2021 Graduate Student Organization Research Symposium

Hosted by

The Texas A&M Institute of Biosciences and Technology Graduate Student Organization

April 16th 2021
# Table of Contents

1: Schedule  
2: Presentation Order  
3: Current Graduate Students  
4: Core Facilities and Centers  
5-7: Publications  
8-35: Abstracts  
36: Acknowledgments
2021 IBT GSO Symposium Schedule

8:45-9:00
Opening Announcements

9:00-9:45
Oral Presentation Session # 1
Oral Presentation #1: Alex Powell
Oral Presentation #2: Shaohai Fang
Oral Presentation #3: John Taylor

9:45-10:00
Break

10:00-10:45
Oral Presentation Session # 2
Oral Presentation #4: Yuhan Yang
Oral Presentation #5: Lauren Lawless
Oral Presentation #6: Yue Yin

10:45-11:00
Break

11:00-12:00
Keynote Speaker Presentation: Dr. Shuvo Roy, UCSF

12:00-1:00
Lunch

1:00-3:00
Poster Presentation Session

3:00-3:30
Raffle

3:30-4:00
Award Ceremony and Closing Announcements

Symposium Zoom Link: https://tamu.zoom.us/j/96965139447
Presentation Order

Oral Presentations
1. Alex Powell
2. Shaohai Fang
3. John Taylor
4. Yuhan Yang
5. Lauren Lawless
6. Yue Yin

Poster Presentations
1. Lian He
2. Kelly Churion
3. RaviKanthReddy Marreddy
4. Abiola Olaitan
5. Chetna Dureja
6. Clara Kjerfve
7. Sabeeta Kapoor
8. Jacob Mardick
9. Dae Chung
10. Victoria Golub
11. Marisa Pinson
12. Bhagath Chirra
13. Aditya Katiyar
14. Purboja Purkayastha
15. You Wu
16. Yi-Tsang Lee
17. Razan Fakieh
18. Yuepeng Ke
19. Ziying Liu
20. Anh Vo
21. Nivedhitha Mohan
22. Jorge Tovar

Poster presentation numbers correspond to the Zoom breakout room in which the presenter will give their poster presentation.
2021 Graduate Students

Jacob Rutherford
John Taylor
Jorge Tovar Perez
You Wu
Yuhan Yang
Yue Yin
Mutian Zhang
Anh Tram Tran Vo
Jonathan Picker
Lauren Lawless
Caleb Whiddon
Razan Fakieh
Shaohai Fang
Amariany Gomez
C’Brionne Hendrix
Linh Huynh
Yuepeng Ke
Ann McKelvey
Yi-Tsang Lee
Ziying Liu
Nivedhitha Mohan
Ahmed Muhsin
Core Facilities

Antibody and Biopharmaceutics Core
  Director – Praveen Rajendran

Center for Advanced Imaging
  Director – Michael Mancini

Flow Cytometry Analysis and Cell Sorting
  Director – Margie Moczygemba

High Throughput Research and Screening Center
  Director – Clifford Stephan

Pre-Clinical Imaging Core
  Director – Jiang Chang

Protein Production Core
  Director – Magnus Höök

Rigor and Reproducibility Core
  Director – Kurt Zhang

Centers

Center for Genomic and Precision Medicine
  Director – Kenneth Ramos

Center for Epigenetics and Disease Prevention
  Director – Rod Dashwood

Center for Translational Cancer Research
  Director – Peter Davies

Center for Infectious and Inflammatory Disease
  Director – Magnus Höök
Publications


O1 – Alex Powell

Classical Complement Pathway Evasion in Lyme Disease Spirochetes, *Borrelia miyamotoi*, and Tick-Borne Relapsing Fever Borrelia

Innate immune evasion is essential for persistence of pathogens. One immune component threatening extracellular pathogens is the classical pathway (CP) of the complement cascade – a catalytic cascade of proteolytic reactions whose components are found in circulation ultimately resulting in formation of a lytic pore in the cell it is activated by. In *Borrelia burgdorferi*, causative agent of Lyme disease (LD), the most common vector-borne disease in the United States, the surface-exposed lipoprotein BBK32 binds and inhibits the initiating protease of the CP, C1r. Orthologues of BBK32, FbpA, FbpB, and FbpC, have been found in relapsing fever (RF) spirochetes, *B. hermsii*, and *B. turicatae*, as well as the *Borrelia miyamotoi* Disease (BMD) spirochete. For RF spirochetes, found in high concentrations in mammalian blood, complement evasion is similarly important. Preliminary data indicates FbpA exhibits similar activity to BBK32. Biochemical and structural analyses, including crystallography and surface plasmon resonance, shed light on key domains/residues for these interactions between BBK32, its orthologues, and C1r. We have found, through testing these Fbp proteins in genetic knock-in systems in serum-sensitive borrelial cells, that FbpA confers some serum resistance in vitro. Genetic knockouts of fbp genes will also be tested for their effect on pathogenicity of these spirochetes via experimental infection. We expect, like bbk32 knockout *B. burgdorferi* strains, fbp-deficient strains of RF and BMD-causing spirochetes will exhibit attenuated infectivity caused by their inability to evade the CP. Using these knock-in and knockout approaches, we aim to further define the role of BBK32, and establish importance of Fbp proteins in persistent infection of LD, relapsing fever, and BMD patients.
DNA methylation and demethylation cycle plays an important role in shaping the epigenetic landscape and chromatin accessibility to control gene expression during mammalian development. Ten-eleven translocation methylcytosine dioxygenases (Tet1, Tet2 and Tet3) have central roles in DNA demethylation. Our previous study showed that cardiac-specific deletion of Tet2 and Tet3 in mice (Tet2/3-DKO) disrupted YY1 mediated long range chromatin interactions during heart development and led to ventricular non-compaction cardiomyopathy (NCC). However, it is still unclear how heart development will be affected when all three Tet enzymes were depleted during heart development.

Cardiac development specific Nkx2.5Cre, Isl1MerCreMer and Mef2cCre were used to deplete all three Tet enzymes (Tet-TKO) during heart development. Phenotype analysis showed that Tet-TKO embryonic hearts showed shorter outflow tract (OFTs) when comparing with control hearts, which indicated that Tet-TKO affected the second heart field development. In order to reveal the underlying mechanisms, bulk and single cell RNA-seq experiments were applied to analyze Tet-TKO embryonic hearts. Results showed that multipotent second heart field (SHF) progenitors were accumulated and myocytes differentiation was impaired in Tet-TKO hearts, which could explain the shorter OFTs phenotype in Tet-TKO embryos. Results also indicated that Sonic hedgehog signaling, epithelial to mesenchymal transition and cell mitosis might be the main targets of Tet during heart development. Lastly, immunostaining experiments confirmed the accumulation of SHF progenitors and loss of myocytes in Tet-TKO hearts and more PHH3 positive mitotic cells were found in the second heart field in Tet-TKO hearts.

