Metabolism in Health and Disease Symposium

A UARK-UAMS Research Collaboration

Nov. 16-17, 2021

Don Tyson Center for Agricultural Sciences' Waldrip Hall
1371 W. Altheimer Drive Fayetteville, AR 72704

UNIVERSITY OF ARKANSAS

UARK-UAMS Research Collaboration
Metabolism in Health and Disease Symposium
Nov 16-17, 2021

Location - Don Tyson Center for Agricultural Sciences
1371 W. Altheimer Drive, Fayetteville, AR 72704

- **DAY 1 – Nov. 16, 2021**
  - 6:00 – 8:30 p.m. - Reception/Meet & Greet
  - Provosts Gardner (UAMS) and Martin (UARK) will welcome everyone

    - 6:00 p.m. - Poster session (all participating UAMS/UAF faculty, administration, postdocs, students)

- **DAY 2 – Nov. 17, 2021**

  - Gather at 8:30 a.m.
    (All participating UAMS/UAF faculty, administration, postdocs, students)

    - 8:40 – 9:00 a.m. - Welcome and Introductions by Drs. English (UARK) and Ho (UAMS)
    - 9:00 – 11:00 a.m. - 8 presentations (4 from UAF; 4 from UAMS: 10 minutes each; 5 minutes for questions)

      (Each presenter will highlight existing collaborations or present opportunities for future collaborations.)
    - 11 a.m. – 11:15 a.m. - Biobreak
    - 11:15 – Noon – 3-minute talks on selected poster abstracts
    - Noon – 1pm - Lunch at assigned tables
    - 1 p.m. – 2:30 p.m. - Specific breakout sessions hosted by presenters
    - 1:30 p.m. – Lab visits as desired
    - 2:30 p.m. - Concluding remarks Drs. English (UARK) and Ho (UAMS)

UAMS-UARK Metabolism Symposium participants

Nov. 16-17, 2021

10 minute talks

- Mario Ferruzzi, PhD, Professor, Pediatrics, UAMS
- Kyle Quinn, PhD, Associate Professor, Biomedical Engineering, UARK
- Marjan Boerma, PhD, Professor, Pharmaceutical Sciences, UAMS
- Shilpa Iyer, Assistant Professor, Biological Sciences, UARK
- Robert Griffin, PhD Professor, Radiation Oncology, UAMS
- Nicholas Greene, PhD, Associate Professor, Health, Human Performance and Recreation, UARK
- Isabelle Racine-Miousse, PhD, Assistant Professor, Biochemistry and Molecular Biology, UAMS
- Narasimhan Rajaram, PhD, Associate Professor, Biomedical Engineering, UARK

3 minute talks

- Justin Leung, PhD, UAMS
- Xuan Zhang, PhD, Biological Sciences, UARK
- Ruud Dings, PhD, UAMS
- Jamie Baum, PhD, Food Science, UARK
- Elijah Bolin, MD, UAMS
- Alison Ramser, Student, UARK
- Jesus Delgado-Calle, PhD, UAMS
- Chenguang Fan, PhD, Biochemistry, UARK

Posters

- Michael Thomsen, PhD (Director of the Center for the Study of Obesity - CoPH)
- Haven Griffin (Grad student - Baldini lab)
- Gunnar Boysen, PhD
- Amy Sato. PhD (Bellido Postdoc) – 3 posters from Bellido lab
- Mario Ferruzzi, PhD (Director of ACH Nutrition Center) – 2 posters
- Young Hye Song, PhD, Biomedical Engineering- 2 posters
- Ishita Tandon- Student/Kartik Balachandran, PhD, Associate Professor, Biomedical Engineering- 1 poster
- Shilpa Iyer, PhD, Assistant Professor, Biological Sciences, 1 poster
- Fibi Meshrkey- Student, Biological Sciences, 1 poster
- Laura Gray, PhD – 2 posters
- Marcos Rodriguez- 1 poster
- Kyle Quinn, PhD- 1 poster
- Leonard Harris, PhD- 1 poster
- Ruben Michael Ceballos, PhD- 1 poster
- Ana Regina Cabrera- 1 poster
- Francielly Morena de Silva- 1 poster
- Ryan Tian, PhD, Biochemsirty- 1 poster

Ryan Tian, PhD, Assoc. Professor, UARK

Abstract Title

New Metabolomic Nanobiosensing Methodologies for Detecting Live Cells in Real-time

Other Authors and Affiliations

Chenguang Fan (UARK), Yuchun Du (UARK), Xuming Zhang (UAMS), Roger Pechous (UAMS)

Introduction

Real-time detections of live viruses and cells have remained grand challenges in general. Here we report two new methodologies, both based on the metabolomics of live cells characteristic interactions with nutrients and with coronaviruses in real-time, all at ultralow cost and simply by someone with a quickly training. The cancer detection was based on the Warburg effect for detecting the cancer cell's sugar metabolites, more for a more aggressive cancer cell type. The same mechanism was proven useful in detecting the bacteria, showing the promise to differentiating the Gram-positive and Gram-negative bacteria using different nutrients and antibiotics. The cell-coronavirus type of specific binding can help differentiate the kinetics at both cell-membrane-binding step and cell-membrane-penetrating step for a coronavirus-variant besides verifying efficacies of both antibodies and virucides.

Materials and Methods

Tian-lab's patented biocompatible nanowires were used to template the growth of graphene-assembled polycrystalline carbon nanotubes, as patented by Tian-lab. The nanotubes were then transferred on the surface of a commercially available wireless communication sensor platform, dried at ambient pressure and temperature overnight, then integrated with a commercially available small gadget and a home-made antenna, forming a new nano-biosensing system.

Results and Discussions

The sensitivity for detecting Gam-negative and Gram-positive bacteria is in the single-cell level, that for detecting human breast cancer cells is in the 1,000 cells level, and that for diagnosing clinical nasal swap samples is in the 50 coronavirus particles level which was recognized and appreciated by NIH Director, Dr. Francis Collins. These new nanobiosensors can work wirelessly.

Conclusions

In comparison with many existing tools in biosensing, nanosensing and wireless sensing fields, this new biosensing methodology has demonstrated for the first time to be truly quick, simple, low-cost, and accurate, which has been long-overdue in clinical, industrial, environmental, and point-of-care settings. The new methodology's wireless communication viability is ideal for integrations with the WiFi tools such as the Bluetooth, 5G- and 6G-cellphones, which is ideal for developing new collaborations involving more labs between UAMS and UARK.

Acknowledgement

The authors acknowledge the partial support from the Provost Offices of UAMS and UARK.

Existing UA-UAMS Collaboration

- Two patents, one between UA-UAMS;
- Two major papers are in final revision for Nature or Nature-sister journal(s), one between UA-UAMS.
- A RO1 proposal submitted, and more of the same size will be submitted soon.

Opportunities for Future Collaboration

- Real-time bioimaging will be involved for verifying the new wireless nano-bio-sensing methodology
- New collaborations with UAMS Cancer Research Institute and Pharmaceutical Sciences will be invited.

Xuan Zhuang, PhD, Assistant Professor, Fulbright College of Arts and Sciences, UARK

Abstract Title

Genetic Basis of Variation in Carbohydrate-rich Diet Induced Diabetic-like Traits in *Drosophila*

Other Authors and Affiliations

Fabio Morgante[2]; Michael Ludwig[1]; Soo Young Park[2]; Yang I Li[2], Matthew Stephens[3], Graeme Bell[2]; Martin Kreitman[1]- 1) Dept. of Ecology and Evolution; 2) Dept. of Medicine; 3) Dept. of Human Genetics, Univ. of Chicago, IL

Introduction

Diabetes mellitus is likely to be the biggest epidemic in human history, the proportion of which is estimated to be 1/11 of the world's adult population. Type 2 diabetes (T2D) making up >90% of cases of diabetes, results from a complex interplay between genes and environment, especially diet. Although previous genome-wide association studies in human have identified a bunch of associated loci for T2D, these loci account for only a small portion of the heritable component of the disease. Understanding the nature of gene-environment interactions in T2D susceptibility is an important goal. However, it is not realistic to strictly control the environment for human populations. Drosophila is a well-established model for investigating quantitative traits, and it provides powerful tools for dissecting the contributions of both genes and environment on metabolism.

