24 hours after the last dose of dexamethasone and blood, white adipose tissue, soleus muscle and liver were collected for RNA preparation and quantitative RT-PCR analysis. Quantitative analysis of trabecular bone parameters was performed by micro-CT. **Results:** Both male and female mice treated with dexamethasone gained less weight over time and surprisingly were more glucose tolerant than the control group. Absence of Nrf2 did not seem to considerably affect the body weight but KO mice tended to have lower body weights after dexamethasone treatment in both genders with the effect on male mice being statistically significant (25% lower, $p<0.05$). Surprisingly, both WT and KO mice of both genders showed lower fasting blood glucose levels after 3 months of treatment and better glucose tolerance. Livers of KO mice showed lower levels (~50%) of the cytoprotective genes Nqo1 and Gclc as expected but no difference was observed after dexamethasone treatment. Sarcopenia muscle markers Mafbx1 and Murf1 showed no significant changes. Male mice showed increased expression of Pnpla3 in white adipose tissue indicating increased lipolysis upon dexamethasone exposure. Micro-CT showed minor changes in the bone parameters without difference between male WT and Nrf2KO mice. **Conclusions:** Dexamethasone unexpectedly led to better glucose tolerance and lower body weight which is uncommon in humans but it has been described previously in mouse models. More analyses are in progress to fully elucidate this phenotype.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Deletion of Nuclear Receptor Constitutive Androstane Receptor CAR Increases Anxiety and Lowers Androgen Levels

Juan P Hernandez, PhD[1], Anjana Asokakumar, MS[2], Rui Xiao, PhD[3], David D. Moore, PhD[4], Sayeepriyadarshini Anakk, PhD[5].
[1]Baylor College of Medicine, Dallas, TX, USA, [2]University of Illinois at Urbana-Champaign, Champaign, IL, USA, [3]Baylor College of Medicine, Pearland, TX, USA, [4]Baylor College of Medicine, Houston, TX, USA, [5]University of Illinois, Urbana, IL, USA.

The orphan nuclear receptor, Constitutive Androstane Receptor (CAR, NR1I3) is primarily known to regulate the transcriptional networks involved in detoxification. We have identified a novel extra-hepatic role of CAR in regulating androgen levels and modulating testis function. Previous data has revealed that CAR activation by estradiol and inactivation by androstanol suggests an intricate link between sex hormones and CAR. We investigated control wild type and CARKO mice and found that the serum testosterone and androstenedione levels were lower in the absence of CAR. As expected, we did not find any induction of the genes in the detoxification machinery including, *Cyp3a, Cyp2b, Cyp2c family, Sult2a1* and *Mrp*. The decrease in the androgen levels in the CARKO mice is consistent with decrease in the anogenital distance, increased anxiety as measured by marble burying and elevated plus maze but no change in testis weight. H&E staining of CARKO mice shows accumulation of fat in the Leydig cells and lower numbers of Leydig cells which are in accordance with the loss of androgen levels. In addition, we will examine the consequence of reduced androgen and the hypothalamus-pituitary-gonadal axis in the CARKO mice.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

DHT Causes Liver Steatosis via Transcriptional Regulation of SCAP in Lean female Mice

Stanley Andrisse, MBA, PhD[1], Jessie Myer, BS[2], Dilip "Bobby" Bogle, MBBS[1], Nicole Eregha, BS, MS[1], Taylor Lofton, BS[1].
[1]Howard University College of Medicine, Washington, DC, USA, [2]University of Missouri, Columbia, MO, USA.

