by ERα, BioID and TurboID screens were performed in two ER+ breast cancer cell lines, T-47D and ZR-75-1. Surprisingly, Cathepsin-D (Cath-D), a lysosomal aspartyl endoproteinase that is an ER target gene, was identified in these screens. Cath-D expression is associated with a poor prognosis and increased metastasis rate in breast cancer irrespective of its catalytic activities {Glondu, 2001 #119}[i]. Cath-D is localized in part to the nucleus where it interacts with TRPS1, a repressor of GATA-mediated transcription and modulator of ERα signaling {Bach, 2015 #117}[ii]. Co-silencing Cath-D and TRPS1 suppressed cell proliferation and inhibited growth under soft agar, suggesting that they cooperate to drive tumorigenesis {Bach, 2015 #117}[ii]. We hypothesized that Cath-D plays genomic as well as non-genomic roles in breast tumor aggressiveness and may alter ERα-mediated transcription. The nuclear localization of Cath-D was confirmed by immunofluorescence using different commercialized antibodies and observed in western blots of chromatin-bound fractions in three different ERα+ breast cancer cell lines, T-47D, ZR-75 and MCF-7. Specificity of the antibodies was confirmed using siRNA-mediated suppression of Cath-D. Moreover, Cath-D was also identified in proximity to TurboID-ERα by LC-MS after chromatin fractionation. The proximity of ERα and Cath-D both in the cytoplasm and nucleus was confirmed by proximity Ligation Assay (PLA) in three ER+ cell lines. Co-immunoprecipitation assays indicated physical interaction of Cath-D with ERα in T-47D cell extracts. Further, Cath-D was detected by ChIP-qPCR on estrogen response elements (EREs) of two ERα target genes, TFF1 and GREB1 in T-47D and ZR-75 cells. These results suggest that Cath-D can interact with ERα on DNA and play genomic roles in ER+ breast cancer cells. [i] Glondu, M., et al. (2001). "A mutated cathepsin-D devoid of its catalytic activity stimulates the growth of cancer cells." *Oncogene* **20**(47): 6920-6929. [ii] Bach, A. S., et al. (2015). "Nuclear cathepsin D enhances TRPS1 transcriptional repressor function to regulate cell cycle progression and transformation in human breast cancer cells." *Oncotarget* **6**(29): 28084-28103.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

The Effect of a Novel Glucocorticoid Receptor Antagonist (CORT113176) on Glucocorticoid and Insulin Receptor Sensitive Hepatic Gene (mRNA) Expression in a Neonatal Rat Model of Human Prematurity

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Preterm birth is a global health problem the sequelae of which are not well understood. Hypoxia, a common stressor with prematurity, can affect blood glucose via stress-induced increases in glucocorticoids (GC). GCs are also administered to preterm infants to improve oxygenation; however, this is controversial. CORT113176 (Corcept Therapeutics) is a novel, selective glucocorticoid receptor (GR) antagonist that does not bind to the progesterone receptor. We have demonstrated that CORT113176 (in our rat model of preterm birth) increases baseline corticosterone (due to loss of GC negative feedback) and attenuates hypoxia-induced increases in insulin resistance implicating endogenous corticosterone in post-natal metabolic adaptations to stress. We now propose that CORT113176 is useful to evaluate the hepatic effects of endogenous GCs in our rat model of preterm birth by measuring critical GC and insulin receptor sensitive gene mRNAs. Postnatal day (PD) 2 rat pups of both sexes (N=5 per treatment/sex) were pretreated with CORT113176 (600 mg/kg IP) or vehicle. After 60 minutes, a group of pups were euthanized with livers collected and preserved in RNAlater (baseline). The remaining pups were separated from their dams, exposed to normoxia (control) or hypoxia (8% O_2) for 60 minutes, and livers obtained. Total hepatic RNA was extracted, and mRNA expression was analyzed (RT-qPCR) for GC and insulin receptor sensitive genes: *GC*: *Fkbp5, Gilz, Nr3c1 (Gr), Nr3c2 (Mr), Per1, Ttpa*. *INSULIN*: *Akt2, G6Pase, Igf1r, Insr, Irs1, Irs2, Pik3cb, Pik3r1, Srebp1c*. CORT113176 decreased the expression of all baseline hepatic insulin receptor mRNAs in both sexes, except for *G6Pase*. *Pik3r1* mRNA expression significantly decreased with 60 minutes of normoxic separation (fasting) in males and females compared to baseline and hypoxic separation; this was blocked by CORT113176. In the GC receptor sensitive panel, CORT113176 decreased basal *Nr3c1 (Gr)* mRNA. Normoxic and hypoxic separation increased *Per1* and *Gilz* mRNA expression; this effect was blocked by CORT113176. Interestingly, *Fkbp5* expression, a proposed clinical marker for GR antagonism, was not altered by CORT113176. The hepatic GC and insulin receptor sensitive gene mRNA panels we developed are sensitive to GR antagonism suggesting they may be a useful addition to *Fkbp5*. The increase in endogenous corticosterone, acting via GR, is critical in the hepatic response to stress in our neonatal rat model of hypoxia and prematurity.

