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Contact Address
Helmut Klocker, PhD
Department of Urology
Division of Experimental Urology
Medical University Innsbruck
Anichstraße 35
6020 Innsbruck - AUSTRIA
Tel: +43 50 50424818, Email: helmut.klocker@i-med.ac.at

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Androgens Biannual Meeting 2016

Invited Lectures

Invited Lectures

Hormone Action

IL1

Determinants and clinical correlates of androgen exposure: phases of life and disease

Jean-Marc Kaufman

Department of Internal Medicine, Section Endocrinology, Ghent University, Ghent, Belgium

The relation between testosterone (T) production and action is complex, involving protein binding in the circulation affecting clearance and tissue availability, T production from precursors in peripheral tissues, deactivating and activating (to dihydrotestosterone and estradiol) metabolism within target tissues, besides factors affecting androgen responsiveness (receptor concentrations, cofactors, genetics,…). Yet, in absence of a useful independent marker of androgen action, serum T remains the main, albeit imperfect tool for assessment of androgen exposure. As many factors affect SHBG levels and thus total T(TT), free T(FT) more closely reflects androgen exposure. There are analytical issues deserving attention. Increased FT is the most reliable marker of hyper-androgenism in women, most frequently part of a PCO syndrome, often with overweight and insulin resistance, and with alopecia, hirsutism and/or acne as clinical expression. Evidence for a female hypoandrogenic syndrome is limited. After menopause, decreasing production of adrenal androgens become the only source of sex steroids. At all ages between men variability in T levels is substantial. (F)T levels follow pubertal development, remain stable until the 6th decade. Thereafter population means decline mildly for TT and sharper for FT due to SHBG increase. Symptoms of severe primary or secondary hypogonadism include sexual dysfunction, muscle waist, increased adipositas, osteoporosis and decreased well-being. Low (F)T in older men is associated with increased mortality, but may be marker rather than cause of poor health. Adiposity is a negative determinant of SHBG and TT. Obesity, diabetes and cardiovascular disease are associated with lower (F)T; although low (F)T may be the consequence of these situations, there is also evidence for bidirectional relationship.

DOI: 10.1530/endoabs.42.IL1

Biographical details

Jean-Marc Kaufman obtained his MD and PhD degrees at the Ghent University, Belgium. He was a senior postdoctoral research fellow (1982–1984) in reproductive physiology with Ernst Knobil at the University of Texas Medical School at Houston. He is board certified in Endocrinology and in Nuclear Medicine. In 1985 he joined the staff of the Ghent University Hospital; he headed the department of Endocrinology from 2003 to 2014 and the Laboratory for Hormonology from 1995 to 2014. He was appointed in 1993 professor of medicine at the Ghent University (1993) and is past chair of the university department of Internal Medicine (2010–2014). From October 1st 2014 he is professor emeritus at the Ghent University. Main research interests are in the assessment, regulation and action of sex steroids with focus on their role in health, disease and aging in men. He is (co)author of 290 publications in international peer reviewed journals; $>$13000 SSCI citations

IL2

Neuro-inflammatory origins of the sexual differentiation of the brain

Christopher Wright

Program in Neuroscience, Department of Pharmacology, University of Maryland School of Medicine, Baltimore, Maryland, USA

During development, surges in testosterone (T) organize the bipotential anlage of the periphery into the male phenotype. The same T surges also act on the preoptic area (POA) and hypothalamus of the brain to prevent the expression of female sex behavior and promote male behavior in adulthood so that the behavioral and anatomical phenotypes match. In the rodent, T actually serves as a prohormone. Once in the brain, T is aromatized to the quintessential female hormone estradiol (E2) to initiate a two-fold increase in dendritic spines, post-synaptic specializations through which an estimated 95% of the excitatory neurotransmission flow. In adulthood, glutamate and dopamine excitatory neurotransmission in the POA are necessary for the ongoing expression of male behavior. The question of what cellular processes trigger this spine formation continues to be surprising. In the periphery, injury and innate immune responses recruit the prostaglandin PGE2 to mediate inflammatory and febrile responses by increasing the levels of PGE2's synthesizing enzymes cyclooxygenase-1 & -2 (COX-1 & -2). During the masculinization of the brain and behavior, perinatal exposure to E2 increases COX-1 & -2 two-fold and PGE2 seven-fold in the POA. PGE2 then mediates the cellular process that forms spines and organizes adult male sex behavior. In fact, as little as one post-natal dose of the labile PGE2 can permanently masculinize a female's adult behavior. Co-administration of the COX inhibitor indomethacin with PGE2 can prevent the behavioral masculinization. Given that PGE2 is involved in a feed-forward mechanism in the brain and given PGE2's peripheral role in inflammation and innate immunity, my colleagues asked whether masculinization could recruit the brain's own resident innate immune cells, microglia. Normally, microglia are implicated in neurodevelopment for their role in cleaning up or phagocytosing errant synapses and cells but they are not known for being in a pro-inflammatory state, at least not at this age and not without injury. My colleagues found that POA microglia change in numbers and morphology across sexes and in response to exposure to hormones post-natally. Males have more ameboid microglia, which are typically known as being their pro-inflammatory state. Females in contrast have more ramified microglia, known for being quiescent sentinels. Neonatal treatment with a microglial inhibitor, minocycline, kept them quiescent, and prevented the formation of spines and the expression of adult male behavior. Post-natal minocycline administration also prevented the increases in PGE2 that would otherwise have been triggered by hormone exposure. This suggests that the ameboid microglia are the source of the feed-forward mechanism. Thus, immune cells and inflammation are crucial for the endocrine system's restructuring of brain circuitry during development.

DOI: 10.1530/endoabs.42.IL2

Biographical details

Christopher Wright obtained his Ph.D. at the University of Maryland, School of Medicine in 2009. After a brief Post-Doctoral Fellowship at the Johns Hopkins University, School of Medicine, he served as Visiting Assistant Professor at Loyola University from 2009 to 2011. Since 2011, he has worked in the laboratory of Margaret McCarthy in the Department of Pharmacology at the University of Maryland, School of Medicine as a Post-doctoral Fellow and Research Associate. The lab's research focuses on the influence of hormones in the developing brain, particularly the cellular mechanisms differentiating the brain into the male and female behavioral phenotypes. He has a particular interest in the role of inflammation and prostaglandin-E2 in affecting neuronal morphology, the organization of sexual behavior by the preoptic area of the brain, and the role of the cerebellum in affecting social behavior. He also received a New Investigator Award from the Organization for the Study of Sex Differences (2008) in addition to awards related to receiving his J.D. from the University of Maryland, Carey School of Law in 2014.

Androgen Receptor

IL3

Three-dimensional structure of homodimeric androgen receptor ligand-binding domain

Eva Estébanez Perpiñá

Department of Biochemistry and Molecular Biomedicine, Institute of Biomedicine (IBUB), University of Barcelona, Barcelona, Spain

The first crystal structure of human androgen receptor (AR) homodimer will be presented. This structure allows for the structure-based rationalization of the largest number of disease-associated mutations described for the AR ligand binding domain (LBD), which have been involved in prostate cancer and androgen insensitivity syndromes. The conservation of essential residues involved in AR self-association in other oxosteroid receptors suggests a more common dimerization mechanism.

DOI:10.1530/endoabs.42.IL3

Biographical details

Eva Estébanez-Perpiñá obtained her degrees in Biochemistry and Psychology (Psychobiology) from the Autonomous University of Barcelona (UAB). She did her Ph.D Thesis under the supervision of the Nobel Laureate Prof. Robert Huber and Prof. Wolfram Bode at the Max-Planck-Institut fuer Biochemie in Martinsried (Germany), where she graduated in 2002. She joined in 2003 the lab of Prof. Robert J. Fletterick at the University California, San Francisco (UCSF) to study the structure-function relationships of human nuclear receptors such as the androgen and the thyroid receptors. She started her Group at the Institute of Biomedicine, University of Barcelona (IBUB) in 2009 and became Associate Professor in 2015.

IL4

Modulation of androgen receptor signaling in prostate cancer cells by SUMOylation and NFκB pathways

Jorma J. Palvimo

Institute of Biomedicine, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

Transcriptional activity of androgen receptor (AR) is governed by androgenic ligands and (thought to be) fine-tuned by post-translational modifications, such as SUMOylation. We have utilized genome-wide tools to study in an unbiased fashion the role of SUMOylation in the regulation of AR-directed gene programs in prostate cancer cells. Our results show that the SUMOylation does not simply repress the AR activity, but the modification modulates the receptor activity in a target gene/pathway and chromatin occupancy site selective manner. Fittingly, SUMO ligase PIAS1 functions as a target gene selective AR coregulator on prostate cancer cell chromatin, cooperating also with the pioneer factor FOXA1. Overall, the SUMOylation regulates gene programs relevant to prostate cancer growth. Androgen signaling also potentiallycrosstalks with other signaling pathways, including proinflammatory signaling. Based on our genome-wide analyses, androgen signaling can significantly modulate the TNFα-induced NF-κB cistrome by exposing latent RelA/p65-binding sites. Conversely, TNFα signaling is able to restrain the AR cistrome. Although the genomic crosstalk between the AR and the NF-κB may in part be accounted for a direct cooperation or competition of the two transcription factors at enhancers, the majority of the androgen and TNFα co-stimulation-specific chromatin binding events of the p65 are likely to result from indirect mechanisms. TNFα in fact affects chromatin binding of FOXA1 and PIAS proteins at p65-binding sites, suggesting their involvement in the crosstalk. Our transcriptome data indicate that the crosstalk between androgen and proinflammatory signaling can lead to activation of a distinct transcription program which may influence prostate carcinogenesis.

DOI: 10.1530/endoabs.42.IL4

Biographical details

Jorma J. Palvimo, PhD, is professor of medical biochemistry at the University of Eastern Finland (UEF), Kuopio. Before becoming a faculty member of the UEF School of Medicine in 2004, JJP worked as an Academy of Finland junior and senior research fellow and university lecturer at the University of Helsinki, Finland. JJP become interested in androgen receptor and transcriptional regulation during his postdoctoral period in the Population Council and the Rockefeller University, NY. JJP is an author in 154 original research articles in international peer-reviewed journals. The publications concentrate on androgen receptor and signaling, transcription, SUMO modifications, nuclear receptor coactivators and corepressors, and chromatin.

Methods and Techniques

IL5

Droplet Digital PCR: Resolving difficult challenges in nucleic acid detection and quantitation

Francisco Bizouarn

The Polymerase Chain Reaction is going through a renaissance with the arrival of new Digital PCR instrumentation. Amongst these, Droplet Digital PCR is embraced by the research community as an affordable, high resolution quantitative and detection analysis solution for nucleic acids. This presentation will cover the concept of digital PCR, absolute and standard free quantitation of nucleic acid targets as well as applications; CNV analysis, Mutation Abundance, Single cell expression analysis and genome editing event screening, that take advantage of high resolution quantitation and partitioning.

DOI 10.1530/endoabs.42.IL5

Hormone and Disease

IL6

Androgens and cardiovascular diseases

Mario Maggi

Sexual Medicine & Andrology, University of Florence, Florence, Italy

Observational studies have consistently shown a relationship between low testosterone (T) and a higher cardiovascular (CV) risk profile. However, it is still obscure whether reduced T levels in the elderly play a direct pathogenetic role in the stratification of CV risk, or if CV diseases (CVD) and low T are concomitant conditions, both associated with the aging process. Much evidence supports both of these possibilities. In the meanwhile, recent reports in the scientific and lay press have suggested that T replacement therapy (TRT) is likely to increase CV risk. In a 2015 release, the Food and Drug Administration (FDA) cautioned that prescribing T products is approved only for men who have low T levels due to primary or secondary hypogonadism resulting from specific problems. The FDA emphasized that the benefits and safety of T medications have not been established for the treatment of low T levels due to aging, because it could be associated to a higher CV burden. However, data from randomized controlled studies and information derived from both observational and pharmacoepidemiological investigations do not support this view. In particular, separate meta-analyses of all the available studies do not support any causal role between TRT and adverse CV events. This is especially true when hypogonadism is properly diagnosed and replacement therapy is correctly performed. Elevated hematocrit represents the most common adverse event related to TRT. Hence, it is important to monitor hematocrit at regular intervals in T-treated subjects in order to avoid potentially serious adverse events.

DOI 10.1530/endoabs.42.IL6

Biographical details

Professor Mario Maggi is Chief of the Sexual Medicine & Andrology Unit at the Department of Experimental and Clinical Biomedical Sciences at the University of Florence, Italy and full Professor of Endocrinology at the same University since September 2000. He got his M.D. from the School of Medicine, University of Florence, Italy in 1981. He served as the secretary of the European Academy of Andrology 2010–2014, Past President of the Società Italiana di Andrologia e Medicina della Sessualità 2012–2014 and president of Andrological Sciences Onlus. He is associate Editor for the Journal of Sexual Medicine and for the International Journal of Endocrinology and board member of the Journal of Endocrinological Investigation. Current H index = 76. i10 index = 326; Total number of citations in peer-reviewed journals = 17707. Prof. Maggi is the author of 414 peer-reviewed manuscripts and has written several book chapters and invited reviews in the fields of human reproduction and sexual medicine. For his work in these fields he has received numerous research grants and presented at both national and international meetings. He chaired, together with Jacques Buvat, The Committee for Endocrine Aspects of Male Sexual Dysfunction of the 3rd International Consultation on Sexual Medicine of ISSM.

IL7

Effects of long-term testosterone therapy on obesity, glycaemic control and other features of the metabolic syndrome

Farid Saad

Global Medical Affairs Andrology, Bayer Pharma AG Berlin, Germany

Epidemiological studies show a high prevalence of hypogonadism in men with obesity, type 2 diabetes and other components of the metabolic syndrome. Restoring physiological concentrations of testosterone in hypogonadal men by long-term testosterone therapy of 5 years and longer has resulted in substantial weight loss and meaningful improvement of glycaemic control. Blood pressure was reduced and lipid pattern improved. The longer the observation time in real-life studies, the more robust the results.

Both magnitude and sustainability of treatment effects, predominantly based on observational studies with and without untreated control groups and a duration of up to 8 years, are of such interest that further studies with an adequate design should be performed. However, conducting placebo-controlled trials over several years in men diagnosed with hypogonadism may not be considered ethical.

A host of studies have shown that adequate testosterone therapy reduces major adverse cardiovascular events and mortality in hypogonadal men. These studies are consistent with the observed beneficial effects of long-term testosterone treatment on the classical cardiovascular risk factors (visceral obesity, hypertension, dyslipidaemia, insulin resistance). In addition, testosterone has anti-inflammatory and anti-coagulatory properties.

According to what is known today, there is sufficient evidence to recommend measuring testosterone in men with obesity and/or type 2 diabetes. If hypogonadism is diagnosed, testosterone therapy should be considered. Adequate therapy, i.e. restoring testosterone levels to the mid- to high normal range for life seems the most promising approach. Medication adherence, as in all chronic diseases, is essential.

DOI: 10.1530/endoabs.42.IL7

Biographical details

Prof. h.c.* Dr Farid Saad is manager of Global Medical Affairs Andrology of Bayer Pharma AG. 1953 born in Alexandria, Egypt; 1973 – 1980 studies of human and veterinary medicine; 1990 – 1998 specialist for reproductive endocrinology, pediatric endocrinology, and andrology, Ferring GmbH, Kiel, Germany; 1998 – 2001 leader of clinical development andrology, Jenapharm, Jena, Germany; specialist in endocrinology of aging, male aging, male hormonal fertility control; 2001 – 2007 leader of product group "Male Health Care", Schering AG, Berlin, Germany; since March 2007 Global Medical Affairs Andrology, Bayer Pharma AG. He has authored and co-authored more than 120 peer-reviewed papers and more than 600 scientific abstracts. In 2005 he received a honorary professorship in clinical research and endocrinology at Gulf Medical University, Ajman, United Arab Emirates and in 2006 a honorary professorship at Men's Health Reproduction Study Center, Hang Tuah University, Surabaya, Indonesia. *Gulf Medical University School of Medicine, Ajman, United Arab Emirates.

Androgen Receptor & Chromatin

IL8

The Androgen Receptor chromatin landscape in prostate tumors: biomarker discovery and beyond

Suzan Stelloo, Ekaterina Nevedomskaya, Karianne Schuurman, Lodewyk FA Wessels, Rui Henrique, Carmen Jerónimo, Andries M Bergman & Wilbert Zwart

Department of Pathology, Erasmus Medical Center, Rotterdam, The Netherlands

The androgen receptor (AR) plays a pivotal role in prostate cancer development, progression and hormone-therapy resistant disease. AR requires a permissive epigenetic state at distinct chromatin regions to facilitate gene expression programs. The vast majority of AR sites are found at active enhancer regions, hallmarked by histone modification H3K27Ac and devoid of repressive markers including H3K27me3. In search for novel biomarkers for prostate cancer prognostication, we performed chromatin immunoprecipitation followed by massive parallel sequencing (ChIP-seq) on AR from surgical specimens. Distinct subsets of AR binding sites were identified that enabled patient stratification on outcome, yielding a novel gene expression-based biomarker that functions synergistically with standard clinicopathological features[1]. To further understand epigenetic regulation and AR genomics in larger patient series, we determined AR chromatin binding in 100 primary prostate cancers, along with histone modifications H3K4me3, H3K27me3 and H3K27Ac, gene expression and copy number profiles. The integrative analysis of these large datasets will provide information on I) distinct profiles of AR and histone modifications that may be bear prognostic potential, and II) the potential existence of distinct epigenetic subtypes in prostate cancer. Ultimately, we aim to further understand epigenetic regulation in prostate cancer along with its clinical implications on a genome-wide scale.

[1]*Stelloo S, Nevedomkaya E, et al. Androgen receptor profiling predicts prostate cancer outcome. EMBO molecular medicine, 2015.*

DOI: 10.1530/endoabs.42.IL8

Biographical details

Wilbert Zwart is a junior groupleader at the Netherlands Cancer Institute, department of Molecular Pathology, where he started his independent lab in 2011. He obtained his BSc and MSc at the University of Utrecht. He received his Ph.D. (cum laude) at the University of Leiden, based on his work at the Netherlands Cancer Institute in the groups of dr. Rob Michalides and prof. dr. Jacques Neefjes. His postdoctoral work was with dr. Jason Carroll at Cancer Research UK, Cambridge Research Institute, where he studied cofactor genomics in breast cancer. His lab studies hormone receptor function and transcriptional regulation in breast, prostate and endometrial cancer, in search for biomarkers and novel targets for therapeutic interventions. E-mail: w.zwart@nki.nl

Metabolism in Prostate Cancer

IL9

Regulating androgen action by steroid synthesis and metabolism

Matti Poutanen

Department of Physiology, Institute of Biomedicine, University of Turku, Finland

Recent results from us and others have shown that concentrations of sex steroids in serum/circulation do not always reflect their intra-tissue levels in hormone-dependent tissues. This proposes that sex steroid metabolism plays a key role in determining the final concentration of active sex steroids in their target tissues. According to the current knowledge, members of three enzyme families, namely cytochrome P450 (CYP), aldo-keto reductase (AKR) and short-chain dehydrogenases/reductase (SDR) enzymes, have a key role in target tissue steroid metabolism. These enzymes potentially regulate the availability of highly potent ligands for the sex steroid receptors, including androgen receptor. A central part of our present studies is connected to our ability to generate novel pre-clinical in vivo models, the use of well-defined clinical cohorts, and the analysis of sex steroid concentrations with high sensitivity mass spectrometric methods (GC-MS/MS, LC-MS/MS) recently developed/under development by us. Accordingly, we have recently shown differential expression of HSD17B and other steroid synthetic enzymes and consequently different metabolism of estrogens and androgens in the eutopic and ectopic endometrium that partially could explain the hormone-dependent growth of endometriosis. Moreover, data from clinical tissue specimens as well as from our preclinical xenograft model of castration resistant prostate cancer indicate that active androgens are synthesized locally in the cancer tissue, and that the intra tissue DHT production is associated with altered expression of CYP, AKR SDR enzymes.