Hyperandrogenemia (HA) and insulin resistance are hallmarks of polycystic ovary syndrome (PCOS). These hallmarks are also integral elements of non-alcoholic fatty liver disease (NALFD). Administering low dose dihydrotestosterone (DHT) induced a lean PCOS-like female mouse model. The molecular mechanism of HA-induced NAFLD has not been determined. We hypothesized that low dose DHT would interrupt hepatic lipid metabolism leading to NAFLD. We extracted white adipose tissue (WAT), liver, and skeletal muscle from control and low dose DHT female mice; and performed histological and biochemical lipid profiles, Western blot, immunoprecipitation, chromatin immunoprecipitation, and real-time quantitative PCR analyses. DHT lowered the 65 kD form of cytosolic SREBP1 in the liver and WAT compared to controls. However, DHT did not alter the levels of the active and inactive forms of SREBP2 in the liver and WAT. DHT increased SCAP protein expression and SCAP-SREBP1 binding via AR binding to intron-8 of SCAP leading to increased SCAP mRNA. FAS mRNA and protein expression was increased in liver of DHT mice. p-ACC levels were unaltered in liver but decreased in WAT. Other lipid metabolism pathways were examined in liver and WAT, but no changes were observed. Our findings suggest that DHT increased de novo lipogenic proteins resulting in increased NAFLD via regulation of SREBP1 in liver. We show that in the presence of DHT the SCAP-SREBP1 interaction is elevated leading to increased nuclear SREBP1 resulting in increased de novo lipogenesis. We propose that the mechanism of action is increased AR binding to an ARE in SCAP intron-8.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Epitranscriptomic Reader HNRNPA2B1 Confers Endocrine Resistance to Breast Cancer Cells

Carolyn M. Klinge, MS, PhD, Belinda J. Petri, M.S., Kellianne M. Piell, B.S.
University of Louisville Schl of Medicine, Louisville, KY, USA.

Despite new combination therapies improving survival of breast cancer patients with estrogen receptor α (ER+) tumors, the molecular mechanisms for endocrine-resistant metastatic disease remain unresolved. HNRNPA2B1

(Heterogeneous Nuclear Ribonucleoprotein A2/B1), an RNA binding protein that functions as reader of the N(6)-methyladenosine (m6A) mark in transcribed RNA, is upregualted in tamoxifen- and fulvestrant-resistant, estrogen receptor (ERα)+ LCC9 and LY2 cells derived from MCF-7 endocrine-sensitive luminal A breast cancer cells (1). The miRNA-seq transcriptome of MCF-7 cells transiently overexpressing HNRNPA2B1 (A2B1) identified gene ontology (GO) pathways including "cellular response to steroid hormone signaling and estradiol" and "positive regulation of protein ser/thr kinase activity". Modest (~ 4.5-fold) stable HNRNPA2B1 overexpression in MCF-7 cells (MCF-7-A2B1) results in ablation of growth inhibition by 4-hydroxytamoxifen (4-OHT) and fulvestrant. This was not due to loss or decrease of ERα; in fact, ERα was increased. Conversely, transient knockdown of HNRNPA2B1 in LCC9 and LY2 cells sensitized the cells to growth inhibition by 4-OHT and fulvestrant while reducing ERα. MCF-7-A2B1 cells showed increased migration, invasion, clonogenicity, soft agar colony size, and markers of epithelial-to-mesenchymal transition. Like LCC9 cells, MCF-7-A2B1 cells showed activation of AKT and MAPK and high androgen receptor (AR). Treatment of MCF-7-A2B1 cells with either PI3K inhibitor Wortmannin or MEK inhibitor PD98059 inhibited soft agar colony formation and reduced colony size. Knockdown of HNRNPA2B1 in MCF-7-A2B1 reduces clonogenicity, but had no effect on the clonogenicity of either LCC9 or LY2 cells. These data suggest a role for HNRNPA2B1 in promoting the initiation of acquired endocrine-resistance by activating ser/thr kinase growth factor signaling pathways. Selective inhibition of HNRNPA2B1 may be a target to prevent acquistion of endocrine therapy resistance, but not treat established metastatic disease. Reference: (1) Klinge CM, Piell KM, Tooley CS, Rouchka EC. HNRNPA2/B1 is upregulated in endocrine-resistant LCC9 breast cancer cells and alters the miRNA transcriptome when overexpressed in MCF-7 cells. Scientific reports 2019; 9:9430

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Estrogen Receptor Alpha Is Required to Protect Daily Metabolic Rhythms From Disruption by High-Fat Feeding in Female Mice

Oluwabukola B. Omotola, B.Sc, Julie S. Pendergast, PhD.
UNIVERSITY OF KENTUCKY, Lexington, KY, USA.

