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Nuclear Receptors: New Roles for Nuclear Receptors in Development, Health and Disease Conference 2018

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Nuclear Receptors: New Roles for Nuclear Receptors in Development, Health and Disease Conference 2018

Plenary Lectures

PL1

Exploiting multiple nuclear receptors in breast cancer

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Estrogen receptor alpha (ER) and progesterone receptor (PR) are widely used predictive and prognostic biomarkers in breast cancer. ER is also a well-established therapeutic target. A major unresolved clinical issue is the development of therapy resistance, especially to ER-targeted therapies. We, and others, have observed somatic ESR1 mutations in up to 40% of metastatic tumors obtained from women who have acquired resistance to endocrine therapies. The two most common mutations are Y537S and D538G, both of which stabilize and/or facilitate the formation of an active AF-2 conformation in the ER LBD. A combination of structural, biophysical, cell and animal studies have helped define the underlying molecular mechanisms that account for AI/SERM/SERD resistance related to ESR1 mutations, which has contributed to the development of novel SERMs and/or SERDs with potential improved clinical utility. The clinical value of PR remains controversial and the role of PR is poorly understood. We have recently observed that PR reprograms and modulates estrogen signaling. Importantly, PR functions as a genomic ER agonist, while acting as a phenotypic antagonist in ER+/PR+ breast cancer models. Animal studies of ER+/PR+T47D human breast tumor xenografts demonstrate that combined treatment with tamoxifen (tam) and certain selective PR modulators (SPRMs) promote tumor regression compared to either treatment alone or to tam plus a progestin. Our results indicate that PR is an essential modulator of ER action and that appropriate co-targeting of ER and PR should be evaluated clinically. It is likely that other steroid receptors (AR, GR, MR) will also become viable co-targets in breast cancers that express these proteins.

DOI: 10.1530/endoabs.54.PL1

PL2

Nuclear receptors and their coactivators: An entre to understanding human diseases

BW O'Malley

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The early work on the existence and function of the estrogen receptor (ER) primed the nuclear receptor field for an eventual explosive development. Subsequent cloning of receptors led to a realization of a giant (48) super-family of related transcription factors. The cloning of coregulators further enhanced this field in terms of mechanism of action. Nuclear receptors control gene expression by recruiting transcriptional coactivators (or corepressors) to target enhancers/promoters. These 'coregulator pre-initiation complexes' can be modulated by histone epigenomic marks. 'Poised initiation complexes' at enhancers then are converted to 'active complexes' by enzymatic posttranslational modifications of the coactivator complex. Recruited coactivators read and write histone marks in the enhancer/promoter locale, and understanding the roles of these reader/writer coactivators permits new understanding of transcriptional mechanisms that can be directed toward novel approaches for disease therapies. The NR-coactivator complexes are 'master regulator complexes' that coordinate the activation of multiple genes and pathways to control physiologies such as reproduction, growth and metabolism. Molecular mechanism studies in diverse tissues have led to the development of new drugs that bind NRs and prevent NR-mediated diseases; studies of coactivators have implicated them in many cancers (e.g., breast and prostate), in metabolic diseases of carbohydrate, lipid, anabolic and energy metabolism and in endometriosis/fertility – consequently, we now can devise new approaches for treatment of many of these pathologies.

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PL3

Targeting DNA repair-AR crosstalk dysfunction in advanced prostate cancer

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Prostatic adenocarcinoma (**PCa**) is the 2nd leading cause of cancer death in US men. Organ-confined PCa can be effectively managed, but there is no durable treatment for advanced disease. Advanced PCa is treated through androgen deprivation therapy, often coupled with direct AR antagonists, as PCa is exquisitely dependent on androgen receptor (**AR**) activity for survival. Furthermore, recent studies identified AR as a major effector of DNA repair, manifest through the ability of the receptor to regulate DNAPK expression and activity. While AR directed therapeutics effectively suppress the pro-proliferative, pro-survival, and pro-DNA repair functions of AR and result in tumor remission, relapse is common. Recurrent disease arises largely due to resurgent AR activity with 2–3 years, and there is no cure for this castration-resistant phase (**CRPC**, castration-resistant PCa). Thus, there is a significant need to develop new means for targeting recurrent AR activity or develop adjuvant therapies in advanced PCa.
Emerging data from our laboratory and others strongly support the concept that AR regulates DNA repair pathway, and that alterations in DNA damage repair (DDR) pathways are more common than previously thought in sporadic PCa, thus uncovering new, potentially more effective means of therapeutic intervention. New studies to be discussed will address underlying mechanisms of action with regard to the pathway, and identify clinically actionable ramifications of AR-DNA repair dysfunction. Major concepts to be considered include new discoveries regarding PARP1 function and activity in lethal disease, and that a newly identified gene signature of PARP1-regulated networks is associated with poor outcome. Further, mechanistic investigation revealed new insight into the means by which PARP1 inhibitors likely act as single agents in advanced prostate cancer, manifest through both DNA repair and transcriptional regulatory functions. Findings to be discussed strongly support a model wherein selected DDR pathways can be developed as therapeutic targets in concert with AR-targeting strategies to tailor treatment for prostate cancer and improve outcome for advanced disease.

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Invited Speaker

IS1

MicroRNA regulation of androgen signalling

Claire E Fletcher[1], Ailsa Sita-Lumsden[1], Alwyn Dart[2], Akifumi Shibakawa[1], Eric Sulpice[3], Stephanie Combe[3], Damien A Leach[1], Johann de Bono[4], SE Lupold[5], SE McGuire[6], Xavier Gidrol[3] & Charlotte L Bevan[1]

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Androgens initially drive prostate tumour growth. Although in advanced disease there is no longer dependence on circulating androgens, the androgen receptor (AR) remains a key driver of this lethal stage thus new ways to inhibit its activity are required. MicroRNAs play vital roles in prostate cancer (PCa) development, progression and metastasis. Previous studies have examined microRNAs dysregulated in PCa, and also identified androgen-regulated microRNAs. We approached microRNAs in PCa from the other angle. Having previously identified microRNA-27a as an androgen-regulated microRNA that in turn affects AR signalling, inhibition of which inhibits PCa cell proliferation, we hypothesised that microRNAs modulating AR activity in lethal castration-resistant PCa represent novel therapeutic targets. Such microRNAs were systematically identified using high-throughput screens to examine effects of ~1000 microRNA inhibitors on AR activity in hormone-responsive and -resistant PCa cell lines. Results were cross-referenced with a database of microRNAs impacting PCa cell growth. Eighty significantly altered AR activity, eight in both cell lines. Upon validation, inhibition of selected identified microRNAs significantly reduced AR activity up to 90%, accompanied by reduced AR mRNA/protein and AR target gene expression. At the cellular level it also increased apoptosis (up to 800%), and reduced cell growth, migration and invasion. Opposing effects were observed upon microRNA overexpression. Inhibition of AR-modulatory microRNAs showed additive effects with AR silencing or anti-androgen treatment, suggesting potential combinatorial applications for PCa treatment.

Pathway analysis of AGO-PAR-CLIP-identified mRNA targets of these microRNAs identified roles in DNA replication and repair, cell cycle, signal transduction and immune function. In addition, inhibition of AR-modulatory microRNAs induced epithelial markers, while reducing levels of mesenchymal markers. Other targets include purported tumour suppressors: silencing of these phenocopies effects of the microRNAs, confirming their physiological relevance. Interestingly, the microRNAs appear to upregulate certain oncogenes as well as AR itself, suggesting novel regulation of the AR 3'UTR and contrary to the accepted dogma that microRNAs reduce expression of their targets via translational repression and/or transcript degradation.

