therefore, we examined the effect of insulin on steroid synthesis in prostate cancer cell lines.

Insulin (10nM) increases *de novo* steroidogenesis in CaP models, via increased expression of SREBP and enzymes within the steroidogenesis pathway (mRNA and protein) resulting in dramatically increased intratumoral steroid production. Intracellular testosterone increased from 0.011 to 0.65ng/g cells and testosterone and DHT secreted into the media 0.0249 and 0.037nM, respectively which is sufficient to reactivate the AR to stimulate PSA expression and secretion; insulin increased PSA mRNA expression with similar potency to 0.16nM DHT. Metformin, is used clinically in obese and diabetic patients to normalise circulating insulin levels and recent studies suggest metformin may improve patient outcomes in prostate cancer. We observed reduced expression of lipogenic and steroidogenic enzyme mRNA in insulin treated PCa cells following metformin treatment. We conclude that insulin can act directly on PCa cells to activate pathways contributing to CRPC progression in part by enhancing steroidogenesis and metformin treatment is sufficient to overcome insulin-stimulated lipogenesis and steroidogenesis.









The Interactions Between Insulin And Androgens In Progression To Castrate Resistant Prostate Cancer

<u>Colleen C. Nelson</u>, Amy A Lubik, Jennifer Gunter, Jennifer A Locke, Melanie Lehman, Michael Pollak, Ian McKenzie, Adrian Herington and Martin E Gleave

Australian Prostate Cancer Research Centre-Queensland Queensland University of Technology, Princess Alexandra Hospital Brisbane, Queensland, Australia

Androgen-dependent pathways regulate maintenance, function, and growth of normal and malignant prostate tissue. Androgen deprivation therapy (ADT) exploits this dependence to treat metastatic prostate cancer, however, regression initially seen with ADT gives way to development of castrationresistant prostate cancer (CRPC). We and others have found that androgen regulated pathways are reactivated in CRPC despite low levels of circulating androgens and this in part is due to de novo intratumoral androgen synthesis1). Targeting this pathway using specific steroid inhibitors (targeting CYP17A1 and SRD5A2) and anti-androgens in LNCaP tumors at different stages of progression to CRPC resulted in an adaptive steroid hormone metabolism pathways in the progression to CRPC2). While ADT generates a therapeutic response for tumor control, it also instigates features of metabolic syndrome including elevated circulating insulin levels. Hyperinsulinaemia appears to precede other metabolic changes and increases in adiposity, and may be a direct result of androgen deprivation. Since prostate cancer cells are capable of synthesizing androgens de novo and express the insulin receptor, we hypothesized that high levels of insulin following ADT may drive prostate cancer progression in part by increasing steroidogenesis in CRPC. We examined this hypothesis by evaluating the effects of insulin in prostate cancer cell lines for steroid synthesis and related pathways. Sustained treatment over 16-48 hours of insulin in LNCaP, 22RV1, and VCaP cell lines increased mRNA and protein expression of steroidogenesis enzymes and upregulated the insulin receptor substrate IRS2. Similarly, insulin treatment upregulated intracellular production of testosterone and secreted androgens, with the concentrations of steroids observed similar to the levels reported in prostate cancer patient tissues3). Insulin treatment under 71 these conditions resulted in increased expression of prostate specific antigen (PSA). This induction by insulin could be blocked by antiandrogens or targeting effectors of the insulin pathway. CRPC progression also correlated with increased expression of IRS2 and insulin receptor in vivo. Taken together, our findings suggest that the elevated insulin levels associated with ADT may exacerbate progression of prostate cancer to CRPC in part by enhancing steroidogenesis and reactivating androgen pathways. Microarray analysis of LNCaP prostate cancer cells reveals differential gene activation by insulin in the presence and absence of androgens. IGF-1 receptor expression is known to be highly upregulated by androgens. In contrast, we have demonstrated that in prostate cancer cells expression of the insulin receptor isoforms is repressed by androgens and thereby in ADT, insulin receptor expression is increased which may facilitate increased insulin signaling. The specific inverse relationship between insulin and testosterone in men has been demonstrated in both men with existing metabolic dysfunction (concomitant increased insulin and decreased testosterone levels) and prostate cancer patients on ADT develop hyperinsulinaemia. This suggests an important metabolic crosstalk exists between these two hormonal axes recently reviewed in Gunter et al4). The complicated interactions of these two axes in men with prostate cancer is highlighted by the clinical observations, diabetes is associated with increased risk of several cancers including breast, kidney and colon and pharmacological treatments such as metformin which neutralizes glucose and insulin levels, are associated with decreased cancer risk. The effect of metformin in men receiving ADT with no history of diabetes is still under investigation. Our in vitro results suggest that metformin is highly efficient at blocking programs of de novo steroid and fatty acid synthesis in androgen-deprived conditions.

