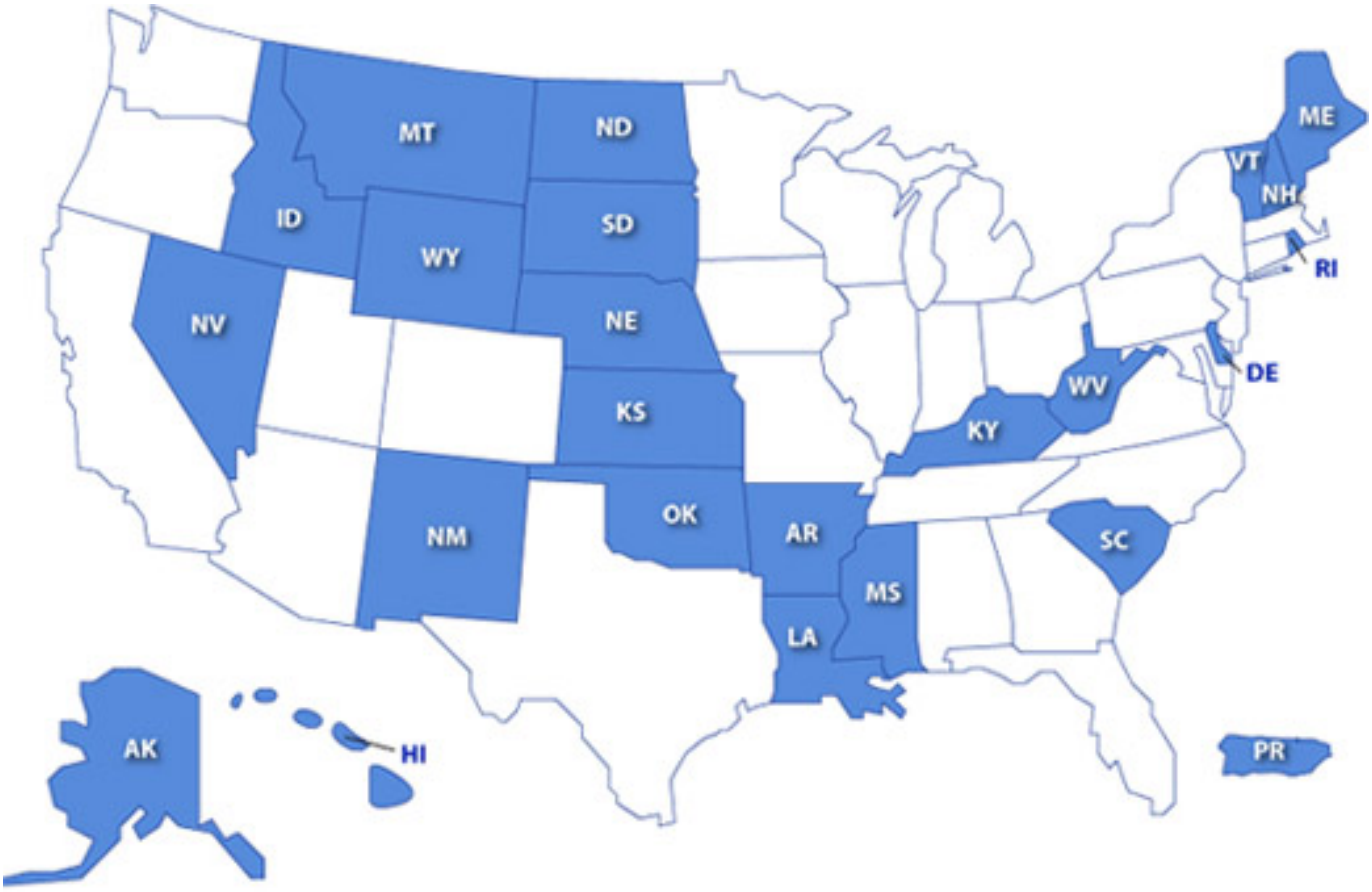

IDeA Program Meeting

Division for Research Capacity Building

NIGMS, NIH

September 22, 2020

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IDeA Program Meeting

Division for Research Capacity Building

NIGMS, NIH

September 22, 2020

2:00 – 5:30 pm ET

Zoom Meeting

Webinar Login Info

Access Zoom Meeting

https://nih.zoomgov.com/j/1618923932?pwd=T1NoUmNuQmJkR3hqa3lVdldZVkxkdz09

Webinar ID: 161 892 3932 Passcode: NIGMS

2:00 - 2:05	**Welcome**
Part I:	**NIH Scientific and Program Overviews**
2:05 – 2:35	**RADx: the NIH response to testing during the COVID-19 Pandemic** *Tara Schwetz, Ph.D., Associate Deputy Director, NIH*
2:35 – 3:05	**Updates from NIGMS** *Jon Lorsch, Ph.D., Director, NIGMS, NIH*
3:05 – 3:25	**IDeA Program Progress and New Initiatives** *Ming Lei, Ph.D., Director, Division for Research Capacity Building, NIGMS, NIH*
3:25 – 3:35	**Q&A**
3:35 – 3:45	**Break**
Part II:	**Research Highlights from IDeA Awardees**

3:45 – 4:30 **Research activities addressing the COVID-19 pandemic**

Moderator: Michele McGuirl, Ph.D., Chief, Research Advancement Programs Branch, DRCB

- **Developing novel strategies to increase COVID-19 testing among underserved and vulnerable West Virginia populations through community and state partnerships**
  Sally Hodder, MD, West Virginia University (CTR PI)
- **Developing a Realtime Monitoring System and Program to Improve COVID-19 Testing for Latinx Populations**
  James Padbury, MD, Brown University (CTR PI)
- **Social and behavioral implications for COVID-19 testing in Delaware's underrepresented communities** *Melissa Harrington, Ph.D., Delaware State University (COBRE PI)*
- **Mississippi CEAL Team: Community-Engaged Research Alliance**
  Caroline Compretta, Ph.D., University of MS Medical Center (CEAL Applicant)
- **Predictive Modeling of COVID-19 Progression in Older Patients**
  Kristin S. Miller, Ph.D., Tulane University (COBRE RPL)
- **Biomarkers of COVID-19 coagulopathy and D-dimer in a biracial cohort study**
  Debora Kamin Mukaz, Ph.D., University of Vermont (Postdoctoral Associate)
- **Center for Modeling Complex Interactions: Modeling COVID-19 in Rural Communities**
  Jennifer Johnson-Leung, Ph.D., University of Idaho (COBRE RPL)
- **Assessing the Efficacy of Home-based Intervention among Patients with Diabetes and COVID-19 related disruption in health and health care in Zuni**
  Vallabh Shah, Ph.D., University of New Mexico Health Science Center (INBRE DRPP Investigator)
- **Genomic epidemiology of COVID-19 in Alaska: multiple independent introductions and community spread of SARS-CoV-2**
  Eric Bortz, Ph.D., University of Alaska (INBRE DRPP Investigator)

4:30 – 5:15 **Progress on non-COVID-19 research**

Moderator: Krishan Arora, Ph.D., Chief, Network and Development Programs Branch, DRCB

- **Retinal Development and Regeneration from a Microglial Perspective**
  Diana M. Mitchell, Ph.D., University of Idaho (INBRE DRPP Investigator)
- **Machine Learning, Genomic Variants, and Osteoporotic Risk Prediction**
  Qing Wu, M.D., Sc.D., University of Nevada (COBRE RPL)
- **Optimization of Quinoline-based HIV-1 Integrase Inhibitors**
  Jacques Kessl, Ph.D., The University of Southern Mississippi (IDeA Co-funded, R01 PI)
- **Conducting Research on the Backbone of IDeA-CTR Infrastructure: LA CaTS and the PROPEL Trial**
  Peter Katzmarzyk, Ph.D., Pennington Biomedical Research Center (CTR Investigator)

- **Epigenetic regulation of mitochondrial biogenesis and myogenesis: roles of AMP-activated protein kinase (AMPK)**
  Shaoning Jiang, Ph.D., Oklahoma University Health Science Center (INBRE DRPP Investigator)
- **Chronic Allergen Exposure Results in Microgliosis and Increased Blood Brain Barrier Permeability in a Mouse Model of Cow's Milk Allergy**
  Danielle Germundson, University of North Dakota (INBRE Graduate student)
- **Overcoming chemotherapy resistance in triple negative breast cancer via targeting lysyl oxidase (LOX)**
  Ozgur Sahin, Ph.D., University of South Carolina (COBRE RPL)
- **IDeA National Resource for Quantitative Proteomics: a new NIGMS National Resource**
  Alan Tackett, Ph.D., University of Arkansas Medical Sciences (COBRE PI)
- **Leveraging SHARPHub Resources to Successfully Spinout a Biotech Company to Accelerate Discovery Research**
  A.J. Mellott, Ph.D., University of Kansas Medical center (Faculty Investigator)

5:15 – 5:30 **Open Forum**

COVID-19 SPEAKERS

Developing novel strategies to increase COVID-19 testing among underserved and vulnerable West Virginia populations through community and state partnerships

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West Virginia Clinical & Translational Science Institute
West Virgina IDeA-CTR U54GM104942 Sally Hodder

West Virginia (WV) is vulnerable to severe SARS Cov-2 impact due to its aging population (20% > age 65 years) and high prevalence of co-morbidities and injection drug use. Through school and university closures in March 2020, along with shutdown of non-essential businesses, WV had relatively few COVID-19 cases and deaths until July 2020 when transmissibility skyrocketed. Testing remains problematic in WV for multiple reasons, including inadequate testing supplies, accessibility to testing sites (given the rurality of the state and lack of widespread public transportation), shortages of personal protective equipment for staff, and lack of insurance coverage for surveillance testing and for uninsured persons. A proposal by the West Virginia Clinical and Translational Science Institute (WVCTSI) in partnership with multiple organizations including: 1) the WV Practice Based Research Network (PBRN), a 107 site primary care network spanning the state, 2) the WV Department of Health and Human Resources, 3) the WV National Guard, and 4) the Partnership of African American Churches (PAAC) is currently under review. Vulnerable populations addressed include individuals in rural communities and African American populations as well as those with comorbidities known to increase risk of severe COVID-19. Given the high prevalence of substance use disorder (SUD) in WV, a cross-cutting theme is ensuring persons with SUD are included in all proposed strategies to increase SARS CoV-2 testing. Three initiatives to increase SARS Cov-2 testing in WV include: 1) COVID-19 testing among rural primary care offices located across WV, 2) mobile vans, and 3) home testing among Black or African American communities. Evaluation of implemented strategies includes assessing numbers of tests performed, uptake of home testing, satisfaction surveys, and structured interviews among Black or African Americans enrolled in the home testing study.

Developing a Realtime Monitoring System and Program to Improve COVID-19 Testing for Latinx Populations

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Indra Neil Sarkar, Philip A. Chan, Yovanska Duarte-Velez
Brown University
Rhode Island IDeA-CTR U54GM115677 James Padbury

Latinx populations face barriers in accessing SARS-CoV-2 tests, receiving and interpreting test results, and getting appropriate follow-up care despite greater impact of the pandemic. Improved understanding of the sources of health equity differences for Latinx populations will better guide allocation of resources and provide essential data for provider organizations seeking to support those in the most need. This RADx-UP project will establish a statewide research and monitoring infrastructure in partnership with community clinics. We will develop community health teams (“Promotoras”) wi th the largest Latinx community organization in Rhode Island to address barriers to COVID-19 testing through patient navigation, health literacy support, and follow-up care.

The overall goals of the proposed study are to improve testing uptake and the understanding of SARS-CoV-2 outcomes among Latinx populations. We will build on partnerships with community clinics that serve a high proportion of Latinx individuals in Rhode Island. The insights gained from the quantitative and qualitative approaches will be used to guide the implementation of community campaigns to improve testing among Latinx populations that are in locations with lower per capita testing than other parts of the state (“testing deserts”) .

This RADx-UP project utilizes a unique approach and infrastructure to monitor SARS-CoV-2 testing rates, including the use of the statewide Health Information Exchange in Rhode Island. The specific aims of this project are to: (1) Identify COVID-19 hotspots and testing deserts using a near-real time geographic information system monitoring system; (2) Determine community and provider barriers that impact access to SARS-CoV-2 testing; and, (3) Implement community-based approaches to improve SARS-CoV-2 testing. This RADx-UP project will develop a community-based infrastructure to enable structured, longitudinal relationships with a historically underserved patient population. Such insights will provide essential data for a population level decision support system guiding testing prioritization and for future consideration of vaccine-based prevention strategies.

Social and behavioral implications for COVID-19 testing in Delaware's underrepresented communities

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Dorothy Dillard, Xuanren Wang Goodman, Nicole Bell-Rogers
Delaware State University
DE COBRE P20GM103653 Melissa Harrington

The COVID-19 pandemic has put a spotlight on our nation's stark disparities in health and burden of disease related to race, ethnicity, socioeconomic status, and literacy. Across our nation, the prevalence of the virus is disproportionately high in minority communities as is the number of COVID-19-related deaths. This project will triangulate data from semi-structured surveys, serology testing and census tract-linked public health and economic data to better understand social and behavioral factors related to COVID-19 testing in minority communities, and develop communication strategies to increase acceptance of testing and a future vaccine.

Social and behavioral factors will be identified through a semi-structured survey, based on the Johns Hopkins University COVID-19 Community Response Survey. The survey will collect detailed information on participants' medical history and current health, family structure and living conditions, employment and socioeconomic status, social distancing knowledge and practices, access to health care, presence of symptoms related to COVID-19 in themselves and among their contacts, their history of virus testing, attitudes toward testing, and interest in receiving a vaccine. Working with community partners we will recruit participants from communities which score poorly on Delaware's community health index, and which have also been hardest hit by the virus. The surveys will be combined with rapid, finger-stick serology tests to assess recent (previous 3 - 4 months) infection with the virus. After the initial survey and test, we will follow the participants over a 12-month period, repeating the survey and serology test every 4 months. Our longitudinal, cohort design will allow us to track participants' attitudes and adherence to mitigation behaviors, referral of contacts based on their test results, and attitudes toward a future vaccine throughout the changing dynamics of the pandemic and public health response.

Our collaborating community partner organizations will identify participants and provide trusted sites in the communities to administer the serology tests and survey questions to participants. Our community partners will schedule initial and follow-up visits, and will maintain the database that includes participant identification information. Data collection will occur at trusted community sites. Delaware State University nursing students will administer the serology tests, and students in the social work and psychology programs will administer the survey. Participants will be given resources for services as appropriate to their test results and health care needs, and will also be compensated for their time and information.

By following the participants' virus-related knowledge, attitudes, testing history and mitigation practices over time and correlating it with an objective measure of virus exposure, our proposed project will identify strategies to make testing more accessible and acceptable, and to increase the use and utility of test results among underserved populations. This will be key not only for reducing the spread of COVID 19, but also for preparing and positioning underserved communities for broad uptake of vaccination when a vaccine becomes available.

Mississippi CEAL Team: Community-Engaged Research Alliance
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University of Mississippi Medical Center
Mississippi IDeA-CTR U54GM115428 Joey Granger

The Mississippi Community-Engagement Research Alliance Against COVID-19 in Disproportionately Affected Communities (CEAL) is a multi-institution alliance that leverages the existing infrastructure within the Mississippi Center for Clinical and Translational Research (MCCTR) to address disparities in COVID-19 prevalence, severity, and outcomes among communities that differ in socioeconomic, geographic, and ethnic/racial factors, and other social determinants of health. This one-year project funded by the National Heart, Lung, and Blood Institute will 1) conduct urgent community-engaged research and outreach focused on COVID-19 awareness and education to address the widespread misinformation about COVID-19 and promote an evidence-based response to the disease, 2) promote and facilitate inclusion of diverse racial and ethnic populations in COVID-19 intervention studies including clinical trials of vaccines and therapeutics, and 3) promote the development, dissemination, and implementation of research findings to reduce the burden of COVID-19 in communities that have been disproportionately affected by the pandemic. The MS CEAL Team will build upon the team's long-standing community partnerships across the state to enhance education, awareness, access, and inclusion of underserved communities in COVID-19 prevention, treatment, and research. Five distinct projects will be pursued that, together, address the spectrum of what are the most urgent issues for addressing the COVID-19 morbidity and mortality disparities that exist within Mississippi.

Predictive Modeling of COVID-19 Progression in Older Patients

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Louisiana COBRE P20GM103629 S. Michal Jazwinski

The objective of this project is to develop a predictive model to identify individuals who are infected with SARS-CoV-2 and at risk of developing severe COVID-19. New Orleans has the third highest caseload and Orleans Parish the highest number of deaths per capita as of March 26th. Severe disease is seen in older individuals and those with underlying complications. The New Orleans population is particularly susceptible to severe COVID-19 as hypertension, diabetes and obesity are rampant. After infection, acute lung injury caused by the virus must be repaired to regain lung function and avoid acute respiratory distress syndrome and pulmonary fibrosis. Mounting evidence suggests that patients with severe COVID-19 have cytokine storm syndrome, which may exacerbate multiorgan injury and risk of fibrotic complications. Lack of effective ways to identify and attenuate severe COVID-19 progression persist due to limited understanding of the biological pathways responsible for cytokine storm syndrome and increased risk in older patients. Therefore, there is a need to determine the critical cytokine profiles responsible for severe COVID-19 progression to develop effective treatments. Further, it is essential to find a way to stage disease trajectory(ies) to identify therapeutic targets with precision to attenuate disease progression and uncover preventive strategies. Towards this end, we seek to leverage a mathematical model of SARS-CoV-2-induced lung damage to predict severity of acute respiratory distress syndrome and pulmonary fibrosis by considering key cytokine-cell interactions. We hypothesize that the model will accurately predict quantitative changes in suites of key cytokines and matrix accumulation with varying COVID-19 progression within 10% accuracy. To accomplish this, we have assembled an investigative team at Tulane University with key expertise in virology, clinical infectious disease research, bioinformatics, and predictive mathematical models of tissue remodeling. In Aim 1 of the proposal, we will identify the critical cytokine markers linked to viral-induced lung damage and pulmonary fibrosis. This will be accomplished by leveraging machine learning to determine the biomarkers and molecular pathways characterizing progression of severe COVID-19 to focus model formulation. In Aim 2, we will predict the severity of COVID-19 in older patients. Model predictions will be compared to blood markers of COVID-19 disease in cohorts of older patients at different stages of disease progression. The model will be refined and informed by cytokine data to discern causal biological pathways and disease processes that can be tested and targeted. Our expected outcome is to have determined the critical cytokine interactions responsible for lung tissue damage and dictating pathways for varying disease trajectories in older patients. These results are expected to have an important impact as the proposed predictive model will open new avenues of research to rationally design pharmaceutical interventions for severe COVID-19 patients.

Biomarkers of COVID-19 coagulopathy and D-dimer in a biracial cohort study

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Vermont COBRE P20GM135007 Mary Cushman

Objective: Coronavirus disease 2019 (COVID-19) coagulopathy is characterized by elevated thrombo-inflammatory biomarkers, especially D-dimer, which predicts thrombosis, critical illness, and death. In the general, non-infected population, these biomarkers are higher in men, older adults and Black individuals. These groups also have higher rates of COVID-19 infection and death. We hypothesized that Black individuals would have an exaggerated correlation between D-dimer and thrombo-inflammatory biomarkers characteristic of COVID-19.

Approach and Results: Correlations of 10 thrombo-inflammatory biomarkers with D-dimer were studied in 1068 participants of the biracial REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. Adverse levels of most biomarkers, especially fibrinogen, factor VIII, C-reactive protein, N terminal pro-B-type natriuretic peptide and interleukin (IL)-6, were associated with higher D-dimer. Several associations with D-dimer differed significantly by race. For example, the association of factor VIII with D-dimer was more than twice as large in Black compared to White participants. Specifically, D-dimer was 26% higher per SD higher factor VIII in Blacks and D-dimer was only 11% higher per SD higher factor VIII in Whites. In Black adults, higher IL-10 and soluble CD14 were associated with higher D-dimer, but not in White adults. In contrast, albumin was negatively correlated with D-dimer in Whites, with no association in Blacks.

Conclusions: Findings suggest a hypothesis that Black persons may have an amplified thrombo-inflammatory response during COVID-19 due to their stronger correlations of key biomarkers with D-dimer prior to infection. Future research should examine the role of thrombo-inflammation in racial disparities in COVID-19.

Center for Modeling Complex Interactions: Modeling COVID-19 in Rural Communities
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University of Idaho
Idaho COBRE P20GM104420 Holly Wichman

The COVID-19 pandemic has been an all-hands-on-deck phenomenon as state and local governments have called on regional scientists to provide analysis and understanding to support decision-making. At the University of Idaho, a multi-disciplinary pandemic-modeling group was formed to consider the particular questions affecting our state and other rural areas. We will give an account of our work to date and an outline of the research which we are currently undertaking to better understand and mitigate SARS-COV-2 transmission in sparsely populated areas of the United States.

Assessing The Efficacy Of Home-based Intervention among Patients with Diabetes and COVID-19 related disruption in health and health care in Zuni

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Objective: Home-Based Care (HBC) is a pragmatic treatment approach that addresses patient preferences and cultural barriers to healthcare. This study investigated the potential for differential efficacy of HBC vs. usual care in diabetic Zuni Indians.

Methods: Linear regression models used to estimate the effect of HBC on improvement in PAM total scores, compared to the control group in this post hoc analysis. We used generalized estimating equations to account for household clustering.

Results: The original study enrolled 63 participants into the HBC group, and 62 into usual care. Twenty-four (38.1%) participants in the HBC group and 32 (51.6%) in usual care had diabetes. The test for interaction between diabetes status and treatment arm suggested a significant differential treatment effect by diabetes status (p=0.022). The treatment effect among those with diabetes showed that PAM total scores increased by 16.0 points (95% CI: 8.8 to 23.1) more in the HBC group than in the usual care group.

We recently received a NM-INBRE pilot funding to explore COVID19 related disruption in health and health care in Zuni using 70-item survey. The impact of COVID-19 has been devastating to many of Zuni Indians who suffer multiple vulnerabilities (food insecurity, food desert location, low socioeconomic status low resource settings, crowded housing, and environmental exposures) which place them at greatest risk for infection and progression to death. They are more likely to live in homes without running water, with poor air quality and with multi-generational family members, that complicate being able to adhere to physical distancing measures associated with more frequent and severe complications from COVID-19. In addition, anxiety and stress about COVID-19 may contribute to poorer mental health outcomes.

Conclusions: The effectiveness of intervention on increasing patient activation is most notable among those patients who also have diabetes. *COVID-19 pilot study is underway in Zuni.*

Genomic epidemiology of COVID-19 in Alaska: multiple independent introductions and community spread of SARS-CoV-2

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University of Alaska INBRE 2P20GM103395 Brian Barnes

Alaska INBRE One Health is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 2P20GM103395.

Project Summary: As of 10 August 2020, there have been 4,473 diagnosed cases of COVID-19 in Alaska (0.6% of the population), 156 hospitalizations, and 26 deaths. More than 60% of cases occurred after the July 4th holiday, when community health measures were relaxed. However, it is unclear how COVID-19 was introduced and spread. We conducted a genomic epidemiology analysis to identify clusters of linked cases and to differentiate local community spread of COVID-19 from novel introductions from other regions in Alaska or out-of-state. Building on the Alaska INBRE-supported Pathogenomics Toolkit, a student-led initiative to build pathogen sequencing capacity in the University of Alaska, we sequenced the complete genomes of SARS-CoV-2, the etiological agent of COVID-19, from early infections in the state.

Results: We specifically amplified cDNA from SARS-CoV-2 by two-step RT-PCR from nasopharyngeal and sputum RNA collected from COVID-19 cases. These cDNA libraries were analyzed by multiplex nanopore sequencing on a MinION platform. The ARTIC bioinformatics workflow was adopted for consensus genome assembly with an overall recovery rate of 75% with 39 genomes of 52 samples sequenced. In parallel, 31 additional genomes were deep sequenced by Illumina short read NGS technology and assembled using CDC's Sequencher- based Applied Molecular Diagnostics pipeline. SARS-CoV-2 genomes were clustered by genetic similarity and maximum likelihood phylogenetics using NextStrain. We identified seven genetic based case clusters of community spread throughout Alaska with this analysis. At least five independent introductions of COVID-19 occurred in Alaska in March and April 2020 with two additional introductions in June 2020. Thus, continual rapid analysis of SARS-CoV-2 genomes provides evidence that despite implementing testing for travellers, and public health measures, COVID-19 was introduced over multiple events, leading to local community spread. We propose that SARS-CoV-2 genomic epidemiology analyses supplement existing contact tracing methods to better inform community intervention against COVID-19.

NON COVID-19 SPEAKERS

Retinal Development and Regeneration from a Microglial Perspective
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Idaho INBRE P P20GM103408 Carolyn Bohach

There is an undeniable connection between microglia, the resident phagocytes in the vertebrate central nervous system (CNS), and neurodegenerative CNS diseases. We are just beginning to gain an understanding of the complex functions of microglia in health and disease. Zebrafish have many advantages as a vertebrate model to study microglia and have an inherent capacity for robust CNS regeneration that does not exist in mammals. Using zebrafish retina, with its well-organized structure and well-defined cell types, we have characterized microglia during development, damage, and regeneration. Transcriptional analysis of microglia isolated from regenerating retinal tissue revealed key timeframes, novel genes, and novel pathways that likely control microglial function in homeostasis and regeneration. In addition, we find evidence of microglial heterogeneity which brings us to lineage tracing and single cell transcriptomics to reveal changes in gene expression over time. We used real-time live imaging to study temporal events and signaling pathways involved in apoptotic cell clearance by microglia *in vivo*. We are beginning to exploit a genetic system of microglial deficiency to determine the effects of microglial absence on retinal development and regeneration with a focus on the effects on the Müller glia. The Müller glia are of particular interest because they are present in both zebrafish and mammalian retinas. While Müller glia act as the source of regenerated retinal neurons in zebrafish, they respond differently to injury in mammals. Collectively, our findings will provide the foundation for successful therapeutic strategies for human retinal damage and disease. This work is a direct result of IDeA funding. Early on, an Idaho INBRE technology access grant supported the transcriptome analysis that was performed in an IDeA-initiated University of Idaho Research Core. An Idaho INBRE Developmental Research Project funded work that resulted in R01 funding by the National Eye Institute through the IDeA co-funding program.

Machine Learning, Genomic Variants, and Osteoporotic Risk Prediction

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Nevada COBRE P20GM121325 Martin Schiller

Developing an accurate predictive model for osteoporosis risk assessment is critical to prevent fracture, a devastating outcome for elders. Recent studies have found thousands of SNPs associated with osteoporosis. However, how to model these variants to create an accurate model for predicting osteoporosis remains unclear. We aimed to develop multiple machine learning models and to identify the best performing model for osteoporosis prediction. Genomic data from the Osteoporotic Fractures in Men cohort Study (N=5,133) was used as the data source. After genotype imputation, we identified 1,103 osteoporosis-associated SNPs for calculating genetic risk. Osteoporosis was defined as a T-score of bone mineral density ≤−2.5. The predicting variables included both conventional osteoporosis risk factors and genomic variants. The data were first normalized and then randomly split into a training set (80%) and a validation set (20%). Synthetic Minority Over-sampling technique was used to account for the low rate of osteoporosis in the data. Osteoporosis prediction models were developed using random forest, gradient boosting, and neural network separately. The model prediction performance was assessed by area under the ROC curve (AUC) and accuracy for each model in the validation set. We found that the performance of gradient boosting in predicting osteoporosis was the best among the three models, with AUC of 0.88 and an accuracy of 0.95. The performance of random forest and neural networks was worse than that of gradient boosting; random forest and the neural network had the AUC of 0.87 and 0.85, and accuracy of 0.92 and 0.85, respectively. The overall difference among the three machine learning models was highly significant ($p<0.0001$) by the Cochran's Q test. McNemar's tests showed that gradient boosting was significantly better than both random forest and random forest (both $p<0.0001$). Thus, we concluded that gradient boosting performed best for osteoporosis prediction in older men.

Optimization of Quinoline-based HIV-1 Integrase Inhibitors

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Mississippi Co-funding R01AI140985 ***Jacques Kessl***

HIV-1 Integrase is a viral enzyme that is essential for the replication of HIV-1. Recent studies have highlighted the vulnerability of the virus to a new class of integrase inhibitors capable of disabling this viral enzyme by triggering its abnormal multimerization at several critical stages of the virus life cycle. We have synthetized a library of active quinoline derivatives in order to better understand the molecular and mechanistic mode of action of these compounds. Our studies combine several approaches such as protein biochemistry, medicinal chemistry and virology.

Conducting Research on the Backbone of IDeA-CTR Infrastructure: LA CaTS and the PROPEL Trial

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The Louisiana Clinical and Translational Science (LA CaTS) Center is a consortium of the major biomedical research centers and academic institutions in the state. The LA CaTS mission is to "*address health disparities and improve health outcomes in our underserved population"*. In 2014 we responded to a PCORI Funding Announcement for "*Obesity Treatment Options Set in Primary Care for Underserved Populations*". We built an application on the LA CaTS Key Component Activities, including Clinical Research Resources, Community Engagement and Outreach, Ethics and Regulatory, Health Literacy, Biostatistics and Epidemiology, and Biomedical Informatics. We successfully obtained funding, and subsequently implemented the Promoting Successful Weight Loss in Primary Care in Louisiana (PROPEL) Trial (Katzmarzyk et al. *Contemp Clin Trials* 2018;67:1-10). PROPEL was a cluster-randomized trial to test the effectiveness of a high intensity lifestyle-based obesity treatment program delivered in primary care clinics serving a high proportion of low-income populations. We randomly assigned 18 clinics to usual care or an intensive lifestyle intervention. A total of 803 adults (67% African American; 65.5% <$40,000 annual income) with obesity were enrolled. The usual care group received normal care from their primary care team. The intensive group received a high-intensity lifestyle program delivered by health coaches in the clinics. The primary outcome was percent body weight loss at 24 months. Percent weight loss at 24 months was significantly greater in the intensive group (-4.99% [95% CI: -6.02, -3.96]) than the usual care group (-0.48% [95% CI: -1.57, 0.61]), with a mean difference of -4.51% (95% CI: -5.92, -3.10) between the groups (P<0.0001). In conclusion, a high-intensity lifestyle-based obesity treatment program delivered in an underserved primary care population produced clinically significant weight loss over 24 months (Katzmarzyk et al. *New Engl J Med* 2020;383:909-18).

Epigenetic regulation of mitochondrial biogenesis and myogenesis: roles of AMP-activated protein kinase (AMPK)

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Oklahoma INBRE GM103447-21 Darrin Akins

Diabetes and obesity can create adverse maternal environments that impact fetal development and long-term health. In humans, children of diabetic mothers later in life have lower skeletal muscle expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), the central regulator of mitochondrial biogenesis. AMP-activated protein kinase (AMPK) is an essential regulator of energy metabolism whose activity is suppressed in diabetes during pregnancy. We hypothesized that AMPK activation will reduce epigenetic maladaptation and consequently improve mitochondrial biogenesis and myogenesis. To examine this hypothesis, the effects of AMPK activators were assessed in WJ-MSCs, which are multipotent mesenchymal stem cells isolated from human umbilical cord. Treatment with an AMPK activator, AICAR, enhanced the differentiation capacity of human WJ-MSCs into myocytes. Further, the AMPK activator AICAR and metformin were also observed to decrease the level of PGC-1α promoter methylation, concomitant with increased PGC-1α expression. Notably, decreased PGC-1α promoter methylation by prior-differentiation treatment of AMPK activators persisted following myogenic differentiation. In addition, AMPK activation prior-differentiation resulted in increased mitochondrial DNA copy number after myogenic differentiation. Similarly, treating WJ-MSC cells with the DNMT1 inhibitor, Azacytidine, resulted in significant increase in mitochondrial DNA abundance, as well as decreased PGC-1α promoter methylation. We found that metformin decreased the abundance of DNA methylation transferase (DNMT1) in WJ-MSCs, which was prevented by AMPK inhibitor Compound C, suggesting specific role of AMPK in inhibiting DNMT1, which likely contributes to decreased PGC-1α promoter methylation and increased PGC-1α expression and mitochondrial abundance. *In vivo*, maternal metformin treatment on high fat diet-fed mice significantly increased mitochondrial DNA copy number in the skeletal muscle of adult offspring. Taken together, these combined observations revealed an important role for AMPK activators in epigenetic regulation of mitochondrial biogenesis and myogenesis, which could lead to potential therapeutics for preventing or reversing fetal mitochondrial programming and long-term adverse outcome.

This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number P20GM103447.

Chronic Allergen Exposure Results in Microgliosis and Increased Blood Brain Barrier Permeability in a Mouse Model of Cow's Milk Allergy

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North Dakota INBRE GM103442-18 Donald Sens

Prolonged peripheral inflammation is thought to pose a risk for the development of neuropsychiatric and neurodegenerative disorders through sustained neuroinflammation. Such chronic inflammation may occur by repeated exposure to an allergen in sensitized individuals. For example, a mildly food-allergic individual who does not experience anaphylaxis may continue to consume the allergen. Under the hypothesis that long-term exposure to an allergen by an individual with subclinical food allergy results in neuroinflammation and associated neuropathology, we investigated glial reactivity, blood-brain barrier integrity, and peripheral immune cell migration into the brain in a mouse model of mild cow's milk allergy. We sensitized 4-week-old male C57BL/6J mice to a vehicle or the vehicle containing a bovine whey allergen, β-lactoglobulin (BLG), for 5 weeks and placed the mice on a whey-containing diet for 2 weeks to simulate repeated allergen exposure. While health and growth were not overtly affected by the sensitization protocol, sensitized mice had significantly increased levels of BLG-specific IgE compared to the vehicle-sensitized sham mice. Immunostaining the brains of sensitized mice for Iba1 showed a greater number of microglia with reactive morphology throughout. Vascular permeability was assessed by the seepage of IgG from blood vessels into brain parenchyma by immunohistochemistry. Diffused IgG staining indicative of a 'leaky' blood brain barrier was observed in BLG-sensitized mice while more defined staining outlined blood vessels of sham mice. Interestingly, we also observed increased numbers of brain mast cells localized around vascularized regions of the brain in BLG-sensitized mice. Taken together, these results indicate that chronic exposure to an allergen, even without overt anaphylaxis, can produce observable neuropathology suggestive of neuroinflammation. Furthermore, our experimental paradigm establishes a novel tool to study food-allergy associated changes in the brain and highlights the possibility of allergen avoidance to prevent the development of neuropsychiatric or neurodegenerative disorders in susceptible individuals.

Overcoming chemotherapy resistance in triple negative breast cancer *via* targeting lysyl oxidase (LOX)

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South Carolina COBRE P20 GM109091 Igor Roninson

Chemoresistance is a major obstacle in triple negative breast cancer (TNBC), the most aggressive breast cancer subtype. Here we develop chemoresistant TNBC tumors *in vivo,* characterize their transcriptomes by RNA-sequencing and identify hypoxia-induced ECM re-modeler, lysyl oxidase (LOX) as a key inducer of chemoresistance. Mechanistically, LOX overexpression in hypoxic tumors treated with chemotherapy, on one hand enhances collagen cross-linking and fibronectin assembly, thereby decreasing drug penetration; and on the other hand, increases the expression of Integrin Subunit Alpha 5 (ITGA5), the major receptor for fibronectin (FN1), leading to activation of Focal Adhesion Kinase (FAK)/Src signaling and chemoresistance. Inhibition of LOX or ITGA5 with shRNA-mediated knockdown, or inhibition of FAK or Src kinases with small molecule inhibitors in combination with doxorubicin led to stronger tumor growth inhibition than individual treatments *in vivo*. The role of LOX in chemoresistance has further been demonstrated using chemoresistant TNBC patient-derived xenografts (PDXs) and organoids, treated with doxorubicin alone or in combination with the LOX family inhibitor, BAPN. Notably, higher LOX, ITGA5, or FN1 levels are associated with shorter survival in chemotherapy-treated TNBC patients. To identify a more potent LOX inhibitor than the currently available ones that suffer from lack of specificity and high toxicity, we performed a high-throughput screen (HTS) of more than 5,000 small molecules. This resulted in identification of several hits that inhibit LOX enzymatic activity without any cytotoxicity. A hit compound was identified after shortlisting of candidates based on their inhibitory effects on the LOX recombinant protein activity and the degree of chemosensitization in collagen-embedded cells. We are currently performing structure-activity relationship (SAR) to optimize the hit compound for more potent activity and better drug-like properties. In addition, we are analyzing the mechanisms leading to enhanced ITGA5 transcription by LOX and the contribution of the enzymatic activity of LOX to transcriptional regulation in more detail. Taken together, these results provide pre-clinical rationale for development and testing of LOX inhibitors to overcome chemotherapy resistance in TNBC patients.

IDeA National Resource for Quantitative Proteomics: A New NIGMS National Resource

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Arkansas INBRE/COBRE Larry Cornett/Alan Tackett

Abstract

Here, we announce the full launch of a newly funded NIGMS National Resource – the *IDeA National Resource for Quantitative Proteomics*. By leveraging NIGMS-IDeA support since 2016, this resource has grown through a regional consolidation of instrumentation, expertise, and bioinformatics resources across Arkansas and Oklahoma. The resource was created because IDeA investigators across the United States can face specific challenges for accessing cutting-edge proteomics resources and bioinformatics expertise for data interpretation. Arkansas and Oklahoma were positioned to unify their respective infrastructures to fill these resource gaps and provide a comprehensive solution to the IDeA network. Now with NIGMS support through PAR-19-301 (R24GM137786), we are transitioning the IDeA National Resource for Quantitative Proteomics to a NIGMS National Resource, which will allow us to expand our capacity to serve the quantitative proteomics needs of the entire IDeA network and others performing research within the mission of NIGMS. We will provide (1) user-friendly services for state-of-the-art, quantitative proteomics that will guide the user from service request to sophisticated bioinformatics analysis to delivery of publication ready data, (2) outreach opportunities for quantitative proteomics, and (3) educational opportunities for state-of-the-art proteomics. By maximizing economy of scale, increasing efficiency, expanding infrastructure and leveraging NIGMS support, we will provide the most cost effective option for proteomics to IDeA investigators. Furthermore, we will offer a peer-reviewed, nation-wide voucher program to provide IDeA investigators fully subsidized access to the resource. Our overall goal is to provide IDeA investigators unmatched access to state-of-the-art quantitative proteomics platforms and skilled bioinformaticians, which will increase the capacity for these 23 states and Puerto Rico to perform cutting-edge research within the mission of NIGMS.

Leveraging SHARPHub Resources to Successfully Spinout a Biotech Company to Accelerate Discovery Research

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Central Hubs P20 GM103418, UT2GM130175

Ronawk is a biotech company that was created for the purpose of producing innovative 3D Bioprinted consumables to accelerate discovery research in the fields of Life Science, Agriculture, and Healthcare. Ronawk's core technology is based off an interlocking 3D matrix developed by Dr. A.J. Mellott at the University of Kansas Medical Center (KUMC). Dr. Mellott has served as the PI on private funding awards and had multiple trainees that were funded through Kansas IDeA Network of Biomedical Research Excellence (K-INBRE) awards, which helped in the development of the technology. Ronawk was one of the inaugural recipients of Sustainable Heartland Accelerator Regional Partnership Hub (SHARPHub) funding. The funding and resources provided by SHARPHub accelerated Ronawk's development and spinout of the KUMC. SHARPHub helped Ronawk gain entry into multiple entrepreneurial programs such as NSF I-CORPS and the prestigious Pipeline Entrepreneurs Program. Since Ronawk's involvement in SHARPHub, Ronawk has raised over $1 million dollars in funding from private equity and debt instruments, and generated revenue in just under 18 months.

Ronawk is helping in the fight against COVID-19 by providing free trial sets of its T-Blocks to help researchers produce 3,600X more cells for reducing material costs by 90% and reducing labor costs by 85%. Ronawk's T-Blocks have already been shipped internationally and are being used to produce billions of mesenchymal stem cells (MSCs) for a variety of research applications that explore the immunomodulation capabilities of different types of MSCs. Thanks to the support of SHARPHub and funding from K-INBRE, Dr. Mellott has been able to position Ronawk to team with several cutting-edge biomedical companies while providing a new technology that helps multiple researchers across the globe.

COBRE

Elevated Mitochondrial Respiration and Cytokines in Female Obese Zucker Rats is Not Affected by Short-Term Metformin Treatment

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Arkansas COBRE 5P20GM109096

Abstract

The incidence of childhood type 2 diabetes is climbing with childhood obesity rates. Thought to target the mitochondria and have anti-inflammatory effects, metformin's effects on inflammation and mitochondrial function in obesity need further study. We used an obese Zucker rat model to investigate effects of obesity and metformin treatment on mitochondrial respiration and inflammatory cytokines.

We fed 5-week old female Zucker rats (*n*=16 lean, *n*=16 obese) AIN-93 G diet for 8 weeks before randomizing to metformin (1 g/kg of feed) for 10 weeks; thus forming 4 groups with n=8 each: lean +/- metformin and obese +/- metformin. We collected serum, spleens, perigonadal visceral adipose tissue (VAT) and skeletal muscle (SM; gracilis). We measured mitochondrial respiration in splenocytes by extracellular flux analysis and in VAT and SM fibers by high-resolution respirometry and serum cytokines by multiplex immunoassays.

We observed obesity effects on mitochondrial respiration in VAT and SM. Obese rats exhibited increased VAT OXPHOS capacity over lean rats with substrates octanoylcarnitine and malate (obese vs lean: 1.33 vs 0.76 pmol O_2/s/mg; SE_{diff} = 0.18, *p*=.005), and after substrates pyruvate (*p*=.012), glutamate (*p*=.009), and succinate (*p*=.045). OXPHOS capacity was increased in SM with substrates octanoylcarnitine and malate (obese vs lean: 12.18 vs 5.45 pmol O_2/s/mg; SE_{diff} = 2.31, *p*=.011) in obese vs lean rats. We observed metformin effects in splenocytes: decreased coupling efficiency (metformin vs no metformin; 56.2% vs 69.8%; SE_{diff} = 4.1%, *p*=0.005) and increased proton leak (*p*<.001) in metformin-treated rats as compared to rats not treated with metformin. We observed effects of obesity, but not metformin, on 10/21 cytokines measured.