In summary, we have identified microRNAs that significantly modulate AR activity in models of hormone-responsive and castration-resistant PCa. Inhibitors of these dramatically reduce AR activity and growth, migration and invasion of PCa cells, thus represent potential novel PCa therapeutics. Given androgen regulation of microRNA expression, our previous findings that androgen signalling can regulate microRNA biogenesis, and the 'hormomiR' hypothesis that microRNAs themselves can mediate long-range effects, this supports two-way crosstalk between these highly influential systems.

DOI: 10.1530/endoabs.54.IS1

IS2

Androgen and estrogen receptors in breast tissues: opponents or teammates?

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The balance of androgen and estrogen hormone activity determines the degree of breast development in males and females. A predominance of androgen action impedes whereas a predominance of estrogen action promotes breast development. This sex hormone antagonism is mechanistically mediated by androgen and estrogen receptors (AR, ER). The alpha form of ER (ERα) is required for normal breast development and is the driving oncogene in the majority of breast cancers. The AR is not required for female breast development but is required for suppression of breast growth in males and modulates post-pubertal breast growth in females. The role of AR in breast cancer is complex, giving rise to ongoing controversies about how best to leverage it as a therapeutic target. In the context of primary ERα positive breast cancer, evidence from previous studies and unpublished data from our laboratory support the concept that AR signalling opposes oncogenic ERα signalling in breast cancer cells and is a viable therapeutic strategy. In contrast, evidence from other studies indicate that AR signalling facilitates ERα signalling so that AR antagonism would also be a viable therapeutic strategy. Similar contradictory findings are present in the arena of endocrine-resistant breast cancers. Currently, both therapeutic strategies (AR agonism and antagonism) are being tested in clinical trials of ERα-positive breast cancer. In this talk, I will present data describing the interaction between ERα and AR on chromatin and its functional consequences in terms of gene regulation and cell cycle control in different models of ERα positive breast cancer, with view to explaining some of the controversies about AR signalling in this form of disease.

DOI: 10.1530/endoabs.54.IS2

IS3

Nuclear receptors, transcriptional enhancers, and gene regulation

Hector L Franco, Shino Murakami, Tim Y Hou, Yasmin M Vasquez, Ziying Liu, Anusha Nagari, Venkat S Malladi, Tulip Nandu & W Lee Kraus

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Transcriptional enhancers, which function as nucleation sites for the assembly of transcription-regulating complexes across the genome, drive cell type-specific patterns of gene expression that underlie the distinct biological properties of different cell types. Although many features of active enhancers (e.g., H3K4me1, H3K27ac, enrichment of p300/CBP and Mediator, and enhancer RNA production) have been defined by genomic assays, the roles of these features in ERα enhancer function are not well understood. The Kraus lab has had a long-standing interest in enhancer biology, in particular the molecular mechanisms and kinetics of enhancer assembly in signal-regulated systems. In particular, we are interested in cell type-specific enhancers that drive biological outcomes in reproductive tissues and in hormone-dependent cancers. We have focused on enhancers formed by estrogen receptor alpha (ERα), a ligand-regulated, sequence-specific DNA-binding transcription factor that nucleates de novo enhancer formation in cells in response to estrogen signaling, as well as other transcription factors (TFs), such as Sox2, FOSL1, and PLAG1. We have used a variety of molecular, biochemical, genomic, genetic, and computational approaches to determine (1) where enhancers are formed by specific TFs across the genome, (2) the kinetics of enhancer formation and disassembly, (3) the influence of genetic variation on enhancer formation and function, and (4) the role of the specific enhancer features noted above, especially enhancer transcription, in enhancer function. In addition, we have developed new computational tools to study enhancer function, such as the Total Functional Score of Enhancer Elements (TFSEE), a robust and unbiased computational pipeline that simultaneously identifies putative subtype-specific enhancers and their cognate TFs by integrating multiple types of genomic information. Collectively, our analyses are providing new insights into enhancer complex assembly and function in a variety of biological systems.

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IS4

Estrogen receptor cistromics in breast tumors: from biomarkers to novel drug targets

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Estrogen receptor α (ERα) is a key transcriptional regulator in the majority of breast cancers. ERα-positive patients are frequently treated with tamoxifen, but resistance is common. Through ChIP-seq analyses, we presviously identified direct target genes of ERα acting in complex with SRC1, SRC2 or SRC3 (Zwart et al., 2011 EMBO J). Only the 111 genes there were under direct control of ERα in conjunction with SRC3 (but not the other two p160s) predicted patient outcome. Here, the 111-gene outcome prediction-classifier was further refined, revealing FEN1 as strongest determining factor in ERα-positive prognostication. We demonstrate FEN1 levels are predictive of outcome in tamoxifen-treated patients, and show FEN1 is required and sufficient for tamoxifen-resistance in ERα-positive cell lines. We show FEN1 dictates the transcriptional-activity of ERα by facilitating the formation and repair of hormone-induced DNA damage, ultimately resulting in DNA methylation changes. FEN1 blockade induced proteasome-mediated degradation of activated ERα, resulting in loss of ERα-driven gene expression and eradicated tumor cell proliferation. Finally, a high-throughput 460.000 compound screen identified a novel FEN1 inhibitor, which effectively blocks ERα-function and inhibits proliferation of tamoxifen-resistant cell lines as well as ex-vivo cultured ERα-positive breast tumors, providing therapeutic proof-of-principle for FEN1 blockade in tamoxifen-resistant breast cancer.

DOI: 10.1530/endoabs.54.IS4

IS5

Rapid, cytoplasmic actions of the glucocorticoid receptor impact on cell movement

Stephen Kershaw, David J Morgan, Andrew Brass, James Boyd, David Spiller, Egor Zindy, Mudassar Iqbal, Laura C Matthews & David W Ray

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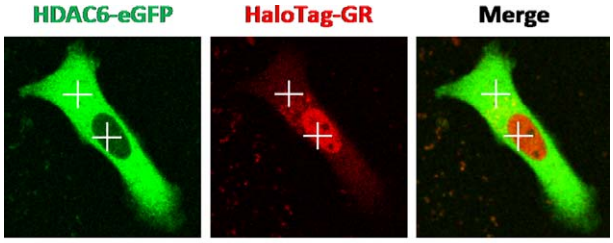
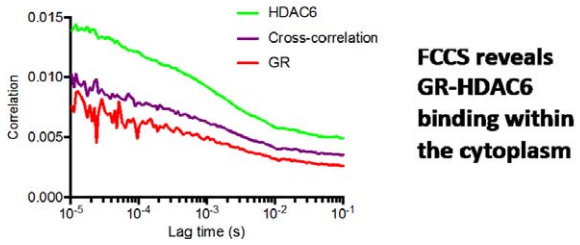
Glucocorticoids (GCs) act through the glucocorticoid receptor (GR) to regulate immunity, energy metabolism, and tissue repair. The inactive GR is held in the cytoplasm in a multi-protein complex, which upon ligand binding undergoes a conformational change. Activated GR translocates to the nucleus to regulate gene expression (over hours), but some effects occur more rapidly. GCs inhibit cell migration through an uncertain mechanism. We now show a very rapid effect, and surprisingly find the GR agonist Dexamethasone, and antagonist, RU486, are equipotent. The migration effect was prevented by GR knockdown, confirming GR specificity, but not by actinomycin D treatment, suggesting a non-transcriptional mechanism. To investigate the GC effect we analysed microtubule network kinetics using plus end microtubule real time assays, which revealed increased tubulin acetylation –a marker of microtubule stability. Inhibition of the cytoplasmic deacetylase HDAC6, which deacetylates tubulin, mimicked the GC effect, and HDAC6 overexpression rescued the GC effect, implicating HDAC6 as the GC effector. We found interaction between GR and HDAC6, using fluorescent cross correlation spectroscopy, and showed HDAC6 intranuclear mobility restriction following GC treatment. We propose that activated GR inhibits HDAC6 deacetylation of microtubules, increasing stability of the microtubule network and reducing cell motility.