Overall, our study demonstrated that complete depletion of Tet enzymes could affect Sonic hedgehog signaling, epithelial to mesenchymal transition and cell mitosis during early heart development and cause the second heart field development defects.
The Type VII secretion system is a virulence determinant of *Streptococcus gallolyticus* subspecies *gallolyticus*

*Streptococcus gallolyticus* subspecies *gallolyticus* (Sgg) has a strong clinical association with colorectal cancer (CRC) and actively promotes the development of colon tumors. However, the molecular determinants involved in Sgg pathogenicity in the gut are unknown. Bacterial type VII secretion systems (T7SS) mediate pathogen interactions with their host and are important for virulence in pathogenic mycobacteria and *Staphylococcus aureus*. Through genome analysis, we identified a locus in Sgg strain TX20005 that encodes a putative type VII secretion system (designated as SggT7SS\(^{T05}\)). We showed that core genes within the SggT7SS\(^{T05}\) locus are expressed *in vitro* and in the colon of mice. Western blot analysis showed that SggEsxA, a protein predicted to be a T7SS secretion substrate, is detected in the bacterial culture supernatant, indicating that this SggT7SS\(^{T05}\) is functional. Deletion of SggT7SS\(^{T05}\)(TX20005\(_{Δ}\)esx) abolished the ability of Sgg to stimulate CRC cell proliferation. Analysis of bacterial culture supernatants suggest that SggT7SS\(^{T05}\)-secreted factors are responsible for the pro-proliferative activity of Sgg. In a murine gut colonization model, TX20005\(_{Δ}\)esx showed significantly reduced colonization compared to the parent strain. Furthermore, in a mouse model of CRC, mice exposed to TX20005 had a significantly higher tumor burden compared to saline-treated mice, whereas those exposed to TX20005\(_{Δ}\)esx did not. Examination of the Sgg load in the colon in the CRC model suggests that SggT7SS\(^{T05}\)-mediated activities are directly involved in the promotion of colon tumors. Taken together, these results reveal SggT7SS\(^{T05}\) as a previously unrecognized pathogenicity determinant for Sgg colonization of the colon and promotion of colon tumors.
Tet deficiency enhances aerobic glycolysis in mouse embryonic stem cells

Ten-Eleven Translocation (TET1, TET2 and TET3) proteins are 2-oxoglutarate (2-OG) and Fe2+-dependent dioxygenases to successively oxidize 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) and cause ultimate DNA demethylation. We report herein a previously unidentified role of TET in modulating intracellular metabolic dynamics in mouse embryonic stem cells (mESCs), with a focus on aerobic glycolysis as one of the major energy-producing pathways in mESCs. In our study, we observed that Tet triple deletion (Tet-TKO) did not exert appreciable effects on mESC proliferation and survival, but led to an elevated extracellular acidification rate (ECAR) and intracellular lactate level, suggesting the augmentation of glycolysis in Tet-TKO mESCs. We also observed that Tet-TKO mESCs displayed enhanced glucose uptake capability than the control. To probe the molecular mechanism, we investigated the transcription of genes that are critical for regulating intracellular glycolysis. We observed increased expression of Glut1, Ldha and Mct4 in Tet-TKO mESC, which might be responsible for enhanced glycolysis in Tet-TKO mESC. In summary, our study suggests that Tet deficiency results in transcriptional alterations of key glycolysis-related genes, thereby causing enhanced glycolysis and glucose uptake in mESCs.
The Effect of Cadmium on Fetal Heart Development: Investigating the Pathogenesis of Left Ventricular Hypertrabeculation

Congenital heart defects are the most common birth defects and are the leading cause of morbidity and mortality in infants and children. Of these, left ventricular hypertrabeculation is a heart defect that is presumably the result of myocardial hyperplasia and/or an incomplete compaction process. Interestingly, only about 13-40% of cases can be attributed to familial genetics, leaving a large gap in understanding other underlying factors, such as the environment or lifestyle, in the pathogenesis of this anomaly. Cadmium, a natural heavy metal and environmental contaminant concentrated by industrial activity, has been found to induce genotoxicity, ROS production, and apoptosis, leading to carcinogenesis, renal dysfunction, and cardiovascular disease. Prenatal cadmium exposure in our mouse model was found to induce ventricular hypertrabeculation in the developing embryo, which was accompanied by significant myocardial hyperplasia. Consistently, upregulation of mRNA and protein levels were observed in the genes known to impact cardiomyocyte proliferation, differentiation, and trabeculae patterning, such as p-AKT/AKT, Pten, and Gata4. A Gata4 germline knockdown model, exposed to the gestational cadmium treatment, demonstrated a phenotypic rescue of hypertrabeculation. Thus, these results suggest the important role of the Gata4-PI3K axis in cadmium induced left ventricular hypertrabeculation. Additionally, female mice exposed to a chronic, low dose of cadmium displayed indications of heart failure at 12 months of age, as evidenced by left ventricular hypertrophy, decreased cardiac output, and poor ejection fraction. In summary, this study generates an excellent mouse model for studying ventricular development and myocardial maturation, and provides compelling evidence for a genetic-environmental interaction underlying the pathogenesis of hypertrabeculation.
LiBis: An ultrasensitive alignment method for low-input bisulfite sequencing

The cell-free DNA (cfDNA) methylation profile in liquid biopsies has been utilized to diagnose early-stage disease and estimate therapy response. However, in typical clinical settings, only very small amounts of cfDNA can be purified. Whole-genome bisulfite sequencing (WGBS) is the gold standard to measure DNA methylation; however, WGBS using small amounts of fragmented DNA introduces a critical challenge for data analysis, namely a low mapping ratio. This, in turn, generates low sequencing depth and low coverage for CpG sites genome wide, which becomes a bottleneck for the clinical application of cfDNA-based WGBS assays. LiBis significantly improves the cost efficiency of low-input WGBS experiments by dynamically removing contamination introduced by random priming. The high sensitivity and cost effectiveness afforded by LiBis for low-input samples will allow the discovery of genetic and epigenetic features suitable for downstream analysis and biomarker identification using liquid biopsy.
Engineering of a bona fide light-operated calcium channel

