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.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

The Transcriptional Function of GRHL2 in Hormone-Dependent Breast Cancers

Rebecca Reese, B.S., Kyle Helzer, PhD, Elaine T. Alarid, PhD.
University of Wisconsin - Madison, Madison, WI, USA.

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.

Steroid Hormones and Receptors

STEROID HORMONES, NUCLEAR RECEPTORS, AND COLLABORATORS

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

Monica Kanki, BBiomedSc(Hons)[1], James Morgan, BSc(Hons)[2], Peter J. Fuller, AM, BMedSci(Hons), MBBS, PhD, FRACP[2], Morag Jennifer Young, PhD[3].
[1]Monash University/Baker Heart & Diabetes Institute, Clayton/Melbourne VIC, Australia, [2]Hudson Institute of Medical Research, Clayton VIC, Australia, [3]Baker Heart & Diabetes Institute, Melbourne, Australia.

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