We therefore report a novel, non-transcriptional mechanism whereby GR agonists and antagonists, through actions on HDAC6, rapidly reorganize cell architecture to change cell function.

DOI: 10.1530/endoabs.54.IS5

IS6

Androgens and endometrial function: replication, repair and regeneration

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The human endometrium is a complex multicellular tissue the prime function of which is to provide a receptive environment during a fertile cycle. The tissue responds to steroid hormones exhibiting dynamic cyclical regeneration, angiogenesis, differentiation (decidualisation) and inflammation. In the absence of an embryo the inner surface is shed and repaired without scarring (menstruation). The endometrium exhitbits spatial and temporal expression of androgen receptors (AR) in stromal fibroblasts and endothelial cells: upregulation of AR in epithelial cells occurs in reponse to progesterone withdrawal (cycle) or administration of PR antagonists.
We used primary cells (human endometrial stromal cells:) and mouse models to investigate the impact of androgens (T, DHT), AR antagonists (Flutamide) and selective AR modulators (SARMs) on key endometrial cell functions.
We discovered that local 'intracrine' biosynthesis of T/DHT by human stromal cells regulates AR and expression of receptivity factors. Notably DHT inhibited cell migration but increased resistance to apoptosis with SARMs exhibiting a range of activities on these functions and on expression of AR-regulated genes.
Administration of DHT to steroid-depleted (ovariectomised) female mice promoted a significant increase in uterine size, induced epithelial cell proliferation, expansion of the glandular epithelium and altered uterine AR immunoexpression. Administration of DHT in a mouse model of endometrial repair ('menstruation') altered regulation of restoration of endometrial tissue homeostasis following endometrial shedding at the time of menstruation. We have used these mouse models to test the impact of SARMs on endometrial proliferation and repair and found evidence for selective effects of different SARMs on these functional processes.
In summary, our studies demonstrate a key role for AR in regulation of endometrial function in health and disease. We believe SARMs may offer a novel way to target the AR for therapeutic benefit.

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IS7

Clinically-relevant contexts for AR variants in prostate cancer

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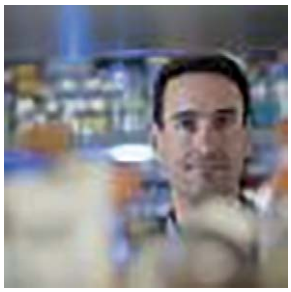
The androgen receptor (AR) functions as a master transcriptional regulator of prostate tissue homeostasis. This master transcriptional regulator function is maintained in prostate cancer. Therefore, prostate cancer is an androgen-dependent disease and suppression of AR transcriptional activity with androgen deprivation therapy (ADT) is an effective systemic therapy. However, development of therapy resistance and transition to castration resistant prostate cancer (CRPC) represents a major clinical challenge. One mechanism by which CRPC may circumvent ADT is by expression of constitutively active AR variants (AR-Vs) that lack the ligand binding domain. The best-studied AR-V is AR-V7, but a controversy in the field is that AR-V7 is broadly expressed and readily detectable in AR-expressing tissues, including normal prostate. To address this controversy, we have been elucidating mechanisms governing the expression of AR-Vs in prostate cancer, with the goal of identifying clinically-relevant contexts in which they may be functioning as drivers of resistance. These studies have revealed heterogeneous, clonally diverse AR gene rearrangements in clinical CRPC. Investigation of several AR gene rearrangement events has demonstrated they converge functionally by driving stable, outlier expression of diverse tumor-specific AR-V species that are functionally equivalent to AR-V7 but undetectable by AR-V7-specific assays currently under clinical development. A second mechanism of AR-V expression is alternative polyadenylation, whereby AR-V7 is co-ordinately expressed in CRPC tissues with AR-V9, AR-V1, and other annotated AR-Vs. This indicates that detection of AR-V7 in CRPC tissue or circulating tumor cells is likely a harbinger of a broader repertoire of AR-V expression. Studies are underway to translate this knowledge to biomarker applications for prostate cancer patients, and also for development of new therapies to combat AR-Vs.



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IS8

Direct and indirect effects of androgens on the musculoskeletal system

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Global knockout models of the androgen receptor (ARKO) illustrates the many roles androgens and their receptor have in the development of male reproductive organs and the gender differences in many features like the musculoskeletal system. However, neither the global ARKO nor orchidectomy models discriminate between direct and indirect effects of androgens. To determine direct and indirect effects of androgens on muscle, we developed a muscle-specific ARKO (called satARKO for satellite cell-specific ARKO). In this model, we found a partial loss of androgen-responsiveness of the levator ani as well as of other muscles. However, there is still an important response to orchidectomy in the muscle of satARKO, which is corrected by administration of testosterone, dihydrotestosterone or the selective androgen receptor modulator Enobosarm. Surprisingly, myostatin is one of the most responsive genes in mouse muscle and we identified the androgen-regulated enhancer involved. This is counterintuitive as myostatin is a well known negative regulator of muscle mass. We propose that myostatin upregulation serves to mitigate the proliferative response to androgens. Similarly, the ARKO in bone cells did not replicate the ARKO phenotype. We are now looking at kidney and brain as suspects for the indirect effects of androgens on the musculoskeletal system.
The observation that in human serum, sex steroids bind with high affinity to sex hormone binding globulin (SHBG) led to the contested free hormone hypothesis. Unfortunately, rodent models do not have the higher serum levels of sex hormone binding globulin seen in humans. We studied the free hormone hypothesis in a mouse model which overexpresses SHBG in its circulation.

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IS9

Progesterone receptor regulation of breast cancer cell translation

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Progesterone receptors (PR) are long recognized to suppress estrogen receptor (ER) mediated transcription in breast cancers. However, a mechanistic basis for this repression has been lacking. Recent reports indicate this occurs, in part, through global repositioning of ER on chromatin in the presence of selective PR modulators (SPRMS), both agonists and antagonists [1, 2]. The goal of our studies was to further understand the mechanisms by which PR impacts estrogen-dependent growth in solid tumor models chronically treated with SPRMs. We grew ER+PR+ breast cancer patient-derived xenografts (PDX) in the presence of E2 alone or E2 plus the natural hormone progesterone (P4) or a synthetic SPRM medroxyprogesterone acetate (MPA) and demonstrated the SPRMs suppress tumor growth similar to tamoxifen. In these tumors P4 and MPA alter up to half of ER regulated genes at the transcript level. However, the majority of these genes ($>80\%$) either show no change in ER chromatin binding by ChIP-seq or have no ER binding sites near their promoter (± 2 kb). We made the interesting discovery via PR ChIP-seq that PR (but not ER) is localized at a large fraction of RNA polymerase III (Pol III) regulated tRNA genes. RIME for PR and subsequent IP found that PR associates with the Pol III complex. Furthermore, select pre-tRNA transcripts and mature tRNA pools are decreased in SPRM treated tumors [3]. We therefore speculate that PR may indirectly impede ER action through regulation of translation. This could occur by reducing the overall bioavailability of tRNAs to reduce protein synthesis rates and curtail tumor growth. Furthermore, PR could alter the tRNA pool to selectively change translational preference through non-optimal codon usage, an increasingly recognized mechanism of cancer cell regulation. Studies are underway to test these hypotheses.