The current optogenetic toolkit lacks a robust single-component Ca2+-selective ion channel tailored for remote control of Ca2+ signaling in mammals. Existing tools are either derived from engineered channelrhodopsin variants without strict Ca2+ selectivity or based on the stromal interaction molecule 1 (STIM1) that might crosstalk with other targets. Here, we describe the design of a light-operated Ca2+ channel (designated LOCa) by inserting a plant-derived photosensory module into the intracellular loop of an engineered ORAI1 channel. LOCa displays biophysical features reminiscent of the ORAI1 channel, which enables precise optical control over Ca2+ signals and hallmark Ca2+-dependent physiological responses. We have demonstrated the use of LOCa to modulate aberrant hematopoietic stem cell self-renewal, transcriptional programming, cell suicide, as well as neurodegeneration in a Drosophila model of amyloidosis.
The opportunistic pathogen *Staphylococcus aureus* (*S. aureus*) is highly human adaptive and has evolved to exploit host proteins to establish infections for survival. It is a highly versatile bacterium that is capable of causing a wide spectrum of diseases ranging from relatively benign skin infections to life threatening diseases including endocarditis, pneumonia and sepsis (Kristinsson, 1989; Lowy, 1998). In addition, it is also a major cause of infections associated with in dwelling medical devices, such as catheters and prostheses. The emergence of antibiotic-resistant strains presents significant therapeutic challenges and no vaccine is yet available to treat any of these types of infections. The development and establishment of disease relies on interactions between *S. aureus* proteins and host proteins. *S. aureus* produces a variety of microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) such as fibronectin binding protein A (FnBPA) that enable the bacteria to colonize and multiply within the host. Host protein fibrinogen (Fg) is one of the major targets of *S. aureus* FnBPA given that this protein is found in abundance in blood plasma and is usually targeted for tissue adherence initiation, host cell invasion, and attachment to implanted materials. The site between FnBPA regions N2 and N3 located in the A domain (Foster 1995, Ganesh 2008), also termed trench binding region, interacts in particular to the C-terminal residues of the fibrinogen γ gamma chain via a dock-lock-latch like mechanism common to all Fg binding MSCRAMMs. Recently, it was found that in addition to its originally identified FgγC trench binding site, MSCRAMM ClfA A domain binds to another distinct site in fibrinogen. Since ClfA and FnBPA are structurally and functionally related MSCRAMMs, it is likely that FnBPA binding to Fg also involves a two-site mechanism. Using techniques such as surface plasma resonance (SPR) and isothermal titration calorimetry (ITC), we were able to determine that FnBPA possesses more than one binding site but different interacting residues when compared to ClfA. Due to the importance of FnBPA as a virulence factor, understanding the molecular mechanism of interaction with an important host protein [fibrinogen], is crucial to potentially explore vaccine candidate development.
Chemical Genetic Exploration of *Clostridium difficile* Toxin Metabolism, Toward Defining Anti-virulent Drug Targets

*Clostridioides difficile* infection (CDI) is the leading cause of hospital-acquired diarrhea, resulting from antibiotic-induced dysbiosis. CDI pathogenesis relies on the biosynthesis of the toxins TcdA and TcdB. While vancomycin is the main recommended treatment, it is not narrow-spectrum and further disrupts the microbiota during therapy. This is thought to contribute to recurrent disease in >20% of patients. Herein, we addressed the urgent need for narrow-spectrum anti-virulent inhibitors that reduce onset of recurrent CDI, by blocking toxin biosynthesis. Screening of a rationally curated phytochemical library identified a molecule (TSI-1) that inhibited toxin production with inhibitory concentration (IC50) of ~16 µM. Interestingly, TSI-1 did not inhibit the growth of other gut bacterial species (MIC >100 µM), suggesting it was narrow-spectrum. To understand the mode of action of TSI-1, we performed click-chemistry proteomics, targeted metabolomics and chemical mutagenesis with ethyl methanesulfonate (EMS). Targeted proteomics identified a key enzyme in purine metabolism, as the molecular target. Consistent with this observation, metabolomics revealed TSI-1 caused intracellular accumulation of adenosine, but depletion of ATP and GTP. Metabolic bypass experiments, in cells exposed to TSI-1, showed toxin production was reinstated by supplementation with purines. TSI-1 target interaction was confirmed biophysically by Isothermal Titration Calorimetry (ITC) and in biochemical enzyme assays. Genome analysis of five EMS mutants that were refractory to TSI-1 revealed mutation of CodY, a global transcriptional regulator. Toxin biosynthesis in a CodY-deletion mutant was not inhibited by TSI-1, indicating the molecule acted through CodY activation. The discovery of TSI-1 and accompanying mechanistic studies reveal new pathways that regulate toxin biosynthesis in *C. difficile*, which can be exploited for narrow-spectrum inhibitors.
Emergence of metronidazole resistance in epidemic *Clostridioides difficile*

Background: Since the 1980s, metronidazole (MTZ) has been used to treat *C. difficile* infection (CDI). MTZ is a nitroaromatic prodrug that is reduced by anaerobes to nitro-radicals that damages cells. Reduced clinical success of MTZ has been widely observed, especially since the epidemic era of *C. difficile*, but there is lack of genetic information and mechanistic study on what drives the emergence of MTZ resistance. To address this question, we analyzed MTZ resistance in a collection of *C. difficile*, including the epidemic 027 ribotype, and performed both transcriptomic and genome-wide analysis to uncover molecular basis of MTZ resistance among epidemic isolates. MTZ resistance was screened for using our improved susceptibility testing by incorporating heme (5 µg/ml). R20291 was exposed to MTZ (2 µg/ml), and MTZ plus heme (5 µg/ml) and subjected to RNA-seq and results confirmed by qRT-PCR alongside historic *C. difficile* CD196. Transposon (Tn) mutagenesis was done in resistant epidemic *C. difficile* R20291 with pRPF215 vector and mutants screened for MTZ susceptibility by standard MIC methods. Genome-wide analysis was performed on >150 isolates, comprising both resistant and sensitive isolates. Heme modulated MTZ resistance accounting for 61% resistance in 113 clinical isolates. Through transcriptomic analysis, R20291 exposed to MTZ displayed DNA-damage responsive genes and extensive perturbation of cellular metabolism, the addition of heme significantly led to the reduction of these oxidative stress response genes. These results suggest that heme promoted MTZ resistance. In contrast, transcriptional perturbation by MTZ in the historic MTZ-sensitive CD196 was not rescued by heme, indicating that epidemic strains have evolved to withstand MTZ. Screening of ~7000 Tn mutants identified two with increased susceptibility (MIC=0.5 µg/ml) compared to WT (MIC=4 µg/ml). In the two mutants, insertions occurred in cysteine protease (cwp84) and 5-nitroimidazole reductase (nimB), respectively. Genome-wide SNP analysis revealed that resistance emerged through the development of unique mutation in the promoter of nimB. Evolutionary analysis shows that mutation in nimB promoter (Prom-nimB) coevolved with gyrA mutation, associated with hypervirulent epidemic *C. difficile*. Promoter strength analysis shows that the unique mutation in Prom-nimB confers higher transcriptional strength (>25X), leading to both constitutive and increased expression of nimB in resistant isolates. Enzymatic analysis shows that nimB can detoxify nitroaromatics to the inactive aromatic amine, explaining the development of MTZ resistance in epidemic *C. difficile*. This study uncovered the molecular mechanisms of heme-induced MTZ resistance owning the evolution of unique SNP in the promoter of nimB, which coevolved with gyrA mutation in epidemic isolates. The mutation causes constitutive expression of NimB, which is a heme-binding nitroreductase, detoxifying MTZ, and resulting in the development of resistance.
Structural and functional impact of Ala 115 of VanR in vanGCD mediated antibiotic resistance