Materials and Methods

Previous studies revealed that a high sugar diet (HSD) has profound effects on cellular physiology and metabolism in *Drosophila*, including insulin resistance, lipid metabolism, impaired lifespan, and reduced body size. We developed a fly model with diabetic-like traits induced by HSD, and examined HSD induced phenotypes in larvae and in adults across a subset of Drosophila Genetic Reference Panel (DGRP). Flies under HSD display an increase in whole body glucose and glycogen level, a decrease in developmental rate, survivorship, body weight, and longevity, compared with flies under low sugar diet (LSD). The examined DGRP lines display a continuous and wide range of these phenotypes and large broad-sense heritability, suggesting great potential for quantitative trait loci (QTL) mapping.

Results and Discussions

We used one of the HSD induced traits (i.e., developmental delay) to perform a bulk segregant mapping analysis in advanced intercross populations we developed. This mapping resource consists of 64 DGRP lines, combined into 16 highly recombinant synthetic populations, each of which is founded by 8 of the inbred lines. Fly embryos from these populations were reared on HSD and LSD. Flies with extreme phenotypes of developmental rate were then individually barcoded and genomes sequenced. Allele frequencies of the extreme phenotypes under each treatment were compared and used to identify single nucleotide polymorphisms (SNPs) associated with the trait. The design of multi-parental advanced intercross populations should greatly increase the power to detect associated variants and provide a greater opportunity for rare variants to be present and for common variants to be represented in the synthetic population, allowing mapping of both rare and common functional variants. We inferred the underlying mosaic founder structure for each sequenced fly using a hidden Markov model (HMM) that takes into account read depth. This allowed us to impute each fly's genotype.

Conclusions

Preliminary genome-wide association studies (GWAS) with mixed liner model show SNPs with suggestive association with variation in high sugar induced developmental delay, but also imply a prevalence of small-effect QTLs. This project is partially supported by Arkansas Biosciences Institute.

Opportunities for Future Collaboration

My lab uses fruit fly to investigate genetic/environmental factors affecting sugar metabolism (e.g. modeling diabetes) and drug toxicity. We seek collaborators in these fields -- (1) whose lab uses human cell lines (e.g. hiPSC) or other animal models (e.g. mouse) to experimentally test candidate genes identified the fly models; (2) whose lab is interested in looking into genetic modifiers of newly discovered disease-causing mutation in human (not limited to diabetes, and perhaps develop a fly model; (3) whose lab is interested in drug toxicity and pharmacogenomics, and we can take advantage of rich genetic variation in fly populations to investigate that.

Jamie Baum, PhD., Associate Professor; Director, Center for Human Nutrition, Bumpers College, UARK

Abstract Title

Breakfast Macronutrient Composition Influences Energy Expenditure and Net Protein Balance, but not Appetite, in Children Ages 7-17 years old

Other Authors and Affiliations

Jamie I. Baum*, Elisabet Børsheim+, Angela M. Tacinelli*, Matthew Cotter+, Aubree L. Hawley*, Sam Walker*
*Center for Human Nutrition, Dept. of Food Science, Univ. of Arkansas System Div. of Agriculture/UARK
+Arkansas Children's Nutrition Center, Arkansas Children's Research Institute, Dept. of Pediatrics, UAMS

Introduction

Currently, 1/5 children in the U.S. is considered obese. More specifically, approximately 40% of Arkansas students in public schools are classified as either overweight or obese. Obesity leads to changes in metabolic health due to the shift in body composition and is associated with an increased risk of chronic disease. This research seeks to determine if higher protein intake at breakfast serves as a potential method to combat childhood obesity by increasing energy expenditure, improving appetite and markers of metabolic health in overweight and obese children ages 7-17 years.

Materials and Methods

This was a 6-week, double-blind, randomized controlled dietary intervention in 7-17 year-old children. A total of 71 participants completed the study (43 males, 28 females). Participants were normal weight (NW; n=35; 22 males, 13 females; 11.5 + 2.5 years) or overweight/obese (OW; n=36; 21 males, 15 females; 13.0 + 2.2 years) and were randomly assigned to either a protein-based breakfast (PRO; 30 g protein) or carbohydrate-based breakfast (CHO; 13 g protein) for 42 days. Participants arrived fasted on day 1 and day 42 to complete two laboratory visit test days. Anthropometrics, metabolic biomarkers, total daily energy expenditure using doubly labeled water, and whole-body protein turner using 15N-alanine were measured at each visit. In a subset of participants (PRO: n=13; 7 male, 6 female; 11.8 + 2.2 years and CHO: n=11; 5 males, 6 females; 12.0 + 2.5 years), energy expenditure, thermic effect of food (TEF), substrate oxidation, and plasma markers were measured at baseline, 30, 60, 120, 180, and 240 minutes postprandial. Appetite was measured at baseline, 15, 30, 60, 90, 120, 180, and 240 minutes postprandial.

Results and Discussions

After controlling for body weight, there was a significant effect of time ($P < 0.05$) and diet intervention x time interaction ($P < 0.01$) on resting energy expenditure. There was a significant effect of time ($P < 0.0001$) and diet intervention ($P < 0.01$) on carbohydrate and fat oxidation. There was no effect of diet on perceived hunger, perceived fullness, perceived desire to eat, and prospective food consumption. There was no effect of diet on metabolic markers. There was an effect of diet ($P < 0.05$) on plasma cholecystokinin and plasma leucine ($P < 0.01$). There was an effect of dietary protein intake on net protein balance. And, when controlled for kg fat-free mass, normal weight participants had higher total daily energy expenditure when compared to participants with overweight or obesity.

Conclusions

Increasing protein intake at breakfast for 6-weeks does not change postprandial meal response in 7-17 year old children who regularly consume breakfast. However, increased protein intake may improve whole body metabolism in 7-17 year-old children. This study was approved by the UAMS IRB and registered as a clinical trial on ClinicalTrials.gov (NCT03602144).

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Existing UA-UAMS Collaboration

With the Center for Human Nutrition, directed by Dr. Baum, there is an opportunity to conduct multi-site dietary intervention trials, clinical trials, and in vitro research related to diet, skeletal muscle, and/or appetite.

Opportunities for Future Collaboration

With the Center for Human Nutrition, directed by Dr. Baum, there is an opportunity to conduct multi-site dietary intervention trials, clinical trials, and in vitro research related to diet, skeletal muscle, and/or appetite.

Laura Gray, PhD, Instructor, Fulbright College, UARK

Abstract Title

Patient Medical Narratives

Other Authors and Affiliations

Washington Regional Medical Center

Introduction

This was first introduced last year in the Health Coaches program to vulnerable patients with lower health literacies in the WRMC system. The purpose was to collect oral health stories from this underrepresented population to learn more about their experiences with health and the medical systems, understandings and correlations with health outcomes.

Materials and Methods

Personal interviews by phone to record their stories. Linguistic analysis of the oral histories to study language markers alongside their experiences to look for dynamic possibilities.

Results and Discussions

Sensitivity toward diverse patient experiences and some reluctance for some populations to be open to share data requires trust-building over time, especially with the phone as the interview medium rather than person to person interaction necessary for patient safety during the pandemic were challenges. The response rate during COVID via phone was low, as expected. The collection is ongoing, though currently I seek ways to expand patient populations and collection opportunities.

