(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

glucocorticoid, or with vehicle as a control, NTC cells had 2039 Dex-regulated genes, while Dex was still able to regulate 1087 genes in GRKD cells. Of these 1087 genes, 895 genes were uniquely regulated by Dex in GRKD cells suggesting that glucocorticoids might be signaling through another receptor in corneal epithelial cells. The top canonical pathways predicted to be altered by Dex in GRKD cells included PI3K/ATK Signaling, ERK5 Signaling, Prostrate Cancer Signaling, Aldosterone Signaling in Epithelial Cells, and PPAR signaling. These findings suggest that Dex could regulate large cohorts of genes through other nuclear receptors in corneal epithelial cells. Given the wide use of ophthalmic Dex in forms including eyedrops, ointments, gels, and implants, it is of clinical significance to understand the molecular actions of synthetic glucocorticoids since they appear to be ligands for multiple nuclear receptors in ocular cells and tissues.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Glucocorticoid Receptor Condensates Link DNA-Dependent Receptor Dimerization and Transcriptional Transactivation

Filipp Frank, PhD, Xu Liu, PhD, Eric Ortlund, PhD.
Emory University, Atlanta, GA, USA.

The glucocorticoid receptor (GR) is a ligand-regulated transcription factor (TF) that controls the tissue- and gene-specific transactivation and transrepression of thousands of target genes. Distinct GR DNA binding sequences with activating or repressive activities have been identified, but how they modulate transcription in opposite ways is not known. We show that GR forms phase-separated condensates that specifically concentrate known co-regulators via their intrinsically disordered regions (IDRs) in vitro. A combination of dynamic, multivalent (between IDRs) and specific, stable interactions (between LxxLL motifs and the GR ligand binding domain) control the degree of recruitment. Importantly, GR DNA-binding directs the selective partitioning of co-regulators within GR condensates such that activating DNAs cause enhanced recruitment of co-activators. Our work shows that condensation controls GR function by modulating co-regulator recruitment and provides a mechanism for the up- and down-regulation of GR target genes controlled by distinct DNA recognition elements.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Improving the Diagnosis, Treatment, and Prevention of Endocrine Diseases Through Accurate and Reliable Laboratory Measurements With CDC Clinical Standardization Programs

Otoe Sugahara, BS, Uliana Danilenko, PhD, Krista Poynter, BS, Lynn Collins, BS, Nasim Khoshnam, MS, Tatiana Buchannan, BS, Clark Coffman, BS, Ashley Ribera, BS, Brandon Laughlin, MS, Komal Dahya, BS, Bianca Smith, BS, Amonae Dabbs-Brown, MS, Fidelia Pokuah, MPH, Douglas Wirtz, MS, Candice Zena Ulmer, PhD, Hui Zhou, PhD, Alicia Lyle, PhD, Li Zhang, PhD, Hubert W. Vesper, PhD.
CDC, Atlanta, GA, USA.

Laboratory measurements are critical for correct diagnosis and treatment of patients with chronic diseases such as hypogonadism, PCOS, and thyroid diseases. Inaccurate measurements of disease biomarkers can lead to misclassification of patients/incorrect treatment and prevent the effective use of research findings in patient care. The CDC Clinical Standardization Programs (CDC CSP) improve the accuracy and reliability of clinical biomarker measurements by assessing and improving the analytical performance of assays. The CDC CSP assist with assay calibration, the certification of analytical performance, and the monitoring of routine patient and research testing. The CDC CSP work with clinical/research laboratories and assay manufacturers to improve laboratory measurements. Its current programs include the following analytes: total testosterone (TT), estradiol (E2), vitamin D (VD), free thyroxine (FT4), total cholesterol (TC), total glycerides (TG), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C). The work is being conducted through certification/monitoring programs and technical assistance. Most assays participating in the certification programs have seen performance improvements and maintain performance over time by continuous participation. Most major commercial laboratories and assays manufactures are enrolled in the certification programs. Currently certified and non-certified assays are available. Assays certified by CDC CSP are listed on the website at https://www.cdc.gov/labstandards/hs.html. The CDC Lipid Standardization Programs and CDC Accuracy-based Monitoring Programs allow for weekly monitoring of analytical performance of routine tests for analytes including TT, VD, TC, TG, HDL-C, apolipoprotein A1 and B. These monitoring programs assist researchers with assessing measurement accuracy of research studies over time and across laboratories. The CDC CSP also support accuracy-based external quality assurance surveys such as those offered by the College of American Pathologists (CAP). The CDC CSP assist researchers and stakeholders with developing and establishing reference intervals and conducting studies to better assess and diagnose patients. Based on the needs and requests from clinical community, programs for new biomarkers such as Lp(a), PTH and glucose are being developed. The CDC CSP work with stakeholders, such as the Endocrine Society and the Partnership for the Accurate Testing of Hormones, to educate the clinical and laboratory communities about the importance of using standardized assays in patient care, research, and public health.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Inhibition of Estrogen Signaling Reverses Established Inguinal Hernias

Tanvi Potluri, ScM, Matthew Joseph Taylor, PhD, Hong Zhao, MD, PhD, Serdar Ekrem Bulun, MD.
Northwestern University, Chicago, IL, USA.