Clostridioides difficile infection (CDI) is a healthcare associated infection usually arise by the disruption of gut microbiota after use of antimicrobial medications. Since, 2010, vancomycin is the first line therapy for CDI and is used regardless of disease severity. In 2017, IDSA and SHEA, recommended fidaxomicin over metronidazole for the treatment of CDI. This will further increase the vancomycin exposure and will promote the selection of vancomycin resistant strains. The genome sequencing of C. difficile 630 in the year 2006 showed the existence of vanG-like gene cluster, with vanR being the regulator of this operon. Previous studies from our lab have shown that the mutation in vanR (Thr115Ala) is responsible for the constitutive expression of the cryptic vanGCD operon and mutant shows reduced susceptibility against vancomycin. In the present study we are investigating the structural-functional impact of the mutated VanR. VanS, the sensor kinase when senses the presence of Vancomycin, phosphorylates the VanR, the phosphorylated VanR dimerizes and regulates the expression of vanGCD operon. Based on the molecular dynamics data we are hypothesizing that the Thr115 mutant is promoting the dimerization independent of the phosphorylation status. So, hereby we have planned to explore the role of Ala115 on the oligomeric status of the VanR and in extending the half-life of VanR-P or in the dephosphorylation of VanR. Further, we will explore whether the mutation at position 115 have changed affinity to the promoter. We are also speculating the mutated VanR regulates the vanGCD operon independent of VanS by getting phosphorylated by cross talk and experiments are under progress to delineate this.
Cell Wall Anchored Protein SesY is Encoded in a Putative Integrated Conjugative Element of Staphylococcus epidermidis

*Staphylococcus epidermidis* is a leading cause of nosocomial infections in patients with a compromised immune system and patients with an implanted medical device. Isolates of *S. epidermidis* have been associated with bloodstream infections, however, there is no current understanding of the mechanism used to survive and replicate in blood. *S. epidermidis* has an open genome with 20% of its' genome comprised of accessory genes. These accessory genes can often be encoded in mobile genetic elements (MGEs) and play a major role in pathogenic adaptation to the host. Our research has identified two cell wall anchored (CWA) proteins: *S. epidermidis* surface protein X (SesX) and SesY present in a putative integrated conjugative element (pICE). Bioinformatic analysis revealed that the pICE contains essential features of an ICE, i.e. an integrase, relaxase, and VirB4-like protein, a type 4 secretion system protein. In a genome wide analysis of 171 blood isolates, the sesY gene was identified in 92% of all sequence type 2 (ST2) strains as the leading ST associated with bloodstream infections globally. Further analysis of publicly available genomes of ST2 isolates from Germany revealed that the sesY gene present in approximately 90% of bloodstream infections isolates compared to 44% catheter, 27% fracture fixation, and 52% prosthetic joining infection isolates. This over-representation of sesY gene in ST2 bloodstream isolates indicates SesY plays a critical role in *S. epidermidis* bloodstream infections. Identification of host ligand targets for SesY proteins was conducted by ELISA-type binding assay with common blood proteins coated in microtiter plate wells. The results show SesY binds to plasmin and plasminogen in a dose dependent manner and with high binding affinities of 23x10^{-9} M and 4x10^{-9} M, respectively. SesY also binds human fibrinogen in a dose dependent manner with a KD 3x10^{-7}M. In conclusion, sesY is present in a pICE that is overrepresented in bloodstream isolates of ST2 *S. epidermidis* infections and binds to common human blood proteins. Fibrinogen, plasminogen and plasmin are crucial components of the fibrinolysis pathway. Studies with other bloodstream infection causing Gram-positive bacteria has shown that bacteria can use these interactions with fibrinogen, plasminogen and plasmin to survive in blood. Studies to understand the significance of SesY interactions with fibrinogen, plasminogen and plasmin are underway.
DNA repair genes have potential clinical value in predicting cancer prognosis and treatment outcomes. Nucleotide excision repair (NER) proteins like ERCC2 play a critical role in maintaining genome integrity by recognizing and unwinding DNA at sites of damage. Aberrant expression of ERCC2 alters NER capacity, influencing treatment outcomes. The current investigation examined the expression of ERCC2 following epigenetic combination treatment in colorectal cancer (CRC) cells and preclinical models. Attention was drawn to ERCC2 based on three observations. First, from online databases, when ERCC2 was overexpressed in colon tumors the corresponding CRC patients had reduced overall survival. Second, ERCC2 was the most highly downregulated gene when dietary histone deacetylase 3 (HDAC3) inhibitor sulforaphane (SFN) was combined with JQ1, an inhibitor of the bromodomain and extraterminal domain family, in human colon cancer cells and in colon polyps from the polyposis in rat colon (Pirc) model. Third, as reported here for the first time, RNA-seq analyses of Pirc colon polyps from rats treated with JQ1 and the SFN analog 6-SFN identified Ercc2 as the most highly downregulated gene. RNA-seq data were corroborated by RT-qPCR and immunoblotting experiments. There is much interest in combination approaches that target epigenetic ‘readers, writers, and erasers’ that are deregulated in cancer and other pathologies. The current work identified promising second-generation inhibitors with enhanced synergy and antitumor efficacy, especially in metastatic cells cultured in three-dimensions. Drug combinations decreased HDAC3, BRD4 and ERCC2 expression, while DNA damage and apoptosis markers were increased both in spheroids and in a mouse xenograft model. This investigation has potential clinical relevance for the identification of robust biomarkers that predict enhanced antitumor outcomes in CRC patients.
Parallel Rap1>RalGEF>Ral and Ras signals sculpt the C. elegans nervous system.

The Ras small GTPase is the most mutated oncoprotein, driving both tumorigenesis and metastasis. Oncogenic Ras effectors Raf and PI3 Kinase cascades are well studied and pharmacologically targeted. Ras directly binds a third oncogenic effector, RalGEF, which in turn activates a “Ras-Like” cousin, Ral. Yet the downstream consequences of RalGEF-Ral activation are poorly understood. Consequently, we are studying the functions of RalGEF-Ral in C. elegans development. We found that mutations of RalGEF and Ral enhance migration phenotypes of mutants in genes with established roles in cell migrations. We tested these findings by using the canal associated neurons (CANs) as our model, while validating with HSN cell migration, neurite guidance, and animal locomotion. We found that Ral functions cell autonomously as a permissive developmental signal. Ras, the canonical activator of RalGEF-Ral in cancer, functions as an instructive signal. Unexpectedly, we identified a function for Rap1, a close relative of Ras, in the same process RalGEF-Ral, suggesting that Rap1 and not Ras activates RalGEF-Ral. These findings demonstrate the role of canonical RalGEF-Ral signaling in development of the nervous system, and implicate an unexpected upstream activator, Rap1.
P9 – Dae Chung

Ethanol effects on fetal neural stem cell-derived extracellular vesicles and its proteome