I expect to record qualitative empowerments in individual narratives and chart these. Those markers in language can then be compared as they may or may not correspond with improvements in health or positive experiences in health events. The sample size is small at present, though IRB protocols and approvals remain open and current from UA and WRMC boards.

Conclusions

The study is ongoing. There is currently much interest and scholarship in this area though there are not others published in this same idea that I have found.

Acknowledgement

WRMC IRB coordinator, Dixie Sharp, for her knowledgeable support.

Opportunities for Future Collaboration

This might be an excellent partnership project between the humanities/arts and the clinical setting. My service-learning pedagogical experience, as well as linguistics and writing background supports working alongside a lab, course or program where this could be an additional collection project.

Sami Dridi, Ph.D., Univ. of Arkansas, Center of Excellence for Poultry Science

Abstract Title

Bacterial Chondronecrosis with Osteomyelitis in Broilers: Translational Model for Human Osteomyelitis

Other Authors and Affiliations

Alison Ramser (presenter), Elizabeth Greene, Robert Wideman, Joshua Flees, Ahmed Dhamad, Adnan Alrubaye, Stephen Hennigan, Jason Pleimann, Mark Smeltzer, Sue Murray, Jennifer Kugel, James Goodrich, Avril Robertson, Douglas Rhoads UARK Ctr. of Excellence for Poultry Science and the Cell and Molecular Biology Program; Washington Regional Medical Ctr.; Dept. Microbiology and Immunology, UAMS; Antisense Drug Discovery, IONIS Pharmaceuticals, Carlsbad, CA; Dept. of Chemistry/ Biochemistry, Univ. of Colorado Boulder; and Institute for Molecular Bioscience, Univ. of Queensland, Brisbane, Australia

Introduction

Inflammatory bone diseases, such as osteomyelitis, are increasingly common and notoriously difficult to treat. Osteomyelitis is characterized by bacterial infection, subsequent inflammation, and bone attrition. Thus far, animal models have been limited in several ways, including their dependence on foreign bodies and bacterial inoculation, as well as their capture of only certain features of acute and chronic osteomyelitis. Our lab has taken the wire-flooring model for spontaneous BCO, in conjunction with human bone tissue and cells and shown that several molecular pathways are involved in osteomyelitis and these pathways are conserved across human and chicken. Taken together, these findings demonstrate the complex molecular pathways involved in the bacterial infection, inflammation, and bone loss seen in osteomyelitis and provide potential therapeutic targets while further solidifying the avian model for human disease and vice versa.

Materials and Methods

BCO-affected tissue and blood from lame birds with severe lesions. Healthy tissue and blood from non-lame chickens without lesions. De-identified human bone samples were collected from age-matched, consenting donor patients with or without osteomyelitis from Washington Regional Medical Center. Plasmid transfection for DICER manipulation. ELISA for dsRNA detection. Human recombinant TNFα, IL-1β and IL-8, chloroquine and 3-MA were used. MTT cell viability assay. Staphylococcus isolates for challenge (MOI of 50:1). Real-time qPCR and Western blot analysis. Statistical analysis, one-way ANOVA, post hoc Student-Newman-Keuls multiple-comparison test, and student t-test, depended on experiment.

Results and Discussions

dsRNA accumulation, coupled with DICER dysregulation and significantly upregulated NLRP3 inflammasome, was seen in both chicken and human affected bone. In vitro analysis revealed DICER dysregulation under bacterial challenge leading to dsRNA accumulation and decreased cell viability. Several autophagy machineries were shown to be down regulated in affected bone. hFOB cells challenged with a known BCO isolate showed dysregulation of autophagy. Dysregulation alone significantly decreased cell viability. Plasma from BCO-affected birds significantly decreased hFOB cell viability. A distinct circulating and local cyto(chemo)kine signature for BCO was found. hFOB cells challenged with upregulated cyto(chemo)kines showed decreased cell viability.

Conclusions

Osteomyelitis shows conserved etiological pathways across chicken and human models. This cross-translational model relationship allows for more in-depth analysis given the chicken model's spontaneity and chicken's fast growth. Additionally, these results speak to common means of infection by causative agents across species. Understanding of the pathways involved and molecular signatures present, make BCO more easily detected, and potential therapeutic targets evident for human medicine and animal welfare.

Acknowledgement

Dr. Charles O'Brien (UAMS) for Ob-6 cells; and Drs. Ronald N. Germain and Naeha Subramanian (Institute for Systems Biology, Seattle, WA) for plasmid NLRP3.

Existing UA-UAMS Collaboration

Mark Smeltzer, UAMS

Opportunities for Future Collaboration

Future investigations into therapeutic targets and characterization of progression of osteomyelitis is critical for treatment and prevention of this disease in human medicine and animal breeding. Integrating human technologies for investigating this disease and comparing different phases or types of human osteomyelitis to the avian model would provide further insight and opportunity for detection, prevention, and treatment of osteomyelitis.

Laura Gray, PhD, Instructor, Fulbright UA

Abstract Title

HealthcARE for All

Other Authors and Affiliations

Dr. Karl Schubert, Dr. Carol Gattis, and the following undergraduate students: Isabel Powers, Lina Patel, Heather Mahoney, Medha Guribelli, Christopher deBin, Mackenzie Hayward

Introduction

We are part of a research group with the Alpha Research Program at the Univ. of Arkansas. This is a volunteer honors undergraduate student research project based in Engineering Philanthropy to find out what Healthcare is available in Arkansas and to whom it is accessible. We want to study adequate healthcare availability and usage for underserved populations, particularly immigrants, those living in rural areas, and veterans. We are including student volunteers to join us from across campus.

Materials and Methods

We are collecting data from the population directly. To accomplish this, we have created a survey being given in interviews and distributed electronically throughout the local region and state using volunteers, word of mouth, non-profits and other affiliations, as well as posters.

Results and Discussions

We have worked with faculty and administrators across campus to create a survey that is sensitive to the needs of underrepresented populations in our state and to those without access to computers and technology; and, we have translated these surveys into languages to match populations we will encounter and hope to serve to support understanding. We have also addressed the possible distrust of the medical system by working with organizations who serve these groups to gain support for the distribution and collection of data through interviews and partnerships. We have placed paper surveys in non-profits, churches, community spaces, along the trail system and public libraries, as examples of ways to be more accessible to reaching our desired targets and to capture more diversity.

Conclusions

We have 617 surveys submitted from yesterday's count, and we are just beginning the collection this fall. We are using volunteers now to expand beyond our regional area and across the state. We are gaining momentum to include more volunteers over Fall and Thanksgiving breaks for paper surveys and posters in more community spaces during these times.

Acknowledgement

Our volunteers and an internal grant from the COE is supporting incidentals from this work

Opportunities for Future Collaboration

The study data will be public and could be a rich planning resource for underrepresented groups for increased medical care and studies for our state.

Michael Thomsen, PhD, Professor; Governor Sidney S. McMath Endowed Chair in Obesity Prevention; Fay W. Boozman College of Public Health, UAMS, Little Rock

Abstract Title

Schools are Protective Against Excess Weight Gain among Early Elementary Children

Other Authors and Affiliations

Di Fang, Assoc. Professor, Dept. of Agricultural Economics and Agribusiness, UARK,
Rodolfo M. Nayga, Jr., Professor, Head, Dept. of Agricultural Economics, Texas A&M Univ.
Kanna Lewis, Assist. Professor, Dept. of Family and Preventive Medicine, UAMS and Assist. Director of Health Policy Research, Arkansas Center for Health Improvement

Introduction

School environment may protect against excess childhood weight gain while providing nutritionally balanced, low-cost/free lunches to children/school day. Meals that meet dietary guidelines are especially helpful for children with poor home food environments. School lowers childhood food insecurity, provides structure, and physical activity. A school's role in preventing weight gain is really important during pandemic-related school closures/disrupted schedules. Understanding childhood obesity is difficult since randomized controlled trials and longitudinal datasets don't provide exogenous variation in school exposure. We study this gap by exploiting an unique Arkansas-based data resource to better understand obesity prevention in schools.

