Androgen-sensitive PCa cell lines (LNCaP and VCaP) and patient-derived-xenografts (PDX), of a prostate tumor (TM00298) were used. In both LNCaP and VCaP cells, western blots and qRT-PCR assays indicated that WBM extract (6~30 mg/mL) suppressed DHT-induced PSA expression and cell proliferation in a dose-dependent manner. Immunofluorescence on AR revealed that the nuclear localization of AR was reduced upon WBM extract treatment, which agreed with the results of a PSA promotor-luciferase assay, suggesting that WBM extract inhibited DHT-induced luciferase activity. RNA-Seq on WBM-treated LNCaP cells confirmed that WBM treatment suppressed androgen response pathways and cell-cycle control pathways. Our prostate cancer PDX showed that oral intake of WBM extract (200 mg/kg/day) significantly suppressed tumor growth, as well as decreased PSA levels in both tumors and serum. Both *in vitro* and *in vivo* studies suggested that chemical(s) in WBM extract behave as AR antagonist(s). We previously identified a conjugated linoleic acid isomer (CLA-9Z11E) as an active component in WBM extract. In the present study, we extended these findings by performing LanthaScreen™ TR-FRET AR Coactivator Interaction Assays for a direct interaction of CLA-9Z11E with AR. We report here that CLA-9Z11E exerts a strong antagonist potency against the recruitment of an AR coactivator peptide towards AR. The inhibitory effect of CLA-9Z11E (IC50: 350 nM) was nearly two times stronger than the known AR antagonist, cyproterone acetate (IC50: 672 nM). The information gained from this study improves the overall understanding of how WBM may contribute to the prevention and treatment of PCa. It also serves as an important, scientific basis for developing diet-based chemoprevention and integrative therapeutic strategies for prostate cancer (supported by NIH R01 CA227230).

Reference: [1] Twardowski P, et al. A phase I trial of mushroom powder in patients with biochemically recurrent prostate cancer: Roles of cytokines and myeloid-derived suppressor cells for Agaricus bisporus-induced prostate-specific antigen responses. Cancer. 2015.121(17):2942-50.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

Differential Genomic Interactions Drive Progesterone Receptor Isoform Specific Functions in Breast Cancer

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Progesterone is critical for normal breast development and function, and has been shown to stimulate proliferation of normal breast epithelial cells by increasing stem and progenitor cell numbers. Breast cancer incidence is increased in women exposed to progesterone analogues in combined estrogen plus progestin hormone replacement therapy, but not in women taking estrogen alone. Classical progesterone signaling is mediated through the nuclear progesterone receptor (PR), which occurs as two related but functionally different isoforms, PRA and PRB. PRA and PRB are co-expressed equally in normal breast tissue but become dysregulated in breast cancer where PRA often becomes predominant. PRA predominance in breast cancer is associated with poorer outcome and higher risk of distant metastasis in tamoxifen treated patients. We show using integrated analysis of ChIP-seq, ATAC-seq and transcriptomic profiling in a breast cancer cell line model of acquired PRA predominance that: 1) PRA and PRB have different requirements with regard to chromatin accessibility; 2) PRA predominance reshapes the PR cistrome and the associated transcriptome to affect genes not normally regulated by PR when PRA and PRB are equivalently expressed, possibly through assisted loading with multiple other transcription factors; 3) Genes regulated by PR only when PRA is predominant are associated with poorer breast cancer outcome and involved in multiple cancer-associated pathways including those that regulate cell proliferation and adhesion. Our data suggest a mechanism for the poorer disease outcome seen in breast cancers with a predominance of PRA.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

Disruption of Adipose Tissue Metabolism by Glucocorticoids Is Attenuated With LXRβ Antagonism

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Excessive exposure to glucocorticoids (GCs), either from endogenous overproduction of cortisol, or exogenous pharmacological GC treatment, potentiates the development of diabetes and obesity in a fat depot-specific manner. Undesirable metabolic side effects resulting from the activation of the glucocorticoid receptor (GR) remain a key limitation to the long-term therapeutic use of GCs as immunosuppressants. GC treatment disrupts the thermogenic function of brown adipose tissue (BAT) and enhances futile cycling within white adipose tissue (WAT). Mice lacking the liver X receptors (LXRs) were previously shown to have smaller AT depots with enhanced BAT activity compared to wildtype (WT) mice. We previously demonstrated that LXRβ is required to mediate the side effects of GCs in the liver but not the beneficial anti-inflammatory effects of GCs. The discovery of this GC/LXRβ crosstalk led to the hypothesis that LXRβ antagonism may be therapeutically beneficial to prevent GC-induced dysfunction in AT. To test this idea, LXRα-/- mice were treated for 5 days with vehicle, 5 mg/kg dexamethasone (Dex, a synthetic GC agonist), and/or 40 mg/kg GSK2033 (GSK, nonselective LXR antagonist). As expected, Dex-treated mice showed significant accumulation of lipids in BAT and were unable to maintain their body temperature when exposed to cold, yet GSK was able to protect against these effects. Dex increased body fat mass and caused adipocyte enlargement in WAT which was not observed in mice co-treated with GSK. At the transcriptional level, GSK attenuated Dex-mediated downregulation of thermogenic genes in BAT, and upregulation of lipogenic genes in WAT. Similar beneficial changes were confirmed in WT mice. The protection afforded by GSK against Dex-induced fat accumulation was confirmed to be cell-autonomous from studies in