The circadian system is a critical regulator of obesity in male mice, but its role in females is poorly understood. In our previous studies we found that estrogen regulates daily rhythms in female mice to confer resistance to diet-induced obesity, but the mechanism is unknown. Estrogen signals via the classical estrogen receptor alpha (ERα) to regulate metabolism and obesity. Therefore, in this study we tested the hypothesis that estrogen regulates daily metabolic rhythms in females via ERα. To do so, we studied daily rhythms in female global ERα knockout (ERα KO) with the circadian reporter, PERIOD2::LUCIFERASE, mice fed high-fat diet for 6 weeks. ERα KO female mice became obese and hyperglycemic when fed high-fat diet, while wild-type females were resistant to diet-induced obesity. Chronic high-fat diet feeding also reduced the amplitude of the daily rhythm of eating behavior in ERα KO, but not wild-type, female mice. In wild-type females, the amplitude of the locomotor activity rhythm increased during high-fat feeding. In contrast, high-fat feeding decreased the amplitude of the activity rhythm in ERα KO females. The temporal relationship between central and peripheral circadian tissue clocks was disrupted by high-fat feeding in ERα KO females since the phase of the liver PERIOD2::LUCIFERASE rhythm was advanced 4 hours by high-fat feeding in ERα KO mice compared to wild-type females. Taken together these results show that estrogen signals via ERα to protect daily metabolic rhythms from disruption by high-fat feeding in female mice.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Glucocorticoid Mediated Transcriptional Activity in Human Corneal Epithelial Cells Lacking the Glucocorticoid Receptor

Andrea Joyce Jonsson, B.S. 20211, Lydia Ennis, B.S. 20211, John A. Cidlowski, PhD[2], Mahita Kadmiel, PhD[1].
[1]ALLEGHENY COLLEGE, Meadville, PA, USA, [2]NIEHS/NIH, Durham, NC, USA.

The cornea is the dome-shaped transparent outermost layer of the eye, forming a physical barrier to protect the internal structures of the eye. Glucocorticoids are a mainstay in the treatment of ophthalmic diseases for their anti-inflammatory and anti-angiogenic properties. However, high doses or chronic use of glucocorticoid therapy can lead to vision-impairing effects such as increase in intraocular pressure and the formation of cataracts. The exact signaling pathways responsible for these undesirable effects of glucocorticoid use is poorly understood. One of the major molecular actions of glucocorticoids is to regulate transcription through its cognate nuclear receptor, the glucocorticoid receptor. We have previously reported the effect of glucocorticoids on global gene expression and their role in wound healing and barrier function in immortalized human corneal epithelial cells (HCE-T). In the current study, we knocked down glucocorticoid receptor using siRNA (GRKD) to determine the function of the glucocorticoid receptor in HCE-T cells. Successful knockdown of glucocorticoid receptor was confirmed by RT-PCR and immunoblot experiments. Genome-wide microarray analysis was performed and an FDR adjusted p value less than 0.01 was considered the cut off to create the list of differentially expressed genes (DEGs). Comparison of GRKD cells to HCE-T cells expressing endogenous glucocorticoid receptor (referred as NTC for No Target Control siRNA) revealed that expression of 2150 genes was altered in HCE-T cells when glucocorticoid receptor was knocked down, indicating that glucocorticoid receptor in corneal epithelial cells regulates a large cohort of genes. Inhibition of matrix metalloproteases, granulocyte adhesion and diapedesis, cyclins and cell cycle regulation were the top canonical pathways predicted by Ingenuity Pathway Analysis (IPA) to be altered in GRKD cells. In a 6-hour treatment with dexamethasone (Dex), a synthetic