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Semaphorin 3C: A Novel Target For Treatment Of Advanced Prostate Cancer

Christopher Ong

Vancouver Prostate Centre, Vancouver, Canada

From two independent lines of investigation involving genome-wide gene expression profiling to study the roles of PTEN and clusterin in PCa, we have recently identified Semaphorin 3C (SEMA3C) as a candidate target gene important in PCa progression. SEMA3C is a secreted member of a family of signaling proteins called semaphorins that are best known as important mediators of cell migration and axon guidance in the developing nervous system. Semaphorins mediate their effects by signalling through plexin receptors. Plexins contain a conserved intrinsic Ras-GAP domain and some plexins stably interact with the tyrosine kinase receptors (RTKs), c-Met and ErbB2 forming a multi-receptor signaling complex. Importantly, binding of semaphorins to plexins can activate the tyrosine kinase activity of these RTKs independently of their respective ligands and lead to activation of downstream signaling pathways including Src, PI3K/Akt, Ras/MAPK pathways. Intriguingly, PlexinB1, a cognate plexin receptor for SEMA3C, has recently been found to be among the most commonly mutated and over-expressed genes in PCa. PlexinB1 has been shown to be over-expressed in over 70% of primary prostate cancers and missense point mutations leading to loss of the intrinsic R-Ras GAP enzymatic activity of plexin B1are found in 89% of bone metastases. This observation points to the critical importance of the SEMA3C/PlexinB1 signalling pathway in advanced PCa. The association of semaphorin bound plexins with ErbB2 and c-Met is intriguing as both proto-oncogenes have been implicated in PCa progression and metastasis.

Through target validation studies, we have found that SEMA3C is upregulated in castration resistant PCa (CRPC) cell lines and importantly, from tumour tissue microarray analyses, we found that SEMA3C is significantly increased in advanced CRPC clinical samples as compared to hormone naïve cancers, and PCa tissue that have been treated with neoadjuvant hormone therapy for less than 6 months. Importantly, SEMA3C was found to be upregulated in a subset of MDV-3100 resistant cell lines and patient tumor samples. To characterize the functional role of SEMA3C, we designed inhibitors of SEMA3C – an antisense oligonucleotides (ASO) against the first 20 nucleotides of the SEMA3C coding sequence and a recombinant protein inhibitor comprised of the SEMA3C sema domain fused to the Fc domain of human IgG1. SEMA3C ASO treatment exhibited dose and sequence specific inhibition of SEMA3C mRNA and protein expression. SEMA3C inhibition led to inhibition of cell growth and induction of apoptosis. Furthermore, suppression of cell growth by SEMA3C blockade could be reversed by addition of recombinant SEMA3C. Moreover, SEMA3C inhibition prevented castration resistant PCa progression of LNCaP xenograft tumors in vivo. Furthermore, we have found that SEMA3C mediates its effects in part through activation of cMet and EGFR/ErbB2 tyrosine kinase signaling. SEMA3C blockade leads to inhibition of c-Met, EGFR/ErbB2, Src, Akt and Erk pathways. We hypothesize that SEMA3C is a critical mitogenic growth factor that can activate invasive growth, metastasis and chemoresistance of prostate cancer cells and mediate SARA resistance in advanced prostate cancer. Thus, therapeutic strategies to inhibit the SEMA3C signaling may be of benefit to delay or inhibit the emergence of SARA resistance.









Identification Of Novel Antagonists That Target Androgen Receptor Surface **Sites**

Nathan A. Lack, Peter Axerio, Eric LeBlanc, Emma Guns, Artem Cherkasov, Paul S. Rennie

Vancouver Prostate Centre, University of British Columbia, 2660 Oak Street, Vancouver, BC V6H 3Z6, Canada