We found obesity was associated with altered cytokines and increased mitochondrial respiration in VAT and SM, and metformin did not affect cytokines or mitochondrial respiration in VAT or SM, but increased proton leak and reduced coupling efficiency in splenocytes.

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Abstract

Background: Arkansas has the largest population of Marshallese Pacific Islanders residing in the continental United States. Marshallese are disproportionately burdened by poorer maternal and infant health outcomes. Exclusive breastfeeding can prevent or help mitigate maternal and infant health disparities. However, exclusive breastfeeding among United States Marshallese communities remains disproportionately low and reasons for are not well documented.

Purpose: This paper describes the protocol of a mixed-methods concurrent triangulation longitudinal study designed to explore the beliefs and experiences that serve as barriers and/or facilitators to exclusive breastfeeding intention, initiation, and duration among Marshallese mothers in northwest Arkansas.

Methods/Search Strategy: The mixed-methods design collects qualitative and quantitative data during simultaneous data collection events, at third trimester, six weeks postpartum, and six months postpartum. Quantitative and qualitative data will be analyzed separately and then synthesized during the interpretation phase. The research team will disseminate results to study participants, research stakeholders, the broader Marshallese community, and fellow researchers.

Findings/Results: Findings and results will be presented in subsequent manuscripts upon completion of the study.

Implications for Practice: This study will be an important first step to better understand beliefs and experiences to exclusive breastfeeding intention, initiation, and duration in this community and will inform tools and interventions to help improve health outcomes.

Implications for Research: This study will aid in filling the gap in research and providing essential information on the infant feeding beliefs and barriers among a Marshallese community in Arkansas.

Sirt3 Deficiency Causes Hyper-acetylation of Mitochondrial Proteins and Mitochondrial Dysfunction in Osteoclasts of Old Mice

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Arkansas COBRE P20GM125503 Ha-Neui Kim

Mitochondrial protein acetylation plays a key role in aging and Sirt3 is the primary mitochondrial protein deacetylase. Sirt3 is indispensable for the increased bone resorption and the accompanying enhanced mitochondrial function in osteoclasts from old mice but the molecular details remain elusive. We analyzed the global proteome of osteoclasts from 16-month-old Sirt3 deficient mice and WT controls by mass spectrometry. Of 4400 identified proteins, 387 were mitochondrial proteins. Gene Ontology enrichment analysis confirmed that several Sirt3 target mitochondrial pathways were down-regulated in Sirt3 null cells. However, the overall protein-fold changes were minimal between genotypes, suggesting that changes in acetylation, rather than protein levels may underlie the mitochondrial dysfunction in osteoclasts of Sirt3 null mice. Following data acquisition in MS/MS mode, we used the Uniprot *Mus musculus* database to identify acetylated peptides. A total of 567 acetylated peptides were identified with a fold change of >2.5 and a *p* value of <0.05. RANKL enhanced 69 acetyl-lysine peptides in WT cells whereas Sirt3 deletion resulted in the hyperacetylation of 305 peptides. In Sirt3 null osteoclasts, 18 mitochondrial proteins were identified with significant hyperacetylation at multiple sites. Importantly, five lysine residues quantified in ATPIF1 – an essential protein for mitophagy – displayed a 3.9- to 213-fold acetylation increase in the absence of Sirt3, indicating that ATPIF1 is a major target of Sirt3 in osteoclasts. Consistent with this, osteoclasts lacking Sirt3 exhibited a striking decrease in the protein levels of mitophagy markers such as Bnip3 and Nix compared to osteoclasts from WT mice. Deletion of Sirt3 also reduced autophagic flux. These results demonstrate that Sirt3 deficiency causes significant changes in the acetylome of osteoclasts and suggest that Sirt3 target proteins are culprits of excessive bone resorption associated with old age.

Piezo1 expression in osteocytes contributes to bone homeostasis

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Arkansas COBRE P20GM125503 Jinhu Xiong

Background and Objective: Mechanical loading plays an essential role in bone growth and homeostasis. The identity of the cells responsible for directly sensing changes in mechanical loading of the skeleton is unclear. In recent studies, deletion of Piezo1, a mechanosensitive ion channel, from Dmp1-Cre-targeted cells decreased cancellous and cortical bone mass and blunted the response of the skeleton to mechanical stimulus, demonstrating the important role of Piezo1 in skeletal mechanotransduction. However, in addition to osteocytes, the Dmp1-Cre transgene used in these studies also causes recombination in mature osteoblasts. The goal of this study is to determine whether Piezo1 promotes bone formation via its expression in osteoblasts, osteocytes, or both.

Methods: We deleted the Piezo1 gene using Sost-Cre transgenic mice, which express the Cre recombinase in osteocytes but not in osteoblasts, and analyzed the skeletal phenotype.

Results: Mice lacking the *Piezo1* gene in SOST-Cre expressing cells, referred to as SOST-Cre;Piezo1$^{f/f}$ mice, exhibited normal body weight but low bone mineral density at 12 weeks of age compared to littermate controls. Micro-CT analysis revealed decreased cancellous bone mass in the femur and vertebra of 12-week-old male and female SOST-Cre;Piezo1$^{f/f}$ mice. Cortical thickness in the femur was also significantly decreased in SOST-Cre;Piezo1$^{f/f}$ mice at 12 weeks of age. Longitudinal femoral length was not affected by the Piezo1 deletion. In contrast to Dmp1-Cre;Piezo1$^{f/f}$ mice, periosteal and endocortical circumferences in the midshaft of femur were unaltered in SOST-Cre;Piezo1$^{f/f}$ mice. In addition, the decreases in cancellous bone mass and cortical thickness in SOST-Cre;Piezo1$^{f/f}$ mice were not as large as those observed in Dmp1-Cre;Piezo1$^{f/f}$ mice.

Conclusions: Together, these results suggest that Piezo1 expression in both osteoblasts and osteocytes contributes to the maintenance of bone mass.

Discovery of Chemical Probes and Therapeutic Leads, Phase II
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Delaware COBRE P20 GM104316 Joseph M. Fox

The goal of this Center of Biomedical Research Excellence is to continue our efforts to develop molecular approaches for probing biology, to discover and apply new chemical biology tools for the study of biological pathways associated with disease, and to develop computational approaches for understanding small molecule interactions with complex macromolecular targets. Our approach is broad-based and highly interdisciplinary with a common theme of developing chemical biology strategies to probe questions centered on infectious diseases and cancer. Our center has recently begun Phase II, with a foundation from Phase I that resulted in independent funding to eight investigators, the recruitment of three new faculty members, and the creation of extensive and broadly utilized instrumentation core. Our phase I investigators were funded through eleven major NIH awards (7 R01s, 2 R35s, 1U01, 1 NIH New Investigator) to five original project leaders, a replacement project leader, and two faculty hires. Active NIH awards by faculty from our center account for a significant fraction of NIH funding to the state of Delaware. The research of our phase I team is having widespread impact, and techniques developed by our Phase I investigators are now used extensively across the drug discovery activities of major pharmaceutical companies and by over 20 research groups internationally. Our center continues to capitalize on the collaborations within the IDeA Network and to leverage regional biomedical collaborations including strong interactions with the NCI Center for Cancer Research. Our center also supports an Analytical Chemsitry Core Facility for molecular characterization and kinetic analysis, and a Synthesis and Discovery Core Facility to support synthesis, catalysis, computation and microscopy. Used by more than 60 research groups and ~300 users, our cores are among the most heavily used facilities in the state of Delaware.

Telomere-like Sequences are Associated with Overall Survival of Cancer Patients Receiving Immune Checkpoint Inhibition Therapy

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Scott Bowler is PhD candidate within the Department of Tropical Medicine at the University of Hawaii at Manoa. Drs Vedbar Khadka and Youping Deng serve as supervising mentor and principal investigator, respectively.
Hawaii COBRE P30GM114737 and INBRE P20GM103466 Scott A. Bowler

As a treatment for cancer, Immune checkpoint inhibition (ICI) therapy shows low efficacy (12.46%) while placing patients at increased risk of immune-related adverse events, highlighting the need to elucidate factors driving these outcomes. Intra-chromosomal telomere-like sequences (ITS) have been implicated in double-stranded DNA breakage, an occurrence commonly observed in cancers. We hypothesized that intra-chromosomal telomere-like sequences (ITS) measured by whole exome sequencing (WXS), will be increased in tumors of cancer patients that respond to ICI therapy and associated with overall survival. This was an *in silico* cancer study of individuals (n=246) who received ICI and underwent tumor and peripheral blood matched WXS. Sequencing and clinical data was made available through the database of genotypes and phenotypes. ITS from somatic chromosomes were assessed using Telomerehunter. Spearman's correlation coefficients assessed relationships between numerical variables and Kaplan-Meier curves were conducted to estimate survival probabilities. The study cohort was predominantly male (64.2%), with median age 63 years, progression free (PFS) and overall survival (OS) of 70 and 443 days, respectively, and 48.8% had melanoma. ITS were expanded within tumor tissue compared to control ($p<0.0001$). Individuals who exhibited clinical benefit from ICI therapy had increased ITS repeats on chr3, 9, and 12 within tumor tissue and chr8, 12, and 15 from control tissue (all $p<0.05$). ITS on 3 cytogenetic bands (8p23.1, 8q24.22, and 12.q24.12) from control tissue was correlated with OS (all $p<0.05$). In conclusion, ITS from healthy tissue are associated with improved OS in individuals diagnosed with cancer receiving ICI. These results are intriguing given their observation from peripheral blood and the need for a complete picture of factors which result in ICI therapy response within this population.

Hypnotics Significantly Improve Clinical Outcomes of COVID-19 Patients

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Hawaii COBRE 5P30GM114737 Richard Yanagihara).

Abstract

Background: Sustained airborne transmission of SARS-CoV-2 has resulted in nearly 22 million cases of COVID-19 and more than 750,000 deaths in 188 countries worldwide. Effective treatments are urgently needed to improve clinical outcomes of COVID-19 patients.

Methods: A retrospective review of 323 COVID-19 patients hospitalized in Wuhan from January 8 to February 20, 2020 was conducted. Patients were classified into three disease severity groups (non-severe, severe, and critical), based on initial clinical presentation. Clinical outcomes were designated as favorable and unfavorable, depending on disease progression and response to treatments. Logistic regression models were performed to identify risk factors associated with clinical outcomes, and log-rank test was conducted for the association with clinical progression.

Results: Standard treatments, including antiviral drugs and oxygen therapy, did not show significant improvement in patient outcomes. Multivariate regression indicated age over 65 years (p<0.001), smoking (p=0.001), critical disease status (p=0.002), diabetes (p=0.025), high hypersensitive troponin I (>0.04 pg/mL, p=0.02), leukocytosis (>10 x 10^9/L, p<0.001) and neutrophilia (>75 x 10^9/L, p<0.001) predicted unfavorable clinical outcomes. By contrast, the administration of hypnotics (dexzopiclone) at a dose of 1.0 mg per day was associated with favorable clinical outcome and survival (p<0.001). The effect of hypnotics was independent of other factors and was even stronger for patients with severe disease.

Conclusions: Hypnotics may be an effective ancillary treatment for COVID-19 by enhancing immune function. We also found novel risk factors, such as higher hypersensitive troponin I, predicted poor clinical outcomes. Overall, our study provides useful data to guide early clinical decision making to reduce mortality and to improve clinical outcomes of COVID-19.

NADH Lifetime Imaging Identifies Unique Metabolic Signatures to Distinguish between Healthy and Disease Adipocyte Populations

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Hawaii COBRE 1P20GM113134 Nicholas G. James

Adipose tissue dysfunction, through energy overload or chronic inflammatory conditions, is associated with several metabolic complications, including obesity and diabetes. Development of a rapid and non-invasive label-free method for monitoring the metabolic state of adipocytes cells in vivo would provide a routine method for managing progress towards disease state(s). In this work we utilized NAD(P)H fluorescence lifetime imaging microscopy (FLIM) to identify metabolic signatures of adipocytes under various energetic states (e.g insulin resistance). We identified distinct phasor trajectories for 3T3-L1 during maturation to adipocytes with the migration trajectory indicating a reduction in bound NAD(P)H and that mature adipocytes were primarily undergoing glycolytic metabolism. This reduction in oxidative phosphorylation within mitochondrial metabolism was confirmed via seahorse analysis. This corroboration in metabolic readouts between methods establishes NAD(P)H FLIM phasor as a sensitive marker for metabolic analysis. Stimulation with of mature adipocytes with TNFα, which was used to simulate insulin resistance under cell culture conditions, produced a metabolic signature that was unique and easily distinguished from healthy mature adipocytes while clearly showing a reduction in metabolic cytokines. Phasor trajectories of healthy adipocytes showed major changes in phasor trajectories, and metabolic rates, when stimulated with IL-4 and FGF-21 indicating that distinct metabolic fingerprints could easily be distinguished among this population. The unique FLIM NAD(P)H metabolic fingerprints provide a novel measurement of adipose energetic status and could be potentially adapted for a quantitative, non-invasive technique for assessing adipose tissue maintenance of energy balance.

A Recombinant Subunit SARS-CoV-2 Vaccine Elicits Strong Antibody and Th1-dominant Cellular Immune Responses in Mice

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Hawaii COBRE P30GM114637 Chih-Yun Lai

Abstract

The recent emergence of the novel human coronavirus, SARS-CoV-2, which causes Coronavirus Disease 2019 (COVID-19) has threatened human health in more than 200 countries and territories. A safe and effective vaccine that will rapidly establish herd immunity is urgently needed to mitigate the ongoing pandemic.

We have previously established a recombinant subunit vaccine platform in which antigens are expressed in Drosophila S2 cells and purified by immunoaffinity chromatography and demonstrated that the filovirus protein antigens with an adjuvanted formulation (CoVaccine HT™) can achieve highly potent immunogenicity and protective efficacy in rodents and non-human primates. Currently, using the same biodefense vaccine platform, we have generated several variants of stabilized recombinant pre-fusion SARS-CoV-2 spike (S) protein and evaluated their immunogenicity in formulations adjuvanted with CoVaccine HT™ in Swiss Webster mice. Our results showed that two doses of recombinant pre-fusion S protein with CoVaccine HT™ elicits high anti-S IgG antibody titers as measured by a Luminex microsphere-based immunoassay. Interestingly, more rapid IgG responses were detected when animals were immunized with S protein in the absence of an adjuvant. Additionally, analysis of anti-S IgG subclass profile revealed that CoVaccine HT™ adjuvanted pre-fusion S protein induced both IgG2a/b and IgG1subclass antibodies, suggesting a balanced Th1/Th2 response whereas low IgG2a/b and high IgG1 seen in mice immunized with protein antigens without adjuvant. Finally, the results of FluoroSpot assay on mouse splenocytes demonstrated that CoVaccine HT™ adjuvanted S protein elicited a robust production of IFN-γ secreting cells, but very low levels of IL-4 secretion, indicating a Th1-dominant cellular immune response. Future studies will further evaluate the levels of serum neutralizing antibodies. Collectively, our findings support that stabilized recombinant pre-fusion S protein formulated with CoVaccine HT™ is a promising vaccine candidate against SARS-CoV-2 infection.

Impact of disrupted exocyst trafficking activity on cardiac metabolism and function

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Hawaii COBRE P20GM113134 Noemi Polgar

The heart metabolizes a variety of fuels to meet its energy requirements. Fatty acid (FA) metabolism generates 70-90% of the ATP in resting conditions, but under certain pathological conditions (i.e. ischemia), the myocardium meets its metabolic demands by increasing glycolysis. In cardiomyocytes, the GLUT4 glucose transporter and the CD36 fatty acid-translocase are responsible for induced glucose and high-affinity long-chain FA uptake, respectively. GLUT4 and CD36 membrane-delivery is triggered by insulin and contraction-induced AMPK signaling, sharing conserved downstream effectors. However, the mechanism of GLUT4 and CD36 membrane-delivery in cardiomyocytes remains poorly understood.

Studies of adipocytes, and our work in skeletal muscle demonstrated that the exocyst trafficking complex is critical for insulin-induced GLUT4 transport. The exocyst also regulates adipocyte FA uptake, and we demonstrated that the exocyst mediates insulin-induced CD36 trafficking in skeletal myoblasts. But it is not known if these mechanisms are conserved in cardiomyocytes, or if the exocyst also regulates cardiac substrate-uptake through differential regulation of GLUT4 and CD36 trafficking.

To determine the role of the exocyst in cardiac muscle, we generated tamoxifen-inducible cardiomyocyte-specific exocyst subunit EXOC5 knockout mice (Exoc5-CMKO). Exoc5 knockout decreases lifespan as Exoc5-CMKO mice die within 3 months of tamoxifen-induced gene deletion. Echocardiography demonstrated increased end-systolic left ventricle internal diameter and volume with a decreased left ventricle wall thickness, pointing to dilated cardiomyopathy. Exoc5-CMKO animals show heart failure with significantly decreased fractional shortening compared to controls. Histological analysis showed cardiac fibrosis in Exoc5-SMKOs, while RNA sequencing revealed disrupted beta oxidation and oxidative phosphorylation, suggesting decreased ATP production downstream of reduced substrate uptake. Ongoing work will further investigate the molecular mechanism of exocyst-mediated fuel uptake in cardiac muscle.

Focused-ultrasound and microbubbles for SARS-CoV-2 splenic vaccination

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Hawaii COBRE GM103341 (PI Shohet) and GM113134 (PI Gerschenson)

The recent emergence and rapid spread of a novel human coronavirus, SARS-CoV-2, which causes Coronavirus Disease 2019 (COVID-19) has threatened human health throughout the world. The development of minimally invasive, anatomically-targeted delivery allows us to evaluate the clinical potential of immunogen expression strategies in the spleen. Modern nucleic acid immunization strategies use viral vector systems to deliver transgenes with high efficiency, or direct injection of expression constructs into muscle or skin, augmented by other strategies that increase uptake of the vector. However, both strategies have important limitations, with the side effects of viral treatment and the poor transfection/expression levels of naked plasmids both limiting the success of nucleic acid vaccines.

Our lab studies a gene delivery approach known as Focused ultrasound targeted microbubble destruction (FUTMD) which can deliver expression constructs to sonographically accessible organs with very high anatomical precision and specificity. We are interested in testing the potential of using FUTMD for spleen-targeted vaccine delivery. The spleen contains a variety of immune cells and provides a cytokine-rich environment and thus can be a promising target for DNA vaccination. Spleen-targeted DNA delivery could allow direct transfection of antigen-presenting cells (APCs) and B cells, which are critical for antigen-specific immune responses.

In preliminary studies, we have used FUTMD to target the delivery of a DNA reporter construct to the mouse spleen. DNA-bound microbubbles were administered retro-orbitally and a high frequency linear array transducer was positioned over the spleen in the transverse plane to visualize the arrival of microbubble contrast agents. The focused ultrasound (FUS) transducer was then scanned over the lateral region of the abdomen guided by this imaging. *In vivo* bioluminescence imaging (IVIS) was used to visualize the position and depth of reporter expression and *ex vivo* imaging confirmed bioluminescence expression in the spleen. Additional experiments are needed to identify and evaluate the cellular distribution of FUTMD transfected spleen cells.

We are currently working on refining this approach to further enhance splenic DNA delivery by optimizing our FUTMD targeting parameters and evaluating transfection efficiency with bioluminescence and histological imaging assays. In future studies, we will collaborate with UH colleague Dr. Axel Lehrer, to use this method to evaluate the delivery and immune responses of expression constructs for a form of the SARS-CoV-2 Spike protein that has been used in previous successful immunization studies.

Nonionic detergent treatment as a novel inactivation method for inactivated virus vaccine

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Hawaii COBRE P30GM114737 Yanagihara

The four serotypes of dengue virus (DENV) cause the most important arboviral disease in humans. Currently the only FDA approved dengue vaccine is Dengvaxia®, a tetravalent live-attenuated dengue vaccine. However, recent studies reported an increased risk of severe disease during breakthrough DENV infection among DENV-naive recipients of this vaccine. Therefore, a safer and more effective dengue vaccine is needed. We developed a novel inactivation method with 1% nonionic detergent Tween 20 treatment, which was found to effectively inactivate DENV1 infectivity but maintain the most neutralizing epitopes comparing with formalin, UV and H_2O_2 inactivation assessed by capture ELISA with a panel of well-characterized human monoclonal antibodies (mAbs) including potent neutralizing domain III (DIII) mAbs, quaternary epitope mAbs and cross-reactive fusion loop (FL) mAbs. Sucrose gradient sedimentation analysis and proteinase K protection assay showed that Tween 20-inactivated DENV maintained membrane integrity. In studying the mechanism of Tween 20 inactivation, we found Tween 20 affected the steps involved in DENV entry including receptor binding and E protein conformational changes required for membrane fusion and also enhanced viral RNA degradation. Moreover, Tween 20-inactivated mature DENV1 particles adjuvanted with Alum induced high titers of neutralizing antibodies to 4 DENV serotypes in BALB/c mice. In competition ELISA, serial dilutions of mouse immune sera competed with the binding of human mAbs to virions. Analysis of IC50 titer revealed high IC50 titer to potent NT human mAbs (EDE1, DI/DII-hinge and DIII) but not FL mAb. Comparable binding to DENV1 wild type and FL-mutant (W101A, F108A) virus-like particles suggested that Tween 20-inactivated mature DENV1 induced undetectable FL antibodies, which have been shown to cause antibody-dependent enhancement. Taken together, these findings suggest Tween 20 for DENV inactivation can be a promising method for future vaccine manufacture.

This work was supported by grants R01AI110769 (Wang) and R21 AI135292-01A1 (Wang) from the National Institute of Allergy and Infectious Diseases, NIH and COBRE grant P30GM114737 (Yanagihara) from the National Institute of General Medical Sciences, NIH.

Nutrient and gut microbiota alter asocialness and repetitive circling in a psychiatric vertebrate model, the Mexican Cavefish.

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Hawaii COBRE P20GM125508 Margaret J Mc Fall-Ngai

In mammals, the gut microbiota is known to regulate brain functions, and its dysbiosis is frequently associated with psychiatric conditions, including autism. However, details of the molecular pathway of how the nutrients, beneficial microorganisms, and the host genes interact and tune the brain function is largely unknown. To address this knowledge gap, we are using *Astyanax mexicanus,* which is composed of cave-dwelling (cavefish) and the surface-dwelling morphs (surface fish). Cavefish show the significant overlaps with autism in behavioral conditions—asocialness, restricted repetitive behavior, imbalanced attention, insomnia, and hyperactivity. In contrast, surface fish show more normative behaviors such as shoaling. Furthermore, cavefish show the similarity in genetic conditions—cavefish exhibit the same directional gene-expression changes seen in the brains of autism patients (>58.5%); in contrast, other proxy systems (BTBR mouse, and iPS cell derived neural cells) have shown much less overlap (<11%). Lastly, the gut of adult cavefish showed dysbiosis: the significant depletion of firmicutes. To reveal the link among the host genes, gut microbiota and brain functions, here, we first address whether nutrient and antibiotic treatment can change asocialness and repetitive circling in cavefish. After the one-month treatment of the ketosis-inducing lipid-rich diet, cavefish significantly increased the social-like interaction. In contrast, the dietary treatment of the antibiotics did not significantly shift the means but increased the variation of the social-like activity in both cave and surface fish. This suggests that gut dysbiosis makes behavioral outputs unstable. We are currently analyzing and will share the results of the16S rRNA gene sequencing of the gut microbiota, and brain transcriptome.

Isolated Nuclei Stiffen in Response to Low Intensity Vibration

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Idaho COBRE P20GM109095 (COBRE) Julia Thom Oxford and P20GM103408 (INBRE)

The nucleus, central to all cellular activity, relies on both direct mechanical input and its molecular transducers to sense and respond to external stimuli. While it has been shown that isolated nuclei can adapt to applied force *ex vivo*, the mechanisms governing nuclear mechanoadaptation in response to physiologic forces *in vivo* remain unclear. To investigate nuclear mechanoadaptation in cells, we developed an atomic force microscopy (AFM) based procedure to probe live nuclei isolated from mesenchymal stem cells (MSCs) following the application of low intensity vibration (LIV) to determine whether nuclear stiffness increases as a result of LIV. Results indicated that isolated nuclei were, on average, 30% softer than nuclei tested within intact MSCs prior to LIV. When the nucleus was isolated following LIV (0.7g, 90Hz, 20min) applied four times (4x) separated by 1h intervals, stiffness of isolated nuclei increased 75% compared to non-LIV controls. LIV-induced nuclear stiffening required functional Linker of Nucleoskeleton and Cytoskeleton (LINC) complex but was not accompanied by increased levels of the nuclear envelope proteins LaminA/C or Sun-2. While depleting LaminA/C or Sun-1&2 resulted in either a 47% or 39% increased heterochromatin to nuclear area ratio in isolated nuclei, the heterochromatin to nuclear area ratio was decreased by 25% in LIV-treated nuclei compared to controls, indicating LIV-induced changes in the chromatin structure. Overall, our findings indicate that increased apparent cell stiffness in response to exogenous mechanical challenge of MSCs in the form of LIV is in part retained by increased nuclear stiffness and changes in chromatin structure. This study was funded, in part, by a COBRE in Matrix Biology Project Investigator Award (P20GM109095) and an Idaho INBRE Developmental Research Pilot Project Grant (P20GM103408). IDeA support directly resulted in current R01 funding from the National Institute on Aging (1R01AG059923-01A1).

An NIGMS-funded career: investigating the blood-brain barrier at each step from an INBRE Graduate Fellow to a Research Project Grant awardee

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Idaho INBRE Program P20GM103408 and COBRE in Matrix Biology P20GM109095

Blood-brain barrier (BBB) dysfunction is a key pathologic component of several inflammation-associated diseases, such as multiple sclerosis, traumatic brain injury, and viral encephalitis. My research interests are focused on elucidating the mechanisms for BBB dysfunction under various neuroinflammatory conditions. More specifically, we are determining the signaling cascades that control endothelial cell-cell tight junction (TJ) complexes during homeostasis and excessive inflammation. I have researched this topic for 10 years, as it has been a common theme throughout my graduate studies, postdoctoral training, and career thus far, all of which were supported by IDeA funding. This career trajectory began with my doctoral training under the mentorship of an INBRE-funded (P20RR016454; current designation P20GM103408) investigator at Idaho State University. This opportunity allowed me to gain vital experience in vascular biology and neuroscience, while studying the role of endothelial glutamate receptors in homocysteine-induced BBB dysfunction. This led to a postdoctoral position at the University of South Florida, where I investigated the role of a signaling cascade that regulates endothelial barrier dysfunction during Systemic Inflammatory Response Syndrome. This postdoctoral position was funded in part through an NIGMS Research Project Grant (R01GM097270) awarded to my mentor. Following my faculty appointment at Boise State University, I was funded through the COBRE in Matrix Biology - Junior Investigator Program (P20GM109095) and an INBRE supplement (P20GM103408-20S2). These funding mechanisms provided me with the resources to establish my lab, develop important collaborations, recruit a competent investigative team, generate significant preliminary data, and develop multiple transgenic mouse models. This trajectory of IDeA-funding resulted in my own R01 award from the National Institute for Neurologic Disease and Stroke (R01NS110934). This research project will investigate the role of two small leucine-rich proteoglycans (SLRP) decorin and biglycan, which have been observed at abnormally high levels in the perivascular matrix of patients with multiple sclerosis.

Plasma Metabolomic Profiling to Identify Metabolic Markers of Methotrexate Response in Juvenile Idiopathic Arthritis

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Kansas COBRE P20 GM130423 Andrew Godwin

Methotrexate (MTX) remains the cornerstone of therapy in juvenile idiopathic arthritis (JIA). However, response to therapy remains variable and unpredictable resulting in the need to identify biomarkers to guide drug therapy in JIA. In this study a global metabolomics strategy is utilized to identify biochemical pathways and metabolomic markers associated with MTX response in the treatment of JIA.

Plasma samples from 30 JIA patients were included in the analysis. Response to MTX was determined based on the ACR Pedi 70 response criteria and non-response was determined by failing to meet ACR Pedi 30 response criteria. Plasma metabolomic profiles were compared immediately prior to the initiation of MTX and after 3-months of MTX therapy. A global metabolomics approach capable of detecting over 800 known metabolites was conducted using three independent metabolomic profiling platforms at the NIH West Coast Metabolomics Center at UC-Davis. The resulting metabolomic data was evaluated by univariate and multivariate analysis using MetaboAnalyst 3.0. Enrichment analyses were conducted using ChemRICH to identify metabolic pathways associated with MTX activity.

Among identified metabolites detected (n=673), 15 were found to be significantly altered following the initiation of MTX. Altered metabolites included those associated with environmental exposure (e.g. isobutylamine), gut microbial metabolism (e.g. dehydrocholic acid), B vitamin metabolism (e.g. biotin) and purine metabolism (i.e. adenine). Chemical enrichment analysis supported significant changes in triglycerides, fatty acids, and pyridines following the initiation of MTX. Of the metabolites found to be significantly altered by MTX therapy, only reductions in plasma dehydrocholic acid and biotin levels were found to be associated with the achievement of ACR Pedi 70 response criteria at 3-months.

This work demonstrates that initiation of MTX therapy is associated with broad alterations in the plasma metabolome of patients with JIA and identifies dehydrocholic acid and biotin as potential biomarker of MTX response in JIA.

A Clinical Phase IIa Study Examining the Role of MAPKAPK-2 (MK2) Pathway Inhibition in Patients with Moderate-severe COVID-19 Infection

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Abstract

A hyperinflammatory state caused by excessive inflammatory cytokine production (*e.g.,* TNF α, IL-1β, IL6, IL-8; cytokine release syndrome (CRS)) is a feature of pathobiology in coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 is associated with hypoxic respiratory failure, lung fibrosis and mortality. MK2 pathway activation has been shown to regulate the same inflammatory cytokines implicated in CRS. *We hypothesize MK2 blockade will attenuate inflammatory cytokines and improve respiratory failure-free survival in moderate-severe* COVID-19. This study builds jointly from two Kansas Institute for Precision Medicine (KIPM) COBRE (NIGMS P20 GM130423)-funded investigators who study inflammatory conditions associated with head and neck cancer and cystic fibrosis, respectively.

We designed an investigator-initiated trial (IND#:149790) of oral MK2 inhibition (ATI-450, Aclaris Pharmaceuticals) in COVID-19. This is a Phase IIa, double-blinded, randomized placebo-controlled proof-of-concept study. SARS-CoV-2 positive hospitalized patients with moderate-severe COVID-19 pneumonia are randomized to oral ATI-450 50mg, or placebo, twice-daily for up to a 2-week period. The primary endpoint of this trial is respiratory failure-free survival at 14 days. Secondary endpoints include clinical endpoints such as change in World Health Organization ordinal scale and circulating inflammatory cytokine levels. ATI-450 safety is also assessed in this acutely ill population. In collaboration with KIPM we are pursuing exploratory translational science endpoints. Because MAPK-pathway activation is linked with SARS-CoV-2 replication, we are examining the impact of MK2-pathway blockade on SARS-CoV-2 viral titers. Given the incomplete understanding of MK2 inhibition on immune cell function, we will characterize the effects of COVID-19 with and without ATI-450 on immune cells via immunophenotyping and 10X Genomics single-cell gene expression analysis. We surmise that myeloid cell activation following SARS-CoV-2 infection contributes to localized and systemic tissue injury and will examine the effect of MK2 pathway blockade on eliciting myeloid cell inflammatory activation-suppression.

Therapeutic potentials of DNA aptamer-based designer DNA nanostructures in COVID-19

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Louisiana COBRE P20GM130555 Samithamby Jeyaseelan

COVID-19, caused by the SARS-CoV-2, has resulted in pandemics that cause severe illness, death and economic crisis. However, there remains no effective therapeutics. In order to develop innovative, affordable, biocompatible clinical antiviral candidates to prevent viral infection and transmission, we exploited the structural characteristics of viral surface proteins that can be matched at nanoscale precision by engineered DNA nanostructure platforms. We have recently designed and synthesized a star-shaped DNA architecture to display 10 dengue viral envelope protein-targeting DNA aptamers into a 2D pattern precisely mirroring the complex spatial arrangement of dengue virus (DENV) epitopes, which resulted in highly potent inhibition of DENV entry in human blood. For SARS-CoV-2, the structure of the trimeric spike protein clusters has been solved, and based on the structural information, we have synthesized a designer DNA nanostructure (DDN) that takes the form of a macromolecular “net” whose vertices are a precise mechanical match to the spacing and positioning of the spike protein matrix displayed on the virus outer surface. We have also screened and found DNA aptamers that are specific for spike receptor binding domain (RBD). The DNA aptamers were incorporated into the ‘knots’ of the DDN net to allow simultaneous binding of multiple DNA aptamers to multiple spikes on the viral surface, in a polyvalent, pattern-matching fashion. The DNA “net”-aptamer construct has afforded dramatic increase in SARS-CoV-2 binding avidity by > 10^5 folds (monovalent aptamer KD: 23 mM; DNA net KD:100 pM). As with DENV, our polyvalent SARS-CoV-2 specific DDN is can work as a decoy, to block virus-host cell interaction, thereby preventing infection and transmission. Cost of treatment with our anti-DENV DNA Nano-complex is ~$10/dose, which could be lower if synthesized in larger scale. Our current data suggest that DDN can be a highly cost-effective antiviral therapy against COVID-19.

Tristetraprolin overexpression in non-hematopoietic cells protects against acute lung injury in mice

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Louisiana COBRE P20GM130555-01 Samithamby Jeyaseelan

Tristetraprolin (TTP) is a mRNA binding protein that binds to adenylateuridylate-rich elements within the 3' untranslated regions of certain transcripts, such as tumor necrosis factor (*Tnf*), and increases their rate of decay. Modulation of TTP expression is implicated in inflammation; however, its role in acute lung inflammation remains unknown. Accordingly, we tested the role of TTP in lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. LPSchallenged TTP-knockout (TTPKO) as well as myeloid cell-specific TTP-deficient (TTPmyeKO) mice exhibited significant increase in lung injury, albeit these responses were more robust in the TTPKO. Mice with systemic overexpression of TTP (TTPΔARE) were protected from ALI, as indicated by significantly reduced neutrophilic infiltration, reduced levels of neutrophil chemoattractants, and histological parameters of ALI. Interestingly, while irradiated WT mice reconstituted with TTPKO hematopoietic progenitor cells (HPCs) showed exaggerated ALI, their reconstitution with the TTPΔARE HPCs mitigated ALI. The reconstitution of irradiated TTPΔARE mice with HPCs from either WT or TTPΔARE donors conferred significant protection against ALI. In contrast, irradiated TTPΔARE mice reconstituted with TTPKO HPCs had exaggerated ALI, but the response was milder as compared to WT recipient that received TTPKO HPCs. Finally, the reconstitution of irradiated TTPKO recipient mice with TTPΔARE HPCs did not confer any protection to the TTPKO mice. These data together suggest that non-HPCs-specific overexpression of TTP within the lungs protects against ALI via downregulation of neutrophil chemoattractants and reduction in neutrophilic infiltration. Our studies could also have implications for the lung hyper-inflammation and potentially life-threatening cytokine storms in the severe coronavirus disease (COVID-19).

ITK regulates IL-10 production by $CD8^+$ T cells and lung immunopathology during influenza infection

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Louisiana COBRE P20 GM130555 Samithamby Jeyaseelan

Influenza (flu) infections cause 250,000 deaths and 3-5 million cases of severe illness during the average flu season. Severe influenza infections are associated with a combination of strong pro-inflammatory and weak anti-inflammatory immune responses. Production of the anti-inflammatory cytokine IL-10 by T cells restricts immunopathology during flu infections, however our knowledge of the signaling pathways regulating IL-10 induction is limited. Using $IL\text{-}10^{GFP}$ reporter mouse models, we found that Interleukin-2 inducible T cell kinase (ITK), a critical component in T cell receptor (TCR) signaling, regulates the development of IL-10-producing $CD8^+$ T cells during influenza A infection. Compared to wild type (WT) mice, $Itk^{-/-}$ mice displayed increased morbidity and mortality after influenza infection, accompanied by a significant reduction of IL-10 producing $CD8^+$ T cells in the airways. Using the model antigen ovalbumin (OVA) and transgenic TCR specific for OVA in $CD8^+$ T cells (OTI), along with an allele sensitive mutation in the ITK kinase domain, we determine that ITK regulates IL-10 production in antigen-specific $CD8^+$ T cells in a kinase dependent manner. Multiparametric flow cytometric analyses revealed that ITK differentially regulates the expression of cell surface markers and transcription factors that are involved in regulating T cell differentiation, effector and memory phenotypes. Following drug candidate screening, we also discover that RAS/MAPK and PI3K signaling pathways are critical in IL-10 production in effector-like $CD8^+$ T cells. Together, our data suggests that ITK is a critical regulator of IL-10 production by $CD8^+$ T cells and regulate immunopathology during influenza infection. Modulating ITK signaling may be a strategy for regulating immunopathology due to viral infections.

Loss of gut mucosal Th17-type immune cell functions is associated with persistent inflammation of aging and chronic treated HIV infection in the rhesus macaque model

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Louisiana COBRE P20GM103629 S. Michal Jazwinski

A chronic low-grade inflammatory status in aging individuals, termed 'inflammaging', has been linked to changes in innate and adaptive immune functions and mucosal barrier mechanisms. This process is reflected in an increase in biomarkers of intestinal epithelial barrier damage (IEBD) and microbial translocation (MT) associated with higher plasma levels of pro-inflammatory cytokines such as IL-6, TNF-α, GM-CSF, etc. and dysbiosis in older individuals. Utilizing the nonhuman primate model of rhesus macaques, the goal of this study is to examine the role of dysregulated gut barrier functions in the sterile low-grade inflammation of aging as well as the persistent inflammation of chronic treated HIV infection. The results demonstrate a similar inflammaging phenotype in aging rhesus macaques with systemic inflammation associated with IEBD/MT biomarkers, and a significant loss of gut mucosal Th17-type immune cell functions. Further, an inflammaging phenotype developed in chronic treated SIV infected macaques (modeling treated HIV infection) following a significant loss in intestinal Th17-type cytokine effector functions. These data indicate that dysregulated gut mucosal Th17-type immune functions contribute to inflammaging phenotype during aging and in chronic SIV infection. These results reveal intricate interactions between mucosal immune function and systemic inflammation, thus providing insights into mechanisms of mucosal immune dysregulation during persistent inflammation of aging and chronic HIV/SIV infection that will inform development of novel therapeutic strategies against inflammaging.

Myeloid-IL4Ra is an indispensable link in IL33-ILCs-IL4Ra axis of eosinophil recruitment in developing mouse lungs.

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Louisiana COBRE P20 GM130555 Samithamby Jeyaseelan

Abstract

Increased eosinophil recruitment is a hallmark feature of eosinophilic disorders. Here, we delineated key molecular and cellular players involved in eosinophilic recruitment during normal postnatal lung development in mice. Physiological eosinophilic recruitment was consistently present in 7, 10, and 15-day-old neonatal mice, but not in adult mice. This feature was completely abolished in interleukin 33 (IL-33)-, interleukin 2 receptor gamma chain (IL2rg)-, and interleukin 4 receptor alpha (IL4Ra)-knockout mice, but not in recombination activating gene 1 (Rag1)-knockout mice demonstrating an indispensable role for IL-33, innate lymphoid cells (ILCs), and IL4Ra in eosinophil recruitment. Interestingly, myeloid-specific IL4Ra deficient (mye-IL4Ra-/-) mice had significantly reduced eosinophils and IL-4 and IL-5 in the airspaces. Eosinophil recruitment was also absent in the airspaces of IL-13-treated mye-IL4Ra-/- mice but was restored upon administration with eosinophil chemoattractants cocktail (IL-5/Eotaxin). These data establish that myeloid-IL4Ra is an indispensable component of IL-33-ILC-IL4Ra-IL-4/13 mediated-eosinophil recruitment.

Direct RT-qPCR Detection of SARS-CoV-2 RNA from Patient Nasopharyngeal Swabs Without an RNA Extraction Step

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Abstract

The ongoing COVID-19 pandemic has caused an unprecedented need for rapid diagnostic testing. The World Health Organization (WHO) recommends a standard assay that includes an RNA extraction step from a nasopharyngeal (NP) swab followed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to detect the purified SARS-CoV-2 RNA. The current global shortage of RNA extraction kits has caused a severe bottleneck to COVID-19 testing. The goal of this study was to determine whether SARS-CoV-2 RNA could be detected from NP samples via a direct RT-qPCR assay that omits the RNA extraction step altogether. The direct RT-qPCR approach correctly identified 92% of a reference set of blinded NP samples (n = 155) demonstrated to be positive for SARS-CoV-2 RNA by traditional clinical diagnostic RT-qPCR that included an RNA extraction. Importantly, the direct method had sufficient sensitivity to reliably detect those patients with viral loads that correlate with the presence of infectious virus. Thus, this strategy has the potential to ease supply chokepoints to substantially expand COVID-19 testing and screening capacity and should be applicable throughout the world.

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Mississippi COBRE P30GM122733 Soumyajit Majumdar

The COBRE Phase III Transitional Center at The University of Mississippi facilitates the study of the interface between natural products and neuroscience (CORE-NPN). Natural products are small molecules derived from several sources including plants, microbes, and aquatic organisms. Some of the most powerful compounds that affect brain and nervous system function are natural products. The Phase III program consists of two cores, the Chemistry/DMPK (CDMPK) Research Core and the Neuropharmacology (NPC) Research Core. The CDMPK core supports lead optimization, analytical expertise, drug metabolism (DM) and pharmacokinetics (PK) and pharmacodynamics (PD), formulation development and evaluation of natural products and related compounds. The CDMPK Core also provides an advisory role for the CORE-NPN investigators that require expertise in the area of exploration of SAR and MAR, analytical method development and validation, chemical modifications to influence PK properties, and pharmaceutical and chemical influences on solubility and formulation, and PK/PD analysis. The NPC Core has established itself as an essential resource for pharmacological screening. The Core has developed a robust array of in vitro and in vivo bioassays providing opioid and cannabinoid screenings in an academic setting. In addition, the availability of Neuropeptide FF receptor screens in academia is limited to our Core facility. The ability to further advance findings from in vitro paradigms directly into rodent/zebrafish behavioral assays is another significant advantage of our program. The close connection of Core scientists facilitates collaboration and sharing of information in a rapid and efficient manner. The in vivo component of the Neuropharmacology Core supports the CDMPK core by performing drug administration and tissue collection for their PK/PD studies, if needed. Thus, the CORE-NPN program supports the transition and development of compounds and products through preclinical studies.