DOI: 10.1530/endoabs.42.IL9

Biographical details

Matti Poutanen, PhD, is Professor Physiology and Director of the Turku Center for Disease Modeling (www.tcdm.fi), Institute of Biomedicine, University of Turku, Finland. Research areas of interest: Biosynthesis and metabolism of steroids and lipids; Disorders in reproductive tissues and hormonal cancer. He has, 182 peer-reviewed publications with a total number of citations 5099.

IL10

Causes and consequences of metabolic changes in prostate tumours

Charles Massie

Cancer Research UK Cambridge Institute, Cambridge Cancer Center, University of Cambridge, Cambridge, UK

Metabolic alterations have long been known to occur in the transition to prostate cancer, from alterations in central metabolism to lipid metabolism and local steroid biosynthesis. However the upstream events that lead to these alterations have not been comprehensively assessed. In this presentation I will draw together genomic, epigenomic and transcriptomic analysis, together with metabolic profiling. Overlaying the effects of androgen signaling on these pathways and metabolite levels will illustrate the circular links between the causes and consequences of these metabolic changes, with the aim of triangulating likely early events, disease markers and weak-points for intervention.

DOI: 10.1530/endoabs.42.IL10

Biographical details

After completing his doctoral studies at the University of Cambridge Department of Oncology, Charlie worked with Prof David Neal and Dr Ian Mills in the Uro-Oncology Group at the CRUK Cambridge Institute. Work there focussed on mapping androgen receptor (AR) binding sites in prostate cancer cells lines and in human tumour samples. Focussing on functional genomics approaches this work revealed direct regulation of central metabolism by the AR in prostate cancer cells and altered AR binding profiles in hormone relapsed prostate cancer tissue. More recent work has focussed on the genetic and epigenetic characterisation of prostate tumours, through collaboration with the International Cancer Genome Consortium. The combination of these efforts has identified a convergence at the pathway level for AR-driven transcriptional programs and epigenetic alterations in prostate cancer, these will be the focus of future efforts.

Castration Resistant Prostate Cancer

IL11

Treatment of castration-resistant prostate cancer

Gero Kramer

Department of Urology, Medical University of Vienna, Vienna, Austria. E-mail: gero.kramer@meduniwien.ac.at

Abstract Unavailalbe.

DOI: 10.1530/endoabs.42.IL11

Biographical details

Gero Kramer is Associate Professor of Urology at the Department of Urology, Medical University of Vienna.

IL12

Identifying drivers of disease progression to personalize prostate cancer therapy

Felix Y Feng

Department of Radiation Oncology, Urology, and Medicine, Helen Diller Family Comprehensive Cancer Center, University of California at San Francisco, San Francisco, USA

We have identified a signature that predicts response to androgen deprivation therapy in localized prostate cancer, and are now using this signature as a stratification variable on biomarker-driven clinical trials. Following up on the Stand-Up-To-Cancer sequencing findings we design a large umbrella trial which will personalize therapy based on actionable genomic alterations in metastatic castration-resistant prostate cancer.

DOI: 10.1530/endoabs.42.IL12

Biographical details

Felix Feng is Associate Professor of Radiation Oncology, Urology, and Medicine and Vice Chair for Faculty Development and Director of Translational Research of the Department of Radiation, University of California at San Francisco. He received his undergraduate training from Stanford University, where he earned a degree in Biological Sciences and received a President's Award for Academic Excellence. He then received his MD from Washington University in St. Louis, where he received numerous awards for his research and academic achievements. He then completed both a postdoctoral research fellowship and his residency in radiation oncology at the University of Michigan, and subsequently joined the faculty there. In addition to being the Director of the Division of Translational Genomics, Dr. Feng also co-led the multidisciplinary clinic for prostate cancer patients and served as Director of the Genitourinary Cancer Program within the Department of Radiation Oncology at the University of Michigan. In 2016 he was recruited to join the faculty at UCSF. Dr. Feng is a physician-scientist focused on clinical and translational research aimed at improving outcomes for patients with prostate cancer. His laboratory focuses on identifying and validating biomarkers associated with treatment resistance in prostate cancer patients, and overcoming radiation or hormone therapy resistance with targeted therapy.

Androgen Receptor in Castration Resistant Prostate Cancer

IL13

Androgen receptor: master contortionist in prostate cancer

Scott Dehm

Departments of Laboratory Medicine and Pathology and Urology and the Masonic Cancer Center, University of Minnesota, Minneapolis, Minnesota, USA

The AR pathway accumulates myriad genomic and transcriptomic alterations during progression of prostate cancer to a lethal castration-resistant phenotype. The best characterized alterations are point mutations or amplification of the AR gene. More recently, our group had identified structural rearrangements in the AR gene as a novel class of alterations that occur frequently in castration-resistant prostate cancer (CRPC) tissues. AR gene rearrangements are associated with outlier expression profiles of a diverse set of truncated AR variants (AR-Vs) lacking the canonical ligand binding domain (LBD). AR-Vs expressed in CRPC share a common basic structure consisting of the transcriptionally active NH2-terminal domain (NTD) and DNA binding domain (DBD), but diverge in COOH-terminal amino acid sequence and length. AR-Vs function as constitutively active transcription factors and represent a mechanism of resistance to AR-targeted therapies whereby the growth of prostate cancer cells can remain AR-dependent, yet uncoupled from endocrine regulation. Tumors that have developed this mechanism of resistance are unlikely to respond to successive generations of endocrine therapies. The absence of a LBD presents a formidable challenge for direct inhibition of AR-Vs. However, our work has shown that targeting alternative non-LBD AR domains and/or factors required for AR-V-mediated transcriptional activation may hold promise for inhibiting AR-Vs in advanced prostate cancer.

DOI: 10.1530/endoabs.42.IL13

Biographical details

Scott Dehm completed his PhD in 2003 at the Saskatchewan Cancer Agency at the University of Saskatchewan in Canada with Dr. Keith Bonham. He conducted postdoctoral training at Mayo Clinic with Dr. Donald Tindall from 2003–2008. Scott is currently Associate Professor and Apogee Enterprises Endowed Chair in Cancer Research in the Departments of Laboratory Medicine and Pathology and Urology and the Masonic Cancer Center at the University of Minnesota. His research is currently funded by the Prostate Cancer Foundation, US Department of Defense Research Program, American Cancer Society, and the National Cancer Institute.

Genetics, Genomics & Epigenetics

IL14

Genomic and epigenomic patterns in prostate cancer

Holger Sültmann

German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany

The international cancer genome projects have provided comprehensive catalogs of molecular alterations in cancer and often led to new molecular tumor stratification. Furthermore, genome sequencing has provided an improved understanding of the mutational processes underlying cancer formation and progression. Interestingly, mutations in genes coding for chromosomal maintenance, DNA methylation and histone modification are frequent in many tumor entities. These epigenomic changes may be among the earliest alterations in cancer. By combining various orthogonal (e.g. genomic, epigenomic, and transcriptomic) data, the processes driving cancer might gradually become clearer. The presentation will highlight examples for the utilization of our recent high throughput molecular data aiming at the development of models how genomic and epigenomic changes can drive prostate cancer and how they can generate the enormous molecular heterogeneity of these tumors in space and time.

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Biographical details

Holger Sültmann graduated in Biochemistry from Tübingen University. After completing his PhD (1994) and postdoctoral studies in Molecular Evolutionary Genetics in the Division of Immunogenetics at the Max-Planck-Institute for Biology (Tübingen), he moved to the German Cancer Research Center (DKFZ) in Heidelberg, where he was an assistant professor in the Division of Molecular Genome Analysis (2000-2010). Since 2010, he has been heading the research group Cancer Genome Research at the DKFZ and the National Center for Tumor Diseases (NCT). In 2012, he was appointed full professor of the Medical Faculty at Heidelberg University.

His main research interest is the application of high throughput technologies to identify, characterize, and translate novel cancer biomarkers into the clinic. He has successfully launched and coordinated several interdisciplinary cancer genome research projects in the German Federal Program for Medical Genome Research. In 2010, he initiated the German ICGC project on prostate cancer genome sequencing.

IL15

The genetics of prostate cancer specific events

Francesca Demichelis

Centre for Integrative Biology, University of Trento, Tento, Italy

Androgens play an important role in the development and the maintenance of the normal prostate gland. Androgen Receptor (AR) signaling is also critical during the initiation and development of prostate cancer that may present with a range of genomic lesions possibly due to DNA repair defects. We first noted a significant correspondence between DNA breakpoints and AR binding sites supporting the inter-related action that hormone regulation plays a role on genomic events. We hypothesized that heritable variants, i.e. individual's genetics, encode predisposition to genomic instability in the context of AR signaling that undergoes changes during men lifetime resulting in early recurrent prostate cancer specific somatic genomic events. Using a mathematical score and interrogating the transcriptome of human prostate tissue cells we identified a polymorphic regulatory element that associates with DNA repair and hormone regulated gene transcripts and with early recurrent prostate cancer specific somatic mutations. The locus showed allele-specific regulatory activity that is concomitantly modulated by the Androgen Receptor (AR) and the CCAAT/Enhancer Binding Protein (C/EBP) beta (CEBPB), altogether suggesting that the locus is involved in a hormone dependent DNA damage response.

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Biographical details

Francesca Demichelis is Associate Professor of Molecular Biology at the University of Trento, Italy. She is expert in the area of Cancer Genomics. Her laboratory focuses on the characterization of tumor evolution and progression through the study of intra- and inter-tumor heterogeneity using single base level information from tissue biopsies or circulating DNA (cell-free DNA). She has longstanding interest in the impact of inherited polymorphisms within transcriptionally active regulatory regions on the initiation of hormone regulated cancer phenotypes.

Oral Communications

OC1

Non-classical testosterone signaling in the testis

Raimund Dietze[1,2], Ahmed Bulldan[1], Mazen Shihan[1], Kai-Hui Chan[2], Lutz Konrad[2] & Georgios Scheiner-Bobis[1]

[1]Institut für Veterinär-Physiologie und -Biochemie, Fachbereich Veterinärmedizin, Justus-Liebig-Universität, Giessen, Germany; [2]Zentrum f. Frauenheilkunde und Geburtshilfe, Fachbereich Medizin, Justus-Liebig-Universität, Giessen, Germany

Although classical and non-classical signaling of testosterone (T) has been observed in the testis, the enigma of the non-classical pathway is not fully resolved. While some researchers favor the androgen receptor (AR), others propose a membrane-bound receptor for non-classical T signaling. Although silencing of the AR in Sertoli cells (SC) resulted in infertility and suggested no involvement of the non-classical pathway, recent experiments using an inhibitor of the non-classical signaling blocked meiosis in prepubertal mice. In this study, we will present data showing that the non-classical T signaling is mediated by the membrane-bound receptor ZIP9, a Zn(2+) transporter from the family of the ZRT, IRT-like proteins (ZRT = zinc-regulated transporter; IRT = iron-regulated transporter), which directly interact with the G-protein Gnα11. The close contacts of both proteins could be demonstrated by an *in situ* proximity assay. Silencing of the expression of either of these two proteins completely blocked all non-classical T signaling effects addressed. Furthermore, we observed that non-classical T signaling activated Erk1/2, and the transcription factors CREB and ATF-1, an effect which was not mediated by the classical AR. We also found that T increased the expression of the tight junction (TJ) proteins claudin-1 and claudin-5 in rat SCs. Furthermore, increased expression of both TJ proteins resulted in TJ formation between neighboring cells. Taken together, we suggest that non-classical T signaling in testis is mediated by ZIP9 and Gn α11 through Erk1/2, CREB and ATF-1 resulting in increased claudin expression. This will contribute to the blood-testis barrier which is essential for male fertility.

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Presenting Author: Lutz Konrad, Center of Gynecology and Obstetrics, Faculty of Medicine, Justus-Liebig-University Giessen, Feulgenstr. 10-12, 35392 Giessen, Germany. Email: lutz.konrad@gyn.med.uni-giessen.de

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OC2

Control of androgen bioactivity by sex hormone-binding globulin

Michael R Laurent [1,2], Leen Antonio[1,3], Marco H Blokland[4], Saskia S Sterk[4], Ferran Jardi[2], V Dubois[5], Jean-Mark Kaufman[6], Tom Fiers[6], Ilpo T Huhtaniemi[7], Geoffrey T. Hammond[8], Dirk Vanderschueren[3] & Frank Claessens[1]

[1]Molecular Endocrinology Laboratory; [2]Gerontology and Geriatrics; [3]Clinical and Experimental Endocrinology, KU Leuven, Belgium; [4]RIKILT-Institute of Food Safety, European Union Reference Laboratory for Residues, Wageningen UR, The Netherlands; [5]Inserm UMR1011, Université de Lille, France; [6]Laboratory for Hormonology, Department of Endocrinology, Ghent University Hospital, Belgium; [7]Institute of Reproductive and Developmental Biology, Department of Surgery and Cancer, Imperial College London, United Kingdom; [8]Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, Canada

Sex hormone-binding globulin (SHBG) is a high-affinity binding protein for androgens and estrogens. According to the "free hormone hypothesis", SHBG regulates the free sex steroid fraction and restricts androgen bioactivity. SHBG has also been independently associated with diabetes, osteoporosis etc., but whether this represents causality or residual confounding remains unconfirmed. We studied mice overexpressing human SHBG. Using multiligand liquid chromatography tandem mass spectrometry (LC-MS/MS) we show that total concentrations of testosterone, DHT and other circulating androgens are ~100-fold increased, whereas their urinary conjugation products were unaltered. Weights of androgen-sensitive organs (seminal vesicles and levator ani muscles) however were slightly (12–20%) but significantly ($P < 0.001$) reduced, indicating suppressed androgen bioactivity in vivo. Also in an *in vitro* androgen reporter bioassay, SHBG suppressed androgen bioactivity. Total estradiol was also increased in male mice but remained strikingly low, indicating that the lack of circulating SHBG in male mice is only slightly responsible for their undetectable circulating estradiol levels. 3H-DHT- and T injections i.v. revealed that SHBG prolongs ligand circulatory half-life. In orchidectomized mice however, SHBG did not prolong the biological actions of androgens. Replacement experiments with anabolic testosterone doses showed that SHBG prevents hypertrophy of sex organ but not muscle, and restricts androgen entry into target tissues like bone. However, glucose sensitivity and bone mass were unaffected in SHBG-Tg mice. Despite 100-fold higher total androgen levels, their bioactivity is reduced in SHBG-Tg mice. This genetically modified mouse model however revealed no independent influence of SHBG on bone or metabolic outcomes.

Presenting Author: Frank Claessens, Molecular Endocrinology Laboratory, KU Leuven, Herestraat 49, 3000 Leuven, Belgium. Email: frank.claessens@med.kuleuven.ac.be.

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OC3

Novel trifluoromethylated enobosarm analogues show very potent antiandrogen activity in prostate cancer cells, and cells with acquired bicalutamide resistance whilst maintaining tissue selectivity *in vivo*

Alwyn Dart[1], Sahar Kandil[3], Serena Tommasini-Ghelfi[2], Charlotte Bevan[2], Wenguo Jiang[1] & Andrew D. Westwell[3]

[1]The Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, CF14 4XN, Wales, UK; [2]Androgen Signalling Laboratory, Department of Surgery & Cancer, Imperial College London, South Kensington Campus, London, W12 0NN, UK; [3]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, CF10 3NB Cardiff, Wales, UK

Prostate cancer often develops anti-androgen resistance, possibly via AR mutations which change AR antagonists to agonists. There is an urgent need for novel therapies which ideally show increased anticancer activity, whilst overcoming current drug resistance. Enobosarm has anabolic effects on muscle and bone tissues whilst having no effect on the prostate – often used to combat cachexia in advanced lung cancer. Here we describe the activity of novel chemically modified Enobosarm analogues. The addition of bis-trifluoromethyl groups, profoundly modified their pharmacokinetics and pharmacodynamic properties. These chemical structural modifications appeared changed their AR ligand binding site affinities – by increasing the molecular occupational volume near the AR helix 12, impeding the agonist conformation. *In vitro*, the analogues SK33 and SK51 showed very potent antiandrogen activity, monitored using LNCaP/AR-Luciferase cells where growth, PSA and luciferase were used as a measure of AR activity. These drugs were 10x more potent than bicalutamide and 100x more potent than the parental Enobosarm. The compounds were also active in LNCaP cells with acquired bicalutamide resistance – cells normally showing active proliferation in response to bicalutamide. *In vivo*, using the AR-Luc reporter mice, these drugs showed potent activity in the prostate and several other AR-expressing tissues e.g. testes, seminal vesicles and brain. These agents did not inhibit AR activity in the skeletal muscle, spleen and bone – thus indicating a selective inhibitory profile – Selective Androgen Receptor Modulatory (SARM) activity. SK33 and SK51 have significantly different activity profiles to enobosarm, and are ideal candidates for prostate cancer therapy as they have increased efficacy and potentially fewer side effects.