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IS10

Modulating glucocorticoid receptor function in breast and prostate cancer

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In normal physiology, glucocorticoid receptor (GR) activation regulates cell type-dependent genes whose products influence metabolism, inflammation, cell cycle and apoptosis/cell survival pathways. Synthetic GR agonists, or glucocorticoids (GCs), are often used to treat hematologic malignancies because of GR's ability to induce proapoptotic gene expression, inhibit nuclear factor–κB, and induce cell cycle arrest. In contrast, recent examination of GR expression and activity in human cancer models and clinical specimens has suggested that GR activity has remarkably diverse roles in breast and prostate cancer subtypes. In estrogen receptor (ER)+ breast cancer, GR appears to modulate ER-regulated transcriptional activity through ER/GR receptor crosstalk resulting in antagonism of ER-associated proliferation. However, in ER-negative breast cancer (including TNBC), high tumor GR expression is associated with poor prognosis, anti-apoptotic signaling, and chemotherapy resistance. In addition, high GR activity in castrate-resistant prostate cancer (CRPC) contributes to therapy-resistance to androgen receptor (AR) antagonism. Recently described selective GR modulators will be described that have been used to dissect divergent GR mechanisms present in breast cancer subtypes and prostate cancer evolution.

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IS11

Protein factors involved in 3D genome organization & transcription regulation

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The human genome is over 2 meters in length, which has to be folded in micrometer-sized nuclear space for proper functions. Although most of our understandings in the human genome are based on linear explanations, it has been speculated that the three-dimensional (3D) and high-order organization of the genome must play important roles in framing the mechanisms of nuclear process such as transcription regulation. Recent advance in 3D genome mapping technologies and sophisticated computational programs have enabled us to reconstitute the 3D models of the genome, and allowed us to investigate the functions of protein factors involved in 3D genome folding and transcription regulation. In this effort, we developed ChIA-PET to comprehensively map specific chromatin interactions mediated by protein factors with haplotype-specificity and nucleotide-resolution. Using ChIA-PET, we have studied the roles of chromatin architecture factors like CTCF, nuclear receptors (NR) such as ER, AR and RARA, and transcription factors (TF) including RNA Polymerase II (RNAPII) in 3D genome organization and transcription regulation. We demonstrated that CTCF-mediated chromatin interaction anchors serve as 3D organizational foci, where constitutive genes are positioned in concordance with the orientation of CTCF binding motifs, whereas RNAPII and other TFs interacts within these structures by drawing cell-type-specific genes towards CTCF-foci for coordinated transcription. We further found that fusion protein PML/RARA could alter the 3D genome configuration of normal cells and become cancerous. In addition, we have shown that haplotype-resolved chromatin interactions have allelic-specific effects on chromatin interactions, thus revise the expression of genes residing in the topological domains, and lead to different traits or diseases. Together, these mechanistic insights establish a topological basis of 3D genome folding and transcription regulation that links genetic variation to phenotype diversity.

DOI: 10.1530/endoabs.54.IS11

IS12

Nuclear receptor networks in male fertility

Emmanuelle Martinot, Lauriane Sèdes, Marine Baptissart, Hélène Holota, De Haze Angélique, Claude Beaudoin & David H Volle

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Male fertility is controlled by complex interactions between hypothalamus, pituitary, and testis. The major functions of the testis include production of spermatozoa and synthesis of hormones. Testosterone is produced by the testicular Leydig cells and ensures male fertility. Testosterone is involved in the development of gonad, the attainment of puberty, the maintenance of secondary sexual characteristics as well as in spermatogenesis process. Many studies have highlighted the complexity of the regulations of testicular homeostasis at tissue and cellular levels. Several nuclear receptors (NRs) have been identified as key regulators of testicular physiology through the control of steroidogenesis and germ cell differentiation. Using both genetic and pharmacologic strategies the roles of the multiple members of the NR superfamily such as the Liver-X-Receptors, the Small Heterodimer Partner and more recently the Farnesol-X –Receptor have been defined. We will give an overview of recent advances highlighting the identification of a complex networks showing the interactions of NRs in the regulation of the exocrine and endocrine functions of the testis.

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IS13

Enhancers mapping uncovers phenotypic heterogeneity and evolution in patients with luminal breast cancer

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The degree of intrinsic and interpatient phenotypic heterogeneity and its role in tumour evolution is poorly understood. Phenotypic divergence can be achieved via the inheritance of alternative transcriptional programs. Cell-type specific transcription is maintained through the activation of epigenetically-defined regulatory regions including promoters and enhancers. In this work, we annotated the epigenome of 47 primary and metastatic oestrogen-receptor (ERα)-positive breast cancer specimens from clinical samples, and developed strategies to deduce phenotypic heterogeneity from the regulatory landscape, identifying key regulatory elements commonly shared across patients. Highly shared regions contain a unique set of regulatory information. In vitro work shows that TF enriched in clonal enhancers are essential for ERα transcriptional activity and defines the critical subset of functional ERα binding sites driving tumor growth in most luminal patients. These transcription factors also control the expression of genes that mediate resistance to endocrine treatment. Finally, we show that H3K27ac levels at active enhancer elements can be used as a surrogate of intra-tumor phenotypic heterogeneity, and to track expansion and contraction of phenotypic subpopulations throughout breast cancer progression. Tracking epigenetically defined clones clones in primary and metastatic lesions, we show that endocrine therapies drive the expansion of phenotypic clones originally underrepresented at diagnosis. Collectively, our data show that epigenetic mechanisms significantly contribute to phenotypic heterogeneity and evolution in systemically treated breast cancer patients.

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IS14

The structural basis of chromatin reprogramming by steroid receptors

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Localized transitions in chromatin structure accompany nuclear receptor binding events in mammalian cells. These remodeling processes are critical to determine the binding landscape for steroid receptors (SRs) in cancer cells. Multiple reports indicate that steroid receptors (ER, GR, AR, PR) can regulate the binding patterns for each other, particularly during cancer progression. Elucidation of the mechanisms by which these 'chromatin opening' processes occur is central to our understanding of steroid receptor cross-talk at the genome level. A widely accepted model suggests that SRs function in concert with pioneer factors to open enhancer chromatin, creating DNase 'hypersensitive' sites (DHS), relieving the inhibitory activity of closed chromatin. These localized sites are often assumed to represent nucleosome free regions (NFRs). We have mapped nucleosome positions at high resolution in mouse mammary adenocarcinoma cells, and characterized GR dependent factor recruitment and changes in nucleosome structure. We find little correlation between the extent of hypersensitivity and nucleosome presence. GR-enhancers exhibit a complex range of states; in some cases, the receptor attacks pre-existing nucleosomes and recruits the Brg1 remodeler, behaving at these sites as a classic pioneering activity. There is also controversy regarding the multimeric status of SRs in their enhancer bound state. GR is typically represented as acting as a monomer or dimer. We described a tetrameric state for GR bound to the MMTV response element in live cells, and tetrameric states for other transcription factors have been reported. Using a mutation that mimics the DNA bound state, we have examined the chromatin binding landscape for a putative tetrameric form of GR. This receptor dramatically increases the binding profile for GR, penetrating chromatin that is closed in mammary cells, but available in other cell types. These findings will be discussed in terms of a model wherein receptors and many transcription factors act to achieve chromatin remodeling and enhancer activation through a highly dynamic mechanism termed 'dynamic assisted loading.'

