University of North Carolina at Chapel Hill
Department of Pharmacology Research Retreat
September 16th, 2022 – William and Ida Friday Center

8:30–9:00 am	Continental Breakfast (Atrium)
9:00–9:10 am	State of the Department Address (Dogwood) – **Henrik Dohlman, PhD**, **Distinguished Professor and Chair**
9:10–9:25 am	Student Survey – **Michael Emanuele, PhD, Associate Professor**
9:25-9:40 am	Pharmacology Diversity, Equity, & Inclusion Update – **Maria Aleman, PhD, Assistant Professor**

Morning Session – all talks include 5 minutes of Q&A

Moderaters: Ryan Sheehy, Graduate Student in the Song Lab & Juan Song, PhD, Associate Professor

9:40–9:55 am	**Meghan Flanigan**, **Postdoctoral Fellow, Kash Lab,** *"Sex-specific regulation of binge drinking, social, and arousal behaviors by subcortical serotonin 5HT2c receptor-containing neurons."*
9:55–10:15 am	**Adam Palmer, PhD**, **Assistant Professor,** *"Accurate prediction of the clinical efficacy of combinations of cancer therapies."*
10:15–10:25 am	**Break**
10:25–10:40 am	**Jeff DiBerto, Graduate Student, Roth Lab**, *"Further Considerations of G Protein Bias in Developing Safer Mu-Opioid Receptor Agonists."*
10:40–11:00 am	**Elizabeth Brunk, PhD, Assistant Professor,** *"Understanding biological ripple effects through multi-faceted omics data."*
11:00–11:15 am	**Tanya Bodrug, Graduate Student, Brown Lab,** *"Neural Network based reconstruction of ubiquitination by the Anaphase- Promoting Complex using time-resolved cryo-EM."*
11:15 am–12:15 pm	**Poster Session 1** (presenters 1-25, Atrium)
12:15–1:15 pm	**Lunch** (Trillium Room)

Afternoon Session – all talks include 5 minutes of Q&A

1:15–2:15 pm	**Poster Session 2** (presenters 26-50, Atrium)
2:15–2:35 pm	**John Morris, PhD, Assistant Professor,** *"The Morris Lab versus p53, the master of the nucleus."*
2:35–2:50 pm	**Amy Pomeroy, PhD, Postdoctoral Fellow, Palmer Lab,** *"A simulation of curative combination therapy explains and predicts clinical trial results in Diffuse Large B-Cell Lymphoma."*
2:50–3:10 pm	**Jessica Walsh, PhD, Assistant Professor,** *"Neural circuit mechanisms underlying social behavior."*
3:10–3:25 pm	**Yadong Li, Postdoctoral Fellow, Song Lab,** *"Hypothalamic modulation of adult hippocampal neurogenesis in mice confers activity-dependent regulation of memory and anxiety-like behavior."*
3:25–3:35 pm	**Break**
3:35–3:55 pm	**Maria Aleman, PhD, Assistant Professor,** *"PCBPs: Regulators of RNA & Iron."*
3:55–4:10 pm	**Emily Fennell, Graduate Student, Graves Lab,** *"Characterization of TR-107, a Novel Chemical Activator of the Human Mitochondrial Protease ClpP."*
4:10–4:25 pm	**Tim Daugird, Graduate Student, Legant Lab**, *"Bridging nano- and mesoscale nuclear organization with single molecule microscopy."*
4:25–4:40 pm	**Marguerite (Bree) Little, Graduate Student, Duncan Lab,** *"Determination of Cross-Reactive Antigens in 4CMenB Against Neisseria gonorrhoeae."*
4:40-4:55 pm	**Gavin Schmitz, Graduate Student, Herman/Roth Labs**, *"Effects of Psilocin on 5-HT2A+ L5P neurons."*

Reception & Award Ceremony

5:00–6:00 pm	Social Mixer and Award Presentations (Magnolia Lounge)

Department of Pharmacology Research – Friday, September 16, 2022

ORAL PRESENTATIONS
(1-9)
(in order of presentation)

1. **Flanigan, Meghan**
   (Kash Lab)
   "Sex-specific regulation of binge drinking, social, and arousal behaviors by subcortical serotonin 5HT2c receptor-containing neurons."

2. **DiBerto, Jeff**
   (Roth Lab)
   "Further Considerations of G Protein Bias in Developing Safer Mu-Opioid Receptor Agonists."

3. **Bodrug, Tatyana**
   (Brown Lab)
   "Neural Network based reconstruction of ubiquitination by the Anaphase- Promoting Complex using time-resolved cryo-EM."

4. **Pomeroy, Amy**
   (Palmer Lab)
   "A simulation of curative combination therapy explains and predicts clinical trial results in Diffuse Large B-Cell Lymphoma."

5. **Li, Yadong**
   (Song Lab)
   "Hypothalamic modulation of adult hippocampal neurogenesis in mice confers activity-dependent regulation of memory and anxiety-like behavior."

6. **Fennell, Emily**
   (Graves Lab)
   "*Characterization of TR-107, a Novel Chemical Activator of the Human Mitochondrial Protease ClpP."*

7. **Daugird, Tim**
   (Legant Lab)
   "Bridging nano- and mesoscale nuclear organization with single molecule microscopy."

8. **Little, Marguerite (Bree)**
   (Duncan Lab)
   "*Determination of Cross-Reactive Antigens in 4CMenB Against Neisseria gonorrhoeae."*

9. **Schmitz, Gavin**
   (Herman/Roth Labs)
   "Effects of Psilocin on 5-HT2A+ L5P neurons."

Sex-specific regulation of binge drinking, social, and arousal behaviors by subcortical serotonin 5HT2c receptor-containing neurons.

M. Flanigan, O.J. Hon, S. D'Ambrosio, K.M. Boyt, L. Hassanein, M. Castle, H.L. Haun, M. Pina, and T.L. Kash.

Binge alcohol drinking is a serious public health issue that is associated with increased risk of developing alcohol use disorder and mood disorders. Using the Drinking-in-the-Dark (DiD) model, I found that chronic binge alcohol consumption selectively reduced social recognition in female mice and increased acoustic startle responses in male mice. DiD increased the intrinsic excitability of lateral habenula serotonin 5HT2c receptor-containing neurons (LHb5HT2c) in both males and females, but decreased excitability of bed nucleus of the stria terminalis serotonin 5HT2c receptor-containing neurons (BNST5HT2c) only in females. Employing multi-site in-vivo fiber photometry in combination with cell-type specific calcium and serotonin sensors, I observed that LHb5HT2c and BNST5HT2c neurons mounted similar calcium responses to alcohol and socio-affective stimuli that were largely reflective of fluctuations in serotonin release onto these neurons. However, compared to water controls, female DiD mice displayed increased serotonin release onto BNST5HT2c neurons upon interaction with a novel social target and enhanced LHb5HT2c calcium responses to alcohol. Male DiD mice displayed increased calcium responses in LHb5HT2c and BNST5HT2c neurons upon exposure to acoustic startle stimuli, but serotonin release onto these neurons during startle was not altered by DiD. While chemogenetic activation of LHb5HT2c neurons reduced social behavior, startle, and drinking in both sexes, chemogenetic activation of BNST5HT2c neurons increased startle behaviors in both sexes but reduced drinking and sociability only in females. Genetic knockdown of LHb5HT2c or BNST5HT2c partially normalized DiD-induced socio-affective disturbances in both sexes, but BNST5HT2c knockdown robustly increased binge drinking only in females. Critically, chemogenetic inhibition of LHb5HT2c fully normalized alcohol-induced behavioral dysfunctions in both males and females. Together, these data suggest that while both LHb5HT2c and BNST5HT2c undergo neuro-plastic changes in response to alcohol, excessive activation of LHb5HT2c is the primary functional mechanism driving both alcohol-induced social deficits in females and alcohol-induced startle potentiation in males.

Further Considerations of G Protein Bias in Developing Safer Mu-Opioid Receptor Agonists.

Jeffrey DiBerto

The G protein-coupled mu-opioid receptor (MOR) is the primary target of pain medications such as morphine. Although MOR agonists are potent and efficacious analgesic drugs, their use is beset by adverse effects like respiratory depression, which contributed to roughly 69,000 deaths in 2020. Previous attempts to develop safer opioids sought to preserve G protein signaling but attenuate beta-arrestin2 recruitment – a concept known as biased agonism -however, such compounds have had mixed pre-clinical and limited clinical success. Recent reevaluation of MOR agonist efficacy and respiratory depression suggests that attenuated G protein signaling may, instead, be more tractable for safer opioid development. Here, we utilized a novel approach to attenuate MOR Gi/o signaling by targeting the receptor sodium binding pocket, through which sodium acts as a negative allosteric modulator. Analogs of the synthetic opioid fentanyl were made that replaced its phenyl group with alkyl linkers of varying lengths connected to a charged guanidine group for engaging the sodium binding pocket. We found two compounds, C5 and C6, displaying potent MOR activation, whose engagements with the sodium binding pocket were confirmed structurally by cryo-electron microscopy, and further shown as having water-mediated and direct interactions with this pocket, respectively, by molecular dynamics simulations. Pharmacological analysis of C5, C6, and a battery of MOR agonists using the TRUPATH bioluminescence resonance energy transfer-based platform for measuring heterotrimeric G protein dissociation and beta-arrestin recruitment revealed C6 as a partial agonist for all Gi/o subunits. Notably, C6 Gz signaling is significantly less efficacious than that of all other tested MOR agonists, including classical partial agonists. Studies conducted in mice found that C6 does not produce respiratory depression at doses equianalgesic to morphine. This study reveals that inter-G protein bias may allow for the development of safer opioids, and emphasizes further evaluation of Gz in mediating MOR-induced respiratory depression.

Neural Network based reconstruction of ubiquitination by the Anaphase- Promoting Complex using time-resolved cryo-EM.Tatyana.

Bodrug., Ellen Zhong, David Haselbach, Nicholas G. Brown.

The Anaphase-Promoting Complex/Cyclosome (APC/C) is a 1.2 MDa RING E3 ubiquitin ligase that orchestrates the cell cycle by polyubiquitinating cell cycle regulators to mark them for proteasomal degradation. Several cofactors work with the APC to dynamically control the timing of its activity, the specificity of its targets, and the extent of ubiquitination. Improvements in our ability to use single-particle cryo-electron microscopy (cryo-EM) to reconstruct 3D structures of the APC/C bound to its cofactors have helped to reveal how the APC/C is activated or inhibited at specific points in the cell cycle and have allowed us to visualize detailed snapshots of its catalytic activity. A comprehensive view of how this highly dynamic molecular machine coordinates its many cofactors and binding partners to direct the cell cycle remains to be determined. Single-particle cryo-EM involves the nearly instantaneous preservation of molecular complexes in a thin film of vitreous ice. This retains their native structure and allows for reconstructions at near-atomic resolution. Conventional 3D reconstruction techniques have focused on providing highly detailed structural information from macromolecules in stabilized conformations. Recent work has demonstrated the feasibility of using neural networks to analyze heterogenous EM datasets to generate distributions of 3D structures and visualize macromolecules as they undergo complex conformational changes. Here, we use conventional cryo-EM sample preparation and data collection methods to reconstitute ubiquitination reactions carried out by the APC/C in a time-resolved manner. We then use cryoDRGN (Deep Reconstructing Generative Networks) in a neural network-based approach to reconstruct the conformational changes that the APC/C undergoes as it interacts with its many binding partners and directly visualize ubiquitination.

A simulation of curative combination therapy explains and predicts clinical trial results in Diffuse Large B-Cell Lymphoma.

Amy E. Pomeroy and Adam C. Palmer.

Diffuse Large B-Cell lymphoma (DLBCL) is the most common blood cancer with more than 18,000 new diagnoses a year in the United States alone. Most cases of DLBCL are cured (do not reoccur in the patient's lifetime) by the 5-drug RCHOP combination therapy. While this combination has been used to treat DLBCL for over two decades, the mechanistic basis for the success of this empirically developed regimen has long been subject to speculation. Recent experimental data has shed new light on the combination's ability to overcome heterogeneity and drug resistance to achieve cures. In this work we apply clinically and experimentally established principles in a mechanistically detailed simulation of kinetics of tumor growth and death to RCHOP in patient populations with DLBCL. Our simulation implements multi-drug dose-response functions in heterogeneous populations of tumor cells, within heterogeneous cohorts of patients, and is calibrated on clinical trials and experimental data with the Metropolis-Hastings algorithm. We find that this simulation reproduces progression-free survival (PFS) distributions for CHOP and RCHOP, differences in cure rate by the number of chemotherapy cycles, and distributions of tumor shrinkage kinetics in patients. Additionally, we used PFS in relapsed/refractory DLBCL patients treated with Polatuzumab-Vedotin (PV) to calibrate the benefit of PV and thereby predicted the PFS benefit of RCHP-BV in the recent POLARIX trial. Together these results show that curative treatments can be understood in quantitative and kinetic detail with simulations that are faithful to clinical data from human trials. These simulations can be applied prospectively to predict the efficacy of novel combination regimens, which can inform trial design to best assess new treatments with curative intent for lymphoma and other cancers.

Hypothalamic modulation of adult hippocampal neurogenesis in mice confers activity-dependent regulation of memory and anxiety-like behavior.

Ya-Dong Li, Yan-Jia Luo, Ze-Ka Chen, Luis Quintanilla, Libo Zhang, Juan Song.

Adult hippocampal neurogenesis plays a critical role in memory and emotion processing, and this process is dynamically regulated by neural circuit activity. However, it remains unknown whether manipulation of neural circuit activity can achieve sufficient neurogenic effects to modulate behavior. Here we report that chronic patterned optogenetic stimulation of supramammillary nucleus (SuM) neurons in the mouse hypothalamus robustly promotes neurogenesis at multiple stages, leading to increased production of neural stem cells and behaviorally relevant adult-born neurons (ABNs) with enhanced maturity. Functionally, selective manipulation of the activity of these SuM-promoted ABNs modulates memory retrieval and anxiety-like behaviors. Furthermore, we show that SuM neurons are highly responsive to environmental novelty (EN) and are required for EN-induced enhancement of neurogenesis. Moreover, SuM is required for ABN activity-dependent behavioral modulation under a novel environment. Our study identifies a key hypothalamic circuit that couples novelty signals to the production and maturation of ABNs, and highlights the activity-dependent contribution of circuit-modified ABNs in behavioral regulation. We will employ this strategy to treat cognitive and noncognitive deficits in Alzheimer's disease.

Characterization of TR-107, a Novel Chemical Activator of the Human Mitochondrial Protease ClpP.

E.M.J Fennell, L.J. Aponte-Collazo, J.D. Wynn, K. Drizyte-Miller, E. Leung, Y.E. Greer, P.R. Graves, A.A. Iwanowicz, H. Ashamalla, E. Holmuhamedov, H. Lang, D.S. Karanewsky, C.J. Der, W.A. Houry2 S. Lipkowitz, E.J. Iwanowicz, L.M. Graves.