Materials and Methods

BMI data were collected from a legislatively mandated statewide BMI screening program for Arkansas public school children. Measurements were taken by trained personnel in the public schools. In addition to BMI, data from the screening program contain information on race, ethnicity, sex, age, grade in school, and school of attendance. The Arkansas Center for Health Improvement facilitated the development of the dataset used in this research. We focus on kindergartners in academic years 2003/2004 through 2018/2019. The outcome variable is %BMI95: the child's BMI as a percent of the BMI at the age-sex specific 95th percentile on age-sex specific reference growth charts from the Centers for Disease Control and Prevention. The treatment variable of interest is length of exposure to school in kindergarten on the date of BMI measurement. The natural experiment arises because this measure is driven by differences in the date of BMI measurement during the academic year leading to differences in the length of school exposure among kindergartners of the same age (in months). Data from 465,235 Arkansas kindergartners over 16-year study period. Statistical models estimated with lfe package v 2.8-6 in R v 4.1.0. Robust standard errors are two-way clustered by year and census tract.

Results and Discussions

Over the full 16-year period, we found negative (beneficial) effects of school exposure that were especially pronounced among lower-income children, those who qualify for free and reduced-price meals. Among these children, a full year of exposure was equivalent to a 0.49 percent reduction in %BMI95 compared to a child of the same age with no exposure. The beneficial effects of school exposure were amplified after improved meal standards mandated by the Healthy, Hunger-Free Kids Act (HHFKA) went into effect with the 2012/2013 academic year with estimates of 0.65 and 0.80 percent reductions in %BMI95 among full and lower-income samples of children, respectively. Finally, school exposure was especially beneficial during the post-HHFKA period among Hispanics and African American children.

Conclusions

Early elementary school is an important age for prevention of excess childhood weight gain and differences in timing of BMI measurement in Arkansas public schools provided an opportunity to assess differences in school exposure. This study provides strong quasi-experimental evidence that schools are non-obesogenic and protective against excess weight gain during kindergarten. While not tested directly, it is likely that school meals are playing a crucial role because we found more pronounced effects among children qualifying for free or reduce-price meals. We also found more pronounced effects after implementation of the HHFKA meal standards. Our findings provide evidence that schools can help reduce disparate rates of childhood obesity that are observed over the socioeconomic gradient. One troubling implication of these findings is that pandemic-related disruptions to school are likely to have further accentuated disparities in childhood obesity as our data indicate schools are especially beneficial to lower-income and minority children in our sample.

Existing UA-UAMS Collaboration

This work spans 12-years and multiple funded projects. Thomsen, new Center for the Study of Obesity director seeks further UARK partnerships to prevent/reverse Arkansas obesity.

Isabelle Racine Miousse, PhD, Assistant Professor, UAMS/Little Rock

Abstract Title

Dietary Methionine Restriction Sensitizes Melanoma to Immune Checkpoint Inhibitors

Other Authors and Affiliations

Lauren C.E. Morehead, Katie Wallis, Tripti Shukla, Alan J. Tackett, Isabelle R. Miousse
UAMS, Dept. of Biochemistry and Molecular Biology, Little Rock, AR

Introduction

Immune checkpoint inhibitors (ICIs) are used in patients with metastatic melanoma to allow the immune system to clear tumor cells. However, only half of the patients respond to therapy. Dietary methionine restriction has been shown to improve the response to chemo- and radio-therapy, but has not been tested in combination with ICIs.

Materials and Methods

We used a syngeneic tumor model to assess the effect of combining dietary methionine restriction with ICIs. C57BL/6 mice were injected with MC38 tumor cells. Mice were then injected with either ICIs or sham, and received a standard laboratory diet or an otherwise identical diet with 83% less methionine. We compared tumor size as well as gene and protein expression in cancer cells in vitro and in vivo.

Results and Discussions

Methionine restriction increases the gene expression of two markers of responsiveness to immune checkpoint inhibitors; MHC-I and PD-L1. This was associated with an increase in the response to ICIs five-fold compared to ICIs alone in the tumor model. In addition to immune markers, our proteomic analysis also identified oxidative stress as a key pathway in the response to methionine restriction both in vitro and in vivo.

Conclusions

Methionine dependence is found in most cancers and our results may apply to multiple cancer types. Accordingly, dietary methionine restriction may improve response rates in metastatic melanoma and expand the use of ICI therapy to multiple other cancer types.

Acknowledgement

The project described was supported by the UAMS Translational Research Institute grant TR003108/UL1 TR003107 (NCATS) and by the Arkansas Integrative Metabolic Research Center grant P20GM139768 (NIGMS). We would like to acknowledge Drs. Issam Makhoul and Brian Koss for helpful advice.

Existing UA-UAMS Collaboration

Kyle Quinn - Arkansas Integrative Metabolic Research Center

Opportunities for Future Collaboration

Tim Muldoon

Elijah Bolin, MD; Associate Professor, Pediatric Cardiology; UAMS and Arkansas Children's Research Institute

Abstract Title

In-hospital Mortality Among Infants of Diabetic Mothers with Hypertrophic Cardiomyopathy

Other Authors and Affiliations

Elizabeth G. Bond, M.S., UAMS and Arkansas Children's Hospital Research Institute
Peter M. Mourani, M.D., UAMS and Arkansas Children's Hospital Research Institute
Craig Porter, Ph.D., UAMS and Arkansas Children's Hospital Research Institute
R. Thomas Collins, II, M.D., Stanford Univ. School of Medicine and Lucile Packard Children's Hospital

Introduction

Infants of diabetic mothers (IDMs) suffer from higher mortality compared to children born to nondiabetic mothers. Hypertrophic cardiomyopathy (HCM) is often observed in IDMs, although it is unclear if HCM contributes to risk of higher mortality in IDMs.

Materials and Methods

We performed a retrospective cohort study of IDMs admitted at ≤ 14 days-old to hospitals in the Pediatric Health Information System (years 2004 – 2019). Multivariable logistic regression was used to evaluate the association between HCM and mortality.

Results and Discussions

Among 32,993 IDMs, there were 204 (0.6%) with HCM. Mortality in patients with HCM was higher than in those without HCM (4.9% vs. 1.3%, $p < 0.001$). Odds of mortality were also higher among those with HCM (adjusted odds ratio [aOR] 2.10, 95% confidence interval [CI]: 1.04 – 4.25; $p = 0.038$). Other factors independently associated with higher mortality were pulmonary hypertension (aOR 7.40, 95% CI: 5.91 – 9.27, $p < 0.001$), prematurity (aOR 2.71, 95% CI: 2.21 – 3.33; $p < 0.001$), moderate/severe congenital heart disease (aOR 2.04, 95% CI: 1.49 – 2.79; $p < 0.001$), and malformations of either the central nervous system (aOR 4.40, 95% CI: 3.24 – 5.96; $p < 0.001$), genitourinary system (aOR 2.24, 95% CI: 1.63 – 3.07, $p < 0.001$), or musculoskeletal system (aOR 1.90, 95% CI: 1.36 – 2.65, $p < 0.001$).

Conclusions

In the largest study to date of IDMs with HCM, we identify HCM as a contributor to mortality . These data reinforce the need both for better prevention of maternal diabetes and effective therapies for HCM in IDMs.

Acknowledgement

The authors wish to thank Julie Nick, who was instrumental in performing data acquisition from the PHIS database.

Opportunities for Future Collaboration

The present study establishes HCM as is a significant contributor to in-hospital mortality among IDMs. Further studies are needed to discover effective treatments for HCM, as well as to elucidate its biological underpinnings. Preliminary studies in murine models from other investigators have suggested that mitochondrial dysfunction is present in the cardiomyocytes of IDMs, although links between maternal diabetes, mitochondrial dysfunction, and HCM have not investigated.