The first line of treatment for advanced, metastatic prostate cancer is some form of androgen withdrawal therapy, which is designed to block either the production of androgens or their binding to the androgen receptor (AR) ligand binding domain. The currently used anti-androgens (eg Casodex and now MDV3100) all inhibit the AR by binding to its hormone/ligand binding site. While treatment with these AR antagonists initially suppress prostate tumor growth, eventually they become less effective with the emergence of the castration- resistant phenotype. We conducted in silico and biological screens to identify compounds that can bind to the AR surface and block binding of co-activators to the Activation Function 2 (AF-2) and to the Binding Function – 3 (BF-3) sites on the AR. Using the combined power of modern computers and methods of artificial intelligence to rationally select new anti-AR drug candidates among millions of existing chemicals, we identified 50 AF-2, and 213 BF-3 candidate binders respectively. After cell-based screening assays for AR inhibition and with Biolayer Interferometry analysis for AR binding, we identified 5 potent AF-2 and 17 BF-3 binders that exhibited significant inhibitory effects on the AR. Furthermore, a fluorescence polarization (FP) assay, which directly monitors blocking of SRC2-3 coactivator peptide (native ligand) to the AF-2 site, revealed IC50 inhibitions in the range of 8-35µM for the 5 AF-2 binders. None of these compounds were able to bind to the hormone binding site. In summary, our AF-2 and BF-3 binders are an entirely new class of antiandrogens that have the potential to effectively inhibit AR transcriptional activity by directly targeting its co-activation. This is a distinctly novel mechanism which does not target the androgen binding site and thereby circumvents treatment resistance seen with conventional anti-androgens. (supported by Genome BC and the PCF with funds from Canada Safeway).









Unraveling The Role Of YB-1 And G3BP In Prostate Cancer Progression - Identification Of RNA Species Bound And Regulated By YB-1 And G3BP

<u>Anja Rockstroh</u>, Morgan Pokorny, Derek Kennedy 1, Susan Moore 2, **Melanie Lehman** 2 and **Colleen** Nelson

Australian Prostate Cancer Research Centre Queensland, Queensland University of Technology, Brisbane, Australia;

- 1. Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia;
- 2. The Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada

The major obstacle for successful treatment of advanced prostate cancer is the development of androgen independence and therapeutic resistance. Identifying the molecular mechanisms underlying these processes is crucial to develop novel therapeutic strategies. Chemotherapy and androgen ablation are known to activate stress induced pathways that can aid towards the survival of the tumour cell and subsequent development of therapeutic resistance. Targeting those stress activated factors has already been proven to be a successful strategy to improve therapeutic outcomes. The stress activated proteins YB-1 and G3BP have been described as biomarkers of poor prognosis and are also promising candidates to become therapeutic targets. They are multifunctional proteins involved in networks regulating proliferation, cellular sensitivity and the cellular stress response. Interesting features of YB-1 and G3BPs are their RNA-binding capacity and their involvement in the formation of stress granules. Therefore they are believed to play important roles in triaging mRNAs in response to changing environmental conditions. Being stress activated and hormone regulated proteins they can act as cellular switches linking signal transduction to adapted gene expression at the post transcriptional level. However, little is known about the identity and fate of the RNAs bound by YB-1 and G3BP and the biological consequences of this interaction. Thus, the focus of our work is to investigate how YB-1 and G3BP function through the binding and regulation of their target RNAs. Employing the RIP-chip assay, we aim to identify the RNA species bound by YB-1 and G3BP in prostate cancer cells. Identifying the specific RNAs interacting with YB-1 and G3BP in adaption to changing environmental conditions will help to uncover their roles in cellular survival and cancer progression. Gaining further insight into the mechanisms underlying therapeutic resistance and androgen independence will also help to find novel therapeutic targets for the treatment of advanced prostate cancer.









Use Of Targeted Magnetic Nanoparticles For Imaging In Prostate Cancer

<u>Russell Pamela J</u> 1, **Soekmadji Carolina** 1, Thierry B 2, Cowin G 3, Strait-Gardner T 4, Verma N 5, Xiaochun Wang 5, Khatri A 5.

- 1. Australian Prostate Cancer Research Centre Queensland, Princess Alexandra Hospital, 199 Ipswich Rd, Brisbane, Queensland, 4102, and Cells and Tissue Domain, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland;
- 2. Ian Wark Institute, University of South Australia, Adelaide;
- 3. National Imaging Facility, Centre for Advanced Imaging, University of Queensland, Brisbane, Queensland;
- 4. National Imaging Facility, University of Western Sydney,
- 5. Prince of Wales Clinical School, Faculty of Medicine, The University of New South Wales, Sydney, NSW, Australia

Objective

Imaging techniques better than those conventionally used are needed to improve prostate cancer (PC) staging and read out of therapeutic effects in real time in treated patients. We aimed to perform preclinical evaluation of newly developed well-characterised, biocompatible, PC-targeted magnetic nanoparticles (MNPs) targeted to prostate cancer by conjugation with J591 (from N Bander, Cornell, USA), an antibody which binds specifically to prostate specific membrane antigen (PSMA) which is expressed on the surface of ~90% of PCs including castrate resistant prostate cancers; this binding results in internalisation. The use of targeted MNPs should enhance the specificity and sensitivity of magnetic resonance imaging (MRI) to enable better staging of patients with PC and future targeted delivery of therapy.