Leveraging predatory awareness of prey signals and physiological features for activation of cryptic biosynthetic space

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Mississippi COBRE P20GM130460 Joshua S. Sharp

As with Actinobacteria and other “gifted” producers of specialized metabolites, myxobacterial genomes are replete with biosynthetic gene clusters (BGCs). The potential metabolites produced by the vast majority of pathways observed in genome data from sequenced myxobacteria remain unknown. Provided the generalist predatory lifestyle of myxobacteria, the utilization of prey quorum signaling metabolites (R15AI137996) and prey membrane features such as lipopolysaccharide (LPS) (P20GM130460) might activate this untapped chemical space and motivate production of novel antimicrobial metabolites with. Using untargeted mass spectrometry and RNA sequencing, we compare the impact of 2 distinct classes of signaling molecules acylhomoserine lactones and quinolone signals as well as isolated LPS from *Pseudomonas putida* on the metabolism of 2 predatory myxobacteria. From these data, we determine that each class of chemical signals elicits a differential response for both myxobacteria. However the development model organism, *Myxococcus xanthus* responds minimally when compared to the more recently isolated *Cystobacter ferrugineus*. We also observed significant overlap in the metabolic response induced by AHLs C6-AHL and 3-oxo-C6-AHL from both myxobacteria and determine that the S-(-)-isomer of the core homoserine lactone moiety elicits a similar response. Utilizing comparative metabolomics, we also determined that acylhomoserine exposure inhibits production of a newly discovered nonribosomal peptide. From comparative transcriptomic data, we observe that both exogenous quorum signals impact the transcription of 22 genes within 20 BGCs. Of these BGC-associated genes, a total of 5 are annotated as regulatory transcription factors and another 3 are annotated as transport related membrane features. Ultimately, we conclude that leveraging predatory awareness of prey provides the opportunity to not only discovery novel specialized metabolites but also explore the sensory and regulatory features associated with the specialized metabolism of predatory myxobacteria.

Effective Inhibition of SARS-CoV-2 Entry by Heparin and Enoxaparin Derivatives

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Mississippi COBRE P20GM130460 Joshua S. Sharp

Abstract

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) has caused a pandemic of historic proportions and continues to spread globally, with enormous consequences to human health. Currently there is no vaccine, effective therapeutic or prophylactic. Like other betacoronaviruses, attachment and entry of SARS-CoV-2 is mediated by the spike glycoprotein (SGP). In addition to its well-documented interaction with its receptor, human angiotensin converting enzyme 2 (hACE2), SGP has been found to bind to glycosaminoglycans like heparan sulfate, which is found on the surface of virtually all mammalian cells. Here, we pseudotyped SARS-CoV-2 SGP on a third generation lentiviral (pLV) vector and tested the impact of various sulfated polysaccharides on transduction efficiency in mammalian cells. The pLV vector pseudotyped SGP efficiently and produced high titers on HEK293T cells. Various sulfated polysaccharides potently neutralized pLV-S pseudotyped virus with clear structure-based differences in anti-viral activity and affinity to SGP. Concentration-response curves showed that pLV-S particles were efficiently neutralized by a range of concentrations of unfractionated heparin (UFH), enoxaparin, 6-*O*-desulfated UFH and 6-*O*-desulfated enoxaparin with an IC_{50} of 5.99 µg/L, 1.08 mg/L, 1.77 µg/L, and 5.86 mg/L respectively. Further characterization of UFH - SGP binding is underway using fast photochemical oxidation of protein (FPOP) method at GlyCORE at the University of Mississippi. The low serum bioavailability of intranasally administered UFH, along with data suggesting that the nasal epithelium is a portal for initial infection and transmission, suggest that intranasal administration of UFH may be an effective and safe prophylactic treatment.

The Delayed Effect of Wildfire Season Particulate Matter on Subsequent Influenza Season in a Mountain West Region of the USA

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Montana COBRE P20130418 Curtis Noonan

Abstract

Background: Particularly in rural settings, there has been little research regarding the health impacts of fine particulate matter (PM2.5) during the wildfire season smoke exposure period on respiratory diseases, such as influenza, and their associated outbreaks months later.

Methods: Our study published in June in Environment International examined the delayed effects of PM2.5 concentrations for the short-lag (1–4 weeks prior) and the long-lag (during the prior wildfire season months) on the following winter influenza season in Montana. We created gridded maps of surface PM2.5 for the state of Montana from 2009 to 2018 using spatial regression models fit with station observations and Moderate Resolution Imaging Spectroradiometer (MODIS) aerosol optical thickness data. We used a seasonal quasi-Poisson model with generalized estimating equations to estimate weekly, county-specific, influenza counts for Montana, associated with delayed PM2.5 concentration periods (short-lag and long-lag effects), adjusted for temperature and seasonal trend.

Results: We did not detect an acute, short-lag PM2.5 effect nor short-lag temperature effect on influenza in Montana. Higher daily average PM2.5 concentrations during the wildfire season was positively associated with increased influenza in the following winter influenza season (expected 16% or 22% increase in influenza rate per 1 $\mu g/m^3$ increase in average daily summer PM2.5 based on two analyses, $p = 0.04$ or 0.008).

Discussion: This is one of the first observations of a relationship between PM2.5 during wildfire season and influenza months later. We are currently investigating this long-lag effect of wildfire exposures for other respiratory infection outcomes.

Undervaccination Patterns and Barriers to Early Childhood Vaccination in a Large, Rural U.S. State

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Abstract

Background: Early childhood vaccination rates are lower in rural versus urban areas of the U.S. Structural barriers, such as a lack of systems for reminding parents when vaccines are due, may contribute to this disparity, as could vaccine hesitancy, which has been understudied in rural areas. Our objective was to estimate the prevalence of patterns of undervaccination consistent with structural barriers or vaccine hesitancy among children ages 0-24 months in Montana.

Methods: We analyzed records from Montana's centralized state immunization information system for children born 2015-2017. We calculated timeliness of vaccine doses from seven vaccine series recommended by the Advisory Committee on Immunization Practices (ACIP). We identified undervaccination patterns previously shown to be consistent with certain barriers to vaccination. Using multivariable log-linked binomial regression, we identified factors associated with not completing the combined 7-vaccine series by age 24 months.

Results: Among n=31,422 children, 33.5% received all recommended vaccine doses on-time, 32.8% received all doses but some or all were late, and 33.7% had not completed the combined 7-vaccine series. Approximately 18.8% of children had an undervaccination pattern indicative of parental vaccine hesitancy, including vaccines spread out over visits ("shot-limiting") and starting some but not all series ("selective" vaccination). An additional 4,967 children (15.8%) had started the seven series but were missing doses. While falling behind on vaccines at all ages was associated with failing to complete the combined 7-vaccine series by age 24 months, being late at ages 12-15 months had the strongest association (adjusted prevalence ratio: 3.4, 95% CI: 3.2-3.6, vs. children with zero days undervaccinated at ages 12-15 months).

Discussion: Only a third of Montana's children received vaccines on-time per the ACIP-recommended schedule. Our results suggest that future work should include initiatives to increase parental vaccine confidence and implementing reminder/recall programs to prompt parents to complete vaccine series.

A Role for COP9 Signalosome Component CSN-5 in Stabilizing Stem Cell Regulators FBF-1 and FBF-2.

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Montana COBRE P20GM103546 Bruce Bowler

Germline stem cell maintenance in *C. elegans* is supported by PUF family RNA-binding proteins FBF-1 and FBF-2; however, the mechanisms regulating FBF protein levels in germ cells have not been explored. We identified an interaction between both FBFs and CSN-5, a subunit of COP9 (constitutive photomorphogenesis 9) signalosome. COP9 is a highly conserved complex that can affect protein stability through a range of mechanisms including deneddylation, deubiquitination, and phosphorylation (Wolf et al., 2003). Our preliminary results suggest that the metalloprotease domain of CSN-5 binds to the RNA-binding domains of FBF-1 and FBF-2 at physiologically relevant (micromolar) concentrations. We found that CSN-5 promotes the accumulation of FBF-1 and FBF-2 proteins in *C. elegans* stem and progenitor cells, therefore it might support germline stem cell maintenance. Quantitative analysis of *fbf-1* and *fbf-2* RNA levels in *csn-5* mutant worms suggests that CSN-5 affects FBF levels post-translationally. Excitingly, protein interaction assays show that human homologs PUM1 and CSN5 interact as well, thus identifying a protein complex that is evolutionarily conserved. Analysis of the interaction between CSN-5 and the FBF proteins will elucidate how assembly of this protein complex is mediated and provide tools to further test our hypothesis that FBF/CSN-5 interaction contributes to the maintenance of germline stem cells.

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Sequence database search is fundamental to modern molecular biology – it allows one sequence to be annotated based on detected similarity to other known sequences. Annotation of metagenomics and metatranscriptomics datasets is incomplete (many observed sequences can't be identified) and rate limited by computational analysis. These conflicting needs for maximal sensitivity and high speed are addressed by the work we describe here.

Profile hidden Markov models (pHMMs) represent an important advance in terms of sensitivity to remote sequence relationships, but their relatively slow speed has limited their application to large annotation projects. Here, we describe two complementary approaches intended to substantially accelerate sequence annotation with pHMMs. We first describe a new, highly optimized, open-source implementation of a string-indexing data structure called the FM-index (10-50x faster than currently-available libraries), and demonstrate that it can be used to substantially reduce the run time for one bottleneck stage of annotation with pHMMs. We then describe a novel approach to constraining the search space, and corresponding run time and memory requirements, of the second bottleneck in pHMM annotation, improving run time for this stage by more than 20x. In sum, these approaches will enable much faster highly sensitive sequence annotation with pHMMs on commodity computer hardware.

Attitudes and Psychological Factors Associated with News Monitoring, Social Distancing, Disinfecting, and Hoarding Behaviors Among US Adolescents During the COVID-19 Pandemic

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Montana COBRE P20GM104417 Aleexandra K. Adams

As COVID-19 spreads across the world, it is critical to understand the psychological factors associated with pandemic-related behaviors. This may be especially important to study among youth, who are less likely to experience severe symptoms but contribute to the spread of the virus. The goal of this study was to examine psychological factors associated with adolescents' behaviors during the COVID-19 pandemic.

Participants were 770 adolescents ages 13-18 (M_{age}=16.34, 75% female) recruited via social media to complete an anonymous survey. Outcomes included COVID-19 news monitoring, social distancing, disinfecting, and hoarding behaviors the 7 days after the US declared a national emergency. The psychological factors were attitudes about COVID-19 severity, social responsibility values, social trust, and self-interest. The *a priori* hypotheses were that attitudes about the severity of COVID-19, greater social responsibility, and greater social trust would be associated with greater news monitoring, social distancing, and disinfecting, whereas greater self-interest would be associated with more hoarding.

Results indicate that many teens reported not engaging in pure social distancing (69%; *n*=528), but were monitoring the news (89%; *n*=688) and disinfecting daily (88%; *n*=676). Some teens reported hoarding (20%; *n*=152). Attitudes that COVID-19 were more severe were associated with more social distancing (*β*=.18; 95%CI=.10,.25), disinfecting (*β*=.16; 95%CI=.08,.23), and news monitoring (*β*=.26; 95%CI=.18,.33), but also more hoarding (*β*=.08; 95%CI=.01,.16). Greater social responsibility was associated with more disinfecting (*β*=.24; 95%CI=.17,.32) and news monitoring (*β*=.14; 95%CI=.07,.22), but less hoarding (*β*=-.07; 95%CI=-.14,-.01). Greater self-interest values were associated with less social distancing (*β*=-.08; 95%CI=-.15,-.01) and more hoarding (*β*=.08; 95%CI=.01,.15). Greater social trust was associated with less hoarding (*β*=-.09; 95%CI=-.16,-.02). Emphasizing the severity of COVID-19 and the social implications of pandemic-related behaviors may be important for teens, particularly for those who are not following preventative health behaviors or who are engaging in hoarding.

Development of a novel therapy for Alzheimer's Disease

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Nevada COBRE P20 GM121325 Martin Schiller

Abstract

Alzheimer’s disease is the world’s leading cause of dementia and the most prevalent neurodegenerative disease. Its major pathological features are amyloid beta (Aβ) plaques, Tau tangles, and neuroinflammation. In the brain, Aβ is primarily removed by immunocompetent cells called microglia through phagocytosis. This process is mediated by pattern recognition receptors including receptor for advanced glycation end products (RAGE) that results to the release of inflammatory factors. Previously, our lab has identified Tubby protein that facilitates the phagocytosis of cellular debris in the retina through Mer Tyrosine Kinase (MerTK) receptor. In contrast to RAGE, phagocytosis through MerTK is considered silent because it does not result to an inflammatory response. To divert the clearance of Aβ from inflammatory RAGE to the noninflammatory MerTK pathway, we created a novel hybrid protein containing the minimal phagocytic domain of Tubby that can recognize MerTK and the Aβ binding peptide that can specifically bind to Aβ. We have shown that our hybrid protein facilitated robust uptake and degradation of Aβ in microglial cells through MerTK receptor. This MerTK-mediated phagocytosis of Aβ led to a reduction in the levels of inflammatory factors and oxidative products resulting to increased cell viability.

Genomic Surveillance of SARS-CoV-2 Through Wastewater and Human Diagnostic Samples

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Nevada COBRE P20GM121325 Martin Schiller

The World Health Organization classified COVID-19 as a global pandemic in March, 2020. Although SARS-CoV-2, the virus responsible for COVID-19, is primarily respiratory in nature, multiple studies confirmed its genetic material can be detected in the feces of infected individuals, suggesting that sewage can be used to surveil community infection rates. Here we investigate the genomic epidemiology and disease emergence of SARS-CoV-2 in a dynamic and urban landscape such as the Las Vegas Valley, a major metropolitan area of >2 million residents and with > 42.5 million tourists each year. Using wastewater and patient samples from public health labs in Southern Nevada, we analyzed the cryptic introduction of viral lineages over the span of six months from March, 2020. In addition, enhanced purification methods and new qPCR probes enabled consistent isolation and quantification of SARS-CoV-2 from wastewater samples. The analysis of novel viral genomes resulted in a robust bioinformatic pipeline and dashboard to document and disseminate information to the public about the evolution of SARS-CoV-2 and emerging variants. Our data suggest that the genomic approach to track the spread of an SARS-CoV-2 across discrete geographic locations and time is both facile and scalable and can be utilized to inform public health decisions and stem interstate dissemination of infectious diseases.

Role of UBR5 Mutations in Mantle Cell Lymphoma

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Nebraska COBRE 1P20GM121316-01A1 Robert Lewis

Mantle cell lymphoma (MCL) is a rare and aggressive non-Hodgkin's lymphoma. Unfortunately limited therapies for MCL are currently available suggesting a need to further unravel molecular mechanisms regulating transformation and progression of the disease. The majority of MCL patients have mutations leading to overexpression of CyclinD1 leading to extensive proliferation and blocks in differentiation originating in the mantle zone of the lymph node, however additional mutations are necessary for transformation. Recently next generation sequencing has identified a number of new novel mutations in MCL patients including the ubiquitin E3 ligase UBR5. E3 ubiquitin ligases serve as the substrate-recognizing component for protein degradation by the ubiquitin proteasome system. Approximately 18% of MCL patients were found to have mutations in UBR5 and more than half are found within the HECT domain of UBR5, which can accept and transfer ubiquitin molecules to the substrate. In order to understand the role of UBR5 HECT domain in B-lymphoid development we generated a conditional mouse using novel CRISPR/Cas 9 technology. Loss of the HECT domain leads to a block in pre-germinal center B cells in the spleen with a reduction of both B1 and marginal B cell subsets. In addition, follicular B cells in the spleen are phenotypically abnormal and functionally impaired. Proteomic studies reveal up-regulation of proteins associated with mRNA splicing via the spliceosome in B cells lacking the HECT domain of UBR5. These studies suggest that understanding molecular mechanism of UBR5 mutations could provide potential therapeutic targets in MCL.

Regulation of SPRTN-mediated DNA-Protein Crosslink Repair Pathway

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DNA-protein crosslinks (DPCs) are toxic DNA lesions that interfere with DNA metabolic processes such as replication, transcription and recombination. SPRTN is a replication-coupled DNA-dependent metalloprotease that cleaves proteins crosslinked to DNA to promote DPC repair. In addition to DPC repair, SPRTN mediates restart of stalled DNA forks, regulates DNA replication and translesion DNA synthesis. Bi-allelic mutations in SPRTN cause RJALS syndrome characterized by genome instability, early-onset hepatocellular carcinoma and premature aging.

Strict regulation of SPRTN protease activity and function is critical to prevent aberrant proteolysis of DNA binding proteins by SPRTN during DNA replication and premature SPRTN auto-cleavage during DPC repair. SPRTN function is tightly regulated by a monoubiquitin switch that controls SPRTN chromatin accessibility and function during DPC repair. We have identified USP11 as a SPRTN deubiquitinase. USP11 interacts with SPRTN and deubiquitinates monoubiquitinated SPRTN in cells and in vitro. USP11 depletion impairs SPRTN deubiquitination in response to formaldehyde-induced DPCs. Loss of USP11 causes an accumulation of unrepaired DPCs and cellular hypersensitivity to treatment with DPC-inducing agents. Our findings elucidate the function of USP11 in the regulation of SPRTN monoubiquitination and SPRTN-mediated DPC repair to maintain genome stability.

Investigating the regulation of SPRTN protease and SPRTN-mediated DPC repair pathway will further our understanding of the DPC repair pathway, identify novel players involved in DPC repair, delineate the mechanism underlying RJALS syndrome, and help develop novel strategies for sensitizing cancer cells to chemotherapy by targeting SPRTN-mediated DPC repair pathway.

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Nebraska COBRE 5P20GM109023-07 Lori Leibold

Funded by the Administrative Core of the Center for Perception and Communication in Children (COBRE grant 5P20GM109023-07), this project developed a web-based application called RAD (Research Administration Database) which consolidates the Boys Town National Research Hospital's (BTNRH) research administration data and data registry for recruitment of human subjects into a single, user-friendly portal.

Project aims were: 1) To eliminate redundancies in data input and storage, 2) To add functionality for researchers, lab staff, and administrative staff who regularly access BTNRH research data, 3) To improve data security with a unified SQL Server database design and Active Directory for managing user authorization and access protocols, and 4) To enable faster implementation of future updates.

RAD is comprised of 3 modules. The first consolidates grant, protocol, and personnel information previously stored in multiple workbooks and databases. The second contains a volunteer registry of over 10,000 individuals who've agreed to be contacted by BTNRH for participation in current or upcoming studies. This registry contains demographic and contact information, medical history and diagnoses, information on hearing, language, and cognitive disorders, plus detailed audiogram and clinical assessment data. The third module is used by staff from BTNRH's 20+ labs to search the registry to identify and contact individuals eligible for studies based on criteria such as age, hearing status, or diagnoses.

RAD's launch in August 2019 has led to more efficient grant management processes and reporting. Re-designs to the volunteer registry have encouraged collaboration between labs and the addition of 50+ fields to the registry. As a result, a broader range of more current and relevant information is available when recruiting study participants. RAD's web-based interface has also made remote access and maintenance considerably easier during the COVID-19 pandemic.

Alzheimer's Disease Risk Gene CD33: the role in the phagocytosis of β-amyloid in Human Microglial Cell Line

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Abstract

Alzheimer's disease (AD) is the leading cause of dementia worldwide, where the patients often experience memory loss and inability to perform daily tasks. β-amyloid (Aβ) aggregation, tau-proteins tangles, and neuroinflammation are the main features of AD. Microglia are the primary immune cells in the brain to remove the extra accumulation of Aβ or tau-proteins via phagocytosis. Genetic studies indicated that risk genes for AD are highly expressed in microglia. Further evidence showed that patients with AD have higher expression of CD33, a transmembrane receptor of microglia. However, the functions of those risk genes are largely unknown during AD development. In this study, we plan to manipulate the CD33 expression in microglia and examine microglia function with different CD33 expression levels. We will first check the CD33 expression patterns during microglial pro-inflammatory (M1) or anti-inflammatory (M2) activation. For this purpose, we stimulate a human microglial cell line (HMC3) with *lipopolysaccharide (*LPS) into M1 phenotype or interleukin (IL)-4 into M2 phenotype. Gene expression of CD33 along other well-known M1/M2 genes will be quantified by real-time-polymerase chain reaction (RT-PCR). The phagocytic capacities under each activation are also be measured with fibril Aβ by confocal imaging. Next, we will knock out CD33 gene expression in HMC3 by the CRISPR-Cas9 system and compare the phagocytic capacities before and after CD33-knockout. Preliminary data from RT-PCR showed that CD33 expression is upregulated during M1 microglial activation with LPS-stimulation, whereas CD33 expression is downregulated during M2 microglial activation with IL-4 stimulation. As compared with HMC3 treated with IL-4, microglia treated with LPS exhibit higher Aβ phagocytic capacity. Our preliminary data suggest that higher CD33 expression in the pro-inflammatory status of a human microglia cell line is a consistent phenotype as seen in the activated microglia of AD patients. With a short-term of M1 activation, microglia may compensate with a higher uptake of amyloid-beta, as compared that with M2 activation. We will further investigate how CD33 knockout affects Aβ uptake in the microglia using the CRISPR-Cas9 technique. All data will need to further validate. An intensive study on this line will warrant to illustrate the role of CD33 in AD development and facilitate novel therapeutic targets for AD treatment.

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Abstract

Targeting new pathogenic biomarkers responsible for diseases in central nervous system is critical as there are limited efficient therapeutics available to control such diseases. The metzincin superfamily, including *matrix metalloproteinases (MMPs)* and *a disintegrin and metalloproteinases (ADAMs),* play multifaceted roles in physiological and pathological processes in the central nervous system and therefore are therapeutic targets to limit neurodegeneration in diseases such as Huntington disease (HD), Parkinson's disease (PD), and Alzheimer's disease (AD). Given the significance of recognizing enzymes that play a central role in neurodegenerative disease progression as novel neurodegenerative therapeutics, enzyme inhibitors with high selectivity are desired. Overexpression of MMP-9 plays a significant role in several neurodegenerative disorders, while ADAM-10 helps block progression of AD. *Tissue inhibitor of metalloproteinases-3 (TIMP-3)* is a natural inhibitor of MMP-9 with pico- and subnanomolar affinity. To overcome the challenges of TIMP-3's wide multispecificity for different classes of MMPs and ADAMs and its interaction with growth factors, a protein engineering approach is needed to tailor a TIMP-3 scaffold to create an outstanding neurodegenerative drug candidate.

Thus, engineering protein-based scaffolds based on the natural enzyme inhibitors that high selectively target a specific metzincin without off-target effects are promising therapeutics for neurodegenerative diseases. We have previously engineered TIMP-1 to improve binding selectivity toward MMP-3 using directed evolution and yeast surface display using a counter-selection strategy. We also studied the mechanism of interaction of these TIMP variants in complex with MMP-3 using X-ray crystallography to understand the underlying mechanism of inhibition. We will expand the state-of-the-art techniques developed to develop highly selective TIMP-3 variants toward MMP-9 binding that avoids binding to ADAM-10 as potential therapeutics. These studies will lay the foundation for preclinical in vivo models and novel therapeutic strategies for neurodegenerative and other metzincin-related diseases.

Anosmia in COVID-19: Underlying Mechanisms and Explanations for the Surprisingly Large Difference in Prevalence Between Western and Asian Populations

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Nevada COBRE GM103554

The COVID-19 pandemic revealed that there is a loss of smell in many patients, including in infected, but otherwise asymptomatic individuals. The underlying mechanisms for the olfactory symptoms are unclear. Using a mouse model, we determined which cells in the olfactory epithelium express the obligatory receptors for entry of the SARS-CoV-2 virus by using RNAseq, RT-PCR, in situ hybridization, Western blot, and immunocytochemistry. We show that the cell surface protein ACE2 and the protease TMPRSS2 are widely expressed in sustentacular cells of the olfactory epithelium, but not in olfactory receptor neurons. These data suggest that sustentacular cells mediate SARS-CoV-2 virus entry and impairment of the sense of smell in COVID-19 patients. Because of the heavy expression of virus entry proteins, the tens of millions of sustentacular cells likely are responsible for the highest viral load in the nasopharynx and contribute to super-spreading of the virus and propagation of the pandemic.

The reported prevalence of chemosensory dysfunction in COVID-19 varies widely between studies. We determined the pooled prevalence of such chemosensory deficits in a systematic review and meta-analysis. Our analysis – of the largest number of studies and cohort number to date – showed that geography/ethnicity was a highly significant factor: Caucasians had nearly three times the prevalence of chemosensory dysfunctions (54.7%) than Asians (19.5%). The finding of geography/population differences points to two, not mutually exclusive, causes. Mutations at the level of the virus (the D614 to G614 substitution) with different infectivity may explain some of the population differences due to the start of the pandemic in Asia with the less infective D614 strain; known genetic, ethnicity-specific variants of the virus-binding entry proteins with differential virus binding affinity could also cause prevalence differences between populations. Both explanations have major implications for infectivity, diagnosis, and management of the COVID-19 pandemic.

Supported by GM103554.

Machine Learning, Genomic Variants, and Osteoporotic Risk Prediction

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Developing an accurate predictive model for osteoporosis risk assessment is critical to prevent fracture, a devastating outcome for elders. Recent studies have found thousands of SNPs associated with osteoporosis. However, how to model these variants to create an accurate modelfor predicting osteoporosis remains unclear. We aimed to develop multiple machine learning models and to identify the best performing model for osteoporosis prediction. Genomic data from the Osteoporotic Fractures in Men cohort Study (N=5,133) was used as the data source. After genotype imputation, we identified 1,103 osteoporosis-associated SNPs for calculating genetic risk. Osteoporosis was defined as a T-score of bone mineral density ≤−2.5. The predicting variables included both conventional osteoporosis risk factors and genomic variants. The data were first normalized and then randomly split into a training set (80%) and a validation set (20%). Synthetic Minority Over-sampling technique was used to account for the low rate of osteoporosis in the data. Osteoporosis prediction models were developed using random forest, gradient boosting, and neural network separately. The model prediction performance was assessed by area under the ROC curve (AUC) and accuracy for each model in the validation set.We found that the performance of gradient boosting in predicting osteoporosis was the best among the three models, with AUC of 0.88 and an accuracy of 0.95. The performance of random forest and neural networks was worse than that of gradient boosting; random forest and the neural network had the AUC of 0.87 and 0.85, and accuracy of 0.92 and 0.85, respectively. The overall difference among the three machine learning models was highly significant ($p<0.0001$) by the Cochran's Q test. McNemar's tests showed that gradient boosting was significantly better than both random forest and random forest (both $p<0.0001$). Thus, we concluded that gradient boosting performed best for osteoporosis prediction in older men.

The Utility of Genetic Risk Score to Improve Performance of FRAX in Fracture Prediction Involving US Postmenopausal Women

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Background: The ability of the fracture risk assessment tool (FRAX) in discriminating fracture and non-fracture in post-menopausal women remains suboptimal. Adding a genetic profile may improve the performance of FRAX.

Methods: Three genetic risk scores (GRSs) (GRS_fracture, GRS_BMD, GRS_eBMD) were calculated for each participant in the Women's Health Initiative Study (n=23,981), based on the summary statistics of two comprehensive osteoporosis-related genome-wide association studies (GWAS). The primary outcomes were incident major osteoporotic fracture (MOF) and hip fracture (HF). The association between each GRS and fracture risk were evaluated in separate Cox Proportional Hazard models, with FRAX clinical risk factors adjusted for. The discrimination ability of each model was assessed by using Area Under the Curve (AUC). The predictive improvement attributable to each GRSs were assessed by using the net reclassification improvement (NRI) and the integrated discrimination improvement.

Results: GRS_BMD and GRS_eBMD were significantly associated with MOF and HF risk, independent of the base FRAX risk factors. Compare to the base FRAX model, the models with GRS_fracture, GRS_BMD, and GRS_eBMD improved the reclassification of MOF by 0.3 % (95% CI, 0.1% to 3.0%, p=0.64), 0.9% (95% CI, 0.6% to 3.1%, p=0.07), and 1.6% (95% CI, 1.0% to 3.0%, p<.01), respectively. The improvement in terms of reclassification by GRSs was generally greater in HF prediction. Compare to the base FRAX model, GRS_BMD and GRS_eBMD improved the reclassification of HF by 1.3% (95%CI, 0.6% to 3.1%, p=0.01) and 2.2% (95%CI, 1.2% to 3.1%, p<0.01), respectively. **Conclusion:** Our study suggested that the addition of genetic profiles did provide meaningful improvements in the reclassification of FRAX for MOF and HF.

Trends in osteoporosis and mean bone mineral density among type 2 diabetes patients: findings from NHANES 2005-2014

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Background: Osteoporosis and T2DM are affected by aging and often coexist in the elderly. This study aimed to examine how bone health changed among T2DM patients in the past decade.

Method: Continuous National Health and Nutrition Examination Survey (NHANES) data from 2005–2006 to 2013–2014 were analyzed to examine BMD, as well as the prevalence of osteoporosis and osteopenia trends among T2DM patients and non-diabetic people aged 40 years and older. The linear trend over the four survey cycles wad examined using orthogonal contrast.

Results: For T2DM patients, the mean age- and BMI-adjusted BMD decreased from 0.813 g/ cm^2 (95%CI, 0.796 g/cm^2-0.829 g/cm^2) to 0.784 g/cm ^{2} (95%CI, 0.771 g/cm^2-0.796 g/cm^2) during 2005-2014. Meanwhile, for non-diabetic, the mean age- and BMI-adjusted BMD also decreased, from 0.795 g/cm^2 (95%CI, 0.786 g/cm^2-0.805 g/cm^2) to 0.773 g/cm ^{2} (95%CI, 0.765 g/cm^2-0.781 g/cm^2). Significant linear trends were observed among the two population (both $P_{\text{linear trend}} \leq 0.009$). During 2005-2014, the prevalence of osteoporosis among T2DM patients and non-diabetic people increased but with no significant linear trend (both $P_{\text{linear trend}} > 0.05$), while the prevalence of osteopenia of the two populations increased linearly (both $P_{\text{linear trend}} < 0.04$).

Conclusion: Age- and BMI- adjusted mean BMD decreased in 2013–2014 in patients with T2DM and non-diabetic people, while the prevalence of osteoporosis and osteopenia increased in both groups.

Investigating the Impacts of COVID-19 on Population Health and Wellbeing

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New Hampshire COBRE P20GM104416 Margaret R. Karagas

Abstract

The Center for Molecular Epidemiology at Dartmouth seeks to: 1) apply novel scientific discoveries and technologies to address major health concerns, 2) identify early indicators of disease pathogenesis and 3) explore common pathways of disease etiology and progression. In addition to research initiatives led by the project and pilot leaders, Center members collectively contribute to the receipt of program project and center grants made possible by our COBRE Biorepository and research infrastructure. Among our Center's focus areas is the impact of exposures during pregnancy, infancy and early childhood on health throughout the life course, especially in rural populations. This includes investigations of the effects of environmental factors i.e., nutrient and toxicant exposures on the developing microbiome and child immune response. Our recent initiatives address the impact of the COVID pandemic on the health and wellbeing of our rural region. Without question, the pandemic and its associated lockdowns and restrictions have resulted in unprecedented changes in behaviors that can serve as a natural experiment to explain the influence of these changes on diet, environmental exposures, lifestyle, stress and other factors, and, in turn, maternal health and child development. Rural regions carry unique characteristics with respect to access to medical care, broad band internet for virtual education, and other stressors that make them more vulnerable to adverse outcomes. Thus, studies being performed in our rural regions will be combined with a national, collaborative efforts to enable comparisons between racial/ethnic groups and urban/rural differences. Our approach utilizes novel sensors and biomarkers along with online surveys. Proposed efforts also will explore critical obstacles to future COVID preventive and treatment efforts. These data will be used to inform policy and practice change to reduce harmful exposures and illuminate solutions for a healthy future.

Gene Regulation and Dynamical Efficacy in Antibiotic Responses
A focus on 'omics', from organisms to single cells
Daniel Schultz, Project Lead
COBRE 1P20 GM130454-01 Michael L. Whitfield

Different selective pressures shape the evolution of antibiotic-resistance mechanisms during their migration from soil bacteria to clinical environments. We test the hypothesis that the dissemination of resistance mechanisms throughout a bacterial population requires the evolution of particular regulatory features that adapt the response to the specific drug regimens in their environment. We constructed a detailed mathematical model to analyze how different selective pressures shape the evolution of a typical drug response: a transcription factor activating a resistance enzyme in the presence of the drug. Optimizing regulation parameters for combinations of different fitness costs, we found that in regimens where expression of resistance is more costly for the cell, the response is optimized by a constitutively expressed repressor; and when drug action is more costly, a self-repressed repressor is preferred. Surprisingly, we found that the complex regulation found in the tetracycline resistance tet operon, with multiple overlapping promoters and operators, is the optimal strategy to provide a quick response upon drug exposure while avoiding an undesirable overshoot in the expression of resistance. We then analyzed the evolution of the aminoglycoside resistance mexXYZ operon in P. aeruginosa chronic infections in the human lung, which are typically initiated by environmental strains. We used a liquid-culture assay to test whether loss-of-function mutations in repressor mexZ, commonly seen in clinical isolates, are able to accelerate the lengthy P. aeruginosa response to aminoglycosides. We found that a mexZ deletion greatly improves the speed of growth recovery (dynamical resistance), without significantly changing the steady-state resistance typically measured in standard MIC assays. We found this dynamical resistance comes at the cost of reduced growth rates for mexZ mutants in the absence of drug. These results, relating the genotype of resistance mechanisms to their phenotype and fitness, help elucidate how antibiotic resistance emerges and is optimized in bacterial populations.

Daniel Schultz
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Role of Protein Dynamics in Inhibitor Recognition by Signaling Proteins

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New Hampshire COBRE P20GM113131 Rick Cote

Regulators of G-proteins Signaling (RGS) proteins bind to the Gα subunits of G-proteins to activate their GTPase accelerating (GAP) activity that leads to accelerated hydrolysis of GTP and termination of signaling by G-Protein Coupled Receptors (GPCRs). RGS proteins are potential drug targets in many therapeutic areas, including in cancer, cardiovascular diseases, and central nervous system disorders. Therefore, inhibiting the RGS-Gα protein-protein interface by targeting an allosteric (distant) site instead of a site directly in the interface represents a promising strategy given that the RGS-Gα protein-protein interface is flat and undruggable. However, inhibitors binding to cysteine amino acids on RGS proteins show differences in their selectivity for various RGS proteins although they inhibit the protein-protein interface via an allosteric mechanism. Presented in this talk will be the details of differences in dynamics of various RGS proteins that correlate with inhibitor potencies, as probed using a judicious combination of molecular dynamics (MD) simulations, Hydrogen-Deuterium Exchange (HDX), and Nuclear Magnetic Resonance (NMR) spectroscopy. Funded by the National Institute of General Medical Sciences under Award Number P20GM113131.

A Non-Canonical Role for the Autophagy Machinery in Anti-Retroviral Signaling Mediated by TRIM5α

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New Mexico COBRE 1P20GM121176-02 Vojo P. Deretic

Abstract

TRIM5α is a key cross-species barrier to retroviral infection, with certain TRIM5 alleles conferring increased risk of HIV-1 infection in humans. TRIM5α is best known as a species-specific restriction factor that directly inhibits the viral life cycle. Additionally, it is also a pattern-recognition receptor (PRR) that activates inflammatory signaling. How TRIM5α carries out its multi-faceted actions in antiviral defense remains incompletely understood. Here, we show that proteins required for autophagy, a cellular self-digestion pathway, play an important role in TRIM5α's function as a PRR. Genetic depletion of proteins involved in all stages of the autophagy pathway prevented TRIM5α-driven expression of NF-κB and AP1 responsive genes. One of these genes is the preeminent antiviral cytokine interferon β (IFN-β), whose TRIM5-dependent expression was lost in cells lacking the autophagy proteins ATG7, BECN1, and ULK1. Moreover, we found that the ability of TRIM5α to stimulate IFN-β expression in response to recognition of a TRIM5α-restricted HIV-1 capsid mutant (P90A) was abrogated in cells lacking autophagy factors. Stimulation of human macrophage-like cells with the P90A virus protected them against subsequent infection with an otherwise resistant wild type HIV-1 in a manner requiring TRIM5α, BECN1, and ULK1. Mechanistically, TRIM5α was attenuated in its ability to activate the kinase TAK1 in autophagy deficient cells, and both BECN1 and ATG7 contributed to the assembly of TRIM5α-TAK1 complexes. These data demonstrate a non-canonical role for the autophagy machinery in assembling antiviral signaling complexes and demonstrate a role for autophagy in the establishment of a TRIM5α-dependent antiviral state.

tRNA Synthetase Inhibitors Increase Lifespan in a *GCN4* / *atf-5* and Autophagy Dependent Manner

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New Mexico COBRE 1P20GM121176-02 Vojo P. Deretic

Abstract

Deletion of genes encoding ribosomal proteins extends replicative lifespan in yeast. This increases translation of the functionally conserved transcription factor Gcn4, and lifespan extension in these mutants is largely *GCN4*-dependent. Gcn4 is also translationally upregulated by uncharged tRNAs, as are its *C. elegans* and mammalian functional orthologs, ATF-5 and ATF4 respectively. We have shown that tRNA synthetase inhibitors upregulate Gcn4 translation, and extend yeast lifespan in a Gcn4-dependent manner. We have also show that tRNA synthetase inhibitors greatly extend lifespan in *C. elegans*, and this depends completely on *atf-5*. We have also shown that there is an increase in autophagy in both *C. elegans* and yeast treated with tRNA synthetase inhibitors. Further investigation into these compounds also reveal that the lifespan extension in *C. elegans* treated with tRNA synthetase inhibitors is dependent on autophagy. These findings establish *GCN4* orthologs as conserved longevity factors and, as several types of long-lived mice exhibit elevated ATF4, leave open the possibility that chemical inhibition of tRNA synthetases will also extend lifespan in mammals. Based on these findings, we believe that the transcriptional targets of Gcn4 are likely effectors that can upregulate autophagy and increase protein turnover. We hypothesize that autophagy plays a key role in the *GCN4*/ *atf-5* dependent lifespan extension by tRNA synthetase inhibitors.

The Dynamics and Causality of Gene Regulation During Erythrocyte-Neutrophil Differentiation

Joanna E. Handzlik and Manu
University of North Dakota, Dept of Biology, 10 Cornell St, Grand Forks, ND 58202 North Dakota COBRE 5P20GM104360-07 Roxanne Vaughan

Abstract

Cell differentiation during hematopoiesis is driven by gene regulatory networks (GRNs), comprising genes that encode transcription factors (TFs) that regulate each other as well as downstream genes. The precise structure of developmental GRNs and the causality of interactions that drive cell fates are unclear due to the recursive wiring of GRNs and limitations of traditional genetic approaches. We took a complementary approach to the construction of GRNs and determination of causality by inferring them from time-series gene expression data using the *gene circuit* approach. We built *in-silico* differential equation models of the dynamics of gene expression of key regulators during the erythrocyte-neutrophil cell-fate decision during murine hematopoiesis. The model's free parameters define both the type and strength of regulatory interactions occurring in time during differentiation. We trained the model on a high-resolution time series of genome-wide gene expression data acquired during the differentiation of a multipotent cell line, FDCP-mix, into erythrocytes or neutrophils (May *et al.,* Cell Stem Cell, 13:754). The obtained GRNs were consistent with known regulatory interactions and predicted the outcomes of perturbation experiments both qualitatively and quantitatively. Analysis of model dynamics allowed us to make predictions about the causality of interactions during differentiation. In particular, the model predicted that *Spi1,* which encodes PU.1, considered a master regulator of the white blood-cell lineage, is in fact activated after *Cebpa* and *Gfi1,* two other TFs necessary for neutrophil formation. We analyzed the transient expression of these three TFs from an independent scRNA-Seq experiment (Tusi *et al.,* Nature, 555:54). These results suggest an alternative view of the regulatory causality during hematopoietic differentiation and demonstrate the utility of the gene circuit framework for understanding the dynamics of developmental processes, which should be applicable even outside hematopoiesis.

Mechanisms of GATA3 mediated cell reprogramming in breast cancer

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North Dakota COBRE P20GM104360 Roxanne Vaughan

Abstract

Transcription factors (TFs) are DNA-binding proteins that control gene expression. They play crucial roles in cell development and reprogramming. However, eukaryotic cells have chromatin structure to package huge DNAs inside of the tiny nuclei. Therefore, TFs need to overcome this physical barrier to initiate cell differentiation and reprogramming. A subset of transcription factors, called pioneer factors, are thought to be special as they are capable of overcoming the chromatin structure by using their ability to bind to nucleosomes (a fundamental unit of chromatin) and induce chromatin opening. Although various pioneer factors have been identified in multiple tissues and human diseases, the fundamental mechanisms underlying their gene-regulatory actions remain elusive. We previously demonstrated that GATA3, one of the most frequently mutated genes in breast cancer, acts as a pioneer factor to suppress tumor growth and metastasis in the MDA-MB-231 human breast cancer cell mesenchymal-to-epithelial transition (MET). We also found that the GATA3 mutation interrupts gene regulation activities of GATA3, leading to more aggressive breast cancer phenotypes in luminal breast cancer cells. In this COBRE funded project, we aim to identify how GATA3 reprograms breast cancer cells, particularly focusing on the roles of chromatin components. To precisely determine nucleosome positions at individual GATA3 binding sites during MET, we established a new technique called Capture MNase-seq. The high-resolution nucleosome mapping by Capture MNase-seq identified the differential nucleosome positioning on the GATA3 binding motifs between productive v.s. non-productive enhancer formation sites. The nucleosome dyad was frequently located near GATA3 motifs within the de novo enhancer regions. The nucleosome mapping data also enabled us to determine the Cryo-EM structure of the GATA3-nucleosome complex. This structure revealed that GATA3 uses its two zinc finger domains to stably bind to nucleosomes. Capture MNase-seq was also used to identify dynamic nucleosome organization during HIV infection.