We have recently described a new class of small molecule activators of the mitochondrial protease ClpP ("TR compounds"), demonstrating their ability to inhibit triple-negative breast cancer cell growth at greater potency than the related compound ONC201. One selected compound (TR-107) demonstrated ClpP-dependent reduction of mitochondrial proteins (including OXPHOS and TCA cycle components), and Seahorse XF analysis confirmed inactivation of OXPHOS and increased glycolysis following TR-107 treatment. Pharmacokinetic properties of TR-107 were investigated and compared to other known ClpP activators (e.g. ONC201, ONC212). TR-107 showed excellent exposure and serum t1/2 following oral administration. MDA-MB-231 xenografts were used to investigate the anti-tumor response of TR-107 in vivo, and demonstrated reduced tumor volume and extension of survival in TR-107 treated mice. In summary, we have identified highly potent ClpP agonists with improved efficacy against TNBC through targeted inactivation of OXPHOS and disruption of mitochondrial metabolism.

Bridging nano- and mesoscale nuclear organization with single molecule microscopy.

T.A. Daugird, Y. Shi, W.R. Legant.

Chromatin in the nucleus is dynamic and heterogenous. At the microscale, chromatin forms 100-300 nm dense domains that are that are visibly distinct and separate from a less-dense interchromatin space. This apparent partitioning of chromatin domains from the interchromatin space is independent of active nuclear processes such as DNA looping or transcription and is posited to have a role in organizing the DNA into 'active' and 'inactive' zones. However, evidence for the functional roles of the dense chromatin domains and the less-dense interchromatin space are largely based on protein localization studies in fixed cells or extrapolated from nuclear-wide single molecule observations of nucleosome dynamics. Moreover, it is not clear how chromatin domains, and more generally, how chromatin density variations at the 100 nm – 1 micron length scale relate to the molecular motions of nucleosomes and nuclear proteins at the nanoscale. Here, we combine high-resolution lattice light sheet microscopy together with single particle tracking to explore to the functional relationships between chromatin density, chromatin domains, and nuclear functions. We observe that on average, individual nucleosomes become more physically constrained as chromatin density increases within the nucleus. Additionally, we find that pharmacological inhibition of various nuclear processes such as transcription or splicing preferentially impact nucleosome dynamics in nuclear compartments with low to intermediate chromatin density whereas histone hyperacetylation preferentially impacts nucleosome dynamics within dense chromatin domains. These data suggest that transcriptional processes stabilize active regions of the genome around the periphery of chromatin domains, potentially maintaining active genes within a transcriptionally competent nuclear microenvironment. Finally, we will present extensions of our imaging approaches to explore the relationship between chromatin density, DNA remodeling proteins, and transcription factor search and binding dynamics within the nucleus.

Determination of Cross-Reactive Antigens in 4CMenB Against Neisseria gonorrhoeae.

Marguerite B. Little, Kristie Connolly, Joshua Tomberg, Weiyan Zhu, Robert A. Nicholas, and Joseph A. Duncan.

Introduction: Recent retrospective studies have shown that rates of gonorrhea have decreased after mass vaccination campaigns with outer membrane vesicle (OMV)-containing meningococcal vaccines such as MenZB and 4CMenB. 4CMenB has also been shown to induce cross-species protection in the mouse model of infection. In addition to OMV, 4CMenB also contains five recombinant antigens, four of which have highly homologous N. gonorrhoeae counterparts. The antigens in 4CMenB or other meningococcal OMV-containing vaccines that are responsible for protective immune responses against N. gonorrhoeae infection are unknown. We investigated the identity of N. gonorrhoeae antigens recognized and functional significance of anti-gonococcal antibodies after vaccination with 4CMenB.

Methods: Serum samples were collected from human participants before and after 4CMenB vaccination and from mice after vaccination with 4CMenB or alum, and were used for immunoblot analysis. Recombinant gonococcal proteins expressed in E. coli and crude N. gonorrhoeae OMV from strain FA1090, and isogenic mutants of the strain were run on SDS PAGE and blotted with mouse and human immune sera to determine whether 4CMenB immunization resulted in recognition of specific gonococcal antigens.

Results: Both mice and humans vaccinated with 4CMenB produce antibodies that cross react with gonococcal NHBA and MtrE. Cross-reactive antibodies to recombinant BamA and PorB from N. gonorrhoeae strain FA1090 were not detected in pre- or post- vaccine sera from either mice or humans using immunoblot assay. Further, cross-reactive antibody production induced by 4CMenB in vaccinated mice does not correlate with rate of N. gonorrhoeae clearance in the mouse model of infection.

Conclusions: Our studies show that 4CMenB vaccination in humans and mice induces cross-reactive antibodies against several gonococcal proteins, but that the level of anti-gonococcal antibodies in 4CMenB-immunized mice does not correlate with rate of bacterial clearance. These studies provide insight into antibody-mediated immunity to N. gonorrhoeae after vaccination with 4CMenB.

Effects of Psilocin on 5-HT2A+ L5P neurons.

Gavin P. Schmitz, Yi-Ting Chiu, Bryan L. Roth, Melissa A. Herman.

Psilocin, the active compound in psilocybin, was granted breakthrough drug status by the FDA due to its potential as a treatment for refractory depression. Despite promising clinical findings, the underlying signaling mechanisms and brain region-specific effects of psilocin and other psychedelic drugs remain unclear. The psychoactive effects of psychedelics are attributed to activation of serotonin 2A receptors (5-HT2ARs). The Prefrontal Cortex (PFC) is a significant hub for these effects. Focal application of psilocin (10 mM) onto undifferentiated Layer 5 Pyramidal (L5P) neurons in the prelimbic PFC of wild-type mice produced variable effects on firing (increase, decrease, or no change). 5-HT2A+ L5P neurons in the mouse prelimbic PFC were identified in transgenic inducible tdTomato labeled mouse. Focal application of psilocin increased firing in all 5-HT2A+ neurons but did not result in any significant changes in inhibitory or excitatory synaptic transmission (sEPSC, mEPSC, or sIPSCs). Application of a 5-HT2C antagonist prior to psilocin administration failed to block the increased firing with psilocin application. However, application of a 5-HT2A antagonist prior to psilocin administration blocked the increase in firing with psilocin administration. Furthermore, application of a Gq inhibitor prior to psilocin administration also blocked the increase in firing with psilocin administration. These results demonstrate that psilocin selectively increases the activity of 5-HT2A+ L5P neurons via a 5-HT2A receptor-dependent mechanism. Additionally, this effect is dependent upon signaling via the Gq protein. Collectively, these data provide important insight into the neurobiological mechanisms underlying psilocin's effects in the PFC that may be implicated in the therapeutic effects of psilocybin for the treatment of anxiety and depression.

Department of Pharmacology Research – Friday, September 16, 2022

POSTER SESSION 1
(1-25)

1. **Alicea Pauneto, Coral del Mar**
   (Gershon/Thaxton Labs)
   Myeloid-directed treatment using the TLR7/8 agonist resiquimod improves the survival of mice with medulloblastoma and enhances efficacy of radiotherapy.

2. **Aponte, Amy**
   (Emanuele Lab)
   Regulation of the retinoblastoma (Rb) tumor suppressor by the ubiquitin-proteasome system.

3. **Bedard, Madigan**
   (McElligott lab)
   The effect of morphine withdrawal on sleep behaviors in mice.

4. **Bellinger, Tania**
   (Reissner Lab)
   Inhibition of Microglial Phagocytosis Reduces Cocaine-seeking in Male Rats Following a Protracted 30-day Abstinence Period.

5. **Bivins, Marissa**
   (Nicholas Lab)
   Elucidating the balance between transpeptidase activity and antibiotic resistance in mutant forms of Penicillin-Binding Protein 2 from N. gonorrhoeae.

6. **Cao, Can**
   (Roth Lab)
   Structure, Function and Pharmacology of Human Itch GPCRs.

7. **Cartaya, Ana**
   (Bahnson Lab)
   Selective delivery of nano-encapsulated Nrf2 activator for atherosclerosis treatment.

8. **Chiu, Yi-Ting**
   (Roth Lab)
   Characterization of mouse 5-HT2A receptor and humanized mouse 5-HT2AR-A242S transgenic mice.

9. **Choi, Mingyu**
   (Hahn Lab)
   *Probing mechanotransduction pathways through visualization and control of vinculin conformation***.**

10. **DeLiberty, Jonathan**
    (Bryant Lab)
    PIKfyve inhibition is a fyve-out-of-fyve strategy for targeting autophagy in pancreatic ductal adenocarcinoma.

11. **Drizyte-Miller, Kristina**
    (Der Lab)
    Targeting Mitochondrial Activity Using Small Molecule Activators of Mitochondrial Protease ClpP for Pancreatic Cancer Treatment.

12. **Edgar, Elise**
    (Song Lab)
    Low level mossy cell activation in dorsal dentate gyrus leads to radial neural stem cell quiescence in multiple dentate gyrus regions.

13. **Effinger, Devin**
    (Herman Lab)
    Sex-Dependent Effects of Psychedelic Drug Exposure on Central Amygdala Reactivity.

14. **Gates, Claire**
    (Bryant Lab)
    Evaluating the Efficacy of Combined ERK and Autophagy Inhibition in RAS-Mutant Cancers.

15. **Gentile, Gabi**
    (Nicholas Lab)
    Impact of the L421P mutation in the ponA gene, encoding Penicillin-Binding Protein 1, on fitness and antibiotic resistance in Neisseria gonorrhoeae.

16. **Gereau, Gray**
    (McElligott Lab)
    Investigating central amygdala neurotensin neurons in the context of ethanol and affective behaviors.

17. **Goriounova, Alexandra**
    (Tarran Lab)
    Super resolution microscopy reveals pro-inflammatory Orai1 activity is elevated in CF and asthma patients.

18. **Graboski, Amanda**
    (Redinbo Lab)
    Gut Microbial Tryptophanases and the Uremic Toxins of Chronic Kidney Disease.

19. **Graves, Adam**
    (Baldwin Lab)
    Roles for the Kinases IKKε and TBK1 in KRAS-Driven Pancreatic Cancer.

20. **Gumpper, Ryan**
    (Roth Lab)
    Structures of Hallucinogenic and Non-Hallucinogenic Analogues of the 5-HT2A Receptor Reveals Molecular Insights into Signaling Bias.

21. **Guzman, Bryan**
    (Dominguez Lab)
    Protein Disorder and RNA Binding.

22. **Hibshman, Priya**
    (Der Lab)
    MYC is a Major Driver of KRAS-Dependent Metabolic Abnormalities in Pancreatic Cancer.

23. **Jenner, Maddy**
    (Yeh Lab)
    Targeting the tumor-stroma in pancreatic cancer.

24. **Kapolka, Nick**
    (Roth Lab)
    Illuminating the pharmacology and structure of the orphan neuropeptide B/W receptors.

25. **Knight, Kevin**
    (Dohlman Lab)
    A universal allosteric mechanism for G protein activation.

Department of Pharmacology Research – Friday, September 16, 2022

POSTER SESSION 2
(26-50)

26. Kothari, Aditi
(Yarbrough/Isaeva Labs)
Interaction between NF-κB and NRF2 signaling pathways leads to better survival in HPV-associated head and neck cancer

27. Landry, Taylor
(Song Lab)
Short-term High Fat Diet Impairs Hippocampal Function and Responsiveness to Feeding Status.

28. Lee, David
(Scherrer Lab)
Uncovering non-opioid GPCR-mediated analgesic approaches through novel pharmacological targets and tools.

29. Lee, Ye
(Der/Cox Labs)
Targeting Valosin-Containing Protein (VCP), a Regulator of the DNA Damage Response, for Pancreatic Cancer Treatment.

30. Li, Yongyi
(Song Lab)
G9a inhibition regulates neuroinflammation and T cell invasion in Alzheimer's disease.

31. Liu, Yongfeng
(Roth Lab)
Activation and allosteric regulation of the Human MRGPRX1 receptor.

32. Maldonado Vazquez, Natalia
(Dominguez Lab)
Characterization of Zinc finger Domain in ZC4H2.

33. Martin, Cole
(Morris Lab)
Investigating the Role of Taz and Yap in p53-mediated Tumor Suppression.

34. Mihalkovic, Abby
(Walsh Lab)
Mapping the neuronal activity patterns of the prosocial effects of MDMA.

35. Pantazis, Jacob
(Palmer Lab)
In Vitro Identification of Molecular Features Defining Drug Resistance in Peripheral T Cell Lymphoma.

36. Patterson, Sarah
(Palmer Lab)
Ultrasensitive response explains the benefit of combination chemotherapy despite antagonism.

37. **Pickett, Julie**
(Roth Lab)
From a selective RARalpha agonist to a first probe for orphan adhesion GPCR ADGRG3/GPR97.

38. **Robb, Ryan**
(Bryant Lab)
Interplay and compensation between autophagy and macropinocytosis in ERK MAPK inhibited pancreatic cancer.

39. **Sandroni, Peyton**
(Jensen Lab)
The Alpha-1A Adrenergic Receptor Regulates Fatty Acid-Dependent Oxidative Phosphorylation in the Mouse Heart.

40. **Schaefer, Antje**
(Der Lab)
The cancer-associated RHOAL57V mutant acts as an oncogene and drives diffuse gastric cancer development through activation of IGF1R-PAK1-YAP signaling.

41. **Sheehy, Ryan**
(Bryant Lab)
A brain-penetrant inhibitor of G9a ameliorates cognitive deficits in Alzheimer's Disease.

42. **Stewart, Mariah**
(Schisler Lab)
Opposing Roles of Co-chaperones in Cancer.

43. **Sturdivant, Michael**
(Kim Lab)
The Mutagenic Effects of APOBEC3A and APOBEC3B in Urothelial Carcinoma.

44. **Wang, Yue**
(Brunk Lab)
Integration of multi-omics data with saturation mutagenesis data to assess biological impact on a systems level.

45. **Welsh, Kaeli**
(Brown Lab)
Functional conservation and divergence of the helix-turn-helix motif of E2 ubiquitin-conjugating enzymes.

46. **Yao, Zhiyuan (Zoey)**
(Kuhlman Lab)
Engineering "two-in-one" antibody of CD19 and CD20 to mitigate tumor antigen escape in CAR-T therapy.

47. **Zewdie, Eden**
(Earp Lab)
TAM Receptor Metabolic Reprogramming of Dendritic Cells.

48. **Zhang, Shu**
(Dohlman Lab)
Decoding signal coordination via crosstalk of MAPK pathways in Saccharomyces cerevisiae.

49. **Zhang, Shicheng**
    (Roth Lab)
    Inactive and active state structures template selective tools for the human 5-HT5A receptor.

50. **Overview of the UNC Proteomics Core Facility – Laura Herring**
    Highlighting current projects and recent publications

Myeloid-directed treatment using the TLR7/8 agonist resiquimod improves the survival of mice with medulloblastoma and enhances efficacy of radiotherapy.