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Oral Communications

OC1

Heterodimerization of retinoid X receptor with xenobiotic nuclear receptors occurs in the cytoplasmic compartment of cell in a ligand independent manner

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The 'Nuclear Receptor (NR) Super-family' is a group of ligand modulated transcription factors with 48 members presently identified in human genome. NRs regulate most of the physiological processes of the body ranging from metabolism to reproduction. Retinoid X Receptor (RXR) is one of the important members of this NR superfamily. It serves as a heterodimeric partner of several other members of this superfamily including two major xenobiotic nuclear receptors i.e. Pregnane and Xenobiotic Receptor (PXR) and Constitutive Androstane Receptor (CAR). The latter two receptors are primarily involved in the regulation of body's metabolism and clearance of endobiotics and xenobiotics (including clinical drugs). In the present study we have investigated the exact subcellular location resulting due to the interaction of RXR with either PXR or CAR. In order to study this event, we have used various GFP- and RFP-tagged receptors and their mutants of nuclear localization signal (NLS) region. The study showed that the initial interaction of RXR-PXR and RXR-CAR occurs in the cytoplasmic compartment of the cell and the NLS of PXR/CAR/RXR play a key role in the import of the heterodimeric complex from the cytoplasm to the nucleus in a ligand-independent manner. Our observtions exhibit that a functional NLS, along with respective ligand(s), are necessary for modulation of the target gene. It is observed that RXR serves as a major driving force in importing the heterodimeric complex to the nuclear compartment. This conclusion is based on the fact that mutation in the NLS region of RXR severly weakens this import process. On the contrary, mutations in the NLS regions of PXR and CAR have little or no significant effect. This RXR-dependent nuclear import of the RXR-PXR and RXR-CAR heterodimeric complex also modulates the individual transcriptional activity of PXR and CAR. Such an enhancement in the basal transcriptional activity of the receptor can be utilized for evaluating the diverse receptor-drug interactions.

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OC2

Stage-specific and global functions of NCOR2 in prostate cancer progression

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The corepressor NCOR2/SMRT regulates multiple nuclear receptors (NRs) and other transcription factors. Disruption to these functions are implicated in prostate cancer (PCa) progression but the details remain enigmatic. Therefore we sought to define the global functions of NCOR2/SMRT using isogenic PCa cell models, PCa mouse models and human PCa cohorts.

We mapped the NCOR2 dependent transcriptome (RNA-seq), miRnome (miRNA-seq), methylome (EPIC methylation array) and cistrome (ChIP-seq) in androgen sensitive (LNCaP) and therapy resistant (LNCaP-C42) PCa cells, both treated with androgen, and stable NCOR2/SMRT knockdown. NCOR2/SMRT knockdown in LNCaP-C42 cells resulted in a striking increase in global hypermethylation (87,078 CpGs $>$ 10% gain, adj.*P*val $<$ 0.01), but a divergent transcriptome (1,491 upregulated, 1,195 downregulated). Similar patterns were observed in LNCaP cells, and included regulation of PPARg expression and sensitivity to PPARg ligands. NCOR2/SMRT genomic binding overlapped with AR and pioneering factor FOXA1, and modulated androgen responses in LNCaP-C42. Ongoing data integration efforts are dissecting NCOR2/SMRT methylome-cistrome-transcriptome relationships within and across models.

Using clinical cohorts we revealed that NCOR2/SMRT-regulated miRNA are significantly altered in men progressing to PCa from high grade PIN, and elevated NCOR2/SMRT expression on a 700 case TMA associated with worse disease free survival ($P<$ 0.04; Log-rank (Mantel-Cox) test). To define NCOR2/SMRT actions *in vivo*, we stably knocked down NCOR2/SMRT in the CWR22 xenograft model. This model simulates androgen dependent primary growth and regression upon androgen deprivation therapy (ADT). NCOR2 loss had no effect on primary tumor growth rate or size of tumor at ADT but significantly reduced regression in response to ADT and tumors recurred significantly quicker ($P=0.0044$). Therefore loss of NCOR2/SMRT during ADT results in more aggressive tumors. These findings suggest NCOR2/SMRT profoundly regulates the methylome, but the consequences are divergent. At early stages, elevated expression may suppress antiproliferative signals from PPARg but during ADT NCOR2/SMRT loss and mutation drives PCa progression.

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OC3

Beyond ligand activation: Disrupting LXRα phosphorylation to reprogram diet induced transcriptomes and modulate progression of metabolic diseases

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The importance of the Liver X receptors (LXRs) as a critical modulators of metabolic homeostasis and immunity in health and disease has been mainly gleaned from studies evaluating the consequences of their pharmacological or genetic manipulation. We previously showed LXRα is phosphorylated upon cholesterol loading. It is however unknown whether post-translational modifications of the receptor modulate diet-induced responses and affect metabolic diseases with an important inflammatory component. To explore the impact of LXRα phosphorylation in disease progression we have generated two models: i) a whole-body phosphorylation-deficient mutant of LXRα at S196A (S196A) in which we explored the progression of non-alcoholic fatty liver disease and ii) a phospho-deficient LXRa mutant in myeloid cells (M-LXRαS196A) on the atherosclerotic LDLR null background (M-LXRαS196A$^{Ldlr\text{-}KO}$) in which we examined the development of atherosclerosis.

S196A mice challenged with a High Fat-High Cholesterol diet exhibit reduced hepatic inflammation and fibrosis associated with a marked protection against cholesterol accumulation. Impaired LXRα phosphorylation in this model uncovers novel diet-specific/phosphorylation-sensitive genes. Furthermore, M-LXRαS196A$^{Ldlr\text{-}KO}$ fed a High-Fat diet display a significant increase in atherosclerosis burden in the absence of altered systemic lipid levels. Reduced LXRα phosphorylation during atherogenesis reprograms the macrophage transcriptome and significantly promotes cell proliferation pathways, which is a feature of developing atherosclerotic lesions. Interestingly, the global gene expression changes observed in response to impaired LXRα phosphorylation are fundamentally different from those revealed by ligand activation highlighting the importance of this post-translational modification in modulating the activity of the receptor in the context of metabolic/inflammatory diseases.

Overall, we show the relevance of manipulating Ser196-LXRα phosphorylation to promote unique transcriptomes, thereby specifically modulating pathways important for the development of metabolic diseases such as non-alcoholic fatty liver disease and atherosclerosis.