Apolipoprotein B Orthologs Function Non-Cell-Autonomously during Adult Stem Cell Differentiation in Planarians

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Oklahoma COBRE GM103636 Linda F. Thompson

Identification of extrinsic (non-cell-autonomous) factors that coordinate stem cell dynamics is a central goal of regenerative medicine. In planarian flatworms, whole body regeneration is achieved by pluripotent somatic stem cells called neoblasts that proliferate and differentiate in response to injury. However, the identities of secreted cues that drive these processes are not well understood. Previously, we showed that RNA interference (RNAi)-mediated knockdown of an intestine-enriched transcription factor, *nkx2.2*, caused dramatic reduction in new tissue production during regeneration, suggesting the intestine may regulate neoblasts. We now report identification of two potential downstream effectors, *apolipoprotein B* (*apob*) orthologs, whose transcripts were dramatically downregulated in the absence of *nkx2.2*. Simultaneous knockdown of *apob-1* and *apob-2* reduced morphogenesis of the wound blastema and delayed multiple aspects of regeneration, including re-establishment of axial polarity, and regeneration of the brain, pharynx, and intestine. Although *apob* mRNA expression was highly enriched in the intestine, we detected robust protein labeling in many cell types outside the intestine, indicating that ApoB proteins are secreted. Consistent with a role for *apob* in export of diet-derived lipids from the intestine (the primary site of neutral lipid storage in planarians) to neoblasts, overall neutral lipid content increased in *apob(RNAi)* planarians, but was reduced in neoblasts. Furthermore, planarian *lipoprotein receptor* orthologs were expressed by stem cells and their differentiating progeny in regenerating tissues. Finally, using flow cytometry, we found that the cell fraction containing post-mitotic early progeny of neoblasts was expanded. Together, these results suggest that ApoB-mediated delivery of neutral lipids and/or other lipoprotein cargos to stem cells is critical for early stages of differentiation during regenerative tissue production. In addition, our findings indicate planarians are a tractable model for elucidating specialized mechanisms by which lipid metabolism is regulated during regeneration.

Childhood Adversity and Susceptibility to Psychosocial Stress due to the COVID-19 Pandemic

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Oklahoma COBRE P20GM109097 Jennifer Hays-Grudo

Given the persistence of SARS-CoV-2/COVID-19 community transmission in the US and the likelihood that the pandemic's psychosocial consequences will be with us for years to come, there is a pressing need to quickly identify phenotypic and biological characteristics that place individuals at elevated risk for psychosocial stress due to the COVID-19 pandemic. Accomplishing this in a timely manner requires ready access to a phenotypically well-characterized cohort of participants who can be tracked through the current COVID-19 pandemic, and for whom biological samples are available from before the advent of the current crisis. Phenotypic data and biospecimens previously collected from 177 participants in the Holistic Assessment of Tulsa's Children's Health (HATCH) Project, a Center for Integrative Research on Child Adversity (CIRCA; P20GM109097) research project at Oklahoma State University, offer a unique opportunity to identify predictors of psychosocial vulnerability to the COVID-19 pandemic. **The central hypothesis of the current research project is that pre-morbid epigenetic changes in key immune and glucocorticoid signaling pathways caused by early life adversity make individuals more susceptible to the psychosocial stresses of the COVID-19 pandemic.** Biomarkers of interest have been identified and analysis will begin in fall, 2020. The current study includes the 107 women who participated through the eighth and most recent survey conducted in April/May, 2020. OLS regression analyses were used to examine the association between childhood adversity and pandemic-related distress. We found that Adverse Childhood Experiences (ACE) scores were associated with higher levels of distress ($b=.08$; $se= .03$; $p<.01$) when controlling for demographic characteristics. The addition of loneliness to the model fully mediates the association between ACE score and distress. Findings suggest that ACEs influence psychosocial stress due to greater social isolation; those with more childhood adversity may be less likely to have the social connectedness needed to reduce distress due to the pandemic.

Chemical Cartography of Influenza Virus Infection
Laura-Isobel McCall, pilot project PI
University of Oklahoma
Oklahoma COBRE GM103648 Lin Liu

Metabolism controls pathogen replication, immune responses and disease pathogenesis. However, relying on systemic metabolomic analyses is insufficient to understand the localized intersection of host and pathogen metabolism. To address this issue, we implement a new approach called “chemical cartography”, which combines spatially-resolved tissue metabolite analysis by liquid chromatography-tandem mass spectrometry, with 3D modeling and big data analytics. In this project, we apply chemical cartography to influenza virus infection. Influenza is a leading cause of respiratory illness and death worldwide, with over 5 million cases and 600,000 deaths annually in non-pandemic years. Although vaccination can prevent influenza, it may not be effective in immunocompromised populations, and is hampered by viral variability. Host-directed therapies may represent a new way to treat this disease, but require improved understanding of IFV pathogenesis and tissue mechanisms of damage *vs* repair. Here, we use chemical cartography to differentiate between influenza virus-induced metabolic alterations in the lung at the site of highest viral load, vs lung metabolic alterations at sites of lower viral load, in comparison to systemic alterations in plasma metabolic profile, in a mouse model of influenza infection. Overall, infection had a strong impact on tissue and plasma metabolic profile. Furthermore, we identified regional differences in metabolic pathways affected by infection, including in immunomodulatory lipids. These results represent a new spatial perspective into virus-host metabolic interactions and their role in disease pathogenesis. Future work will focus on developing these results into new therapeutics for influenza virus infection.

Genetic variants in lysyl-tRNA synthetase 1 (KARS1) cause neurological phenotypes recapitulated by the kars1 knockout zebrafish

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Oklahoma COBRE GM103636 Linda F. Thompson

Aminoacyl-tRNA synthetases (ARSs) are essential enzymes that catalyze the aminoacylation of specific amino acids onto their cognate tRNA. Pathological variants in lysyl-tRNA synthetase 1 (KARS1) are associated with distinct clinical manifestations. Here, we identify five novel and two known biallelic missense variants in KARS1 in 11 patients displaying hearing loss, brain white matter abnormalities and additional phenotypes. We generated homozygous kars1$^{-/-}$ zebrafish larvae, which recapitulated key patient phenotypes. kars1$^{-/-}$ zebrafish showed increased expression of genes related to p53 signaling and apoptosis. TUNEL assays revealed elevated apoptosis in kars1$^{-/-}$ larvae compared to wild-type, and knockdown of p53 rescued several defects in kars1$^{-/-}$ larvae. These data suggest that loss of Kars1 induces p53-mediated apoptosis. Zebrafish overexpressing patient-specific kars1 variants phenocopied kars1$^{-/-}$ knockout animals. Our work provides a novel animal model for human diseases related to Kars1 and suggests that the brain, eye, ear, heart, and skeletal muscle are particularly sensitive to Kars1 deficits.

Calcium Signaling as a Novel Regulatory Mechanism of *Pseudomonas aeruginosa* Virulence

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Oklahoma COBRE P20GM103648 Lin Liu

Abstract

Pseudomonas aeruginosa is a human pathogen responsible for severe acute and chronic infections. It is one of the primary organisms infecting the airways of patients with cystic fibrosis (CF), causing fatal airway blockage. Calcium (Ca^{2+}) regulates host responses to bacterial infection and accumulates in pulmonary and nasal liquids of CF patients. Earlier we have established that *P. aeruginosa* responds to elevated Ca^{2+} by enhancing the production of secreted virulence factors and infectivity in plant and animal models. We have shown that this response is controlled by two regulatory pathways, involving two-component regulatory system CarSR and the intracellular Ca^{2+} (Ca^{2+}_{in}) transients. The Ca^{2+}-regulated CarSR controls the transcription of several genes in Ca^{2+}-dependent manner. This includes a periplasmic protein CarP, required for resistance to elevated Ca^{2+} and Ca^{2+} regulation of several virulence traits and interaction with host. We have identified several transporters required for generation of the Ca^{2+}_{in} transients, including Ca^{2+} channel, CalC, responsible for letting Ca^{2+} into the cell. By using RNA-seq analysis of the *calC* deletion mutant, we showed that the Ca^{2+}_{in} transients are required for Ca^{2+} regulation of a large number of pathways, including production of virulence factors, transition to sessile mode of growth, and cellular communication. We also identified a Ca^{2+} sensor, EF-hand protein, EfhP, specifically binding Ca^{2+} and mediating Ca^{2+} regulation of infectivity and resistance to oxidative stress. By using pull-down assay, we identified that EfhP interacts with a global regulator of iron sequestration, Fur, in a Ca^{2+}-dependent manner. This observation was validated by measuring the production of high-affinity iron siderophore, pyoverdine. Overall, we determined that Ca^{2+} signaling regulates *P. aeruginosa* virulence and mapped the essential components of the Ca^{2+} signaling network in the pathogen that can serve as drug targets in future developments of new means of preventing or controlling *P. aeruginosa* infections.

SARS-CoV-2 nucleocapsid protein undergoes phase separation with RNA and co-partitions with human hnRNPs associated with stress granules and disease

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Rhode Island COBRE 5P30GM122732-03 Qian Chen

Abstract

Tightly-packed complexes of nucleocapsid protein and genomic RNA form the core of viruses and assemble within viral factories, dynamic compartments formed within the host cells that are associated with physiological structures known as stress granules (SGs). Here, we test the possibility that the multivalent RNA-binding nucleocapsid protein (N) from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) condenses with RNA via liquid-liquid phase separation (LLPS) and that N can be recruited in phase separated forms of human RNA-binding proteins associated with SG localization. We find that N phase separates avidly by addition of non-specific RNA. In addition, N enters in vitro LLPS condensates of human hnRNPs (TDP-43, FUS, hnRNPA2) that contribute to phase separation of stress granules and also aggregate in human diseases of neurons (ALS and frontotemporal dementia), muscle (inclusion body myopathy), and bone (Paget's disease of bone). These results provide a potential mechanism for the role of N in SARS-CoV-2 viral genome packing and host protein co-opting necessary for viral replication and infectivity.

COBRE for Skeletal Health and Repair (PI: Qian Chen)

COVID-19 related research abstract for IDeA meeting September 2020

Elucidating novel roles for the transcription factor Sox9b in cardiovascular development

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Rhode Island COBRE P20GM103652-06 Sharon Irene Smith Rounds

Congenital heart defects (CHD) are the most common type of birth defect and the leading cause of infant death in the US. CHD result from disruptions to cardiac and great vessel development. Pharyngeal arch arteries (PAA) are transient vessels that give rise to the great vessels, the vascular structures that connect the heart to the periphery. Clinical data suggest that human mutations that disrupt SOX9 function are associated with great vessel and cardiac defects. Thus, to study Sox9b functions in cardiovascular development, we have generated transgenic tools to manipulate Sox9b function in a cell-type specific manner. Zebrafish are a great model for studying cardiovascular development as embryos obtain oxygen through passive diffusion from the water in which they develop. As a result of passive oxygen diffusion, embryos can survive with severe vascular malformations that would otherwise be embryonically lethal in mammals. Zebrafish have two *SOX9* orthologues, *sox9a* and *sox9b*. Our findings indicate that global loss of Sox9b function severely disrupts PAA development. Specifically, we observed stenosis of the PAAs, blockage of the outflow tract, and coarctation of the ventral aorta. Currently, we are working to determine where Sox9b lies in the transcriptional hierarchy of known regulators of PAA development and are performing experiments to determine whether endothelial-specific loss of Sox9b is sufficient to produce the observed vascular phenotypes. Given that *sox9b* is expressed broadly in the developing pharyngeal arches, we have begun characterizing the other cell types surrounding the PAAs that may contribute to the observed phenotypes. We found that following global loss of Sox9b function disrupts neural innervation of the pharyngeal arches. Additional experiments will address how loss of Sox9b function affects the developing musculature and pericytes. Together, these findings reveal novel functions for Sox9b and contribute to our understanding of the molecular mechanisms underlying CHD.

Embryonic macrophages can influence cardiac conduction and chamber formation in developing zebrafish

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Rhode Island COBRE P20GM103652-06 Sharon Irene Smith Rounds

Despite the unremitting research surrounding heart development and disease, congenital heart defects (CHD) re main the most common birth defect worldwide. Arrhythmias are the leading cause of morbidity and mortality in CHD patients, though the etiologies of arrhythmia are not well understood. Notably, there have been considerable advances in our understanding of cardiac macrophage biology in both regulating developmental processes and modulating adult cardiac conduction. However, it is not known 1) when macrophage-derived signals influence heart electrical function, nor 2) if macrophages are integral to the development and function of the embryonic or juvenile conduction system. Using transparent zebrafish, a genetically tractable and well-established developmental model, we employ advanced microscopy techniques to visualize cardiac macrophages in real-time and *in vivo* throughout heart development. We demonstrate here that embryonic cardiac macrophages display synchronous calcium activity in-time with heartbeat. Using optogenetics to stimulate macrophage electrical activity via light-gated ion channels, we show that macrophage-specific expression of channelrhodopsin, a cation channel, or halorhodopsin, a chloride channel, can directly influence heartrate. Thus, embryonic macrophages can functionally manipulate cardiac rhythm in developing zebrafish. Further, loss of embryonic macrophages, either through drug-inducible ablation or embryonic macrophage genetic mutation, disrupts organization of both the embryonic and adult ventricular myocardium. We therefore hypothesize cardiac macrophages are functionally important in embryonic conduction and fetal heart development, such that embryonic macrophage loss negatively impacts long-term cardiac health. This work will provide novel insight into potential contributing factors of cardiac developmental dysregulation and macrophages as targets of intervention for arrhythmia.

A New Paradigm in Inflammo-Fibrosis based on the Identification of Human Primary Cardiac Fibroblast Cells with Dual Mesenchymal Myofibroblast and Lymphoidal CD4 features

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Rhode Island COBRE P20GM103652 Sharon Irene Smith Rounds

Abstract

Fibrotic maladaptive remodeling of the right ventricle (RV) due to ischemia or pressure overload is believed to lead to heart failure in patients with pulmonary hypertension. Heterogeneous and poorly characterized, activated resident cardiac myofibroblasts, may be the principal drivers of cardiac fibrosis and cardiac dysfunction. Moreover, the biology of these cells is closely aligned with that of various immune cells and secreted cytokines, although the mechanism underlying this interaction is unknown. Multiplex immune lineage characterization of cardiac fibroblasts using mass cytometry identified a novel sub-population of cardiac myofibroblasts expressing both mesenchymal markers (SMA, Vimentin) and lymphoidal markers (CD4, CCR6 and CD183). CD4 expressing spindle shaped cells were identified in human RV tissue from both unaffected and individuals affected with pulmonary hypertension. The expression of these CD4/SMA co-expressing cells increased in fibrotic regions of SUGEN/ hypoxia rat model of cardiac fibrosis and in the RV determined using flow cytometry. Primary human cardiac fibroblast cells were rounded, non-adherent, and expressed CD4 upon treatment with IL-1 ng/mL. IL-1 increased the expression of T cell homing receptors CD44, IL-1R and CCR2 on cardiac fibroblasts in addition to the phosphorylation of p38 and NFkB. Transcription of inflammatory genes (*PLCG1, PTGIS, NR3C1, MAPK14*) and extracellular matrix genes (*COL1A1*, *COL4A2, COL8A1, COL12A1*) increased with IL-1 treatment. The secretome of the cardiac fibroblasts treated with IL1 was comprised of immune associated cytokines, chemokines, immunomodulators, and metabolites. In summary, we identified a cardiac fibroblast cell subpopulation expressing the Treg cell marker CD4 in human and rat RV. IL-1 potentiates the immune cell associated structure and function of cardiac fibroblast through the modulation of the secretome (genome, proteome and metabolome). We postulate that resident cardiac fibroblast may transdifferentiate into immune cells as a “first response” to diverse forms of myocardial damage and orchestrate inflammation and tissue repair in the stressed myocardium.

Non Standard Abbreviations: *CD*; Cluster of Differentiation, *SMA*; Alpha Smooth Muscle Actin*, IL-1* Interleukin 1 Beta *COL1A1*; Collagen Type I Alpha 1 Chain, *COL4A2*; Collagen Type IV Alpha 2 Chain, *COL8A1*; Collagen Type VIII Alpha 1 Chain, *COL12A1*; Collagen Type XII Alpha 1 Chain, *PLCG1*; Phospholipase C Gamma 1, *PTGIS*; Prostaglandin I2 Synthase, *NR3C1*; Nuclear Receptor Subfamily 3 Group C Member 1, *MAPK14*; Mitogen activated kinase

Dependency of the aryl hydrocarbon receptor in indole-3-carbinol-mediated attenuation of colitis and prevention of colitis-associated microbial dysbiosis.

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South Carolina COBRE P20GM103641 Prakash S. Nagarkatti

Abstract

Colitis is an inflammatory bowel disease (IBD) characterized by chronic inflammation of the colon with significant microbial dysbiosis. In our previous reports using mouse models of colitis we showed indole-3-carbinol (I3C), a natural product in cruciferous vegetables and ligand for aryl hydrocarbon receptor (AhR), reduced colitis severity and prevented colitis-associated alterations in the gut microbiome/metabolome. In addition, *in vivo* neutralization of IL-22, a key regulatory cytokine involved in host-microbiome interactions and linked to AhR activation, also prevented I3C-mediated beneficial effects. The aims of the current COBRE-funded Target Faculty project were as follows: 1.) Determine the dependency of AhR in I3C-mediated effects during colitis and prevention of colitis-associated microbial dysbiosis, as well as 2.) Investigate the source and regulation of IL-22 after I3C treatment during colitis. 10x Genomics single cell analysis and flow cytometry validation experiments revealed IL-22 production was significantly increased in innate lymphoid type 3 (ILC3) cells after treatment with I3C during colitis. Additionally, colonic epithelial cells (CECs) in I3C-treated mice showed an increase in host defense responses to microbes, such as mucin (muc2) and anti-microbial peptide (AMP) production. Increased AMP production included mainly beta-defensins (BDs) and regenerating islet-derived protein 3 (Reg3) types. Gene transcriptome array and PCR validation experiments confirmed the results obtained from the single cell analysis. Lastly as AhR is expressed in several cell types, colitis experiments during I3C treatment were also performed in conditional cre-flox knockout mouse strains. Ahr knockout in both immune cells (Rorc-cre x Ahr-flox) and CECs (Vil-cre x Ahr-flox) both resulted in negating the beneficial effects of I3C treatment during colitis. These results revealed I3C prevention of colitis and colitis-associated microbial dysbiosis was dependent on AhR expression in both immune cells as well as CECs. These studies were supported in part by the COBRE Center for Dietary Supplements and Inflammation (P20GM103641).

The Rapid Detection of SARS-COV-2 and Spreading Analysis in SC Communities

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South Carolina COBRE P20 GM109091 Igor B. Roninson

The rapid global spread of SARS-CoV-2, the virus causing the COVID-19 pandemic, is placing hospitals, universities, under immense stress. At the beginning of the pandemic, when testing capacity in South Carolina was limited, it was critical to develop rapid and effective measures to detect carriers of the virus and determine routes of spreading. The Functional Genomics Core of the University of South Carolina Center for Targeted Therapeutics, supported by COBRE grant P20 GM109091, has established a rapid procedure for virus detection with less than 24 h turnaround through local IRB-approved research testing. We performed more than 6000 thousand tests of Prisma health care professionals, and members of the local Columbia SC community. We compared testing technologies: RT-qPCR, Loop-Mediated isothermal amplification (LAMP), and cas13a based detection (SHERLOCK). We analyzed and compared the results of samples collected from saliva and nasopharyngeal swabs, and detection with or without viral RNA purification. We did not detect any significant difference in the detection of SARS-CoV-2 between saliva and nasopharyngeal swabs and agree with a growing scientific consensus that the collection of the saliva appears to be much more convenient for self-collection and rapid screening. Further, despite the higher sensitivity of an RNA-purification based approach, our analysis of the distribution of virus levels strongly suggests that virus detection from raw saliva or swabs provides sufficient power to identify all virus carriers who pose the highest risk to spread the virus through the community. Thus, we have established and implemented a rapid and inexpensive procedure for RT-qPCR based epidemiological testing of raw saliva samples to prevent the virus from spreading. To further speed up the tests and make them available to local sites (schools, athletic competitions, etc.) we established LAMP-based testing to deliver results in less than an hour. Additionally, to monitor routes of virus spreading, we established nanopore-based sequencing and analysis of viral genomic RNA. Through this analysis we have delineated several different routes by which the virus entered South Carolina communities.

Overcoming Chemotherapy Resistance in Triple Negative Breast Cancer *Via* Targeting Lysyl Oxidase (LOX)

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South Carolina COBRE P20 GM109091 Igor B. Roninson

Chemoresistance is a major obstacle in the treatment of triple negative breast cancer (TNBC), the most aggressive breast cancer subtype. As we have recently reported (*Nature Commun.* 5;11(1):2416), we have selected TNBC tumors for chemotherapy resistance *in vivo,* characterized their transcriptomes by RNA-sequencing and identified hypoxia-induced ECM remodeler, lysyl oxidase (LOX) as a key inducer of chemoresistance. LOX overexpression has two distinct effects in hypoxic tumors treated with chemotherapy. As an ECM remodeler, LOX enhances collagen cross-linking and fibronectin assembly, thereby decreasing drug penetration. In addition, LOX exerts a surprising novel effect on transcription, increasing the expression of Integrin Subunit Alpha 5 (ITGA5), the major receptor for fibronectin (FN1), leading to activation of Focal Adhesion Kinase (FAK)/Src signaling and chemoresistance. Inhibition of LOX or ITGA5 with shRNA-mediated knockdown or inhibition of FAK or Src kinases with small molecule inhibitors in combination with doxorubicin greatly enhanced tumor growth inhibition *in vivo* relative to individual treatments. The role of LOX in chemoresistance has further been demonstrated using chemoresistant TNBC patient-derived xenografts (PDXs) and organoids, treated with doxorubicin alone or in combination with the LOX family inhibitor, BAPN. Notably, higher LOX, ITGA5, or FN1 levels are associated with shorter survival in chemotherapy treated TNBC patients. Currently available LOX inhibitors suffer from lack of specificity and high toxicity. To identify a more potent and selective LOX inhibitor, we performed a high-throughput screen (HTS) of a diversified small-molecule library. HTS resulted in identification of several hits that inhibit LOX enzymatic activity without any cytotoxicity. A lead compound was identified after the screening of hits based on their inhibitory effects on LOX enzymatic activity and the degree of chemosensitization in collagen-embedded cells. We are currently performing structure-activity relationship (SAR) analysis to optimize the lead compound for more potent activity and better drug-like properties. In addition, we are analyzing the mechanisms through which LOX enhances ITGA5 transcription and how the enzymatic activity of LOX contributes to transcriptional regulation. Our study provides a pre-clinical rationale for the development and testing of LOX inhibitors to overcome chemotherapy resistance in TNBC patients.

Prototyping a Rapid SARS-CoV-2 Detection Device

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South Carolina COBRE P20 GM121342 Hai Yao

Introduction/Background: There is an essential need to develop rapid and inexpensive tools= for innovative diagnostic of SARS-CoV-2 infection and mass-production to halting the spread of this disease; hence, easing the societal and economic impact of COVID-19 pandemic. Current testing approaches rely on pharyngeal secretion viral RNA detection using reverse transcription polymerase chain reactions that are often time-consuming, limited to supply chain and limited access to approved laboratories. Alternatively, blood serology testing that identifies serum antibodies verifying past infection is labor-intensive. Both testing approaches require large amounts of reactants that are not amenable for mass production and require complex instrumentation for the detection or specialized technicians to perform these tests. Moreover, the readily available US tests have a wide range of accuracy, thus delivering significant false positives and false negatives.

Goal of Study: We aim to prototype a reliable testing device capable of detecting SARS-CoV-2= infections in saliva samples with the following advantages: i) can be used for high throughput screening, ii) uses easily acquired saliva samples iii) simultaneously screen for multiple protein markers of infection, iv) economic when mass-produced, v) fast and easy readout without the need of sophisticated instrumentation, specialized laboratories, or the need of specialized trained personnel.

Methods and Results: Our prototype device targets SARS-CoV-2 proteins using an= engineered aptamer switch sensor capable of Förster Resonance Energy and Electron Transfer for fast, easy, and on-site readout from a salivary sample. Due to its simplicity, the concept devices could be mass-produced as the DNA/RNA aptamer domain can be easily manufactured and amplified. All reaction chemistries used for the functionalization of the quantum Dots have been established and widely used in many different applications.

Conclusions: The device does not require sophisticated optical arrangements to detect= fluorescence or faint ELISA readouts. It can be used on blood, saliva, or urine patient samples. All these properties make this device desirable for commercialization beyond the current need of the COVID-19 pandemic.

Overview of the Advanced Fabrication and Testing (AFT) Core of SC-TRIMH

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South Carolina COBRE P20 GM121342 Hai Yao

Background: As the engineering innovation engine of the South Carolina COBRE for Translational Research Improving Musculoskeletal Health (SC-TRIMH, P20GM121342), the Advanced Fabrication and Testing (AFT) Core has established and maintained the state-of-the art facilities that contain centralized state-of-the-art equipment and engineering expertise to support the researchers at the TRIMH center, Clemson University, South Carolina, and beyond. This presentation will review the current progress of the AFT Core in achieving its goals and the plan to improve the capabilities, continue the success, and expand the services to future achievements.

Goals of Project: The AFT Core aims to aid the SC-TRIMH investigators by 1) assuming a role in 3D model-based design and rapid prototyping, 2) assuming a role in the provision of sensor, instrumentation, and testing services, and 3) promoting the core as a leading centralized bioengineering resource with a special emphasis in musculoskeletal related research.

Methods and Results: The AFT Core has over 4,000 square feet of space in one centralized location where all equipment is maintained and improved. The facility contains specialized equipment for advanced manufacturing, rapid prototyping, optics, electronics, sensors, instrumentation, and testing. The AFT Core also offers engineering supports in optical, mechanical and electrical designs, modeling and simulation related to musculoskeletal research and beyond.

Conclusions: The AFT has been successful at providing advanced fabrication capabilities to SC-TRIMH investigators and accomplishing the goals set forth. In the future, the AFT will continue to maintain, expand, and advance the technological services as a centralized manufacturing and testing resource for bioengineering research.

Overcoming Chemotheraphy Resistance in Triple Negative Breast Cancer *Via* Targeting Lysyl Oxidase (LOX)

Ozge Saatci[1], Ozge Akbulut[1], Abdol-Hossein Rezaeain[1], Carolyn E. Banister[1], Vitali Sikirzhytski[1], Sercan Aksoy[2], Aytekin Akyol[3], Aysegul Uner[3], Phillip J. Buckhaults[1], Campbell McInnes[1], Yasser Riazalhosseini[4,5], Ozgur Sahin[1]

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South Carolina COBRE P20 GM109091 Igor B. Roninson

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Interrogating the Role of IL-17A in the Etiology of Autism

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South Carolina COBRE 5P20GM103641-07 Nagarkati, P, Co-PI: Lizarraga, S

Autism spectrum disorder (ASD) is a group of behaviorally heterogeneous disorders characterized by impairments in social interaction, verbal and nonverbal communication, and repetitive behaviors. ASD has a highly complex etiology, and recent evidence from animal models and epidemiological studies suggest that maternal immune inflammation during pregnancy could be an environmental risk factor for ASD. Our research focuses on understanding how maternal inflammation from severe viral infections during pregnancy contributes to the etiology of ASD. During maternal immune activation (MIA), IL17A levels become elevated in the developing offspring and promote ASD-like phenotypes. IL17A is a Th17- lymphocyte related cytokine that is known to be detected in the immune cells and plasma of autistic children. Currently, how exposure to IL17A could impact the early development of human neuronal circuitry and contribute to ASD pathogenesis is unknown. We developed human cellular models of neuronal development to interrogate the effect of IL-17A in human neurons. Our preliminary results show that exposure to IL17A alters the expression of known ASD and cytokine genes. These changes in gene expression correlate with a decrease in synaptic activity in neurons exposed to IL-17A. We propose that IL17A could be a key mediator of Autism pathogenesis through its influence on gene regulation of ASD and cytokine genes, and modulation of synaptic activity.

Testing a wearable telemedicine-controllable taVNS device for NeuroCovid Recovery and Rehabilitation

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South Carolina COBRE P20GM109040 Steven A Kautz

Coronavirus disease (COVID-19) can enter and directly infect the brain, creating direct neurologic and psychiatric problems. This has led researchers to ask “Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19” (NeuroCovid)? Innovative therapeutic approaches, particularly home-based, are desperately needed.

Transcranial auricular Vagus Nerve Stimulation (taVNS) is a new non-invasive neuromodulation technique that can directly stimulate the brain and has important anti-inflammatory and other neuromodulatory functions that likely aid with long-term recovery from COVID or NeuroCovid. It offers the ability to directly treat the brain, reducing neuroinflammation and potentially improving and facilitating recovery from a variety of COVID disorders.

A team from the COBRE in Stroke Recovery has received a pilot grant from the Delaware-CTR to test a home based taVNS protocol for feasibility and efficacy in NeuroCovid patients. We will recruit 30 adults who are now COVID antibody positive and at home. They must also have a residual new neuropsychiatric symptom that started after COVID developed. This study is pioneering as it is entirely online and home-based.

Importantly, once initial feasibility and efficacy of the taVNS home therapy protocol has been established it can be readily applied to a number of post-COVID disabilities and rehabilitation needs (e.g., COPD and other complications treated through immunomodulatory and respiratory therapy amenable to vagal nerve action). We simply don’t know what is in store one year from now, but it is likely that the after-shocks of this pandemic will be the defining backdrop of the next decade. This research will allow us to pivot in the directions of greatest need in one year with a multitude of potential therapeutic targets and benefits.

Characterization of the glucokinases from pathogenic free-living amoebae

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South Carolina COBRE P20GM109094 Steven A. Kautz

Naegleria fowleri and *Acanthamoeba castellanii* are free-living amoebae that are capable of establishing life-threatening central nervous system infections in humans. In an effort to identify new targets for therapeutic intervention, we have started to score the importance of glucose metabolism on these parasites. Supporting the suitability of this pathway for target identification, *N. fowleri* trophozoite growth is severely impaired when cultured without glucose. The *N. fowleri* and *A. castellanii* genome encodes a single glucose phosphorylating enzyme, a glucokinase (NfGlck and AcGlck), that generates glucose-6-phosphate, an important intermediate for both glycolysis and the pentose phosphate pathway. Following cloning and heterologous expression, NfGlck was found to be a monomer with an apparent molecular mass of 47.7kDa, while AcGlck was found to be 43.9kDa. The substrate range of both enzymes was limited, with greatest activity observed with glucose or glucosamine. NfGlck had apparent K_m values of 42.5 ± 7.3 μM and 142 ± 9.9 μM for glucose and ATP, respectively, and the structure of the enzyme in complex with the ATP analog AMPPNP and G6P has been resolved to 2.2Å (PDB 6da0). AcGlck had apparent K_m values of 45.9 ± 2.3 μM for glucose and 472 ± 32 μM for ATP. Since NfGlck and AcGlck share limited identity with the host enzyme (hGlck), we anticipate inhibitors specific to the parasite with anti-amoeba activity could be generated. To that end, we have screened a collection of potential inhibitors against NfGlck and AcGlck and have identified several small molecules with IC_{50} values < 5μM that may serve as lead chemotypes for future therapeutic design.

Cathepsin C-like protease 1 post-translationally modifies *Toxoplasma gondii* secretory proteins for optimal invasion and egress

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South Carolina COBRE P20GM109094/NIH R01AI143707 Steven A. Kautz

The protozoan parasite *Toxoplasma gondii* infects one-third of the global human population and is particularly dangerous for immunocompromised individuals. *T. gondii* must synthesize, process, and secrete specialized effector proteins to establish infection. For example, *Toxoplasma* utilizes microneme proteins for attachment and invasion of host cells. Previous work revealed that some of these micronemal invasion effectors are proteolytically processed by an aspartyl protease (TgASP3) within a post- Golgi compartment. Additionally, the endolysosomal system in *T. gondii* is a hub for protein trafficking and processing which includes the lysosome-like vacuolar compartment/plant-like vacuole (VAC/PLV) and the endosome-like compartment (ELC). Housed within these organelles are cathepsin proteases which also play an important role in proteolytic maturation of micronemal invasion effectors. For example, the VAC/PLV-localized, cathepsin L-like protease (TgCPL) maturates TgMIC3 and TgMIC2- associated protein (TgM2AP). Additionally, *Toxoplasma* expresses a cathepsin B-like protease and two cathepsin C-like proteases during acute infection. Cathepsin C-like protease 1 (TgCPC1) was previously identified as a dense granule protein. In this study, by two independent strategies of epitope tagging TgCPC1 at different locations, we found that this protease largely localized to the ELC but could also be detected within the VAC/PLV. Furthermore, *Tgcpc1* was genetically ablated and the resulting *Δcpc1* mutant displayed multiple defects within the lytic cycle, specifically invasion, egress, and migration. Additionally, *Δcpc1* displayed defective secretion and trimming of several microneme proteins, most notably TgM2AP, TgMIC5, and perforin-like protein (TgPLP1), while protein trafficking was unaltered. Interestingly, processing of subtilisin protease (TgSUB1) was completely blocked within *Δcpc1.* Defective trimming of TgM2AP and TgSUB1 was also observed in parasites treated with an inhibitor targeting the *Plasmodium* ortholog of TgCPC1, further suggesting that TgCPC1 is involved in processing microneme proteins. Overall, these results indicate that TgCPC1 plays a significant role in processing secreted effector proteins which are crucial for successful invasion and egress.

Studies resolving unexpected functions and regulation of fructose 1,6-bisphosphatase in the parasite *Trypanosoma brucei*

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Glucose-6-phosphate (G6P) is a key metabolite, required for both glycolysis and the pentose phosphate pathways. This critical compound is generated by either glycolysis or gluconeogenesis (GNG), with the former utilizing glucose while the later utilizes non-carbohydrate sources. *T. brucei* alternates between a source of high (~5 mM) glucose in the mammalian bloodstream as the bloodstream form (BF) and the tsetse fly vector as the procyclic form (PF) where glucose is scarce. BF parasites generate ATP predominately via glycoslysis. Presumably, gluconeogenesis (GNG) is active under low-glucose conditions. However, the presence of an active GNG pathway in culture under low-glucose conditions has yet to be demonstrated. Fructose 1,6 bisphosphatase (FBPase), the key enzyme in gluconeogenesis, has been identified in *T. brucei* and is expressed in both PF and BF parasites. FBPase activity has been difficult to detect while the recombinant enzyme has robust activity, leading to the speculation that that a post-translational modification inhibits its activity. In previous work, FBPase-deficient cells had no growth phenotype in culture but were unable to establish an infection in tsetse flies. Western analysis using known amounts of rFBPase reveal that expression is very low (xx). Using a sensitive, fluorescence-based FBPase assay, we have been able to measure activity and found that it is influenced by cell density and extracellular glucose levels. In glucose-deplete media FBPase activity was low in log-phase culture and increased in stationary phase. Conversely, in glucose-rich media FBPase activity was highest in log-phase culture and decreased in stationary phase. We were surprised to find that when cells are in log-phase growth, FBPase activity is higher in glucose-rich media; conditions we anticipate gluconeogenesis would not be required for generation of glucose-6-phosphate. This observation suggests that FBPase may play a novel role in *T. brucei* metabolism.

Regulatory Roles of Resveratrol in Skin Inflammation

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South Carolina COBRE P20GM103641 Nagarkatti Mitzi, Prakash Nagarkatti

Atopic dermatitis (AD)/eczema, a chronic inflammatory disease characterized by pruritic skin lesions, affects 2 of 10 children worldwide out of whom about 60% will experience some AD symptoms in adulthood. Little is known about the alterations occurring in the skin and the implications of skin mast cells (MC), innate tissue-resident cells, in the development of skin lesions featured in eczema. Using a variation of a well-established human AD-like mouse model, our lab previously reported a lesion-enabling function of MC and MC-derived sphingosine-1-phosphate (S1P), a pro-inflammatory sphingolipid metabolite, in skin inflammation and cell infiltration observed in the prelesional phase of eczema. To induce skin lesions, this preclinical model of AD consists in three epicutaneous (EC) applications of antigen ovalbumin (OVA) or saline vehicle controls through gauze patches applied on the shaved and tape-stripped dorsal skin of mice for seven-day periods. One 7-day OVA (or saline) EC exposure triggered prelesional skin alterations. Resveratrol (RSV) is a naturally occurring polyphenol endowed with health-benefiting functions. However, contrasted reports on MC activation and S1P production remain roadblocks to therapeutic indications for AD. We hypothesized that MC-initiated early skin inflammation, driven by local S1P elevation, will be alleviated by RSV. When repeating the model in the presence of RSV (EC 2.5 µg applied daily), local cellular infiltration was prevented. Moreover, whereas MC numbers were similar in RSV/OVA- or vehicle/OVA-treated skins, RVS significantly inhibited local MC activation, decreased OVA-induced cellular infiltration and CCL2 and CCL5 chemokine skin-associated mRNA levels. Our findings indicate that topical RSV may prevent the development of AD by reducing cell recruitment and inflammation through its suppressive effects on chemokine gene expression. Future studies will delineate the anti-inflammatory mechanisms of RSV in skin and in MC through a regulatory signaling network engaged in skin inflammation, involving MC activation and S1P signaling.

Piezo1 channels are mechanosensors in CNS capillaries

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Vermont COBRE P20GM135007 Mary Cushman

Cerebral blood flow (CBF) is exquisitely controlled to meet the ever-changing demands of active neurons. This activity-dependent blood delivery process in the brain is rapidly and precisely controlled through molecular mechanisms collectively termed 'neurovascular coupling'. Our recent work provided evidence that brain capillaries act as a neural activity-sensing network. In particular, brain capillary endothelial cells (cECs) sense neurovascular coupling agents released from neurons onto the outer capillary wall and initiate different signaling cascades to control CBF. The lifelong flux of blood cells and plasma through narrow-diameter capillaries imposes shear stress on the inner capillary wall. However, whether—and if so how—CBF could be mechanically sensed in capillaries is not known. Here, we propose that the mechanosensitive Piezo1 channels operate as mechanosensors in brain capillaries to ultimately regulate CBF. Electrophysiological and immunohistochemical analyses confirmed the expression and function of Piezo1 channels in CNS cECs (brain and retina). Mechanical or pharmacological activation of Piezo1 channels evoked currents that were sensitive to Piezo1 channel blockers or the genetic deletion of the channel. Using genetically engineered mice with an endothelial-specific Ca^{2+} indicator, we observed that Piezo1 channel activation triggered Ca^{2+} signals in cerebral and retinal cECs. The mechanosensitivity of capillary Piezo1-mediated Ca^{2+} signals was confirmed using an *ex vivo* pressurized retina preparation. Focal stimulation of Piezo1 in brain capillaries increased CBF, consistent with Piezo1-mediated Ca^{2+} signals facilitating the synthesis of the endothelium-derived vasodilator, nitric oxide. In conclusion, this study shows that Piezo1 channels act as mechanosensors in CNS capillaries and that these channels initiate crucial Ca^{2+} signals that are involved in CBF control. We further speculate that this mechanism of CBF control is altered in disorders characterized by altered hemodynamics, such as hypertension.

Personalized Pacing: a New Paradigm for Diastolic Heart Failure Treatment

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Background: Without evidence-based guidance the lower heart rate (HR) setting of permanent pacemakers is typically left at the factory setting of 60 beats-per-minute (bpm). This one-size-fits all approach does not take into account that adult resting HR averages between 71 to 79bpm and is predicted by body size. Increasingly, evidence suggests that patients with diastolic dysfunction and/or heart failure with preserved ejection fraction (HFpEF) may benefit from a higher resting HR.

Methods: In this prospective, double-blinded randomized controlled pilot study, patients with pacemakers, diastolic dysfunction and/or HFpEF were randomized to either a personalized pacemaker lower rate setting or to the conventional 60bpm setting. Personalized HRs were derived from an algorithm, validated in large cohorts, predicting individual resting HRs based on height and cardiac function. At planned interim analysis of this study, change in Minnesota Living with Heart Failure Questionnaire (MLHFQ) scores and N-terminal brain natriuretic peptide (NTproBNP) levels were compared from baseline to 1-month follow-up.

Results: The primary outcome of change in MLHFQ scores from baseline improved in the personalized HR group (n=45) by 10.6 points (95% confidence interval [CI] 6.3, 14.9; $p<0.001$) at 1 month follow-up using paired t-test. MLHFQ scores did not significantly change in the control group (n=41). Per-individual change in NTproBNP levels from baseline to 1 month were on average 779.45 nanogram/liter lower in the treatment group than the control group by paired then unpaired t-tests but this was not statistically significant ($p=0.26$). Blinding was assessed at 1 month by asking participants if they believed that their pacemaker lower rate was changed: 80.2% (69/86) were uncertain or guessed incorrectly, while 19.8% (17/86) guessed correctly.

Conclusions: At planned 1 month interim follow-up of this 12 month study, the primary outcome of quality of life scores significantly improved in the personalized HR group compared with the control group.