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Presenting Author: Alwyn Dart, The Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, CF14 4XN, Wales, UK. Email: dartd@cardiff.ac.uk

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OC4

The use of apolipoprotein D as a biomarker for androgen sensitivity identifies a new type of androgen insensitivity syndrome that is not associated with a mutation in the androgen receptor gene

Nadine C Hornig[1], Martine Ukat[1], Hans-Udo Schweikert[2], Olaf Hiort[3], Ralf Werner[3], Stenvert LS Drop[4], Martine Cools[5], Ieuan A Hughes[6], Laura Audi[7], S Faisal Ahmed[8], Jeta Demiri[1], Pascal Rodens[1], Lisa Worch[9], Gaby Wehner[2], Alexandra E Kulle[1], Desiree Dunstheimer[10], Elke Müller-Roßberg[11], Thomas Reinehr[12], Ahmed T Hadidi[13],

Anne K Eckstein[14], Christof van der Horst[15], Christoph Seif[16], Reiner Siebert[17], Ole Ammerpohl[9] & Paul-Martin Holterhus[1]
[1]Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Campus Kiel, Schwanenweg 20, 24105 Kiel, Germany; [2]Department of Medicine III, Institute for Biochemistry and Molecular Biology, University Bonn, Nussallee 11, 53115 Bonn, Germany; [3]Department of Pediatrics, Division of Experimental Pediatric Endocrinology, University Luebeck, Ratzeburger Allee 160, 23538 Luebeck, Germany; [4]Department of Pediatrics, Division of Pediatric Endocrinology, Sophia Children's Hospital, Erasmus MC, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands; [5]Department of Pediatric Endocrinology, Ghent University Hospital, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium; [6]Department of Pediatrics, University of Cambridge, Hills Road, Cambridge CB2 0QQ, UK; [7]Pediatric Endocrinology Research Unit, VHIR (Vall d'Hebron Institut de Recerca), Hospital Universitari Vall d'Hebron, CIBERER (Center for Biomedical Research on Rare Diseases), Instituto de Salud Carlos III, Passeig Vall d'Hebron 119 – 08035 Barcelona, Spain; [8]Developmental Endocrinology Research Group, School of Medicine, University of Glasgow, Yorkhill Glasgow G3 8SJ, UK; [9]Institute of Human Genetics, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Campus Kiel, Schwanenweg 24, 24105 Kiel, Germany; [10]Kinderklinik, Klinikum Augsburg, Stenglinstr. 2, 86156 Augsburg, Germany; [11]Klinikum Esslingen, Hirschlandstr. 97, 73730 Esslingen, Germany; [12]Department of Pediatrics, Division of Pediatric Endocrinology, Diabetes and Nutrition, University. Witten/Herdecke, Dr. F. Steiner Str. 5, 45711 Datteln, Germany; [13]Hypospadiezentrum, Frankfurter Str. 51, 63500 Seligenstadt, Germany; [14]Gemeinschaftspraxis für Kinderchirurgie, Eichkoppelweg 74, 24119 Kronshagen, Germany; [15]Urologische Gemeinschaftspraxis, Prüner Gang 15, 24103 Kiel, Germany; [16]UROLOGIE Zentrum Kiel, Alter Markt 11, 24103 Kiel, Germany; [17]Institute of Human Genetics, University Hospital of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Although androgen insensitivity syndrome (AIS) is commonly suspected as a cause of a 46,XY disorder of sex development (DSD), only about half of these cases can be attributed to an inactivating mutation within the coding sequence (CDS) of the androgen receptor (*AR*) gene. This led to the hypothesis that disrupted AR activation in AIS may also be caused by a defect in a co-factor of AR-activity. However, so far mutations in AR co-factors leading to AIS have not been identified. To further investigate this discrepancy between genotype and phenotype, we have evaluated the dihydrotestosterone (DHT) dependent AR-induced expression of apolipoprotein D (*APOD*) in cultured genital skin fibroblast (GF) as a measure of the AR transcriptional activity in a total of 169 individuals. Using this "APOD assay" we were able to define a cut-off value that distinguishes AR transcriptional activity in GF from individuals with genetically confirmed AIS bearing a mutation in the *AR-CDS* and a male control group without any DSD with high significance ($P<0.0001$). When this cut-off was applied to GF derived from individuals with suspected AIS who did not have a mutation in the *AR-CDS*, we were able to identify a subgroup ($n=17$) with significantly reduced AR function. This subgroup strongly supports the existence of genetic factors outside the *AR,* e.g. in regulatory regions or co-factors, compromising AR-activity in AIS.

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Presenting Author: Nadine C. Hornig, Department of Pediatrics, Division of Pediatric Endocrinology and Diabetes, Christian-Albrechts-University Kiel & University Hospital Schleswig-Holstein, Campus Kiel, Schwanenweg 20, 24105 Kiel, Germany. Email: nhornig@medgen.uni-kiel.de

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OC5

What is the impact of AR modulation in the decidualisation of hESCs from women with endometriosis?

Ioannis Simitsidellis[1], Douglas A. Gibson[1], Olympia Kelepouri[1], Andrew W. Horne[2] & Philippa T. K. Saunders[1]
[1]MRC Centre for Inflammation Research, The University of Edinburgh, Edinburgh, United Kingdom; [2]MRC Centre for Reproductive Health, The University of Edinburgh, Edinburgh, United Kingdom

Endometriosis is a hormone-dependent disorder, characterised by growth of endometrial tissue outside the uterus. It has been reported that 30–40% of women with infertility have endometriosis. Transformation of human endometrial stromal fibroblasts (hESC) (termed decidualisation) is accompanied by increased steroid synthesis and is fundamental to the establishment of a receptive endometrial microenvironment, which can support and maintain pregnancy. Evidence suggests that women with endometriosis have an impaired decidualisation response. In the current study, we have compared the decidualisation response of women with and without endometriosis, examined the temporal expression of decidualisation factors and steroidogenic enzymes and explored the impact of steroid receptor ligands. Primary hESCs from women with and without endometriosis were recovered during the proliferative phase of the menstrual cycle and incubated with progesterone and cAMP to model decidualisation in vitro. Co-treatment with androgen receptor ligands (DHT, flutamide) was performed. Culture media, RNA and protein samples were recovered on days 1, 2, 4 and 8 of treatment. Expression of decidualisation markers (*IGFBP1, PRL, FOXO1*) and steroidogenic enzymes (*AKRIC3, SRD5A1*) was determined and concentrations of secreted IGFBP1, PRL and DHT measured by ELISA. Results revealed time-dependent changes in gene and protein expression, with evidence that local (intracrine) biosynthesis of androgens may a role in regulation of decidualisation. Decidualisation of hESCs from women with endometriosis was characterised by different patterns of expression of the key androgen-metabolising enzymes *AKR1C3* and *SRD5A1* compared to hESC from women without endometriosis. Flutamide appeared to differentially affect decidualisation of hESCs from women with and without endometriosis.

Presenting Author: Ioannis Simitsidellis, MRC Centre for Inflammation Research, The University of Edinburgh, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ, Scotland. Email: I.Simitsidellis@sms.ed.ac.uk

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OC6

Transgender: biological model for steroid action

Ludwig Wildt, Katharina Winkler-Crepaz & Bettina Boettcher
Department of Gynecological Endocrinology and Reproductive Medicine, Medical University of Innsbruck, Innsbruck, Austria

Transgender patients are treated by down-regulation oft he pituitary gland and the gonads with GnRH- analogues and the opposite male or female sex steroids, followed by gonadectomy. After gonadectomy, the GnRH analogue is discontinued while the treatment with sex steroids (estrogen or testosterone) is continued. This offers the unique possibility to study the effects of sex steroids on bone density, metabolism, and other regulatory systems in the opposite sex. We present data in a large group of male to female and female to male transsexuals treated at our Department over the last 13 years.

Presenting Author: Ludwig Wildt, MD. Department of Gynecologic Endocrinology and Reproductive Medicine, Innsbruck Medical University, Innsbruck, Austria. Email: Ludwig.wildt@i-med.ac.at

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OC7

Identification of AR genomic targets in mesenchymal cell subsets during prostate development

Claire Nash, Nadia Boufaied & Axel A. Thomson
Division of Urology, Department of Surgery, McGill University Health Centre Research Institute, Montreal, Quebec, Canada

Sexually dimorphic organogenesis of the prostate is a key function of androgens during mammalian development. Mesenchymal AR signalling is essential for prostate development, while epithelial AR is not required. Within the mesenchyme there are distinct mesenchymal subsets that show differential effects of androgens; androgens regulate the thickness of the urethral smooth muscle layer, while in the inductive mesenchymal pad, androgens stimulate branching and growth of prostate epithelia. However, there is little knowledge of AR target genes in mesenchyme or mesenchymal subsets. We used ChIPseq to define AR binding sites (ARBs) in microdissected mesenchymal subsets during prostate development. Our focus was upon urethral smooth muscle (SM, female), inductive pad mesenchyme (MP, female), ventral prostate (VP, male) and dorsolateral prostate (DDLP, male). Tissues were microdissected from day of birth rats where AR expression is largely restricted to mesenchymal cells. Western blotting for AR showed higher levels in males (VP and DDLP) than females (SM and MP). ChIPseq was performed on pooled tissue, and ARBs co-identified with both MACS2.0 and HOMER. We validated promoter ARBs

using RNAseq transcript profiling of the same tissues used for ChIPseq, to restrict our focus to tissue expressed genes and to define a mesenchymal AR cistrome. 80% of promoter specific ARBs were validated by transcript expression. Mesenchymal pad and smooth muscle cistromes showed a 70% and 68% overlap respectively with developing human fetal prostate transcripts suggesting that a high proportion of our defined mesenchymal ARBs may play a functional role *in vivo* during human prostate organogenesis.

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Presenting Author: Axel Thomson, Department for Surgery, Division of Urology, MUHC Research Institute, 1001 Decarie Blvd Montreal, Quebec H4A 3J1 1 514 934 1934 ext 76307, Canada. Email: axel.thomson@mcgill.ca

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OC8

Fibroblast AR signalling in prostate cancer: Unique regulation of AR signalling, and associations with patient outcomes by influencing cancer progression and invasion

Damien A. Leach[1,2], Eleanor F. Need[1] & Grant Buchanan[1]

[1]Department of Surgery, The University of Adelaide, Adelaide, Australia; [2]Department of Surgery and Cancer, Imperial College London, London, UK

The stromal compartment changes with cancer, with an emergence in activated fibroblasts. These stromal changes, which occur with cancer initiation and throughout progression, are known to influence the hallmarks of cancer and are becoming increasingly studied for prognostic and therapeutic purposes. Androgen receptor (AR) signalling in stromal cells is important in prostate cancer and these cancer stromal communications, yet the mechanisms underpinning stromal AR contribution to disease development and progression remain unclear. Using tissue microarrays from dual cores of benign and cancerous tissue from each of 64 patients, we found an association between low stromal expression of AR and the androgen regulated protein FKBP5 with increased prostate cancer specific mortality over 5-years post diagnosis. In prostatic fibroblasts, microarray and ChIP analysis revealed cell-lineage specificity in AR binding and signalling, potentially resulting from differential co-regulator and pioneer factor expression. The lineage specific AR-signalling culminates in control of proliferation, secretion of paracrine factors, adhesion, invasion, and ECM production. The resulting ECM environment is able to alter the genetic profile of cancer cells as well as being able to impede cancer cell migration and invasion. Proteomic analysis revealed a potential role for LOXL2, which when silenced, affects ECM pore size and cancer cell invasion, and the expression of which was associated with prostate cancer mortality. In conclusion, when AR is disrupted in the stroma, it creates an environment that enables cancer invasion influencing patient outcome, providing prognostic markers and therapeutic targets.

Presenting Author: Damien A. Leach, Department of Surgery and Cancer, Imperial College London, UK. Email: damien.leach@imperial.ac.uk

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OC9

Chromatin relaxation is a feature of advanced prostate cancer

Alfonso Urbanucci[1,2], Stefan Barfeld[1], Ville Kytölä[3], Daniel Vodák[4], Liisa Sjöblom[5], Teemu Tolonen[6], Sarah Minner[7], Christoph Burdelski[8], Kati K. Kivinummi[3], Steven Kregel[9,10], Mandeep Takhar[11], Mohammed Alshalalfa[11], Elai Davicioni[11], Nicholas Erho[11], R. Jeffrey Karnes[12], Ashley E. Ross[13], Edward M. Schaeffer[13], Donald J. Vander Griend[9], Stefan Knapp[14,15], Teuvo L.J. Tammela[16], Guido Sauter[7], Thorsten Schlomm[17], Matti Nykter[3], Tapio Visakorpi[5] & Ian G. Mills[1,2,18]

[1]Centre for Molecular Medicine Norway, Nordic European Molecular Biology Laboratory Partnership, Forksningsparken, University of Oslo, Oslo, Norway; [2]Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [3]Prostate Cancer Research Center, Institute of Biosciences and Medical Technology (BioMediTech), University of Tampere and Tampere University of Technology, Tampere, Finland; [4]Department of Tumor Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; [5]Prostate Cancer Research Center, Institute of Biosciences and Medical Technology (BioMediTech), University of Tampere and Fimlab Laboratories, Tampere University Hospital, Tampere, Finland; [6]Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Tampere, Finland; [7]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [8]General, Visceral and Thoracic Surgery Department and Clinic, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [9]Department of Surgery - Section of Urology, University of Chicago, Chicago, Illinois, USA; [10]Michigan Center for Translational Pathology, University of Michigan, Ann Arbor, Michigan, USA; [11]Research and Development, GenomeDx Biosciences, Vancouver, British Columbia, Canada, V6B 1B8; [12]Department of Urology, Mayo Clinic, Rochester, MN, USA; [13]Brady Urological Institute, Johns Hopkins Medical Institute, Baltimore, MD, USA; [14]Nuffield Department of Clinical Medicine, University of Oxford, Old Road Campus, Roosevelt Drive, Oxford OX3 7DQ, UK; [15]Institute for Pharmaceutical Chemistry, Goethe-University Frankfurt, Campus Riedberg, Max-von Laue Str. 9, 60438 Frankfurt am Main, Germany; [16]Prostate Cancer Research Center and Department of Urology, University of Tampere and Tampere University Hospital, Tampere, Finland; [17]Martini-Clinic, Prostate Cancer Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [18]PCUK Movember Centre of Excellence, CCRCB, Queen's University, Belfast, UK

Epigenetic reprogramming including altered transcription factor binding and altered patterns of chromatin and DNA modifications are now accepted as the hallmark of aggressive cancers. We show that global changes in chromatin structure and chromatin accessibility in prostate tumour tissue can define castrate-resistant prostate cancer and be used to inform the discovery of gene-level classifiers for therapy. In addition, we show that the androgen receptor overexpression alone, which is a hallmark of progression on its own, is a primary driver for chromatin relaxation, which can lead to promiscuous binding of other key transcription factors important to disease progression. We identify mediators of chromatin relaxation that can be used as disease biomarker and show that such mediators identify DNA stretches enriched in low p-value GWAS-significant disease/tissue-specific susceptibility loci.

Presenting Author: Urbanucci Alfonso Urbanucci, Centre for Molecular Medicine Norway, Nordic European Molecular Biology Laboratory Partnership, Forksningsparken, University of Oslo, Oslo, Norway and Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway. Email: alfonso.urbanucci@gmail.com

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OC10

Glycosylation is a global target for androgen control in prostate cancer cells

Jennifer Munkley, Karen E. Livermore, Daniel Vodak, Katherine James, Brian T. Wilson, Urszula L. McClurg, Bridget Knight, Paul MCcullagh, John Mcgrath, Malcolm Crundwell, Lorna W. Harries, Hing Y. Leung, Craig N. Robson, Sebastian Oltean, Ian G. Mills, Prabhakar Rajan & David J. Elliott

Institute of Genetic Medicine, Newcastle University, Newcastle-upon-Tyne, NE1 3BZ, UK

Changes in glycan composition are common in cancer and can play important roles in all of the recognised hallmarks of cancer (1). We recently identified glycosylation as a global target for androgen control in prostate cancer cells and further defined a set of 8 glycosylation enzymes (GALNT7, ST6GalNAc1, GCNT1, UAP1, PGM3, CSGALNACT1, ST6GAL1 and EDEM3), which are also significantly up-regulated in prostate cancer tissue (4). These 8 enzymes are under direct control of the androgen receptor (AR) and are linked to the synthesis of important cancer-associated glycans such as sialyl-Tn (sTn), sialyl Lewis^X (SLe^X), O-GlcNAc and chondroitin sulphate (4). Glycosylation has a key role in many important biological processes in cancer including cell adhesion, migration, interactions with the cell matrix, immune surveillance, cell signalling and cellular metabolism. Our results suggest that alterations in patterns of glycosylation via androgen control might modify some, or all of these processes in prostate cancer (2,3). The prostate is an abundant secretor of glycoproteins of all types, and alterations in glycans are, therefore, attractive as potential biomarkers and therapeutic targets. Emerging data on these often overlooked glycan modifications have the potential to improve risk stratification and therapeutic strategies in patients with prostate cancer.

Presenting Author: Jennifer Munkley, Institute of Genetic Medicine, University of Newcastle, Newcastle, UK. Email: jennifer.munkley@ncl.ac.uk

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OC11

Sex steroid deficiency alters renal calcium transporter expression independently of its effect on bone resorption

Rougin Khalil[1], Ferran Jardi[1], Michaël Laurent[2], Frank Claessens[2], Dirk Vanderschueren[1] & Brigitte Decallonne[1]
[1]Clinical and Experimental Medicine, KU Leuven, Leuven, Belgium; [2]Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium

It is well established that sex steroid deficiency induces bone loss, resulting in osteoporosis. Consequently, global androgen receptor knock out (ARKO) mice have trabecular and cortical osteopenia. Bone cell-specific ARKOs however, have a much less pronounced bone phenotype, suggesting that androgens have an influence on processes in other systems or organs which in turn have an impact on bone metabolism. The kidney is a likely candidate, as it plays an important role in calcium homeostasis, through reabsorption/excretion and synthesis of vitamin D. Therefore, we hypothesize that androgens regulate renal calcium homeostasis, hereby indirectly affecting bone resorption. To test this hypothesis, adult male C57BL6/J mice were orchidectomized (ORX vs SHAM) and treated with the antiresorptive drug risedronate (RIS vs vehicle), in order to study the effects of sex steroid depletion on renal calcium homeostasis independent of bone resorption. Orchidectomy resulted in a decreased kidney weight (2 weeks post-ORX), hypercalciuria (1 week post-ORX) which was normalized 2 weeks post-ORX along with normal serum levels of serum calcium, 1.25(OH)2D3, PTH, and FGF23. Orchidectomy combined with prior bone antiresorptive treatment abolished the early hypercalciuric phase and even resulted in transiently decreased serum calcium levels 1 week post-ORX. Compared to control mice, a significant upregulation of renal calcium transporters (TRPV5, PMCA, NCX1, CaBP9K and CaBP28K) was observed in both the ORX and ORX + RIS group, while intestinal calcium transporters (TRPV5, TRPV6, PMCA, CaBP9K) remained unchanged, suggesting that sex steroid deficiency might impact renal calcium homeostasis independent of its effect on bone resorption.

Presenting Author: Rougin Khalil, Clinical and Experimental Medicine, KU Leuven, Herestraat 49, 3000, Leuven, Belgium. Email: rougin.khalil@kuleuven.be

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OC12

Rapid cycling high dose testosterone (Bipolar Androgen Therapy) as therapy for men with metastatic castrate-resistant prostate cancer (mCRPC)

Samuel R. Denmeade, Emmanuel Antonarakis, Channing Paller, Hao Wang, Ben Teply, Charles Drake, Michael Carducci, Jun Luo & Mario Eisenberger
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore MD, USA

Prostate cancer (PC) becomes resistant to chronic castration via an adaptive increase in androgen receptor (AR) axis activity. AR overexpression, however, is a liability that can be exploited therapeutically through rapid cycling between high supraphysiologic and low castrate levels of serum testosterone (T), (Bipolar Androgen Therapy (BAT)). In a pilot study, 14 men with CRPC treated with BAT showed a 50% PSA and objective response. A larger study was initiated in which asymptomatic men with CRPC and progression on abiraterone (A) and/or enzalutamide (E) (30/cohort) receive T cypionate 400 mg every 28 days. At BAT progression men are rechallenged with A or E. Co-primary endpoints include PSA response after 3 cycles of BAT and after re-treatment of E or A. To date, 37 have completed $\geq$3 cycles of BAT: 11/37 (30%) had $\geq$ 50% PSA decline. 4/17 (23%) had RECIST responses. Post-BAT, 8/25 (32%) had $\geq$ 50% PSA response to retreatment A or E. Six men were AR-V7+ and all became AR-V7 negative after BAT with 2/6 having PSA response. BAT has generally been well tolerated with no DLT's thus far. 1 patient had a self-limited increase in pain and 1 had urinary retention, otherwise there were no bone/soft tissue AE's with BAT to suggest disease flare. This preliminary data demonstrates the safety and activity of BAT in patients with CRPC post-A and/or E with PSA and objective responses, including responses in AR-V7+ men. An ongoing multi-center randomized trial is testing BAT vs E in the post-A CRPC population.