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OC4

Rationale targeting cell plasticity in treatment resistant prostate cancer

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Resistance to newly developed androgen receptor pathway inhibitors (ARPIs), such as abiraterone and enzalutamide, rapidly emerges and patients generally die within 2 years. In particular, a subset of patients who relapse following ARPI therapy exhibit lineage switching whereby tumours shed their dependence on AR signaling and emerge with neuroendocrine features. These tumours, termed treatment induced neuroendocrine prostate cancer (t-NEPC), carry an extremely poor prognosis and, to date, treatment remains decades old cytotoxic chemotherapy which carries a short-lived response at the cost of significant toxicity. Thus, the need to develop targeted treatments for this devastating disease is of paramount importance. Dr Zoubeidi will discuss how cell plasticity including cancer stem cells and neuroendocrine are mechanisms of ENZ resistance that could be in part governed by changes in the epigenome and why the transcription factor BRN2 is a major regulator/driver and a promising target for t-NEPC.

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OC5

The pathogenic role of estrogen receptor beta drives in endometriosis

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The defining feature of endometriosis is that endometrial tissues are deposited and grown onto sites outside of the uterine cavity. The pathogenesis of endometriosis, however, remains controversial despite extensive research. Since the endometriosis has been known as an estrogen-dependent inflammatory disease, the alterations in estrogen-mediated cellular signaling play an essential role in the pathogenesis of endometriosis. In addition to higher estrogen receptor (ER)β levels, enhanced ERβ activity was detected in endometriotic tissues compared to the normal, and the inhibition of enhanced ERβ activity by an ERβ-selective antagonist suppressed mouse ectopic lesion growth. Notably, gain of ERβ function stimulated the progression of endometriosis. As a mechanism to evade endogenous immune surveillance for cell survival, ERβ interacts with cellular apoptotic machinery in the cytoplasm to inhibit TNFα-induced apoptosis. ERβ also interacts with components of the cytoplasmic inflammasome to increase interleukin-1β and thus enhance its cellular adhesion and proliferation properties. Finally, the integration of the ERβ-regulated transcriptome and the ERβ-cistrome revealed that the gain of ERβ gene function directly enhances gene signatures of epithelial-mesenchymal transition and Reactive Oxygen Species in ectopic lesions in addition to cell cycle gene signature, thereby increasing the invasion and proliferation activities of endometriotic tissues for the establishment of ectopic lesions. Collectively, we reveal how endometrial tissue generated by retrograde menstruation can escape immune surveillance and develop into sustained ectopic lesions in part via gain of ERβ function.

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OC6

Chemical systems biology analyses reveal dissociated glucocorticoid signaling networks in skeletal muscle

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Glucocorticoids (GC) are catabolic in skeletal muscle to provide nutrients during fasting or other stressors, including inhibiting insulin-mediated glucose uptake, protein synthesis, and mitochondrial function, while stimulating proteosomal breakdown of proteins. Published work in cultured myotubes required μM GC dosing to see these effects. We found GCs to show low nM activity in myotubes by using a more physiologically relevant setting, including nutrient deprivation and insulin challenge. In order to understand the molecular basis for these phenotypic activities we generated quantitative, statistically robust bioassays in myoblasts and myotubes and characterized a set of GCs designed to perturb the glucocorticoid receptor with several distinct structural mechanisms. The ligands displayed a full range of variance across the skeletal muscle bioassays, allowing us to identify ligand-specific gene expression patterns that were highly predictive for their effects on insulin-mediated phosphorylation of AKT, glucose disposal, mitochondrial function, and protein balance, including protein synthesis and proteosomal degradation. In vivo validation reveals that our approach, called ligand class analysis, can tie chemical and receptor structure to specific transcriptional signaling outcomes that define glucocorticoid action in skeletal muscle. In doing so we identified a dissociated glucocorticoid with full anti-inflammatory activity that is slightly anabolic for protein balance and mitochondria, and a full antagonist with strong anabolic activity.

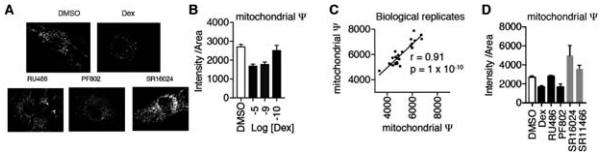

Figure 1 Identification of glucocorticoids with beneficial effects on mitochondria. A) C2C12 myoblasts were starved for 24 hr during treatment with the indicated compounds and analyzed with high content imaging after labeling using mitotracker dye. B) Mitochondrial potential was quantitated as the intensity of mitotracker dye/mitochondrial area. C) Assay reproducibility with 22 novel glucocorticoids + controls. D) Quantitation of A) shows that PF802, a dissociated glucocorticoid for which a prodrug is in clinical trials (Pfizer) inhibits mitochondrial potential similarly to Dex. The two SR compounds (Scripps Research) have pM affinity for GR and improve mitochondrial function. Not shown, SR1466 has better *in vitro* anti-inflammatory activity than PF802.

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OC7

Checkpoint kinase 2 and androgen receptor cross-talk regulate the DDR and prostate cancer growth

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It has long been known that the AR is regulated not only by its cognate steroid hormone, but also by interactions with a constellation of co-regulatory and signaling molecules. Checkpoint kinase 2 (CHK2) is a serine/threonine protein kinase whose main function is regulating the DNA damage response (DDR) triggered by double-strand DNA breaks. The androgen receptor (AR) is a major driver of prostate cancer, even at the castration–resistant stage of the disease. Our research suggests a CHK2–CDC25C–CDK1–AR phospho–S308 signaling pathway in the regulation of AR activity and prostate cancer cell growth. We have now uncovered novel molecular interactions between CHK2 and AR that provide mechanistic insight into our observation that CHK2 regulates prostate cancer growth. The AR directly interacts with CHK2, and that interaction increases with radiation. We found that the interaction of CHK2 and AR occurs at sites of DNA damage. The binding of CHK2 with AR can be disrupted with CHK2 kinase inhibitors suggesting that the kinase activity of CHK2 is required. This was verified using kinase–impaired CHK2 variants, including the K373E variant associated with 4.2% of prostate cancer. Furthermore, the radiation–induced increase in CHK2–AR requires AR phosphorylation on both serine 81 and serine 308. Interestingly, CHK2-depletion in LNCaP cells increases ionizing radiation induced AR expression, AR regulation of DDR genes, and DNA damage. Together, these data provide the rationale for targeting the CHK2–AR signaling axis to improve the effectiveness of prostate cancer therapies. The combination of CHK2, Aurora, or CDK1 inhibitors with androgen deprivation therapy (ADT) and radiation enhances repression of tumor cell growth. Our data substantiates a new role for CHK2 signaling and directly links a critical member of the DDR with AR–mediated transcription and proliferation in prostate cancer. The data suggest that the CHK2–AR interaction functions to downregulate the AR mediated DDR. These findings are clinically relevant since nearly every patient with disseminated prostate cancer will relapse following ADT and develop incurable castration–resistant prostate cancer. These data may assist in the rational application of existing therapies and lead to the development of novel prostate cancer therapeutics.