Chronic Hypertension Impairs Hippocampal Vascular Function and Memory in Male and Female Rats

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Hypertension and psychological stress are major risk factors of dementia; however, their effects on the vasculature of the cognition-centric hippocampus remain unclear. This study investigated hippocampal vascular function and memory using a novel model of neuroendocrine stress and hypertension in which vector mediated brain-derived neurotrophic factor (BDNF) overexpression in the paraventricular nucleus of the hypothalamus (PVN) induces chronic stimulation of the major hypothalamic stress pathways leading to significant long-term elevation of blood pressure. Male and female Sprague Dawley rats received PVN injections of AAV2-BDNF vector to overexpress BDNF or AAV2-GFP vector for control (n=4-8/group). Four weeks later, hippocampal-dependent memory function was determined with an object recognition task. Hippocampal arterioles (HAs) were then isolated and studied pressurized in an arteriograph chamber. Vasodilator responses of HAs to mediators of functional hyperemia were measured, including small- and intermediate-conductance calcium-activated potassium (SK/IK) channel activation via NS309, and activation of inward rectifier potassium (K_{IR}) channels by increasing extracellular K^+ from 3-15mM. Memory function was significantly impaired by hypertension (twoway ANOVA, Tukey's post hoc test, $F_{1,20}$=11.41; p=0.003), but sex had no effect ($F_{1,20}$=0.39; p=0.54). Object recognition was lower in both male and female hypertensive rats that spent 49±3% and 52±6% of the time with the novel object, compared to normotensive male and female rats that spent 66±3% and 68±5% of the time investigating the novel object. HAs from male and female hypertensive rats dilated significantly less to NS309 than controls ($F_{1,20}$=12.35; p=0.002), suggesting endothelial damage. Activation of K_{IR} channels with 15mM K^+ caused a 20-30% vasodilation of HAs from normotensive rats that did not occur in HAs from hypertensive rats that underwent a 3-5% vasoconstriction ($F_{1,20}$=24.90; p<0.001). Overall, the blunted vasodilation of HAs during hypertension suggests functional hyperemia is impaired in the hippocampus that could potentiate neuronal dysfunction and contribute to impaired memory and cognition.

Class-Effect of Antihypertensive Treatment on the Restoration of Functional Hyperemia Deficits in a Mouse Model of Chronic Hypertension

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Objectives: Functional hyperemia is the process underlying moment-to-moment adjustments in local blood flow to match the ever-changing neuronal activity within the brain. A growing body of evidence indicates that hypertension attenuates functional hyperemia in human patients and animal models of hypertension. However, the pathological mechanisms causing functional hyperemia deficits remain to be elucidated. Recently, we have demonstrated the involvement of a novel signaling pathway, capillary-to-arteriole signaling, in functional hyperemia. Our goal in this study was to examine the impact of chronic hypertension and the beneficial effect of antihypertensive treatment on functional hyperemia, specifically capillary-to-arteriole signaling, using a murine model of polygenic hypertension.

Methods: Functional hyperemia and capillary-to-arteriole signaling were examined 8-month-old hypertensive (BPH) and normotensive (BPN) mice. Antihypertensive treatment was started at 3-month-old, employing three clinically-used antihypertensive drugs with distinct pharmacological mechanisms (amlodipine; a Ca^{2+} channel blocker, losartan; an angiotensin receptor blocker, or atenolol; an adrenergic β blocker). Functional hyperemia was measured as cerebral blood flow increase in response to whisker stimulation by laser Doppler flowmetry. Capillary-to-arteriole vasodilatory signaling was evaluated using an ex vivo preparation of a parenchymal arteriole with intact capillary branches, and patch-clamp electrophysiology.

Results: Functional hyperemia was significantly blunted in BPH mice compared to BPH mice. Capillary-to-arteriole signaling was also attenuated in BPH mice. Remarkably, antihypertensive treatment with amlodipine or atenolol restored the whisker stimulation-induced functional hyperemia as well as capillary-to-arteriole signaling in BPH mice. Interestingly, losartan treatment had less benefit on functional hyperemia restoration despite the similar effects on blood pressure.

Conclusions: We first demonstrated the impairment of functional hyperemia, caused by the dysfunction of capillary-to-arteriole signaling, in a mouse model of chronic hypertension. Further, our data suggest that antihypertensive treatment can prevent the deficits in functional hyperemia, although the efficacy may differ among classes of antihypertensives.

Biomarkers of COVID-19 Coagulopathy and D-dimer in a Biracial Cohort Study

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Objective: Coronavirus disease 2019 (COVID-19) coagulopathy is characterized by elevated thrombo-inflammatory biomarkers, especially D-dimer, which predicts thrombosis, critical illness, and death. In the general, non-infected population, these biomarkers are higher in men, older adults and Black individuals. These groups also have higher rates of COVID-19 infection and death. We hypothesized that Black individuals would have an exaggerated correlation between D-dimer and thrombo-inflammatory biomarkers characteristic of COVID-19.

Approach and Results: Correlations of 10 thrombo-inflammatory biomarkers with D-dimer were studied in 1068 participants of the biracial REasons for Geographic And Racial Differences in Stroke (REGARDS) cohort. Adverse levels of most biomarkers, especially fibrinogen, factor VIII, C-reactive protein, N terminal pro-B-type natriuretic peptide and interleukin (IL)-6, were associated with higher D-dimer. Several associations with D-dimer differed significantly by race. For example, the association of factor VIII with D-dimer was more than twice as large in Black compared to White participants. Specifically, D-dimer was 26% higher per SD higher factor VIII in Blacks and D-dimer was only 11% higher per SD higher factor VIII in Whites. In Black adults, higher IL-10 and soluble CD14 were associated with higher D-dimer, but not in White adults. In contrast, albumin was negatively correlated with D-dimer in Whites, with no association in Blacks.

Conclusions: Findings suggest a hypothesis that Black persons may have an amplified thrombo-inflammatory response during COVID-19 due to their stronger correlations of key biomarkers with D-dimer prior to infection. Future research should examine the role of thrombo-inflammation in racial disparities in COVID-19.

Structural Neural Correlates of Coordination in Persons with Chronic Stroke

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Currently, little is known about structural connectivity damage and fine motor deficits post-stroke. The purpose of our study was to examine the relationship between structural connectivity within key brain motor areas and upper/lower extremity (UE/LE) coordination in chronic stroke. Data were collected from 55 participants (20 female; mean age 60.0 $\pm$ 10.2 years; time post-stroke 60.7 $\pm$ 56.0 months) and retrospectively analyzed. Participants underwent MRI and a comprehensive behavioral assessment that included UE/LE dexterity tasks (unilateral/ bilateral tapping). Interrater reliability of tapping counts was assessed using intraclass correlation coefficients (ICCs), and relationships between structural connectivity amid a sensorimotor subnetwork and UE/LE coordination tasks were examined using Spearman's correlations. Interrater reliability for tapping counts was excellent, with ICC values ranging from 0.97-1.00. Correlations between structural connectivity and UE/LE coordination tasks were strongest involving cortico-subcortical connectivity between ipsilesional primary motor cortex (M1)/supplementary motor area (SMA) and the cerebral peduncle/thalamus, and between the primary sensory and anterior cingulate cortex and the thalamus ($p \leq 0.002$). M1-SMA connections were specific to LE coordination ($p \leq 0.002$). Structural connectivity between key motor regions, especially cortico-subcortical connectivity, is positively related to UE/LE coordination tasks post-stroke. There is a large degree of overlap between gross motor neural connections and those related to coordination, suggesting these tasks share similar motor networks.

Adenylate Cyclase Activated by Near-Infrared Window Light and Optimized for Optogenetic Applications in Mammals

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Light in the spectral region known as the near-infrared optical window (NIRW) penetrates deep through mammalian tissues, including the skull and brain tissue. Here we engineered a NIRW light-activated adenylate cyclase (NIRW-AC), IlaM5, for optogenetic control of cAMP-dependent pathways in mammals. In favorable contrast to the photoactivated adenylate cyclases generated previously, IlaM5 has significantly higher activity at 37 ºC and is better expressed in mammalian cells. We verified the ability of IlaM5 to photoactivate (i) cAMP-dependent gene expression in HEK293 cell culture and (ii) Hyperpolarization-activated cyclic nucleotide–gated (HCN) channelsin thalamic relay neurons. IlaM5 delivered into the ventral basal thalamus of mice was used suppress spindle oscillations, a HCN channel-dependent wave pattern of the sleeping brain hypothesized to function in memory consolidation and refinement of brain circuits. Spindle waves in mice during sleep can be suppressed robustly and reversibly via an external IR-light source, i.e. transcranially. Because cAMP signaling is a prevalent mode of regulation, the NIRW-AC adapted for mammals is expected to enable optogenetic control of cAMP signaling pathways in deep mammalian tissues in a noninvasive, temporally resolved and cell type-specific manner.

INBRE

Genomic epidemiology of COVID-19 in Alaska: multiple independent introductions and community spread of SARS-CoV-2

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University of Alaska INBRE 2P20GM103395 Brian Barnes

Alaska INBRE One Health is supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number 2P20GM103395.

Project Summary: As of 10 August 2020, there have been 4,473 diagnosed cases of COVID-19 in Alaska (0.6% of the population), 156 hospitalizations, and 26 deaths. More than 60% of cases occurred after the July 4th holiday, when community health measures were relaxed. However, it is unclear how COVID-19 was introduced and spread. We conducted a genomic epidemiology analysis to identify clusters of linked cases and to differentiate local community spread of COVID-19 from novel introductions from other regions in Alaska or out-of-state. Building on the Alaska INBRE-supported Pathogenomics Toolkit, a student-led initiative to build pathogen sequencing capacity in the University of Alaska, we sequenced the complete genomes of SARS-CoV-2, the etiological agent of COVID-19, from early infections in the state.

Results: We specifically amplified cDNA from SARS-CoV-2 by two-step RT-PCR from nasopharyngeal and sputum RNA collected from COVID-19 cases. These cDNA libraries were analyzed by multiplex nanopore sequencing on a MinION platform. The ARTIC bioinformatics workflow was adopted for consensus genome assembly with an overall recovery rate of 75% with 39 genomes of 52 samples sequenced. In parallel, 31 additional genomes were deep sequenced by Illumina short read NGS technology and assembled using CDC's Sequencher-based Applied Molecular Diagnostics pipeline. SARS-CoV-2 genomes were clustered by genetic similarity and maximum likelihood phylogenetics using NextStrain. We identified seven genetic based case clusters of community spread throughout Alaska with this analysis. At least five independent introductions of COVID-19 occurred in Alaska in March and April 2020 with two additional introductions in June 2020. Thus, continual rapid analysis of SARS-CoV-2 genomes provides evidence that despite implementing testing for travellers, and public health measures, COVID-19 was introduced over multiple events, leading to local community spread. We propose that SARS-CoV-2 genomic epidemiology analyses supplement existing contact tracing methods to better inform community intervention against COVID-19.

Challenges and opportunities for undergraduate engagement in biomedical research in Arkansas

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Introduction: Evaluation often fails to account for the way in which institutional and broader social, cultural, economic and political contexts affect program outcomes. Qualitative methods are uniquely suited to identifying institution- and context-level features which impact program outcomes. Findings can be used to inform the development of realistic program improvements.

Objectives: Identify features of the Arkansas INBRE (IDeA Network of Biomedical Research Excellence) program, institutions, and broader contexts which impact undergraduate engagement in biomedical research at two primarily undergraduate institutions (PUIs) in Arkansas. Methods: Nine (N=9) faculty and staff from two PUIs in Arkansas were invited by email to participate in a 60-minute telephone interview. Qualitative data was collected using interview guides containing open-ended questions informed by domains from the Context, Input, Process and Product (CIPP) evaluation model. Interviews transcripts were analyzed using qualitative content analysis with assistance from MAXQDA software.

Results: Participants expressed that the INBRE program was critical for biomedical research at their respective institutions. Initial analysis of interview transcripts revealed program-, institution-, and context- level challenges to engaging undergraduate students in biomedical research. Participants at both institutions cited the declining pool of college applicants with corresponding increased teaching loads as a barrier to maintaining viable research programs. Participants recommended classroom-based research as a potential strategy to overcome this challenge and to continue to engage undergraduates in biomedical research.

Conclusion: The Arkansas INBRE was perceived as having had a significant positive impact on engaging faculty and undergraduate students in biomedical research at the two PUIs. The program will likely need to adapt to changes arising from broader contexts to remain relevant in the future. These findings suggest a need for additional qualitative and CIPP-informed evaluation of INBRE programs. Data collection and analysis are ongoing for this evaluation.

The human pathogen *Candida glabrata* is uniquely susceptible to killer toxins produced by the Brewer's yeast *Saccharomyces cerevisiae*

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Combatting the spread of drug-resistant fungi is a serious threat to human health and requires the discovery of new antifungal compounds with novel mechanisms of inhibition. Alternatives to existing antifungal drugs are "killer toxins" that are proteins produced by many different species of "killer yeast", including the Brewer's yeast *Saccharomyces cerevisiae*. Toxigenic strains of *Saccharomyces cerevisiae* can inhibit the growth of many fungal pathogens but are often strain-specific in their antifungal activities, limiting their usefulness as therapeutics. After testing over 10,000 interactions between toxigenic yeasts and fungal pathogens we have determined that *Candida glabrata* is uniquely susceptible to ionophoric killer toxins that attack the fungal plasma membrane. Despite the lack of natural resistance, mutant strains of *C. glabrata* that are able to grow in the presence of killer toxins were selected, followed by whole genome sequencing to determine mutations responsible for resistance. From 20 resistant strains of *C. glabrata*, we identified mutations in 10 genes, including *TAO3*, *FKS1*, and *VPS54*. These three genes are important for resistance to echinocandins and azoles, which are the frontline therapeutics used against invasive fungal disease. We found that *VPS54* mutants that impart killer toxin resistance lost prior resistance to fluconazole and became hypersensitive to the drug. Mutations in *TAO3* and *VPS54* became hypovirulent in an animal model for yeast infection, with *TAO3* mutations more than doubling the time to 50% lethality compared to the parental strain of *C. glabrata*. Overall, the universal susceptibility of *C. glabrata* to killer toxins and the fitness tradeoffs associated with resistance suggest that killer toxins have potential as therapeutics to treat the growing challenge of drug-resistant candidiasis.

Key Perspectives from Obstetric Patients and Providers to Inform the Design of a Mobile App for Exercise During Pregnancy and Postpartum in a Rural Setting

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Background: Mobile health technology offers the opportunity for women to engage with physical activity promotion programs without many of the challenges commonly associated with exercise during and after pregnancy (which may be especially relevant during the COVID-19 pandemic). Such technology, including apps, may be especially useful in under-resourced rural environments. However, for a mobile health app to be effective, it must be tailored to the needs of the population for whom it is designed. We conducted the first known study on perspectives of pregnant women, postpartum women, and obstetric health care providers in a rural setting on needs related to the development of a mobile app designed to increase physical activity.

Methods: Focus groups and in-depth face-to-face personal interviews were conducted with 14 pregnant women, 13 postpartum women, and 11 health care providers in a rural community. Semi-structured questions were asked utilizing constructs of the Health Belief Model. Recordings of all in-depth interviews and focus groups were transcribed and standard content analyses were conducted.

Results: Participants expressed several key perspectives about physical activity during and after pregnancy which encapsulated two main themes: 1) physical activity as critical for weight control and 2) the need for evidence-based exercise information. These perspectives contributed to developing a next phase in physical activity promotion: the development of a mobile app designed to increase physical activity during and after pregnancy. Key desired features of this app, identified by study participants, include goal setting/progress tracking, evidence-based exercise guidance tailored to specific time points of pregnancy and postpartum, social support via community-based forum, symptom tracking, time-efficient workouts, and push notifications.

Conclusions: The perspectives identified by participants in the study are currently integrated into a newly developed mobile app, and early app testing is underway. Future directions include a pilot study to determine feasibility and potential efficacy.

Distinct roles for Notch1 and Notch3 in human adipose-derived stem/stromal cell adipogenesis

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Louisiana INBRE GM103424-19 Konstantin Kousoulas

The role of the Notch signaling pathway in adipogenesis has long been controversial as the role of individual Notch receptors appears to vary with experimental conditions. Here we demonstrate that in human adipose-derived stem/stromal cells (hADSCs), Notch1 and Notch3 have distinct expression profiles and roles during adipogenesis. Expression of these Notch receptors changed during adipogenesis with Notch3 expressed prior to the formation of lipid vesicles and Notch1 only appearing after vesicle formation. In addition, the siRNA-mediated Notch3 knockdown demonstrated an increased expression of PPARγ, an adipogenic marker that was complemented by a marked decrease in expression of β-catenin, the key functional component of the canonical Wnt/β-catenin signaling pathway. This study deepens the understanding of Notch signaling by clarifying the distinct roles of Notch1 and Notch3 during adipogenesis offering a novel therapeutic target for research aimed at obesity and diabetes.

Targeted Delivery of Doxorubicin Liposomes for Her-2+ Breast Cancer Treatment Anup Kundu
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Purpose: The adverse side effects and toxicity caused by the non-targeted delivery of doxorubicin has emphasized the demand of emerging a targeted delivery system. The goal of this study is to enhance the delivery of doxorubicin by formulating an aptamer-labeled liposomal nanoparticle delivery system that will carry and deliver doxorubicin specifically into Her-2+ breast cancer cells.

Methods: Twelve liposomal batches were prepared using different saturated (HSPC and DPPC) and unsaturated (POPC and DOPC) lipids by thin film hydration. The liposomes were characterized for their particle size, zeta potential, and drug encapsulation efficiency. The particles were also assessed for in vitro toxicity and DOX delivery into the breast cancer cells. Results: The formulations, F1 through F12, had a small particle size of less than 200 nm and a high entrapment efficiency of about 88±5%. The best formulation, F5, had a particle size of 101 ±14 nm, zeta potential of 5.63±0.46 mV, and entrapment efficiency of ≈93%. The cytotoxicity studies show that the DOX-loaded liposomal formulations are more effective in killing cancer cells than the free DOX in both MCF-7 and SKBR-3 cells. The uptake studies show a significant increase in the delivery of DOX by aptamerlabeled F5 into Her-2+ breast cancer cells compare to non-aptamer-labeled nanoparticles.

Conclusions: This preliminary study indicates that aptamer-labeled F5 nanoparticles among several batches showed the highest uptake as well as the targeted delivery of doxorubicin into Her-2+ breast cancer cells. Thus, aptamer targeted approach results in substantial reduction in the dose of DOX and improves the therapeutic benefits by promoting the target specificity.

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Computational Modeling of Blood Metabolomics Biomarkers Addressing Racial Disparity

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Alcohol use disorders are among the most devastating disorders contributing to the global burden of disease (139 million disability-adjusted life-years). In the United States, over 8% of the population meets the criteria for alcohol dependence with alcohol-related problem cost exceeding 223 billion dollars, 88,000 deaths, and nearly 10,000 driving fatalities per year. Furthermore, alcohol contributes to over 200 diseases such as alcohol dependence, liver cirrhosis, cancers, and injuries. The FDA approved Acamprosate in 2004 to treat alcoholism (anti-craving drug). Multiple clinical trials have demonstrated that Acamprosate is effective in the maintenance of abstinence from alcohol in identified populations of alcohol-dependent individuals. A meta-analysis of 17 studies, which included 4087 individuals, showed continuous abstinence rates to be significantly higher in Acamprosate- treated subjects when compared to placebo. In this meta-analysis, the continuous abstinence rate after 6 months of Acamprosate treatment was 36.1%, which was significantly greater than the placebo rate of 23.4%. Given the variable efficacy of Acamprosate, the identification of biomarkers or genetic factors that could help predict treatment outcomes would represent a major public health benefit. We hypothesize that a metabolite in the blood of alcohol dependent patients could associate with anxiety based heavy drinking patterns and/or race, which may play an important role in explaining Acamprosate efficacy in subpopulations of alcohol dependent patients. In this proposal, we will develop biomarker paradigms to be applied to metabolomics and clinical measurement data using computational modeling.The results will allow us to establish a prediction algorithm between drinking patterns and depression in Acamprosate efficacy. Additionally, we will test whether racial disparity between Caucasian and African-American alcohol dependent populations influences the drinking pattern. The results from this study will suggest a prediction paradigm for the identification of a sub-population in alcohol dependence.

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Mechanism of Translation Initiation in the protozoan parasite *Giardia lamblia*
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Abstract

Translation initiation factor eIF4F is essential for cap-dependent translation initiation in eukaryotes. eIF4F is a trimeric complex consisting of a scaffold protein eIF4G, cap-binding protein eIF4E and DEAD--box RNA helicase eIF4A. eIF4F binds to the 5' cap structure of the mRNA through eIF4E and facilitates the binding of the preinitiation complex (PIC) via protein-protein interactions of eIF4G with eIF3 in mammals or with eIF5 in yeast. In Giardia, homologs for eIF4E (GleIF4E2) and eIF4A (GleIF4A) have been identified but not for eIF4G. To address how PIC is recruited to the 5' end of the mRNA in the absence of eIF4G homolog, we have used yeast two-hybrid assays to identify potential interactions of GleIF4E2 with the components of the PIC. The results show that GleIF4E2 can interact with the β subunit of the initiation factor GleIF2, a `component of the PIC. ZDOCK modeling of the GleIF4E2--GleIF2β complex revealed that the dorsal side of GleIF4E2 is likely involved in binding to GleIF2β. Site-directed mutagenesis of the ZDOCK predicted residues of GleIF4E2 disrupted of its interaction with GleIF2β. These results suggest that GleIF4E2 can facilitate the recruitment of the PIC to the 5'end of the mRNA by interacting directly with PIC. The role of GleIF4A in translation initiation in Giardia is not clearly understood. Interestingly, Pateamine A, a specific inhibitor of human eIF4A, inhibited the growth of Giardia in a dose-dependent manner, suggesting that the activity of GleIF4A is probably required for translation. Using yeast two-hybrid assays, we have identified a novel interaction of GleIF4A with i subunit of the initiation factor GleIF3 (GleIF3i), another component of the PIC. Site-directed mutagenesis of ZDOCK predicted residues in N-terminal domain of GleIF4A disrupted its interaction with GleIF3i. These results indicate that the GleIF4A can also interact directly with the components of the PIC.

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Establishing a Protocol for Activating the Massive Transfusion Protocol for Air Medical Service Trauma Patients

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Trauma is the leading cause of death world-wide in persons under the age of 40 and accounts for approximately 10% of all deaths in general. Massive hemorrhage is a major cause of early death in trauma patients in both civilian and military trauma care. In the initial management of trauma patients both interventions in hemostasis and proper preparation of blood products are crucial to prevent hemorrhagic shock, which can easily lead to early death.

Massive Transfusion Protocols (MTPs) are initiated per established policies in many trauma centers, but too many centers still rely heavily on subjective clinical judgment of patient's initial vital signs. As familiarity with MTP triggers has increased, there is a growing interest and need in applying these in the civilian and military populations to initiate them earlier and to identify easy and fast ways to predict the need for MTP.

From previous studies of MTP protocols and determined that many of them are very complex and require variables that are not available until trauma arrival and are thus not usable for air medical service and no single measurement of vital signs appeared to be are a good predictor in determining the need for MTP activation. We focused on identifying a reliable and easy-tocalculate MTP trigger. Our goals were to (1) evaluate the reliability of air medical blood product transfusion as a trigger for MTP, (2) determine the reliability of air medical calculation of Shock Index (SI) as a trigger for MTP and (3) identify a rapid and simple scoring system for MTP based on a comparison across existing scoring systems.

Louisiana

Detecting Race-Relevant Molecular Biomarkers with Clinical Utilities Using Multi-omics Data Across Tumor Types

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To date, significant progress has been made in our understanding of the role of socioeconomic factors in cancer racial disparities. Increasing evidence now suggests that a number of intrinsic molecular factors specific to malignant cells must also partly account forthe observed health inequalities. Although research has begun to explore the biological basis of cancer disparities, most existing work is limited to several common cancer types and does not methodically explore whether the observed genetic and molecular differences represent the clinically-meaningful racial disparities in other fatal human cancers. Moreover, massive amounts of multi-faceted omics data generated by high-throughput technologies have not been fully utilized and well integrated with clinical data to search for race-specific molecular characteristics, biomarkers or potential drug targets. The goal of this LBRN research project is therefore to address these significant limitations by performing an in-depth, data-driven, pan-cancer study to investigate the cancer-specific mutome, epigenome, and RNA-Seq transcriptome differences in different racial groups. The proposed study will focus on the eight TCGA cancer types, with pertinent cancer data from other sources (E.g. dbGaP, GEO, ICGC, etc.) being systematically utilized for methodology development and/or empirical validation throughout the entire project. For a specific cancer, in connection with clinical data, we will develop new bioinformatics algorithms and pipelines to analyze these multiple types of omics data individually. As such, we will establish a pan-cancer, race-relevant assemblage of coherent genes, modules and biological pathways, some of which will hold significance and promise for clinical use. This will provide large-scale direct molecular level evidence for the biological mechanism underlying racial disparities in cancer, which is practically impossible using the approaches of *in vitro, in vivo* and/or population follow-up. Furthermore, we will biologically validate the identified signatures for prostate cancer using clinical samples. A database for all pinpointed signatures will be constructed so that cancer disparity researchers can interrogate how various levels of molecular variations may alter gene functions in different cancers and races. A set of efficient and powerful analytical tools for the proposed data-driven analyses of health disparities in cancer will also be made publicly

Inhibition of Molecular Pathway of Tumor Progression in 3D Cell Cultures of Prostate Cancer Cells

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Anticancer drugs are powerful chemicals that kill cancer cells by arresting their growth at one or more checkpoints in their cell cycle or induce apoptosis. However, metabolic changes occurring in cancer cells are considered to be fundamental for the resistance to different types of chemotherapeutic drugs. Therefore, resistance to chemotherapy represents a major problem in the treatment of cancer. Resveratrol (RES) is a component of Asian traditional medicine used to treat cardiovascular diseases. Recently, RES has gained considerable attention as an anticancer agent potential use in chemoprevention and chemotherapy for various cancer forms relies on its effects on cell growth, apoptosis, and cancer metastasis. As RES appears to have many anti-tumor effects on different cancer cell types, the molecular basis of these effects needs to be extensively studied using a cell culture model that best resembles the tumor environment in the body. To identify RES target genes involved in the activation of cell apoptotic pathways, we exposed different concentrations of RES to prostate cancer cells DU145 and performed transcriptome analyses by proteome profiler. There were 16 proteins (such as Bcl2 ,Bcl-x, cIAP1 and 2, xIAP) down-regulated at least twofold (P. <0.05) in response to RES and 4 proteins(such as Pro-Caspase-3 , p21) up- regulated in 3D cell cultures of prostate cancer cells by human apoptosis array. Further analysis by human XL oncology array, there were 22 proteins (Such as VEGF, FGF basic, ErB2/3/4) down-regulated at least twofold (P. <0.05) in response to RES. Under-expression of anti-apoptotic genes and over-expression of pro-apoptotic genes can result in the increase of cancel cell death. Inhibition of oncology genes enhances the effects of apoptotic signals and tumor progression in prostate cancer cells. These will allow us to better understand RES mechanism of action and its potential use as a coadjuvant drug for established cancer treatments.

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Sunflower trypsin inhibitor template peptides for immunomodulation

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Louisiana INBRE P20GM103424 Konstantin Kousoulas

Naturally occurring cyclic peptides such as sunflower trypsin inhibitor (SFTI) with disulfide bonds are resistant to thermal, chemical, and enzymatic degradation. The objective is to use the SFTI template to design stable peptide molecules to modulate the protein-protein interactions (PPI) of CD2-CD58 (CD48 in mice) adhesion/costimulatory molecules. Blocking of CD2-CD58 molecules interactions results in inhibiting co-stimulatory signals required for the generation of the immune response that ensures the production of pro-inflammatory cytokines and inflammation. A grafted peptidomimetic (SFTI-DBF) from the SFTI template was generated to inhibit the PPI of CD2-CD58 interactions. The peptide exhibited cell adhesion inhibition activity in a cellular assay with an IC50 value of 0.6 nM. *In vitro* stability studies of this peptide using simulated gastric and intestinal fluid indicated that the peptide is stable up to 6 h in these fluids. Pharmacokinetic studies of this peptide in DBA/1 mice suggested that peptide has a terminal half-life of 30 h in mice. Binding studies of this peptide to CD58 protein using surface plasmon resonance and flow-cytometry indicated that SFTI-DBF binds to the CD58 protein adhesion domain. To evaluate the ability of this peptide to inhibit protein-protein interactions, proximity ligation assay (PLA) was carried out. Peptide SFTIDBF was shown to inhibit the adhesion of HFLS-RA cells and Jurkat T cells, presumably by inhibiting CD2-CD58 costimulatory molecules. To understand the molecular mechanism of signaling inhibition by peptides, the co-culture of Jurkat cells with HFLS-RA cells were used. Coculturing of these cells and treating with anti-CD3 significantly increases the calcium concentration in Jurkat cells. By treating the cells with different concentrations of SFTI-DBF, calcium flux was shown to be decreased as detected with calcium sensitive dye. Thus the peptide SFTI-DBF inhibits the PPI of CD2-CD58, resulting in inhibition of T cell signaling for inflammatory kinase and modulate the immune response. This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under the grant number P20GM103424-18.

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Topically applied fisetin ameliorates psoriasiform dermatitis in Balb/c mice: Involvement of mTOR-regulated signaling pathways

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Louisiana INBRE P20GM103424 Konstantin Kousoulas

Psoriasis is a very common chronic and currently incurable inflammatory skin disease with complex and incompletely understood molecular pathogenesis. Designing novel psoriasis management approaches require a deeper understanding of its signaling and pathogenesis, as treatment for moderate to severe psoriasis remains elusive. Dysregulation of the central mTOR signaling has emerged, as a clinically relevant target for psoriasis and due to the paucity of effective treatments, identifying agents that can abrogate their activities could potentially meet the need for novel and cost-effective approaches to achieve significant long-term benefits. We identified fisetin, a dietary polyphenolic ingredient, as a potent mTOR/P70S6K kinase inhibitor (Biochem Pharmacol.;89(3):349-60.), which attenuates psoriasis-like features *in vitro* (Cells. 2019 Sep 15;8(9). pii: E1089.). Herein, we examined the effect of fisetin *in-vivo* using an imiquimod (IMQ)-induced psoriasis-like disease model in Balb/c mice, and begun generating a myeloid lineage mTOR knockout mouse model for validating the concept. Topical application of IMQ cream induced mouse psoriasisiform dermatitis lesions characterized by increased erythema, ear swelling (acanthosis and hyperkeratosis), and scaling; immunohistopathological analysis revealed the activation of Akt/mTOR pathway when compared to matched control tissues. Lesional skin tissue sections of mice topically treated with fisetin (1mg/cm2 of shaved skin/ear, daily) exhibited significant decrease in i) psoriasiform hyperplasia including ear swelling and epidermal thicknesses, ii) erythema, iii) levels of inflammatory mediator and cytokines (IL-22 and iNOS) and iii) proliferation (Ki-67) when compared with control mice. Furthermore, fisetin-treated lesional skin tissue sections showed decrease in the phosphorylated forms of Akt and mTOR and downstream targets. Collectively, our data affirm mTOR involvement in psoriasis and suggest fisetin as a modulator alone or as an adjuvant to current therapies. Validating these in the myeloid lineage mTOR deficient mice may provide data that could be useful for treating psoriasis and other inflammatory skin diseases.

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Conformationally Constrained Multicyclic Grafted Peptidomimetic as an Immunomodulator

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Immune system mechanism can be regarded as double edge sword; one edge is for protecting our body against foreign antigens and eliminating them whereas the other edge on activation has detrimental effect leading to self-destruction of our tissues and cells. The balance towards the beneficial effect of immune system is maintained by co-stimulatory and co-inhibitory receptors expressed on T- cells and antigen presenting cell (APC).CD58 is a co-stimulatory molecule found to be over-expressed in APC in autoimmune disease like rheumatoid arthritis. Inhibition of CD2- CD58 protein-protein interaction (PPI) that occurs between T-cells and APC can be a potential therapeutic intervention in treatment of such autoimmune disease. From our previous studies by alanine scanning followed by grafting on sunflower trypsin inhibitor (SFTI) we obtained a potent CD2-CD58 PPI inhibitor peptide (SFTI-AS1) with multiple conformations having an IC50 of 37 nM in lymphocyte epithelial cell adhesion assay. In this study our objective is focused on conformational constraining and locking of SFTI-AS1 into a major bioactive conformer peptide. The designed peptide SFTI-DBF has been found to be conformationally locked into major single conformer evident by NMR studies. SFTI-DBF inhibited adhesion between T-cells and RA cells with an IC50 of 3 nM. Binding study of SFTI-DBF with CD58 is confirmed further by molecular docking, flow cytometry and surface plasmon resonance. SFTI-DBF was able to inhibit CD2-CD58 PPI evident by Proximity Ligation Assay (PLA) and inhibits T-cell activation. SFTI-DBF was found to be stable in-vivo with half-life of 30 hours in pharmacokinetic study. In an in-vivo mice model of collagen induced arthritis, SFTI-DBF was able to significantly reduce the arthritis incidence, arthritis score and collagen antibody level. To summarize, a potent conformationally constrained grafted peptidomimetic is designed and studied both in-vitro and in-vivo as a potential therapeutic agent in rheumatoid arthritis.
This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under the grant number P20GM103424-18.

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Distinct roles for Notch1 and Notch3 in human adipose-derived stem/stromal cell adipogenesis

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The role of the Notch signaling pathway in adipogenesis has long been controversial as the role of individual Notch receptors appears to vary with experimental conditions. Here we demonstrate that in human adipose-derived stem/stromal cells (hADSCs), Notch1 and Notch3 have distinct expression profiles and roles during adipogenesis. Expression of these Notch receptors changed during adipogenesis with Notch3 expressed prior to the formation of lipid vesicles and Notch1 only appearing after vesicle formation. In addition, the siRNA-mediated Notch3 knockdown demonstrated an increased expression of PPARγ, an adipogenic marker that was complemented by a marked decrease in expression of β-catenin, the key functional component of the canonical Wnt/β-catenin signaling pathway. This study deepens the understanding of Notch signaling by clarifying the distinct roles of Notch1 and Notch3 during adipogenesis offering a novel therapeutic target for research aimed at obesity and diabetes.

Kifunensine compromises lung endothelial barrier function

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The pulmonary endothelium mediates key regulatory processes to maintain lung homeostasis. Disruption of this endothelial monolayer causes endothelial hyper-permeability and contributes to the pathogenesis of Acute Respiratory Distress Syndrome (ARDS). We have previously shown that Hsp90 inhibitors protect the lung endothelial barrier by inducing the unfolded protein response (UPR). In this study, we investigated the effects of the UPR suppressor kifunensine in the lung endothelial barrier integrity. Kifunensine induced the phosphorylation of myosin light chain 2 (MLC2) and activated the actin-severing activity of cofilin in bovine pulmonary artery and human lung microvascular endothelial cells. Measurements of transendothelial resistance demonstrated that the aforementioned kifunensin-triggered events increased the lung endothelial permeability in a dose-dependent manner. Our results suggest that UPR suppression compromises the function of the lung endothelial barrier. Thus, UPR induction may serve as a promising therapeutic approach against ARDS, including the COVID-19 related ARDS. Our study was supported by 1) The Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (5 P20 GM103424 and3 P20 GM103424-15S1) and 2) The R&D, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents through the Board of Regents Support Fund (LEQSF(2019-22)-RDA- 26) to NB (PI).

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Effects of Heat Shock Protein 90 Inhibition in the Lungs

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The inhibition of the heat shock protein 90 (Hsp90) is associated with anti-inflammatory effects and supports lung endothelial barrier integrity. In the current study we employed human lung microvascular endothelial cells to investigate the effects of the Hsp90 inhibitors 17-AAG, AUY-922 and 17-DMAG in the unfolded protein response (UPR) and viability of lung cells. Our observations indicate that moderate doses of those compounds trigger the activation of the UPR without inducing lethal effects in vitro. Indeed, AUY-922 triggered UPR activation in the lungs of C57BL/6 mice. UPR has been previously involved in the enhancement of the lung endothelial barrier function. Thus, the present study suggests that the barrier protective effects of Hsp90 inhibition in the lung microvasculature are highly probable to be associated with the activation of the UPR. Hence, the development of novel compounds which stochastically capacitate the repairing elements of UPR, may deliver new therapeutic possibilities against the severities of the acute respiratory distress syndrome.

Funding: This study was supported by 1) The Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (5 P20 GM103424-15 and 3 P20 GM103424-15S1) and 2) The R&D, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents through the Board of Regents Support Fund (LEQSF(2019-22)-RD-A-26) to NB (PI).

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P53 Deficiency Potentiates LPS-Induced Acute Lung Injury *in vivo*

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) represent a significant cause of morbidity and mortality in critically ill hospitalized patients. Emerging evidence suggest that the expression levels of P53 in the lungs are associated with the supportive effects of heat shock protein 90 inhibitors and growth hormone releasing hormone antagonists in the endothelium. In the current study, we employed an *in vivo* model of intratracheal administration of lipopolysaccharides (LPS)-induced ALI to investigate the role of P53 in counteracting LPSinduced lung inflammatory responses. In wild type mice, LPS induced the expression of IL-1α, IL- 1β, and TNFα in the lungs. Moreover, this endotoxin increased the bronchoalveolar lavage fluid protein concentration, and activated the actin-severing activity of cofilin. Those responses were more potent in P53 knockout mice, suggesting the crucial role of P53 in orchestrating rigorous endothelial defenses against inflammatory stimuli.

Funding: This study was supported by 1) The Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (5 P20 GM103424-15 and 3 P20 GM103424-15S1) and 2) The R&D, Research Competitiveness Subprogram (RCS) of the Louisiana Board of Regents through the Board of Regents Support Fund (LEQSF(2019-22)-RD-A-26) to NB (PI).

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Surveillance of Sewage Systems to develop an early warning system for identifying SARS-CoV-2 outbreaks in East Baton Rouge Parish and Ruston, Louisiana

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Sewage systems have long been monitored for presence of pathogens such as Poliovirus and Anti-microbial resistance. The SARS CoV-2 virus, initially believed to be limited to the respiratory system, is now confirmed to cause digestive disorders in addition to infection of multiple other organs. According to the World Health Organization detection of non-infective RNA fragments of SARS-CoV-2 in untreated wastewater and/or sludge has been reported in a number of countries. We have been tracking SARS CoV-2 in flow composite samples from the wastewater treatment plants in East Baton Rouge Parish since late May of 2020. We have standardized the extraction of Total RNA from sewage samples, conversion into cDNA, and used CDC authenticated Primer Probe Mixes to quantify the number of SARS CoV-2 Genome units in the various samples. Based on our initial studies we were able to establish a 14-day timeline between the enforcement of a mask mandate and fall in the number of Genome Units in sewage samples. We were also able to detect a spike in SARS CoV-2 Genome Units in sewage samples at least 7 days before the Louisiana Department of Health was able to report a spike in clinic testing. Recently we have added pumping stations near LSU A&M Baton Rouge, to compare the levels before and after arrival of students for the fall semester. Dr. Jamie Newman (LBRN – Pilot PI) at LA Tech University will be doing the same at the college town of Ruston, Louisiana. We will be presenting our observations on the effect of (i) the mask mandate and (ii) the impact a large influx of students on Campuses has on the prevalence of SARS CoV2 in the population. We will also test our ability to predict a spike in cases based on detection of SARS CoV-2 in campus adjacent sewage samples.

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Remote undergraduate research experiences involving bioinformatics and computational approaches

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Biomedical research is increasingly defined by a data explosion enabled by high throughput "-omics" approaches. This has enabled many biomedical research questions to be addressed using existing datasets. A barrier to such research is the computational skill needed to interrogate the data, which many experimental biologists lack. In response to COVID-19, which necessitated the cancelation of our on-site summer student research programs, Maine INBRE focused on developing resources that address the need for computational expertise that would facilitate remotely mentored research involving omics-scale datasets. Although Maine INBRE was not able to retool all of its planned summer fellowships into remote research experiences, we were able to do so for many through the coordinated efforts of the Bioinformatics and Research Training and Resources Cores and in collaboration with INBRE and COBRE investigators with omics data that invited further analysis. To that end the Bioinformatics Core (BC) developed online resources (e.g. Amazon Web Service working machines) to enable remote bioinformatics-based student research. Students were trained by BC staff via Zoom through structured presentations and met with BC staff online through biweekly office hours and in regular joint meetings with the research mentors. The BC generated reusable training modules consisting of markdown materials with accompanying short videos. As part of the summer undergraduate program the Research Training and Resources Core (RTRC) offered a "Communicating Science" class, to which BC leadership contributed presentations on data visualization. BC staff were also available to answer student questions via e-mail and on the LabCentral online platform developed and maintained by the RTRC. While we are still in the process of evaluating the results of the remote summer program, student presentations and feedback thus far indicate that it was a success and can be used as a model going forward for remote mentored research that interrogates omics-scale data.