Prenatal alcohol exposure (PAE) can result in craniofacial abnormalities, growth deficits, and is the leading cause of neurodevelopment disability worldwide. Neural stem cells (NSCs) are particularly vulnerable to alcohol (ethanol) exposure during the late first through the second trimester, when they are most extensively involved in neurogenesis. NSCs reside in a complex microenvironment rich in sub-200 nanometer-sized extracellular vesicles (EVs), which are shown to traffic protein, lipid, and RNA cargo between cells, that can serve as a mode of intercellular communication. Using fetal mouse derived cortical neuroepithelium, cultured ex-vivo as non-adherent neurosphere cultures, we previously found that ethanol exposure resulted in significant elevation of miRNA cargo like miR-140-3p in EVs, which direct NSCs towards an aberrant astroglial lineage. Subsequently, EVs may be amplifying PAE’s temporal and spatial effects in the stem cell niche to result in a neurogenic capacity decline. For this study, we further investigated the impact of ethanol on the proteome of NSC-EVs by employing quantitative proteomics to profile the protein expression across treatment groups of 18 EV and its 18 parent NSCs. Analyses of our EVs identified ~86% of proteins needed for eukaryotic translation initiation, implying that EVs carry with them the ability to translate EV-chaperoned mRNAs in recipient cells. Statistical and pathway overrepresentation analyses showed that moderate ethanol, ~26 mM, resulted in a significant increase in proteins of the Nonsense-Mediated Decay (NMD) pathway in EVs, whereas a higher dose, ~70 mM, resulted in EV overexpression of mitochondrial proteins that constitute a Danger-Associated Molecular Pattern (mito-DAMP) pathway. NMD is an important surveillance pathway that reduces errors in gene expression by eliminating premature stop codon-containing mRNA transcripts. Consequently, NMD pathway proteins sequestered in EVs are hypothesized to transfer neuroprotection to cells where the capacity to correct errors in protein translation may be depleted. Eukaryotic cells under high stress, expel mitochondrial proteins as a ‘danger’ signal that activates ‘pattern recognition’ receptors and pro-inflammatory responses in target cells. Consequently, mito-DAMPs in EVs from heavy-ethanol-exposed NSCs are predicted to spread inflammation through the NSC microenvironment, compromising NSC growth and differentiation. Collectively, these studies identify EVs as a novel source for communicating stress responses, in an ethanol dose-related manner, to cells within the fetal neural stem cell niche. Ongoing studies are focused on verification of ethanol-sensitive EV proteins through immunoblot staining and the effects of ethanol-exposed EVs on naïve NSCs’ cell cycle, proliferation, and death.
Hyperactivation of epigenetic HDAC pathway in an experimental model of acquired epilepsy

Epigenetic modifications have been implicated in a diverse set of neuronal processes, both salubrious and pathological, including learning and memory, normal aging, and maladaptive inflammatory responses. Increasing evidence suggests histone deacetylase inhibitors (HDACi) have roles in modulating neurogenesis, inflammation, and necrosis following CNS injury, though the underlying mechanisms are still uncertain. In this study, we investigate whether broad spectrum HDACi therapy following traumatic brain injury (TBI) provides neuroprotective effects in the early stages of post-traumatic epilepsy (PTE) development. Sodium butyrate (600 mg/kg) was administered twice daily for 21 days following severe controlled cortical impact model of TBI in mice. To assess HDAC activity levels throughout the study, we utilized the fluorometric Boc-Lys(Ac) HDAC enzyme assay. Enzyme-linked immunoassays (ELISA) were used to determine alteration of cytokine profiles after epigenetic modification by sodium butyrate. Immunohistochemistry for astrocytes (GFAP) and microglia (IBA1) was performed at days 1, 7, and 30 post-injury to determine the extent of glial expression. Results: TBI induced a robust increase (35-61%) in HDAC activity in both the cortex and hippocampal tissues beginning 4 hr post-injury, which persisted throughout the 30-day period. Sodium butyrate treatment resulted in a significant (~70%) decrease in TBI-induced HDAC activity. Immunohistochemistry for astrocytes and microglia revealed a clear peak in activated glial expression at 7 days post-injury in non-treated mice, which was visibly ameliorated by sodium butyrate. Furthermore, ELISA indicated reduction in pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β in plasma following treatment with HDACi. These results correlated with reduced lesion volume and improved functional behavior in treated mice following TBI. Conclusions: Our results confirm a state of hyperactivity of HDAC pathways following TBI, leading to astrogliosis and microgliosis. These maladaptive responses can promote the development of post-traumatic seizures and impaired functional outcomes in affected patients. HDAC inhibitors, such as sodium butyrate and the FDA-approved Vorinostat, represent a promising line of therapeutics for TBI by minimizing inflammation and promoting the protection and regeneration of damaged tissues.
Maternal Circulating miRNAs and Alcohol Impact Fetal Vascular Dynamics And Placental Transcriptome

Previous work conducted in our lab identified 11 microRNAs (miRNAs) which were significantly elevated in the plasma of mothers whose infants were affected by maternal alcohol consumption (Heavily Exposed Affected: HEa) compared to infants who were apparently unaffected by alcohol exposure (Heavily Exposed Unaffected: HEua) or unexposed (UE) controls. We found that a single systemic administration of HEamiRNAs, to pregnant mice, resulted in decreased fetal and placental growth and inhibited core members of the epithelial-mesenchymal transition (EMT) pathway in the placenta. In this study, we investigated whether HEamiRNAs might also contribute to prenatal alcohol exposure’s (PAE) deleterious effects on fetal vascular dynamics and any persistent alterations of the placental transcriptome. We compared litters exposed to HEamiRNAs via tail vein injection to litters exposed to alcohol prenatally via gavage. For each pregnant dam, blood flow imaging was conducted on a fetus in the upper left and upper right quadrant of the uterine horn for repeated imaging on gestational day 12 (GD12), GD14, and GD18. Color and pulse wave Doppler measurements for umbilical arteries and ascending aorta were obtained using a high-frequency ultrasound imaging. At GD18, dams were euthanized and fetal size measurements taken before tissues (including placenta) were snap-frozen for later RNA isolation. Fetal sex was determined by PCR analysis on tail samples. Total RNA was extracted from the nondecidual (labyrinthine with junctional zone) portion of mouse placetas and RNA-sequencing and subsequent analysis was conducted on samples. Our data suggests that both HEamiRNAs and PAE impair umbilical artery blood flow, potentially resulting in the observed growth restrictions observed in both the HEamiRNAs and PAE groups. These changes in blood flow dynamics may be mediated by persistent placental transcriptomic changes, suggesting underlying mechanisms contributing to the pathology of Fetal Alcohol Spectrum Disorders.
Antimicrobial Efficacy and Targeted Nanoparticle Delivery of Silver Ibuprofen to Treat Multi-Drug Resistant Gram-positive and Gram-negative Pathogens

Chronic respiratory infections with Pseudomonas aeruginosa (PA) are common in patients with cystic fibrosis (CF). With the emergence of multi-drug resistant (MDR) strains coupled with the steady decline in the development of novel antimicrobials in the past 30 years, it has become increasingly difficult to treat these infections. To address this clinical need, through a simple precipitation reaction, we have we developed a groundbreaking new antimicrobial compound, silver ibuprofen (Ag+IBU). Silver is a broad-spectrum antibiotic that is widely used to treat burn and wound infections. We have previously shown that silver is bactericidal against MDR-PA and bacteriostatic against methicillin-resistant Staphylococcus aureus (MRSA) strains. In addition, we have determined that ibuprofen not only has anti-inflammatory properties, but also, at high concentrations, antimicrobial activity against both MDR-PA and MRSA strains.