C.Alicea Pauneto, D.Hwang, C.Park, M.Skolsky, T.Gershon. Dept. Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC

We show the therapeutic potential of myeloid-directed immunomodulatory treatment for medulloblastoma. Patients with medulloblastoma, the most common malignant pediatric brain tumor, need new treatments, as standard therapy produces disabling neurotoxicities and fails 20% of patients. About 30% of medulloblastomas show hyperactivation of the SHH (Sonic Hedgehog) signaling pathway, and these tumors have large myeloid populations in the tumor microenvironment (TME). We analyzed whether activating toll-like receptors on these myeloid cells would slow tumor growth. Our prior single-cell RNA sequencing studies in both patient samples and mouse models showed that 10% of the cells in SHH medulloblastoma are myeloid cells and that these cells uniquely express TLR7 and TLR8. We treated mice genetically engineered to develop SHH medulloblastomas with the TLR7/8 agonist resiquimod, administered systemically either as free drug or in polyoxazoline nanoparticles (POx-resiquimod), and compared POx-resiquimod+radiotherapy to radiotherapy alone. We found that POx-resiquimod extended the survival time of mice with medulloblastoma, while free drug failed to show benefit. PK studies showed that POx-resiquimod increased tumor drug exposure, consistent with increased efficacy. Mechanistically, POx-resiquimod increases tumor myeloid populations and decreases the fraction of myeloid cells that express IGF1. POx-resiquimod plus radiation therapy was superior to either POx-resiquimod alone or radiotherapy alone. Together our data show that the TLR7/8 agonist resiquimod, delivered in nanoparticle formulation, produced a significant anti-tumor effect in SHH medulloblastoma and increased the efficacy of radiotherapy. As radiotherapy is the mainstay of current medulloblastoma treatment, we propose that TLR7/8-agonist therapy such as resiquimod may be added to current regimens to reduce the radiation dose needed for efficacy and to increase the fraction of successfully treated patients.

Regulation of the retinoblastoma (Rb) tumor suppressor by the ubiquitin-proteasome system.

A. Aponte, M. Emanuele. Department of Pharmacology and Lineberger Comprehensive Cancer Center, University of North Carolina, NC

The retinoblastoma (Rb) protein is a prototypical tumor suppressor due to its role in restricting proliferation. Active Rb functions as the key regulator of the G1/S transition by physically binding to E2F family of transcription factors, which inhibits activation of genes necessary for DNA replication. To relieve transcriptional repression and promote cell cycle progression, Rb is inactivated through phosphorylation by the cyclin-dependent kinases 4 and 6 (Cdk4/6), and this mechanism is the direct target of the Cdk4/6 inhibitor Palbociclib, a drug currently used for treatment of advanced hormone-receptor positive breast cancer (BC). Despite our extensive understanding of Rb phosphorylation, not much is known about additional mechanisms that may regulate the stability of Rb and thus mediate sensitivity to Cdk4/6 inhibition. I have found that in both normal and BC cells, acute Palbociclib treatment results in decreased Rb protein expression without a significant change in RB1 mRNA. This finding challenges the current model of Rb in G1 phase and highlights a previously undescribed response to Cdk4/6 inhibition. Moreover, my data shows that the proteasome inhibitor Bortezomib and cullin-RING ligase inactivator MLN-4924 rescue Rb levels in cells that are treated with Palbociclib, suggesting that Rb is subjected to degradation by the ubiquitin-proteasome system. To date, the most extensively studied mechanism of Rb degradation is that of DNA tumor virus oncoproteins, yet its nonviral-mediated degradation in human cells and the proteins involved in this system remain unknown. By combining biochemical and genomic approaches, I am currently attempting to identify the ubiquitin ligase that promotes Rb degradation. Future experiments will be performed to investigate phenotypic outcomes and determine the biological significance of Rb degradation. Collectively, data from my studies will provide us with a more comprehensive model of Rb regulation in both normal physiology and in the context of cancer.

The effect of morphine withdrawal on sleep behaviors in mice.

Bedard, MB. McElligott, ZA. Dept. Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC, USA.

The opioid epidemic has increased dramatically over the last few decades resulting in many suffering from opioid use disorder (OUD). The prevalence of opioids and opioid overdose has been driven by the development of new synthetic opioids, increased availability of prescription opioids, and more recently, the COVID-19 pandemic. As we see increases in exposure to opioids, the United States has also seen increases in the frequency of instances of Narcan (naloxone) administration as a life-saving measure for respiratory depression, and, thus, consequently, naloxone-precipitated withdrawal. Sleep dysregulation is one of the main symptoms of OUD and opioid withdrawal syndrome, and therefore should be a key facet of animal models of OUD. Here we examine the effect of precipitated and spontaneous morphine withdrawal on sleep behaviors in C57BL/6J. We find that morphine administration and withdrawal dysregulates sleep, however not equally across morphine exposure paradigms and not qualitatively the same across sexes. Furthermore, many environmental triggers promote relapse to drug-seeking/taking behavior, and the stress of disrupted sleep may fall into that category. We find that sleep deprivation dysregulates sleep in mice that had previous opioid withdrawal experience. These data suggest that the 3-day precipitated withdrawal paradigm has the most profound effects on opioid-induced sleep dysregulation and that further validates the construct of the 3-day precipitated withdrawal model as a model for opioid dependence and OUD.

The opioid epidemic has increased dramatically over the last few decades resulting in many suffering from opioid use disorder (OUD). The prevalence of opioids and opioid overdose has been driven by the development of new synthetic opioids, increased availability of prescription opioids, and more recently, the COVID-19 pandemic. As we see increases in exposure to opioids, the United States has also seen increases in the frequency of instances of Narcan (naloxone) administration as a life-saving measure for respiratory depression, and, thus, consequently, naloxone-precipitated withdrawal. Sleep dysregulation is one of the main symptoms of OUD and opioid withdrawal syndrome, and therefore should be a key facet of animal models of OUD. Here we examine the effect of precipitated and spontaneous morphine withdrawal on sleep behaviors in C57BL/6J. We find that morphine administration and withdrawal dysregulates sleep, however not equally across morphine exposure paradigms and not qualitatively the same across sexes. Furthermore, many environmental triggers promote relapse to drug-seeking/taking behavior, and the stress of disrupted sleep may fall into that category. We find that sleep deprivation dysregulates sleep in mice that had previous opioid withdrawal experience. These data suggest that the 3-day precipitated withdrawal paradigm has the most profound effects on opioid-induced sleep dysregulation and that further validates the construct of the 3-day precipitated withdrawal model as a model for opioid dependence and OUD.

Inhibition of Microglial Phagocytosis Reduces Cocaine-seeking in Male Rats Following a Protracted 30-day Abstinence Period.

T.J. Bellinger, A. Testen, J.W. VanRyzin, K.J. Reissner. Dept. of Pharmacology, University of North Carolina School of Medicine, and Dept. of Psychology and Neurosci., Chapel Hill, NC, USA

The activated microglial response to drugs of abuse has been implicated in the development and maintenance of substance use disorders. Phagocytosis is a prominent function of activated microglia, which is associated with behavioral consequences in both development and pathology. Given this, we sought to determine whether microglial phagocytosis contributes to cocaine-seeking behaviors during abstinence. To investigate this question, we utilized long-access (6h/day) cocaine self-administration in adult male Sprague Dawley rats for ten days, followed by a 30-day home cage abstinence. Starting on abstinence day 1, rats received intra-NAc microinjections of neutrophil inhibitory factor peptide (NIF, 0.5 uL per hemisphere) or vehicle every 7 days (4 microinjections total). The NIF peptide binds to the CD11b subunit of the microglial C3 receptor, inhibiting the initiation of microglial phagocytosis (Moyle et al., 1994, Kopec et al., 2018). On abstinence day 30, all rats underwent cue-primed behavioral testing to measure drug-seeking behavior. Rats that received intra-NAc NIF peptide demonstrated a statistically significant reduction in lever-pressing (Vehicle = 254.0 +/- 26.25, NIF = 183.8 +/- 19.95, p-value = 0.0380). Ongoing experiments will analyze microglia morphology as well as lysosomal activity and evidence of phagocytosis in these samples.

Elucidating the balance between transpeptidase activity and antibiotic resistance in mutant forms of Penicillin-Binding Protein 2 from N. gonorrhoeae.

Marissa M. Bivins, Gabriella L. Gentile, Caleb Stratton, Christopher Davies, Robert A. Nicholas

Neisseria gonorrhoeae is a Gram(-) bacterium that is the causative agent of gonorrhea, a sexually transmitted infection that can cause pelvic inflammatory disease, infertility, and increased susceptibility for HIV infection if untreated. Over the last 75 years, N. gonorrhoeae has become resistant to virtually every antibiotic used to treat infections, and there are emerging strains resistant to multiple antibiotics. H041 is one such multidrug-resistant N. gonorrhoeae strain and was the first isolate fully resistant to ceftriaxone, the only remaining recommended antibiotic for gonorrhea. Ceftriaxone and other β-lactam antibiotics are substrate analogs that formed long-lived complexes with penicillin binding proteins (PBPs). In N. gonorrhoeae, these antibiotics target Penicillin Binding Protein 2 (PBP2), a peptidoglycan transpeptidase (TPase) that is essential for cell wall synthesis and cell division in N. gonorrhoeae. PBP2 from H041 has undergone extensive recombination with non-pathogenic Neisseria species and has more than 60 mutations compared to wild-type PBP2, but it is still able to maintain its essential function. We have identified 8 mutations that, when introduced into wild-type PBP2, confer ~80% of the resistance of PBP2(H041) to ceftriaxone. Based on structural analysis of PBP2, these mutations likely increase resistance through altered dynamics, but there is minimal data on how these mutations impact the native, essential TPase activity of PBP2. My project is to investigate the effects of these 8 mutations, individually and in groups, on PBP2 through an in vitro TPase assay. Furthermore, when the gene encoding PBP2 in a wild-type strain is replaced by homologous recombination with the gene encoding PBP2(H041), there is a significant reduction in growth and fitness. We will also assess the influence of these 8 mutations in N. gonorrhoeae strain growth, fitness, and morphology. We predict that mutations in PBP2 that significantly increase resistance to ceftriaxone will proportionally hinder TPase activity.

Structure, Function and Pharmacology of Human Itch GPCRs.

Can Cao, Hye Jin Kang, Isha Singh, He Chen, Chengwei Zhang, Wenlei Ye, Byron W. Hayes, Jing Liu4, Ryan H. Gumpper, Brian J. Bender, Samuel T. Slocum, Brian E. Krumm, Katherine Lansu, John D. McCorvy, Wesley K. Kroeze, Justin G. English, Jeffrey F. DiBerto, Reid H. J. Olsen. Xi-Ping. Huang, Shicheng Zhang, Yongfeng Liu, Kuglae Kim, Joel Karpiak, Lily Y. Jan, Soman N. Abraham, Jian Jin, Brian K. Shoichet, Jonathan F. Fay* and Bryan L. Roth**

The MRGPRX family of receptors (MRGPRX1-4) represent an unusual family of Mas-related G protein coupled receptors that have appeared relatively recently in evolution1. Of these, MRGPRX2 and MRGPRX4 are important physiological and pathological mediators of itch and related mast-cell mediated hypersensitivity reactions2-5. Intriguingly, MRGPRX2 appears to couple to both Gi and Gq in mast cells6. Here we describe agonist-stabilized structures of MRGPRX2 coupled to both Gi and Gq, in ternary complex with the endogenous peptide cortistatin-14 and with a synthetic agonist probe, respectively, and potent antagonist probes for MRGPRX2. We also describe a specific MRGPRX4 agonist and structure complexed with MRGPRX4 and Gq. Together, these findings will accelerate the structure-guided discovery of therapeutics for pain, itch and mast-cell mediated hypersensitivity.

Selective delivery of nano-encapsulated Nrf2 activator for atherosclerosis treatment.

A.E. Cartaya, S. Maiocchi, N.E. Buglak, S. Torzone, A. Peterson, A. Ackerman, and E.M. Bahnson.

Cardiovascular disease (CVD) remains the leading cause of death and disability worldwide. Atherosclerosis is the underlying cause of CVD. Oxidative stress and inflammation have been implicated as drivers of atherosclerosis development. However, clinical studies based on anti-inflammatory and redox-active therapies for treatment of CVD have been largely unsuccessful. A main challenge for systemic delivery of redox-based therapies is their inability to reach and maintain bioavailability at sites of redox dysregulation, which underscores the need for selective delivery of these therapies. Nrf2 activators have emerged as a promising class of therapeutics for prevention of atherosclerosis progression through the expression of Nrf2 regulated antioxidants. Therefore, we propose that selective delivery of Nrf2 activators to atherosclerotic plaque will prevent atherosclerotic progression. To selectively deliver Nrf2 activators to atherosclerotic regions, we first developed nanoparticles encapsulating the potent Nrf2 activator, CDDO-Me. Recently, we showed that these nanoparticles accumulate in atherosclerotic plaque and activate Nrf2 in vivo. Here, we investigate the therapeutic effect of CDDO-Me nanoparticle administration on atherosclerotic burden in high fat diet fed Ldlr -/- mice. Adipo-Clear and immunolabeling coupled with light-sheet fluorescent microscopy were employed to image aortas and associated branches of the arch allowing 3D visualization and analysis. Together with histological analysis of the aortic sinus, these methods were employed to measure atherosclerotic plaque burden and macrophage infiltration. Overall, our studies highlight the feasibility of targeting the atherosclerotic plaque as an effective way to selectively deliver redox-active therapies.

Characterization of mouse 5-HT2A receptor and humanized mouse 5-HT2AR-A242S transgenic mice.

Yi-Ting Chiu, Kunjie Hua and Bryan Roth

Department of Pharmacology, University of North Carolina School of Medical, Chapel Hill, North Carolina

5-hydroxytryptamine 2A receptor (5-HT2AR) is involved in a lot of physiological functions such as smooth muscle contraction and modulations of mood and perception, as well as psychiatric disorder including schizophrenia and anxiety. CRISPR technology was used here to generate two 5-HT2AR mouse lines. One is inducible mouse 5-HT2AR (mHTR2A-eGFP-CT-IRES-CreERT2, 2A-eGFP-CreERT2) and the other one is humanized mouse HTR2AR at one point mutation to human receptor serine residue at alanine 242 residue (mHTR2A-A242S-eGFP-CT-IRES-Cre, A242S-eGFP-Cre). The advantage of this transgenic mouse design can reveal “5-HT2A receptor” itself via GFP-fusion protein and “5-HT2A positive cells” via Cre-dependent reporter. The distribution of 5-HT2AR in the brain was characterized and we also further identified some interested brain regions as well as receptor trafficking on these two mouse lines.