*Presenter, Co-director of the Maine INBRE Bioinformatics Core

Mechanisms of Immune Suppression by Metastatic Cancer Stem Cells

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Mississippi INBRE P20GM103476 Mohamed Elasri

A critical issue for cancer treatment is the existence of cancer stem cells (CSCs), that drive the inexorable growth of malignant tumors, resist to treatments and render the opportunity for cancer recurrence. Eradication of CSCs is therefore a major challenge. Unfortunately, our understanding of cellular and molecular mechanisms that underlie CSC properties is limited. Our research on metastatic CSCs (mCSCs) has identified three novel mechanisms used to escape immune surveillance. 1) mCSCs but not bulk tumor cells miRNAs which suppress both activation and proliferation of effector T cells in response to immune surveillance. 2) mCSCs recruit T regulatory cells through secretion of CCL5/CCL17 and form physical interactions with recruited Tregs. 3) mCSCs drive macrophage polarization towards M2 through the generation of an arginine metabolite, asymmetric dimethylarginine (ADMA). In this study we will analyze whether secretion of immunoediting miRNAs by mCSCs is common in molecular subtypes of breast cancers. We will also study how effector T cells perceive the signals from these miRNAs. We will also examine whether blockage of CCL5/CCL17 with neutralizing antibodies prevents the recruitment of Tregs and the subsequent immune suppression driven by the recruited Tregs. Using a human protomicroarray, we will further identify proteins involved in the molecular interactions between mCSCs and Tregs. Finally, we will address the molecular mechanisms of how ADMA is accumulated in the microenvironment by mCSCs and how ADMA affects macrophage polarization by regulating mitochondrial function. With the completion of this proposal, the information collected from this work will not only provide mechanistic insights of tumor progression mediated by mCSCs to escape from immune surveillance but may also lead to more effective therapeutic interventions specifically for mCSCs.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

SCUBE3 is a Therapeutic Target that Drives Breast Cancer Progression and Resistance to Doxorubicin to TNBC

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Triple-negative breast cancer is the most aggressive subtype of breast cancer. Characterized by its lack of expression of hormone receptors, TNBC is commonly diagnosed in younger women below 50 years of age. Patients diagnosed with TNBC often have worse treatment outcomes. This is due to the lack of clear molecular targets associated with the disease. Chemotherapy remains the main choice of treatment for TNBC. However, with chemotherapy there are concerns such as toxicity, adverse side effects, resistance, and death. We have identified Signal peptide-CUB-EGF-like domain-contain protein 3 (SCUBE3), through a genome-wide loss of function study, as a molecular target that can improve TNBC treatment in combination with doxorubicin, a chemotherapeutic agent. The suppression of SCUBE3 expression sensitized TNBC cell lines to doxorubicin, compared to control. In our pre-clinical mouse study, SCUBE3 knockdown significantly improved doxorubicin treatment, compared to controls. SCUBE3 overexpression conferred TNBC growth and metastatic advantages. Also, we demonstrated that SCUBE3 is a crucial regulator of DNA damage repair genes, as ectopic or knockdown of SCUBE3 significantly altered the expression of BRCA1, BRCA2, RAD51, and EXO1, and Foxm1, compared to control. Lastly, our result showed that SCUBE3 regulates EGFR activation, which is commonly overexpressed in greater than 50% of TNBC cells. SCUBE3 was found to bind EGFR through immunoprecipitation, and ectopic SCUBE3 expression elevated EGFR phosphorylation. Similarly, knockdown of SCUBE3 reduced the levels of EGFR phosphorylation in TNBC cells. These findings demonstrate SCUBE3 could be an important target for TNBC and that modulators of SCUBE3 expression may offer an effective strategy for treating TNBC patients.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Quinacrine Triggers Chemosensitivity in Drug-Resistant Ovarian Cancer

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Mississippi INBRE P20GM103476 Mohamed Elasri

Quinacrine (QC), a quinine derivative, has been widely used in human civilization for the treatment of malaria during World War-II. Post second world war, scientists have evidenced QC as an anti- inflammatory and antioxidant agent. QC is currently approved by FDA for the treatment of lupus. It has been shown that QC treatment inhibits the proliferation of different cancer cells by targeting multiple pathways and triggers apoptosis in a caspase dependent mechanism. Efficacy of QC as an anti-cancer agent has been reported by several investigators and placed QC as a promising drug. Clinical trials with QC in combination with other antitumor drugs are ongoing for treating solid tumors as well. Our data shows that QC treatment induces autophagy in ovarian cancer cells by p62/SQSTM1 downregulation, LC3B-II accumulation and autophagosome formations. QC sensitizes the isogenic chemoresistant C13 and HeyA8MDR cells in an autophagy dependent manner when treated in combination with chemotherapeutic agents. *In vivo* studies demonstrate that QC alone and in combination with carboplatin suppresses tumorigenesis compared to carboplatin treatment alone. Unfortunately, irrespective of all the benefits, QC suffers from clinical limitations like poor bioavailability and therapeutic range in micromolar scale. Recently, some small molecules (e.g. curaxin, CBLC137) are screened that mimic QC's actions with a better bioavailability and structural stability. Nano-formulations of QC are designed to enhance the pharmacokinetic profile and bioavailability of QC.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

A novel epigenetic silencing mechanism on Ac/Ds transposons in maize

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Transposable element (TE) activity results in genome instability in a wide variety of organisms, including humans. This instability has been associated with several diseases, including neurofibromatosis, hemophilia and cancer. Epigenetic silencing is an efficient mechanism for the initiation and maintenance of TE repression on a genome-wide scale. Here we analyzed two independent cases of spontaneous silencing of the active maize Ac/Ds transposon system. This silencing was initiated by Alternative Transposition (AT), a type of aberrant transposition event that engages the termini of two nearby separate TEs. When AT occurs during DNA replication it can generate Composite Insertions (CIs) which contain inverted duplications of transposon sequences. We show that inverted duplications of two CIs are transcribed to produce dsRNA that trigger the production of siRNAs. These siRNAs include two distinct classes: a 24-nt class complementary to the TE terminal inverted repeats (TIRs) and sub-terminal regions, and a 21-22 nt class corresponding to the TE transcribed regions. Plants containing these siRNA-generating CIs exhibit decreased levels of Ac transcript and heritable repression of Ac/Ds transposition. This study documents the first case of TE silencing attributable to transposon self-initiated AT and may represent a general initiating mechanism for silencing of DNA transposons.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

MMPs as Therapeutic Targets in the Neurodegenerative Disease Spinocerebellar Ataxia Type 1

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Matrix metalloproteases (MMPs) are endopeptidases and therapeutic targets in Huntington's disease, a poly-glutamine disease. We suspect MMPs play a similar role in another poly-glutamine disease, Spinocerebellar Ataxia Type 1 (SCA1). SCA1 is a fatal neurodegenerative disease caused by a mutation in the ATXN1 protein. We hypothesize that MMPs degrade poly-glutamine aggregates enhancing mutant ATXN1 neural toxicity. In cell culture models, we found MMPs localize to mutant ATXN1 inclusions and increase degradation. Further, inhibition of MMPs enhanced inclusion body formation and reduced mutant ATXN1 degradation. Real-time PCR and transcriptomic analysis reveal that MMPs are upregulated in SCA1 animal models. Treating SCA1 mice with an MMP substrate competitor, an Elastin-Like-Polypeptide (ELP), improved balance and coordination and increased neuronal marker proteins. Together, our data suggests that MMPs may play a role in the SCA1 disease. To further validate MMPs as therapeutic targets, we will test the efficacy of ELP alongside chemical MMP inhibitors in SCA1 drosophila and mouse models. This work will identify MMPs as therapeutic targets in SCA1 and validate new MMP inhibitors, such as ELP, to treat MMP-involved diseases.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Improving the Reliability of Eye Tracking to Diagnose Concussion.
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Mild Traumatic Brain Injury (mTBI) or concussion affects more than a million of Americans each year and represents a great societal burden. Unfortunately, reliable biomarkers are still lacking. Probing oculomotor behavior, by measuring eye movements, has been hailed as a promising strategy to detect concussion, because circuits in the brain that are devoted to vision, vestibular or oculomotor function are omnipresent in the brain and almost certainly affected by any blow to the head. The results from studies, using eye movements as a biomarker to predict concussion, are encouraging, albeit not sufficiently strong to prove clinical utility. We aim to increase the prognostic power of eye tracking by introducing the following innovations: (1) test individuals, using a novel multifaceted oculomotor task whereby a large array of oculomotor variables are measured (2) expand the continuum of eye-movements that will be measured to include microsaccades, tiny eye movements that are made when the gaze is fixed, and (3) use novel metrics that focus on measuring deviation from normal oculomotor behavior rather than on the identification of an "oculomotor signature" of concussion. Using data collected from student athletes at Millsaps, we are recording eye movements from ~300 individuals while they are conducting a ~25-minute oculomotor task. Athletes who sustain a head injury during the following sports season will then be invited to retake the test. While this project is a longitudinal study that will culminate after several years of data collection, a pilot study where the proposed innovations were introduced, shows promising results.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Comparing Preventative Behaviors for COVID-19 among Individuals with and without Diabetes in South Mississippi

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The coronavirus disease COVID-19 spread rapidly affecting millions of people worldwide. Individuals with certain pre-existing chronic diseases, such as diabetes, are more likely to be impacted by COVID-19 and have an increased risk for health complications. The high diabetes prevalence in Mississippi (13.6%) and the current COVID-19 pandemic might pose a greater risk to Mississippians. Behaviors such as social distancing and handwashing help prevent contracting COVID-19. The purpose of this study was to compare the degree to which South Mississippians with and without self-reported diabetes complied with stay-at-home guidelines. Data were collected using an online survey that was promoted via text messages, email, and social media announcements. Participants included in the study were adults who were 18 years or older and lived in South Mississippi (N=108). Preventative behaviors assessed social distancing behaviors outlined in stay-at-home guidelines. Using linear regression analysis, it appeared that engagement in preventative behaviors for COVID-19 did not differ for diabetes status but differed significantly for age (p=.002), race (p=.003), and COVID-19 risk attitudes (p=.004). Older age and those who self-reported as African American predicted greater stay-at-home adherence. Greater COVID-19 risk perception was associated with lesser stay-at-home adherence. Our findings provided insight about engagement in COVID-19 preventative behaviors in South Mississippi and can help public health officials identify focused groups for which to provide increased education and awareness of preventative measures. Future studies are needed and should include a larger sample size and consider evaluating additional preventative behaviors for COVID-19.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Diet, Physical Activity, and Preventable Diseases: Examining the Relationship between Community Cultural Values and Health Behaviors among Louisiana Native Americans

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Mississippi INBRE P20GM103476 Mohamed Elasri

Preventable chronic diseases resulting from poor diet and lack of physical activity are major health concerns throughout the southeastern United States. Native Americans in the Deep South are a relatively underexplored population with regard to healthy behaviors that reduce preventable chronic diseases. Cultural values that promote healthy diets and physical activity have been identified as key determinants of diet and physical activity, but little is known about the relation in this population. The purpose of this study was to examine the relationship between cultural values that promote healthy diet and physical activity among self-identified Native American adults (18+ years) living in Louisiana (N=68). Data were collected online via Qualtrics survey regarding disease status, barriers and facilitators of healthy diet, and physical activity levels. A five-question measure was used to assess community cultural values for healthy dietary and physical activity behaviors. The Dietary Screener Questionnaire was used to assess dietary intake. The single question Global Physical Activity Questionnaire was used to assess weekly levels of physical activity. Demographic variables that served as covariates included age, income, gender, and education. Results from a regression of cultural values onto physical activity and dietary intake controlling for demographic covariates indicated no significant effects of cultural values on any of the diet variables or physical activity. Future interventions should consider more local networks, family, friends, and other kinships for their support and encouragement of healthy diet and physical activity behaviors that reduce preventable chronic diseases at the community level in this population.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

COVID19 Prevention Behaviors & Chronic Disease Status among Southeastern Native Americans in Mississippi

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Early on during this pandemic, it quickly became apparent that individuals with pre-existing health conditions, such as diabetes, high blood pressure and others are at a greater risk for complications related to COVID-19. Native Americans have higher rates of chronic diseases (e.g., diabetes, high blood pressure) and have also been affected by COVID-19 at higher rates. COVID-19 related deaths have also been greater among Native Americans in Mississippi compared to other race/ethnic groups, making recommendations of social distancing even more imperative for this population. In the current study, we examined the relationship between self and household adult medical history and COVID-19 preventative behaviors among Native Americans who reside in Mississippi. With approval of the USM IRB and tribal partners, data were collected from participants (N = 93) who were administered an online questionnaire and were awarded an e-gift card as an incentive for completion. Results from COVID-19 risk behaviors regressed onto self and household adult disease status and demographic variables revealed a positive relation between COVID-19 risk behaviors and self-disease status and a negative relation of risk behaviors with household adult disease status. Additionally, older participants and those with more education reported fewer risk behaviors. These results point to cultural differences among Native American community members who may respond more readily to COVID-19 prevention messages that focus on care for household members rather than messages that target individuals.

Acknowledgement: This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Adaptation of Mississippi INBRE Summer Scholars Programs to Online Research Format in Response to COVID-19

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Mississippi INBRE seeks to increase competitiveness in biomedical research through the provision of experiential learning opportunities for Mississippi undergraduate students by preparing them for health-related careers serving Mississippians who suffer from a wide range of health disparities, such as cancer, diabetes, obesity, and infectious diseases. To date, 736 students have been training through three Mississippi INBRE Summer Scholars programs, which have increased student exposure and interest in STEM fields, built professional relationships, and increased the retention of students within the state for biomedical graduate programs that directly promote Mississippi's biomedical workforce. The presentation of COVID-19 this past Spring brought a unique challenge to many research training programs. Like others, we were not able to offer our Mississippi INBRE Research Scholars program, which is our laboratory-based biomedical research experience. However, we were able to successfully train 48 scholars this summer through the adaptation of our most recently developed community-based public health and nutrition scholars programs to a fully online format: the Mississippi INBRE Service Scholars program, established in 2013 to offer community-based public health research experience with our partner, My Brother's Keeper, Inc of Jackson, MS; and the Mississippi INBRE Outreach Scholars program, established in 2019 to offer nutritional intervention training to Native American and African American populations of Mississippi and Louisiana facilitated through our Mississippi INBRE Telenutrition Center at USM. Through these two programs, scholars engaged in survey-based research to address the nutrition, physical activity, and mental health concerns related with the COVID-19 pandemic affecting the vulnerable populations of Mississippi, which have been disproportionally impacted through reported infection and deaths in our state. Through the structured virtual program facilitated through Zoom meetings and Canvas platform, students followed workshops in sequence with the research process of literature review, research design, survey distribution, data collection, statistical analysis, poster presentation, abstract writing, and professional development.

***Acknowledgement*:** This work was supported by the Mississippi INBRE, funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103476.

Fetal-Derived Human Lung Organoids to Model SARS-CoV-2 Infection

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Fetal-Derived Human Lung Organoids to Model SARS-CoV-2 Infection. To date the SARS-CoV-2 pandemic has resulted in over 775,000 deaths worldwide and has disproportionately affected medically-underserved, rural, and minority communities. SARS-CoV-2 infects the respiratory tract, which is comprised of highly-differentiated polarized epithelial tissue including many distinct cell types and primarily infects ciliated cells which express its receptor, angiotensin-converting enzyme 2 (ACE2). Modelling the respiratory tract is complicated by the complexity of respiratory tissue, and traditional cell culture models typically include a single cell type and fail to sustain cellular differentiation, leading to a loss of ACE2 expression. To better recapitulate a human lung environment, we employed fetal-derived human lung organoids, which are 3-dimensional cellular structures complete with a lumen, sustained cellular differentiation and polarization, and a differentiated cell population. Our organoids spontaneously assemble from multipotent basal progenitor cells and exhibit complex morphology including ciliary beating within 20 days of culture, with differentiation sustained for at least 45 days. Upon infection with SARS-CoV-2 (WA01) at MOI = 10 for 24 hours, a subset of cells in multiple organoids were positive for SARS-CoV-2 nucleocapsid protein (SARS-CoV-2 N). A population of infected cells appeared adjacent to one another within the organoid indicating a productive, spreading infection, whereas another population of SARS-CoV-2 N-positive cells dissociated from the main organoid body. Importantly, dissociated cells lacked indicators of cell death suggesting recapitulation of the migratory behavior observed *in situ* in response to epithelial damage. Quantification of viral titers and the induction of organoid antiviral responses to the infection are in progress. This preliminary work suggests that our fetal-derived human lung organoids recapitulate hallmarks of human lung tissue, and thereby offer a superior system for the study of lung epithelial responses to SARS-CoV-2 infection.

Exploring a Rural Latinx Population's Perspectives of the COVID-19 Pandemic

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Objectives: The purpose of our study was to understand the perception of the Latino community in a rural state regarding COVID-19. In December 2019, a new strain of a SARS coronavirus emerged from Wuhan, China and spread across the globe as COVID-19, creating a pandemic. Since June 2020, nearly 2 million Americans have become infected and over 110,000 people have died. Rates among minority populations are disproportionately high when compared to Whites. Over one quarter of the COVID-19 cases are among Latinos.

Design: Respondents were recruited using snowball sampling as part of a previous effort to establish an academic-community partnership with Latinos in the area. In April 2020, we conducted 14 semi-structured interviews in Spanish with participants over the phone. Interviews were audio-recorded, transcribed into Spanish, and translated to English. We conducted a thematic analysis using NVivo 12 to identify common themes in participant responses.

Results: Common themes were a wariness of news appearing on social media, generalized worry, and the use of natural medicines to maintain health. Respondents followed recommended guidelines to protect their own health, though expressed concern that members of their community were not.

Conclusions: We offer insights into the perception of Latinos of the COVID-19 pandemic in a rural state. Our findings may influence communication techniques of local health departments and offer a way to understand how this often-overlooked community deals with the pandemic.

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I, Dr. Kelly Shepardson, am a research scientist at Montana State University and am the project leader on a pilot grant that aims to identify the role of the type I interferon (IFN) receptor 2 (IFNAR2) subunit, part of the IFNAR1/2 receptor, in the damage response to *Aspergillus fumigatus* (Af). Each year over 300,000 cases of Af infection are due to invasive pulmonary aspergillosis (IPA) in patients with altered, either hypo- or hyperactive, immune systems. A main factor contributing to pathological outcome of IPA is the level of damage the host incurs. We recently discovered that type I interferon (IFN) signaling, via IFNAR2 of the IFNAR1/2 receptor, regulates susceptibility to and damage from influenza. We found that IFNAR2 deficiency (Ifnar2-/- mice) resulted in increased cellular damage and morbidity at 24 hrs post-*Af* compared to WT and Ifnar1-/- mice. Ifnar2-/- mice cleared spores more efficiently than both WT and Ifnar1-/- mice but were unable to control invasive disease and maintain pulmonary architecture, evidenced by hyphal growth and fibrosis-like tissue at 48 hrs post-*Af*. Recently, we have found an atypical increase in eosinophil infiltrates occurring early at 18hrs post-A.f. and two-fold higher inflammatory cytokines in Ifnar2-/- mice compared to WT and Ifnar1-/- mice. Additionally, although neutrophil recruitment was unaltered, we found that Ifnar2-/- neutrophils produced more external ROS in response to *Af* suggesting altered effector cell function may be involved in the IFNAR2 regulated damage response. Together, these results and ongoing experiments are identifying the cellular mediators affecting the altered lung environment regulated by IFNAR2 that are involved in both the damage response and anti-fungal immunity to Af pulmonary infection. By understanding how IFNAR2 regulates the damage response during pulmonary *A.f.* infection, this will allow us to understand role of type I IFN signaling in anti-fungal immunity and controlling pulmonary tissue damage.

Sensory Integration in Native American Youth

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Behavioral challenges in children are estimated at 3-6%, but in low-income families, that number increases to 30%. Children exposed to early adversities such as environmental stressors, poverty, parental stress or substance abuse problems have a higher likelihood of behavioral difficulties. Child behavioral difficulties may be due to their inability to process sensory input, such as touch, sound, balance, and body awareness. Although there is a relationship between low-income households and behavior problems in children, there are significant gaps in our understanding of the connection between behavior and sensory processing difficulties as well as how sensory-based environmental modifications in classrooms and at home may assist in emotional regulation. In addition, there is no research in this area in American Indian children at high risk of adverse childhood experiences and subsequent behavioral challenges. Occupational therapists (OT) use child-centered and culturally appropriate approaches to understand the specific and unique needs of each child. A sensory program is a child-centered intervention that addresses sensory processing difficulties and provides a child with individualized sensory-based activities throughout the day to optimize behavioral regulation. The purpose of this study is to investigate the relationship between implementation of a sensory program and emotional regulation and behavior. This study is a single-case design in which the participant is a three year old male born with substances in his system consistent with a diagnosis of Neonatal Abstinence Syndrome, thereby exhibiting sensory seeking behaviors. Using a mixed methods design including observations, semi-structured interviews, pediatric OT assessments and sensory-based interventions we will analyze pre and post test data to create a culturally-appropriate sensory program for the participant to enhance family-based social participation in community events.

Early signaling pathways that dictate a universal TLR2/6-based anti-viral response to conserved viral architectures.

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Throughout history, viral infections have emerged that caused several epidemics and now we are facing a viral global pandemic that poses a serious threat to human health highlighting our need to further understand mechanisms of universal viral recognition and anti-viral response pathways. The field of innate viral pattern recognition primarily focuses on innate recognition of viral nucleic acids, but some extracellular viral pattern recognition receptors (PRRs), such as C-type lectin receptors bind glycoproteins present in the viral envelope. Our recent discovery suggests there may be a more common pattern, namely the repeating protein subunit pattern (RPSP) that serves as a likely conserved mechanism of extracellular pathogen associated molecular pattern (PAMP) recognition for viruses. Recognition of the RPSP is dependent on the cell-surface PRR Toll-like receptor 2 (TLR2), which regulates an anti-viral response through type I IFNs, a proinflammatory response, and improved clearance of a subsequent bacterial infection that occur in some post-respiratory viral infections, including influenza. TLRs are commonly known to signal from either the cell surface, through MyD88/Mal, or from the endosome, through TRAM/TRIF, for the induction of inflammatory responses or type I IFNs, respectively. In this regard, we found that both MyD88 and TRAM were required for RPSP-mediated *S. aureus* clearance by macrophages and for the induction of type I IFNs through IFN-β in response to RPSP relative to WT macrophage. In addition, we found that *tram*$^{-/-}$ macrophages exhibited diminished RPSP internalization by microscopy and flow cytometry compared to WT macrophages. However, while we found that TRAM, a canonically endosome associated signaling adaptor, is involved in the RPSP anti-viral response, blocking formation of endosomes by Latrunculin A did not reduce *S. aureus* killing by RPSP-exposed macrophages. Our data therefore suggests that a non-canonical signaling pathway involving crosstalk between MyD88 and TRAM is induced upon RPSP exposure

Algal Blooms Community Health Literacy Program – ABC HeLP
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Freshwater habitats comprise some of the most altered ecosystems. Nutrient enriched waters often support Harmful Algal Blooms (HABs), outbreaks that may lead to cyanotoxin poisoning. Fertilizer and animal wastes leached from agricultural lands drive freshwater algal blooms. Our research investigates a novel and functional approach for algal bloom identification in rural freshwater systems. We propose innovative solutions that lead to more timely health official responses and increased public awareness. Our *ecological study* establishes monitoring locations on a high-use recreational and agricultural waterway, potentially transforming current sampling protocols and public warning systems. A Public Health Efficacy Model tailors algal bloom epidemiology and ecology information, as well as response efforts, through the Algal Bloom Community Health Literacy Program (ABC HeLP). Using the ABC Toolkit, *disseminated information is designed to meet the participants' unique needs, economic resources, cultural responsiveness and geographic challenges.*

Our initial results identified parameters associated with early formation of HABs. Instrumentation that quickly delimits chlorophyll measures by algal class, in addition to toxin quantification and microscopy, also reveal early HAB identification. Unexpectedly, our watershed sampling discovered human sewage release in associated privately-held land drainages. Additionally, within the 44-52 km2 of public land encompassed in our study, >125 cabins exist. These sewage systems range from contained septic tanks to privies that leak sewage directly into recreational waters. With recent documentation of SARS-CoV2 in urban sewage systems, our discovery suggests another avenue with the potential to quickly pass viruses through recreational water use. Under- or poorly-funded county health enforcement of sewage standards may indirectly contribute to the movement of viruses through ecosystems. Linking scientific results to health systems and increasing community awareness to modify public response to HABs, our research fills a gap in understanding ecological and public health threats of algal blooms and addresses environmental occurrences that are expected to increase with climate change.

Molecular Epidemiology of SARS-CoV-2 in Nevada

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Nevada INBRE GM103440-18 Jonathan Baker

The novel coronavirus (Covid-19) has been declared a pandemic by the World Health Organization. Currently in the state of Nevada there are over 60,000 confirmed cases with more than 1,000 deaths caused by Covid-19 infections. Identifying local variants of SARS-CoV-2 in relation to disease pathology and Nevada health disparities will help to inform statewide prevention and treatment efforts. NV INBRE supports and helps to coordinate a statewide molecular epidemiological study involving both of Nevada's research institutions (University of Nevada, Las Vegas and University of Nevada, Reno), the Nevada State Public Health Laboratory, the Southern Public Health Laboratory, and three INBRE-supported scientific cores. Genetic sequencing analysis of over 190 Nasopharyngeal swabs from Nevada patients from both Reno and Las Vegas identified specific variants of SARS-CoV-2 and show that most patients in NV are infected with the clade C variant of the virus. These studies have resulted in three manuscripts in preparation (mutation in RdRp (nsp12) of SARS-CoV-2 detected at high frequency from patient nasopharyngeal swab samples; reinfection with SARS-CoV-2; and assessment of viral specimens from fatal COVID-19 cases) and six grant applications. These studies are providing research opportunities for both graduate and undergraduate students and are creating and strengthening statewide research collaborations.

Evolutionary Conservation of Ornithine Decarboxylase Antizyme Pseudoknot RNA Binding to Spermine

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Nebraska INBRE 5P20GM103427 GM103427-19

Nearly all organisms possess the capability to synthesize polyamines, which are essential for cell growth and differentiation. Not surprisingly, the transport and metabolism of polyamines are highly regulated by complex feedback mechanisms. Ornithine decarboxylase (ODC) is the key regulatory enzyme in polyamine biosynthesis. Both ODC and cellular uptake of polyamines are inhibited by Ornithine Decarboxylase Antizyme (OAZ). Mammalian OAZ mRNAs further possess a pseudoknot (PK) structure. Although the role of the OAZ pseudoknot RNA element (further designated OAZ-PK) in polyamine biosynthesis has been investigated, it has not been examined as a distinct polyamine “sensor”.

Riboswitches are elements within noncoding regions of mRNAs that directly bind to cellular metabolites and modulate gene expression. Many riboswitches provide a mechanism of feedback regulation for gene products within the biosynthetic pathway of the cognate metabolite. Although riboswitches are widespread among bacteria, no riboswitches have been found in animals. We propose that the highly conserved OAZ-PK RNA functions as a riboswitch. Utilizing in-line probing and equilibrium dialysis, apparent binding affinity and specificity for polyamines was determined. The mouse OAZ1-PK RNA binds to spermine with greater affinity than to other polyamines, and spermine binding to OAZ1-PK RNA specifically elicits conformational change, a fundamental property of riboswitches. Closely related spermine analogs (with identical or greater overall positive charge) have lesser affinity and specificity for the OAZ1-PK RNA.

Current work is focused on investigating OAZ-PK RNAs from other organisms. Variations in the structures of these RNAs may or may not result in similar functioning to the mouse OAZ1-PK RNA with regards to spermine binding affinity and specificity and ligand-dependent conformational changes. The function of OAZ-PK RNA as a spermine “sensor” suggests a substantially broader distribution of riboswitches among eukaryotic organisms and represents a potential new drug target in a key metabolic process relevant to cellular and organismsurvival.

Screening for Inhibitors of SARS-CoV-2 using a Spike Pseudotyped MLV Virus Particles
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Nebraska INBRE 5P20GM103427 GM103427-19

This is a newly initiated collaboration where my lab is working in collaboration with Dr. St. Patrick Reid and his lab at UNMC to screen potential inhibitors of SARS-CoV-2. Our goal is to set up experiments that will screen the potential inhibitors for their impact on SARS-CoV-2 spike protein-mediated entry into target cells. Specific inhibitors to be investigated at UNO are pending selection and will be chosen together with Dr. Reid based upon his existing collaborations and BSL3 findings.

Dr. Reid has established protocols and will be performing BSL3 level investigations of inhibitors using infectious virus. In my lab at UNO, we will be using pseudotyped virus particles to discover inhibitors that are capable of blocking virion entry. The reason we are unable to look at other aspects of the virus replication cycle is that we are not using SARS-CoV-2 in our work. Rather than using infectious virus, we are going to be working with a Moloney mouse leukemia virus vector system that allows us to create virion particles pseudotyped with spike protein. These pseudotyped vectors are only capable of virus entry and marker gene (EGFP) insertion into the transfected cell. There are no preliminary data to report as we are still establishing the system in my lab.

We will use a flow cytometer (Beckman-Coulter Cytoflex) in my lab at UNO and Perkin Elmer Operetta in Dr. Reid's lab at UNMC to quantify the effects of the potential inhibitors. By working together in this partnership, we will more rapidly identify potent inhibitors of SARS-CoV-2 and gain critically important insights into the mechanistic actions of such inhibitors of this virus.

A New Method for Calculation of Transfer Entropy from Biomolecular Simulation Provides Rapid and Reliable Insight into Information Flow Pathways in ERK2

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Transfer entropy methods provide an approach to understanding asymmetric information flow in coupled systems. In biomolecular systems, transfer entropy can be useful for identification of “driving” and “responding” residues as well as pathways or networks of residues that are coupled in their information flow. Unfortunately, most methods for calculating transfer entropy require very long simulations and almost equally long calculations of joint probability histograms to compute the information transfer. Available approximate methods based on graph/network theory approaches are rapid but lose sensitivity to the chemical nature of the biomolecules and thus are not applicable in mutation studies. We show that reliable estimates of the transfer entropy can be obtained from the variance-covariance matrix of atomic fluctuations, which converges quickly and retains sensitivity to the full chemical profile of the biomolecular system. We validate our method on ERK2, a well-studied kinase involved in the MAPK signaling cascade for which considerable computational, experimental, and mutation data are available. We show that our method is consistent with the results of computational and experimental studies on ERK2, and we present a method for interpreting networks of interconnected residues in the protein from a perspective of allosteric coupling. Our results highlight the advantages and disadvantages of various methods for calculating transfer entropy and show the important role of transfer entropy analysis for understanding allosteric behavior in biomolecular systems.

Differential Brain Pathway Activation in a Non-Anaphylactic Mouse Model of Cow's Milk Allergy

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Food allergy has been associated with various neuropsychiatric disorders, such as anxiety and autism spectrum disorder, although, the mechanism by which the peripheral immune disorder affects behavior is not well understood. We have previously demonstrated that sensitization of male C57BL/6J mice to the milk allergen, beta-lactoglobulin (BLG; Bos d 5), results in anxiety-like behavior without anaphylactic response to allergen challenge. We hypothesize that in response to food allergy mice would express increased circulating cytokines and stimulate pathways relating to inflammation and glial cell function within the brain. To evaluate pathogenic influence of food allergy on brain function, we performed transcriptomic analysis of different brain regions in a mouse model of cow's milk allergy. Male mice were sensitized over 5 weeks to BLG alongside unsensitized sham mice, then challenged with the allergen. Despite the lack of clinical symptoms upon BLG challenge in sensitized mice, increased serum BLG-specific IgE was observed, confirming acquired immunity to the allergen. Increased plasma eotaxin-2, CCL9, and CXCL4 were also detected, while IL-6, CXCL5, and CCL5 were reduced. Transcriptomics analysis using Ingenuity Pathway Analysis platform generated a list of core regulators including *Dio2*, *Slc16a2*, *Bdnf*, *Psen1*, and *Eomes*. In the region that included the striatum, synaptogenesis, eNOS signaling, endocannabinoid neuron developing, and G protein α_s pathways were differentially activated. In a region containing the thalamus and hypothalamus, netrin and Th17 signaling pathways were activated in BLG-sensitized mice, while the Fcε receptor and ALS pathways were highlighted in the midbrain. Our results demonstrate allergic biomarkers without anaphylactic symptoms, despite the lack of symptoms, mice had altered cytokine abundances and changes in pathway activation associated brain function. Results indicate neuroinflammation caused by allergy potentially via an IgE-Fcε mechanism. The resulting inflammation likely causes changes in the number and structure of glia and neurons in addition to altering neuronal signaling

The C-terminus of Polymerase Epsilon is Important for Interaction with Mcm10 and in the Maintenance of Genome Stability

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A hallmark of cancer is a high rate of mutation and genomic instability caused by genetic changes. Many of these changes are caused by errors during DNA replication. Our laboratory studies Minichromosome maintenance protein 10 (Mcm10), an essential replication protein that is important for maintaining genome stability. Studies have shown that Mcm10 levels are upregulated in cervical cancer and glioblastomas; in fact, the expression of Mcm10 correlates with the stages of cancer progression, suggesting that it might contribute to tumor aggressiveness. We hypothesize that Mcm10 functions during replication and checkpoint activation pathways to maintain genome stability. Yeast two-hybrid (Y2H) results from our laboratory show a robust interaction between Mcm10 and Pol2, the catalytic subunit of Polymerase epsilon (Pol). Pol plays a crucial role in DNA replication, DNA damage repair, and chromatin remodeling pathways. To substantiate the Y2H results, we examined the physical interaction between Mcm10 and Pol2 through co-immunoprecipitation. Interestingly, we found that this protein-protein interaction is cell cycle dependent but independent of DNA interaction. Moreover, Mcm10 interacts with the essential C-terminus domain of Pol2. We have identified mutants in the C-terminal domain of Pol2 that inhibit its interaction with Mcm10 but does not inhibit its formation of the Pol holoenzyme. We expect that the combined studies will reveal the importance of these Pol2 mutants that reveal the relevance of this interaction during DNA replication and DNA damage.

This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number P20GM103447.

Antiproliferative properties of Ethanolic and Aqueous Graviola Leaf Extracts on Tongue Squamous Cell Carcinoma cell line 25 (SCC-25).

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Background: *Annona muricata,* commonly known as Graviola, soursop or guanabana, is an evergreen tree native to the tropics with a long history of use in ethnomedicine in indigenous communities in Africa and South America. Its active phytoconstituents have provided medicinal benefits against various ailments and diseases such as arthritis, parasitic infection, hypertension, fever, or diabetes. Studies conducted *in vitro* and *in vivo* have concluded that Graviola phytocomponents have anti-cancer and anti-tumor properties. One of the characteristics of cancer cells is their uncontrolled proliferation rate. In that sense, molecules that inhibit cell proliferation offer potential therapeutical benefits.

Methods: We prepared ethanolic and aqueous extracts from dried Graviola leaves and tested their respective antiproliferative activities on tongue Squamous Cell Carcinoma-25. We treated the cells with increasing concentrations of the extracts for 24 h. The respective doses leading to a 50% inhibition of cells growth (GI50) was determined.

Results: Our results showed that the ethanolic was 4 times more active in inhibiting the growth of SCC-25 than the aqueous extract (respective GI50 of 61.7g/mL, and GI50 of 274.6 g/mL).

Conclusion: We hypothesize that some organic compounds involved in the antiproliferative/ cytotoxicity of Graviola leaves were selectively extracted by Ethanol. Future plans include characterizing those bioactive compounds and assessing their bioactivity on SCC- 25 vs. non-cancerous oral cells. Our hope is to discover natural molecules to be used as alternative treatment for oral Squamous Cell Carcinomas.

A Pilot Cohort study of SARS-CoV-2 prevalence, incidence, transmission and symptom severity in high-risk groups in Puerto Rico to serve as a repository for microbiome studies.

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As SARS-CoV-2 is sweeping through Puerto Rico, healthcare workers (HCW) are our first line of defense. Susceptible to becoming infected, with concomitant loss in productivity, their lack of quality of life and possibility about transmission to patients and family members, are of great concern. Our study focuses on HCW groups at the University of Puerto Rico including personnel taking care of SARS-CoV-2-infected patients, and those who are not. Additionally, we will include another high-risk group, pregnant woman who are frequently attending the hospital, and hospitalized patients. In fact, pregnant women experience immunologic and physiologic changes, which might make them more susceptible to viral respiratory infections. These high-risk groups are more exposed to SARS-CoV-2 from the hospital environment itself. Our specific objective is to establish a prospective six-month cohort to characterize the factors related to viral transmission and disease severity in both healthcare settings and workers' households as well as pregnant women (PW), non-health care workers (Medical Sciences Campus personnel) and other patients attending the clinics. Our central hypothesis is that HCW and PW are at higher risk of acquiring and transmitting SARS-CoV-2 compared with non- healthcare workers (NCHW). We propose to detect SARS-CoV-2 in saliva and prepare a biorepository of saliva, blood and rectal swabs for future microbiome studies to associate to COVID-19 status and disease severity. The proposed cohort study will produce immediate, actionable, and translatable knowledge to assess prevalence of SARS-CoV2 in this high risk group, and will help understand the role of the microbiome during the COVID-19 pandemic. Our repository of several thousand biological samples will serve as a foundation for future mechanistic studies.

Characterization of Five Lead Compounds with Influenza Antiviral Activity

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Influenza A virus (INF-A) is responsible for a well-known infectious disease that affects humans and is implicated in all INF related seasonal epidemics and pandemics. The treatments are limited, and the emergence of resistant strains highlights the need for the development of new and effective therapies. Using In-Silico drug discovery we identified a group of drug-like compounds with predicted affinities in the sub-micromolar range. Results from an *in vitro* bioassay with our top compounds were consistent with viral inhibition as predicted by In-Silico analysis. We hypothesized: *Selected morpholinyl quinoline "lead compounds" are highly potent, efficient, and relatively safe with low risk of resistance development, and broad-spectrum activity against a variety of influenza virus strains by acting through a mechanism that includes inhibition of the interaction between polymerase acidic protein-basic protein 1 (PA-PB1) sub-units and therefore assembly of the polymerase complex*. MDCK cells were infected with A/PR/8/1934 H1N1 strain of INF-A virus, and viral replication assays performed. Initial screening revealed that all selected quinoline analogs have significant antiviral activity at 100nM with relatively low toxicity at 100μM/4 days exposure. Dose-response curves for DIPA-253 and ribavirin antiviral activity indicated EC_{50} values of 73nM and 12.5μM respectively. The effects of 10μM of our selected "lead compounds" on viral replication, measured by expression levels of the viral nucleoprotein (NP) via Western Blot analysis, demonstrated a significant reduction. Similar levels of inhibition were obtained with concentrations as low as 1-5 nM for two of the tested compounds (DIPA102 and 193). Preliminary results from in cell-ELISA, with NP as a marker, revealed IC_{50} of ≈6.3μM for the positive control ribavirin and IC_{50}s of two of our "lead compounds" DIPA-102 and 004 in the nanomolar range. The identification of these five "lead compounds" will allow us to determine their pharmacological parameters and therefore, therapeutic potential against INF.

Quassinoids from *Simarouba tulae* and their cytotoxic activities.

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Puerto Rico INBRE GM103475-18 Jose Rodriguez-Medina

Plants belonging to the *Simarouba* genus are well known for producing quassinoids, a group of diterpenes possessing anti-malarial, anti-cancer, and antiviral activities. *Simarouba tulae,* is a small tree endemic from Puerto Rico. In a previous study related with the evaluation of the cytotoxic properties of several species of tropical plants, the chloroform extract of this plant was the most active extract. This extract was purified using different chromatographic techniques to afford the quassinoid Simalikalactone D (SKD) and a SKD derivative. In cancer cells, SKD showed high cytotoxicity activity, with an IC_{50} of 55, 58, and 65 nM in A2780CP20 (ovarian), MDA-MB-435 (breast), and MDA-MB-231 (breast) cell lines, respectively. Exposure to SKD led to 15% inhibition of the migration of MDA-MB-231 cells. Recent results showed that SKD can activate the cell death endpoints such as apoptosis induction, DNA fragmentation, and mitochondrial permeabilization. In addition, potentiation effects were observed when SKD was combine with camptothecin on MDA-MB-231 cell line. Based on our results, we demonstrate the strong anti-proliferative activity of the quassinoid SKD isolated from *Simarouba tulae*. Research reported in this publication was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the NIH under grant number P20GM103475.

First Insights into the Microbiota of HPV-induced penile cancer

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Puerto Rico INBRE GM103475-18 Jose Rodriguez-Medina

Penile cancer (PeCa) is a disease with a high morbidity and mortality, among developing countries. Although penile cancer is a relatively uncommon cancer, its incidence is nearly four times higher in Puerto Rico (PR) when compared with other racial and ethnic groups in the United States (US). Infection with human papillomavirus (HPV) has been identified as a risk factor for an average of 48% of PeCa cases. To date, the role of the microbiota in the pathogenesis of HPV positive PeCa is unknown, as there are no studies on the microbiome of penile cancer. We hypothesized that the penile bacterial communities changed according to HPV infections and cancer lesions. Genomic DNA was extracted from biopsies of 51 patient biopsy samples, followed by HPV typing and microbiota analyses using 16S rRNA genes with the Illumina MiSeq platform. Demultiplexed data was deposited in QIITA for quality control and bioinformatic analyses with a rarefaction level of 2,000 sequence reads, involved alpha and beta diversity analyses, taxonomic characterization and biomarker analyses according to HPV status and tumor histology.

We found no significant differences in alpha diversity according to HPV status or lesion. However, we found clear differences in community composition. HPV positive samples had higher levels of Actinobacteria, including *Actinomyces europaeus, Mobiluncus* sp., *Corynebacterium* sp., *Corynebacterium simulans.* For the histology category we found a reduction of Proteobacteria in high grade lesions, and an increase in Firmicutes and Actinobacteria in the intermediate and high grade lesions, including *Veillonella parvula, Corynebacterium kroppenstedtii* and *Actinomyces.* We thus found lipophilic and anaerobic bacteria associated to HPV and high grade tumor lesions, that may be involved in triggering inflammatory responses and oncogenesis. Although many challenges must be overcome to dissect the specific interactions of coinfecting bacteria during the penile cancer infections process, our findings demonstrate that microbes maybe involved in these cellular processes.

Program Name: Advancing Competitive Biomedical Research in Puerto Rico
Program Grant Number: 5P20GM103475-18
Presenter: Filipa Godoy Vitorino

Anogenital Mycobiota Analyses Reveals an Increase in *Malassezia* yeast associated to HPV infections

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Puerto Rico INBRE GM103475-18 Jose Rodriguez-Medina

Characterization of bacterial biomes has revealed bacterial populations associated with HPV infection and dysplasia, but several unanswered questions still remain. Fungi represent a large component of the microbiome that has been significantly neglected, yet it has the ability to form biofilms in the mucosa of the host and likely modify the epithelial microenviroment.