Our goal was to determine the antimicrobial activity of Ag+IBU against strains of MDR-PA and MRSA. Further, because Ag+IBU is aqueously insoluble, we developed poly(lactic-co-glycolic acid)/poly(lactic-co-glycolic acid)-polyethylene glycol (PLGA/PLGA-PEG) nanoparticles to allow for depot delivery of Ag+IBU, as well as aerosolization for direct delivery to the lung. We confirmed the structure and mass of Ag+IBU using NMR and Mass Spectrometry. Using the standard CLSI broth microdilution method, we determined the minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of Ag+IBU. Further, cellular toxicity at therapeutic concentrations was evaluated against Human Bronchial Epithelial cells (HBEs). Nanoprecipitation was utilized to formulate PLGA/PLGA-PEG nanoparticles loaded with Ag+IBU. The size and percent loading of the nanoparticles was confirmed using direct light scattering and spectrophotometric measurements, respectively. Ag+IBU inhibited the growth of 90% of the MDR-PA and MRSA strains tested at concentrations of 4 and 12 µg/mL, respectively. Ag+IBU showed bactericidal activity against PA strains at 12 µg/mL, but only bacteriostatic activity against MRSA. Cytotoxic assays indicated that the lethal dose (LD50) of Ag+IBU was ~100 µg/mL for HBE cells, which is well above the therapeutic concentration. By pre-treating HBEs with N-acetyl cysteine (NAC), we were able to significantly increase the LD50 to >5 mg/mL. Finally, the size of our Ag+IBU-loaded, PLGA/PLGA-PEG nanoparticles was ~300 nm with ~10% Ag+IBU (w/w). We developed a simple, cutting-edge compound, Ag+IBU with antimicrobial and anti-inflammatory properties that is effective against multidrug resistant Gram-positive and Gram-negative pathogens, while exhibiting no toxicity towards eukaryotic cells in the presence of NAC. Thus, Ag+IBU-loaded, PLGA/PLGA-PEG nanoparticles may serve as effective treatment of chronic pseudomonal pulmonary infections in CF patients.
Liquid like nuclear deformation around extracellular obstacles during cell migration

Mechanical forcing techniques have revealed the nucleus to be a visco-elastic organelle. Whether viscous behavior is relevant for nuclear deformation in migrating cells is not clear. Here we show that a nucleus in a migrating cell flows like a liquid around micro-post obstacles, such that micro-obstacles penetrate and pass through completely through the interior of the nucleus without getting exposed to nuclear contents. The integrity of the nuclear envelope is maintained through fusion between apposing nuclear surfaces which prevents rupture of the envelope. Intranuclear structures like the nucleolus fragment as they flow past the obstacles. The nuclear lamina, generally considered highly stable, deforms, fuses, and disassembles around the obstacle during the migration. Lamin A/C-/- nuclei deform around the post without re-fusing in its wake, behaving like a liquid drop with reduced surface tension. These results suggest that in migrating cells, the nucleus behaves like a liquid drop surrounded by a tensed nuclear surface that can remodel around obstacles.
P14 – Purboja Purkayastha

Rapid evolution by clonal selection within populations of fibroblasts propagated on a novel soft substrate

Mechanical properties such as substrate stiffness are a ubiquitous feature of a cell’s ecological and evolutionary context, and may be a significant source of natural selection. Many types of animal somatic cells exhibit canonical phenotypic plasticity when grown on substrates of differing stiffness, both in vitro and in vivo. Whether such plasticity is a multivariate optimum due to hundreds of millions of years of animal evolution, or instead is a compromise between conflicting selective demands, is unknown. We addressed these questions by means of experimental evolution of replicate populations of mouse fibroblasts propagated for ~90 cell generations on soft or stiff substrates. The ancestral cells grow twice as fast on stiff substrate as on soft substrate, and exhibit the canonical phenotypic plasticity. Soft-selected lines derived from a genetically diverse ancestral population increased growth rate on soft substrate to the ancestral level on stiff substrate, and evolved the same multivariate phenotype. Conversely, growth rate and phenotypes did not change in cell populations derived from clonal cells. These results imply that the changes were the result of genetic evolution and not phenotypic plasticity. Whole-transcriptome analysis revealed consistent differentiation between ancestral and soft-selected populations, and that both emergent phenotypes and gene expression tended to revert in the soft-selected lines. However, the selected populations appear to have achieved the same phenotypic outcome by means of at least two distinct transcriptional architectures related to mechanotransduction and proliferation.
P15 – You Wu

 Relatives of Ras regulate function of the C. elegans exocyst complex in development

Among three Ras effectors of roughly equivalent oncogenicity, Raf and PI3K are well studied but the downstream mechanisms of Ras-RalGEF-Ral signaling remain poorly understood. Ral (Ras-like) is a small GTPase related to Ras. Ral uses the exocyst complex, a heterooctameric protein complex that mediates targeting and tethering of transport vesicles to the plasma membrane, as a signaling intermediary (as exocyst components-Exo84 and Sec5 are Ral effectors) and also performs essential activities to regulate exocytosis functions of the exocyst, which precludes conventional biochemical bootstrapping to identify signal transduction components downstream of the exocyst. Rap1 (Ras proximal) is another small GTPase that sometimes signals in parallel with Ras. Delineating the mysterious functions of Ral in signaling and Ral and Rap1 in functions of the exocyst are important for therapeutic targeting of oncogenic Ras and understanding the cell biological functions of the exocyst complex. We are using genetic, biochemical and cell biological analyses in C. elegans to identify roles of Ral and Rap1 in control of exocyst functions during development. The three aims of this project are: 1. Determine the contribution of Ral to functions of the exocyst complex. 2: Investigate whether the Ral signaling function works to regulate exocytic functions of the exocyst separately from the signaling-independent functions. 3: Delineate Rap1 contributions to exocyst functions. We found that Ral is needed for the exocyst function, as deletion of Ral aggravates developmental defects conferred by maternally rescued (M+Z-) deletion of Sec5. Yet signaling-defective Ral does not alter the phenotype of M+Z- Sec5 mutant animals. Furthermore, constitutively activated Ral reduces the severity of M+Z- Sec5 mutant defects, implicating signaling-dependent and -independent contributions of Ral to exocyst-dependent development. Rap1 similarly interacts with the M+Z- Sec5 mutant and the M+Z- Ral mutant, suggesting a model similar to that of Ral. We will continue to test the contributions of Ral and Rap1 to exocyst-dependent development using the chemogenetic tool Auxin-Inducible degron (AID). We will also use development of elaborate arborization of the axons of PVD neurons as a more precise readout of exocyst function, and also assay the impact of Sec5, Ral and Rap1 depletion on transport of marked exocytic cargoes. Besides, we will use biochemical tools (Co-immunoprecipitation, yeast-two-hybrid) and will do confocal imaging to measure the physical interaction and colocalization of HA and fluorescent tagged Ral and exocyst components. In conclusion, this study will further investigate the role of two Ras relatives, Ral and Rap1, in the exocyst. This will help delineate the downstream effectors of the poorly understood Ras>RalGEF>Ral pathway, and help better understand the relationship of Rap1 with the exocyst, as well as the crosstalk of Rap1 and Ras effectors.
Expanding the Chemogenetic Toolbox by Circular Permutation

To expand the repertoire of chemogenetic tools tailored for molecular and cellular engineering, we describe herein the design of cpRAPID as a circularly permuted rapamycin-inducible dimerization system composed of the canonical FK506-binding protein (FKBP) and circular permutants of FKBP12-rapamycin binding domain (cpFRB). By permuting the topology of the four helices within FRB, we have created cpFRB–FKBP pairs that respond to ligand with varying activation kinetics and dynamics. The cpRAPID system enables chemical-controllable subcellular redistribution of proteins, as well as inducible transcriptional activation when coupled with the CRISPR activation (CRISPRa) technology to induce a GFP reporter and endogenous gene expression. We have further demonstrated the use of cpRAPID to generate chemically switchable split nanobody (designated Chessbody) for ligand-gated antigen recognition in living cells. Collectively, the circular permutation approach offers a powerful means for diversifying the chemogenetics toolbox to benefit the burgeoning synthetic biology field.
The RAP-2 Small GTPase and MIG-15 MAP4 kinase promote tertiary fate in C. elegans VPC Patterning