Probing mechanotransduction pathways through visualization and control of vinculin conformation

Mingyu Choi*, Joe Szulczewski*, Shiqiong Hu, Klaus Hahn

Vinculin is important in multiple contexts where cells must measure and respond to mechanical force. It acts via a conformational latch mechanism where stretching produces an open conformation that enables binding to downstream effectors. We are specifically studying how cells use vinculin-containing structures called podosomes to probe the rigidity of potential targets during phagocytosis, and how cancer cells navigate between tumors and the vasculature by sensing the rigidity of aligned collagen. Podosomes oscillate like pistons against phagocytic targets, with frequency dependent on target stiffness. Metastatic cells use vinculin-containing focal adhesions to monitor and respond to the rigidity of collagen fibers. In each case it is difficult to probe how force-induced changes in vinculin induce downstream signals, because forces are transient and localized. We are therefore developing vinculin analogs for use in living cells to 1) report vinculin conformational state, and 2) control vinculin activity with light.

To visualize the exposure of hidden binding sites in vinculin, a short peptide chain was inserted where it is exposed only in the open, "stretched" conformation. When exposed, the tag can be accessed by a small fluorescent protein, so simple colocalization of vinculin and this protein indicates the open conformation. To control vinculin's transmission of force, we sought to allosterically modulate vinculin-actin interaction. Previous studies have shown that protein activity can be controlled by inserting the LOV2 domain, which undergoes conformational changes when irradiated. We used this to make photoinhibitory and photoactivated vinculin analogs. We will show initial application of these analogs and fluorescent biosensors to study cell motility on micropatterned substrates that mimic the tumor microenvironment.

PIKfyve inhibition is a fyve-out-of-fyve strategy for targeting autophagy in pancreatic ductal adenocarcinoma.

J.M. DeLiberty, E.G. Schechter, N.L. Pieper, R. Yang, C.J. Der, A.D. Cox, K.L. Bryant

Pancreatic ductal adenocarcinoma (PDAC) is characterized by KRAS- and autophagy-dependent growth. Autophagy is a lysosomal-mediated process whereby cells degrade and recycle macromolecules to sustain growth. We and others have demonstrated that inhibition of the RAS-RAF-MEK-ERK pathway resulted in upregulated autophagic flux, and that dual treatment with the autophagy inhibitor hydroxychloroquine (HCQ) and ERK-MAPK inhibitors synergistically blocked PDAC growth. HCQ is limited in terms of specificity and potency, and we sought to identify a more efficacious autophagy inhibition strategy. We performed a CRISPR/Cas-9 mediated genetic loss-of-function screen with a library targeting cancer signaling pathways and identified PIKfyve as an essential autophagy-related gene in PDAC cell lines. PIKfyve is a lipid kinase critical for the recycling dynamics of endosomes and lysosomes. We hypothesized that PIKfyve inhibition could be a more potent anti-autophagy therapy in PDAC. Accordingly, inhibition of PIKfyve with apilimod (PIKfyvei) resulted in an accumulation of the canonical autophagy markers p62 and LC3B-II, suggestive of autophagy inhibition. Additionally, we observed a build-up of large intracellular vacuoles staining positive for LAMP1, a marker of lysosomes and late endosomes. Since autophagy is a lysosomal-mediated process, we sought to determine if these enlarged lysosomes were still functional and capable of degrading cargo. Indeed, PIKfyve inhibitor treatment significantly reduced the acidity of lysosomes. When we inhibited MEK (with mirdametinib (MEKi)) and PIKfyve concomitantly, we observed a decrease in MEKi-induced autophagic flux. This suggested that combined PIKfyve and MEK inhibition could be an efficacious strategy for PDAC treatment. Accordingly, dual MEKi and PIKfyvei showed substantial synergy across a panel of PDAC cell lines, due in part to a significant induction of apoptosis following combination treatment. This synergistic relationship was maintained in patient derived PDAC organoids. Taken together, these findings suggest dual MEK and PIKfyve inhibition is a potentially efficacious therapeutic strategy and warrants further investigation in more advanced models of PDAC.

Targeting Mitochondrial Activity Using Small Molecule Activators of Mitochondrial Protease ClpP for Pancreatic Cancer Treatment.

K. Drizyte-Miller, A. A. Amparo, C. A. Stalnecker, J. A. Klomp, R. Yang, C. Cerda-Smith, E. J. Iwanowicz, L. M. Graves, K. C. Wood, K. L. Bryant, A. D. Cox, and C. J. Der.

The KRAS oncogene is activated in ~95% of pancreatic ductal adenocarcinoma (PDAC) patients and reprograms tumor metabolism to support the bioenergetic and biosynthetic demands of cancer cells. Emerging evidence suggests that PDAC cells rely on altered mitochondrial function for their survival and that inhibiting mitochondrial activity could be a viable therapeutic option. However, there is a lack of effective mitochondrial inhibitors to assess for PDAC treatment. Here, we evaluated ONC201, a clinical candidate agonist of the mitochondrial matrix protease ClpP, and a more potent, next generation analog, TR-107, as potential therapeutics for PDAC. Both ONC201 and TR-107 hyperactivate ClpP, causing proteolytic degradation of a spectrum of mitochondrial proteins such as those involved in oxidative phosphorylation (OXPHOS), the tricarboxylic acid cycle, heme biosynthesis, and mitochondrial translation in a ClpP-dependent manner. We found that ONC201-induced hyperactivation of ClpP inhibited the growth of PDAC cell lines and organoids and impaired mitochondrial respiration and mitochondrial ATP production. However, treatment also resulted in a compensatory increase in glycolysis to offset the deleterious consequences of impaired OXPHOS. We found that concurrent treatment with a selective KRASG12D inhibitor (MRTX1133) or a selective ERK1/2 inhibitor (SCH772984), both of which suppressed glycolysis, further enhanced ONC201 inhibition of PDAC cell growth. KRAS-ERK inhibition additionally promoted mitochondrial fusion and suppressed expression of mitochondrial biogenesis genes. Our ongoing studies are evaluating the consequences of ONC201 treatment on other metabolic activities and cancer cell signaling pathways in PDAC. Additionally, we are using our custom-designed mitochondrial-focused CRISPR/Cas9 library to perform a loss-of-function CRISPR screen to identify metabolic genes that modulate PDAC sensitivity to ONC201 and to identify combination treatment strategies that would enhance the long-term efficacy of ONC201. In summary, our results support a therapeutic value in targeting mitochondrial function using ClpP agonists for KRAS-mutant PDAC treatment.

Low level mossy cell activation in dorsal dentate gyrus leads to radial neural stem cell quiescence in multiple dentate gyrus regions

E. Edgar, J. Song.

The hippocampus is widely considered to be a crucial brain region involved in learning and memory processes. The dentate gyrus (DG) is the first sub-region within the hippocampus to receive inputs from the entorhinal cortex, and the DG passes these signals to the CA regions. The DG is known to contribute to spatial navigation, spatial orientation, and pattern separation. Mossy cells (MCs) are the primary excitatory cell type within the hilus of the DG which modulate both GABAergic and glutamatergic circuitry throughout the hippocampus. MCs are one of the few cell types which can project both ipsilaterally and contralaterally within the DG, which may have implications on coordinating bilateral hippocampal activity states. MCs are additionally morphologically and functionally distinct between dorsal and ventral DG. Optogenetic studies found that dorsal MCs excite ventral DG granule cells, with more granule cells excited ipsilaterally than contralaterally. However, little is known about the pathways mediated by dorsal vs ventral MCs. We previously determined that low level MC activation resulted in radial neural stem cell (rNSC) quiescence due to MC activation of GABAergic interneurons (INs). This was only studied in contralateral, dorsal DGs, and not in any ventral DG region. We hypothesized that low level MC activation would lead to rNSC quiescence in dorsal and ventral regions. order to examine this in both dorsal and ventral DG regions, mice expressing Cre-dependent labelling of MCs were injected with a designer receptor exclusively activated by designer drugs (DREADD) to activate MCs. Immunohistochemistry was then conducted to examine neural stem cells in different phases of maturation and differentiation. MC-activated mice displayed rNSC quiescence in both contralateral dorsal and ipsilateral ventral DG, but not in contralateral ventral DG. This data provides the framework for future studies investigating the role of MC activity-mediated rNSCs in disease models.

Sex-Dependent Effects of Psychedelic Drug Exposure on Central Amygdala Reactivity

***D.P. Effinger**, S.G. Quadir, M.C. Ramage, M.G. Cone, M.A. Herman.*

Psilocybin, and its active metabolite psilocin, have been shown to elicit rapid and long-lasting symptom improvements in a variety of affective psychiatric illnesses. However, the region-specific alterations underlying these therapeutic effects remain relatively unknown. The central amygdala (CeA) is a primary output region within the extended amygdala that is dysregulated in affective psychiatric disorders. Here, we measured CeA activity using the activity marker cFos and CeA reactivity using fiber photometry and an aversive air puff stimulus. We found that psilocin administration acutely increased CeA activity in both males and females and increased stimulus-specific reactivity in females, but not males. In contrast, psilocin produced time-dependent decreases in reactivity in males, but not females, as early as 2-days and lasting to 28-days post administration. We also measured behavioral responses to the air puff stimulus and found sex-dependent changes in threat responding but not exploratory behavior or general locomotion. Repeated presentations of the auditory component of the air puff were also performed and sex-specific effects of psilocin on CeA reactivity to the auditory-alone stimulus were also observed. This study provides new evidence that a single dose of psilocin produces sex-specific, time-dependent, and enduring changes in CeA reactivity and behavioral responding to specific components of an aversive stimulus. These data are an important step towards dissecting the dynamic effects of psilocin on CeA plasticity and the role of the amygdala in the potential therapeutic effects of psychedelics.

Evaluating the Efficacy of Combined ERK and Autophagy Inhibition in RAS-Mutant Cancers.

Claire Gates, Noah Pieper, Kirsten L. Bryant.

It is well-established that oncogenic RAS supports tumor proliferation through activation of the ERK MAPK signaling cascade. While ERK MAPK inhibition limits growth of RAS mutant cancers in preclinical models, patients treated with inhibitors targeting any single node in this pathway rapidly acquire resistance. Therefore, identifying combination-based therapeutic strategies that mitigate acquired resistance to ERK MAPK inhibition is a major priority in the treatment of RAS-mutant cancers. Our group and others recently determined that ERK inhibition results in upregulation of the nutrient scavenging pathway macroautophagy (herein referred to as autophagy), a process of "self-eating" by which cells degrade proteins and organelles to obtain monomeric subunits for use in anabolic processes. We hypothesized that increased autophagic flux may promote resistance to ERK-inhibitor induced growth suppression and demonstrated that dual ERK and autophagy inhibition synergistically promoted growth inhibition and enhanced apoptosis in human PDAC cell lines, organoid models of PDAC, and PDX-derived mouse models. These observations formed the basis for two currently active clinical trials assessing the combination of the MEK inhibitor binimetinib (NCT04132505) and the ERK inhibitor LY3214996 (NCT04386057) in combination with hydroxychloroquine in PDAC.

While PDAC is almost exclusively driven by RAS mutation (97%), lung adenocarcinoma (LAC) and colorectal cancer (CRC) also have high rates of RAS mutations (52% and 32% respectively.) Despite striking differences in the genetic drivers of initiation and progression of these cancers, they have all been demonstrated to depend on autophagy for growth. Thus, we hypothesized ERK inhibition would induce increased autophagic flux in models of LAC and CRC, and that concurrent ERK and autophagy inhibition would suppress growth. We demonstrate dual ERK and autophagy inhibition effectively suppresses growth in LAC and CRC cell lines with multiple drivers including mutant KRAS, NRAS, EGFR, and BRAF. Ongoing studies in the laboratory are aimed at assessing the effect of ERK inhibition on mitochondrial dynamics and the utilization of macropinocytosis, an extracellular nutrient scavenging pathway, in LAC and CRC.

Impact of the L421P mutation in the ponA gene, encoding Penicillin-Binding Protein 1, on fitness and antibiotic resistance in Neisseria gonorrhoeae.

G. Gentile, C. Davies, C. Stratton, Y. Grad, K. Ma, T. Mortimer, and R. Nicholas.

Chromosomally mediated resistance to beta-lactam antibiotics in Neisseria gonorrhoeae is driven primarily by 4 mutated alleles: penA, mtrR, penB, and ponA1. The roles of penA, mtrR, and penB in facilitating resistance to beta-lactams are more clearly defined than that of ponA1. ponA1 introduces an L421P mutation into Penicillin-Binding Protein 1 (PBP1), a bifunctional transglycosylase (TGase)/transpeptidase (TPase) enzyme involved in peptidoglycan synthesis. The L421P variant is present in a large majority of penicillin-resistant strains and has a 3-fold lower acylation rate for penicillin G (PenG). Thus, ponA1 appears to be involved in resistance to beta-lactam antibiotics, but its role is unclear. To assess the role of ponA1 in antibiotic resistance in N. gonorrhoeae, we investigated the biochemical and phenotypic effects on the gonococcal cell incurred through acquisition of the L421P mutation. Phylogenetic analysis of sequenced N. gonorrhoeae isolates revealed that, in addition to penicillin-resistant strains, ponA1 is also present in a large majority of ceftriaxone-resistant strains harboring a mosaic penA allele, which encodes highly mutated variants of PBP2, but rarely in antibiotic-susceptible strains. The L421P mutation, unlike resistance-conferring mutations in PBP2, is located far from the active site on the hinge region between the OB domain and the penicillin-binding domain, and introduction of a proline could alter interactions between the TPase domain and the other domains of PBP1. Transformation studies with active-site mutants in either the TGase or TPase domains indicate that these mutants are not viable, indicating both activities are essential for cell viability. These data suggest that ponA1 is involved in beta-lactam resistance in some capacity, but the extent and nature of the role ponA1 plays in resistance is not completely understood. The essentiality of both the TGase and TPase domains and lack of PBP1-specific beta-lactam antibiotics suggest that PBP1 could be a heretofore untapped target for drug development.

Investigating central amygdala neurotensin neurons in the context of ethanol and affective behaviors.

G. Gereau.

Alcohol use disorder (AUD) is a pervasive and costly neuropsychiatric disorder. Each year, AUD kills around 95,000 people and represents a substantial cost to society at about $2.05 in lost productivity per drink consumed in excess in the US. Increasing amounts of evidence have shown that the drive to consume alcohol is regulated by brain regions associated with reward and valence. The central nucleus of the amygdala (CeA) is a component of a plethora of relevant neural circuitry in this space. The CeA is host to a population of neurons that express neurotensin, a 13 amino-acid neuropeptide that has been implicated in consummatory behaviors. Data from our lab shows that neurotensin neurons in the CeA are activated during the drinking of rewarding fluids like ethanol. We have also demonstrated that ablating these neurons reduces ethanol drinking. These data implicate the CeA as a key hub for the control of ethanol consumption, and we seek to further characterize this neuronal population with this work. Here, we make use of a DIO vGAT shRNA virus to knock down GABA release in this population or a DIO caspase virus to ablate these neurons completely in an effort to determine the roles of different neurotransmitters released by CeA neurotensin cells in altering ethanol preference, affective behaviors, and pain perception. We find that the knock-down of GABA release in CeA neurotensin cells results in modulation of approach/avoidance behaviors, including an increase in avoidance behavior in males that is less apparent in females as a result of a possible sex-dependent neophilia phenotype that may be skewing data in the assays we chose to use. We also see differences in thermal hypersensitivity as a result of GABA knockdown in these neurons.