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STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

The Impact of a Single Phosphorylation Site Mutation in the Glucocorticoid Receptor on the Molecular and Cellular Development of the Cerebral Cortex

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Premature birth leads to a significant increase in adverse clinical outcomes, including Respiratory Distress Syndrome, Bronchopulmonary Dysplasia, Necrotizing Enterocolitis and Intraventricular Hemorrhage. Synthetic Glucocorticoids (sGC) are administered prenatally to pregnant mothers at risk to reduce the chance of these complications. However, there is a correlation between long-term neurological defects in the infant and the clinical use of sGC prenatally. The use of the sGCs have been linked to the development of cerebral palsy and deficits in attention and concentration. To investigate the cellular basis of these abnormalities, we examined the consequences of sGC administration of the developing murine brain. Our studies demonstrated that premature exposure to sGC alters neural stem cell biology and has long term consequences for adult behavior in mice. In humans, site-specific phosphorylation of the Glucocorticoid Receptor (GR) on Serine 211 versus Serine 226 is associated with activated or repressed transcriptional states and clinical studies indicate that the ratio of S220/S226 phosphorylation is associated with increased predisposition to specific psychiatric disease states, including Major Depressive Disorder and Bipolar Disorder. To examine the role of these phosphorylation sites in the development of behavioral abnormalities, we utilized a knock-in mouse model where Serine 220 (equivalent to human Serine 211) was replaced with an alanine (S220A). In-vitro microarray analysis of neural stem cells and QPCR validation were performed to examine the expression changes in individual transcripts in critical pathways that may correlate with long-term neurologic disorders. Our results indicated that changing the phosphorylation status of GR alters the expression of 2570 genes. Ingenuity Pathway Analysis indicated that the major pathways altered include those involved in cellular proliferation, mitochondrial function, Valine degradation and G-coupled protein receptors involved in neurotransmission. Both in-vitro and in-vivo experiments indicated that the S220A mutation alters the cells response to sGC administration by impacting proliferation and differentiation. The long-term goal of these experiments was to demonstrate a role for S220 phosphorylation in the development of neuropsychiatric disorders.

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STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

The Transcriptional Function of GRHL2 in Hormone-Dependent Breast Cancers

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The Grainyhead-like protein family, composed of GRHL1, GRHL2, and GRHL3, are nuclear transcription factors that regulate epithelial differentiation. GRHL2 has been associated with several nuclear hormone receptors, including progesterone receptor (PR), androgen receptor (AR), and more recently, estrogen receptor (ER). In breast cancer, GRHL2 has been shown to both activate ER-dependent enhancers through FOXA1 and MLL3-mediated deposition of the activating histone mark H3K4me1 and repress enhancers via inhibition of the histone acetyltransferase p300. Cistromic analysis by our group of ER phosphorylated at serine 118 (pS118-ER), a form of transcriptionally active ER, found an enrichment of the GRHL motif near pS118-ER binding sites. Despite these findings, the direct relationship between GRHL2 and ER transcriptional function and how that relationship influences ER-positive breast cancer growth and differentiation is not well-defined. To explore the relationship between GRHL2 and pS118-ER further, we used transcriptomic and cistromic analysis of ER-positive cells lacking GRHL2 to determine the impact of the loss of GRHL2 on cellular and transcriptional responses to estrogen. This analysis identified a subset of genes that are controlled by both GRHL2 and estrogen. In addition, CRISPR engineered T47D cells lacking a portion of the GRHL2 transactivation domain (ΔTAD) demonstrate reduced nuclear ER levels and reduced ER chromatin occupancy. Gene expression analysis of ΔTAD-GRHL2 cells showed increased *GRHL3* expression, and ChIP analysis revealed increased Pol II occupancy at the *GRHL3* promoter, suggesting that there may be a compensatory mechanism within the GRHL family to regulate the transcriptome. Finally, ΔTAD-GRHL2 mutants reduced growth and colony formation relative to wild-type controls. Together, this work will provide an understanding of how transcriptionally active ER and GRHL2 selectively cooperate to regulate transcription, growth, and differentiation in ER-positive breast cancer.

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STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

Time of Day Regulates Renal Mineralocorticoid Receptor Transcriptional Control of Electrolyte Balance

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The mineralocorticoid receptor (MR) has an established role in blood pressure control and cardiovascular homeostasis via many actions in the heart and kidney. We recently identified a role for the MR in controlling the circadian clock in cardiac cells and demonstrated that time-of-day impacts MR activation in the heart. While time dependent behaviours such as upright posture and fluid intake control aldosterone release via the renin-angiotensin-aldosterone system (RAAS), we hypothesise that the circadian clock controls aldosterone signalling by modifying MR transcriptional outcomes. Two established MR target genes and core circadian clock genes are *period 1* (*Per1*) and *period 2* (*Per2*). We have previously shown that a bolus dose of aldosterone (i.p.) induced cardiac expression of *Per1* and *Per2* in wildtype mice treated at 8AM (start of rest period) but not when administered at 8PM (start of active period). Whether MR activation in the kidney is similarly dependent on time of day and aligns with MR actions in the heart remains to be assessed. We also sought to determine