Methods

MNPs were prepared, engineered to the appropriate size and conjugated with J591. There was no compromise in J591 cell binding or specificity for PSMA positive cells due to the conjugation, and Inductively Coupled Plasma Optical Emission Spectrometry and Prussian blue staining for iron indicated increased uptake of MNPs that were conjugated with the antibody. *In vivo* studies were performed in immunodepressed nude mice with subcutaneous LNCaP-LN3 (PSMA-positive) xenografts (post euthanasia using an 11.7T NMR system) or on live mice with orthotopic LNCaP xenografts, using a 16.4T MRI imaging system following intravenous injection of MNPs.

Results

Similar enhancement of MRI was obtained by NMR after injection of MNP or J591-MNP conjugates. Live imaging of mice given systemic J591-MNPs showed uptake into the prostate tumours.

Conclusions

The data indicate the promise of this technique in enabling imaging of small clusters of human prostate cancer cells. This should enable the effects of therapy to be determined by imaging in real time, improving patient management.









Using A Novel Model Of Bony Metastasis In Immunocompetent Mice To Assess Effects Of Combining Docetaxel And Zoledronic Acid Treatment

Power C A 1,3, Hung T-T 1,3, Chan JC 2, Russell Pamela J 4

- 1. Prince of Wales Clinical School, Prince of Wales Hospital, Randwick, New South Wales, Australia,
- 2. Centre for Vascular Research, University of New South Wales, Sydney, New South Wales, Australia,
- 3. Faculty of Medicine, University of New South Wales, Sydney, New South Wales, Australia,
- 4. Australian Prostate Cancer Research Centre-Queensland, Princess Alexandra Hospital, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia

Objective

Bone metastatic prostate cancer remains a major cause of morbidity and mortality in men. The taxane, docetaxel (DOC) and the bisphophonate, zolodronic acid (ZOL), have shown efficacy in independent clinical trials for advanced disease but the influence of DOC treatment on bone has not been determined; interactions between drug, bone and tumour require further study. Here, we used a mixed osteolytic/osteoblastic murine model of bone-metastatic prostate cancer, RM1(BM), to assess the effects of DOC and ZOL treatment on bone growth and metastasis (integrity of tumour-bearing vs non-tumour-bearing bones) and the survival of the tumour-bearing mice.

Materials/Methods

The model involves intracardiac injection for arterial dissemination of the RM1(BM) cells (transfected to express green fluorescence protein, GFP) in C57BL/6 mice. Mice were treated with three doses on days -1, +3 and +7 of ZOL (100 mg/kg) (given subcutaneously) or DOC (20 mg/kg) (intravenous injection) singly or in various combinations: 5, 10 or 20 mg/kg DOC with 20 mg/kg ZOL or 20 mg/kg DOC with 100 mg/kg ZOL. Age matched mice were used as controls. Bone integrity was assessed in legs with tumors in the distal femur and/or proximal tibia by micro-computed (CT) tomography and compared to untreated mice. Bones were decalcified and paraffin-embedded histological sections stained for TRAP activity. The osteoclast and osteoblast activity was determined by measuring serum tartrate-resistant acid phosphatase 5b (TRAP 5b) and osteocalcin, respectively. Mice were euthanased according to predetermined criteria and survival was assessed using Kaplan Meier plots.

Results

Micro-CT and histological analysis showed that both DOC and ZOL treatments affected bone structure and metabolic activity. ZOL inhibited tumour-induced bone lysis, maintained bone volume and reduced the calcification of tumour induced endochondral osteoid material. ZOL treatment also led to a decreased serum osteocalcin and TRAP 5b levels. However, ZOL treatment did not inhibit the cells ability to metastasise to bone as the number of bone-metastases was similar in both treated and untreated mice. DOC decreased the number of bone-metastases, but altered the morphological appearance of bones, reducing bone surface area and bone volume compared to control mice. Importantly, ZOL preserved bone integrity when used in combination with DOC.

Conclusions

Our results demonstrate that ZOL and DOC have differential effects on bone and tumour-mediated bone pathology. Importantly, while ZOL preserved bone integrity, DOC alone did not. Despite the fact that ZOL increased the survival of tumour-bearing mice, it did not prevent establishment of bone-metastases in this model. These results support the use of ZOL as an adjunct to DOC, to preserve bone structure.