Super resolution microscopy reveals pro-inflammatory Orai1 activity is elevated in CF and asthma patients.

Alexandra S. Goriounova, Rodney C. Gilmore, Joe A. Wrennall and Robert Tarran.

Orai1, a plasma membrane Ca2+ channel, is involved in store operated calcium entry (SOCE). In pulmonary cells, SOCE regulates gene expression and stimulates cytokine, mucin, and protease secretion. Activation of Orai1/SOCE results in the recruitment of neutrophils to the lungs. Orai1 activation is also upstream of Nuclear Factor of Activated T cells (NFAT), which facilitates the onset of inflammation. In CF and asthma, the immune response is dysregulated and the lung is chronically inflamed. However, Orai1 lung expression is poorly understood.

We performed RNAscope analysis and immunostaining on lung sections from normal, asthma, and CF donors (4M/4F per group). We imaged sections by confocal and super resolution microscopy, and analyzed Orai1 and STIM1 expression, colocalization, and particle size in different pulmonary cell types.

Orai1 must interact with STIM1 in order to activate SOCE. We therefore used STIM1/Orai1 colocalization as a marker of Orai1 activity. Using this approach, we found significantly increased colocalization between these proteins in both CF and asthma lung epithelia (CF, 50%; asthma, 54%; normal, 15%), interstitia (CF, 57%; asthma, 49%; normal, 16%) and luminal immune cells (CF, 66%; asthma, 70%; normal, 38%). Orai1 also aggregates as part of its interaction process. Using super resolution microscopy, we found significantly more Orai1 and STIM1 aggregation in immune cells from CF and asthmatic lungs (average Orai1 particle size: CF, 52 nm; asthma, 63 nm; normal, 28 nm; average STIM1 particle size: CF, 77 nm; asthma, 59 nm; normal, 14 nm).

This is the first comprehensive analysis of Orai1 and STIM1 expression in lungs from normal, CF, and asthma donors. We found evidence that Orai1 was more active in CF and asthma than normal lungs. Therefore, these data suggest Orai1 has a key role in CF and asthma lung inflammation and attest to the potential of anti-inflammatory therapeutics that target Orai1.

Gut Microbial Tryptophanases and the Uremic Toxins of Chronic Kidney Disease.

Amanda L. Graboski, Mark E. Kowalewski, Joshua B. Simpson, Matthew R. Redinbo.

Chronic kidney disease (CKD) afflicts nearly 500 million people worldwide and is one of the fastest growing causes of mortality. A key consequence of a diseased kidney is the serum retention of toxic compounds that have a broad impact on human physiology. One of the most dangerous uremic toxins is indoxyl sulfate (IS), a metabolite produced solely from the breakdown of tryptophan by gut microbial tryptophanases (TPases). High IS levels in preclinical and clinical models have been correlated with altered mitochondrial oxidative phosphorylation, renal fibrosis, and six different phenotypes of cardiovascular disease. Recent studies showed the genetic elimination of TPase in an artificial microbiome of germfree mice prevented the formation of IS and reduced biomarkers of kidney injury, suggesting that inhibition of TPase could prevent or reduce uremic toxicity. In this study, we investigate the structural and functional landscape of gut microbial TPases to gauge its drugability and to guide inhibitor design. First, we selected a group of diverse TPases from the 124 sequences present in the Integrated Genome Catalog of human fecal metagenomes and examined their activity against tryptophan using substrate-turnover assays. We then elucidated the crystal structures of these TPases, revealing highly conserved tertiary structure and active site architecture. Using this structural information and the well-characterized catalytic mechanism, we designed and synthesized a panel of structure-based and mechanism-inspired inhibitors. A handful of these novel tryptophan-like compounds display inhibitory activity against our subset of TPase enzymes. In the future, we will continue to evaluate this structure-activity relationship to optimize inhibitor potency before bringing lead candidates forward into cell and animal studies. The discovery of a potent TPase inhibitor will help to both unravel the molecular basis of increased serum IS levels in CKD and may serve as therapeutic avenue for IS toxicity.

Roles for the Kinases IKKε and TBK1 in KRAS-Driven Pancreatic Cancer.

A. Graves, A. Baldwin.

Pancreatic ductal adenocarcinoma (PDAC) is a disease with an abysmally low 5-year survival rate, due to therapy resistance and poor early detection. Approximately 90% of PDAC express mutant KRAS, the key driver of oncogenesis in this cancer. MEK/ERK signaling is a critical effector pathway downstream of oncogenic KRAS, promoting proliferation and other cancer-associated mechanisms. How chronic MEK/ERK signaling promotes the oncogenic phenotypes is not fully understood, which is the focus of my project.

The IKK-related kinases TBK1 and IKKε have been extensively studied in the context of innate immunity, where they promote interferon gene expression. Both kinases have been studied in different cancers, including pancreatic cancer, although their roles and potential redundancy is unclear. Knockdown studies in the MIA PaCa-2 pancreatic cancer cell line have shown a significant decrease in 2D growth. I am currently analyzing growth defects potentially associated with apoptosis, cell-cycle arrest, and senescence, relative to TBK1 and IKKε knockdown. I have found KRAS or ERK inhibition leads to the loss of IKKε levels in pancreatic cancer cell lines, suggesting the importance of KRAS-associated signaling in controlling TBK1/IKKε kinase activity. Additionally, I have found that IKKε and TBK1 interact in pancreatic cancer cells suggesting shared and cross-regulatory functions. Our genomic studies have shown significant overlap between genes regulated by ERK1/2, oncogenic KRAS, and TBK1/IKKε. Our phosphoproteomic and genomic works indicate that TBK1/IKKε may regulate cellular translation, possibly through the translation factor EIF4G1. Ongoing work and future plans relate to: identifying key substrates for TBK1 and IKKε in the RAS/ERK pathway, performing syngeneic PDAC tumor studies to analyze in vivo effects of loss of these kinase on tumor progression and tumor immunity, identifying distinct and potential redundant functions of these kinases in PDAC, and dissecting potential translational control mechanisms associated with TBK1 and IKKε in the KRAS-driven pathway.

Structures of Hallucinogenic and Non-Hallucinogenic Analogues of the 5-HT2A Receptor Reveals Molecular Insights into Signaling Bias.

Ryan H. Gumpper, Jeff DiBerto, Manish Jain, Kuglae Kim, Jonathan Fay, and Bryan L. Roth.

Recently, there has been a resurgence in utilizing classical psychedelics to treat depression, addiction, anxiety disorders, and cluster headaches. The biological target of these compounds, and the route of its therapeutic actions, is the 5HT2A receptor (5HT2AR). It has been hypothesized that the hallucinations and therapeutic actions can be separated through biased agonism and G-protein activation. Here we present 8 new cryoEM structures covering all major compound classes for the 5HT2AR including a novel arrestin biased compound RS130-180. Utilizing the structural and functional data we noticed a correlation between ligand bias and the placement of the canonical “toggle-switch” tryptophan. These findings lead to a broader mechanistic understanding of 5HT2AR activation as well as potential for the development of biased ligands.

Protein Disorder and RNA Binding.

B.B. Guzmán, G.A. Goda, M.M. Aleman, D.I. Dominguez.

RNA bindings proteins (RBPs) interact and tightly regulate the fate of messenger RNAs. Despite their essential role in normal biology, how RBPs bind their cognate RNA targets is not well understood. Generally, RBPs are known bind short RNA motifs through their well-folded RNA binding domains. Many RBPs also contain low complexity domains (LCDs) which are regions that characterized to have lack of structure and repeats of 1-2 amino acids. LCDs are thought to participate in non-specific protein-protein and protein-RNA interactions. However, recent work from our lab and others has demonstrated that RBPs utilize disordered regions interact with RNA. Furthermore, frequent mutations in LCDs are associated with human disease, such as amyotrophic lateral sclerosis. Given the role of RBPs in gene regulation and the prevalence of LCDs in RBPs, it raises the question of whether the disordered regions recognize and bind specific RNA sequences or structures. Thus, understanding LCDs as a functional domain is crucial to decipher protein function. Here, we study HNRNPR, an RBP with three RNA binding domains and an arginine-glycine rich LCD. HNRNPR functions as a translation regulator and is involved in mRNA transport. Interestingly, mutations to the LCD of HNRNPR are associated with cranio-facial malformations and developmental delays. However, is unclear how HNRNPR recognizes and interacts with RNA. Through in vitro binding assays, we found that the LCD of HNRNPR is the main driver for RNA interaction and binds guanine-rich RNAs which form G-quadruplexes (G4), important structural elements involved in RNA regulation. Interestingly, we demonstrate that HNRNPR-G4 interaction are dependent both on G4 structure and LCD presence. These results suggest that disordered regions in RBPs can serve as a functional domain and display specificity towards RNA. This work highlights our misunderstanding of protein-RNA interactions and motivates future investigations to address LCDs as a functional domain within RBPs.

MYC is a Major Driver of KRAS-Dependent Metabolic Abnormalities in Pancreatic Cancer.

Priya S. Hibshman, J. Nathaniel Diehl, Clint A. Stalnecker, Richard G. Hodge, Jeff A. Klomp, Craig M. Goodwin, Sen Peng, Nhan L. Tran, Emanuel F. Petricoin III, Adrienne D. Cox, and Channing J. Der.

Mutationally activated KRAS drives pancreatic ductal adenocarcinoma (PDAC) growth predominantly through activation of the ERK mitogen-activated protein kinase (MAPK) cascade. However, how ERK supports KRAS-dependent cancer growth remains to be established. While ERK phosphorylates and regulates a complex phosphoproteome (>1000 direct/indirect substrates), we hypothesized that the MYC transcription factor is a key ERK substrate critical for supporting KRAS-dependent PDAC growth. To delineate the contribution of MYC to KRAS-driven PDAC, we first applied RNA-Seq analyses to establish a system-wide profile of the MYC-dependent transcriptome. We determined gene transcription changes at 24 h after treatment with siRNA targeting MYC. We identified ~2000 significantly downregulated and ~2400 significantly upregulated genes. GO/KEGG/Reactome pathway analyses determined that MYC-deregulated genes control diverse cellular processes. Next, we validated key MYC-dependent processes using acute genetic suppression of KRAS or MYC expression. We found that MYC is a major driver of KRAS-dependent cell proliferation and metabolism. In particular, MYC depletion suppressed KRAS-regulated processes such as nucleotide synthesis, glucose metabolism, mitochondrial function, and nutrient scavenging. Furthermore, consistent with MYC-dependent regulation of genes involved in autophagy and lysosomal function, we observed compensatory upregulation of autophagic flux. KRAS or ERK inhibition similarly altered the metabolic landscape, supporting a critical role for KRAS-regulated MYC function in PDAC. For example, both KRAS and MYC suppression promoted mitochondrial fusion. In contrast, we determined that other KRAS-dependent processes, such as steroid biosynthesis and fatty acid metabolism, may be regulated independently of MYC. In summary, our studies have revealed that MYC facilitates diverse KRAS-driven cellular activities, and have also identified KRAS-dependent, MYC-independent mechanisms of PDAC growth. Our studies support the provocative concept that inhibiting MYC function may be an effective strategy for targeting KRAS for PDAC treatment.

Targeting the tumor-stroma in pancreatic cancer.

M.R. Jenner, B. T. Golitz, S.M. Gomez, J. Yeh.

Pancreatic cancer remains one of the deadliest cancers with only 10% of patients surviving 5 years post-diagnosis. Cytotoxic therapies are the standard of care, but these only benefit a subset of patients before ultimately encountering resistance. It is widely accepted that the tumor microenvironment plays a critical role in modulating drug response and enabling resistance mechanisms. One of the major contributing cell types to the microenvironment are CAFs, or cancer-associated fibroblasts. While it is still unknown which CAF populations are tumor-supportive versus tumor-suppressive, there is established reciprocal signaling between the tumor and CAFs. CAFs can secrete soluble factors, metabolites, and extracellular matrices that can feed tumor cells to support proliferation or alter tumor signaling cascades. For these reasons, targeting the tumor stroma (CAFs and connective tissue) is an emerging therapeutic approach.

There have been a few stroma-targeting therapies in clinical trials for pancreatic cancer. However, none have been approved to date. This is partially due to CAF heterogeneity; current approaches are not selective against normal tissue or tumor-suppressive CAFs. Therefore, there is an unmet need to understand tumor-CAF biology to harness its therapeutic potential.

My work will identify CAF-induced kinase vulnerabilities in tumor cells. Patient-derived xenograft pancreatic cancer cell lines will be co-cultured with CAF lines to represent the tumor microenvironment and cultivate tumor-CAF cell signaling. The cell viabilities of tumor and CAF cells can be collected simultaneously after drug treatment using a novel, dual luciferase system. This allows for simultaneous cell viability readings of both cell types in the same well. Kinase inhibitor hits will maximize tumor cell sensitivity and minimize CAF sensitivity. Characterized hits will identify specific kinase drug targets that differ in drug sensitivity between tumor and CAF cells. This work will elucidate tumor-stroma vulnerabilities and signaling components and identify kinase inhibitors to treat pancreatic cancer.

Illuminating the pharmacology and structure of the orphan neuropeptide B/W receptors.

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Our cells utilize more than 800 unique G protein-coupled receptors encoded in the human genome to sense a remarkably diverse repertoire of extracellular stimuli such as protons, neuropeptides, and hormones. As such, GPCRs are involved in virtually all physiological processes and are the most common target of FDA-approved drugs. Despite their success in the clinic, many opportunities for novel GPCR drug development remain. This is especially true for the ~100 understudied (e.g. orphan) receptors currently listed by the NIH common fund initiative called the Illuminating the Druggable Genome (IDG) project. The neuropeptide B/W receptors 1 and 2 (NPBWR1 and NPBWR2, respectively) are two orphan GPCRs implicated in neuropathic pain, stress, and appetite. These receptors are activated by the endogenous peptide agonists neuropeptide B (NPB) and neuropeptide W (NPW), both of which have multiple isoforms (NPB-23, NPB-29, NPW-23, and NPW-30) with conserved sequence similarity among the longer variants. Here, we describe the pharmacological profiles of NPBWR1 and NPBWR2 with their endogenous peptide agonists against 14 G protein transducers and summarize preliminary efforts to purify and determine the cryo-EM structure of both receptors. Our results confirm that NPBWR1 and NPBWR2 couple to Gαi/o-type G proteins and indicate that the longer isoform of both peptide agonists (NPB-29 and NPW-30) is more potent than the shorter isoform. This suggests that the conserved sequence similarity shared between the two longer isoforms is important for agonist binding and receptor activation. Our efforts toward purifying both receptors in complex with their peptide agonist(s) and G protein transducers for cryo-EM structure determination are ongoing. Once complete, we intend to use structural insight to confirm key residues for agonist activity and selectivity, and identify novel small molecule modulators to contribute to the community's effort to better understand the role(s) of NPBWR1 and NPBWR2 in health and disease.