Marjan Boerma, PhD, Professor, UAMS College of Pharmacy

Abstract Title

Metabolomics in the identification of radiation biomarkers

Other Authors and Affiliations

Vijayalakshmi Sridharan, UAMS College of Pharmacy
Amrita Cheema, Georgetown Univ.
Keith Unger, Georgetown Univ.
Brian Fish, Medical College of Wisconsin
Heather Himburg, Medical College of Wisconsin

Introduction

Accidental exposure to ionizing radiation can cause early and delayed adverse health effects. Moreover, patients who receive radiation therapy may develop early and late toxicities due to normal tissue exposure to radiation. Current treatment of these toxicities is mainly symptomatic and starts only when the toxicities are advanced. Methods that identify individuals at risk for normal tissue radiation toxicities may aid in starting treatment early and tailoring treatment to the individual patient and the type of toxicities that are expected. We perform studies in animal models and cancer patients to identify panels of metabolites in plasma or urine that can be used in early diagnosis of radiation toxicities.

Materials and Methods

Plasma and/or urine samples are collected from male and female mice and rats before and after exposure to whole body irradiation or localized irradiation of certain organ systems, or from cohorts of cancer patients who receive radiation therapy. Plasma and urine samples are subjected to metabolomics, and metabolite panels are related to functional and tissue endpoints of normal tissue radiation toxicities.

Results and Discussions

Early post-irradiation biomarker panels are identified that predict or diagnose normal tissue toxicities with high sensitivity and specificity.

Conclusions

Metabolomics is a powerful tool in the prediction and early diagnosis of normal tissue radiation toxicities. Studies are ongoing to identify the influence of species, sex and age on these biomarker panels.

Acknowledgement

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Gunnar Boysen, PhD, Associate Professor, UAMS College of Public Health

Abstract Title

Glutamine drives glutathione synthesis and contributes to radiation sensitivity of A549 and H460 lung cancer cell lines.

Other Authors and Affiliations

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Univ. of Arkansas for Medical Science, Little Rock AR 72205

Introduction

Increased glutamine uptake is known to drive cancer cell proliferation, making tumor cells glutamine-dependent. Studying lymph node aspirates with malignant lung tumor cells showed a strong correlation between glutamine consumption and glutathione (GSH) excretion. Subsequent validation in A549 and H460 lung tumor cell lines gave evidence that glutamine drives GSH synthesis and excretion at µmolar amounts. We studied GSH metabolism to understand the possible mechanistic link between glutamine consumption and GSH excretion.

Materials and Methods

Using cell culture/mouse xenograft models, the efficacy of glutaminase inhibitors, BPTES and CB-839, are found based on colonic viability and tumor growth delay. Effects on GLS activity and glutamine metabolism are determined by mass spectrometry-based metabolomic and our stable isotope labelled glutamine (trace metabolite).

Results and Discussions

Inhibition of glutaminase with BPTES and CB-839, two known inhibitors, essentially abolished GSH synthesis and excretion in lung tumor cells. Surprisingly, using stable isotope labeled glutamine as tracer metabolite, demonstrated that the glutamate group in GSH is directly derived from glutamine, linking glutamine utilization to GSH syntheses. Effect of GLS inhibitor could be rescued using GSH ester, the bio-available form of GSH demonstrating the importance of GSH synthesis for cell viability. Inhibition of glutaminase markedly radiosensitizes the lung tumor cell lines, suggesting an important role of glutamine-derived GSH in determining radiation sensitivity. In subsequent mouse xenografts, short term CB-839 treatment reduced serum GSH by >50% and increases response to radiation therapy of H460 derived tumors by 30%, suggesting a clinical relevant mechanism. Inhibition of glutaminase with BPTES and CB-839, two known inhibitors, essentially abolished GSH synthesis and excretion in lung tumor cells. Surprisingly, using stable isotope labeled glutamine as tracer metabolite, demonstrated that the glutamate group in GSH is directly derived from glutamine, linking glutamine utilization to GSH syntheses. Effect of GLS inhibitor could be rescued using GSH ester, the bio-available form of GSH demonstrating the importance of GSH synthesis for cell viability. Inhibition of glutaminase markedly radiosensitizes the lung tumor cell lines, suggesting an important role of glutamine-derived GSH in determining radiation sensitivity. In subsequent mouse xenografts, short term CB-839 treatment reduced serum GSH by >50% and increases response to radiation therapy of H460 derived tumors by 30%, suggesting a clinical relevant mechanism. GSH is the most abundant and most widely studied endogenous antioxidant and concentrations in tumor tissue have been reported to be as high a 10 mM. Extracellular GSH is metabolized by γ-glutamyl-transferase (GGT) to various γ-glutamyl-amino acid derivates.

Conclusions

Results offer evidence that lung tumors utilize glutamine to promote GSH synthesis to increase defense against radiation injury and excrete GSH to recruit neighboring cells producing a heterogeneous tumor phenotype.

Acknowledgement

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Kyle Quinn, PhD, Associate Professor, UA Fayetteville

Abstract Title

Deep Learning Segmentation of Cutaneous Skin Wound Sections and In Vivo Images

Other Authors and Affiliations

Jake D. Jones, Marcos R. Rodriguez, Kyle P. Quinn

Introduction

Histological analysis is a gold standard technique for studying impaired skin wound healing. Label-free multiphoton microscopy (MPM) can provide natural image contrast similar to histological sections and quantitative metabolic information using NADH and FAD autofluorescence. However, MPM analysis requires time-intensive manual segmentation of specific wound tissue regions limiting the practicality and usage of the technology for monitoring wounds. The goal of this study was to train a series of convolutional neural networks (CNNs) to segment MPM images of skin wounds to automate image processing and quantification of wound geometry and metabolism.

Materials and Methods

CNNs with a 4-layer U-Net architecture were trained to segment unstained skin wound tissue sections and in vivo z-stacks of the wound edge. The wound section CNN used 380 distinct MPM images while the in vivo CNN used 5,848 with both image sets being randomly distributed to training, validation, and test sets following a 70%, 20%, and 10% split. The accuracy of each network was evaluated on the test set of images, and the effectiveness of automated measurement of wound geometry and optical redox ratio were compared with hand traced outputs of six unstained wound sections and 69 wound edge z-stacks from eight mice.

Results and Discussions

The MPM wound section CNN had an overall accuracy of 92.83%. Measurements of epidermal/dermal thickness, wound depth, wound width, and % re-epithelialization were within 10% error when evaluated on six full wound sections from days 3, 5, and 10 post-wounding that were not included in the training set. The in vivo wound z-stack CNN had an overall accuracy of 89.66% and was able to isolate the wound edge epithelium in z-stacks from eight mice across post-wound time points to quantify the optical redox ratio within 5% of what was recorded by manual segmentations.

Conclusions

The CNNs trained and presented in this study can accurately segment MPM imaged wound sections and in vivo z-stacks to enable automated and rapid calculation of wound geometry and metabolism. Although MPM is a noninvasive imaging modality well suited to imaging living wound tissue, its use has been limited by time-intensive user segmentation. The use of CNNs for automated image segmentation demonstrate that it is possible for MPM to deliver near real-time quantitative readouts of tissue structure and function.

Acknowledgement

This research was funded by NIH grant numbers R00EB017723 and R01AG056560, as well as the Arkansas Biosciences Institute.

Existing UA-UAMS Collaboration

Several through the COBRE-funded Arkansas Integrative Metabolic Research Center.

Opportunities for Future Collaboration

Several through the COBRE-funded Arkansas Integrative Metabolic Research Center.