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Exosomes As Biomarkers For Prostate Cancer Progression And Drug Resistance

Carolina Soekmadji, Colleen C Nelson and Pamela J Russell

Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Over 20,000 Australian men are diagnosed with prostate cancer with over 3,500 deaths/year from this disease. Worldwide, 910,000 cases were recorded in 2008, accounting for 14% of men's cancer and these figures are predicted to reach 1.7 million by 2030. Early prostate cancer (PCa) is treated by surgery or radiation; androgen depletion therapy (ADT) is subsequently used for those who fail treatment, as prostate cancer is androgen regulated. Among these, 25-40% of cases develop castrate resistant prostate cancer (CRPC) with a rising PSA, an androgen regulated gene, despite low androgen levels in the serum and continue to progress with metastatic disease. The underlying mechanisms for progression to CRPC are complex. PCa cells that survive ADT arrest adapt to a low androgen environment through various mechanisms which maintain Androgen Receptor (AR) signalling and continue to proliferate. Chemotherapeutic drugs (eg docetaxel) are commonly used to treat CRPC patients in the clinic, but ~30% of patients who receive docetaxel therapy relapse and suffer from severe side effects.

Biomarkers that could define whether patients will response towards ADT and drugs are needed. We believe that exosomes may provide novel biomarkers that will alter with treatment, potentially providing markers that reflect PCa progression and drug resistant disease. We investigated the effects of DHT treatment on exosome secretion using AR positive LNCaP and 22RV1 cell lines as an in vitro PCa model. DHT changes the protein profile of exosomes isolated from both cell lines, implying that in PCa, the AR plays a role in selecting the content of exosomes and confirming that exosome analysis has the potential to provide novel biomarkers for PCa progression and drug resistance.









The EphB4 Receptor And Its Ligand, EphrinB2, Are Primary Substrates For The Serine Protease Kallikrein 4 - All Key Regulators Of Epithelial Cancer Progression

Adrian C. Herington 1,2, Scott H. Stansfield 1, Carson R. Stephens 1, Judith A. Clements 1,2 and <u>Sally-</u> <u>Anne Stephenson</u> 1,2

- 1. Institute of Health and Biomedical Innovation and the Faculty of Science and Technology, Queensland University of Technology, Kelvin Grove, Queensland
- 2. Australian Prostate Cancer Research Centre, Queensland, Queensland University of Technology, Princess Alexandra Hospital, Brisbane, Australia

Topic

Identification of functionally important genes: new prevention and treatment targets

The receptor tyrosine kinase EphB4 is over-expressed in cancer cells from several different tissue origins, and is a key contributor to tumour development by promoting angiogenesis and cancer cell survival and facilitating invasion and migration. Recent studies suggest cancer-promoting EphB4 signalling pathways are ephrinB2 ligand-independent, as addition of soluble ephrinB2 causes tumour suppression in cancer cells over-expressing EphB4. Both EphB4 and ephrinB2 contain predicted recognition sites for the kallikrein serine protease KLK4, another key regulator of epithelial cancer progression. We hypothesise that KLK4, over-expressed and secreted from cancer cells, degrades surface EphB4 and/or ephrinB2, preventing EphB4-ephrinB2 interaction and activation of signalling pathways that normally suppress tumour formation, and thereby facilitating cancer progression. To determine whether ephrinB2 and EphB4 are substrates of KLK4, recombinant proteins were incubated together with a dilution series of KLK4 and cleavage of the substrate proteins visualised by SDS- and native PAGE with silver staining and western analysis. KLK4 cleaved both proteins into several fragments at low concentration. The primary KLK4 cleavage site in ephrinB2 was verified by N-terminal sequencing and shown to be one of the predicted sites between R178 and N179. Native PAGE showed that pre-formed EphB4/ephrinB2 complex remained intact after KLK4 cleavage, but released the cleaved fragments on denaturation and reduction. Current experiments are aimed at determining whether cleaved EphB4 and/or ephrinB2 retain or lose biological activity post-KLK4 cleavage. These data demonstrate the first evidence for interactions between the Eph/ephrin axis and KLK4 and provide new insights into the potential control mechanisms underlying the apparently conflicting tumour-suppressive and tumour-promoting actions of EphB4-ephrinB2 signaling.









Role Of Stress-activated GADD45G Gene In Prostate Cancer Cell Survival

Raj S Vasireddy 1, Dawn C Cochrane, Colleen C Nelson 1

1. Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation, Queensland University of Technology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

Prostate tumours depend on androgen hormones and androgen-receptor (AR) signalling for their growth and survival and therefore tumours regress during androgen-deprivation therapy (ADT). Tumours undergo stress during ADT resulting in upregulation of stress-responsive genes that promote prostate cancer (PCa) cell survival. Recent studies have implicated a central role for DNA damage in mediating the AR-signalling in PCa. Drugs that target either AR-signalling (e.g. Bicalutamide) or stress-activated genes alone show a good therapeutic response but unable to completely eradicate the tumours. We observed that androgen induced and stress/DNA damage-activated GADD45G gene (Growth Arrest and DNA Damage Inducible-45 Gamma) is critical for PCa cell survival. Knock-down of GADD45G attenuates the activation of androgen-activated genes (e.g Fatty acid synthase). This study will determine the potential of therapeutically targeting of GADD45G to inhibit the progression of PCa to castrate-resistant stage.