A universal allosteric mechanism for G protein activation.

Kevin M. Knight, Soumadwip Ghosh, Sharon L. Campbell, Tyler J. Lefevre, Reid H.J. Olsen, Alan V. Smrcka, Natalie H. Valentin, Guowei Yin, Nagarajan Vaidehi, and Henrik G. Dohlman.

G proteins play a central role in how cells sense and respond to their environment. Hormones, neurotransmitters, odorants, and drugs bind a large class of receptors called G protein coupled receptors (GPCRs) transverse the plasma membrane. The receptors activate intercellular G proteins, which then initiate proper cellular signaling responses. The heterotrimer consists of G-alpha subunits and an obligate dimer called G-betagamma. Structural studies have revealed how G-alpha proteins interact with G-betagamma proteins, receptors, RGS proteins and downstream effectors. However, the molecular events that precede heterotrimer dissociation remained poorly understood. Using cell-based assays, biochemical and structural analysis, and computational simulations, we identified a conserved triad of amino acids that governs G protein activation and subunit dissociation. Our work reveals the “G-R-E motif” exists in all known G-alpha subunits and controls the final committed step of G protein activation.

Interaction between NF-κB and NRF2 signaling pathways leads to better survival in HPV-associated head and neck cancer.

Aditi Kothari, Travis Parke Schrank, Wendell Gray Yarbrough, Natalia.

Incidence of HPV associated head and neck squamous cell carcinoma (HNSCC) is on the rise, displaying a significantly favorable prognosis and overall better survival as compared to HPV negative HNSCC. Intense radiotherapy is the primary medical care leading to treatment associated morbidity in patients, thereby generating a need for de-intensification strategies. Biomarkers that can identify patients with less aggressive tumors as candidates for de-escalation therapy would revolutionize treatment.

We identified two intrinsic subtypes of HPV+ HNSCCs from three independent patient cohorts. These subtypes distinguished based on high NF-κB activity have a specific mutation pattern (e.g. TRAF3/CYLD inactivation) and tumor microenvironment, episomal HPV, and improved patient survival. We also found that tumors with increased NF-κB activity had a reduced nuclear factor erythroid 2-related factor (NRF2) signaling, which is often associated with radio-resistance in cancer.

Our studies show that NF- κB -driven intrinsic tumor characteristics contribute to increased sensitivity to radiation, providing patients survival benefits. Indeed, TRAF3 or CYLD deletion activated NF-kB and dramatically increased radiation sensitivity of HPV+ head and neck cancer cells. In line with our RNA seq analysis, we found that activation of NF-κB via TRAF3/CYLD deletion significantly correlated with marked downregulation of NRF2 activity and reduced nuclear localization in HPV+ HNSCC cells. Interestingly, TRAF3 CRISPR KO cells had lower NRF2 protein levels that were restored by MG132 treatment, indicating an involvement of KEAP1/CUL3 mediated proteasomal degradation of NRF2.

In summary, our data unveils a unique relation between NF-κB pathway and radiosensitivity in HPV+ HNSCC. Our NF-κB activity classifier would be an invaluable tool for clinicians to identify patients with HPV-associated HNSCC with favorable prognosis as candidates for de-escalated therapy, as well as identification of patients who need intensive therapy.

Short-term High Fat Diet Impairs Hippocampal Function and Responsiveness to Feeding Status.

Taylor Landry, Seth Tart, Libo Zhang, & Juan Song.

Epidemiological studies demonstrate metabolic diseases such as diabetes and obesity are associated with increased incidence of cognitive decline. However, the mechanistic link between these diseases and cognitive dysfunction remains unclear. Rodent models of metabolic disease exhibit aberrant hippocampal neurogenesis, decreased synaptic complexity, and increased apoptosis in the dentate gyrus (DG) of the hippocampus, a critical brain region involved in cognitive function. Therefore, we hypothesize that DG function is finely regulated by whole-body energy status, and becomes dysregulated in response to high fat diet (HFD), leading to cognitive deficits. Using immunohistochemistry in the DG of healthy mice, we observed decreased neural progenitor proliferation and reduced cFos expression in granule cells (GC) in response to fasting, which were robustly increased after refeeding. Similarly, in vivo fiber photometry revealed long-lasting increases in GC activity after refeeding, while GABAergic interneurons experienced opposite effects. These effects were also observed in response to an intraperitoneal (IP) glucose injection. Interestingly, just 5 days of HFD blunted the GC response to refeeding and IP glucose injection, despite the interneuron response remaining intact. These changes in DG responsiveness to feeding corresponded to impaired performance during hippocampus-dependent learning and memory tasks. Fiber photometry also revealed impaired GC and interneuron responses during memory tasks in the HFD mice. Strikingly, one-night fast followed by refeed before memory encoding completely rescued memory performance in HFD mice. Overall, these data demonstrate GC's and interneurons in the DG dynamically and robustly respond to feeding status and glucose levels. Importantly, even short-term dietary changes, such as high fat diet, or an acute fast/refeed, can have significant impacts on DG function and subsequently modulate memory processing.

Uncovering non-opioid GPCR-mediated analgesic approaches through novel pharmacological targets and tools.

D.F. Lee, D.J. Berg, J. Krzeski, T. Matsubara, G. Scherrer.

Opioid analgesics are broadly used to manage severe pain, however they are responsible for harmful side effects like addiction, hyperalgesia, constipation, and lethal opioid-induced respiratory depression (OIRD). Today, over 20% of the US population experiences chronic pain, with 1.6 million of the population having an opioid use disorder in the past year. This dual epidemic of chronic pain and opioid misuse urgently requires a better understanding of opioid receptor neurobiology and novel pharmacological targets to develop safer therapeutic alternatives. To reveal novel analgesic targets devoid of opioids' harmful effects, I aim to both target novel G protein-coupled receptor (GPCR) candidates for non-opioid analgesia and characterize the proteome of opioid receptors across diverse neuron-types and circuits. First, I describe the pharmacological targeting of non-opioid analgesic GPCRs that are expressed in neurons encoding the negative emotional valence of pain (i.e., pain unpleasant quality). Our lab previously described a discrete ensemble of neurons in the amygdala that encodes pain unpleasantness. Single cell RNA sequencing (scRNA-seq) of these neurons has revealed non-opioid GPCR targets, which I have pharmacologically targeted in mice using systemic administration of selective agonists. I found that these compounds decrease pain affective-motivational behaviors, which indicates unpleasantness, in the hotplate, post-operative, and formalin pain assays. Next, I outline the process of uncovering in vivo opioid receptor dynamics and interactomes across diverse neural populations. Recent advances in proximity labeling tools have allowed for the determination of receptor proteomes and ultrastructural localizations with high spatial and temporal resolution. Here, I describe the development and use of novel adeno-associated viruses (AAVs) expressing ascorbate peroxidase 2 (APEX2)-conjugated opioid receptors in a conditional, Cre recombinase-dependent manner, to uncover differential receptor interactions and trafficking across opioid receptor subtypes, neuron-types, circuits, and opioid treatments. This combination of approaches is enabling the discovery of GPCR-mediated analgesic alternatives to opioids to end the dual epidemic of chronic pain and opioid addiction.

Targeting Valosin-Containing Protein (VCP), a Regulator of the DNA Damage Response, for Pancreatic Cancer Treatment.

Ye S. Lee, Jennifer E. Klomp2, Craig M. Goodwi, Clint A. Stalnecker, Yanzhe Gao, Gaith N. Droby, Cyrus Vaziri, Kirsten L. Bryant, Channing J. Der, and Adrienne D. Cox.

Our recent proteomics and genetic functional screens identified components of the DNA damage response (DDR; e.g., WEE1, CHK1) as critical for pancreatic ductal adenocarcinoma (PDAC) cell growth in vitro. To further elucidate the role of the DDR more thoroughly, we performed a comprehensive and DDR-focused CRISPR-Cas9 loss-of-function screen targeting 504 genes involved in DDR regulation. One gene identified to be essential for PDAC growth encodes valosin-containing protein (VCP), an ATPase with pleiotropic functions in protein degradation pathways, autophagy, cell cycle, and more. We observed that genetic and pharmacologic inhibition of VCP inhibited PDAC growth and induced apoptotic death, in the absence of consistent or significant cell cycle perturbations. Next, we addressed the mechanistic basis for VCP-dependent growth. First, we addressed the role of VCP in the DNA damage response and found that genetic and pharmacologic inhibition of VCP resulted in the accumulation of DNA double-strand breaks. Secondly, we addressed the role of VCP in protein degradation and found that genetic and pharmacologic inhibition of VCP activated the unfolded protein response (UPR) in response to the accumulation of ubiquitinated proteins. Recent studies identified a role for VCP in promoting autophagy, a metabolic process essential for PDAC growth. Surprisingly, we found that genetic or pharmacologic inhibition of VCP increased autophagy in KRAS-mutant PDAC. We speculated that this increase may represent a compensatory response to the growth suppression induced upon loss of VCP. Addressing this possibility, we determined that combining VCP inhibition with the autophagy inhibitor chloroquine caused synergistic apoptotic cell death. We conclude that concurrent targeting of autophagy can enhance the efficacy of VCP inhibitors in KRAS-mutant PDAC.

G9a inhibition regulates neuroinflammation and T cell invasion in Alzheimer's disease.

Y.Y. Li, R.N. Sheehy, and J. Song.

Neuroinflammation is a hallmark pathology in the development and progression of Alzheimer's disease (AD). It is often characterized by the aberrant activation of microglia, resident macrophages responsible for the clearance of pathogens in the brain. Activated microglia in the AD brain will adopt a pro-inflammatory and neurotoxic phenotype and, consequently, damage and kill neurons. In the hippocampus, microglia-mediated neuronal death putatively exacerbates cognitive decline in AD. Recent work has suggested T cell infiltration to contribute to microglial activation and neuroinflammation. Though the exact effects of T cell invasion remain unclear, these studies highlight the interplay between the nervous system and the immune system during AD progression. For this project, we studied neuroinflammation and T cell invasion in AD with G9a inhibition. G9a is a lysine methyltransferase that canonically represses gene expressions via epigenetic histone modifications. It has been shown that acute pharmacological inhibition of G9a could restore synaptic functions and rescue behavioral deficits in AD mice. We wanted to explore the effects of chronic G9a inhibition and its impact in the dentate gyrus (DG) of the hippocampus, a critical brain region for learning and memory that shows accelerated AD pathology. To address this, we took advantage of the 5XFAD mouse model, which rapidly develops features of AD pathology. We injected AD mice with a novel G9a inhibitor and performed immunohistochemical analyses on mouse brain sections. Interestingly, we found that G9a inhibition significantly increased neuroinflammation. We also found an increase in CD8 T cell invasion in 8-month, but not in 5-month, AD mice with G9a inhibition. However, G9a inhibition didn't lead to any changes in CD4 T cell invasion. Altogether, these data suggested that while acute G9a inhibition is shown to rescue cognitive decline in AD mice, chronic treatments might produce adverse effects and potentially worsen AD pathology.

Activation and allosteric regulation of the Human MRGPRX1 receptor.

Y. Liu, C. Cao, X.P. Huang, R.H. Gumpper, S.L. Shih, B.K. Krumm, S. Zhang, J.F. Fay, B.L. Roth.

The human MAS-related G protein-coupled receptor X1 (MRGPRX1) is preferentially expressed in the small diameter primary sensory neurons and involved in the mediation of nociception. Central activation of MRGPRX1 by the endogenous opioid peptide fragment BAM8-22 and its positive allosteric modulator ML382 has been shown to effectively inhibit persistent pain, making MRGPRX1 a promising target for non-opioid pain treatment. However, the activation mechanism of MRGPRX1 is still largely unknown. Here, we report two high resolution cryo-electron microscopy structures of MRGPRX1-Gαq in complex with a synthetic agonist compound-16 and an endogenous opioid peptide fragment BAM8-22, respectively. We also report the cryo-electron microscopy structure of MRGPRX1-Gαq signaling complexes bound simultaneously to BAM8-22 and its positive allosteric modulator ML382. These structures reveal the agonist binding mode for MRGPRX1 and illuminate the structural requirements for positive allosteric modulation. Collectively, our findings provide a molecular understanding of the activation and allosteric modulation of the MRGPRX1 receptor, which could facilitate the structure-based design of non-opioid pain-relieving drugs.

Characterization of Zinc finger Domain in ZC4H2.

Maldonaro Vazquez, M.

Deficiency and mutations in zinc finger (ZNF) domain containing ZC4H2 commonly lead to different X-linked inherited disorder phenotypes in patients. This has led to a spectrum of congenital diseases commonly referred to as ZC4H2 associated rare disorders (ZARD). ZNFs are short motifs that contain cysteine and histidine residues that are coordinated by zinc ions and adopt a finger like structure. ZNF domains are involved in various cellular processes that are important for cell differentiation and survival. ZC4H2 has been reported to interact with E3-ubiquitin ligases and transcription factors involved in neuronal and muscle development. However, there is no information on how structural domains like the ZNF in ZC4H2 influence ubiquitination or transcriptional regulation pathways relevant to development. We hypothesize that the ZNF domain in ZC4H2 mediates protein-protein interactions that influence ubiquitination of transcription factors that regulate genes involved development. To examine how the ZNF domain in ZC4H2 influences protein-protein interactions we used an inducible mammalian expression vector to transfect HEK-293T (Human Embryonic Kidney) and CAD (Cath-a-differentiated) for expression ZC4H2 and co-immunoprecipitations with known interactors like RNF220 and Phox2a. We were able to immunoprecipitate FLAG tagged-ZC4H2 using FLAG antibodies and protein A/G beads. To determine if ZC4H2 was able to bind nucleic acid or change the transcription of other genes we used HEK-293T cells for RNA Immunoprecipitation and sequencing. Our results suggest that ZC4H2 changes the expression of a few genes, suggesting that this gene may not be a transcriptional regulator but may still be interacting with RNA or DNA. To further examine the interactions between ZC4H2 and nucleic acids we performed RNA bind-n-seq using a randomized RNA and DNA oligo pool. Our results suggest that ZC4H2 can interact with both RNA and DNA. However, sequencing results are inconclusive and a specific motif for ZC4H2 was not identified.

Investigating the Role of Taz and Yap in p53-mediated Tumor Suppression.

Cole P. Martin, William Sullivan, Jacqueline Brinkman, & John P. Morris IV.

Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer characterized by aberrant cell plasticity and the exploitation of transcriptional programs involved in normal organ development and homeostasis. The tumor suppressor TP53 is mutated in ~75% of PDACs and p53 inactivation is associated with progression to advanced invasive and metastatic disease. The paralogous Hippo pathway transcriptional coactivators Taz and Yap are also important factors in PDAC development, essential for cancer initiation and maintenance. Previous work has linked Hippo pathway signaling with p53-triggered cell fate decisions, but the role of Taz and Yap in neoplastic cells harboring functional wildtype p53 is poorly understood. By using a mouse model of PDAC where tumor suppressive levels of p53 can be restored, we demonstrate that p53 paradoxically promotes the accumulation of active nuclear Taz and upregulation of Taz/Yap target genes in a Taz/Yap-dependent manner. p53 restoration in PDAC cells promotes actin polymerization and cytoskeleton remodeling which is known to stabilize Taz. Indeed, disrupting the actin cytoskeleton uncouples p53 from its ability to upregulate active Taz protein and Taz/Yap target genes. Taz or Yap suppression in PDAC cells results in distinct patterns of differential gene expression dependent on p53 status. The transcriptional programs controlled by Taz or Yap in a p53-dependent manner include apoptosis, epithelial-to-mesenchymal transition, and cell identity, critical cell fate programs governed by p53. Specifically, Taz and Yap suppress a mucinous gastric-like gene program licensed by p53 that is reminiscent of a premalignant gastric cell identity observed in the neoplastic pancreas. Our work uncovers a novel link between wildtype p53 function and the activation of Taz likely through actin cytoskeleton remodeling. Our work identifies unexpected coordination of p53-dependent cell fates by the Hippo effectors and highlights the role that developmental signaling pathways play in transcriptional networks established by oncogene-tumor suppressor interplay during PDAC development.

Mapping the neuronal activity patterns of the prosocial effects of MDMA.

Abrianna Mihalkovic, Khondamir Imomnazarov, Jessica J. Walsh.

Social behavior is composed of a variety of distinct types of interactions that play a critical role in reproduction, survival and overall well-being. Deficits in sociability is a major source of morbidity in a host of psychiatric and neurodevelopmental disorders, notably autism spectrum disorder (ASD). Individuals with ASD consistently show dysfunction in the neurobiology, physiology and genetics of the serotonin system. Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed to treat behaviors associated with ASD, such as anxiety, depression and obsessive-compulsive disorder. However, they have variable efficacy and there is a paucity of evidence that they aid in the amelioration of core ASD symptoms, such as social deficits.

MDMA, known as an entactogen or empathogen, as well as the recreational drug “ecstasy,” robustly releases serotonin, in addition to other monoamines such as norepinephrine and dopamine. In contrast to SSRIs, which are only capable of increasing serotonin in an activity dependent manner, MDMA robustly releases serotonin in an activity-independent manner, which significantly raises serotonin levels beyond the ability of SSRIs. It has undergone a resurgence of investigation as an adjuvant in psychotherapy due to its robust prosocial effects.

We recently found that enhancing serotonin activity via systemic administration of a single dose of MDMA rapidly and acutely reverses social deficits present in four etiologically distinct mouse models for ASD. However, these behavioral effects are not long-lasting and utilizing a schedule 1 drug as a chronic therapeutic has many limitations. Yet, our preliminary data indicates a sustained reversal of social deficits one week following a 2-dose MDMA regimen in a genetic mouse model for ASD, but not control mice. Here, we utilized MDMA as a powerful tool to investigate how robust enhancement of serotonergic tone, via a 2-dose MDMA regimen, affects neural activity patterns in a genetic mouse model versus control mice.

In Vitro Identification of Molecular Features Defining Drug Resistance in Peripheral T Cell Lymphoma

J. Pantazis, A. Palmer

Peripheral T Cell Lymphoma (PTCL) is a group of highly heterogeneous hematological malignancies for which prognosis is generally poor. The standard first line treatment regimen for this disease, CHOP, (Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone) can promote greater than 10-year progression free survival (PFS) for a minority of patients, but 70% of PTCL patients will experience relapse within two years of treatment. When patients relapse from CHOP, there are several targeted therapies recommended by the National Comprehensive Cancer Network that can similarly provide remissions for small populations of patients. However, there is currently not much known about the molecular features that define the responsiveness of PTCL to the library of available therapies. Biomarker stratification would improve treatment outcomes in PTCL, allowing patients to be better matched to effective chemotherapies. We present in vitro gene expression and dose response data for a heterogeneous panel of 27 PTCL cell culture models. We identify some preliminary associations between gene expression and the sensitivity of PTCL cultures to 25 currently available PTCL treatments. Our preliminary data and existing literature support the role of multi-drug efflux pump ABCC1, DNA mismatch repair gene MSH2, and anti-apoptotic factor BCL2 on the cytotoxic activity of clinically effective therapeutic agents, doxorubicin, pralatrexate, and gemcitabine, respectively. Our proposed biomarker discovery framework intends to verify these associations and identify other gene expression patterns that could inform the precision-based administration of therapies to PTCL patients.

Ultrasensitive response explains the benefit of combination chemotherapy despite antagonism.

S. Patterson and A. Palmer.

For over 50 years aggressive lymphomas have been treated with combination chemotherapy, most often as several cycles of concurrent drug administration. Concurrent administration is in theory optimal when combination therapies have synergistic (more than additive) drug interactions. We investigated pharmacodynamic interactions in the 4-drug 'CHOP' regimen for Peripheral T-Cell Lymphomas (PTCL) by isobologram analysis in 7 PTCL cultures. We found that CHOP consistently exhibits antagonism between some of its drugs, but never synergies, which is unexpected given that it cures approximately half of patients with PTCL. We next used month-long in vitro models of treatment cycles to test whether staggered treatment schedules could enhance tumor cell kill by avoiding antagonism, relative to concurrent treatment using the same doses. Surprisingly, we observed that tumor cell kill is maximized by concurrent drug administration despite antagonistic drug-drug interactions. We propose that an ultrasensitive dose response, as described in radiology by the linear-quadratic (LQ) model, can reconcile these seemingly contradictory observations. The LQ model describes the relationship between cell survival and dose, and in radiology has identified scenarios favoring hypofractionated radiation – the administration of fewer, larger doses rather than multiple smaller doses. Specifically, a large 'quadratic' component in a dose-response describes cells requiring an accumulation of DNA damage in order to die, which we also observed for the DNA-damaging chemotherapies in the CHOP regimen. By adapting the LQ model to combination chemotherapy, we find that even when chemotherapies have antagonistic interactions, tumor cell kill is maximized by concurrent administration of multiple drugs, explaining the clinical efficacy of CHOP. Thus, our study identifies a new mechanism by which combination therapy can be clinically advantageous that does not depend on drug-drug interactions.

From a selective RARalpha agonist to a first probe for orphan adhesion GPCR ADGRG3/GPR97.

J. E. Pickett, S. G, W. Kroeze, X. Wan, T. Che, B. K. Shoichet, and B. L. Roth.

G-protein coupled receptors make up the largest and most diverse class of membrane proteins in the human genome and are highly druggable. Many GPCRs, however, remain orphans, a large number of which are adhesion-GPCRs. GPR97/ADGRG3 is a prototypical adhesion receptor, with the exception that it is the only adhesion-GPCR with a known small molecule ligand--the glucocorticoid beclomethasone dipropionate. This potent activity at glucocorticoid receptors substantially inhibits its utility as a chemical probe. Herein we report the first potent and selective small molecule agonist of GPR97, Z5126457289. Functional assay screening of GPR97 against more than 17,000 compounds identified a selective Retinoic Acid Receptor Alpha agonist which also had affinity for GPR97. Iterative hit-to-lead optimization led to Z5126457289, a novel compound with Retinoic Acid Receptor activity eliminated and selectivity against the GPCRome. Site-directed mutagenesis has illuminated the binding poses of beclomethasone dipropionate and Z5126457289, in addition to revealing amino acids important for Galpha/beta-arrestin recruitment and agonist versus inverse agonist function. In addition to Z5126457289, two negative probe controls were also identified, constituting a selective probe set with which to study the function of GPR97/ADGRG3.

Interplay and compensation between autophagy and macropinocytosis in ERK MAPK inhibited pancreatic cancer.

Ryan Robb, Alex Z. Marler, Kirsten L. Bryant.

Alteration of essential metabolic pathways is a major mechanism by which oncogenic KRAS promotes tumor development and growth in pancreatic ductal adenocarcinoma (PDAC). KRAS-driven PDAC is dependent on nutrient scavenging pathways, including macropinocytosis and autophagy to fuel the high metabolic demand of rapid proliferation. Thus, these metabolic processes are attractive targets for the development of treatments for PDAC. KRAS loss results in downregulation of macropinocytosis in PDAC. Additionally, our lab demonstrated that KRAS loss or inhibition of ERK MAPK signaling decreased glucose consumption and glycolysis but increased autophagy, thereby enhancing dependency on autophagy for survival and growth. Accordingly, dual ERK MAPK and autophagy inhibition (via chloroquine) synergistically enhanced anti-tumor efficacy in PDAC. Early clinical data has demonstrated that resistance to this treatment arises over time through unknown mechanisms. My preliminary data indicates that following ERK MAPK inhibition both autophagy induction and macropinocytosis downregulation is transient—with autophagic and macropinocytic activity returning to/surpassing basal levels after prolonged treatment. The underlying mechanistic and signaling crosstalk between autophagy and macropinocytosis remains poorly understood. We hypothesize that there is compensatory regulation between autophagy and macropinocytosis signaling following ERK MAPK inhibition. We propose that prolonged activation of autophagy upregulates macropinocytosis over time, consequently abrogating dependency on autophagy and therefore reducing sensitivity to autophagy inhibition. We demonstrate an inverse temporal relationship between autophagic flux and macropinocytosis following genetic loss of KRAS or pharmacological inhibition of the KRAS-ERK MAPK pathway. Furthermore, we show that chloroquine, which is commonly thought of as a lysosomotropic drug, does not prevent degradation of proteins engulfed via macropinocytosis. Together our data suggest that upregulation of macropinocytosis may mediate resistance to the combination of ERK MAPK inhibition and chloroquine. A better understanding of the signaling underlying these metabolic resistance pathways will inform future ERK MAPK inhibitor combinations.

The Alpha-1A Adrenergic Receptor Regulates Fatty Acid-Dependent Oxidative Phosphorylation in the Mouse Heart.

Sandroni, Peyton B., Huang, Wei, Jensen, Brian C.

Adrenergic receptors (ARs) are critical regulators of cardiomyocyte physiology in both the uninjured and injured heart. Acute β-AR activation increases heart rate and contractility. However, chronic β-AR activation causes maladaptive alterations in cardiomyocyte metabolism which contribute to the pathobiology of heart failure. In contrast, mounting evidence suggests that α1-ARs, particularly the α1A subtype, are cardioprotective. These receptors may mitigate the deleterious effects of chronic β-AR activation by the shared endogenous ligands epinephrine and norepinephrine. The mechanisms through which α1A-ARs adaptively regulate cardiomyocyte function remain unclear. Here, we characterize the effects of global α1A-AR genetic deletion on mitochondrial function and metabolism in the uninjured mouse heart using respirometry, substrate-specific electron transport chain (ETC) enzyme assays, electron microscopy, proteomics, and lipidomics. We then compare the effects of the α1A- and β-AR agonist treatment on ETC activity and oxidative stress in vivo and in vitro. Mice with global α1A-AR genetic deletion (α1A-KO) exhibited increased left ventricular mitochondrial size and density with marked ultrastructural abnormalities. Isolated cardiac mitochondria from α1A-KO mice had deficits in fatty acid oxidation (FAO)-dependent respiration and ETC activity that were accompanied by decreased abundance of critical FAO regulators. Conversely, the selective α1A-AR agonist A61603 (100 ng/kd/d, 3d) enhanced FAO-dependent respiration in isolated cardiac mitochondria and increased expression of FAO mediators without inducing hypertrophy. The β-AR agonist isoproterenol induced pathological hypertrophy in vivo and enhanced oxidative stress in vitro. The α1A-AR agonist A61603 mitigated isoproterenol-induced increases in mitochondrial superoxide production and glutathione oxidation in vitro. The selective α1A-AR agonist increased Complex I activity and preserved fractional shortening following myocardial infarction by left anterior descending coronary arterial ligation. Collectively, these findings may define a novel mechanism through which α1A-AR activation supports cardiomyocyte metabolism in the uninjured heart and protects against cardiac injury induced by β-AR activation.

The cancer-associated RHOAL57V mutant acts as an oncogene and drives diffuse gastric cancer development through activation of IGF1R-PAK1-YAP signaling.

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Diffuse gastric cancer (DGC) is a highly lethal cancer lacking effective therapies. Missense mutations in the RAS homologous RHOA gene were identified in 15-26% of DGC. In contrast to the hotspot RAS mutations at G12, G13 and Q61 in cancer, RHOA alterations are, unexpectedly, localized at different residues. It is unknown whether mutant RHOA functions as tumor suppressor or oncogene and driver of gastric neoplasia. The most common RHOA mutations are Y42C and L57V. Recently, we identified RHOAY42C as oncogene that promotes DGC through activation of focal adhesion kinase (FAK) which stimulates YAP/TAZ, PI3K-AKT and β-catenin signaling (Zhang, Schaefer et al., Cancer Discovery, 2020). In the present study, we determined that RHOAL57V displays a gain-of-function phenotype and is an oncogene in promoting DGC, but through a molecular mechanism distinct from RHOAY42C. We generated a mouse gastric organoid model where expression of RHOAL57V together with inactivation of the tumor suppressor Cdh1 (encodes E-cadherin) disrupted the three-dimensional architecture of organoids and induced a phenotype similar as in DGC patients. RHOAL57V, like RHOAY42C, promoted stress fiber formation, cell migration and adhesion. RHOAL57V exhibited reduced intrinsic and GAP-catalyzed GTP hydrolysis activities leading to an impaired RHOA inactivation. In contrast to RHOAY42C, RHOAL57V retained interaction with the effectors ROCK, Rhotekin and mDia. Reverse phase protein array (RPPA) analyses of Cdh1-/- gastric organoids revealed that expression of RHOAL57V, but not of RHOAY42C, stimulated IGF1R, PAK1 and YAP1 signaling. We found that Cdh1-/- RHOAL57V expressing gastric organoids were sensitive to combined pharmacological inhibition of IGF1R and PAK1. In summary, RHOAL57V and RHOAY42C are oncogene drivers in DGC through distinct biochemical and signaling mechanisms. KRAS mutations at residues analogous to RHOA Y42 and L57 did not result in oncogenic phenotypes, revealing the distinct mutational and mechanistic paths that RAS and RHOA take in cancer.

A brain-penetrant inhibitor of G9a ameliorates cognitive deficits in Alzheimer's Disease.