Kyle Quinn, PhD, Associate Professor, UA Fayetteville

Abstract Title

Monitoring skin wound healing through non-invasive metabolic imaging

Other Authors and Affiliations

Jake D. Jones, Hallie E. Ramser, Alan E. Woessner, Kyle P. Quinn

Introduction

With advanced age, skin wound healing is often delayed, leaving patients at risk for developing chronic wounds. However, it is challenging to discriminate age-related delays from the numerous possible etiologies of wound chronicity. We have recently identified quantitative biomarkers capable of discriminating delayed healing in diabetic and non-diabetic wounds based on the autofluorescence of metabolic cofactors (NADH and FAD). With the increasing prevalence of non-healing wounds in the elderly, there is also a critical need to understand how these metrics are sensitive to altered healing due to intrinsic aging. The objective of this study is to utilize in vivo label-free multiphoton microscopy (MPM) to characterize differences in wound metabolism between aged and young wounds.

Materials and Methods

All procedures were approved under Univ. of Arkansas IACUC Protocol #17063. Male and female C57BL/6J mice at 24 months and 4 months of age were given 6 mm full-thickness excisional wounds. On days 1, 3, 5, 7, and 10 post-wounding, in vivo multiphoton microscopy was performed to non-invasively quantify cell metabolism within the epithelium at the wound edge. Multiphoton microscopy was performed using a Bruker laser-scanning microscope equipped with a Ti:Sapphire laser. NAD(P)H autofluorescence was isolated using 755nm excitation and a 460±20nm emission filter, while FAD was isolated using 900nm excitation and a 525±25nm emission filter. Multiphoton image z-stacks were acquired at the wound edge and an optical redox ratio of FAD/(NADH+FAD) autofluorescence was computed as in previous work. NAD(P)H fluorescence lifetime images were also acquired to evaluate the ratio of free to protein-bound NADH. After day 10 post-wounding, tissue was harvested and sectioned for direct ex vivo comparisons between autofluorescence imaging and histology.

Results and Discussions

In vivo MPM enabled the spatial isolation of epithelial keratinocytes in both aged (24 month) and young (4 month) mice. Temporal changes in the optical redox ratio were consistent with previous findings with a decrease in redox ratio from day 1 to days 3 and 5 ($p < 0.01$), followed by an increase in redox ratio by day 10 ($p < 0.05$) for both groups. However, aged mice exhibited a higher overall redox ratio ($p < 0.001$) than the young mice, and females had a higher redox ratio than males ($p < 0.01$). NADH fluorescence lifetime images also revealed temporal changes in the mean lifetime, which correlated ($R=0.78$, $p<0.01$) with the optical redox ratio. These findings indicate an optical sensitivity to the accumulation of free NADH caused by the biosynthetic demands of epithelial proliferation, and that aged skin may have reduced proliferative capacity leading to delays in healing.

Conclusions

Our work demonstrates label-free MPM is sensitive to age-related changes in wound metabolism. These findings suggest that MPM can distinguish age-related delays from the hyperproliferative epidermis of diabetic wounds and may be used to guide specific treatment strategies in the clinic. Future studies will be aimed at exploring the underlying biological mechanisms that drive the differences in wound metabolism produced by age, sex, and the multiple phases of the wound healing process.

Acknowledgement

Supported by NIH grant numbers R01AG056560 and R00EB017723.

Existing UA-UAMS Collaboration

Several through the COBRE-funded Arkansas Integrative Metabolic Research Center.

Opportunities for Future Collaboration

Several through the COBRE-funded Arkansas Integrative Metabolic Research Center.

Kyle Quinn, PhD, Associate Professor, UA Fayetteville

Abstract Title

Quantitative metabolic imaging through label-free multiphoton microscopy

Introduction

Label-free multiphoton microscopy can provide a variety of non-invasive quantitative biomarkers of cell metabolism, collagen fiber organization, and tissue composition. By quantifying the naturally present fluorescence of NADH and FAD without our cells, we can non-invasively measure dynamic changes in the metabolism of live cells, tissues, and organisms.

Materials and Methods

Our primary efforts focus on developing quantitative biomarkers for non-invasive, real-time assessments of tissue structure and function to diagnose disease or trauma and guide therapies. Our research efforts span a wide range of biomedical applications, but we have a strong interest in skin wound healing and aging. To investigate the different dynamic processes involved in skin aging, disease, and tissue repair, we utilize a combination of label-free multiphoton microscopy and advanced image analysis algorithms, such as deep learning convolutional neural networks.

Results and Discussions

Through the use of these technologies, we can obtain quantitative readouts from three-dimensional images of live cells and tissue at the microscale without the need for fixation, mechanical sectioning, or staining.

Conclusions

I will cover recent results that detail dynamic changes in mitochondrial structure and function in a variety of applications.

Acknowledgement

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Existing UA-UAMS Collaboration

Several UAMS faculty participate in our COBRE-funded Arkansas Integrative Metabolic Research Center.

Jesus Delgado-Calle, PhD, Assistant Professor, College of Medicine, UAMS, Little Rock

Abstract Title

Targeting Notch Inhibitors to the Myeloma Bone Marrow Niche Decreases Tumor Growth and Bone Destruction without Gut Toxicity

Other Authors and Affiliations

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Introduction

Multiple myeloma (MM) is a plasma cell malignancy characterized by the growth of clonal plasma cells in the bone marrow (BM) and is currently incurable. Crosstalk between MM cells and host cells in the bone/BM niche plays a critical role in MM onset and progression (2). Communication via Notch signaling favors MM progression by increasing MM proliferation, promotes chemotherapy resistance, and stimulates angiogenesis. The multifunctional role of Notch communication in MM provides a strong rationale to block Notch signaling in the MM tumor niche. However, the specific role of Notch components in MM is poorly defined, and Notch receptors can have distinct functions. Thus, signaling inhibition downstream of the four Notch receptors with γ-secretase inhibitors (GSIs) has been used to inhibit Notch in MM. GSIs decrease MM growth and mitigate the bone disease in animal models of MM. Despite these promising results, the use of GSIs in the clinic has been prevented because of undesirable, dose-limiting severe toxicities, particularly gastrointestinal toxicity, derived from the systemic inhibition of Notch in different tissues.

Materials and Methods

To circumvent the unwanted side-effects of GSI while retaining its ability to block Notch communication in the MM niche, we generated a bone-targeted Notch inhibitor (BT-GSI). We used a modified bisphosphonate with a high affinity for bone as a bone-targeting moiety and linked it to GSI-XII using a hydrazine acid-sensitive linker designed to be cleaved in areas of the bone/BM with low pH. We characterized BT-GSI using in vitro, ex vivo, and in vivo models of MM disease.

Results and Discussion

BT-GSI administration decreased Notch target gene expression in the bone marrow, but it did not alter Notch signaling in intestinal tissue or induce gut toxicity. In mice with established human or murine MM, treatment with BT-GSI decreased tumor burden and prevented the progression of MM-induced osteolytic disease by inhibiting bone resorption more effectively than unconjugated GSI at equimolar doses.

Conclusion

In summary, we show here that targeted inhibition of Notch signaling to the MM niche with BT-GSI is a promising therapeutic approach with dual anti-MM and anti-resorptive properties, enabling simultaneous inhibition of tumor growth and prevention of bone destruction in MM. Because BT-GSI is tissue-specific and selectively blocks Notch signaling in bone, it lacks gut toxicity and circumvents the deleterious side effects that limit the clinical use of GSIs for MM and potentially other cancers that grow in bone.

Opportunities for Future Collaboration

We are interested in collaborations related to drug development, cancer, and bone biology.