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Presenting Author: Samuel R. Denmeade, M.D. Department of Oncology, The Johns Hopkins University School of Medicine, Cancer Research Building 1, 1650 Orleans Street, Baltimore, Maryland, USA. Email: denmesa@jhmi.edu.

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OC13

The role of nuclear steroid receptors in castration-resistant prostate cancer (CRPC)

Karolina Nowakowska[1], Ruth Riisnaes[2], Daniel Nava Rodrigues[2], Daniel Wetterskog[1], Anuradha Jayaram[1,3], Zafeiris Zaferiou[3], Joaquin Mateo[2,3], Johann S de Bono[2,3] & Gerhardt Attard[1,3]
[1]Division of Molecular Pathology, Centre for Evolution and Cancer, The Institute of Cancer Research, Sutton, Surrey, UK; [2]Division of Clinical Studies and Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, UK; [3]The Royal Marsden NHS Foundation Trust, Sutton, Surrey, UK

With the wide-spread use of abiraterone/enzalutamide for CRPC, there is an urgent need to understand and reverse resistance to these treatments. Studies have implicated glucocorticoid receptor (GR) as activating androgen receptor (AR) signalling and driving resistance. Here we studied progesterone receptor (PR), which is phylogenetically most closely related to the AR. We first used digital droplet PCR in prostate cancer (PCa) cell lines and observed >10 times higher levels of expression in the abiraterone/enzalutamide resistant cell line, LNCaP95 compared to LNCaP, VCaP and 22RV1. We confirmed increased PR expression on western blots. We then proceeded to develop a PR immunohistochemistry assay for studying PCa tissue, including biopsies of bone metastases. We tested 110 biopsies and detected PR-positive cells in 15 (30%) patients including 4 (8%) with >50% of cancer cells PR-positive. ER IHC on an adjacent slide was mutually exclusive. To study the relationship between AR targeting and PR expression, we treated AR-positive PCa cells with AR SiRNA or enzalutamide and observed a 6- and 7- fold rise in PR mRNA respectively, suggesting a close inter-play between these 2 signalling axes. We have developed Tet-On PR-A/PR-B, ER-alpha and GR model for evaluation of induction of treatment resistance.Small molecular PR antagonist, onapristone, inhibited T878A-AR (recently implicated in resistance to abiraterone in 15% of CRPC patients), when co-transferred with an ARE-reporter assay in AR-negative PC3 cells. Our data confirms the presence of PR in a molecular sub-set of PCa. Inhibition of PR with onapristone in combination with abiraterone in abiraterone-resistant CRPC is currently undergoing evaluation in a Phase I/II clinical trial (NCT02049190).

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Presenting Author: Karolina Nowakowska, The Institute of Cancer Research, Centre for Evolution and Cancer, Treatment Resistance Team, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK. Email: Karolina.nowakowska@icr.ac.uk

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OC14

Optimization of an engineered microrepressor for the treatment of castration-resistant prostate cancer

Flavia Marialucia Fioretti[1], Chun Fui Lai[1], Sue Powell[1], Simak Ali[1], Greg N. Brooke[2] & Charlotte Bevan[1]
[1]Department of Surgery and Cancer, Imperial Centre of Translational and Experimental Medicine, Imperial College London, London, UK; [2] School of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, UK

Prostate cancer is currently treated with hormonal therapies, which aim to block the production and/or action of androgens. However, tumours eventually progress to castration-resistant prostate cancer and there is a great need for new therapeutic approaches. We have designed and tested engineered repressors which could be effective in circumstances where current therapies fail. These consist of two modules: an interaction domain, which binds directly to the androgen receptor (AR), and a transcriptional co-repressor domain, that promotes the formation of a transcriptional inhibitory complex. The most effective interaction domain tested is part of the AR N-terminus itself (aa 1-54), containing the ^{23}FQNLF^{27} motif, which has been fused to Krüppel associated box (KRAB) or MAD-SID dominant transcription repression domain. These engineered repressors have been shown to suppress AR activity through disruption of the AR N-/C-terminal interaction, destabilization of the AR protein and promoting the recruitment of histone deacetylase (HDACs) to AR. Expression of engineered repressors in LNCaP cells decreases active histone modification which leads to down-regulation of target gene expression. We have also demonstrated that the repressors are effective in models of castration resistance, for instance presence of mutants of the AR and increased co-activators expression. The ultimate goal is to create a novel therapeutic effective in resistant stages of the disease, that can be administered systemically or delivered specifically to the prostate, therefore minimizing the deleterious side-effects associated with current therapies used to target the androgenic axis.

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Presenting Author: Flavia Marialucia Fioretti, Department of Surgery and Cancer, Imperial Centre of Translational and Experimental Medicine, Imperial College London, London W12 0NN, UK. Email: f.fioretti12@imperial.ac.uk
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OC15

In vivo imaging reveals prostate pathology in the PTEN knockout murine model of prostate cancer

Alysha Bhatti[1], Gilberto Serrano de Almeida[1], Serena Tommasini Ghelfi[1], Alwyn Dart[2], Anabel Varela-Carver[1] & Charlotte L. Bevan[1]
[1]Department of Surgery and Cancer, Imperial Centre for Translational and Experimental Medicine, Imperial College London, London, UK; [2]The Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, CF14 4XN, UK

Prostate cancer (PCa) is driven by the androgen receptor (AR) signalling axis and begins with prostatic intraepithelial neoplasia (PIN), progressing to invasive adenocarcinoma and eventually metastatic disease. It is treated with androgen deprivation therapies, to which, in late-stage disease, tumours often become resistant and proliferation occurs in a low androgen environment. Mutation of the PTEN tumour suppressor gene is found in approximately 30% of primary human prostate adenocarcinomas and is commonly implicated in metastatic and treatment-refractory disease. In this study, transgenic murine models recapitulating the sequence of human PCa development were used, having mono- or bi-allelic PTEN deletion. Incorporation of an androgen-responsive luciferase reporter construct allowed visualisation of AR activity through bioluminescent imaging, whilst T_2-weighted Magnetic Resonance Imaging (MRI) was performed to determine the effects of PTEN deletion on murine prostate pathology. In non-castrate male mice, pelvic bioluminescence was significantly greater in those with probasin-mediated prostate-specific PTEN deletion compared to age-matched controls ($P=0.0285$). 10, 14 and 17 month old mice heterozygous for PTEN deletion showed an age-dependent trend towards increasing pelvic bioluminescence (by bioluminescent imaging), prostatic enlargement and a greater prostate volume (by MRI imaging), while no gross structural prostate abnormalities were evident. This study demonstrates the efficacy of bioluminescent imaging to non-invasively investigate AR action in this transgenic murine model of PCa.
Funding: Prostate Cancer UK and Imperial College London
Presenting Author: Alysha Bhatti, Department of Surgery and Cancer, Imperial Centre for Translational and Experimental Medicine, Du Cane Road, W12 0NN, London, UK. Email: alysha.bhatti12@imperial.ac.uk
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OC16

Genomic analysis of Enzalutamide-resistant cells

Stefan Prekovic[1], Thomas Van den Broeck[1], Elien Smeets[1], Martin van Royen[2], Adriaan Houtsmuller[2], Biswajyoti Sahu[3], Päivi Pihlajamaa[3], Olli A. Jänne[3], Gerhardt Attard[4], Frank Claessens[1] & Christine Helsen[1]
[1]Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2]Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands; [3]Department of Physiology, University of Helsinki, Helsinki, Finland; [4]The Institute of Cancer Research & Royal Marsden NHS Foundation Trust, London, UK

Enzalutamide (Enza) is a second-generation antiandrogen currently used in the clinic for treatment of metastatic prostate cancer. It significantly prolonged survival of men with metastatic castration resistant prostate cancer after chemotherapy by a median of 4.8 months in comparison to the placebo group. However, in a subset of patient's beneficial effect of Enza cannot be observed, while others who initially respond eventually develop resistance towards the treatment. Understanding molecular pathways that mediate Enza resistance is important for future therapy and drug design. Thus, to study mechanisms that lead to resistance we generated Enza resistant LNCaP cells. The resistance to Enza was confirmed both *in vitro*, and *in vivo* in nude mice. Furthermore, we performed RNA sequencing, copy number analysis, FAIRE sequencing, and AR ChIP sequencing to study androgen receptor signaling and resistance mechanism. Our data provide a deeper insight into Enzalutamide resistance and point to specific mechanisms that occur in these resistant cells.
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Presenting Author: Stefan Prekovic, Department of Cellular and Molecular Medicine, KU Leuven, Herestraat 49, 3000, Leuven, Belgium. Email: stefan.prekovic@med.kuleuven.be
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OC17

miR-32 promotes replicative changes in prostate epithelium *in vivo*

Leena Latonen[1], Andrew Gillen[1], Mira Valkonen[1], Pekka Ruusuvuori[1], Fuping Zhang[2], Matti Poutanen[2] & Tapio Visakorpi[1]
[1]Prostate Cancer Research Center and BioMediTech, University of Tampere, Finland; [2]Department of Physiology and Turku Center for Disease Modeling, Institute of Biomedicine, University of Turku, Finland

The androgen receptor (AR) signaling pathway is central to the emergence of castration-resistant prostate cancer (CRPC). miR-32 is an androgen regulated miRNA which is differentially expressed in CRPC compared to benign prostatic hyperplasia (BPH) and able to provide a significant growth advantage to LNCaP cells. To study how increased miR-32 expression contributes to prostate cancer formation and/or progression *in vivo*, we have established transgenic mice expressing miR-32 specifically in the prostate epithelium post-puberty. Expression of miR-32 transgene increases replicative activity in prostate epithelium, as demonstrated by Ki-67 staining. In aged mice, miR-32 overexpression increases incidence of goblet cell metaplasia. To provoke neoplastic lesions in prostate epithelium, the miR-32 mice were cross-bred with mice heterozygous for tumor suppressor Pten or mice overexpressing oncogenic Myc in the prostate. In both models, increase in Ki-67 staining by miR-32 overexpression was observed indicating increased replicative activity of prostate epithelium. Although incidence of PIN lesions in Pten heterozygous background was only modestly affected, the lesions with miR-32 overexpression are histologically different according to an image feature-based random forest classification. In contrast, the prostates of Myc-overexpressing mice were significantly larger when miR-32 transgene was present. Histological changes provoked by miR-32 expression in the preneoplastic lesions and tumors in the prostates of these models are presented. Our data show that miR-32 is able to affect replication potential of prostate epithelium and contribute to prostate tumor development dependently on the genetic background of the tumors.
Presenting Author: Leena Latonen BioMediTech, PL100, 33014 University of Tampere, Finland. Email:leena.latonen@uta.fi
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Poster Presentations

P1

Inhibition of NADPH oxidase 4 attenuates stromal activation associated with prostate cancer

Natalie Sampson[1], Cédric Szyndralewiez[2] & Helmut Klocker[1]

[1]Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria; [2]Genkyotex S.A., Geneva, Switzerland.

Carcinoma-associated fibroblasts (CAFs) within the stromal tumor microenvironment play a key role in promoting the development, progression and therapy resistance of prostate cancer (PCa). However, the stromal compartment is not targeted by current treatment strategies. Elevated secretion of transforming growth factor beta (TGFβ) by epithelial cells in prostate intraepithelial neoplasia (PIN) lesions and tumor cells induces fibroblast activation to the CAF phenotype and is associated with poor prognosis. We previously showed that elevated production of reactive oxygen species (ROS) by NADPH oxidase 4 (Nox4) is essential for TGFβ1-mediated stromal activation of primary prostate fibroblasts. Moreover, Nox4 mRNA levels are elevated in PCa patients that experienced biochemical relapse. This study aimed to determine whether pharmacological inhibition of Nox4 attenuates fibroblast activation as a potential therapeutic strategy. Nox4 inhibition attenuated TGFβ1-induced ROS production and fibroblast activation of primary normal tissue-associated prostate fibroblasts (NAFs) as indicated by abrogated induction of reactive stromal markers. Moreover, Nox4 inhibition attenuated fibroblast migration. Microarray analyses of primary patient matched NAF and CAFs reveal two molecularly distinct CAF subtypes with one subtype exhibiting elevated Nox4 mRNA levels. Similarly to TGFβ-activated fibroblasts, pharmacological inhibition of Nox4 in CAFs also leads to down-regulation of reactive stromal markers at both the mRNA and protein level. Similar results were obtained using Nox4-specific shRNA-mediated silencing. Collectively, these data indicate that targeting Nox4 attenuates key molecular and phenotypic hallmarks of activated fibroblasts. Further investigation of Nox4 inhibition as a potential treatment strategy for PCa is warranted.

Presenting author: Natalie Sampson, Department of Urology, Medical University of Innsbruck, Anichstraße 35, A6020, Innsbruck, Austria. Email: natalie.sampson@i-med.ac.at.

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P2

Functional analysis of the AR LBD dimerization surface

Christine Helsen[1], Stefan Prekovic[1], Martin E van Royen[2], Adriaan B Houtsmuller[2], Pablo Fuentes-Prior[3], Eva Estébanez-Perpiñá[4] & Frank Claessens[1]

[1]Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2]Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands; [3]Molecular Bases of Disease, Biomedical Research Institute Sant Pau, Barcelona, Spain; [4]Department of Biochemistry and Molecular Biology, Institute of Biomedicine from the University of Barcelona (IBUB), University of Barcelona (UB), Barcelona, Spain.

The androgen receptor (AR) is a multidomain transcription factor consisting of an aminoterminal domain (NTD), a DNA binding domain (DBD) and a ligand binding domain (LBD). Binding of androgens marks the start of a sequence of intra- and interdomain communications, DNA binding, coregulator recruitment and gene activation. Besides the DBD-mediated dimerization on the DNA, there is a functionally relevant N/C interaction. The contribution of the intra- and intermolecular N/C interactions to AR function and its spatiotemporal organization have been well documented. Most recently, the first crystal structure of AR-LBD dimer was solved (Gallastegui *et al*. in preparation). Here, we investigated the functional relevance of the LBD dimerization by mutational analysis. Several mutations that are predicted to either disrupt or stabilize AR LBD–LBD interactions were introduced in the full size AR, and the resulting mutant receptors were investigated for their ability to transactivate, for their capacity to bind androgens and for their ability to bind DNA. Since the mutations that disrupt the LBD dimer also decrease DNA binding, the involvement of the AR DBD is tested via swapping experiments with ER DBD and GR DBD. Furthermore, we have confirmed the presence of the LBD-dimer in a cellular context using biochemical approaches. At the moment, we are testing the influence of dimer disrupting and dimer stabilizing mutations on the outcome of these biochemical assays.

Presenting author: Christine Helsen, Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, KU Leuven, Herestraat 49, 3000 Leuven, Belgium. Email: christine.helsen@med.kuleuven.be.

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P3

Diminished response of prostate cancer cells to antiandrogens upon co-culture with cancer-associated fibroblasts as shown in a 3-dimensional prostate cancer epithelial-stromal organoid model

Theresa Eder[1,2,3], Anja Weber[1], Hannes Neuwirt[4], Georg Grünbacher[1], Georg Schäfer[1], Christian Ploner[5], Helmut Klocker[1], Natalie Sampson[1] & Iris E Eder[1]

[1]Department of Urology, Medical University Innsbruck, Innsbruck, Austria; [2]Department of Radio Oncology and Radiotherapy, Charité University Hospital, Berlin, Germany; [3]German Cancer Research Center (DKFZ), Heidelberg and German Cancer Consortium (DKTK), Partner Site Berlin, Berlin, Germany; [4]Department of Internal Medicine IV – Nephrology and Hypertension, Medical University of Innsbruck, Innsbruck, Austria; [5]Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Innsbruck, Innsbruck, Austria.

The use of more tissue-mimetic 3-dimensional (3D) cell culture models for *in vitro* drug screening has significantly increased in the past with the expectation to overcome with over-interpretation of drug effects obtained with conventional 2D cultures. In this study, we characterized 3D prostate cancer (PCa) organoids where PCa epithelial cells (LNCaP, DuCaP, LAPC4) were cultured alone or with PCa-associated fibroblasts (CAFs) in 96 well hanging drop plates in order to investigate the influence of the stromal cells on the therapeutic effects of two commonly used antiandrogens (bicalutamide, enzalutamide). All tested PCa cell lines formed so-called organoids and displayed a significant increase in E-cadherin expression, as determined by Western blotting, indicating strong cell-to-cell adhesion upon 3D culture. Interestingly, CAFs seemed to disappear in co-culture organoids by day 8, suggesting that the PCa cells replace the CAFs over time. Organoids displayed androgen responsiveness in 3D culture, however, the effects were less pronounced in PCa/CAF co-culture organoids. Similarly, PCa organoids were less sensitive to the growth-inhibitory effects of the antiandrogens bicalutamide and enzalutamide than 2D cultures, an effect that was further enhanced in co-culture organoids. In LNCaP cells, which are negative for the tumor suppressor gene PTEN, 3D organoids showed increased Akt signaling along with resistance to antiandrogens while AR levels were unchanged. In line with this alteration the phosphatidyl-inositol 3 kinase (PI3K) inhibitor LY294002 significantly inhibited LNCaP organoids, suggesting that targeting Akt could be used to overcome antiandrogen resistance in PTEN negative PCa cells.

Presenting author: Iris E. Eder, Department of Urology, Medical University Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. Email: iris.eder@i-med.ac.at.

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P4

Expression of a novel androgen-regulated long noncoding RNA correlates with progression-free survival in prostate cancer patients

Annika Kohvakka, Kati Kivinummi, Ville Kytölä, Antti Ylipää, Matti Annala, Alfonso Urbanucci, Matti Nykter & Tapio Visakorpi

Prostate Cancer Research Cancer, BioMediTech, University of Tampere and Tampere University Hospital, Tampere, Finland.