To shed light on the complex microepithelial communities of anogenital fungi, we performed an unparalleled in-depth fungal diversity assessment from self-collected vaginal and anal samples and related these to HPV infections. A total of 253 self-collected cervical and anal samples from Puerto Rican women between the ages 16-64, were amplified and sequenced using ITS-2 primers and tested for HPV using MY09/MY11 consensus HPV L1 primer. Data was analyzed using qiime1 and R to understand microbial shifts between samples according to BMI, HPV status, and menopause status.

Although overall sample analysis showed no significant structural or compositional differences of the fungal communities, when considering only pre-menopausal women with a normal BMI (n=57), we found a decrease of *Candida* sp. and an increase of *Malassezia* sp. in HPV positive samples both in the cervix and anus.

Our analyses reveal a significant increase in lipophilic yeast associated to HPV infections that is likely involved in polymicobial and immunomodulatory changes of the anogenital tract.

Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103475. Partially supported by Award Number U54 MD007600 from the National Institute on Minority Health and Health Disparities. Parent grant: NIAID 1 SC2 AI090922-01. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Unnarmicin D, an anti-inflammatory cyanobacterial metabolite that binds to delta and mu opioid receptors

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Rhode Island INBRE P20GM10343

To combat the bottlenecks in early-stage drug discovery, a pipeline to identify neuropharmacological therapeutic candidates using *in silico*, *in vitro*, and receptor specific assays was devised. A library of pure compounds isolated from blooms of the cyanobacterium *Trichodesmium thiebautii* was evaluated using this approach. *In silico* analysis of drug likeliness and *in vitro* permeability analysis using the parallel artificial membrane permeability assay (PAMPA) highlighted multiple metabolites of interest with potential blood-brain barrier (BBB) permeability. Murine macrophages were used to assess if these BBB permeable compounds could reduce nitric oxide levels after LPS induced inflammation. Compounds that significantly lowered NO levels were further analyzed for the ability to modulate pro-inflammatory cytokines TNFα, IL-6, and sTLR-2 in the cellular supernatant. The nontoxic metabolite unnarmicin D was identified as an early candidate and it was further evaluated due to its moderate permeability in the PAMPA assay, promising ADME data, modulation of all cytokines tested, and prediction as an opioid receptor ligand. Molecular modeling of unnarmicin D to the mu and delta opioid receptor showed binding potential for both opioid targets. *In vitro* binding assays validated this pipeline showing micromolar binding affinity for both the delta and mu opioid receptors opening the potential for further analysis of unnarmicin D derivatives for the treatment of pain and neuroinflammation related diseases.

Effect of Radiation Energy, Nanoparticle Size, Targeting Molecules and Time on Reactive Oxygen Species Production Using Irradiated Copper-Cysteamine Nanoparticles

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Rhode Island INBRE P20GM103430 Bongsup Cho

Copper-cysteamine nanoparticles have the potential to increase the effectiveness of radiation therapy. When irradiated, these nanoparticles stimulate the production of reactive oxygen species such as singlet oxygen. If the nanoparticles are located within a tumor, the reactive oxygen species produced could cause additional damage to the tumor. In the work presented here, we measured the effect of four experimental variables on the amount of reactive oxygen species generated, using the RNO-ID method. These variables are radiation energy (90, 250, and 350 kilovolts peak), nanoparticle size (40, 100 and 200 nm), time (5-30 minutes after irradiation) and the addition of hyaluronic acid (known to target specific types of cancer) to the nanoparticles. Measurements were made across two separate repeats of the experiment that used different batches of nanoparticles. A linear regression analysis was done on the first repeat (data largely from one time point), and a linear mixed effect model was used in the second repeat to capture the dependencies of nanoparticles over time. 90 kilovolts peak had significantly higher reactive oxygen species output than 250 kilovolts peak in both repeats, and significantly higher output than 350 kilovolts peak in one repeat. The difference between the 40 nm and 200 nm nanoparticle sizes was found to be insignificant in both repeats, although both were significantly increased compared with 100 nm (in the one repeat with 100 nm) and all sizes were significantly increased from control. In the one repeat where all samples were measured at different times, the output increased significantly with time. The use of hyaluronic acid reduced the output by a statistically significant amount in one of the two measurement sets.

Exploring a Rural Latinx Population's Perspectives of the COVID-19 Pandemic

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Montana INBRE GM103474-20 Brian Bothner

Objectives: The purpose of our study was to understand the perception of the Latino community in a rural state regarding COVID-19. In December 2019, a new strain of a SARS coronavirus emerged from Wuhan, China and spread across the globe as COVID-19, creating a pandemic. Since June 2020, nearly 2 million Americans have become infected and over 110,000 people have died. Rates among minority populations are disproportionately high when compared to Whites. Over one quarter of the COVID-19 cases are among Latinos.

Design: Respondents were recruited using snowball sampling as part of a previous effort to establish an academic-community partnership with Latinos in the area. In April 2020, we conducted 14 semi-structured interviews in Spanish with participants over the phone. Interviews were audio-recorded, transcribed into Spanish, and translated to English. We conducted a thematic analysis using NVivo 12 to identify common themes in participant responses.

Results: Common themes were a wariness of news appearing on social media, generalized worry, and the use of natural medicines to maintain health. Respondents followed recommended guidelines to protect their own health, though expressed concern that members of their community were not.

Conclusions: We offer insights into the perception of Latinos of the COVID-19 pandemic in a rural state. Our findings may influence communication techniques of local health departments and offer a way to understand how this often-overlooked community deals with the pandemic.

Enzyme Upregulation Linked to Autophagic Failsure as a Potential Biomarker for Neurodegenerative Lysosomal Storage Disease

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South Carolina INBRE P20GM103499 Edie Goldsmith

Introduction: GM1 Gangliosidosis is a fatal neurodegenerative lysosomal storage disease characterized by lack of production of β-galactosidase (βgal). With no βgal production, other hydrolases are upregulated to compensate, but the relationship between this and disease progression is unknown. Autophagic failure is the first step in neurodegeneration. When autophagy is impaired in GM1 Gangliosidosis, fusion between autophagosomes and lysosomes does not occur, leading to accumulation of normally digested substrates.

Goal of Study: This project determines the association between autophagic failure and lysosomal enzyme upregulation as a biomarker of GM1 Gangliosidosis. Simultaneously, polymersomes are developed to respond to enzyme upregulation to quantify enzyme activity levels *in situ*, providing real-time information on patient health.

Methods and Results: Fibroblasts from GM1-affected (GM1SV3) and normal (NSV3) felines modeled GM1 Gangliosidosis. Immunofluorescence identified the level of co-localization and correlated with activities of hexosaminidase A (HexA), mannosidase, and βgal. To correlate impaired autophagy with enzyme upregulation, starved NSV3 cells were compared to disease. Low starvation times had autophagosomal-lysosomal fusion, while high starvation times lost autophagosomal-lysosomal fusion, similar to disease. Quantification of co-localization confirms this and shows that 48-hour starvation is most similar to GM1SV3. Enzyme activities increase with increasing starvation time, indicating a correlation between autophagic failure and hydrolase upregulation. Polymersomes of hyaluronic acid-b-polylactic acid (HAPLA) responded to upregulated HexA. Polymersomes of HAPLA form at diameters of 108.5 ± 18.3 nm and degrade more rapidly in the presence of hyaluronic acid (cognate similar to upregulated HexA) versus incubation in non-cognate enzyme βgal, which causes zero payload release.

Conclusions: These results suggest a relationship between lysosomal enzyme upregulation and impaired autophagy, helpful for diagnostics development. Working towards a diagnostic tool, HAPLA polymersomes form at deliverable, consistent diameters and degrade more rapidly in the presence of cognate enzymes.

The DNA Methylation Signature of EPCAM-/CD49F- Breast Cancer Stem Cells Correspond with Worse Clinical Severity in Basal-Like Breast Cancers

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The metastatic potential in breast cancer can correspond with the uncontrolled expansion of mammary stem cells identified by loss of epithelial markers EpCAM and CD49f. These populations identified as breast cancer stem cells (CSCs) can be important from a prognostic standpoint, thus characterizing the CSC-specific epigenetic events would be of great interest with DNA methylation playing such a critical role in cell fate. To accomplish this characterization, we compared the 450K DNA methylation profile of EpCAM-/CD49f- cells from the isogenic MCF10A p53-/PTEN- breast cell line against the corresponding EpCAM+/CD49f+ and EpCAM-/CD49f+ subpopulations to determine a CSC-specific DNA methylation signature. Additionally, we overlapped the profiles from 16 established breast cancer cell lines of varying aggressiveness to determine how these cells relate epigenetically with the isolated CSCs. Based on unsupervised PCA and matrix dissimilarity clustering, we identified 3 distinct groups that cluster based on EpCAM-/CD49f- enrichment status. From these groups, we performed differential DNA methylation analysis between varying genomic regions and discovered that DNA methylation changes varied by location between CSC-rich and CSC-poor cell lines (ANOVA FDR *p*-value <0.001). Specifically, CpG islands and promoter-associated regions were differentially hypermethylated in CSC-rich cells, yet non-CpG islands and gene body regions demonstrated extensive hypomethylation in CSCs. Furthermore, correlation between differentially methylated regions and EpCAM-/CD49f- enrichment showed that gene body hypomethylation significantly correlated with an increase in EpCAM-/CD49f- positive cells. Additionally, these hypomethylation events were present in the TCGA breast cohort with gene body hypomethylation being preferentially enriched in both basal-like tumors and tumors harboring *TP53* mutations. Lastly, several genes with extensive gene body hypomethylation corresponded with worse progression-free survival including *BACE2*, *MED12L*, *MLLT3*, *OVOL1*, and *MCF2L*. In conclusion, we identified a DNA methylation signature in EpCAM-/CD49f- breast CSCs and demonstrated that these CSC-associated epigenetics events served as surrogate markers for clinical severity.

Induction of Phenotypic Changes in HER2-Positive Breast Cancer Cells in vivo and in vitro

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Introduction: The influence of breast cancer cells on normal cells of the microenvironment, such as fibroblasts and macrophages, has been heavily studied but the influence of normal epithelial cells on breast cancer cells has not. Here using in vivo and in vitro models we demonstrate the impact epithelial cells and the mammary microenvironment can exert on breast cancer cells. Under specific conditions, signals that originate in epithelial cells can induce phenotypic and genotypic changes in cancer cells. We have termed this phenomenon “cancer cell redirection.”

Hypothesis: Once breast cancer cells are redirected, either in vivo or in vitro, they lose their tumor forming capacity and undergo a genetic expression profile shift away from one that supports a cancer profile towards one that supports a non-tumorigenic epithelial profile.

Methods and Results: HER2+ breast cancer cells were grown alone or in specific ratios with normal breast epithelial cells in vitro and transplanted in vivo. Attenuation of tumor formation was found in co-transplantations indicating cancer cell redirection. Tissue staining and bioinformatic studies demonstrated that redirected HER2+ cells assumed a normal genetic expression phenotype.

Conclusions: These findings indicate that epithelial cells and the normal microenvironment influence breast cancer cells and that under certain circumstances restrict proliferation of tumorigenic cells.

Sex-dependent impacts of environmental enrichment on Angelman Syndrome mice
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Angelman Syndrome (AS) is a rare neurodevelopmental disorder caused by mutations or deletions of the maternal allele of UBE3A. AS in humans is marked by intellectual disability, ataxia, autism-like behaviors, and a happy, excitable demeanor, among other symptoms; in mice (*Ube3a*$^{m-/p+}$, C57B/6J, AS mice for remainder of abstract), these symptoms are displayed as deficits on a variety of behavioral assays including the open field test, marble burying, rotarod, novel-object recognition, and forced swim, as well as physiological defects associated with learning. Notably, there are no effective therapeutic approaches for treating AS. As a project funded by the SD BRIN Faculty Fellowship Program, I worked with undergraduate students on a project investigating the ability of a behavioral manipulation known as environmental enrichment (EE)—long-term, post-weaning exposure of AS mice and wildt-type littermates to increased cage space, toys, treats, and running wheels—to rescue these phenotypes. Importantly, we examined whether there are sex-dependent differences in treatment outcomes on behavioral tasks. We found that EE for male AS mice reduced the motor coordination deficits usually seen in rotarod performance and restored species-specific marble burying behavior. We also examined alterations of behavior in the open field test and in the forced swim task. EE also ameliorated the weight phenotype in AS mice. Interestingly, female AS mice did not respond to environmental enrichment in the same way as the male mice. Ongoing experiments are examining whether EE can rescue well-documented deficits in AS mice related to plasticity, including reduced spine density and dampened long-term plasticity. Future experiments may examine molecular changes in the brain as a result of post-weaning EE.

Lipoprotein Sorting as a Potential Target of Resazomycins, A Novel Family of Antibiotics Against Francisella tularensis and Neisseria gonorrhoeae

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West Virginia INBRE GM103434-19 Gray Rankin

Antibiotic resistance is one of the top threats to global public health. In the United States alone, over two million people each year are infected with antibiotic resistant bacteria which results in approximately 23,000 deaths and billions of dollars in health care costs. The development of new antibiotics is essential to combat this crisis and prevent the loss of additional lives from these once curable diseases. Our laboratory discovered that resazurin, the active component of the commonly used viability dye Alamar Blue, exhibits antimicrobial activity against a select family of Gram-negative bacteria including the human pathogens *Neisseria gonorrhoeae, Helicobacter pylori,* and *Francisella tularensis.* Resazurin and derivatives of this compound, which we collectively call resazomycins, are capable of killing *N. gonorrhoeae* and *F. tularensis* in broth culture as well as inside host cells. One resazomycin, resorufin pentyl ether, significantly reduces vaginal colonization by *N. gonorrhoeae* in a mouse model of infection. Most of the Gram-negative bacteria that are sensitive to resazomycins possess a unique lipoprotein sorting complex (LolDF) that differs from other Gram-negative bacteria. Since the antimicrobial activity of resazomycins appears to selectively target bacteria with LolDF, we hypothesized that this sorting machinery is a potential target of these compounds. To test this hypothesis, we measured differences in the expression of the major *F. tularensis* lipoprotein LpnA in the absence and presence of resazomycins. Treatment with resazomycins resulted in reduced expression of LpnA in the outer membrane of *F. tularensis* suggesting improper sorting of lipoproteins by LolDF. Furthermore, *F. tularensis* bacteria treated with resazomycins are more sensitive to select detergents and antibiotics likely due to increased permeability of the outer membrane correlating with reduced lipoprotein expression. Together, these data suggest resazomycins alter lipoprotein sorting in *F. tularensis*.

High-Fat Dietary Cholesterol Uptake and ROS Response in Enterocytes in the Larval Zebrafish

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West Virginia INBRE GM103434-19 Gray Rankin

Abstract

Vertebrate dietary lipid absorption occurs primarily in the small intestine. The process of dietary lipid absorption involves a complex interplay between nutrients, microorganisms, bile, and mucus that determine intestinal luminal environment. In this study we describe cholesterol uptake and transcriptome response to highly defined diets. This reductive approach allows us to examine specific dietary component's impact on lipid processing and oxidative stress response within the intestine. Utilizing in vivo imaging of whole larval zebrafish to model dietary lipid absorption within intestinal enterocytes, we demonstrate that dietary fatty acids oleic acid and alpha-Linolenic acid, promotes BODIPY-cholesterol absorption and diets without fatty acid do not. When zebrafish larvae were fed increasing amounts of C18:1 or C18:3, a concentration dependent increase in dietary cholesterol was imported into intestinal enterocytes. An experiment comparing oleic acid and oleic acid plus cholesterol diets identified 57 genes responsive to cholesterol metabolism. Transcriptome response to these diets with and without cholesterol reveal differences in pathways regulating cholesterol efflux and lipoprotein formation, ROS, OXPHOS, and mitochondrial biogenesis and fusion. Genes were then mapped using Cytoscape and literature databases to identify network genes involved in oxidative stress and cardiovascular disease. Specifically, mitochondrial associated genes GCLM and GSTO1 linked to regulating gluconeogenesis were up-regulated in high-fat diets without carbohydrates. These data illustrate the power of the zebrafish system to address longstanding questions in vertebrate digestive physiology. *This work was supported by NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence and NIH Grant P20GM103434 awarded to Bluefield State College.*

CTR

Clinical Nurse Perspectives on Implicit Rationing of Nursing Professional Standards in COVID-19: A Qualitative Study

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Delaware IDeA-CTR U546M104941 Monica F. Rochman

Background: In a pandemic situation such as COVID-19, nurses are likely to find themselves in an environment with competing demands and goal conflicts due to time and resource constraints. This will lead to the need to balance crisis management and professional nursing standards of care. Implicitly rationed care occurs when nurses' are unable to complete all nursing care activities for patients. It is characterized by scarcity of resources, time, or an abnormal work environment. Although an emerging body of evidence exists exploring implicit care rationing and similar concepts, nurse perspectives on implicit rationing of nursing care during COVID-19 pandemic is absent.

Purpose: The purpose of this study is to explore clinical nurse and nurse manager experiences of implicit rationing of nursing care with COVID-19 patients and to identify perceived barriers and facilitators to care delivery for COVID-19 patients.

Methods: The study utilized an exploratory qualitative descriptive study design. Qualitative data was obtained through four focus groups containing six to eight clinical nurses or unit nurse managers. Purposive sampling was used to select participants in COVID-19 units and the Emergency Department in a large health system in the Northeast. Our theoretical framework is derived from grounded theory. A thematic analysis will be performed by a single investigator doing an iterative memoing process in NVivo12 until a coding scheme emerges; discovering major themes related to nursing care during the COVID-19 pandemic.

Conclusion and Implications for nursing: Understanding the barriers and facilitators will aide in the development of pandemic specific tailored interventions for care delivery. Workflows can be streamlined for pandemic situations and also to inform daily practice to promote better patient outcomes. As a result of streamlined care, the reduction of moral distress as it relates to shifts in patient care and increased reliance on nurses can be mitigated.

Inhalable biomaterials-based microparticles for the treatment of COVID-19 within the upper airspaces

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The recent COVID-19 pandemic is caused by the SARS-CoV-2 virus. One of the major hurdles in controlling spread of this disease is the virus' ability to be transmitted via asymptomatic carriers prior to the appearance of symptoms. This makes virus infectivity extremely difficult to minimize and control. During the initial state of infection, the virus infects cells of the upper airway where significantly more viral replication occurs compared to several other viruses including SARS-CoV-1. This replication pattern results in limited initial symptoms but a high transmission rate between individuals. The virus eventually descends into the lower pulmonary space, leading to the more severe, hallmark symptoms of COVID-19 disease. To target this early phase of high viral replication and infectivity, we are developing an inhaler compatible microparticle therapeutic for virus sequestration. This system is engineered to deposit microparticles into the mucosal layers of the nose, mouth, and upper airways to bind free virus and allow safe clearance from the respiratory tract without initiating infection or an adverse immune response. This therapeutic can be used early in COVID-19 to slow infection in the upper airway, decrease free viral load, person to person transmission, and potentially decrease COVID-19 severity. Due to the nature of action, this microparticle therapeutic can be used as a prophylactic treatment for high risk individuals including front-line health care workers and family members with a COVID-19+ household member to decrease infection and asymptomatic viral transmission. Lastly, this strategy serves as a platform therapeutic as the microparticles can be easily modified to target a variety of antigens, thus making it adaptable to other upper respiratory viruses.

Circadian Melatonin Signal Disruption by Exposure to Artificial Light at Night Promotes Bone Lytic Breast Cancer Metastases

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Breast cancer (BC) metastasis to bone is most common in patients with advanced metastatic BC. Erosion of bone by BC metastases increases bone fragility, risk of fracture and mortality in BC patients. Bone metastatic BC cannot be surgically removed and can only be treated with chemotherapy and/or radiation therapy. Circadian rhythms are daily cycles of ~24h that control most physiologic processes, disruption of these processes by exposure to artificial light at night (LAN) has been shown to be strongly associated with the development of cancer, particularly breast cancer. Disruption (suppression) of melatonin (MLT) production by LAN is considered as a risk factor for BC. The present study addressed the hypothesis that *circadian MLT disruption by dim LAN (dLAN) promotes osteolytic bone metastatic BC.* Our research shows that the disruption of the anti-cancer circadian hormone melatonin signaling by exposure to dLAN can significantly enhance the metastatic potential of BC cells. This supports the report of the International Agency for Research on Cancer that night shift work is a “probable human carcinogen” and highlights the association between exposure to LAN and invasive BC. Our present results show that moderately metastatic estrogen receptor alpha (ERα) positive MCF-7 BC cells, when inoculated into the tibia (to mimic bone metastatic disease) of MLT-producing Foxn1^{nu} athymic-nude mice and housed in dLAN (suppressed nocturnal melatonin production), developed robust bone metastatic tumors that were highly osteolytic as determined by IVIS bioluminescent imaging and μCT analysis. Administration of nighttime melatonin at physiological levels was able to partially reduce the bone metastatic tumor burden. Melatonin receptor (MT1 and MT2) antagonist luzindole blocked the inhibitory effect of nighttime melatonin on bone metastatic tumor growth supporting a receptor-mediated mechanism. These findings demonstrate for the first time the importance of intact nighttime MLT anti-cancer signaling in suppressing bone metastatic breast tumor growth.

Metabolic Syndrome and COVID-19 Mortality

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Introduction: Coronavirus disease 2019 (COVID-19) mortality is high in patients with hypertension, obesity and diabetes mellitus, yet a plausible mechanism remains unknown. We examined the association between hypertension, obesity, hyperlipidemia, and diabetes, individually and clustered as metabolic syndrome (MetS), and COVID-19 outcomes.

Methods: A retrospective, observational study of consecutive COVID-19 patients hospitalized at two academic tertiary hospitals in New Orleans from March 30th to April 5th, 2020. Patients were identified as MetS using WHO criteria and compared to patients without MetS. The primary outcome was hospital mortality. Secondary outcomes included ICU admission, invasive mechanical ventilation (IMV), a diagnosis of ARDS defined by Berlin Criteria, hospital length of stay (LOS), and hospital readmission. Multivariable regression models included age, sex, race, individual hospital site, and Charlson Comorbidity Index.

Results: Among 287 patients (mean age, 61.5 years; female, 56.8%; non-Hispanic Black, 85.4%), MetS was present in 188 (66%). MetS was significantly associated with mortality (adjusted odds ratio [aOR]: 3.42, 95% confidence interval [CI]: 1.52-7.69), ICU (aOR: 4.59, CI: 2.53-8.32), IMV (aOR: 4.71, CI: 2.50-8.87) and ARDS (aOR: 4.70, CI: 2.25-9.82), compared with non-MetS. Multivariable analyses of hypertension, obesity and diabetes individually showed no association with mortality. Obesity was associated with ICU (aOR, 2.18, CI, 1.25-3.81), ARDS (aOR, 2.44, CI, 1.28-4.65), and IMV (aOR, 2.36, CI, 1.33-4.21). Diabetes was associated with ICU (aOR, 2.22, CI, 1.24-3.98) and IMV (aOR, 2.12, CI, 1.16-3.89). Hypertension was not significantly associated with any outcome. Inflammatory biomarkers associated with MetS, C-reactive protein (CRP) and lactate dehydrogenase (LDH) were associated with mortality [CRP (aOR, 3.66, CI, 1.22-10.97), LDH (aOR, 3.49, CI, 1.78-6.83)].

Obesity the Most Common Co-Morbidity in SARS-CoV-2: Is Leptin the Link?

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Obesity is a major risk factor for diabetes, cardiovascular disease, and lung disease. These diseases most commonly predispose individuals with SARS-CoV-2 infections to require hospitalization including intensive care unit admissions. Elevated circulating leptin concentrations are a hallmark of obesity. Leptin is secreted by adipocytes in proportion to body fat and regulates metabolism. Leptin signaling plays a pivotal role in the development of lung fibrosis. Through receptors on leukocytes, leptin signals through many pathways including the Jak/STAT pathway to mediate immune cell number and function.

During an infection, T-cell activation is accompanied by high energy requirements to support biosynthesis of intracellular components. Leptin is especially important for activated T-cells to upregulate glucose metabolism to meet the demands of the cell. While early studies show that starvation and leptin deficiency are associated with decreased immune reactivity, hyperleptinemia has also been shown to have detrimental effects on the immune response. Based on evidence from patients with obesity and pneumonia, and rodent models of hyperleptinemia with and without obesity, high baseline levels of leptin drive immune defects. Impairments in neutrophil response, and an insufficient antiviral response predispose to increased susceptibility to, and severity of, respiratory infections. In SARS-CoV-2 infections, lymphopenia appears to be a consistent finding, and occurs in approximately 80% of patients. Immune insufficiency or misdirection may increase viral replication or render its clearance ineffective which can cause tissue damage, stimulation of further macrophage activation, and an uncontrolled loop of self-amplification. The resulting cytokine-storm syndrome can precipitate multi-organ failure.

By altering the metabolic environment, obesity and its attendant condition of hyperleptinemia disrupts T-cell function resulting in a suppressed T-cell response to infection. We propose that leptin may be the link between obesity and its high prevalence as a comorbidity of SARS-CoV-2 infections (Rebello *et al*, Int J Obes, 2020; Jul 9:1–8).

Population Level Spread of SARS-CoV-2 Across Two Metro Areas in Louisiana: What Do Seroprevalence Studies Catch that our Community Testing Efforts Miss?

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Background: New Orleans was hit particularly hard early in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, possibly due to travelers and crowds throughout Mardi Gras, and a summertime uptick in positive testing in Baton Rouge coincided with reports of super spreader events at re-opened bars. Testing for the novel virus has been limited worldwide by supply chain issues and testing capacity. To estimate the true spread of infection, two high-throughput prevalence studies were performed in New Orleans at the end of a “stay-at-home” order (May 9-15) and Baton Rouge after two phases of reopening (July 15-31).

Methods: Subjects were targeted according to census demographics and iterative recruitment ensured a highly representative sample. Subjects were given a survey, nasopharyngeal swab and a blood draw. Paired PCR (Abbott m2000, 100 copies/μL limit of detection) and antibody (Abbott i2000, specificity 99.6%, sensitivity 100%) testing were used to determine whether someone had a new/contagious infection, a late infection, or a past infection.

Results: Although over all exposure was similar between New Orleans and Baton Rouge (7.8% and 6.6%, respectively), the distribution of new versus old infections was different. There was a high rate of asymptomatic infection and distinctively high odds of testing positive if anosmia was reported. Racial disparities were apparent across both cities and positivity ratios were highly variable by ZIP.

Conclusions: Although some study outcomes are specific to these two cities, several valuable lessons can be applied to other areas of the world; asymptomatic infection makes this virus particularly insidious, anosmia should be integrated into screening practices, and prevalence studies should strive to recruit a demographically representative sample.

Adequate Sleep Duration Enhances Cardiovascular Benefits of a Physical Activity Intervention in Older African Americans

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African Americans are at a greater risk for cardiovascular disease and inadequate sleep than corresponding whites. Age-associated declines in sleep duration, cardiovascular health, and physical activity (PA) highlight the importance of the relationship among these variables in African Americans. While PA is thought to be beneficial for promoting sleep quality, it remains unknown how habitual short sleep during a PA intervention influences the interventional response.

Sedentary older African Americans (n=27; 65-85 years old; 74% female) participating in the intervention arm of a 12-week PA trial (NCT03474302) were categorized by sleep. Habitual sleep was determined using commercial activity monitors. Short sleep (n=12) was defined as averaging <6 hrs of sleep during the intervention. Participants wore validated activity monitors at baseline and 12 weeks. Differences in cardiovascular outcomes at baseline and 12 weeks were assessed between sleep categories using sex-adjusted linear mixed models.

Daily steps via accelerometer increased (P=0.007) with no between group differences (P=0.780). Moderate to vigorous activity (MVA) duration increased (p<0.001), and time spent in MVA was 9 minutes higher if sleeping ≥6 hrs (P=0.047). Body weight did not significantly change (-0.71 kg; p=0.11) and was similar between groups (p=0.55). Only those sleeping ≥6 hrs experienced improvements in systolic blood pressure (-10 4.5 mmHg; p=0.03), and a trend existed for between group differences (P=0.096). Total (TC) and LDL cholesterol (LDL) decreased overall. Only those sleeping ≥6 hrs experienced significant declines in TC (-30 11 mg/dL) and LDL (-23 9 mg/dL) (P<0.012, all) with differences noted between sleeping categories (TC: P=0.044; LDL: P=0.095). Diastolic blood pressure, HDL cholesterol, and triglycerides were not significantly different over time or between groups.

Adequate sleep during a PA intervention may be important to elicit cardiovascular benefits. Thus, research evaluating sleep extension complementary to increased physical activity is warranted in short sleepers.

Support: BrightFocus (A20175472); National Institute of General Medical Sciences of the National Institutes of Health (U54-GM104940)

The radiosensitizing effects of androgen receptor (AR) blockade on glioblastoma

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Purpose: To explore the radiation (RT) sensitizing effects of AR antagonists on glioblastoma (GBM).

Methods: Apoptosis assay and cell cycle assay were performed on established GBM cell lines after AR blockade with enzalutamide or bicalutamide treatment. Combenefit study was performed when combining various concentrations of enzalutamide and doses of irradiation on human and mouse GBM cell lines. The correlations of mRNA expression levels between AR and DNA repair genes were studied in The Cancer Genome Atlas (TCGA) database. RNAseq was performed on U87MG after the treatment of enzalutamide. Syngeneic orthotopic GBM mouse model was used to compare the treatment effects and overall survivals of brain RT, AR antagonists, alone or combined.

Results: Enzalutamide induced apoptosis in U87MG and Ln229 cell lines but not in U138MG and increased the G2/M cell cycle in all three human GBM cell lines. For U87MG cells, the most synergy was observed with 80 μM enzalutamide and 2 Gy of RT. RNAseq results from TCGA database demonstrated strong positive correlations of the expression levels between AR and most DNA repairing enzymes in GBM patients. From our RNAseq results after treating U87MG cells with enzalutamide for various time periods, the expression level of DNA repair enzymes decreased significantly. All the mice in the enzalutamide plus RT group or bicalutamide plus RT group survived at the end of the experiment but none of the single treatment group did, demonstrating the synergistic effects of AR antagonists and brain RT on tumor control. Rechallenging of the survived mice showed no tumor growth indicating durable immunologic effects of the dual modality treatment.

Conclusions: AR antagonists have significant radiosensitizing effects on GBM both *in vitro* and *in vivo*. When combined with RT, AR antagonists increases anti-GBM immunologic effects.

Nuclear to Cytoplasmic analysis of Kaiso (ZBTB33) as a predictor of overall breast cancer survival

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North Dakota GM128729-02 Marc Basson

Background: Our understanding of clinical relevance of subcellular distribution of Kaiso (nuclear/ cytoplasmic) in the growth and survival of human Breast cancer (BC) is limited. We apply artificial intelligence (AI) approach to quantitatively profile the subcellular distribution of the multi- functional transcriptional regulator Kaiso in the tumors of a large racially diverse BC cohort from a designated health disparities region in the United States. These findings identify more effective modalities of Kaiso biomarker assessment while uncovering unanticipated insights into Kaiso's role in BC progression.

Methods: We analysed a cohort of BC patients (n=555) who underwent surgery for their primary BC in Greenville, NC using AI and SM approach.

Results: The sub-classification BC shows, cytoplasmic Kaiso is differentially enriched in ER- BC (p=0.001) compared nuclear Kaiso (p=0.8) and is significantly enriched in the more aggressive classes LumB (p=0.0017), HER2+ (p=0.05) and TNBC (p=6.1e-07) with respect to less aggressive class LumA BC patients. Additionally, the survival analysis of different compartments of Kaiso demonstrates that high cytoplasmic Kaiso (HR = 16.29 (7.6 – 34.8), p = 5.5e−13) is much more predictive of poor survival compared to nuclear Kaiso (HR = 2.83 (2.02 – 3.8), p = 6.1e−11).

At gene expression level, ZBTB33 mRNA levels do not correlate with either nuclear (Spearman correlation: -0.03157, p= 0.7267) or cytoplasmic levels (Spearman correlation: -0.03526, p= 0.6962) of Kaiso. Surprisingly, ZBTB33 mRNA abundance is predictive of poor overall BC survival as demonstrated in two independent publicly available BC cohorts named as Metabric (HR = 2.14 (1.49 − 3.08), p = 2.7e−05) and Gyorffy B et al. (HR = 1.81 (1.55 − 2.12), p = 2.5e−14). Nuclear and cytoplasmic levels of Kaiso do not show significant differences based on race p=0.27 and p=0.1 respectively.

Conclusion: Our AI based results shows the subcellular distribution of high Kaiso is associated with poor prognosis of BC survival and subcellular localizations of Kaiso may play differential biological roles in BC prognosis.

Efficacy of RAGE inhibitors with dacarbazine in PDX models of melanoma Estelle Leclerc, Sakshi Taneja and Daniel Tuvin.
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Metastatic melanoma (MM) remains a malignancy very difficult to treat. For many years, the standard of care for MM was the cytotoxic drug dacarbazine. However, the response rate with dacarbazine was only 22%. In the last decade, new drugs with improved efficacy have been approved: BRAF kinase mutant and immune check- point inhibitors. These new drugs are now standard of care but are not miraculous drugs: not all patients respond to them, recurrence is common and they are associated with severe adverse effects. Clinical studies have shown that anti-cancer treatments are more effective when several drugs are combined and many of the current standard of care drugs are now tested in combination therapies.
Interestingly, many of the new combinations include dacarbazine, showing regain in interest for this old drug.

A few years ago, we showed in a mouse model of melanoma that the cytotoxicity of dacarbazine could be enhanced when combined with an inhibitor for the receptor for advanced glycation endproducts (RAGE). RAGE is a pattern recognition receptor that responds to cellular stress and is activated by ligands generally described as damaged associated molecular patterns (DAMPs). We and others have shown that RAGE is upregulated in some melanoma tumors and that targeting RAGE signaling could be a valid approach for the treatment of melanoma.

In the awarded DaCCoTA Ready-To-Go Pilot Grant, we propose to test the combination of dacarbazine, and other standard of care for MM, with RAGE inhibitors, in clinically relevant Patient-Derived-Xenograft (PDX) mouse models. For this project, we are working in collaboration with Dr. Daniel Tuvin from Sanford, a Surgical Oncologist, for obtaining melanoma tumors from patients. These tumors will be implanted in severely immune compromised mice, and their responses to different drug treatments will be investigated. In this short presentation, we will discuss progresses made on this project.

Prognostic and Therapeutic Value of two IDG-Focus Kinases in Breast Cancer Treatment
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Resources generated through the NIH initiative “Illuminating the Drugable Genome - IDG” (NIH Common Fund) were used to identify two IDG kinases with high predictive value in breast cancer. High transcription levels of either DYRK2 or STK3 correlated strongly with shorter survival in breast cancer patients. The objectives of the DaCCoTA funding pilot project are to evaluate first whether (1) protein expression levels of STK or DYRK2 in breast cancer tumor tissue have comparable prognostic value as mRNA level. The second aim is to correlate STK3 and DYRK2 protein expression in patient derived breast cancer specimen with treatment outcomes. The long-term objective is to use STK3 or DYRK2 protein expression levels as novel biomarkers to inform clinical treatment decisions and to potentially target both kinases with molecule kinase inhibitors as a novel approach to breast cancer treatment.

The presentation will discuss early results regarding reagent validation, results from gene expression studies and how the COVID-19 pandemic has impacted research in the laboratory.

Aunt Bertha Community Connections: Enhancing connections between healthcare and community **Lisbeth Wierda, MPH, Project Manager, Center for Outcomes Research and Evaluation; Kerri Barton, MPH, Rural Research Navigato; Dr. Neil Korsen, Co-Director NNE-CTR Rural Core**
Maine IDeA-CTR U54GM115516 Gary Stein

Background: The Aunt Bertha Community Connections (ABCC) program was launched in summer 2020 to engage high school, college, and medical students in translational research. The students provided an intervention to enhance the implementation of a directory of community resources in a county in rural Maine. The information gathered by students will be included in Aunt Bertha, a community asset directory integrated into the electronic health record of the local health system to connect patients to resources in their community. While creating this community resource inventory, students learned about social determinants of health (SDOH) impacting rural communities. Students also learned about educational and career paths through a tiered mentoring system comprised of a medical student, college student and high school students. The aim of this mentorship was to encourage students to enter into health-related careers. This model was based on the MAPSCorps© model and has been adapted to meet the needs of this project.

Methods: The high school and college students worked with community- based organizations to provide training on how to claim and maintain their listing in the Aunt Bertha platform. They also identified gaps in assets not included in the directory and worked with Aunt Bertha staff to add those assets. At the same time, the medical student worked with the local medical practice to create and implement a workflow, which will ultimately strengthen the referral process from primary care providers to these community organizations.

Results: The field team found over 200 local community assets not yet included in the resource directory. In the month of July, 25 community organizations claimed their program in the Aunt Bertha platform across MaineHealth, and over 45 MaineHealth staff members utilized the platform. The medical student trained 15 clinicians from the local medical practice on the use of the platform and its referral capability.

Conclusion: This intensive summer program enhanced an existing community asset directory to match the needs of the population that it is meant to serve, and created a pathway of community referrals from the local healthcare system for patients who screen positive for SDOH needs.

Learning objectives:
Describe how SDOH can be addressed through referrals to, and use of, assets within a community.
Understand strategies to enhance a community asset directory to be more relevant to local users.
Evaluating the effectiveness of inter-professional collaboration to address SDOH.

Nuclear DNA Damage as a Potential Tool to Predict Cervical Cancer Risk

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Background: Cervical cancer (CC) is the fourth most frequent cancer in women. Infection by the human papillomavirus (HPV) is considered a necessary event. Yet, only a small subset of HPV infected woman will develop high-grade cervical dysplasia, also referred as CIN 2/CIN 3, and an even smaller and unpredictable subset will progress to cervical cancer. HPV infection increases DNA damage potentially contributing to cancer progression. Our lab has developed a novel highly sensitive DNA damage quantification assay (q-PADDA). Based on q-PADDA preliminary data, we hypothesize that patients with the highest levels of DNA damage have a higher chance to progress to cervical cancer.

Aims: (1) Determine whether the level of DNA damage correlates with the grade of cervical dysplasia and cancer. (2) Assess whether risk factors for development of cervical dysplasia and cancer correlate with levels of DNA damage.

Methods: Following IRB approval and patient consent, cervical epithelium samples were collected by cytobrush during pelvic exams. Genomic DNA was extracted and damage quantified by q-PADDA. Patient demographic and clinicopathologic data were collected. ANOVA and regression analysis were performed.

Results: A total of 105 participants have been enrolled into the study and divided into 4 groups based on pathology reports: No dysplasia (CIN0; n=33), low dysplasia (CIN1; n=30), moderate/high dysplasia (CIN2/3; n=37) and CC (n=5). DNA damage quantification has been completed on the first 30 cases enrolled. We observed that, compared to cases without dysplasia, CIN1 cases have a 2-fold increase ($p<0.05$) and CIN2/3 cases have approximately a three-fold increase ($p \leq 0.02$) in DNA lesions. Regression analysis shows no significant correlation between age, race, smoking and drinking status compare with pathology risk.

Conclusion: Our preliminary data shows that patients without dysplasia have the lowest levels of DNA damage, and suggest that DNA damage increases with escalating grade of dysplasia and cancer risk.

Grant support: This work was supported by OSCTR and by The Peggy and Charles Stephenson Cancer Center. Dr. Queimado holds a Presbyterian Health Foundation Endowed Chair in Otorhinolaryngology.

Innovation During COVID-19: Telebuprenorphine
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The opioid epidemic continues to be one of the most pressing public health crises in the United States and during the coronavirus disease (COVID-19) pandemic, overdose deaths are rising nationwide. COVID-19 has increased health-related risks associated with opioid use, including overdose and overdose death, in part due to disruptions in access to addiction treatment. Risk of overdose within the context of the COVID-19 pandemic may be further intensified due to stress caused by social isolation, using drugs alone, and resumed use among people in recovery who have had loss of opioid tolerance. Recognizing limited treatment access during COVID-19, in March 2020 the Substance Abuse Mental Health Services Administration (SAMHSA), the US Drug Enforcement Administration (DEA), and the US Department of Health and Human Services released a series of policy changes which have allowed for use of telehealth for starting buprenorphine treatment without requiring an in-person evaluation or video interface. With these new allowances, in April 2020, we established an on-demand buprenorphine telehealth phone line to expand buprenorphine access in Rhode Island, which has one of the highest rates of opioid overdose deaths in the United States. The Buprenorphine Hotline is a 24/7 telephone service where people can call to receive consultation with a physician about starting buprenorphine treatment and, if appropriate, prescribed buprenorphine and linked to follow up for maintenance treatment. To date, 48 people have received buprenorphine treatment through the Buprenorphine Hotline. Planned research about the hotline includes 1) Telephone administered surveys with patients to assess hotline feasibility, acceptability, and patient experiences and 2) Evaluation of patient outcomes including treatment follow-up and opioid overdose. Outcome comparisons will be made with a matched control group that has engaged in addiction treatment through an in-person encounter.

UBXN2A Suppresses Colorectal Cancer Growth and Metastasis by Suppressing mTORC2-AKT Signaling Pathway

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The mTORC2/pAKT signaling pathway plays a critical role in promoting the tumor growth and metastasis in human colorectal cancer (CRC); therefore, it is a promising target for novel anti-cancer therapeutics in CRC patients. Our previous studies have identified a ubiquitin-like (UBX) domain-containing protein, UBXN2A, as a novel tumor suppressor protein in CRCs. In this current study, we first used murine models to show haploinsufficiency of UBXN2A significantly increases colon tumorigenesis. Consistent with a tumor suppressor role, CRC tissues with worse histological grade exhibit decreased UBXN2A protein expression. Here, we report that induction of UBXN2A significantly reduces AKT phosphorylation particularly at Ser473 and Thr308 which is essential for a plethora of cellular processes including cell growth and migration. Mechanistic studies revealed that UBXN2A targets Rictor protein, a key component of the mTORC2 complex and the specific kinase for AKT phosphorylation on Ser473, for the 26S proteasomal degradation. Meanwhile, mTORC1 activities stay unchanged in the presence of UBXN2A. Finally, a set of genetic and pharmacological studies showed the presence and the absence of UBXN2A significantly affect the cancer cell proliferation, motility, migration and invasion. These findings provide new insights into functions of a ubiquitin-like protein in the inhibition of a dominant oncogenic pathway and support the notion that UBXN2A is an attractive and promising target for treatment of both primary and metastatic forms of CRC.