A Novel Simulation For Advanced Prostate Cancer Patient Journey

Amol Wagholikar a,b, , Nicholas Graves b and Colleen Nelson c

- a. The Australian e-Health Research Centre, CSIRO ICT Centre, Brisbane, Australia
- b. School of Public Health, Queensland University of Technology, Brisbane, Australia
- c. The Australian Prostate Cancer Research Centre-Queensland, IHBI, Queensland University of Technology, Brisbane, Australia

Objective

To report a novel health economic model that can simulate the change in cost and health outcomes due to new therapeutic interventions for advanced prostate cancer.

Background

Prostate cancer is the second most diagnosed male cancer in the world [1,2]. It is important to have effective disease management strategy and care model particularly at advanced stages of the disease progression. It is difficult to understand the prostate cancer patient journey due to variable treatment patterns and economic impact of treatment interventions to achieve desired health outcomes. The issue is more complicated for the advanced stages of prostate cancer. The aim of the research is to develop simulation models to suggest improvements for management of advanced prostate cancer.

Methods

A health economic model was developed using Markov model. The advanced prostate cancer stages were determined through consultation with advanced prostate cancer patient support groups and guidelines from Andrology Australia [3]. The model comprises of eight unique states of advanced prostate cancer patients. The research used the data collected from the prostate cancer patients through an on-line survey and consultations with clinical researchers.

Findings

The simulation using a baseline model describes a method to evaluate the changes in the management of advanced prostate cancer. Accurate information about healthcare costs and health outcomes can be used to determine the impact of any change. The change can be evaluated against increase in Quality Adjusted Life Years (QALYs) for a given cost associated with the change. The baselines model facilitates evaluation of changes such as introduction of new treatment intervention and prostate cancer care delivery in remote as well as urban areas.

Conclusion

The initial findings suggest that the proposed simulation can be used to evaluate changes and influence policy making decisions for improved health services to advanced prostate cancer. This research provides a better holistic understanding of advanced prostate cancer journey.

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Patient Identification For Advanced Prostate Cancer Clinical Trials

Dr. Amol Wagholikar 1, Dr. Anthony Nguyen 1, Dr. Maggie Fung 2

- 1. The Australian e-Health Research Centre, CSIRO
- 2. The Australian Prostate Cancer Research Centre-Queensland

Objective

Identification of patients for Advanced Prostate cancer clinical trials is a challenging task for clinicians as well as clinical data custodians. The process of assessing suitability of the patient against inclusion and exclusion criteria of a clinical trial is highly labour-intensive. The data usually resides in disparate electronic or paper-based systems both at Urologists and Oncologists. Due to this the clinicians find it difficult to refer a patient for a clinical trial and in some cases patients may not have the opportunity in participating in a relevant clinical trial. This may put certain limitations on the treatment of patients with advanced prostate cancer. Some advances have recently been made with web-based clinical trial finders (Patel et. al, 2007, TrialX 2008); however, this requires clinicians manually going through a checklist of common inclusion/exclusion criteria for each and every candidate patient to assess their eligibility for a clinical trial. This problem of patient identification presents an opportunity to apply a software solution for automatically identifying patients for clinical trials.

Methods

The clinical data describing patient findings and patient characteristics usually resides in patient investigation, reports and clinician correspondence. This data is mostly in free-text form. The patient data should be matched against the inclusion and exclusion criteria of clinical trials. The Australian e-Health Research Centre (AEHRC) team at Commonwealth and Industrial Research Organisation (CSIRO) in collaboration with The Australian Prostate Cancer Research Centre-Queensland (APCRC-Q) has proposed: 1) data integration service for linking patient data across disparate information sources, and 2) text-mining algorithms and the use of clinical terminologies and their semantics for matching inclusion/exclusion criteria with urological and/or oncological patient data. Common (and/or discriminating) sets of inclusion/exclusion criteria will be used to extract semantically equivalent clinical information from the patient's free text record. That is, clinical concepts used to describe the inclusion/exclusion criteria can be used to test for subsumption of clinical concepts identified in the free text. An aggregate score resulting from the inclusion/exclusion criteria matching process can be used to identify or rank patients for possible enrolment in clinical trials. This effectively automates the short-listing of patients suitable for clinical trials, whereby clinicians will be then able to evaluate the patients for a given clinical trial.

Results

The proposed solution is under development and will be demonstrated through a solution prototype. It is anticipated that the proposed solution will simplify the process of patient identification for clinical trials.