Kartik Balachandran, PhD, Associate Professor, Dept. of Biomedical Engineering, Univ. of Arkansas

Abstract Title

Label-free metabolic metrics are sensitive to early calcific aortic valve disease progression

Other Authors and Affiliations

Ishita Tandon (1), Ngoc Thien Lam (1), Olivia I. Kolenc (1), Shelby Johns (1), Delaney Cross (1), Alan Woessner (1), Isaac Vargas (1), Asya Ozkizilcik (1), Jessica Perez (1), Srikanth Vallurupalli (2), Timothy J. Muldoon (1), Kyle P. Quinn (1), Kartik Balachandran (1)

1 Univ. of Arkansas
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Introduction

Heart valves are elegant dynamic structures that maintain a unidirectional flow in the heart, and experience harsh hemodynamic and mechanical forces. The most common heart valve disease - calcific aortic valve disease (CAVD) is a progressive disease with a complex pathophysiology. CAVD is associated with 50% elevated risk of fatal cardiovascular pathologies resulting in 15,000 annual deaths in North America alone. The only standard of care currently available is valve replacement surgery as early detection, prevention, and mitigation strategies are still lacking. There is a critical need to develop strategies to detect and monitor early CAVD progression. Two-photon excited fluorescence (TPEF) microscopy has shown potential in providing label-free, non-invasive, quantitative metrics that have been extensively employed in stem cell and cancer research. TPEF metrics have shown potential in detecting CAVD markers like osteogenic differentiation, calcification, and collagen remodeling. We hypothesized that TPEF metrics will correlate with the pathophysiological changes during early CAVD progression in simple in vitro and ex vivo models.

Materials and Methods

For in vitro assessment, Porcine aortic valve interstitial cells (VICs) were cultured on 2D substrates and in 3D collagen-based hydrogels to simulate healthy and diseased conditions. For ex vivo assessment, A novel wild-type early CAVD model was developed by feeding 20-week old C57BL/6J mice a control vs pro-calcific diet. TPEF imaging was used to obtain autofluorescence intensities at 755nm and 860nm excitation with 460nm±40nm and 525nm±45nm emission wavelengths to assess TPEF 755-860 ratio ((A860/525) / (A755/460) + (A860/525). The 755-860 ratio was then correlated with established biomarkers of VAD progression.

Results and Discussions

TPEF 755-860 ratio decreased by day 14 for 2D osteogenic cultures and correlated with functional and phenotypic markers like osteocalcin, RUNX2, TGFβR1, and osteopontin gene expression. Mitochondrial clustering, quantified from A755/460 intensity maps correlated with structural markers like RhoA and nuclear morphology. TPEF 755-860 ratio was also reduced with pathological stretching of the VICs and correlated with increased proliferation. Additionally, VICs in 3D cultures under elevated cyclic stretch showed reduced TPEF 755-860 ratio and had increased proliferation which was mediated by Akt/mTOR pathway. The pro-calcific mice showed lipid deposition and calcium deposition, collagen remodeling, proliferation, osteogenesis, and inflammation in aortic valves but did not show altered left ventricular function. TPEF 755-860 ratio decreased with disease progression and correlated with increased calcium deposition, proliferation, and RUNX2 expression.

Conclusions

These in vitro and ex vivo studies show that the TPEF autofluorescence markers identified the pathological changes and correlated with the early CAVD progression suggesting it is a promising tool to look at the structural, functional, and metabolic changes occurring in cells and tissues during the CAVD progression.

Acknowledgement

This work was supported by the National Science Foundation [CMMI-1452943 to K.B., CBET-1846853 to K.P.Q.], and the American Heart Association [18AIREA33900098 to K.B., 19PRE34370061 to I.T.]. This work was also supported by funding from National Institutes of Health [R00EB017723, R01AG056560 to K.P.Q.]. The custom-built multiphoton imaging setup was supported by the Arkansas Biosciences Institute to T.J.M.

Existing UA-UAMS Collaboration

Collaborations with Dr. Vallurupalli (Division of Cardiology).

Ana Regina Cabrera, PhD Student, UARK - Fayetteville, AR

Abstract Title

A Biological Sex Comparison of Muscle Contractile Function in the Onset of Colorectal Cancer-induced Cachexia

Other Authors and Affiliations

J. William Deaver[1], Seongkyun Lim[1], Francielly Morena da Silva[1], Eleanor Schrems[2], Landen Saling[2], Tyrone A. Washington[2], Nicholas P. Greene[1]

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Introduction

Cancer cachexia (CC) represents 20-40% of mortality among cancer patients. It is clinically defined by involuntary loss of >5% weight in a six-month window, despite nutritional interventions. Skeletal muscle is one of the primarily affected tissues in CC and is associated with weakness and fatigue. Impaired muscle function is associated with lower quality of life in cancer patients. During CC, muscle mass and fiber cross-sectional area are controlled by protein turnover, among others. Protein imbalance via exceeding protein degradation of myofibrillar proteins, over synthesis will result in muscle wasting. In addition, evidence from our research group suggests biological sex differences. No effective therapies are yet available to treat CC, and to date, it remains an untreated condition. The critical mechanisms of how this pathology evolves and how it affects muscle function remain incompletely known.

Materials and Methods

Eight-weeks old BALB/c mice (69 male and 60 female) were separated into 5 groups (PBS, 10-, 15-, 20-, and 25-day). The 10-25-day groups received colorectal cancer cell injections at day 0 and while control mice received a PBS administration. Forty-eight hours prior to tissue harvest, skeletal muscle contractility measures (in vivo peak isometric torque and fatigability) were performed of the anterior crural muscles, in which TA is the largest muscle component of the group for this type of contraction. Torque frequency curves were determined across frequencies from 10-300 Hz. Afterward, fatigability was measured at 40 Hz with 120 continuous stimuli. TA muscle was collected at each time point and mRNA content of REDD1, Atrogin, and Deptor were measured as part of protein turnover markers. A one way-ANOVA across time points within each sex was performed as the global analysis with $\alpha=0.05$

Results and Discussions

Male mice had lower body weights and higher fatigue than females. Before normalization, both male and female mice presented less force at higher torque frequencies. mRNA content of REDD1, atrogin, and muRF1 in TA muscle were elevated after 25 days of tumor implantation.

Conclusions

Male mice waist more skeletal muscle and have more fatigue than female mice during C26 induced-CC. Our results reinforce the theory that muscle weakness can be attributed to the decline in muscle mass and may not be attributed to alterations in the intrinsic contractile properties of the myofibers, while markers for ubiquitin proteolysis are increased and synthesis repressed.

Acknowledgement

This study was funded by the National Institutes of Health, Award: 5 R01 AR075794-02.

Existing UA-UAMS Collaboration

With the Center for Musculoskeletal Disease Research and Isabelle Racine-Miousse

Nicholas Greene, PhD, Assoc. Professor, Interim Director - Exercise Science Research Center; College of Education and Health Profession / UARK

Abstract Title

Mechanisms in Onset of Cancer-Induced Cachexia

Introduction

Our laboratory's primary research goal is to characterize key steps in the initial development of cancer-induced cachexia to determine mechanisms in onset of this condition which can then be utilized to target efficacious therapies. Cancer cachexia, is defined primarily by an ongoing loss in skeletal muscle mass, which may be accompanied by losses in total body and fat mass. Cachexia occurs in up to 80% of patients and can account for 20-40% of the cancer related mortality depending upon type of cancer. We utilize time course approaches in pre-clinical models of cancer cachexia to assess the pre-cachectic signature of muscle, which identifies changes to the muscle prior to development of overt muscle loss. Subsequently, this signature is utilized to test mechanisms of cachexia using genetic and pharmacologic approaches to mitigate cachexia.