Prostate cancer (PC) is the second most frequently diagnosed cancer in men worldwide. 10–20% of the PC patients develop castration-resistant prostate cancer (CRPC) that has no curative therapies. There are also no effective prognostic markers to predict emergence of CRPCs. Long noncoding RNAs (lncRNAs) are a recently found group of RNAs that are not translated into proteins. Many of them are found to be differentially expressed in cancer, and shown to have a regulative role in tumorigenesis and tumor development. In addition, some lncRNAs have been associated with cancer progression and/or survival, making them potentially interesting as prognostic markers. Previously, we performed RNA sequencing of 28 hormonally untreated PC, 13 CRPC and 12 non-cancerous, benign prostatic hyperplasia tissue samples, out of which 145 novel PC-associated lncRNAs (PCATs) were discovered. Subsequently, the expression of 39 PCATs were analyzed in 87 samples from prostatectomy-treated PCs by qRT-PCR on Fluidigm Biomark HD, and the results were associated with clinical data. Some of the PCATs had a significant correlation with progression-

free survival. One of these PCATs was also found to be a target of androgen receptor (AR) regulation. According to publicly available AR-ChIP-seq data, there is an AR binding site in the transcription start site of this novel PCAT in prostate cancer. Thus, we validated the AR binding by ChIP-qPCR in AR-expressing LNCaP and LuCaP cells. In addition, when AR was silenced by siRNAs, the expression of the PCAT was significantly diminished.
Presenting author: Annika Kohvakka, BioMediTech, University of Tampere, Biokatu 8, 33520, Tampere, Finland. Email: Annika.Kohvakka@staff.uta.fi.
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P5

Androgen pathway regulating microRNAs in prostate cancer progression and therapy

Foteini Kalofonou, Claire Fletcher, Jonathan Waxman & Charlotte Bevan
Androgen Signalling Laboratory, Department of Surgery and Cancer, Imperial College London, London, UK.

Prostate cancer is an androgen dependent malignancy that initially responds well to androgen ablation therapy. However treatment, castrate resistant prostate cancer eventually emerges. Even in that phase of the disease, the androgen receptor (AR) still seems to play a role. MicroRNAs are small (19–25nt) non-coding RNAs that modulate gene silencing through inhibition of translation and mRNA degradation. They are considered to be master regulators of gene expression and act both as oncogenes and tumour suppressors, as well as possible biomarkers and therapeutic targets for prostate cancer. We have identified a number of miRs that modulate AR activity in AR-dependent and castrate resistant cell lines and may have roles in human progression. We have investigated the effect of miR modulation on AR activity and identified pathways that could be used as possible therapeutic targets in hormone unresponsive prostate cancer. Prostate cancer cell lines that stably expressed an AR reporter element were transfected with specific miR inhibitors and mimics. We investigated the effect of miR modulation on potential alteration in AR activity (through a luciferase assay), on cell growth by an SRB assay and on apoptosis, using a caspase 3/7 assay on AR-dependent and castrate resistant cell lines. Of three miRs tested, two mimics significantly increased AR activity in the castrate resistant cell line and one inhibitor significantly reduced AR activity in the castrate resistant cell line. In cell growth assays, an inhibitor of one of these miRs reduced growth of the AR dependent cell line. For two miRs, mimic increased while inhibitor repressed AR activity and growth, suggesting they are potential oncomiRs in prostate cancer. Predicted targets include genes with roles in growth arrest, apoptosis or DNA repair. Future studies will be directed towards further characterisation of those miRs, through alteration of mRNA and protein levels of AR and AR target genes.
Presenting author: Foteini Kalofonou, Division of Cancer, Department of Surgery and Cancer, Imperial Centre for Translational & Experimental Medicine, Imperial College London, Hammersmith Hospital Campus London W12 0NN, UK. Email: f.kalofonou15@imperial.ac.uk.
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P6

The bi-directional interaction of AR and IL6 signalling in the response to enzalutamide in prostate cancer cells

Florian Handle[1], Holger HH Erb[1,2], Birgit Luef[1], Julia Hoefer[1], Dimo Dietrich[3], Walther Parson[4,5], Glen Kristiansen[3], Frédéric R Santer[1,†] & Zoran Culig[1†]
[1]Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Innsbruck, Austria; [2]Yorkshire Cancer Research Unit, University of York, York, UK; [3]Institute of Pathology, University Hospital Bonn, Bonn, Germany; [4]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria; [5]Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania, USA; [†]Joint senior authors.

Chronic inflammation and high expression of pro-inflammatory cytokines, such as Interleukin-6 (IL6), are well-known risk factors for prostate cancer (PCa). IL6 is known to activate the androgen receptor (AR) and has been implicated in development of castration resistance. Therefore, we wanted to investigate the interaction of AR and IL6 signalling and the effect on anti-androgen treatment. We could confirm IL6 mediated activation of the AR on 3 AR target genes in presence of R1881 by qPCR in LNCaP ($P<0.001$). In contrast to previous publications we could not detect AR activation in absence of androgens. Mechanistically, IL6 did not change AR protein expression nor nuclear localization, which suggests that the transactivation potential of the AR is enhanced. Interestingly, anti-androgen treatment of LNCaP and DuCaP cells led to increased IL6 signalling and an upregulation of the IL6 induced negative feedback-regulator *SOCS3*. This effect could be linked to a direct AR mediated suppression of *IL6ST* and *JAK1*, two key components of the IL6 receptor complex. Normally, induction of SOCS3 expression would counteract the increase in IL6 activity, but we found that the promoter region of *SOCS3* is hypermethylated in more than 85% of PCa patients ($n=269$), which has been linked to reduced mRNA expression previously. Functionally, SOCS3 knock-down was able to abrogate the effect of enzalutamide on *PSA* expression in presence of IL6. In conclusion, we could show a bi-directional interaction of the AR and IL6 pathways and this effect could play an important role during anti-androgen treatment, especially under low SOCS3 conditions.
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Presenting author: Florian Handle, Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Anichstr 35, A-6020, Innsbruck, Austria. Email: florian.handle@i-med.ac.at.
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P7

μ-Crystalline as hormone antagonist in prostate cancer

Olaf Merkel[1,†], Osman Aksoy[1,†], Martin Suzani[1,2,3], Melanie Hassler[1,4], Karin Schlangen[5], Theresa Balber[6], Markus Mitterhauser[6], Ali Moazzami[7], Michaela Schlederer[1], Suzanne Turner[1,8], Gerda Egger[1], Gregor Hörmann[9], Markus Hacker[6], Zoran Culig[10], Jan Pencik[1,2] & Lukas Kenner[1,2,11]
[1]Department of Clinical Pathology, Medical University Vienna, Vienna, Austria; [2]Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria; [3]Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria; [4]Department of Urology, Medical University of Vienna, Vienna, Austria; [5]Department of Biosimulation and Bioinformatics, Medical University Vienna, Vienna, Austria; [6]Department of Nuclear Medicine, Medical University Vienna, Vienna, Austria; [7]Department of Chemistry and Biotechnology, Swedish University of Agricultural Sciences, Uppsala, Uppsala, Sweden; [8]Division of Molecular Histopathology, Department of Pathology, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; [9]Department of Laboratory Medicine, Medical University Vienna, Vienna, Austria; [10]Department of Urology, Medical University Innsbruck, Innsbruck, Austria; [11]Unit of Pathology of Laboratory Animals, University of Veterinary Medicine Vienna, Vienna, Austria. [†]These authors contributed equally.

Prostate cancer (PCa) is the most frequent cancer in men in the western world. PCa growth is highly dependent on androgens and androgen ablation is the cornerstone of current therapeutic approaches. μ-Crystalline (CRYM) is the main component of the kangaroohs eye's lens. CRYM binds thyroid hormone (T3) in a NADPH dependent manner thereby sequestering it from being transcriptionally active in the nucleus. The role of CRYM in prostate cancer is largely unknown. This study identifies low CRYM as a negative prognostic factor in PCa using IHC and Kaplan-Meier analysis. In PCa CRYM expression was reduced, an effect that is further pronounced in metastases. In contrast, thyroid hormone receptor ß (TRβ) showed high expression in PCa that is again increased in metastases. Overexpression of CRYM in PCa cell lines led to increased uptake T3 and reduced invasive capacity. RNA sequencing transcriptome analysis of CRYM overexpressing PCa cell lines reveals androgen receptor and dihydrotestosteron induced genes to be highly specifically suppressed, identifying CRYM as a key hormone antagonist in PCa. Moreover, high CRYM expression leads to deregulated lipid metabolism. Finally, metabolome analysis using nuclear magnetic resonance (NMR) shows that high CRYM expression drastically reduces intracellular choline levels and is able to mask T3 effects in metastatic PC3 PCa cell line. Using PET/MRI we recently described choline levels as non-invasive biomarker for PCa surveillance. This study identifies CRYM as a prognostic factor and key antagonist to T3 and androgen signalling in PCa.
Presenting Author: Olaf Merkel, Department of Clinical Pathology, Medical University Vienna, Währinger Gürtel 18-20, 1090 Wien, Austria. Email: olaf.merkel@meduniwien.ac.at
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P8

Antiandrogens reduce intratumoral androgen concentrations and induce androgen receptor expression in castration-resistant VCaP xenografts

Matias Knuuttila[1,2], Arfa Mehmood[3], Riikka Huhtaniemi[1,2,4], Riikka Oksala[4], Merja Häkkinen[5], Teemu D Laajala[6,7], Tero Aittokallio[6,7], Seppo Auriola[5], Claes Ohlsson[8], Laura Elo-Uhlgren[3,6], Petra Sipilä[1,2], Sari Mäkelä[2,9] & Matti Poutanen[1,2,8]
[1]Department of Physiology, University of Turku, Turku, Finland; [2]Turku Center for Disease Modeling (TCDM), Institute of Biomedicine, University of Turku, Turku, Finland; [3]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland; [4]Orion Pharma, Turku, Finland; [5]School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [6]Department of Mathematics and Statistics, University of Turku, Turku, Finland; [7]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland; [8]Institute of Medicine, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [9]Functional Foods Forum, University of Turku, Turku, Finland.

The growth of prostate cancer (PCa) can be suppressed by androgen deprivation therapy (ADT). However, in a significant proportion of men receiving ADT PCa progresses to castration-resistant prostate cancer, associated with activation of intratumoral androgen biosynthesis and induced androgen receptor (AR) expression. Accordingly, we have recently shown that the castration-resistant VCaP (CR-VCaP) xenografts express high level of AR and retain detectable intratumoral androgen concentrations, and respond to antiandrogens, enzalutamide and ARN-509, as evidenced by the reduced circulating PSA concentration. In the present study we show that the expression of full-length AR (AR-FL), as well as the splice variants AR-V1 and AR-V7, was further increased 2 to 3-fold by the antiandrogens, both mRNA and protein levels, while the AR-FL still remained by far the most abundantly expressed AR form. Interestingly, the antiandrogen treated tumors presented with markedly reduced (tenfold) intratumoral testosterone and DHT concentrations as compared with the vehicle treated tumors, while no such drop was detected for androstenedione and for the precursors for androgen synthesis, such as pregnenolone, progesterone and 17-hydroxyprogesterone. For all the steroids measured the tumor concentrations were higher than that measured in the serum, indicating local synthesis. Despite of the low intratumoral concentrations, antiandrogen treatment induced only minor changes on the expression of classical androgen-regulated genes, including TMPRSS2 and KLK3, likely due to the induced expression of full-length AR and AR variants. However, global transcription analysis revealed altered expression of 291 genes, and of those, several AR-interacting genes and enzymes involved in steroid metabolism were included.

Presenting author: Matias Knuuttila, Department of Physiology, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, 20520 Turku, Finland. Email: matias.knuuttila@utu.fi.

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P9

The deubiquitinating enzyme USP12 controls prostate cancer cell survival by regulating the AR-AKT-p53 signalling network

Urszula L McClurg[1], Nay C T H Chit[1], Sirintra Nakjang[1], Joanne Edwards[2], Stuart R McCracken[1] & Craig N Robson[1]
[1]Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK; [2]Unit of Experimental Therapeutics, Institute of Cancer, University of Glasgow, Glasgow, UK.

We previously demonstrated that USP12 functions as an AR co-activator by directly deubiquitinating the AR and stabilising its protein levels. Additionally, we showed that USP12 targets the PHLPP AKT phosphatases leading to decreased levels of activated, phosphorylated AKT (pAKT) and as such indirectly stabilises the AR preventing its phosphorylation at serine 213. We further investigated the role of USP12 in prostate cancer by analysing the transcriptome of the LNCaP prostate cancer cell line following depletion of USP12 using siRNA. We discovered that in addition to regulating the AR signalling cascade, USP12 controlled the p53 signalling pathway. Further analysis determined that USP12 directly targets the E3 ubiquitin ligase MDM2, stabilising MDM2 protein and consequently controlling p53 protein levels. Clinical importance of USP12 was confirmed in clinical prostate cancer where increased USP12 was found to be a marker of poor prognosis that correlated with shorter relapse-free survival and reduced overall survival. Additionally we observed that USP12 protein levels were significantly increased in castration resistant prostate cancer (CRPC) patients for two independent clinical cohorts that we investigated, suggesting that USP12 may play a role in the development of CRPC. These findings reveal that a deubiquitinating enzyme that stabilises the AR, namely USP12, additionally controls the p53 pathway and that USP12 protein levels are elevated in prostate cancer and associated with decreased relapse-free survival and overall survival. This identifies USP12 as a promising therapeutic target in prostate cancer and CRPC.

Presenting author: Urszula McClurg, Northern Institute for Cancer Research, Newcastle University, Framlington Place, NE2 4HH, Newcastle upon Tyne, UK. Email: urszula.mcclurg@Newcastle.ac.uk.

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P10

Metastases-prone localized prostate cancer: a genomic analysis

Thomas Van den Broeck[1,2], Thomas Gevaert[2], Stefan Prekovic[1], Bram Boeckx[3], Elien Smeets[1], Kaye Ong[4], Jonathan Lehrer[4], Zaid Haddad[4], Nicholas Erho[4], Christine Helsen[1], Diether Lambrechts[3], Christine Buerki[4], Elai Davicioni[4], Steven Joniau[2] & Frank Claessens[1]
[1]Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2]Department of Urology, University Hospitals Leuven, KU Leuven, Leuven, Belgium; [3]Laboratory of Translational Genetics, KU Leuven, Leuven, Belgium; [4]GenomeDx Biosciences, Vancouver, British Columbia, Canada.

Clinical features of the primary prostate tumor remain insufficient for clinicians to accurately define patients at highest risk of developing metastases. However, the primary tumor is the source of these metastases, and thus should contain information on its metastatic potential. We hypothesized that the combination of a primary tumor's copy number alterations (CNAs) and genome-wide transcriptome information would give us more insight in the biology of metastases-prone localized disease. We designed a retrospective clinically matched cohort study of patients with high-risk localized prostate cancer who have been treated with radical prostatectomy. A cohort of patients who have developed metastases during long-term follow-up (M+ cohort) was matched with patients who did not develop clinical recurrence (M− cohort). Paraffin embedded tumor blocks were retrieved for tissue collection and used for DNA and RNA extraction. CNAs were analyzed using GISTIC and transcriptome analysis was performed using the Decipher GRID platform. Forty-four patients were withheld with both high quality CNA and gene expression data of which 19 are part of the M+ cohort and 25 of the M− cohort. GISTIC analysis showed distinct CNA profiles, with significantly more focal amplifications in the M+ cohort, whilst the M− cohort harbors a 6q15 deletion. Transcriptome-wide gene expression analysis allowed us to identify a selection of genes in the amplified or deleted regions that were up- or downregulated, respectively. Ongoing investigations are focused on identifying which of these genes are driving the primary tumors' metastatic potential.

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Presenting author: Thomas Van den Broeck, Department of Cellular and Molecular Medicine, KU Leuven, Herestraat 49, 3000 Leuven, Belgium. Email: thomas.vandenbroeck@med.kuleuven.be.

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P11

Switch to succinate-mediated mitochondrial respiration associated with HIF-1α stabilization in PTEN negative prostate cancer cells

Anja Weber[1], Jan Pencik[2], Lukas Kenner[2], Helmut Klocker[1] & Iris E Eder[1]
[1]Department of Urology, Medical University Innsbruck, Innsbruck, Austria; [2]Clinical Institute for Pathology, Medical University Vienna, Vienna, Austria.

Metabolic reprogramming resulting in significant alterations of the energy metabolism is a hallmark of prostate cancer (PCa). In this study we performed a comprehensive metabolic analysis of various human (LNCaP, DuCaP, PC-3, Du145) and murine PCa cell lines differing in the expression of the tumor suppressor phosphatase and tensin homolog (PTEN). In line with previous studies we found that $PTEN^-$ PCa cells (LNCaP, PC-3) had a higher glycolytic activity than $PTEN^+$ PCa cells (DuCaP, Du145) with increased lactate production and elevated expression of hexokinase 2 (HK2) mRNA. $PTEN^-$ PCa cells also exhibited lower activity of pyruvate dehydrogenase (PDH) and higher expression

of the PDH inhibitor PDK1 (pyruvate dehydrogenase kinase), thereby attenuating pyruvate flux into Krebs cycle and pyruvate-fuelled oxidative phosphorylation (OXPHOS). Overall, mitochondrial routine respiration was higher in $PTEN^-$ compared to $PTEN^+$ cells, with a significant switch towards succinate-(complex II) fuelled respiration at the expense of pyruvate (complex I) mediated respiration. Notably, sodium – dependent dicarboxylate cotransporter (NaDC3/SLC13A3), a transporter protein that mediates succinate uptake, was elevated in $PTEN^-$ PCa cells as shown by Western blotting. In line with an increased succinate level, which is known to stabilize the hypoxia-inducible factor HIF1α immunofluorescent staining confirmed increased expression of HIF1α in $PTEN^-$ compared to $PTEN^+$ cell lines. In conclusion, our data suggest that the uptake of succinate via NaDC 3 enhances a hypoxia-response and oxidative phosphorylation in order to fulfil increased energy requirements of PCa cells. An intervening with this pathway may offer a new way for the treatment of PCa.

Presenting author: Anja Weber, Department of Urology, Anichstraße 35, 6020 Innsbruck, Austria. Email: anja.weber@i-med.ac.at.

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P12

Exploiting pioneer factors of androgen receptor variants for novel prostate cancer therapies

Lewis Chaytor & Luke Gaughan

Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK.

Prostate cancer (PC) growth is androgen-dependent, hence, the mainstay for treatment is hormone-ablation therapy using anti-androgens, and/or androgen-deprivation therapies. Unfortunately, after a median time of 18 months, the cancer reappears in an androgen independent form, termed castrate-resistant PC (CRPC), which is largely fatal. To date, many molecular mechanisms have been suggested to be responsible for persistent AR signalling in CRPC. AR variants (AR-Vs), short forms of the AR which lack the ligand binding domain (LBD), have been identified as a major mechanism of maintaining AR signalling in castrate conditions. Underpinning mechanisms of AR-V regulation is essential to ablate the transcriptional effects of these transcription factors. Overexpression of pioneer factor such as GATA2, which act to facilitate AR loading on chromatin, have also been reported in CRPC. Taken together, depletion or inhibition of the pioneer factor - AR-V axis could prove useful in ablating AR-V-dependent transcription in castrate conditions. We report that GATA2 knockdown reduces AR-V recruitment to chromatin at specific target gene promoters/ enhancers and regulates ~10% of the AR-V transcriptome. Concordantly, depletion of GATA2 results in reduced proliferation of AR-V driven prostate cancer cell lines. Additionally, the interaction between GATA2 and chromatin was disrupted by a BET inhibitor, JQ1. Data presented here indicates that AR-Vs remain reliant on the pioneer factor GATA2 for maximal transcriptional activity. Removing GATA2 from *cis*-regulatory elements using BET inhibitors or GATA2-targeted therapies may contrite to creating an inaccessible chromatin environment and attenuate advanced prostate cancer cell growth.

Presenting author: Lewis Chaytor, Northern Institute for Cancer Research, Newcastle University, NE2 4HH, Newcastle upon Tyne, UK. Email: l.chaytor@ncl.ac.uk.