DaCCoTA funding: Research reported in this abstract was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number U54GM128729.

A Systems Approach for Evaluating Centers for Translational Research (CTR)

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The goals of this presentation are to a) increase awareness of the CTSI and CTR network partners of the innovative evaluation approach being used by the DaCCoTA CTR, and b) refer those interested to our supporting peer-reviewed publications that describe in greater detail the key lessons learned and how to apply our approach to other systems.

The presentation will begin by making the case that the CTR organizational structure does not fully capture the coordination needed between and among KSA's (i.e., cores) that is critical for success. We will then discuss why *a systems evaluation approach* is needed to compliment and supplement the NIH required logic model approach to evaluation. Participants will then be given an overview of the science behind the approach and shown how the three steps of System Evaluation Theory, or SET, (Renger, 2015) allowed us to define and evaluate the DaCCoTA system showing how SET fills in the gaps in logic models and assists with critical day-to-day decision-making. CTSI's and CTRs interested in learning more will be provided contact information and links to additional resources.

Relevant publications:

Renger, T., Renger, J., Basson, M.D., Van Eck, R., Renger, J., Souvannasacd, E., & Hart, G. (under revision). Using the Homeland Security and Exercise Evaluation Program (HSEEP) Building Block Approach to Implement System Evaluation Theory (SET) in Evaluating a Center for Translational Research (CTR). *American Journal of Evaluation*.

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Renger, R., Hart, G., Van Eck, R., Souvannasacd, E., Renger., J., & Basson, M.D. (2020). Lessons Learned in Evaluating a NIH Centre for Translational Research (CTR). *Evaluation Journal of Australasia*, *20*(1), 1-17

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Renger, R. (2015). System Evaluation Theory (SET). *Evaluation Journal of Australasia, 15*(4), 16-28.

Elastic Powder-Based Lung Tissue Sealant for Pleural Defects in a Mouse Model

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Damage to the pleural tissue lining the lung can lead to a fluid leak into the pleural cavity, which can result in mortality. Few tissue sealant options are available and even fewer are effective. Thus, we have developed a powder sealant capable of adhering to tissue and crosslinking to form an elastic hydrogel. Methacrylated alginate (AMA) was synthesized and to enable gelation, visible green light crosslinkers were incorporated into the aqueous-based system. The solution was lyophilized and processed into a powder. Gelation kinetics and viscosity of a reconstituted powder solution were collected. Burst pressure experiments were conducted. Mice were anesthetized and tracheostomized with an 18-gauge cannula, and paralyzed. Mice were connected to a computer-controlled small animal mechanical ventilator
(flexiVent, Scireq). Once the mice adjusted to the ventilators the thoracic cavity of the animal was open and lungs were punctured, followed by another series of baseline measurements to measure mechanics testing, and verify injury. The injury was sealed using AMA powder and green light crosslinking; tests were repeated to compare lung mechanics pre-injury and post-repair, allowing for real-time pressure data collection of lung mechanics. After acute traumatic injury, there was a loss in pressure. A successful post-repair test (i.e., application of the AMA powder, hydration and crosslinking) indicated that the sealant maintained pressure and returned lung capacity to pre-injury values, and that the sealant did not fail. The data indicates efficacy of a novel powder-based elastic sealant for recapitulating lung mechanics post injury. The powder-based sealant has the advantages of adhering to tissue upon application *in situ* and subsequent hydration, and remaining in place while crosslinking took place. Future work will explore long term *in vivo* studies to investigate lung tissue healing after repair with the novel powder sealant material.

This work was funded in part by NIH R01EB020964 (Oldinski).

Increased Expression of CXCR4 Leads to Enhanced Cell Migration in CALM-AF10 Driven Leukemia

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Maine IDeA-CTR U54GM115516 Gary Stein

The bidirectional nature of interactions between leukemic cells and the bone marrow stromal components is increasingly recognized as important in controlling cell proliferation, quiescence, and chemoresistance in aggressive leukemias. Adhesion of leukemic blasts to the bone marrow stroma through cell surface and secreted proteins, including the G-protein coupled receptor CXCR4, is proposed as a mechanism by which leukemia evades chemotherapy, resulting in disease relapse. *CALM-AF10* is a leukemogenic chromosomal translocation found in 15% of T-ALL, and is associated with bulky mediastinal disease and a propensity for CNS relapse, phenomena related to leukemia cell adhesion. Utilizing two independent systems, including murine and human leukemia cell lines characterized by the presence or absence of *CALM-AF10* (CALM-AF10+ or CALM-AF10-), we compared expression of CXCR4. We identified increased CXCR4 expression in CALM-AF10+ leukemias, in comparison to CALM-AF10- leukemias. This is evident at both the mRNA transcript and protein levels. We specifically identified an increased expression of CXCR4 at the plasma membrane. We *hypothesize* that CALM-AF10 modulates the leukemic cell interactions with the bone marrow stroma via CXCR4, and that this can be targeted to therapeutic effect. We then assessed whether differences in cell adhesion, migration, or proliferation are altered by activating CXCR4 with its ligand CXCL12, or inhibiting it with the small molecule inhibitor, AMD3465. Importantly, we found that CALM-AF10+ cells expressing high levels of cell surface CXCR4 demonstrated a two-fold increase in cell migration towards a CXCL12 stimulus; the control CALM- AF10- cells did not exhibit this change in migration. Mechanistically, we identified that inhibition of CXCR4 signaling is accompanied by a decrease in pERK/ERK, and are currently investigating the functional consequences of this perturbation. Future studies will dissect the mechanisms of overexpression, and will assess possible synergy between targeted CXCR4 inhibitors and traditional chemotherapy.

Acknowledgements: The Children's Leukemia Research Association, Pediatric Cancer Research Foundation, Northern New England Clinical and Translational Research Network (U54GM115516), Emily M. Lyman Pediatric Leukemia Fund, and Keegan Bradley Charity Golf Classic funded this work.

Prescription Opioid Policy Changes Impact on Opioid Overdose and Related Adverse Effects

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This work was supported by the NIH NIGMS Northern New England Clinical Translational Research

Maine IDeA-CTR U54GM115516 Stein Gary

(**Instructions:** "Abstracts should be no more than 300 words and include the name of the presenter and their role in the funded program (e.g., PI, research project leader, graduate student, etc.).")

Presenter: Valerie Harder (Research Project Leader)

Background: Policies aimed at mitigating the opioid epidemic limited quantities of prescribed opioids to decrease opioid overuse and diversion. The impact of these policy changes on opioid-related overdoses and adverse effects has not been evaluated.

Objective: Examine the impact of opioid prescribing policy change on emergency department (ED) and hospital utilization for opioid overdose and related adverse effects.

Methods: Patients aged ≥15 years from Maine (N= 1,370,960) and Vermont (N= 598,784) statewide all-payer claims. We examined 1) rate of opioid overdoses and 2) rate of opioid-related adverse effects per 100,000 person-months using interrupted time series models to assess the impact of the policy initiation in Maine (07/2016) and Vermont (07/2017), using multilevel mixed-effects negative binomial regression models. Analyses were stratified by age categories and rural/urban designations to examine possible moderating effects.

Results: In Vermont, the opioid overdose rate significantly increased following the prescribing policy change (Incidence rate ratio (IRR: 1.28, 95% Confidence Interval (CI): 1.12-1.42), and the opioid-related adverse effect rate significantly decreased (IRR: 0.75, 95%CI: 0.60-0.93). Stratified analyses identified 25-34 year olds and those living in isolated rural areas with highest overdose rates, and those 65 years and adults living in large rural cities with lowest adverse effect rates. In Maine, no significant changes were seen for either outcome over time.

Discussion: While Vermont's decrease in adverse effects is promising, the increase in opioid overdoses for specific groups following the prescription policy change is concerning and should be monitored for more than one year post policy.

Medication-assisted treatment and postpartum health care utilization among pregnant women with opioid use disorder in Maine, 2010-2018

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Maine IDeA-CTR U54GM115516 Stein Gary

Our objective was to estimate the prevalence of medication-assisted treatment, and its association with 12-month postpartum hospitalization and emergency department (ED) visits, among pregnant women with opioid use disorder (OUD) in Maine. We used data from the Maine All Payer Claims Database, a repository of healthcare claims data for the majority of residents with health insurance in Maine. We restricted the analysis to women covered by Medicaid because substance use disorder-related claims are currently not available from commercial payers. OUD was identified among pregnant women if they had at least one OUD diagnosis code (ICD-9/10) or medication-assisted treatment code during the 5 months leading up to delivery month. Consistent treatment (evidence of buprenorphine or methadone treatment for each of the 5 months prior to delivery) was compared with inconsistent and no treatment for risk of hospitalizations and ED visits in the first 12 months postpartum using log binomial regression. Risk ratios (RR) were adjusted for age at delivery, rurality, delivery type, hospital level of care, and any prescription claim during pregnancy for benzodiazepines and antidepressants, separately. During 2010- 2018, 43,480 pregnancies in Maine were paid for, in part, by Medicaid. OUD among pregnant women increased from 7.4% (SE=0.4%) in 2010 to 11.0% (SE=0.5%) in 2018; consistent treatment among women with OUD increased from 42.6% (SE=2.4%) in 2010 to 49.8% (SE=2.4%) in 2018. Among women with continuous Medicaid coverage (n=3668), consistent treatment was associated with lower risk of hospitalization compared with inconsistent treatment (aRR=0.69, 95% CI: 0.53-0.89) and no treatment (aRR=0.83, 95% CI: 0.63-1.09), and lower risk of ED visits compared with no treatment (aRR=0.87, 95% CI: 0.81-0.94) (comparison with inconsistent treatment showed no difference in risk (aRR=1.00, 95% CI: 0.92-1.09)). Consistent medication-assisted treatment during pregnancy results in decreased 12-month postpartum emergency health care utilization among women with OUD covered by Medicaid.

UV LAVE™, An automated viral inactivation system for the purpose of inactivating viral particles on Filtered Face Respirators

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Maine lDeA-CTR U54GM115516 Stein Gary

Background: This project will demonstrate the feasibility of using broad spectrum UV-C light to effectively inactivate viral particles on the most commonly used filtered face respirators (FFR) models for the purpose of reuse up to three times by the original user.

Objective: To examine the use of UV-C light to inactivate viral particles on commonly used FFRs.

Method: Two portable prototypes currently under production: a countertop unit for long-term and skilling nursing, dental offices and clinics and a larger unit for hospitals specifically, operating room. Collaboration with Maine Medical Center, Bowdoin College and University of New England an automated prototype will be tested and verified in September, 2020 at University of New England.

Results: Transmission of UV-C through mask layers from bidirectional UV sources combined with reflections off FFR layers determines internal intensities inside FFR models and predicts the overall transmission and UV-C dose required to inactivate specific viral particles on FFRs.

Significance: Testing will demonstrate using UV-C to inactivate viral particles is feasible and effective in recycling FFR thereby conserving resources. We are building and conducting testing on two innovative FFR viral inactivation systems which may prove more cost effective in inactivating viral particles on FFRs.

Discussion: We are in the midst of a pandemic overshadowed by national unrest and tenuous global diplomacy. The intelligence behind this project is preparedness amidst uncertainty. When prepared, there is a degree of control over an outcome. The UV LAVE™ provides national and state autonomy and preparedness should a supply chain disruption recur. In building and testing the UV LAVE™ we provide options for Maine and the nation by ensuring the UV LAVE™ is positioned alongside ventilators in our nation’s stockpile.

COVID-19 in Rural America: Testing Patterns and Clinical Characteristics in West Virginia

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Background: There are limited data describing the COVID-19 epidemic in rural America. Methods: This retrospective observational analysis of clinical and demographic data was conducted using extracted electronic medical record data including patient age, sex, ethnicity, race, testing dates and results, testing site location, as well as information on chronic comorbidities, among patients tested for SARS CoV-2 in the West Virginia University Hospital systems between March 13, 2020 and July 12, 2020. To receive testing, patients had symptoms consistent with COVID-19 or a history of potential exposure to a person infected with SARS CoV-2.

Results: Of 22,197 persons tested, 777 (3.5%) tested positive, of whom 56% were women, 88% white, and 26% obese. One third of all tests (n=7,329) were conducted at drive up testing sites, of which only 0.4% were positive. Black patients were significantly more likely to test positive compared with whites (10% vs. 3% respectively, $p<0.001$). Overall, 126 patients required hospitalization and those hospitalized were more likely to be >60 years (69.8%) and male (54.8%). Among obese patients testing positive, 27% were hospitalized. Black patients required hospitalization more often compared with whites (27.9% vs. 16.8% respectively, $p<.001$). Those with cardiovascular disease were 3 times more likely to require hospitalization ($p=0.005$). Death was more frequent among hospitalized patients (15.9%) compared with outpatients (0.6%).

Conclusions and Relevance: Our findings suggest rural patients who were Black were more likely to test positive for SARS CoV-2. Blacks, obese persons, and those with cardiovascular disease, are at increased risk for severe COVID-19, suggesting a critical need to expand testing among persons in the risk groups and their families. Drive-up testing sites were popular but detected a small proportion of the positive tests suggesting that testing tents are an inefficient venue for detecting infected persons.

Daily Transmissibility, Rt, provides an Early Warning of COVID-19 Outbreaks

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Background: The basic reproduction number, R0, describes the transmissibility of an infectious agent as measured by the number of secondary cases that one case would produce in a susceptible population. If R0 > 1, an outbreak will continue and if R0 < 1, the outbreak will not be sustainable. Transmissibility of an infectious agent can change over time, particularly with initiation of control measures which alter contact rates (e.g., social distancing, mask wearing).

The instantaneous basic reproductive number (ℝ) estimates time specific changes in transmissibility and can be interpreted similarly to the basic reproductive number (0). In practice, it represents the expected total number of secondary cases from infected individuals consistent with conditions at the specified time. As such, it is an effective tool for charting the evolution of the pandemic and monitoring effectiveness of control measures.

Methods: Daily PCR testing results by county are obtained from the West Virginia (WV) Department of Health and Human Resources. The value of is calculated daily for the state of WV as well as for each WV county, using cases over a seven-day window and the EpiEstim R package.

Results: Findings demonstrate the ability of to predict near- term increases in COVID-19 incidence. Here an increase in (red line) at the end of June is followed by a subsequent spike in COVID-19 incidence in early July (blue bars) (see Figure).

Conclusions: Preliminary data suggest may be an effective early warning tool to complement existing public health control measures.

CO-FUNDING

Development and Testing of an Injectable Matrix Gel for the Treatment of Rotator Cuff Degeneration Jeff Wolchok

University of Arkansas

Arkansas IDeA Co-funding 1R15 AR073492-01 Jeff Wolchok

Introduction: The muscles of the rotator cuff undergo progressive degeneration following tendon tear. We suggest that a minimally invasive injectable matrix gel prepared from decellularized skeletal muscle could be used to regenerate rotator cuff muscle.

Materials and Methods: Human tibialis anterior (TA) muscle was decellularized and pepsin digested to produce injectable matrix gel for testing. The host response to matrix gel injection was examined using a rat model. Matrix gel efficacy was examined using a delayed rotator cuff repair model in a rabbit.

Results:

Host Response: The peak TA contractile force was significantly increased (44%) in the matrix gel injection group (3.25±0.41 N/kg) when compared to PBS controls (2.25±0.70 N/kg). TA mass was also elevated (20%) in the matrix gel injection group (2.43±0.14 g/kg) when compared to PBS controls (2.05±0.07 g/kg). Three days following matrix gel injection, the expression levels for several key wound healing cytokines (IL-6 and IL-10), extracellular matrix molecules (Col1, Col3, and TGFβ1), and the macrophages marker CD68 were elevated.

Efficacy: Although repair+matrix gel (2.17±0.16 g/kg body weight) and repair only (1.86 ±0.17 g/kg) supraspinatus muscles were atrophied when compared to uninjured controls (2.77±0.22), the amount of atrophy was significantly reduced ($p<0.01$) in the repair +matrix gel treatment group (23.5±3.4% atrophied) when compared to repair only (31 ±5.8% atrophied). Histological examination revealed a noticeable trend towards lower (32%) fatty infiltration in the repair+matrix gel group. Expression levels for the key muscle synthesis regulating gene MURF-1 was significantly decreased in the repair only group, but were similar to normal in response to matrix gel injection.

Conclusions: The rabbit shoulder cuff results suggest that the short-term activation of wound healing pathways observed in the rat model is complemented by a longer-term positive effect on muscle atrophy and fatty infiltration.

Dynamically Controlled Plasma Scalpel for Wound Debridement

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Idaho IDeA Co-funding 1R15EB024930-01A1 Jim Browning

Cold Atmospheric pressure Plasma (CAP) is being used to kill and remove bacterial biofilms in chronic wounds. This work describes a CAP source fabricated using a robust ceramic materials system to enable fast inactivation of biofilms in wounds. The device operates at 20 kHz and 1-4 kV with a discharge gap of 0.75 mm. Typical discharge currents for this source are 0.2 – 1 mA. Experiments have been performed using an Ar/O2 gas mixture. The CAP can reduce Colony Forming Units (CFUs) by >90% in < 5 s for pathogens such *Staphylococcus aureus* and *Pseudomonas fluorescens* and by >99% in 60 s. Discharges which add water vapor through a dreschel tube have etched through biofilms though inconsistently. Ongoing work imaging Trypan-blue stained biofilms on collagen gels indicates that it might be feasible to selectively etch biofilms while minimizing plasma exposure to healthy cells in wounds. An XY-stage has been developed to move biofilm samples under a CAP source to demonstrate selective treatment of imaged biofilms. Results on pathogen treatment and imaging work will be presented

Targeting tumor and its microenvironment using nanotherapeutics for pancreatic cancer

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Nebraska IDeA Co-funding 1R01CA247763-01A1 Satyanarayana Rachagani

Pancreatic cancer (PC) is lethal with a five-year survival rate of less than 9.2 % and a median survival of 5-6 months. The limited efficacy of traditional therapies has led to the exploration of combination therapies with limited success because of challenges associated with dose-limiting side effects, drug-associated toxicities, drug resistance, and poor pharmacokinetics. This study addresses these challenges by determining functional and clinic-pathological significance of miR-345 as well as developing a dual delivery nanoscale device (DDND) for combined delivery of miR-345 and GEM for the treatment of PC. Our previous studies showed that miR-345 targets several important genes, including sonic hedgehog (Shh), Kras, MUC4 mucin and its downstream targets, playing critical roles in tumor growth and metastasis by promoting epithelial to mesenchymal transition, PC stem cells, angiogenesis, desmoplasia, which limit the delivery and efficacy of chemotherapy. We hypothesized that downregulation of miR-345 contributes to PC pathogenesis by upregulation of Kras, SHH, and MUC4. Its restoration, combination with GEM through the DDND, enhances GEM sensitivity in PC through modulation of SHH/Kras/MUC4 pathways, resulting in inhibition of desmoplasia, pancreatic stellate cells, and PC stem cells leading to an improved therapeutic outcome of GEM through improving its tumor perfusion. DDND was based on temperature and pH responsive copolymer electrostatically complexed with miR-345 and subsequently self-assembled with GEM encapsulated layers. Our results indicated that the DDND design allowed effective co- incorporation of miRNA/GEM combination; facilitated cellular entry; enhanced stability; provided miRNA protection; facilitated endosomal escape in cancer cells; and allowed dose-sparing of the cytotoxic drugs. This novel DDND design also holds promise for delivery of other therapeutics as well to be used in other cancer types in the future.

Rearing environment and sex impact the effectiveness of n-acetylcysteine to decrease amphetamine cue-induced relapse

Mary E Cain and Troy D. Fort

Kansas IDeA Co-funding R15DA035435 Mary Cain

Relapse remains a significant barrier to psychostimulant treatment. While N- acetylcysteine (NAC) has shown potential to reduce relapse, there is variability in its effectiveness across human experiments. Given that genetic factors contribute 30-60% to the development of substance use disorders (SUDs), it is imperative we understand the contribution of environmental factors to SUDs. In pre-clinical animal models, the early rearing environment results in numerous effects on drug-taking behavior due to marked adaptations in neuronal plasticity and glial cell density. Environmental enrichment (EE) results in decreased self- administration of low-unit doses of amphetamine during short access sessions, but the ability of EE to reduce the escalation of amphetamine self-administration of a high unit dose and resulting cue-induced relapse is not established. The current experiment raised male and female Sprague-Dawley rats in EE, isolated (IE), or standard (SE) environments from postnatal days 21-51 and following implantation of an indwelling jugular catheter, trained the rats to lever press for amphetamine (0.1 mg/kg/infusion) during 6-hr sessions. Following 12 sessions, rats had a 14-day forced abstinence period with NAC (60 mg/kg) or vehicle (saline) injections daily. Rats were then returned to the operant chambers for a final cue-induced relapse test. To date, EE prevents against the escalation of amphetamine self-administration. While there is no sex effect during self-administration, females respond more than males during the cue-induced relapse test. In males, there is a trend for NAC to reduce cue-induced relapse in IE and SE rats.

Interestingly, in females, NAC to date is not effective in SE rats. Our data suggest that sex and rearing condition may impact the effectiveness of NAC treatment during the abstinence period to decrease cue-induced relapse and these factors may explain some of the variability observed in human experiments.

The role of Tcf21 in the development and expansion of visceral adipose tissue

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Louisiana IDeA Co-Funding 1R15DK122383-01 Xing Fu

Obesity and type-2 diabetes are increasingly prevalent in the US. A strong correlation has been identified between visceral adipose tissue (VAT) and insulin resistance, suggesting that VAT is an excellent target for treating T2D. Evidence also suggests some beneficial effects of subcutaneous AT (SAT) on insulin sensitivity. Thus, knowledge about the VAT-specific regulation of adipogenesis is needed to facilitate the specific and effective targeting of VAT. Recent studies revealed that Tcf21 was exclusively expressed in VAT. Using tamoxifen-inducible *Tcf21* lineage-tracing mouse lines, we found that Tcf21 is restrictively expressed in a subset of PDGFRα+ progenitor cells residing in VAT but no *Tcf21* lineage-traced cells were identified in SAT. Tamoxifen treatment of *Tcf21* lineage-tracing mice at gestation day 11.5 labeled all visceral adipocytes (VAPs) in 1-month-old offspring mice, which indicates that developmentally the *Tcf21* lineage gives rise to all VAPs. Treatment of *Tcf21* lineage-tracing mouse pups with tamoxifen at postnatal day 2 labeled a subset of VAPs at 1 month of age, suggesting that the *Tcf21* lineage also actively participate in the neonatal development of VAT. However, *Tcf21* lineage- tracing mice treated with tamoxifen at 2 months of age only labeled a small number of adipocytes after a long-term high-fat diet (HFD) challenge, indicating that the *Tcf21* lineage in adult mice has lost most of its adipogenic potential. Instead, in these mice, we identified a large number of *Tcf21* lineage-traced fibroblast-like cells co-localized with crown-like structures. We then employed a tamoxifen-inducible *Tcf21* lineage-specific *Tcf21* KO mouse line. Pups of this line treated with tamoxifen at postnatal day 2 showed an increase in the number of *Tcf21* lineage-traced adipocytes at 1 month of age and after a long-term HFD treatment as compared to their WT littermates. Our result suggests that Tcf21 may play a negative role in the development and expansion of VAT.

Optimization of Quinoline-based HIV-1 Integrase Inhibitors
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Mississippi Co-funding R01AI140985 Jacques Kessl

HIV-1 Integrase is a viral enzyme that is essential for the replication of HIV-1. Recent studies have highlighted the vulnerability of the virus to a new class of integrase inhibitors capable of disabling this viral enzyme by triggering its abnormal multimerization at several critical stages of the virus life cycle. We have synthetized a library of active quinoline derivatives in order to better understand the molecular and mechanistic mode of action of these compounds. Our studies combine several approaches such as protein biochemistry, medicinal chemistry and virology.

Offspring from HELLP but not preeclamptic rats have impairment in spatial memory during early adolescence

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Mississippi Co-Funding R0MH116027 Kedra Wallace

Children born to women with hypertensive conditions during pregnancy such as preeclampsia or HELLP syndrome are at an increased risk of delays in neurodevelopment, learning and memory and increased cardiovascular problems. The objective of the current study was to determine if suppression of the immune system during hypertensive pregnancies prevents behavioral deficits in offspring. To induce preeclampsia (PreE) and HELLP rats were implanted with mini-osmotic pumps secreting sFlt-1 or sFlt-1+sEng (respectively) beginning on gestational day 12 until delivery. Orencia (prevents T cell activation) was infused the following day to a subset of rats. Sensorimotor development was assessed by surface righting and negative geotaxis during the first week of life and spatial memory was assessed via the Barnes maze during week 7. Blood pressure was obtained and pups were euthanized. At birth PreE ($p=0.04$) and HELLP pups ($p=0.03$) were smaller compared to NP pups; which was improved in Orencia treated rats ($p=0.0003$; $p<0.0001$). PreE pups were delayed in surface righting and negative geotaxis ($p=0.05$), whereas HELLP pups did not have a difference compared to NP pups ($p>0.05$). Orencia had no effect in PreE pups; however, it did decrease performance in HELLP pups. When assessed during the adolescent period there were no differences between PreE and NP pups ($p>0.05$). HELLP had significant impairments in spatial memory compared to NP pups ($p=0.001$), however immune suppression did not improve outcomes ($p=0.91$). There was no significant different between the groups in blood pressure ($p=0.98$).

Offspring from PreE dams were more affected by development delays during the immediate growth period, whereas HELLP rat pups only had impairments during the adolescent period. Immune suppression during pregnancy did not improve outcomes in either group. This work is still in progress and we are currently examining differences between male and female offspring.

Craniofacial cartilage organoids from human embryonic stem cells via a neural crest cell intermediate

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Montana IDeA Co-funding 1R15DE028434-01 Mark Grimes

During neurulation, neural crest cells (NCCs) are specified at the neural plate border, located between the neural plate and non-neural ectoderm. NCCs delaminate and migrate throughout the developing embryo, differentiating into several different lineages of cells including neurons and glia of the peripheral nervous system, secretory cells, melanocytes, and the majority of craniofacial cartilage and bone. Here we describe a new protocol for NCSC differentiation from both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). From differentiated NCSCs we have further derived craniofacial cartilage organoids. Cartilage organoids self-organized in culture dishes and expressed several collagen isoforms and collagen receptors. Organoids also expressed markers indicative of neural crest lineage and were continuously cultured for one year, reaching up to one centimeter in diameter. Histological staining of cartilage organoids revealed tissue architecture typical of hyaline cartilage.

Organoids were composed of rounded aggregates of glassy, gray matrix that contained scattered small nuclei in lacunae. Mass spectrometry analysis of cells at different stages of differentiation indicated that growth factors secreted by organoids may contribute to the formation of an autocrine loop that promotes chondrocyte differentiation. Organoids treated with combinations of specific ligands identified from mass spectrometry exhibited accelerated growth, supporting the hypothesis that a positive feedback loop may promote craniofacial cartilage differentiation. These results provide further insight into the mechanisms driving neural crest differentiation into craniofacial cartilage, which is important for the understanding of normal human development and diseases that originate from neural crest tissues.

Variation profile of the coronavirus genome

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Nebraska IDeA Co-funding R01GM120108-01 Hernan Garcia-Ruiz

The causal agent of the COVID-19 pandemic is the *Severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2) first reported in Wuhan, China, in December of 2019. SARS-CoV-2 belongs to the genus *Betacoronavirus*, which includes two other species that infect humans: *Severe acute respiratory syndrome coronavirus* (SARS-CoV), and *Middle East respiratory syndrome coronavirus* (MERS-CoV). COVID-19 management strategies are largely dependent on diagnostic, vaccines and antiviral drugs under development. Diagnostic tests detect the presence of viral RNA, viral proteins, or antibodies developed to viral proteins. Vaccines and antiviral drugs inhibit critical parts of the infection cycle, such as cell entry or virus replication. Mutations in the genome of SARS-CoV-2 have the potential to affect both the precision of diagnostic tests and the efficacy of vaccines and antiviral drugs. To gain insight on their evolution, in this study, we profiled genomic variation in all species the genus *Betacoronavirus*. Nucleotide variation analyses revealed that Betacoronaviruses harbor hypervariable areas at homologous locations. The most variable cistrons are the spike S protein and open reading frame 8. Spike S protein is a the most common vaccine target and antibodies against the S protein are the target of antibody-based diagnostic tests. These findings highlight the remarkable ability of coronaviruses to accumulate mutations and are important for the development of SARS-CoV-2 diagnostic tests, and for the deployment of vaccines and antiviral drugs.

Access to Kidney Transplantation in Minority Populations (AKT-MP)
Larissa Myaskovsky
University of New Mexico
New Mexico IDeA Co-funding 1R01MD013752-01A1 Larissa Myaskovsky

Significant disparities exist in kidney transplantation (KT) for members of disadvantaged groups [e.g., Hispanic/Latino (HL), American Indians (AI), low income]. Although HL and AI are referred for KT evaluation equally with non-Hispanic whites (WH), they are less likely to be wait-listed or to undergo KT than WH. Thus, it is important to focus on disparities in the evaluation process occurring after referral for KT. The KT evaluation process is lengthy, time consuming, and burdensome, requiring patients to complete numerous tests. Rather, than focusing on educating or changing patient behaviors, our study focuses on reducing the burden of the evaluation process, and eliminating external barriers that may prevent patients from completing KT evaluation. We will compare two approaches to delivering care to patients to determine which approach will significantly reduce KT disparities: (1) Kidney Transplant Fast Track (KTFT), a streamlined KT evaluation process; or, (2) peer navigators who were former KT patients who will help patients “navigate” their way through KT evaluation. After culturally and contextually adapting the two interventions, we will conduct a pragmatic randomized trial of 398 kidney failure patients a university-affiliated transplant center with large HL and AI patient populations, and compare results to previous local and national KT populations. We will assess facilitators and barriers to widespread implementation and conduct a cost effectiveness analysis. Although it is expected that KTFT will be more effective than peer navigators, KTFT may be more costly, requiring significant administrative and clinical changes in the transplant center, which may be impractical to maintain. KTFT also may lead to more patient ambivalence because the shortened evaluation period will give patients less time to consider their treatment options. Ultimately, our study will inform transplant programs faced with disparities in KT about which disparity-reducing intervention to use given their particular needs and resources.

Critical Roles of NK Cells and Group3 ILCs in Carbapenemase (KPC)-Producing *Klebsiella Pneumoniae* Pulmonary Infection

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Louisiana IDeA Co-funding Jay Kolls

Objective: Infections with carbapenem-resistant *Klebsiella pneumoniae* (KPC) is a global threat due to it's wide-spread antimicrobial resistance particularly in immunocompromised patients. Thus, understanding of the pathogenesis of this opportunistic infection are still warranted and would allow for the innovative treatments.

Methods: At first, we examined the host susceptibility in genetically engineered mice, *Rag2-/-* and *Rag2Il2rg-/-* mice, challenge with an ST258 C4, KPC-2 clone. To mimic this clinically, we treated wild-type C57Bl/6 mice with FK506, a widely used calcineurin inhibitor in solid organ transplant recipients. In each model, the single cell RNA seqencing (scRNAseq) was performed to determine which genes were required for host resilience to ST258 infection. Finally, we tested the efficacy of IL-22 in both model to pursue the possibility of the adjunctive immunotherapy.

Results: Both wild-type C57Bl/6 mice and *Rag2-/-* mice, which lack B cells and T cells, were resistant to infection. In contrast, *Rag2Il2rg-/-* mice were susceptible to pulmonary infection and bacteremia suggesting that cells that develop under *Il2rg* signaling are critical for host resistance. Using single-cell RNA-seq in infected *Rag2-/-* mice, we identified that host resistance was associated with distinct clusters of Ifng+ NK cells and Il17a+, Il22+, and inducible T-cell costimulatory molecule (ICOS)+ group 3 innate lymphoid cells (ILCs) were critical for host resistance. FK506 also conferred susceptibility which was associated with reduce activation of NK cells and gamma delta T cells confirmed by scRNAseq, and the decline of ILC3 proliferative function confirmed by flow cytometry. Treatment with recombinant IL-22 ameliorated ST258 pulmonary infection in both models via hepatic IL-22ra1 signaling and improved the survival.

Conclusions: These data support the host directed immunotherapeutic strategies to treat or prevent this opportunistic infection.

Vasopressin Activates TRPV1 and Depresses GIRK Channels to Excite Subicular Neurons via Multiple Signaling Mechanisms

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University of North Dakota

North Dakota IDeA Co-funding R01MH118258 Saobo Lei

Arginine vasopressin (AVP) is a nonapeptide that serves as a neuromodulator in the brain and a hormone in the periphery that regulates water homeostasis and vasoconstriction. The subiculum is the major output region of the hippocampus and an integral component in the networks that processes sensory and motor cues to form a cognitive map encoding spatial, contextual, and emotional information. Whereas the subiculum expresses high densities of AVP- binding sites and AVP has been shown to increase the synaptic excitability of subiculum neurons, the underlying cellular and molecular mechanisms have not been determined. We found that activation of V1a receptors increased neuronal excitability in the subiculum via activation of TRPV1 channels and depression of the GIRK channels. The functions of phospholipase C, protein kinase C and degradation of phosphatidylinositol 4,5-bisphosphate (PIP2) were required for V1a receptor-induced excitation of subicular neurons. Our results provide a cellular and molecular mechanism to explain the physiological functions of AVP in the brain.

(Supported by R01MH118258)

Q&As from three NIH Speakers:

Dr. Tara Schwetz:

Q: For diagnostic assays that get EUA, given the accelerated process, what is the planned 'post marketing' evaluation to assure that we are using assays we can trust?

A: Each of the RADx efforts has connections to/interactions with FDA colleagues. Specific to RADx-Tech, FDA has been working with NIH and the RADx-Tech external advisors to provide general advice on test validation and is prioritizing the review of EUA for RADx-Tech tests. Additionally, this is also an opportunity for linkage between RADx-Tech and RADx-UP (which will only utilize those tests with an EUA) to ensure that the right tests are being distributed to the right populations and delivered in the best way possible.

Q: Is Sep 30th the very last deadline for RADx-rad? Or might the program be extended in future?

A: It is the last deadline for now. There are discussions surrounding some additional efforts as part of RADx-rad in the future. Please continue to check grants.nih.gov or the RADx website for more information, which will be posted as it becomes available. https://www.nih.gov/research-training/medical-research-initiatives/radx

Q: Can you clarify if the comment made about having an overwhelming number of applications/not processing more was in reference to just a single area within RADx or overall?

A: The RADx Tech program received a very robust response and is no longer accepting applications. RADx-UP will have a second phase next year and RADx-rad may also publish additional opportunities in the future.

Q: How do we become part of the Collaborative Clinical Research Network?

A: The network will be formed by supporting applications received through these two NOSIs:

- https://grants.nih.gov/grants/guide/notice-files/NOT-OD-20-121.html
- https://grants.nih.gov/grants/guide/notice-files/NOT-OD-20-120.html

It will be expanded in Phase II - look for more information on Phase II next year.

Q: Please define a "diverse background".

A: Here is the NIH definition of those underrepresented in science: https://grants.nih.gov/grants/guide/notice-files/NOT-OD-20-031.html

Q: When will the awards be made for NOT OD-120?

A: September 2020 and November 2020

Dr. Jon Lorsch:

Q: Could you share an approximate breakdown between the numbers of ESI R01 vs. R35 vs. DP awards? Also, is there approximate information available on the relative success rates of the different mechanisms for ESIs?

A: The 2019 numbers for ESI R01 and ESI R35 awards with success rates in parenthesis are:

- 69 ESI R01s (24%)
- 150 ESI R35s (42%)

DP2 is a program funded by the NIH OD instead of NIGMS.

Q: Can we send the samples for cryo-EM? Or we have to be there to process the samples ourselves?

A: No, unfortunately users cannot send samples and have the centers take care of the rest. Due to COVID-19 restrictions, users currently are not allowed to be on-site at any of the centers either.

However, there is a lot of support for users to receive training in the theory and practice of cryo-EM. The Cryo-EM centers are eager to help researchers from the IDeA states access cryo-EM resources. The centers are set up to provide access for data collection or to provide training in the techniques through helping the user solve a cryo-EM structure. Currently all three centers are only accepting specimens for remote data collection; no in-person visits are allowed.

The first step is to apply for access or training. Generally, there are two tracks:

Data collection access: User has specimens that are data-collection ready, meaning grids are prepared and have produced promising preliminary analysis on a screening microscope.

Training: user wishes to learn the process on their own sample. The center will help/teach specimen prep, data collection and initial analysis through production of an electron density map.

Both tracks are accessed through a peer-reviewed application process. The potential user should contact staff at one of the centers to get started, as they will be able to guide them through the process and answer any questions. https://commonfund.nih.gov/cryoem/sites.

Besides links to the three centers, there are links to the four curriculum development projects that have many online materials for self-paced training.

Q: cryo-EM opportunities for IDEA states (applications to common-fund sites) requires facilities for EM screening. These facilities are limited in IDEA states. Montana investigators will be able to access NSF-funded Arctica at MSU, but generally - screening facilities are limited.

A: Investigators are encouraged to look into the NIH S10 instrumentation funding opportunities at https://orip.nih.gov/construction-and-instruments/s10-instrumentation-programs.

Q: Does NIGMS fund R56 (or RO1 bridge funding)?

A: No.

Q: Thank you for your support of basic science research. What is your opinion on IDeA states providing financial support to investigators doing basic science research, but not purely biomedical?

A: NIGMS encourages IDeA state investigators to conduct basic research as it is the foundation of all advances in science.

Dr. Ming Lei:

Q: Can you also breakdown the IDeA co-funding for each NIH institute? There are only two R01 fundings for NCI, from how many applications. What is the percentage for funding for NCI?

A: We typically receive ~50 nominations each year, with a given IC submitting anywhere between 0 and 7 for consideration. We fund ~25 nominations each year. Because applications nominated by each individual IC are in small numbers, and the number of applications selected for IDeA co-funding varies, there is no meaningful percentage for each IC.

Q: Since R15 is divided to AREA and REAP. Can IDeA Co-Fund REAP R15 grants?

A: Yes, REAP awards are eligible as long as all of the other eligibility requirements (such as performance sites entirely in IDeA states, among others) are met.

Q: Some "institution leaders" are not very interested in COBRE funds, except insofar as they can benefit from them. Many of us have exhorted NIGMS and the IDeA program to hold IDeA state "institutional leaders'" feet to the fire, at the very least in insisting that they make good on their institutional commitments. Making good on such institutional commitments has been lacking in many cases over the years.

A: Thank you. We keep on trying from our end and are open to your ideas.

Q: What do you define as an "INBRE" or "COBRE" investigator? Are you just talking about the pilot investigators? INBREs and COBREs have way larger impact than the small handful of pilot grant awardees

A: In the context of the administrative supplements to INBREs for collaboration, INBRE and COBRE investigators refer to research project leaders as well as pilot project investigators, who are eligible to lead a collaborative project.

Q: For the collaborations, could they be between a COBRE project leader and a co-funded investigator?

A: Not in the past. We plan to expand intra-program collaborations to include co-funded investigators in the future, should funds be available.

Q: Why is there a prohibition for a COBRE to collaborate with multiple IDeA-CTRs? This doesn't align with the goal of increased collaboration.

A: There is no such prohibition. There must be some miscommunication or misunderstanding. Please follow up with us for clarification.

Q: Are COBRE project leaders given deference when applying for co-funding?

A: No

Q: How successful were INBRE investigators funded by the Women's health program, collaborations with non-IDeA programs?

A: INBRE investigators were very successful for Women's Health supplements . Received 10 applications from INBREs and 6 were funded, so the success rate was 60%. The success rate across all applications was 51%.

Q: Can you comment on the Core Directors training program? Is this a certificate type of training, or a seminar series?

A: It is 4-hour workshop taking place on Monday, October 5, 1-5 pm. Registration ends on October 1. The link to the meeting is at https://www.eventbrite.com/e/core-business-virtual-meeting-tickets-118641450773 . A Feedback Loop post was made and can be found at: https://loop.nigms.nih.gov/2020/09/attend-the-idea-core-business-virtual-workshop/

Q: Are COBRE junior project leaders who have recently graduated eligible for some of the collaborative mechanisms (e.g. COBRE-CTR; INBRE-COBRE etc.?) If not, could they be?

A: No. The collaborative supplements are to support collaborations between currently IDeA-funded investigators.

Q: Why not make the minimal institutional commitments explicit in the RFA?

A: It is practically difficult to do that because institutions the IDeA Program supports have very different financial footings.

Q: Is the collaboration between IDeA and non-IDeA states only on women's health?

A: No. Not limited to that. For example, there are collaborations between the IDeA-CTRs Registry and the NCATS N3C program. Another one is the collaboration with NIA on Alzheimer's disease research. These opportunities are not in the forms of standing FOAs available every year. But we make a great effort to seek such opportunities for IDeA researchers whenever possible.

Q: Can INBRE, CTR, and COBRE award NOT count in the limit for R15 eligibility?

A: The institutional $ cap for R15 eligibility is a NIH-wide policy that is not set by the IDeA program.

Q: For geographically isolated IDeA states - is there an interest to growing emerging investigators by organically growing them - in addition to what you mentioned about working alongside research intensive institutions?

A: Yes, that is one of the primary objectives of the IDeA COBRE and INBRE programs.

Q: May I ask that if any investigators here are interested in getting involved in NIH funded pragmatic randomized trials of antithrombotic interventions, the ACTIV 4 program. We are looking for sites for 3 trials - in the in-hospital, post-discharge, and ambulatory settings. Happy to discuss with you - mary.cushman@uvm.edu

A: This message has been shared with the IDeA-CTR PIs.

Q: Any predictions on NIH funding levels in the next year? Are there any hints from budget discussions in either the House or Senate?

A: Sorry to disappoint. We are not in a position to predict.