During C. elegans development, graded EGF signal from the anchor cell (AC) induces the six equipotent vulval precursor cells (VPCs) to assume a pattern of 3˚-3˚-2˚-1˚-2˚-3˚ cell fates. The VPC closest to the AC is induced via the Ras-Raf-MEK-ERK MAP kinase cascade to assume 1˚ fate. Presumptive 1˚ cells generate DSL ligands to induce the two neighboring cells via the Notch receptor to assume 2˚ fate. 1˚ and 2˚ developmental programs have been shown to be mutually antagonistic. Our lab showed that lower EGF dose causes Ras to switch effectors, from Raf to RalGEF-Ral, which functions to promote 2˚ fate in support of Notch. We further showed that Ral signals through GCK-2, a member of the Ste20 family of mitogen-activated protein kinase kinase kinase kinases (MAP4Ks), to trigger a p38 MAP kinase cascade to promote 2˚ fate (Shin et al., 2018). 1˚ and 2˚ cells execute distinct and stereotyped division patterns to form the vulva. In contrast, 3˚ fate is typically referred to as the “ground” or “uninduced” cell fate; 3˚ cells divide once and fuse with the surrounding epithelium. We have found that a paralog of GCK-2, MIG-15, also plays a role in VPC patterning. Upon mutation or RNAi depletion of MIG-15 or RAP-2, we observed an increase in ectopic 1˚ as well as ectopic 2˚ cells. MIG-15 is also required for expression of a putative cell fate reporter in 3˚ cells. Both RAP-2 and mig-15 are necessary for full expression of the 3˚ biomarker. Thus, we hypothesize that, like 1˚- and 2˚-promoting signals, 3˚-promoting signals antagonize other vulval cell fates. Using CRISPR-Cas9, we engineered an insert of fluorescent protein and epitope tag into the 5’ end of the endogenous mig-15 gene, revealing ubiquitous expression in the animal, localized to the cytosol and cell-cell junctions. We also inserted auxin inducible degron (AID), which mediates conditional degradation of tagged proteins. We will use complementary degradation experiments and tissue-specific transgenic rescue to test whether MIG-15 functions in the VPCs to repress 1˚- and 2˚-signals. We will also use CRISPR to mutationally activate MIG-15, as we did previously with the paralogous GCK-2. Preliminary data suggest that RAP-2 functions similarly to MIG-15, and RAP-2 has been shown to activate MIG-15 in other systems. We hypothesize that RAP-2-MIG-15 promotes 3˚ fate, counter to the notion of 3˚ fate as “uninduced.” Our work presents positions us to explore signals that promote “ground” developmental state and perhaps informs the relationship cancers and surrounding stromal cells.
Prostate cancer (PCa) is the second leading cause of cancer death in men, with no cure so far available for patients in the advanced stage. The new hotspot for the PCa is to combine antibody treatment targeting various cellular signaling cascades with immunotherapy. To date, this new strategy starts to gain momentum, and understanding the cellular mechanism of PCa progression will accelerate the path of it by facilitating the development of novel clinical combinations. There are various cellular signaling cascades that regulate PCa development, and fibroblast growth factor receptor (FGFR) signaling is one of them that serves as a major regulatory signal in normal and pathological processes in the prostate. FGFRs express in a cell type-specific manner, while the acquisition of FGFR1 expression in epithelial cells is usually highlighted among others. Despite that the relevance between aberrant activation of FGFR1 signaling and PCa development is established, the underlying mechanism of how FGFR1 contributes to PCa progression is not fully understood. Here we focus on the cell metabolism that shapes the immune response in the tumor microenvironment. A recent paper showed that ablation of FGFR1 reduced aerobic glycolysis via regulating lactate dehydrogenase in PCa cells. We reported that ablation of FGFR1 signaling promotes oxidative phosphorylation and suppresses aerobic glycolysis, suggesting that FGFR1 involves in the metabolic reprogramming in PCa cells. Increasing evidence showed that the metabolic reprogramming and metabolites changes in the tumor microenvironment could potentially mediates immune cell functions during immune response, and may facilitate the immune escape. Aerobic glycolysis in the cancer cells could be a potential target to improve immune therapy via reducing oxygen and nutrition competition, and lactic acid associated acidification. Taking the low frequency of FGFR1 positive immune cells in peripheral blood and the overexpression of FGFR1 in prostate tumors into consideration, selectively suppress FGFR1 in cancer cells may serve as an ideal strategy to inhibit cellular aerobic glycolysis, and may further increase the immune response in the tumor microenvironment.
Determine the Mechanism of How FGFR1 Signaling Promotes Prostate Cancer.

Prostate cancer (PCa) is the most common cause of cancer death in American men over age 75. The progression of PCa is a multiple-step process and is driven by various genetic abnormalities. One of them is the aberrant Fibroblast Growth Factor receptor (FGFR) signaling pathway. FGF/FGFRs are widely expressed in humans. There are 22 FGF with three subtypes: Paracrine, intracrine, endocrine. The stimulation of these ligands will trigger the dimerization and auto-phosphorylation at phosphorylation tyrosine sites of the four monomer receptors (FGFRs) located on the cell membrane, and thus activating the downstream signaling pathways to further contributes to cell proliferation, differentiation, apoptosis, and angiogenesis. To date, the underlying mechanism of how FGFR1 regulates PCa progression is not fully understood. Increasing evidence showed that abnormal FGFR1 activation regulates PCa progression through multiple canonical downstream signaling pathways. The downstream signaling of the specific phosphorylation tyrosine sites are partially determined. For example, Y463 phosphorylation will recruit CRK, FRS2, GRB2, SOS complex binds to FGFR1 and activate RAS-RAF signaling; Y730 phosphorylation will activate AKT-mTOR signaling; and Y766 phosphorylation will activate PLCy1-IP3-calcium signaling. Besides, the importance of the non-canonical downstream pathways was recently highlighted, as it is reported that ectopic FGFR1 activation promotes NF-κB signaling pathway via TAK1 phosphorylation; and that aberrant FGFR1 activation promotes tumor progression by regulating lactate dehydrogenase (LDH). Here, we focus on the discovery of novel FGFR1 non-canonical downstream signaling and to further determine the underlying mechanism of how FGFR1 regulates PCa progression. We took advantage of the RNA-seq technology and found 1620 genes differentially expressed in FGFR1 knockout DU145 cells. By GO annotation, we found that in the top 10 enriched biological processes, four are relevant to the sterol/cholesterol process, including sterol metabolic and biosynthesis, cholesterol metabolic, and biosynthesis, which is supported by the decreased cholesterol level in FGFR1 knockout DU145 cells.
**P20 – Anh Tran Tram Vo**

**Mitochondrial spatial dynamics and the efficacy of oxidized carbon nanoparticles to protect cells from oxidative injury**