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P13

Lysine demethylase 7A (KDM7A) as a potential therapeutic target in prostate cancer

Veronika M Metzler[1,2], Simone de Brot[1], Jonathan Whitchurch[1,2], Brian D Robinson[3], Stephen Boorjian[4], Lorraine J Gudas[5], Martin Johannson[6], Jenny L Persson[7], David M Heery[2] & Nigel P Mongan[1,4]
[1]Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Science, University of Nottingham, UK; [2]School of Pharmacy, University of Nottingham, UK; [3]Department of Pathology, Weill Cornell Medicine, New York, New York, USA; [4]Department of Urology, Mayo Clinic, Rochester, Minnesota, USA; [5]Department of Pharmacology, Weill Cornell Medicine, New York, New York, USA; [6]Department of Laboratory Medicine, Lund University, Malmö, Sweden; [7]Clinical Research Center, Lund University, Malmö, Sweden.

Androgen Receptor (AR) is central to prostate cancer (PCa) tumorigenesis and metastases. The genomic actions of androgens are mediated by the AR in complex with multiple chromatin modifying coregulators. We and others have identified lysine demethylases (KDMs) as important mediators of androgen signaling and increased expression of specific coregulators, including KDM1A, are implicated in PCa recurrence. There is an urgent need for new PCa treatments. While androgen deprivation therapies (ADT) impede tumor progression, castrate resistant PCa (CRPC) typically arises within ~18 months. CRPCs escape androgen dependency and are incurable. The mechanisms involved in CRPC include AR mutations/splice variants and/or alterations in AR-coregulators. However AR-coregulators, in particular 'druggable' enzymes including KDMs, offer alternative targets to circumvent resistance to existing ADTs. We reported that the androgen-induced *microRNA-137 (miR137)* acts as a suppressor of an extended network of transcriptional coregulators, including *KDM1A* and *KDM7A*, in normal prostate cells. However loss of *miR137* in PCa contributes to increased expression of these coregulators. Here we report that androgen induces *KDM7A* expression in hormone responsive LNCaP cells. siRNA-mediated functional depletion of *KDM7A* expression impairs androgen induction of *KLK3/PSA* and the pro-angiogenesis factor *VEGFA* in LNCaP and its hormone-refractory derivative, LNCaP:C4-2. We examined KDM7A expression in PCa specimens and correlate expression with key clinical parameters. Collectively our data supports a role for KDM7A in androgen dependent and refractory PCa. Thus KDM7A, like KDM1A, represents a novel potential drug-target to inhibit androgen signaling in hormone dependent and refractory contexts and thereby potentially circumvent resistance to existing ADTs.

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Presenting author: Veronika Metzler, Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Health Sciences, University of Nottingham, UK. Email: stxvm8@nottingham.ac.uk.

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P14

Androgen receptor variants and microenvironment in prostate cancer

Edwige Schreyer[1], Eva Erdmann[1], Félicie Cottard[1], Marine Delbecque[1], Pauline Berthélémy[1], Jean-Emmanuel Kurtz[1,2] & Jocelyn Céraline[1,2]
[1]INSERM U1113, Team 3 "Cell signalling and communication in kidney and prostate cancer", University of Strasbourg, Strasbourg, France; [2]Haematology and Oncology Unit, Strasbourg University Hospital, Strasbourg, France.

Androgen ablation therapy remains the most common treatment for patients with advanced prostate cancer (PCa). However, most patients relapse and develop a castration-resistant PCa. The emergence of androgen receptor (AR) variants, such as constitutively active ARs, has been involved in this failure to androgen deprivation. Nevertheless, the tumour microenvironment is another necessary feature driving PCa progression. Cancer associated fibroblasts (CAFs) are one of the specialized stromal cells that favour tumour progression. They can be derived from different cell types, and 25% of CAFs originate from bone marrow-derived mesenchymal stem cells (MSCs). In this study, we investigated the effects of AR variants on the surrounding prostate tumour microenvironment by focusing on MSCs differentiation into CAFs. We used an *in vitro* co-culture system of human MSCs together with LNCaP cells, expressing or not AR variants, to analyse CAFs differentiation markers expression in MSCs by RT-qPCR. These differentiation markers were also analysed with a FISH approach in MSCs exposed to conditioned medium of LNCaP cells expressing or not AR variants. RT-qPCR data revealed an upregulation of several CAFs differentiation markers in MSCs such as FSP-1. These results were confirmed with a FISH approach showing an increase in FSP-1 fluorescent spot number for MSCs exposed to conditioned medium from LNCaP cells expressing AR variants. Together, our data would highlight an unknown property of AR variants in prostate tumour cells that is their ability to induce MSCs differentiation into CAFs. Studies are going on to validate these data using an *in vivo* PCa model.

Presenting author: Edwige Schreyer, INSERM U1113, Team 3 "Cell signalling and communication in kidney and prostate cancer", University of Strasbourg, 1 Place de l'Hôpital, 67091, Strasbourg, France. Email: schreyer.edwige@gmail.com.

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P15

Altered steroid profiles in prostate cancer xenograft model with low ADRB2 levels

Håkon Ramberg[1], Ralf Kellman[2], Peder Rustøen Braadland[1], Elin Stærli[1], Stein Waagene[1], Gunnar Mellgren[2,3], Gunhild Mari Mælandsmo[1,4] & Kristin Austlid Taskén[1,5]
[1]Department of Tumor Biology, Oslo University Hospital, Oslo, Norway; [2]Hormone Laboratory, Haukeland University Hospital, Bergen, Norway; [3]Department of Clinical Science, University of Bergen, Bergen, Norway; [4]Department for Pharmacy, University of Tromsø, Tromsø, Norway; [5]Institute of Clinical Medicine, University of Oslo, Oslo, Norway.

It has been established in recent years that androgens are involved in the progression of castration resistant prostate cancer (CRPC). Although androgen deprivation therapy reduces the level of androgens and inhibits the androgen receptor, there are studies reporting that the androgen signaling axis is still involved in the development of CRPC. We have in a previous study shown that low level of β_2-adrenergic receptor (ADRB2) is associated with shorter time to CRPC in a patient cohort. One possible mechanism is the role of ADRB2 in regulating the level of glucuronidation of androgens in prostate cancer cells, altering the levels of steroids in favour of development of CRPC. In this study, we determined the steroid profile in tissue and serum samples from LNCaP xenograft models expressing high or low levels of ADRB2. Using a multi-steroid LC-MS/MS assay the levels of testosterone, dihydrotestostoren, progesterone and aldosterone were measured. Serum samples were taken at the day of castration and weekly up until termination. Tumor samples were homogenized to extract steroids for determination of the intratumoral concentration. The intratumoral cholesterol level was also measured, as this is an important precursor in the androgen synthesis. PSA was measured to follow the progression of the development of CRPC in the xenograft mice after castration. The results from our analysis indicated that the intratumoral levels of dihydrotestosterone, progesterone and aldosterone were increased in xenograft tumors with low level of ADRB2. The level of total cholesterol was also higher in xenograft tumors with low expression of ADRB2. The steroid profile from serum samples showed no difference in the levels of the measured steroids at the day of castration. We observed a decrease in serum levels of androgens in the time course analysis for both groups. Whereas the level of progesterone showed an initial increase after castration, followed by decreasing levels three weeks post castration.

Presenting author: Håkon Ramberg, Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Ullerenchausseen 70 0379 Oslo, Norway. Email: hakon.ramberg@rr-research.no.

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P16

Identification of protein kinases involved in AR transcriptional regulation in prostate cancer

Scott Walker, Peter Banks, Craig Robson & Kelly Coffey
Northern Institute for Cancer Research, Paul O'Gorman Building, The Medical School, Newcastle University, Newcastle upon Tyne, UK.

Phosphorylation of the Androgen Receptor (AR), has been shown to play an important role in directly modulating AR activity. However, the full extent of which protein kinases are involved in the regulation of the AR remains unknown. In order to address which kinases are important in the regulation of AR activity in both androgen sensitive and independent prostate cancer (PCa), a comprehensive siRNA kinome screen was performed. AR transcriptional regulation was evaluated using an AREIII-PSA Luciferase gene reporter assay in LNCaP and LNCaP-AI cell lines. Following parameter optimisation, the siRNA kinome screen identified multiple novel kinases involved in the regulation of AR transcriptional activity. Subsequent candidate validation led to the identification of a novel threonine/serine kinase, found to be critically involved in AR transcriptional activation. Both siRNA mediated KD and pharmacological antagonism, suppressed AR mediated transcriptional activation, as well as inhibiting PCa cell proliferation, including colony formation potential. Our comparative analysis of androgen sensitive and independent cells, indicates that this kinase is a promising therapeutic target in models of PCa. Subsequent evaluation of these kinase targets will help to discern novel mechanisms involved in AR regulation, as well as facilitate the development of more effective treatments for PCa.

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Presenting Author: Scott Walker, Northern Institute for Cancer Research, Paul O'Gorman Building, The Medical School, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK. Email: Scott.walker@ncl.ac.uk.

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P17

Next generation sequencing panels to predict response to hormonal therapy in prostate cancer

Heini M L Kallio[1], Matti Annala[1], Anniina Brofeldt[1], Reija Hieta[1], Kati Kivinummi[1], Teuvo Tammela[2], Matti Nykter[1], Hans G Lilja[1], G Steven Bova[1] & Tapio Visakorpi[1]
[1]Prostate Cancer Research Center, BioMediTech, University of Tampere and Fimlab Laboratories, Tampere University Hospital, Tampere, Finland; [2]Prostate Cancer Research Center, Department of Urology, Tampere University Hospital and School of Medicine, University of Tampere, Tampere, Finland.

Prostate cancer (PC) is the most common malignancy and third most common cause of cancer-related death among men in Europe. Although most PCs grow slowly, 20–25% of the patients believed to have organ-confined disease will experience biochemical recurrence already during 5-years of follow-up. The standard treatment against advanced PC is androgen deprivation (ADT). Unfortunately, androgen deprivation treatment eventually fails leading to the emergence of castration resistant PC (CRPC) that is lethal. However, also CRPCs are dependent on androgens. Owing to this understanding, several drugs have recently emerged for the treatment of CRPC including enzalutamide and abiraterone, but approximately 20–40% of patients have no response to these agents. One explanation to this could be the expression of constitutively active androgen receptor splice variants (AR-Vs). The aim of our project is to interrogate all possible AR aberrations in PC and CRPC. We have set up two NGS panels using Agilent's SureSelect Target Enrichment system allowing detection of all AR transcript variants as well as AR mutations, copy number variations and rearrangements. The assays have been validated with our existing whole-genome sequencing and RNA-seq data. We are now running samples representing hormone-naïve PC, neoadjuvant ADT treated PCs as well as CRPCs. The data will be presented in the meeting.

Presenting author: Heini M.L. Kallio, BioMediTech, University of Tampere, Lääkärinkatu 1, 33520 Tampere, Finland. Email: heini.kallio@uta.fi.

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P18

Altering androgen precursor availability impacts on endometrial function

Douglas A Gibson[1], Olympia Kelepouri[1], Ioannis Simitsidellis[1], Hilary O D Critchley[2] & Philippa T K Saunders[1]
[1]MRC/University of Edinburgh Centre for Inflammation Research, Edinburgh, UK; [2]Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK.

The establishment of pregnancy requires dynamic remodelling of the endometrium. Decidualization, a key part of this process, is characterised by differentiation of endometrial stromal fibroblasts (ESF) which secrete factors that regulate implantation and placental development. We recently discovered that ESF synthesise androgens which modulate the expression of endometrial receptivity and decidualization markers. Utilisation of the circulating androgen precursor DHEA within target tissues requires local action of the enzyme 3β-hydroxysteroid dehydrogenase (3βHSD) which we have previously reported is expressed in ESF. Although DHEA is abundant in the circulation, whether changes in the bioavailability of DHEA can affect endometrial function is not known. We hypothesised that modulating the bioavailability of DHEA could alter local tissue androgen concentrations and impact on decidualization and endometrial receptivity. Primary human endometrial stromal cells were isolated from endometrial biopsies collected from women during the proliferative phase of the cycle ($n=18$). Decidualization was induced *in vitro* (Progesterone + cAMP); some cells were co-treated with DHEA or with the 3βHSD inhibitor trilostane. DHEA increased testosterone biosynthesis ($P<0.001$) and was associated with increased expression of the decidualization marker *IGFBP1* ($P<0.05$). Trilostane significantly reduced biosynthesis of testosterone and secretion of IGFBP-1 ($P<0.001$) and decreased expression of the receptivity marker osteopontin (*SPP1*; $P<0.01$).These data demonstrate that changes in the bioavailability of

DHEA impact on the expression of decidualization and endometrial receptivity markers. These findings suggest a previously unrecognised role for tissue androgen bioavailability in the regulation of the endometrium which may impact on the establishment of pregnancy in women.

Presenting author: Douglas Gibson, MRC Centre for Inflammation Research, The Queens Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ. Email: D.A.Gibson@ed.ac.uk.

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P19

The AR/NCOA1 signaling regulates prostate cancer migration by involvement of PRKD1

Birgit Luef[1], Florian Handle[1], Gvantsa Kharaishvili[2], Martina Hager[3], Johannes Rainer[4], Günter Janetschek[5], Stephan Hruby[5], Christine Englberger[5], Jan Bouchal[2], Frédéric R Santer[1,†] & Zoran Culig[1,†]

[1]Division of Experimental Urology, Department of Urology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria; [2]Department of Clinical and Molecular Pathology and Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic; [3]Departments of Pathology, Paracelsus Medical University, Salzburg, Austria; [4]Division of Molecular Pathophysiology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria; [5]Urology, Paracelsus Medical University, Salzburg, Austria; [†]Joint senior authors.

Due to the urgent need for new prostate cancer (PCa) therapies, the role of androgen receptor (AR) interacting proteins should be investigated. In this study we aimed to address whether the AR coactivator nuclear receptor coactivator 1 (NCOA1) is involved in PCa progression. Therefore, we tested the effect of long-term *NCOA1* knockdown on processes relevant to metastasis formation. [^{3}H]-thymidine incorporation assays revealed a reduced proliferation rate in AR-positive MDA PCa 2b and LNCaP cells upon knockdown of *NCOA1*, whereas AR-negative PC3 cells were not affected. Furthermore, Boyden chamber assays showed a strong decrease in migration and invasion upon *NCOA1* knockdown, independently of the cell line's AR status. In order to understand the mechanistic reasons for these changes, transcriptome analysis using cDNA microarrays was performed. Protein kinase D1 (PRKD1) was found to be prominently up-regulated by *NCOA1* knockdown in MDA PCa 2b, but not in PC3 cells. Inhibition of *PRKD1* reverted the reduced migratory potential caused by *NCOA1* knockdown. Furthermore, PRKD1 was negatively regulated by AR. Immunohistochemical staining of PCa patient samples revealed a strong increase in NCOA1 expression in primary tumors compared to normal prostate tissue, while no final conclusion could be drawn for PRKD1 expression in tumor specimens. Thus, our findings directly associate the AR/NCOA1 complex with *PRKD1* regulation and cellular migration and support the concept of therapeutic inhibition of NCOA1 in PCa.

Presenting author: Frédéric R. Santer, Division of Experimental Urology, Department of Urology, Medical University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. Email: frederic.santer@i-med.ac.at.

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P20

Combined AR phosphorylation at serine 81 and serine 213 are associated with decreased survival in Castrate Resistant Prostate Cancer

Milly J McAllister[1], Pamela McCall[2], Mark A Underwood[3], Hing Y Leung[4] & Joanne Edwards[1]

[1]Institute of Cancer Sciences, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, UK; [2]University of Strathclyde, Glasgow, UK; [3]Department of Urology, Southern General Hospital, Glasgow, UK; [4]Cancer Research UK Beatson Institute, The Beatson Institute of Cancer Research, Glasgow, UK.

Current therapies for locally advanced or metastatic prostate cancer aim to inhibit androgen receptor (AR) activation directly or by depleting androgens via androgen deprivation therapy. However this therapeutic approach eventually fails in ~80% of patients, leading to development of castrate resistant prostate cancer (CRPC). There are currently few therapeutic options available for CRPC with limited prognostic or predictive biomarkers. The aim of the current study was to determine whether AR phosphorylation at serines 81 (pAR^{ser81}) and 213 (pAR^{ser213}) could be exploited as biomarkers. Immunohistochemistry of pAR^{ser81} and pAR^{ser213} was performed on 73 patients with CRPC and protein expression assessed using the weighted histoscore method. The relationship between pAR^{ser81}, pAR^{ser213} and cancer specific time to death from relapse (TTDR) was determined. High pAR^{ser81} and high pAR^{ser213} expression were associated with decreased TTDR (4.3 years vs 2.6 years, $P=0.013$ and 4.7 years vs 2.1 years, $P=0.000107$). Prognostic significance was further increased when AR phosphorylation at both serine residues were considered together with 5 year survival being stratified from 47 to 7% ($P=0.000042$). Patients with dual low expression had the longest TTDR (5.7 years vs 2.02 years, $P=0.000057$) when compared to those with dual high expression. On multivariate analysis, dual phosphorylation was independently associated with TTDR ($P=0.040$) when combined with known clinical parameters. Considering both pAR^{ser81} and pAR^{ser213} in combination may serve as a prognostic biomarker for CRPC and potential novel therapeutic targets.

Presenting author: Milly J McAllister, Institute of Cancer Sciences, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Garscube Estate, Switchback Road, G12 8QQ, Glasgow, Scotland. Email: m.mcallister.1@research.gla.ac.uk.

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P21

Investigating the role of SUMOylation of androgen receptor splice variants by SUMO1 in castration resistant prostate cancer

Evangelia E Kounatidou & Luke Gaughan

Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK.

Prostate cancer (PC) is currently the most commonly diagnosed non-cutaneous cancer affecting UK men. Androgen deprivation therapy (ADT) has traditionally been used as the gold standard treatment for advanced PC. Despite the initial response to androgen ablation, tumours relapse and become refractory to clinically approved anti-androgens, resulting in castration-resistant PC (CRPC). In CRPC, androgen receptor (AR) signalling is inappropriately restored by AR splice variants (AR-Vs). AR-Vs lack the C-terminal ligand-binding domain, that is the target of anti-androgens, but retain the N-terminal transactivation domain and DNA-binding domain, and are thus capable of sustaining the androgenic signalling programme in castrate conditions, remaining unchallenged by the current anti-AR agents. Regulatory post-translational modifications are highly implicated in PC progression. Modification of the full-length AR (FL-AR) by SUMO1 compromises AR transcriptional activity and cell proliferation *in vitro*. Consistent with FL-AR, we report that the CRPC-relevant AR-V7 and V1/2/3/2b variants are modified by SUMO1 at lysines 386 and 520 within the N-terminal domain. K386 is the major site for polySUMOylation, whilst K520 is likely the substrate for monoSUMOylation. The presence of other potential acceptor lysines as well as the role of AR-V SUMOylation in CRPC are currently being investigated. A comprehensive understanding of how these AR-Vs are regulated by upstream signals such as SUMOylation is of great significance in the finding of more effective treatments. This project aims to highlight key regulatory processes of AR-Vs and conceivably identify potential targets that may be exploited in the clinic to greatly benefit patients with advanced PC.