adipose-specific LXRβ-/- mice (AdβKO). Dex-dependent increases in lipolysis in gonadal WAT and plasma free fatty acids (FFA) were also attenuated by GSK co-treatment. With GSK co-treatment, Dex-induced liver steatosis was diminished suggesting that LXRβ antagonism attenuated FFA shuttling to the liver. The lipolytic and lipotoxic effects of Dex in AT and liver were largely abrogated in AdβKO along with improved systemic insulin sensitivity. Overall, our data suggest that LXRβ antagonism prevents disruption of BAT and WAT (and indirectly liver) function caused by GC treatment in an *in vivo* model, highlighting the potential role of LXRβ antagonists in combating the negative effects of excessive GC exposure on the development of diabetes and obesity. The identification of this novel mechanism of interrupting GC adipose tissue action suggests therapeutic targeting of LXRβ with an antagonist could improve the health of patients currently taking GCs to control inflammation but suffer the detrimental side effects of drug treatment.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

LBD Dimerization of the Androgen Receptor but Not N/C Interaction Is Crucial for Normal Male Development in Mice

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The androgen receptor (AR) is a nuclear receptor with a main role in the development and maintenance of the male phenotype. To execute its role as transcription factor, the AR forms homodimers. Three dimerization modes have been described for the AR: one via the DNA binding domain, a second via the ligand binding domain (LBD) and a third via interactions between the LBD and the aminoterminus of the AR (N/C). Based exclusively on *in vitro* data, all three dimerization modes seem to contribute to full AR activity, albeit to a different extent. The *in vivo* role of the dimerization modes, however, remains unknown. To study the physiological relevance, we generated two mouse models using a CRISPR/Cas9 approach, in which either the N/C interaction (AR^{NoC}) or LBD dimerization (AR^{Lmon}) was disrupted. Surprisingly, the male AR^{NoC} mice have a normal phenotype, indicating that the N/C interaction is not crucial for male development. In contrast, AR^{Lmon} males have an external female phenotype with cryptorchid testes and high levels of circulating testosterone (T), androstenedione and luteinizing hormone (LH) (6-, 13- and 45-fold higher, respectively). They have no prostate, seminal vesicles or epididymis, illustrating the importance of LBD dimerization during male development. Phenotyping the AR^{Lmon} model furthermore provided evidence of a crucial role for the AR in bone homeostasis as well as steroidogenesis. The AR^{Lmon} males display a severe bone phenotype, similar to that of complete AR knockout (ARKO) mice. The bone phenotype of ARKO was postulated to be mainly due to lower estrogen levels. However, in contrast to ARKO mice, AR^{Lmon} mice have high circulating levels of T, which can still function as prohormone for estradiol and support bone function via the ERα. Immunohistological analysis of AR^{Lmon} testes showed hyperplasia of the Leydig cells and residual spermatogenesis. Analysis of the steroidogenic pathway revealed that while the expression of most genes is increased, the expression of *Hsd17b3*, encoding the enzyme responsible for conversion of androstenedione into T, is low in AR^{Lmon} testis. Reporter assays confirmed that the promotor of this gene is indeed upregulated by the AR itself. In conclusion, our work uncovers the physiological role of the N/C interaction and LBD dimerization of the AR. It furthermore demonstrates a direct role for AR in male bone development independent of T aromatization into estrogens. Finally, we show that the AR controls the final step in the synthesis of its own ligand. In contrast to the *in vitro* data, N/C interaction is not crucial for male development *in vivo*. The AR^{Lmon} model illustrates that LBD dimerization could be an excellent new therapeutic target for inhibiting AR activity for example in advanced prostate cancer that has developed resistance to the current AR-targeting therapies.

Steroid Hormones and Receptors

STEROID RECEPTORS IN DEVELOPMENT AND DISEASE

Mapping of Corticosteroids in Murine Kidneys Using Mass Spectrometry Imaging

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Renal sodium reabsorption is important for blood pressure homeostasis and is physiologically regulated by aldosterone; glucocorticoids may also contribute. Abnormal steroid hormone activity within the kidney contributes to hypertension but the mechanisms are not fully defined. Molecular profiling of receptors and metabolising enzymes indicates that steroid hormone action is compartmentalised within the kidney. Ambient steroid concentrations are a critical factor governing bioactivity at a cellular level, but this is largely unknown, and the kidney remains a "black box". Mass spectrometry imaging (MSI) was applied recently to localise steroids in brain and testes, and here is applied to kidney. Image reconstruction permits characterisation and co-registration of kidney histological regions based on regional markers detectable by MSI. Our aim was to map and quantify glucocorticoids and aldosterone in different histological zones (cortex, medulla) of murine kidneys, using an optimised MSI method. This approach has the potential to map steroids within functional zones of the kidney, providing fundamental new information relevant to hormone action in health and in disease. Cryosections of male C57BL6 mouse kidneys (age 12 weeks, n=6) were subject to MSI following derivatisation using Girard T reagent and α-cyano-4-hydroxycinnamic acid matrix application. Images were reconstructed, and methods optimised to enhance signal and limit diffusion of analytes of interest. Matrix assisted laser desorption/ionisation (MALDI) was used as a sampling method, coupled to Fourier Transform Ion cyclotron mass spectrometry. Ions with m/z 458.3010,