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OC8

Structural basis of specific DNA recognition by the estrogen-related receptor ERR

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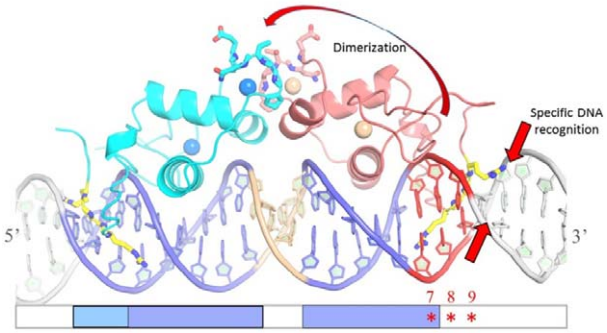
Like other steroid hormone receptors (SHRs), the ERR binds to IR3 response elements (REs). However, the naturally occurring ERR binding sites are



composed of single, extended half-sites (ERREs) that are recognized by the homodimeric ERR. In order to unravel the specific structural reasons and the related functional consequences related to the binding of a homodimer to single extended half-site REs, we conducted a series of complementary structural and biophysical experiments and investigated the binding behavior of the isolated DBD to different REs. ERR DBD binds as a monomer to IR3, in contrast with what is observed in the case of ER DBD. However, we also identified target DNA sequences where stable DBD dimer binding is observed. Using a series of chimera and mutant DNA sequences of ERREs and IR3 REs we found the key determinants for the binding of dimeric ERR DBD. Our results suggest that the sequence-directed DNA shape is more important than the exact nucleotide sequence for the binding of ERR DBD to DNA as a dimer. This underlines the importance of the shape-driven DNA readout mechanisms based on minor groove recognition and electrostatic potential. These conclusions may apply not only to ERR, but also to other members of the steroid NR family, such as AR or GR, for which a strong well conserved half-site is followed by a weaker one with a degenerated sequence. We further extended our work to full ERR receptor complexes bound to DNA and present results of structural and functional studies that can be extrapolate to other SHRs. In fact, while the isolated DBD and LBD domains can fulfil their prime functions of transactivation, DNA- and ligand binding, allosteric communication between the different domains is thought to be essential for the cellular function.

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Poster Presentations

P1

Single-molecule analysis of peroxisome proliferator-activated receptor γ2 and α reveal subtype specific differences in chromatin binding dynamics

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The peroxisome proliferator-activated receptors (PPARs) display a high degree of conservation in the DNA- and ligand-binding domains. Despite these sequence similarities, the PPARs show distinct functions, even when co-expressed. Many experimental approaches have been employed to determine the molecular mechanisms that underlie their subtype-specific characteristics. However, these approaches have largely relied on cell population based studies, such as ChIP-seq. Here we have used single-molecule tracking (SMT) to investigate the unexplored intranuclear dynamics of two PPAR subtypes. Using HILO illumination, HaloTags, and the bright and stable fluorophore JF549, we have examined the behavior of PPARγ2 and PPARα *in vivo* at the single-molecule level. We detect slow and fast stops which we hypothesize to be functional- and non-functional binding events, respectively. Consistent with this model, we find that most long-lived binding events are lost upon mutation of the PPAR heterodimerization- and DNA-binding domains. The residence time and bound fraction (BF, slow stops) of both PPARs are unaffected by agonist or antagonist treatment. Interestingly however, both the BF and the residence time are greater for PPARγ2 than for PPARα, indicating that PPARγ2 and PPARα display subtype-specific dynamic binding behavior at the single-molecule level. This subtype specificity is found to be dependent on the N-terminal domain. Furthermore, we show that the BF and residence time of PPARγ2 significantly increase in the presence of C/EBPα, which we have previously shown can facilitate PPARγ binding to chromatin. The ability of C/EBPα to facilitate PPARγ binding is dependent on the AF-2 domain, consistent with a model wherein the interplay between multiple TFs relies on the recruitment of coactivators and chromatin remodelers. Overall, we have demonstrated that SMT provides a unique ability to resolve unanswered questions concerning the highly dynamic properties of transcription factors, including properties that are linked to specific subtypes of closely related transcription factors.

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P2

Next generation glucocorticoid receptor modulators

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Synthetic glucocorticoids bind to the glucocorticoid receptor (GR) and have been used for nearly 70 years to treat inflammatory diseases. However, their use is limited by adverse effects such as diabetes, muscle wasting and osteoporosis. High throughput screening identified a novel non-steroidal scaffold with great potential for chemical optimization. Through rational design we developed the indazole ether series which combines high potency with structural motifs that provide vectors to key functional areas of the receptor. Exploitation of novel pockets within receptor enabled us to generate AZD7594, a GR agonist with properties optimized for inhaled administration with high potency, long lung retention and minimal systemic exposure. AZD7594 is currently in a phase II trial for asthma.
To identify an oral agent, we modified the screening strategy to search compounds with good bioavailablility and with different mechanistic properties. Again, we started from the indazole ether series and used rational design to generate partial agonists with potential for differentiation. Evaluation in both human *in vitro* systems and in rat *in vivo* models led to the discovery of AZD9567, a compound that exhibited full inhibition of TNFα release in human whole blood after LPS stimulation but with no induction of gluconeogenic enzymes in primary hepatocytes. AZD9567 is currently evaluated vs the effects of prednisolone in clinical studies in healthy volunteers.

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P3

Interactions between AR coregulators, TRIM24 and TRIM28, in Castrate Resistant Prostate Cancer (CRPC)

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Castrate Resistant Prostate Cancer (CRPC) is the inevitable outcome of hormone treatment for advanced disease. Although no longer dependent on high levels of androgens, the androgen receptor (AR) remains active and there is evidence that other nuclear receptors (NRs) can drive CRPC progression and/or therapy resistance. NRs share a repertoire of essential coregulators: proteins possessing the ability to aid or repress NR action and have been proposed as a potential mechanism for driving this inevitably lethal disease. Using publically available datasets we have found distinct patterns of coregulator expression between CRPC and hormone naïve disease. Importantly, we could identify a group of coregulators that were consistently differentially expressed across all cohorts. These could be further categorise into distinct function clusters or pathways.
One such comprises TRIpartite Motif (TRIM) proteins TRIM24, TRIM28, and TRIM33. These form a unique subgroup of the larger TRIM family, in that only these three have Bromo-domains. Co-immunoprecipitation assay for endogenous proteins reveals that these proteins interact with each other and AR. Using ChIP-seq data we identified AR regulated genes which are also potentially TRIM24 and TRIM28 targeted. Using ChIP and RT-qPCR we were able to validate TRIM24 and TRIM28 binding independently or concurrently to AR target genes VEGFA, SLC45A3, CXCR7. Silencing TRIMs can alter androgen regulation of such genes, and was also able to reduce proliferation and response to androgen in AR-expressing prostate cancer cells. Intriguingly in one such cell line (22RV1), silencing individual TRIMs made no difference to anti-androgen response, but simultaneous silencing resensitized cells to the antiandrogen enzalutamide. Furthermore their expression in TCGA data sets could be used together to predict biochemical relapse. Our data suggest that TRIM24 and TRIM28 proteins interact, in gene specific manners, to regulate AR activity and may provide a potential target to increase effectiveness of anti-androgen therapy.