Presenting author: Evangelia K. Kounatidou. Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK. Email: e.e.kounatidou2@ncl.ac.uk.

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P22

The cellular and molecular effects of the androgen receptor agonist, Cl-4AS-1, on breast cancer cells

Mamoun Ahram, Ebtihal Mustafa, Shatha Abu Hammad, Mariam Hodhod & Malek Zihlif

Department of Physiology and Biochemistry, Faculty of Medicine, The University of Jordan, Amman, Jordan.

The notable expression of the androgen receptor (AR) in breast cancer suggests an important biological role and, hence, a window of utilizing it as a therapeutic target. Due to the undesirable side effects of AR agonists, attempts have been undertaken to develop tissue-selective androgen receptor modulators (SARMs). One such SARM is Cl-4AS-1, which has previously been shown to behave similar to the natural AR agonist, dihydrotestosterone (DHT). We aimed to examine the effects of this drug more closely at the molecular and cellular levels. Different breast cancer cell lines were utilized, namely the luminal MCF-7 cells, the molecular apocrine MDA-MB-453 cells, and the basal MDA-MB-231 cells. There was high and significant concordance in the regulation of gene expression between DHT and Cl-4AS-1. In addition, both drugs caused a similar morphological change of the MDA-MB-453 cells into a fibroblast-like phenotype. Treatment of cells with DHT resulted in increased proliferation of the MCF-7 and MDA-MB-453 cells, but not effect was observed on the growth of the MDA-MB-231 cells. On the other hand, increasing doses of Cl-4AS-1 induced an identical dose-dependent inhibition on the growth of the three cell lines. This inhibition appeared to be a result of stimulation of apoptosis. Cell cycle analysis revealed that Cl-4AS-1 blocked cell progression into the S-phase, which was followed by DNA degradation. These results indicate that Cl-4AS-1 has unique properties making it a possible drug for the treatment of breast cancer.

Presenting author: Mamoun Ahram, Department of Physiology and Biochemistry, Faculty of Medicine, The University of Jordan, Amman 11942, Jordan. Email: m.ahram@ju.edu.jo.

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P23

Characterising mechanisms of aberrant androgen receptor signalling in advanced prostate cancer

Wenrui Guo, Craig Robson & Luke Gaughan

Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK.

Although new treatments for castrate-resistant prostate cancer (CRPC), such as enzalutamide and abiraterone, have shown promise, moderate response rates and development of resistance to these agents has limited their clinical effectiveness. It is therefore vital we improve our understanding of androgen receptor (AR) re-activation in advanced disease, focusing particularly on regulatory processes governing activity of AR mutants and splice variants (AR-Vs), to enable the development of patient-orientated treatments in CRPC. Our previous research utilising an AR replacement model in LNCaP cells demonstrated that the bicalutamide-activated AR_{W741L} mutant selectively regulates a gene-set distinct from endogenous AR_{T877A} offering new insights into discriminate functionality and opportunities for selective drug targeting of AR mutants. In order to enable more physiological modelling of aberrant AR activity, and to define their global transcriptome and cistrome, we are currently generating CRISPR-edited LNCaP and CWR22Rv1 cell lines expressing AR_{W741L}, and respective abiraterone- and enzalutamide-activated AR_{H874Y} and AR_{F876L} mutants. We have verified our designed sgRNA/Cas9 targeting AR exons 5 and 8 to enable knock-in mutations at positions W741 and H874Y/F876L, respectively in both cell lines. The activity of each AR mutation in the two cell lines will be assessed using (i) candidate AR target genes expression analysis, (ii) AR immunofluorescence and (iii) RNA- and chromatin immunoprecipitation sequencing. The expected outcomes for this project is to comprehensive profile aberrant AR signalling that is vital for therapeutic exploitation to ultimately benefit in advanced PC patients.

Presenting author: Wenrui Guo, Newcastle University Northern, Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington place, Newcastle upon Tyne, NE2 4HH, UK. Email: w.guo4@newcastle.ac.uk.

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P24

Identification and characterization of a CRM1/XPO1-dependent nuclear export signal in the human androgen receptor

Stefanie V Schütz[1], Axel Merseburger[2], Anca Azoitei[1] & Marcus V Cronauer[2]

[1]Department of Urology, University of Ulm, Ulm, Germany; [2]Department of Urology, UKSH Campus Lübeck, Lübeck, Germany.

There is experimental evidence that inhibition of the glycogen synthase kinase-3β (GSK-3β) by small molecule inhibitors induces a rapid, CRM1/XPO1-dependent nuclear export of the AR protein in human prostate cancer cell lines. By contrast nucleo-cytoplasmic shuttling of Q641X, a C-terminally truncated AR-mutant, remains unaffected by GSK-3β inhibition. *In silico* analysis of the AR C-terminus (amino acids [aa] 641-920) predicted two putative NES-sites, located between aa 790-840 of the human AR. Using site-directed mutagenesis we were able to identify 3 aa involved in XPO1/CRM1 mediated nuclear export of the AR. A comparative analysis of the resulting NES peptide sequence showed that it is evolutionary highly conserved among vertebrate species (fish, amphibia, modern sauropsida, mammalia). The latter suggests that this NES plays an important role in the regulation of AR-function and/or signaling.

Presenting author: Marcus Cronauer, Department of Urology, UKSH Campus Lübeck, Lübeck, Germany. Email: marcus.cronauer@uksh.de.

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P25

Mitochondrial function and mitochondrial heteroplasmy levels differ between benign and malignant prostate tissue.

Bernd Schöpf[1], Georg Schäfer[2,3], Hansi Weissensteiner[1], Erich Gnaiger[4] & Helmut Klocker[2]

[1]Division of Genetic Epidemiology, Department of Medical Genetics; [2]Division of Experimental Urology, Department of Urology; [3]Department of Pathology; [4]D. Swarovski Research Laboratory, Department of General and Transplant Surgery, Medical University of Innsbruck, Austria

Mitochondria play a vital role in cellular bioenergetics, providing energy via oxidative phosphorylation (OXPHOS) and metabolic intermediates. Aerobic ATP production is orchestrated by a multi-enzyme complex, which uses the electrons gained during oxidation of energy substrates to generate an electrochemical potential across the mitochondrial (mt) membrane that drives ATP synthase. Although there is evidence that mitochondrial function as well as mtDNA mutations might play a role in tumor formation and progression, combined analysis of mt-physiology and DNA alterations are lacking. We analyzed mitochondrial respiration using high-resolution respirometry (OROBOROS Oxygraph-2k). By applying various mitochondrial substrate-uncoupler-inhibitor combinations we dissected the contribution of mitochondrial electron transfer pathways to oxidative phosphorylation. Relative mtDNA copy numbers were determined by duplex qPCR, mtDNA mutations by next-generation sequencing (Ion Torrent Proton). Sequencing data were analyzed using our newly developed mtDNA-Server analysis pipeline (http://mtdna-server.uibk.ac.at). Significant differences in BE and CA tissue were found with respect to the respiratory capacities of Complex I- and Complex II-linked pathways (CI and CII). CA tissue exhibited lower CI-linked respiration which was compensated by higher CII-linked capacities. No correlation was found between mt-function and mtDNA copy number. A comparison of mtDNA sequence variations in BE and CA tissue revealed a significantly higher overall mutation rate in malignant tissue. Most interestingly, deleterious mutations in regions coding for Complex I components strongly correlated with a relative loss of CI-linked respiration in both CA and BE tissue. No statistically significant correlation of mt-mutation burden was found to tumor Gleason scores or patients' serum PSA levels.

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Presenting Author: Bernd Schöpf, Division of Genetic Epidemiology, Department of Medical Genetics, Medical University of Innsbruck, Schöpfstraße 4, 6020 Innsbruck, Austria. Email: Bernd.schoepf@gmail.com

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P26

Mechanisms behind tumor relapse in 22Rv1 xenografts after treatment with abiraterone or cabazitaxel

Erik Bovinder Ylitalo & Pernilla Wikström

Department of Medical Biosciences, Umeå University, Umeå, Sweden

Castration-resistant prostate cancer (CRPC) develops after androgen deprivation therapy of advanced PC, often with metastatic growth in bone and very poor prognosis. Standard treatment is docetaxel, followed by androgen-synthesis (abiraterone) or androgen receptor inhibition, or treatment with the novel taxane cabazitaxel. Novel drugs are constantly under development and molecular properties of individual tumors will determine treatment effectiveness in individual cases. The aim of this study was to identify possible response and resistance mechanisms towards Abiraterone acetate and Cabazitaxel in the human PC 22Rv1 xenograft. Xenografts were established by subcutaneous inoculation of 22Rv1 PC cells in nude mice and treated with vehicle and/or sham operation ($n=23$), castration by surgical incision ($n=7$), abiraterone ($n=9$), cabazitaxel ($n=7$), castration plus abiraterone ($n=8$), or castration plus cabazitaxel ($n=11$). Therapy response and tumor progression/relapse were monitored by measurement of tumor volume. Animals were followed until tumor progression and sacrificed when tumor size reached $\sim 1000\ mm^3$. Preliminary results showed very little effect of abiraterone in hindering tumor progression while cabazitaxel induced complete tumor regression in 3 animals and tumor regression followed by relapse in 15 animals. Cell lines from xenografts relapsing after cabazitaxel treatment were established. Analysis of mechanisms behind response and resistance is ongoing; analysis of AR, AR-V7, steroidogenic enzymes and whole genome expression patterns. Results are probably related to 22Rv1 cell expression of constitutively active AR variants, including the AR-V7, making tumor cells resistant to androgen-deprivation (castration, abiraterone) but, due to high proliferation rate, sensitive to cabazitaxel.

Presenting Author: Erik Bovinder Ylitalo, Department of Medical Biosciences, Umeå University, By 6M Sjukhusområdet, 901 85, Umeå, Sweden. Email: erik.bovinder@umu.se

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P27

The amino terminal domain of steroid hormone receptors as a novel drug target: Identification of small molecule inhibitors

Amy E. Monaghan[1], Stuart McElroy[2] & Iain J. McEwan[1]

[1]Institute of Medical Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK; [2]Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, UK

The role of the androgen receptor (AR) in the progression of prostate cancer (PCa) is well established. Competitive inhibition of the AR ligand binding domain (LBD) has been the staple of antiandrogen therapies employed to combat the disease in recent years. However their efficacy has often been limited by the emergence of resistance, mediated through point mutations and receptor truncations. As a result the prognosis for patients with malignant castrate resistant disease remains poor. The amino terminal domain (NTD) of the AR has been shown to be critical for AR function. Its modular activation function (AF-1) is important for both gene regulation and participation in protein-protein interactions. However due to the intrinsically disordered structure of the domain, its potential as a candidate for therapeutic intervention has in the past been dismissed. The recent emergence of the small molecule EPI-001 has provided evidence that the AR-NTD can be targeted therapeutically, independent of the LBD. We have developed a phenotypic cell based assay for novel AR modulators and used this to screen a library of over 7,000 chemically diverse molecules. We have identified and begun characterising four novel compounds with the potential to inhibit the AR. Targeting of the AR-NTD has the potential to disrupt multiple inter-molecular interactions between the AR and its coregulatory binding partners, in addition to intra-molecular cross-talk between the domains of the AR. Therapeutics targeting these protein-protein interactions, or the NTD directly should also have efficacy against emerging AR splice variants which may play a role in PCa progression.

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Presenting Author: IJ McEwan, Institute of Medical Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, Scotland, UK. Email: iain.mcewan@abdn.ac.uk

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P28

Evidence for neuroendocrine progenitor cells in a transgene mouse model of prostate cancer

Simon Udovica[1], Alexander Otahal[1], Erwin Tomasich[1], Gerwin Heller[1], Michael Schwarz[1], Andreas Spittler[2], Reinhard Horvat[3], Peter Horak[4], Maximilian Marhold[1] & Michael Krainer[1]

[1]Department for Internal Medicine I, Oncology, Medical University of Vienna, Vienna, Austria; [2]Department of Surgery, Medical University of Vienna, Vienna, Austria; [3]Institute for Pathology, Medical University of Vienna, Vienna, Austria; [4]DKFZ, Heidelberg, Germany

In developed countries, prostate cancer (PCa) is the most prevalent cancer and the second most common cause of cancer related death in males. Tumors from patients exhibiting disease progression after systemic androgen deprivation treatment (ADT), referred to as castration resistant prostate cancer (CRPC), often show differentiation towards an aggressive phenotype – neuroendocrine prostate cancer or NE-PC. In our study, we used the Simian-Virus 40 (SV-40) T-antigen driven model of the mouse prostate (TRAMP) as a model for studying carcinogenesis of NE-PC on a cellular level. By isolation of cell populations of both basal and non-basal compartments of the murine prostate epithelium using flow cytometry (FACS), we traced down the roots of neuroendocrine differentiation in TRAMP mice: When successfully engrafted subcutaneously in NSG mice or grown as spheroids *in vitro*, non-basal cells harboring upregulation of neuroendocrine markers showed growth advantages when compared to cells of the basal cell compartment. Further, by means of RNA sequencing, we found potential novel therapeutical targets and biomarkers for these cells.As a conclusion, we isolated and further molecularly characterized non-basal cells exhibiting neuroendocrine and progenitor functions from TRAMP tumors, thus creating preclinical models for NE-PC *in vivo* and *in vitro*. Our findings may help in developing novel treatment approaches as well as biomarkers within this entity.

Presenting Author: Maximilian Marhold, Department for Internal Medicine I, Oncology, Medical University of Vienna, Währinger Gürtel, 1090 Vienna, Austria. Email: maximilian.marhold@meduniwien.ac.at

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Potential of Metabolome Analysis for new Insights into System Biology and Relevance for Translational Medicine

Therese Koal

BIOCRATES Life Sciences AG, Innsbruck, Austria

All characteristic metabolic properties of a bio-fluid, cell, tissue or an organism are described by its metabolome, which is the functional readout of the genome and proteome. Metabolomics is the newest member in the "omics" cascade investigating the metabolome within a specific biological matrix (e.g. serum, tissue, cells or CSF) offering the possibility of assessing the relationship of a genetic modification to a specific desired phenotype in an effort to determine the critical biochemical pathways involved. Today the emerging technology of (targeted) metabolomics contributes extremely successful in new insights of metabolic phenotyping (metabotypes) and as a logical consequence in improved metabolic understanding of complex diseases and related biomarker discovery (e.g. metabolic disorders, oncology, cardiovascular diseases) for disease development, progression, treatment, and drug function and assessment. Over 600 metabolites can be identified, quantified easily and quality controlled on a newly developed metabolomics platform and kits with high reproducibility and accuracy. The set of analytes consists of the following classes i.e. acylcarnitines, amino acids, biogenic amines, eicosanoids, lipids, steroids, neurotransmitters, bile acids, energy metabolism, and oxysterols, in which the appropriate pathways are partly linked to androgens. The developed metabolomics platform and kits products represents a powerful tool for the standardized metabolome analysis allowing a global interlaboratory comparibility of the sample and sample data analysis for more than 20 different tested biological matrices in more than 15 different species. Current metabolic biomarker studies covering different application areas will be presented underlying the power and clinical future of metabolome analysis.

Presenting Author: Therese Koal, BIOCRATES Life Sciences AG, Eduard-Bodem-Gasse 8, A-6020 Innsbruck, Austria. Email: therese.koal@biocrates.com

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P30

Critical Role of Androgen Receptor Level in Prostate Cancer Cell Resistance to New Generation Antiandrogen Enzalutamide

Julia Hoefer[1], Mohammady Akbor[1,2], Florian Handle[1], Philipp Ofer[1], Martin Puhr[1], Walther Parson[3,4], Zoran Culig[1,5], Helmut Klocker[1] & Isabel Heidegger[1]

[1]Department of Urology, Division of Experimental Urology, Medical University of Innsbruck, Innsbruck, Austria; [2]School of Biosciences and Veterinary Medicine, University of Camerino, Italy; [3]Institute of Legal Medicine, Medical University of Innsbruck, Innsbruck, Austria; [4]Forensic Science Program, The Pennsylvania State University, University Park, Pennsylvania, USA; [5]Center of Biomolecular and Cellular Engineering, International Clinical Research Center, St. Anne's Hospital Brno, Czech Republic

Enzalutamide is an androgen receptor (AR) inhibitor approved for therapy of metastatic castration resistant prostate cancer. However, clinical application revealed that 30 to 40% of patients acquire resistance after a short period of treatment. Currently, the molecular mechanisms underlying such insensitivities are not completely understood, partly due to a lack of model systems. In the present study we established three different cellular models of enzalutamide resistance including a cell line with wild type AR (LAPC4), DuCaP cells which overexpress wild-type AR, as well as a cell which has been adapted to long term androgen ablation (LNCaP Abl) and harbors the AR T878A mutation. After 10 months of cultivation in the presence of increasing concentrations of the enzalutamide (up to 8 μM], sustained growth of resistant sub cell-lines was achieved. When compared to controls, resistant cells exhibit significantly decreased sensitivity to enzalutamide as measured with ^{3}[H]thymidine incorporation and WST assay. Moreover, these cell models exhibit partly re-activated AR signaling despite presence of enzalutamide. In addition, we show that enzalutamide resistant cells are insensitive to bicalutamide but retain considerable sensitivity to abiraterone. Mechanistically, enzalutamide resistance was accompanied by increased AR full length and AR-V7 mRNA and protein expression as well as AR gene amplification, while no additional AR mutations have been identified.

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Presenting Author: Julia Hoefer, Department of Urology, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria. Email: Julia.hoefer@i-med.ac.at

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Mediators of stress resistance in prostate cancer cells

Adam Pickard[1], Francesca Amoroso[1], Lorna May-Stewart[1], Jonathan McComb[1] & Ian G. Mills[1,2,3]

[1]Prostate Cancer UK/Movember Centre of Excellence, Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UK; [2]Prostate Cancer Research Group, Centre for Molecular Medicine (Norway), University of Oslo and Oslo University Hospitals, Oslo, Norway; [3]Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospitals-Radium Hospital, Montebello, Oslo, Norway

The emergence of prostate cancer and the progression of the disease are significantly driven by the androgen receptor (AR) in combination with other transcription factors. The evolution of aggressive disease requires tumours to become resistant to metabolic stress and subsequently therapeutic stress. Given the role of the prostate gland has a secretory organ characterised by high rates of protein synthesis particularly in AR-positive luminal epithelial cells there are inherent biologies that contribute to stress resistance, notably protein folding. This presentation will outline some example of the AR-dependent pathways which contribute to stress resistance and how these evolve as metastatic disease emerges culminating in pro-inflammatory signalling and immune evasion. At each stage in this process there are opportunities to repurpose therapeutics which may restrict disease progression.