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Application Of Case-based Reasoning For Chronic Disease Management Using **Personally Controlled Electronic Health Record**

Dr. Amol Waqholikar 1, Dr. Maggie Fung 2, Prof. Colleen Nelson 2

- 1. The Australian e-Health Research Centre, CSIRO
- 2. The Australian Prostate Cancer Research Centre-Queensland

Objective

To identify online information system requirements and demonstrate case-based reasoning (CBR) approach for improving self-care of advanced prostate cancer patients in a Personally Controlled Electronic Health Record (PCEHR) environment.

Background

Research suggests that prostate cancer patients exercise self-care treatment options such as over -thecounter (OTC) complementary medications. There is always a need to improve the quality of self-care practices to improve the health outcomes as well as reduce economic burden on the health care systems. The PCEHR initiative in Australia presents an opportunity to develop data-driven approaches for improving prostate cancer patient care [1]. Our research presents an approach that can be implemented to leverage data stored in the PCEHR.

Methods

The study was conducted to understand self-care pattern through an on-line survey. The survey was conducted to identify requirements for an online information system. The non-identifying questionnaire was distributed to the patients through prostate cancer support groups in Queensland, Australia. The pilot study was carried out between August 2010 and December 2010. A case-base of 52 patients was developed. We developed a research prototype for modeling the prostate cancer patient journey.

Results

The research made important observations about the self-care patterns among the advanced prostate cancer patients. The data analysis also showed that selenium was the common complementary supplement (55%) used by the patients. The most common OTC used by the patients was Paracetamol (about 45%).

Conclusion

The results of the study specified requirements for an online case-based reasoning [2, 3] information system. The outcomes of this study are being incorporated in design of the proposed AI driven patient journey browser system. A basic version of the system is currently being used at the advanced prostate cancer MDT meetings.

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Androgen And Nutrient Signaling Pathways Regulate Amino Acid Transporter Expression To Direct Prostate Cancer Cell Growth

<u>Qian Wang</u> 1,2, Charles G Bailey 2,3, Cynthia Ng 2, Annora Thoeng 2, Vineet Minhas 1,2, **Melanie L.** Lehman 4, Stephen C. Hendy 4, Grant Buchanan 5, Ladan Fazli 4, Estelle Li 4, Martin E. Gleave 4, Colleen C. Nelson 4,6, John E.J. Rasko 2,3,7 and Jeff Holst 1,2,3.

- 1. Origins of Cancer Laboratory
- 2. Gene & Stem Cell Therapy Program, Centenary Institute
- 3. Cell and Molecular Therapies, Royal Prince Alfred Hospital, Camperdown
- 4. Sydney Medical School, University of Sydney, Sydney, New South Wales
- 5. Molecular Ageing Laboratory, University of Adelaide, Adelaide, South Australia
- 6. Australian Prostate Cancer Research Centre-Queensland, Queensland University of Technology, Brisbane, Queensland, Australia
- 7. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Nutrient transport, including amino acid supply, plays an important, but poorly understood role in the development and progression of cancer. It is known that mRNA expression of the L-type amino acid transporters SLC7A5 (LAT1) and SLC43A1 (LAT3) are increased in prostate cancer, however the pathways regulating their expression, and whether they play a functional role in prostate cancer, have not been determined. Using tissue microarrays of primary and hormone ablated prostate cancers, we confirmed the expression of both LAT1 and LAT3 protein in human prostate cancer. Here we show that inhibition of L-type amino acid transporters using the leucine analague BCH, inhibits leucine uptake, leading to decreased growth and reduced mTOR signalling in LNCaP and PC3 prostate cancer cell lines. Microarray analysis shows an inverse expression pattern in primary prostate cancer (high LAT3, low LAT1) and after hormone ablation and in metastasis (high LAT1, low LAT3). Using chromatin immunoprecipitation and luciferase assays, we have determined the transcription factors that regulate the expression of these transporters, showing that LAT3, also known as prostate cancer overexpressed gene 1, is directly regulated by the androgen receptor. Conversely, LAT1 is directly regulated by amino acid deprivation, via the transcription factor ATF4. Studies using shRNA identified LAT3 as the major leucine transporter in LNCaP cells, and LAT1 in PC3 cells, both leading to a decrease in cell growth and clonogenicity. Our data suggest that L-type amino acid transporters and their regulatory pathways may provide a novel target for therapeutic intervention, designed to "starve the cancer".







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Applying Patient Derived Cancer Xenografts For Personalized Cancer Therapy

Yuzhuo Wang, Ph.D.