Brain injury, including intracerebral hemorrhage (ICH), involves oxidative injuries from ischemia/reperfusion and the blood breakdown products. Our lab discovered that iron-containing porphyrin, hemin induced reactive oxygen species, widespread and persistent double-strand breaks, and a robust DNA Damage Response (DDR) in mitochondria and the nucleus. Consistent with the association between DDR and the pathological cellular phenotype of senescence, hemin also induced multiple cellular pathways known to occur in senescence. In hemorrhage, late-stage senescent cells assume an inflammatory secretory phenotype, injuring neighboring cells and amplifying the neurological damages. Moreover, senescent cells are resistant to various types of cell deaths, including iron-mediated ferroptosis, resulting in persistent pathology-promoting cells. We inhibited the transformation to senescence and resistance to ferroptosis in ICH by administering a novel carbon nanoparticle (CNP). The nanozyme possessed the catalytic superoxide-dismutase mimetic activity and was conjugated to the iron-chelating drug deferoxamine to enhance its application. This finding is intriguing because our CNPs are excluded from the nucleus yet protect it from hemin. Notably, our CNPs, likely because of their cationic charge, co-localize with the mitochondria. These dynamic organelles exhibit network and morphological plasticity in response to diverse cellular stimuli. The abilities to fuse and communicate with the nucleus emphasize the essential role of mitochondria in cellular activities. The overall hypothesis is that our CNPs can protect the nucleus by promoting pro-survival pathways within the mitochondria, which rely on inter-organelle communication with the nucleus.

Here, we present the initial test of the hypothesis by examining the mitochondria movement after exposure to the oxidant, hydrogen peroxide (H2O2), with and without our CNPs. We developed an image analysis method to detect the spatial organization of mitochondria relative to the nucleus. A radial scan in ImageJ was designed to detect mitochondria distribution in the cytoplasm. Our preliminary data showed that mitochondria congregated in the perinuclear region within 60 minutes of exposure to H2O2. Surprisingly, CNPs appeared to enhance the presence of perinuclear mitochondria, while co-treatment with both agents prevented this H2O2-induced rearrangement. These results suggest that CNPs, perhaps through metabolic changes, promote mitochondrial perinuclear movement and facilitate defense pathways in the nucleus. This possibility will be examined in the future to explore CNPs mechanism of action and further optimize their structural, electrochemical, and biological activities through varying their synthesis parameters.
Epigenetic regulation of alternative RNA splicing in BAZ1A

BAZ1A (Bromodomain Adjacent to Zinc finger domain 1A) is a non-catalytic subunit of ACF (the ATP-dependent chromatin assembly factor), and is involved in several cellular processes including DNA replication, chromatin assembly, DNA repair and transcriptional gene regulation. Defective DNA repair is one of the hallmarks of tumor progression, and BAZ1A is one of the highly expressed proteins in colorectal cancer tissues. BAZ1A exists in two main isoforms, a full-length form, and a truncated short form, generated by the alternative splicing (AS) of exon-13. Sulforaphane (SFN), a dietary histone deacetylase (HDAC) inhibitor, modulates AS in human colon cancer cells, increasing the expression of the short splice form of BAZ1A transcript and BAZ1A protein. CCAR2 (Cell Cycle and Apoptosis Regulator 2) is a master regulator of metabolism, aging and cancer (1), and interacts with ZIRD (Zinc finger protein 326), a factor involved in alternative mRNA splicing with potential links to BAZ1A. The DBIRD complex (CCAR2 + ZIRD) integrates various cellular processes, including alternative splicing and the rate of transcript elongation (2). Knockdown of DBIRD members via siRNA, including ZIRD and splicing factor hnRNPA1, downregulated the expression of BAZ1A. Conversely, BAZ1A knockdown downregulated both ZIRD and hnRNPA1, without affecting CCAR2 expression. Previous studies showed that SFN induced acetylation of CCAR2 and downregulated Wnt/β-catenin signaling, and that the BAZ1A bromodomain recognized K97 acetylation in CCAR2 peptide arrays (3). On investigating the involvement of CCAR2 in BAZ1A AS, SFN treatment increased the formation of the short form of BAZ1A in parental HCT116 cells, but not in CRISPR/Cas9 engineered CCAR2−/− HCT116 cells. In addition, SFN upregulated the expression of other DBIRD members, including hnRNPA1 and ZIRD in HCT116 cells. On the other hand, there was no significant changes in the expression of hnRNPA1 and ZIRD in SFN-treated CCAR2−/− HCT116 cells. Analyses via RT-qPCR in parental, CCAR2 KO, and CCAR2 acetylation mutant HCT116 cell lines revealed that SFN-induced CCAR2 acetylation promoted BAZ1A AS, whereas genetically-encoded CCAR2 acetylation did not induce BAZ1A AS. Co-immunoprecipitation assays identified CCAR2+BAZ1A direct interactions, in the presence and absence of SFN treatment. Overall, this work provided mechanistic insights into the epigenetic regulation of BAZ1A AS, which might implicate a ‘switch’ of functionalities via alternative splice forms in colorectal cancer.
Epigenetic regulation of alternative RNA splicing in BAZ1A

Low tumor immunogenicity has been a mechanism widely associated with cancer immune escape, inducing immunotherapy resistance and poor patient outcomes [1]. The major histocompatibility complex (MHC) antigen presentation pathways (APPs) play a crucial role promoting tumor T-cell recognition and initiating antitumoral immune responses [2]. However, MHC downregulation is a common mechanism used by cancer cells that leads to low tumor immunogenicity and immune evasion [3]. This investigation examined the effects of epigenetic drugs on the expression of MHC APP components using colorectal cancer (CRC), small intestine cancer (SIC) and melanoma cell culture models. Aberrant cancer epigenetic alterations, that act as gene repressors, have been shown to downregulate MHC components [4]. Therefore, a mouse melanoma cell line (B16/OVA) and human CRC (LOVO), SIC (HUTU80) and normal colon (CCD841) cell lines were treated with a variety of epigenetic drugs that inhibit distinct epigenetic effectors, leading to a decrease in epigenetic gene repression marks, or a diminished recognition of such marks. Here we show that inhibition of histone methylation repression marks with polycomb repressive complex 2 (PRC2) inhibitors, EED226 or EPZ011989, is a common mechanism that can be targeted to increase the expression of both MHC-I and MHC-II APP components across the panel of different cancer cell lines tested, as evidenced by RT-qPCR and immunoblotting experiments. While the inhibition of other epigenetic modifications such as histone deacetylation and DNA methylation can also upregulate MHC APP elements in SIC and melanoma cells. Moreover, normal colon epithelial cells show resistance to all previous treatments, indicating cancer cell specificity when using these epigenetic approaches. The current investigation has the capability for clinical application, as it sheds light into new epigenetic drugs that can be used to increase tumor immunogenicity, improving patient outcomes, and increasing the effectiveness of current immunotherapeutic strategies.
We would like to thank Cindy Lewis for all the things she does to make our graduate program function, and to ensure that this annual event is a success!

From the Graduate Student Organization to participants like you...

Thanks for your participation in the 2021 Graduate Student Organization Research Symposium!