Sheehy, Ryan

Alzheimer's Disease (AD) is a neurodegenerative disease that characterized by aberrant processing of amyloid precursor protein (APP) to form neuroinflammatory Aβ plaques and progressive cognitive failure. G9a (EHMT2), a histone/lysine methyltransferase, has recently been implicated in AD. Recent work showed that short-term enzymatic inhibition of G9a for 3 days rescued synaptic and some cognitive functions in AD mice without an effect on Aβ plaques. Despite the established role of G9a in acute treatment of AD, the long-term effects of G9a inhibition remain unknown. Recent data from our lab implicated increased activity of G9a in chronically inflamed microglia during AD. We hypothesized long-term inhibition of G9a would significantly ameliorate microglial activation in AD and thus modulate plaque deposition and further improve behavior. To test this hypothesis, we utilized 5xFAD mice, an Aβ AD mouse model, and treated these mice with a novel brain penetrant G9a inhibitor or vehicle control for 6 weeks. After inhibitor treatment, mice underwent behavioral testing and tissue was collected for both immunofluorescence and proteomic analysis. Inhibitor-treated mice displayed significantly altered synaptic markers and behavioral changes. These results implicate a prominent role for G9a and its downstream effectors for multiple facets of AD pathogenesis. Overall, our data suggest that targeting G9a to treat AD has putative therapeutic value.

Opposing Roles of Co-chaperones in Cancer.

M. Stewart, J. Schisler.

Background: Protein quality control (PQC) involves a network of proteins that maintain cellular protein homeostasis (proteostasis) and requires control of protein folding, maintenance, and degradation. In cancer cells, the components of PQC are dysregulated or inactivated, leading to proliferation and uncontrolled growth. Two co-chaperones, carboxyl terminus of heat shock 70 interacting protein (CHIP) and heat shock organizing protein (HOP) are essential components of PQC; however, CHIP, via its ubiquitin ligase function, promotes a pro-degradation complex, whereas HOP promotes protein refolding.

Hypothesis: Higher CHIP expression will be associated with a better cancer prognosis, and higher expression of HOP will be associated with a worse cancer prognosis.

Methods: Using the DepMap portal and the Human Protein Atlas, I compared CHIP and HOP expression levels and their associations with outcomes in various cancer types. I created renal and gallbladder cancer cell lines with a transient shRNA knockdown of endogenous CHIP to establish a cell-based model.

Results: Four of the eighteen cancer types included in the analysis had highly expressed CHIP and low HOP expression and had better outcome data, including renal and ovarian cancers. Other cancer types had higher HOP and lower CHIP expression and a worse prognosis. Interestingly, higher CHIP expression was associated with a worse prognosis in some types of cancer, like gallbladder and glioma. I successfully knocked down CHIP expression in renal cancer cell lines and confirmed by western blot.

Conclusions and Future Directions: CHIP's role in cancer cell proliferation and prognosis is complicated and cancer type dependent. Further experiments exploring CHIP and HOP in PQC will shed more light on their roles in cancer proliferation. Ongoing experiments include incorporating different CHIP mutants to determine the most critical components of CHIP in these cancer cell lines and to assess the effect on proliferation.

The Mutagenic Effects of APOBEC3A and APOBEC3B in Urothelial Carcinoma.

M. Sturdivant, A. Truong, M. Zhou and W.Y. Kim.

As the most common malignancy of the urinary tract among men and women, bladder cancer is estimated to surpass 80,000 new cases and 17,000 deaths is 2022. Accounting for most bladder cancers cases, urothelial carcinoma has a dismal survival rate of approximately 5% in the metastatic setting. APOBEC3A(A3A) and APOBEC3B(A3B) are members of a family of cytidine deaminase enzymes that catalyze the removal of an amino group from cytosine nucleotides generating an uracil in its place that serve as a source of somatic mutations. A3A and A3B enzymes are aberrantly overexpressed in numerous cancer types, including urothelial carcinoma, with the APOBEC mutational signature seen in most cases of this cancer type. Currently, it is unclear if both A3A and A3B drive APOBEC3-induced mutagenesis, or if one enzyme plays a larger mutagenic role than the other. Previous work has postulated ortholog specific mutagenesis in breast and liver cancers, implicating A3A and A3B respectively as the source of APOBEC3 mutagenesis in these cancer types. We hypothesize that A3A and A3B enzymes differ in mutagenic activity leading to differences in promotion of intratumoral heterogeneity and response to anti-PD-1 immune checkpoint inhibition. To address this hypothesis, a murine bladder cancer cell line, that is representative of human muscle invasive bladder cancer, has been developed to express either A3A or A3B in the presence of doxycycline that will allow for a direct comparison of these two enzymes. Subsequent experiments comparing A3A and A3B are aimed to elucidate the driver of the APOBEC mutational signature, promotion of intratumoral heterogeneity, and influence on treatment response to anti-PD-1 therapy, which will result in the identification of the driver of APOBEC3 mutagenesis in urothelial carcinoma. Delineation of the driver of APOBEC3 mutagenesis can provide a predictive biomarker for immunotherapeutic treatment response in urothelial carcinoma patients.

Integration of multi-omics data with saturation mutagenesis data to assess biological impact on a systems level.

Yue Wang, Stephanie Ting, Elizabeth Brunk

Multiplexed assays for variant effects (MAVEs) enable saturation mutagenesis and functional analysis of regulatory elements or proteins of interest at nucleotide precision. In recent years, MAVEs demonstrate great clinical potential for determining the significance of variants of unknown significance (VUS). In cancer research, the majority of variants have VUS status, and it remains a considerable challenge to distinguish functionally relevant somatic mutations from benign mutations. In one of the most established cancer omics databases, the Cancer Cell Line Encyclopedia (CCLE), over 1000 cancer cell lines have been mutationally profiled and the collective mutational landscape consists of over 1.05 million unique protein-coding variants. The CCLE also houses the largest repository for functional omics data (e.g. transcriptomics, proteomics, metabolomics, CRISPR loss-of-function screens, and drug response assays) in cancer cell lines. In this study, we aim to integrate the recently published MAVE data for two oncogenes, BRCA1and TP53, with multi-omics data from the CCLE. Through integration of these complementary data types, we infer the impact of gene variation in BRCA1 and TP53 genes on downstream biochemical and regulatory networks in breast cancer cell lines. Using a semi-supervised approach, we develop inference models that predict association between the functionally impactful variants (from MAVE assays) and activated pathways (from CCLE transcriptomics, proteomics and metabolomics data). We verify functional connections between variants and their impacted pathways through analysis of CRISPR-mediated loss of function screens. Integration across MAVE and multi-omics data presents novel opportunities for utilizing saturation mutagenesis to assess biological impact on a systems level.

Functional conservation and divergence of the helix-turn-helix motif of E2 ubiquitin-conjugating enzymes.

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Polyubiquitination by E2 and E3 enzymes is crucial to cell cycle control, epigenetic regulation, and development. The hallmark of the E2 family is the ubiquitin (Ub)-conjugating (UBC) domain that forms a dynamic thioester conjugate with ubiquitin (E2~Ub). Numerous studies have focused on E2 surfaces, such as the N-terminal and crossover helices, that directly interact with an E3 or the conjugated ubiquitin to stabilize the active, "closed" state of the E2~Ub. However, it remains unclear how other E2 surfaces regulate ubiquitin transfer. Here, we demonstrate the helix-turn-helix (HTH) motif of the UBC tunes the intrinsic polyubiquitination activity through distinct functions in different E2s. Interestingly, the E2HTH motif is repurposed in UBE2S and UBE2R2 to interact with the conjugated or acceptor ubiquitin, respectively, modulating ubiquitin transfer. Furthermore, we propose that Anaphase-Promoting Complex/Cyclosome binding to the UBE2SHTH reduces the conformational space of the flexible E2~Ub, demonstrating an atypical E3-dependent activation mechanism. Altogether, we postulate the E2HTH motif evolved to provide new functionalities that can be harnessed by E3s and permits additional regulation to facilitate specific E2-E3-mediated polyubiquitination.

Engineering “two-in-one” antibody of CD19 and CD20 to mitigate tumor antigen escape in CAR-T therapy

Zhiyuan(Zoey) Yao, Gianpietro Dotti, Brian Kuhlman.

CD19 or CD20 chimeric antigen receptor (CAR) T therapies have produced unprecedented achievements to treat relapsed B cell leukemia and lymphoma. However, a significant number of patients still have therapy-resistant relapses caused by CD19 or CD20 antigen loss. One strategy to mitigate this problem is developing dual targeting CARs that have the tandem scFv antibody recognition domain. While the tandem CARs have been proved to prevent antigen negative relapse in pre-clinical trials, the cytolytic efficiency encountering each single antigen was attenuated. This reduction of activity is potentially caused by the tandem scFv features. Thus, to develop a recognition domain for dual targeting CAR, we propose to design a “Two-in-One” scFv that can bind to both the CD19 and CD20 receptors for dual specific CAR recognition domain.
To develop the “two-in-one” antibody for structurally unrelated antigens CD19 and CD20, we design to distribute two paratopes on complementarity-determining regions (CDRs) loops in a single scFv. We are using CDR libraries designed by computational and rational antibody design methods based on Rosetta, rather than using the randomized CDR library. The designed library will then be screened with yeast cell surface display (YSD) to find the most promising antibody candidates. the successful design of “two-in-one” scFvs that efficiently recognize CD19 and CD20 antigens will be applied to CAR-T studies. This “two-in-one” scFv antibody fragment can generate more reliable CD19 and CD20 dual targeting CAR-T therapy and improve treatment of B cell malignancies with antigen loss.

TAM Receptor Metabolic Reprogramming of Dendritic Cells.

E.Y. Zewdie, A.H, Hanks, H. Shelton Earp.

Recent progress in immunotherapy, specifically the anti-PD-1 checkpoint inhibitor, which works by blocking tumor cell evasion of immune cell attack, has shown some success in treating aggressive metastatic melanoma. However, similar to other cancer treatments, tumor cells develop resistance to this drug rendering its use ineffective in some patients. Studies have shown the key role Dendritic Cells (DCs) play in the tumor microenvironment as antigen presenting cells, for initiating treatment efficacy. Their significance makes them potential targets of inhibition as tumors evolve to escape immune elimination. We explored the relationship between MerTK, a receptor tyrosine kinase responsible for clearance of apoptotic cells and the induction of metabolic stress in DCs, as a potential mechanism of DC inhibition in the tumor microenvironment. What we found was that in anti-PD-1 resistant tumors, there were high levels of MerTK expressing DCs. We also observed that treatment with apoptotic melanoma cells in vitro results in increased MerTK expression and downstream increase in mitochondrial respiration of DCs. Furthermore, inhibition of MerTK in DCs resulted in the reduction of mitochondrial respiration, specifically fatty acid oxidation, with and without treatment of apoptotic melanoma cells. DCs lacking MerTK expression also increased CD8+ T cell proliferation with and without treatment of apoptotic melanoma cells. In addition, treatment of mertk-/- mice with anti-PD-1 resulted in a significant reduction in tumor volume and IL-10 serum concentration, and an increase in survival rate and IL-12 serum concentration. These results show that importance of targeting MerTK in DCs for melanoma. We propose that inhibition of MerTK can potentially reduce DC metabolic reprogramming and inhibition, and reverse anti-PD-1 resistance in patients and restore immune cell attack.

Decoding signal coordination via crosstalk of MAPK pathways in *Saccharomyces cerevisiae*.

Zhang, Shu

Background: Cryptic transcription is the process by which transcripts are initiated at intragenic promoters. Emerging studies indicate that transcription fidelity relies on the suppression of cryptic transcription, and this process is fine tuned in concert with signaling proteins and transcription regulators1. The two well conserved yeast MAPK pathways, mating and osmosensing (HOG), both share the gene STE11 which is well known to undergo cryptic transcription. The product of this gene is a protein kinase that relays signal to multiple MAP kinases of the pathways, including Fus3 (mating), Hog1 (HOG) and a shared MAPK Kss1. Both MAPK pathways are conserved in humans, but yeast has advantages that include unequalled genetic tractability.
Hypothesis: Cells exposed to different and simultaneous external stimuli undergo adaptations that include cryptic transcription. In particular, we propose that yeast exposed to mating pheromone and osmotic stress prioritize the osmosensing pathway. We propose further that cryptic transcription coordinates both mating and osmosensing pathways by phosphorylation, activation or inactivation of the MAPKs Fus3, Hog1 and Kss1.
Approach: We directly measured phosphorylation and transcriptional outputs of the MAPKs in yeast strains that increase or decrease cryptic transcription, and upon stimulation of one or both pathways.

Result: Our data indicate that cryptic transcription primarily regulates osmostress-dependent transcription with small effects on the mating pathway and MAPK activity. We suggest that cryptic transcription helps to coordinate responses to multiple stimuli and operates at multiple levels of the signaling pathways.

Inactive and active state structures template selective tools for the human 5-HT5A receptor.

Shicheng Zhang, He Chen, Chengwei Zhang, Ying Yang, Petr Popov, Jing Liu, Brian E. Krumm, Can Cao, Kuglae Kim, Yan Xiong, Vsevolod Katritch, Brian K. Shoichet, Jian Jin, Jonathan F. Fay, and Bryan L. Roth.

Serotonin receptors are important targets for established therapeutics and drug development as they are expressed throughout the human body and play key roles in cell signaling. There are 12 serotonergic G protein-coupled receptor members in the human genome, of which the 5-HT5AR is the least understood and lacks selective tool compounds. Here, we report four high-resolution (2.73-2.80 Å) structures of human 5-HT5A receptors, including an inactive state bound to an antagonist AS2674723 by crystallization, and active states bound to a partial agonist lisuride, and two full agonists 5-carboxamidotryptamine (5-CT) and methylergometrine by cryo-EM. Leveraging the new structures, we developed a highly selective and potent antagonist for 5-HT5AR. Collectively, these findings both enhance our understanding of this enigmatic receptor and provide a roadmap for structure-based drug discovery at 5-HT5AR.

An Overview of the UNC Proteomics Core Facility Highlighting Current Projects and Recent Publications

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The UNC Proteomics Core provides services for the analysis of proteins from tissues, cells or other biological samples, using mass spectrometry as the primary technique. We are available to the UNC community, as well as external customers worldwide. We're committed to educating students and researchers in the field of proteomics and will work with you from initial experimental design through publication. An array of sample preparation, instrumentation and data analysis services are offered, as well as method development, in-depth consultation to help researchers optimize their experimental design and grant support. In the past six years, the UNC Proteomics Core has collaborated with 150 labs from basic science and clinical laboratories at UNC, as well as 50 external academic and 15 industry collaborators. UNC Proteomics Core's bibliography from 2015-present can be found on our website.

The facility is equipped with four state-of-the-art systems: Thermo QExactive HF, Thermo QExactive HF Biopharma, Thermo Fusion Lumos Tribrid and Thermo Exploris480. In addition to the mass spectrometers, we have one Thermo Ultimate3000, three Thermo Easy nLC 1200s and a 908 Devices ZipChip capillary electrophoresis device for up-front separation. We have an Agilent 1260 Infinity II HPLC for offline peptide fractionation. We offer several services including: protein identification/characterization, phosphoproteomics and other PTMs, large-scale proteomic profiling, chemoproteomics, intact mass analysis, and protein-protein interaction analyses. We can perform these services quantitatively using labeled (SILAC or TMT10/18plex) or label-free approaches. Comprehensive data analysis is provided through a variety of database search engines and software packages. We can also aid in bioinformatics & statistical analysis.

This presentation will highlight several of the proteomics projects in progress or recently published.