Living Tumor Laboratory, The Vancouver Prostate Centre & BC Cancer Agency Department of Urologic Sciences, the University of British Columbia, Vancouver, Canada

When a cancer is detected, it is hoped that clinical doctors can truthfully prescribe the right therapy, to the right cancer patient, at the right time. Recently a number of studies have demonstrated the use of molecular information, as clinical prognostic factors in cancers.

In addition, we, at the Living tumor laboratory (<u>www.livingtumorlab.com</u>), report here that a biological system will likely allow us to direct the use of currently available anti-cancer drugs, develop novel targeted therapeutics, and provide an opportunity to better match the most effective drug or drugs with the biological and molecular characteristics of the individual patient's cancer.











My Road Ahead: An Online Psychological Support Program For Men With Prostate Cancer

Dr Addie C Wootten, Australian Prostate Cancer Research Centre Epworth Dr Jo M Abbott, Australia, Swinburne University of Technology A/Prof David Austin, Swinburne University of Technology A/Prof Britt Klein, Swinburne University of Technology Prof Anthony J Costello, Australian Prostate Cancer Research Centre Epworth A/Prof Declan Murphy, Australian Prostate Cancer Research Centre Epworth Prof Marita McCabe, Deakin University Ms Katherine Chisholm, Deakin University

Objective

The objective of this study is to develop and assess the efficacy of a unique online psychological intervention that is accessible, user friendly and engaging to men with prostate cancer and that reduces the stigma of psychological distress in the context of prostate cancer.

Methods

This randomised controlled trial will examine the efficacy of an online CBT-based self-directed intervention which aims to provide experiential as well as information and psycho-educational material to men diagnosed with prostate cancer across a range of topic areas delivered across 6 modules. The topics explored include identifying emotions and feelings; an introduction to CBT and the role of thought processes and beliefs; communication; coping with physical changes; sexuality and masculinity; sexuality and intimacy and relationships; fear of recurrence and planning for the future.

Participants will be randomly assigned to one of three (3) intervention arms. Group 1: online intervention, group 2: online intervention plus access to the moderated bulletin board; Group 3: moderated bulletin board only. Participants will be assessed utilising the Depression Anxiety and Stress Scale (DASS; Lovibond & Lovibond, 1995), the Prostate Cancer-Related Quality of Life scale (PCa-QoL; Clark et al., 2003), the International Index of Erectile Function (IIEF; Rosen et al., 1997), the Dyadic Sexual Communication Scale-short form (Catania, 1998), the Communication Patterns Questionnaire - Short Form (CPQ-SF; Christensen & Heavy, 1990) and the Kansas Martial Satisfaction Survey (Schumm, Nichols, Schectman & Grigsby, 1985).

Conclusions

This novel online psychological intervention for men with prostate cancer could provide a way in which support can be delivered to the majority of men diagnosed with prostate cancer despite geographic location. The anonymity of the online medium could also provide a forum for men to access appropriate support without fear of stigma that still surrounds psychological or emotional distress in the wider community.









Alterations In Cellular Energy Metabolism Associated With The Antiproliferative Effects Of The ATM Inhibitor KU-55933 And With Metformin

<u>Mahvash Zakikhani</u> 1, 2, Miguel Bazile 2, Daina Avizonis 2, Shiva Javeshghani 2, Julie St Pierre 2, Sina Hashemi 2, and Michael N. Pollak 1, 2,

- 1. Segal Comprehensive Cancer Center, Lady Davis Institute, SMBD-Jewish General Hospital
- 2. McGill University, Montreal, QC, Canada

KU-55933 is a specific inhibitor of the kinase activity of the protein encoded by Ataxia telangiectasia mutated (ATM), an important tumor suppressor gene with key roles in DNA repair. Unexpectedly for an inhibitor of a tumor suppressor gene, KU-55933 reduces proliferation. In view of prior preliminary evidence suggesting defective mitochondrial function in cells of patients with Ataxia Telangiectasia (AT), we examined energy metabolism of cells treated with KU-55933. The compound increased AMPK activation, glucose uptake and lactate production while reducing mitochondrial membrane potential and coupled respiration. The stimulation of glycolysis by KU-55933 could not compensate for the reduction in mitochondrial functions leading to decreased cellular ATP levels and energy stress. These actions are similar to those previously described for the bigunanide metformin, a partial inhibitor of respiratory complex I. Both compounds decreased mitochondrial coupled respiration and reduced cellular concentrations of fumarate, malate, citarate, and alpha- ketogluterate. Succinate levels were increased by KU-55933 levels and decreased by metformin, indicating that the effects of ATM inhibition and metformin are not identical. These observations suggest a role for ATM in mitochondrial function and show that both KU-55933 and metformin perturb the TCA cycle as well as oxidative phosphorylation







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