Presenting Author: Ian Mills, Centre for Cancer Research and Cell Biology (CCRCB), Queen's University of Belfast, BT9 7AE, Belfast, UK. Email: I.Mills@qub.ac.uk

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Differential expression and androgen regulation of microRNA molecules in breast cancer cells

Mamoun Ahram, Rand Zaza, Ebtihal Mustafa, Heba Jarrar, Razan Al-Saber, Shatha Abu Hammad & Malek Zihlif

Department of Physiology and Biochemistry, Faculty of Medicine, The University of Jordan, Amman, Jordan

MicroRNA molecules (miRNAs) have important roles in regulating cell behavior. In addition, their expression levels can be used in tumor classification. The expression of miRNAs has been to be regulated by the androgen receptor (AR), which seems to play an interesting role in the tumorigenic process of breast cancer. We hypothesized that AR may control the behavior of breast cancer cells via modulating the expression of miRNAs. Using PCR arrays, we examined the differential expression of 84 miRNAs in three breast cancer cell lines, the luminal MCF-7 and T47D cells and the molecular apocrine MDA-MB-453 cells. Each cell line had distinct miRNA expression with let-7a and -7b as markers of the MDA-MB-453 cells, and miR-205 as a marker for the luminal cell lines. Treatment of the cells with the AR agonist, CI-4AS-1, resulted in dissimilar alterations in miRNA expression among the three cell lines. CI-4AS-1 reduced the expression let-7a, let-7d, and miR-205-5p in the MDA-MB-453 cells. These three molecules are known to block epithelial-to-mesenchymal transition (EMT) and, interestingly, their reduction paralleled a dramatic morphological alteration of the molecular apocrine cells into fibroblast-like shape. The expression of two other miRNAs, miR-100-5p and miR-125-5p, was also decreased. These two molecules have previously been shown to down-regulate the expression of the metalloprotease-13 (MMP-13), which was up-regulated in the MDA-MB-453 cells following AR activation. Collectively, these data indicate that miRNA molecules can differentiate breast cancer cells and that AR may control the biological behavior of breast cancer cells and protein expression via regulating the expression of miRNAs.

Presenting Author: Mamoun Ahram, Department of Physiology and Biochemistry, Faculty of Medicine, The University of Jordan, Amman 11942, Jordan. Email: m.ahram@ju.edu.jo.

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P33

Phosphorylation of androgen receptor at serine 81 by cyclin-dependent kinases

Zuzana Skrášková, Radek Jorda, Eva Řezníčková & Vladimír Kryštof

Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Olomouc, Czech Republic

The androgen receptor (AR) is a steroid-hormone receptor that plays a crucial role in the normal development of male reproductive tissues. Its high expression and/or relaxation of its regulation are strongly implicated in prostate cancer (PCa). Androgen binding induces conformational changes of AR that influence its subcellular localization, interactions with other proteins, DNA binding and transcriptional activity. Androgen dependent is also AR(S81) phosphorylation. Phosphorylation of AR at position of serine 81 was first associated with CDK1, in another study, it was found that CDK9 can mediate AR(S81) phosphorylation as well. In both studies, experiments were performed with pan-selective CDK inhibitors such as roscovitine or flavopiridol, therefore we decided to elucidate effects of CDKs on AR phosphorylation at serine 81 in detail. In our study, we transiently silenced CDK1, 2, 4, 7 and 9 by transfecting PCa cells with CDK-specific siRNA. Observed effect was compared with changes in AR phosphorylation at S81 after chemical inhibition using CDK inhibitors with confirmed selectivity against particular CDKs. According to our results, phosphorylation of AR at S81 can be mediated by several CDKs.

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Presenting Author: Zuzana Skrášková, Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacky University & Institute of Experimental Botany ASCR, Slechtitelu 27, 78371 Olomouc, Czech Republic. Email: skraskova.zuzana@gmail.com

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Mechanisms of radioresistance in prostate cells

Fabian Guggenberger[1], Holger Erb[2], Ira-Ida Skvortsova[3], Zoran Culig[1] & Frédéric R. Santer[1]

[1]Division of Experimental Urology, Medical University of Innsbruck, Innsbruck, Austria; [2]YCR Cancer Research Unit, Department of Biology, University of York, York, UK; [3]Department of Therapeutic Radiology and Oncology, Medical University of Innsbruck, Innsbruck, Austria

Among androgen deprivation therapy (ADT), radiation therapy is an approved treatment either for early stage local PCa, but also for metastatic M1 stage PCa. However, tumour relapse is a frequent event that affects about 80% of patients undergoing prior treatment. There is increasing evidence that the occurrence of cancer stem cells (CSC) may play an important role in therapy resistance, in particular also in radioresistance. However, our knowledge on the mechanisms of radioresistance of the prostatic basal layer is still limited. The aim of this study is to identify a novel molecular mechanism underlying radioresistance in the prostate basal cell layer containing stem cells. In this study benign primary prostate basal epithelial cells (PrEPs) obtained from 5 patients were isolated and cultured in the presence of a feeder layer. The PrEPs were characterised by clonogenic assays and by label retention assay using FACS analysis subsequent to PKH67 membrane labelling, which might indicate the presence of stem cells within the cultures. The PrEPs were irradiated for 21 times following a therapeutic schedule with 0, 0.5 or 1 Gray using a linear particle accelerator (LINAC). NextGen transcriptome sequencing was performed and bioinformatical analysis, including pathway analysis, is in progress. Once it is finished a careful review of literature is performed. Based thereon a candidate gene, whose expression is shown to be strongly altered by irradiation, is chosen for further experiments (e.g. knock-down, over-expression, inhibition) that will test the candidate's involvement in radioprotection. This may improve co-treatment strategies for future irradiation therapy.

Presenting Author: Fabian Guggenberger, Department of Urology, Innsbruck Medical University, Anichstrasse 35, A-6020 Innsbruck, Austria. Email: fabian.guggenberger@i-med.ac.at

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P35

Isolation, propagation and characterisation of primary prostate cancer epithelial cell lines from prostate specimens

Samantha Patek[1,2], Pamela McCall[1], Mark A Underwood[3] & Joanne Edwards[1]

[1]Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow G12 8QQ, UK; [2]Academic Department of Surgery, School of Medicine, University of Glasgow, Walton Building, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, G4 0SF, UK; [3]Department of Urology, Queen Elizabeth University Hospital, Glasgow G31 2ER, UK

Prostate cancer is the most common male cancer in the UK. Currently there is a lack of pre-clinical models to predict patient's response to treatment for prostate cancer. Identifying which patients will respond best to treatment avoids exposing patients to treatment side effects unnecessarily. Primary cell culture provides a translational model to predict individual patient's response to drug treatments. In this study, we develop a technique for isolation, propagation and characterisation of primary prostate cancer cells in 2-D culture from prostate specimens. Presence of cytokeratin (CK) 18, a luminal epithelial cell marker, and absence of CD90 (fibroblast cell marker) was confirmed using flow cytometry. The presence of CK 8/18, androgen receptor (AR) and prostate specific antigen (PSA) and absence of high molecular weight cytokeratin (HMWCK) on immunofluorescent imaging suggests a luminal epithelial phenotype with functioning AR. RNA was extracted from the cells and RT-PCR performed. Expression of alpha-methylacyl-CoA racemase (AMACR), fatty acid synthase (FASN), Golgi membrane protein 1 (GOLM1), AR and kallikrein related peptidase 3 (KLK3 – gene for PSA protein) was increased in both primary cell lines compared to PNT2 cells (benign) and comparable in both to that of two established prostate cancer cell lines (LNCaP and VCaP) suggesting a malignant phenotype with functioning AR. We have established a method to develop and characterise primary prostate cancer cell lines, which is of high translational relevance. This method has potential for use to predict a patient's response to prostate cancer therapies, progressing towards personalised prostate cancer treatment.

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Presenting Author: Samantha Patek, Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow G12 8QQ, SCOTLAND. Email: s.payne.2@research.gla.ac.uk

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Androgen and Estrogen Receptor Co-regulation of Human UDP-glucuronosyltransferases 2B15 and 2B17 in Breast Cancer

Dong Gui Hu[1,†], Luke Selth[2,†], Gerard Tarulli[2], Robyn Meech[1], Dhilushi Wijayakumara[1], Apichaya Chanawong[1], Roslin Russell[3], Carlos Caldas[3], Jessica LL Robinson[4], Jason Carroll[4], Wayne Tilley[2], Peter Mackenzie[1] & Theresa Hickey[2]

[1]Department of Clinical Pharmacology, Flinders University School of Medicine, Flinders Medical Centre, Bedford Park SA 5042, Australia; [2]Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, The University of Adelaide, Adelaide, SA 5005, Australia; [3]Breast Cancer Genomics Group, Cancer Research UK, Cambridge Institute, Cambridge University, Cambridge, UK; [4]Nuclear Transcription Factor Laboratory, Cancer Research UK, Cambridge Institute, Cambridge University, Cambridge, UK; [†]Authors provided equal contribution.

Glucuronidation is an enzymatic process that terminally inactivates steroid hormones, including estrogens and androgens, thereby influencing carcinogenesis in hormone-dependent cancers. While estrogens drive breast carcinogenesis via the estrogen receptor alpha (ERα), androgens play a critical role as prohormones for estrogen biosynthesis and ligands for the androgen receptor (AR). Herein, the expression and regulation of two androgen inactivating UDP-glucuronosyltransferase (UGT) enzymes, UGT2B15 and UGT2B17, was assessed in breast cancer. In large clinical cohorts, high *UGT2B15* and *UGT2B17* levels positively influenced disease-specific survival in distinct molecular subgroups of breast cancer. Expression of these genes was highest in ERα+ cases, and in cell line models, ERα, FOXA1 and AR co-operatively increased transcription via tandem binding events at their proximal promoters. ERα activity was dependent on FOXA1, facilitated by AR activation, and potently stimulated by estradiol as well as estrogenic metabolites of 5α-dihydrotestosterone. AR activity was mediated via binding to an estrogen receptor half site 3' to the FOXA1 and ERα binding sites. Although AR and FOXA1 bound the UGT promoters in AR+, ERα-negative breast cancer cell lines, androgen treatment did not influence basal transcription levels. *Ex vivo* culture of human breast tissue and ERα+ tumors provided evidence for up-regulation of *UGT2B15* and *UGT2B17* by estrogen or androgen treatment. ERα binding was evident at their promoters in a small cohort of primary tumors and distant metastases. Collectively, this data provides insight into sex steroid receptor-mediated regulation of androgen inactivating enzymes in ERα+ breast cancer, which may have subtype-specific consequences for disease progression and outcomes.

Presenting Author: Theresa Hickey, Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, DX Number 650 801, The University of Adelaide SA 5005, Australia. Email: theresa.hickey@adelaide.edu.au

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Abstract withdrawn.

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Histological modeling of prostate cancer

Pekka Ruusuvuori, Mira Valkonen, Kimmo Kartasalo, Tapio Visakorpi, Leena Latonen & Matti Nykter
BioMediTech, University of Tampere, Tampere, Finland

Prostate cancer is multifocal in nature, and histological grading is the key clinical prognostic factor. To build non-subjective histological analysis tools, and to model the multifocality of prostate cancer within the organ, we use analysis of histological images to quantitatively describe prostate cancer. Our current effort shows how heterogeneity in prostate tissue due to cancer or spatial location can be quantified with image-derived features. We use high-resolution digital whole slide images of serial sections of H&E stained tissue, enabling to use data from whole organ for quantitative analysis. We use automated image analysis for extracting several (hundreds) local descriptors capturing the characteristics of each spatial location. The descriptors include several common image morphology, texture and intensity features as well as features specifically engineered for characterizing the spatial context of the region. We then use these descriptors for building a discriminative model for normal and cancerous tissue, as well as for separating spatial locations within prostate. Specifically, we use machine learning for building a classifier model, yielding a subset of informative features related to spatial heterogeneity. Our hope is that this will lead to increased knowledge of the histological changes in prostate. Our aim is to histologically model prostate cancer in 3D, and in the future, combine sequencing based measurements in the three-dimensional spatial context. Furthermore, we will correlate genomic measurements with histological features.

Presenting Author: Pekka Ruusuvuori, BioMediTech, University of Tampere, FI-33014, Tampere, Finland. Email: pekka.ruusuvuori@uta.fi

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Androgen receptor variant 7 induces cellular senescence

Zeynep Kaya & Nathan A. Lack
Koç University, School of Medicine, Istanbul, Turkey

Androgen receptor (AR) signaling is critical at all stages of prostate cancer including chemically castrated late-stage forms of the disease. How the AR can initiate transcription in the absence of androgens has been proposed to occur through several mechanisms including the production of constitutively active androgen receptor variants. Lacking a ligand binding domain, these variants are intrinsically resistant to all clinically approved AR antagonists and have been found to correlate to drug resistance. Yet many fundamental questions still remain about their mechanism of action. Given the proposed clinical importance, a better understanding of ARV7 activation is critical to treating late-stage prostate cancer patients. Surprisingly, in LNCaP single cell colonies expressing a tetracycline-inducible ARV7 we found that overexpression of the variant rapidly causes marked cellular senescence and the degree of senescence was dependent on the level of expression. Senescence was independent of full-length AR and was mediated by AR transcriptional activity. ARV7 was found to induce senescence via ROS production which leads to the activation of the p53 pathway. Demonstrating that this phenotype was not limited to LNCaP cells, we observed a similar senescence in several androgen responsive prostate cancer cell lines. Interestingly, we were able to identify several LNCaP clones that were resistant to expression of ARV7. Overall our results demonstrate that constitutive activity of ARV7 is not tolerated in AR dependent cells and may represent a “barrier” for the cancer to overcome.

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Presenting Author: Nathan A. Lack, Koç University, School of Medicine, Istanbul, Turkey. Email: nlack@ku.edu.tr

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Segment-specific enrichment of AP-2 and Runx motifs within caput- and IS-preferred androgen receptor binding sites in the mouse epididymis

Päivi Pihlajamaa[1], Biseajyoti Sahu[1], Olli A. Jänne[2], Matti Poutanen[3] & Petra Sipilä[3]
[1]Genome-Scale Biology, Research Programs Unit, University of Helsinki, Helsinki, Finland; [2]Institute of Biomedicine, University of Helsinki, Helsinki, Finland; [3]Department of Physiology and Turku Center for Disease Modeling, Institute of Biomedicine, University of Turku, Turku, Finland

Epididymis is an androgen dependent organ with four main segments; initial segment (IS), caput, corpus and cauda. It is an essential site for post-testicular sperm maturation, as spermatozoa acquire fertilizing capacity during epididymal transit. Post-testicular maturation is dependent on the proteins expressed and secreted to the lumen by epididymal cells. Androgens regulate gene expression in epididymis in a segment-specific manner. Caput-specific genes are androgen regulated, whereas IS-specific genes are mainly regulated by other testicular factors. However, the basis of differential androgen responsiveness of IS and caput genes is still unknown. We have performed in vivo ChiP-seq analyses on androgen receptor (AR) in IS and caput epididymides. Our results demonstrated differential AR binding in the two segments with 20,138 and 7,072 androgen receptor-binding sites (ARBs) in caput and IS, respectively. Motif enrichment analyses for the cis-elements within the ARBs revealed that 100% of IS-preferred sites had adjacent Runx motif, whereas caput-preferred ARBs showed no enrichment for Runx motif. Instead, caput-preferred sites were enriched with contiguous AP-2α cis-elements. We then compared genome wide distribution of activating histone marks; H3K27ac and H3K4me2. Majority of genomic locations in IS and caput showed similar patterns of activating histone marks around the ARBs. However, there were still marked differences in some individual genes. In conclusion, differential enrichment for cis-elements within caput- and IS-preferred ARBs suggest that collaborating transcription factors, AP-2 and Runx, might affect the segment-specific androgen regulation of gene expression. Furthermore, differences in activating histone marks may explain some differences in gene expression profiles.

Presenting Author: Petra Sipilä, Department of Physiology, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, 20520 Turku, FINLAND. Email: petra.sipila@utu.fi

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Characterisation of Androgen and Estrogen Receptor Cross-Talk

Mohammad Alkheilewi, Rosie Bryan & Greg Brooke
School of Biological Sciences, University of Essex, Colchester, CO4 3Q20, UK

Worldwide, breast cancer is the most common cancer to develop in women. Steroid receptors play a key role in the progression of the majority of breast cancer subtypes. Estrogen Receptor-α (ERα), for example drives the growth of approximately 70% of tumours and is therefore a useful therapeutic target for this disease. However, the Androgen Receptor (AR) is the highest expressed receptor in normal breast and appears to also play an important role in breast cancer. In contrast, in ERα positive disease the crosstalk between ERα and AR is inhibitory to tumour growth. The mechanisms underlying this cross-talk occurs are not completely understood and warrant further investigation. In growth assays and reporter assays, AR and ERα were demonstrated to inhibit each others activity. To characterise this further, ERα mutants that are unable to translocate to the nucleus or bind DNA were co-transfected with the AR. The data suggest that competition for coactivators could explain the inhibitory cross-talk. It is hoped that this work will further our understanding of AR/ERα signaling in breast cancer and could identify strategies to utilise this cross-talk for therapeutic gain.

Presenting Author: Mohammad A M Alkheilewi, School of Biological Sciences, University of Essex Wivenhoe Park Colchester, CO4 3Q20, UK. Email: mamalk@essex.ac.uk

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High levels of the AR-V7 splice variant and co-amplification of the Golgi protein coding YIPF6 in AR amplified prostate cancer bone metastases

Erik Djusberg[1*], Emma Jernberg[1*], Elin Thysell[1], Irina Golovleva[2], Pia Lundberg[2], Sead Crnalic[3], Anders Widmark[4], Anders Bergh[1], Maria Brattsand[1] & Pernilla Wikström[1]
[1]Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden; [2]Department of Medical Biosciences, Medical and Clinical Genetics, Umeå University, Umeå, Sweden; [3]Department of Perioperative Sciences, Orthopedics, Umeå University, Umeå, Sweden; [4]Department of Radiation Sciences, Oncology, Umeå University, Umeå, Sweden
*Contributed equally

The relation between androgen receptor (AR) gene amplification and other mechanisms behind castration-resistance in prostate cancer, such as increased expression of constitutively active AR variants and steroid-converting enzymes have been poorly examined. Specific aims of this study were to examine AR amplification in treatment-naïve and castration-resistant prostate cancer (CRPC) bone metastases and to explore molecular and functional differences. AR gene amplification was assessed and verified in 16 (53%) of the CRPC bone metastases ($n=30$), and in none of the untreated bone metastases ($n=10$). AR gene amplification was associated with increased AR mRNA levels and its constitutively active AR-V7 splice variant, as well as with co-amplification of genes in the AR proximity at Xq12, such as YIPF6. Therefore, the paper focused on evaluating functional effects of YIPF6 overexpression. Members of the Yip1 protein family are mainly localized to the ER and Golgi apparatus and are thought to be involved in vesicle transport. Here we demonstrate YIPF6 protein expression in the Golgi of PC cells, and show that overexpression leads to reduced cell proliferation and colony formation. Interestingly, high YIPF6 levels also increased production of extracellular vesicles (EVs) containing coagulation factors such as tissue factor, fibrinogen and factor XIII. While investigating the EVs *in vitro* an ability to stimulate blood coagulation was demonstrated, and we therefore suggest that YIPF6 amplification and overexpression in CRPC bone tumors may lead to a local and systemic stimulation of coagulation mediated by pro-coagulant EVs.

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Presenting Author: Erik Djusberg, Umeå universitet, Institutionen för medicinsk biovetenskap, Umeå Sweden. Email: erik.djusberg@umu.se

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