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P4

Selective disruption of ERα expression in dendritic cells of lupus prone mice results in female-specific reduced survival

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Systemic lupus erythematosus (SLE) is a disease that disproportionately affects females. We previously showed that a functional knockout of estrogen receptor alpha (ERαKO) resulted in significantly reduced renal disease and increased survival in murine lupus. The mechanism of this effect, which requires estrogen, is not known. Interestingly, an ERα−/− (null mutant) mouse is not similarly protected. We and others have demonstrated a role for ERα in dendritic cell (DC) development and Toll-like receptor (TLR) responsiveness. Here we show that selective genetic disruption of ERα in DCs of lupus prone mice results in a survival difference, but unexpectedly only in females, who die prematurely compared with intact females. Floxed-ERα and Cre-CD11c strains were backcrossed onto the NZM2410 lupus-prone background for 12 generations. Males and females were studied ($n=24$). There was no significant difference in survival between NZM CrePos/Floxed-ERα (DC-specific ERαKO) mice and NZM CreNeg/Floxed-ERα mice. Considered separately, however, female survival was significantly different. Median age at death was 30.0 weeks (± 1.8) for the CrePos and 40.4 weeks (± 3.9) for the CreNeg females ($P<0.04$). Spleen cells were isolated and flow cytometry was performed to determine number and subset of DCs. Preliminary flow cytometry results revealed a significantly reduced percent of MHCII+F480-CD11c+CD11b+ DCs and

MHCII + B220 + SiglecH + pDCs in CrePos vs. CreNeg mice. There was a trend towards increased percent of MHCII + CD11c + CD11b-CD8a + cells in CrePos mice. In summary, while selective deletion of ERα in DCs of female lupus-prone mice results in female-specific reduced survival, the etiology of this unexpected accelerated disease phenotype is not clear. This data joins a growing body of evidence that ERα plays an important role in modulating immune cell function.

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P5

Glucocorticoid receptor inhibits ER-mediated pro-proliferative gene expression

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Early-stage ER + breast cancer (BC) with high tumor glucocorticoid receptor (GR) expression is associated with improved long term relapse-free survival compared to tumors with low GR expression. In addition, GR activity inhibits ER-mediated BC cell proliferation. We therefore hypothesized that GR and ER engage in nuclear receptor crosstalk to influence pro-proliferative gene expression, thus contributing to a better outcome in ER + /GR + breast cancer. To understand the mechanisms by which ER/GR co-activation contribute to a more indolent ER + BC phenotype, we performed ChIP-sequencing and gene expression analyses in ER + /GR + BC cell lines. We found that activation of GR with the synthetic agonist, dexamethasone (dex), led to decreased ER + BC cell proliferation. Furthermore, ER/GR co-activation dampened cell cycle gene expression (e.g. *CDK6*, *CDK2*, and *CCND1*) compared to ER-activation alone. GR and ER ChIP-sequencing revealed co-localization of ER and GR at a known downstream enhancer for ER-mediated *CCND1* transcription suggesting direct GR-mediated antagonism of ER. The highly selective GR modulators (SGRMs), CORT125134 and CORT118335 also reduced ER-driven cell proliferation and E2-mediated pro-proliferative gene expression. Moreover, MCF-7 cells engineered to express ER ligand-binding domain mutations (Y537S and D538G) similarly demonstrated decreased proliferation with either dex or SGRM treatment. Taken together, these studies suggest that GR modulation deserves further investigation as an approach for inhibiting ER-driven BC.

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P6

The impact of 27-hydroxycholesterol on endometrial cancer proliferation

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Endometrial cancer (EC) is the most common gynaecological malignancy. Obesity is a major risk factor for EC and is associated with elevated cholesterol. 27-Hydroxycholesterol (27HC) is a cholesterol metabolite that functions as an endogenous agonist for Liver X Receptor (LXR) and a selective estrogen receptor modulator (SERM). Exposure to estrogenic ligands increases risk of developing EC however the impact of 27HC on EC is unknown.

Samples of stage 1 EC ($n = 126$) were collected from post-menopausal women undergoing total abdominal hysterectomy according to a method approved by the local institutional ethics committee. Expression of LXRs (NR1H3, LXRα; NR1H2, LXRβ) and enzymes required for the synthesis (CYP27A1) or breakdown (CYP7B1) of 27HC was assessed in all grades of EC. LXRα and LXRβ expression was detected in EC but did not change with grade. Expression of CYP7B1 decreased with disease severity ($P < 0.05$) consistent with an association between increased bioavailability of 27HC and progression of EC.

The impact of 27HC or the LXR agonist GW3965 on cell proliferation or expression of an LXR- or ER-dependent luciferase reporter gene was assessed in cell lines originating from well-, moderate- and poorly-differentiated endometrial cancers (Ishikawa, RL95, and MFE 280 respectively). Incubation with 27HC or GW3965 increased transcription via LXRE in Ishikawa, RL95 and MFE 280 cells ($P < 0.01$). 27HC selectively increased ERE reporter activity in Ishikawa cells ($P < 0.001$) and promoted proliferation of both Ishikawa and RL95 cells ($P < 0.001$). Selective targeting of LXR with GW3965 significantly reduced cell proliferation of RL95 and MFE280 cells ($P < 0.0001$).

Altered cholesterol metabolism and exposure to 27HC may contribute to endometrial cancer risk by altering cell proliferation. These novel results suggest that 27HC can promote proliferation of endometrial cancer epithelial cells and highlight LXR as a potential therapeutic target in the treatment of advanced disease.

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P7

MIR-96 regulates retinoic acid receptor gamma cross-talk with the androgen receptor to drive aggressive prostate cancer

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The retinoic acid receptor gamma (RARg, encoded by *NR1B3/RARG*) is significantly downregulated but not mutated in the MSKCC and PRAD prostate cancer (PCa) cohorts in the Cancer Genome Atlas (TCGA) data. We investigated the consequences of modifying RARg expression.

Independent of ligand, stable RARg knockdown stimulated RWPE-1 and LNCaP cells proliferation, changed the cell cycle profile and significantly altered global gene expression patterns that were enriched for AR and NF-kB signaling. RARg ChIP-Seq in RWPE-1 cells identified ∼1300 significant binding sites, in the absence of ligand. ∼800 of these sites significantly overlapped with active enhancer histone modifications (H3K27ac, H3K4me1), AR and p65 (unit of NF-kB) binding and were coincident with the ONECUT2 motif. Combining RARg cistrome and transcriptome data and applying bootstrapping demonstrated RARg sustained target gene expression. Reducing RARg levels in HPr-1AR cells disrupted AR-dependent gene regulation; ∼1700 genes including MYC targets. RARg directly bound MYC, suggesting RARg directly regulates MYC, and co-regulates the AR capacity to repress MYC networks. Reduced RARg target gene expression in the TCGA-PRAD cohort associated with higher Gleason grade PCa.

RARg reduction was driven by elevated miR-96 in PCa cells, mouse models, and TCGAPRAD, which associated with worse disease free survival. MiR-96 mimics stimulated cell cycle progression and biotin-miR-96 pulldown identified 360 miR-96 targets. The most altered miR-96 target genes in TCGA-PRAD consisted of a RARg-centric network. Finally, tumors with low RARg-network and high miR-96 expression displayed repression of RARg target genes (e.g. SOX15) and significantly worse disease free survival.

Together, these findings support the concept that, ligand independent RARg binds at gene enhancer regions to govern AR signaling, including MYC network repression, and control proliferation. Elevated miR-96 represses the RARg-centered network and drives aggressive PCa. Conceptually, identifying miRNA that govern NR genomic functions, independently of ligand, has the capacity to change how these key transcription factors are studied and therapeutically exploited.

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