Existing UA-UAMS Collaboration

Current collaborations with UAMS:

Center for Musculoskeletal Disease Research
Isabelle Racine-Miousse

Francielly Morena da Silva, PhD Student, Univ. of Arkansas, Fayetteville

Abstract Title

Comparison of the Phenotypic Development of C26 Colorectal Cancer-induced Cachexia between Biological Sexes

Other Authors and Affiliations

J. William Deaver[1], Seongkyun Lim[1], A. Regina Cabrera[1], Eleanor Schrems[2], Landen Saling[2], Tyrone A. Washington[2], Nicholas P. Greene[1]
[1]Cachexia Research Laboratory, Dept. of Health, Human Performance, and Recreation, UARK
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Introduction

Cachexia is a multifactorial syndrome commonly experienced by cancer patients. Cachexia is clinically defined by involuntary weight loss greater than 5% in six-months and is generally not responsive to nutritional interventions alone. Cancer cachexia (CC) is associated with resistance to anti-cancer treatment and is responsible for 20-40% of cancer-associated deaths. Atrophy, muscle weakness, and fatigue are the primary hallmarks of CC. Mechanisms of cachexia are not fully understood, and current interventions lack efficacy. Studies from our group and others suggest differences in CC between biological sexes. However, direct comparisons of the phenotypic development of cachexia between biological sexes are scarce. PURPOSE: Therefore, the purpose of this study was to characterize phenotypic differences between biological sexes during the development of CC in a time-course manner in a preclinical C26 colorectal cancer model.

Materials and Methods

A total of 129 (69 males and 60 female) 8-wk old BALB/c mice were separated into PBS control, 10-, 15-, 20- and 25-day of tumor-bearing group (~10-12 animals/group). Cancer groups were injected with a total 1 million C26 cells bilaterally to the hind flanks, while equal volume of PBS was injected in PBS control (age-matched with 25-day animals). Tissue collection was performed at each designated time point represented by each group; Gonadal-Fat, Liver, Spleen, Heart, Soleus, Plantaris, Gastrocnemius, EDL and TA muscles were weighed and snap frozen in liquid nitrogen. All tissue weights were normalized to tibia length to account for differences in body size. A one way-ANOVA across timepoints within each sex was performed as the global analysis with $\alpha=0.05$.

Results and Discussions

Tumor free body weight was significantly lower in male 25-day mice by 15% ($p<0.0001$) when compared to PBS control, while no differences in body weight were noted in female mice across groups. For muscle weights: soleus, gastrocnemius, and TA muscle weights were 9.4%, 16.8%, and 19.4% ($p<0.0001$) lower in male 25-day mice compared to PBS group, respectively. No statistical differences in muscle weights were observed in female mice. In males, 25- and 20-day groups showed 49% ($p<0.0001$) and 23% ($p=0.0165$) lower fat content compared to PBS control. Accordingly, female 25-day fat content was reduced by 55% compared to PBS control ($p=0.0136$). Spleen weight was significantly greater in 20- (87%, $p<0.0001$) and 25-day (155%, $p<0.0001$) groups when compared to PBS control in males. In females, a similar pattern was noted, 20- and 25-day had a significantly heavier spleen by 61% and 118% respectively, compared to PBS control ($p<0.0001$).

Conclusions

Despite demonstrating classic cachexia phenotypes regarding splenomegaly and losses in fat mass, female mice appear to protect skeletal muscle wet weights relative to males during development of cancer cachexia.

Acknowledgement

This study was funded by the National Institutes of Health, Award: 5 R01 AR075794-02.

Existing UA-UAMS Collaboration

Existing collaboration with the Center for Musculoskeletal Disease Research and Isabelle Racine-Miousse

Emory Gregory, Senior Graduate Assistant, College of Engineering, Univ. of Arkansas, Fayetteville

Abstract Title

Examining Breast Cancer Response to Metformin Using Tissue-Engineered *in vitro* Testbeds

Other Authors and Affiliations

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[1]Biomedical Engineering, [2]Food Science, [3]Chemical Engineering, UARK

Introduction

In the United States, 1 in every 8 women will develop breast cancer in their lifetime. Sadly, the American Cancer Society estimates 280,000 new cases of invasive breast cancer will occur in 2021 alone [1]. Recent studies show that breast tumor innervation, in which nerves invade adjacent breast tumors, plays a critical role in the acceleration of cancer invasiveness and contributes to poor patient prognosis [2,3]. Metformin has been identified as a breast cancer therapy; however, this treatment contributes to patient vitamin B12 deficiency [4]. In response, doctors prescribe supplementation, but vitamin B12 over-supplementation is linked to special cases of increased neural cell survival and neurite outgrowth [5, 6]. While there lacks evidence of vitamin B12-induced axonal outgrowth in breast cancer, we hypothesize that this event promotes breast tumor innervation and cancer metastasis [7]. Here, we establish a tissue-engineered platform for modeling breast cancer in vitro, evaluate the effect of Metformin on cancer cell viability, and determine the concentration needed to deplete cancer cell metabolism and cytokine secretion most significantly. These discoveries will aid in future studies investigating dose-dependent vitamin B12-induced neurite outgrowth and metastatic potential in breast cancer and the potential identification of viable targets for prospective breast cancer therapies.

Materials and Methods

Subcutaneous adipose tissue from male Sprague Dawley rats was harvested, decellularized based on a previously described protocol [8], and digested into a hydrogel to create a biomimicking platform of mammary extracellular matrix (ECM). This gel was combined with a rat tail collagen-I hydrogel 1:1 to fabricate a model more relevant to the stiffness of fibrous breast tumor tissue. Gelation kinetics and immunofluorescence were performed to establish collagen fibril polymerization. Normal (HC11) and cancerous (4T1) mammary epithelial cells were embedded in the engineered hydrogel with and without 3T3-L1 preadipocytes to replicate the environment of early- and late-stage breast cancer, respectively. The cell-containing hydrogels were cultured for 1 week in complete media supplemented with either 0, 1, 2.5, 5, or 10mM of Metformin. Cell metabolism after Metformin treatment was evaluated using alamarBlue, and VEGF secretion was determined via Luminex. Immunofluorescence was performed using anti-N-cadherin and anti-alpha-SMA primary antibodies. Preliminary co-cultures with BALB/c mouse dorsal root ganglia (DRG) were conducted to establish neurite outgrowth before vitamin B12 supplementation, and immunofluorescence against beta-III tubulin was performed.

Results and Discussions

The composite hydrogel undergoes complete thermal polymerization, making it a viable platform for cell culture. Cultures with 3T3-L1 cells show stable cell metabolism and significantly increased VEGF levels with increased Metformin dosage. Moreover, 3T3-L1 cells exhibit higher alpha-SMA and significantly higher N-cadherin levels in cultures with 4T1 cancer cells and with increased Metformin dosage. This data can be used to determine that 3T3-L1 cells express myofibroblastic phenotypes with 4T1 culture and increased Metformin concentration, and the cells act as cancer-associated fibroblasts (CAF) to support cancer cells. Immunofluorescence against beta-III tubulin indicated early neurite outgrowth of DRGs after 3 days of culture, predicting the ability for future successful neurite outgrowth studies.

Conclusions

Here, we demonstrated the successful models of normal and malignant breast tissue in vitro using an engineered ECM-mimicking hydrogel, the differentiation of 3T3-L1 cells into CAFs in 4T1 cultures and increased Metformin treatment, and the ability to perform successful neurite outgrowth studies. Future studies should determine how vitamin B12 supplementation affects neurite outgrowth via DRG co-cultures and whether vitamin B12 supplementation leads to a brain metastatic phenotype via breast tumor innervation.

Acknowledgement

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Young Hye Song, PhD, Assistant Professor, Engineering/Fayetteville

Abstract Title

Tissue engineering strategies to modulate extracellular matrix presentation in vitro

Other Authors and Affiliations

Inha Baek, Nikolas Ala-Kokko, Gabriel David, Mackenzie Lewis, Luis Pinzón-Herrera, Alan Woessner, Kyle Quinn, Jorge Almodovar

Pinzon-Herrera and Almodovar: Chemical Engineering, Univ. of Arkansas, Fayetteville, AR
everyone else: Biomedical Engineering, Univ. of Arkansas, Fayetteville, AR

Introduction

Tissue engineering approaches provide valuable tools and techniques to study cell behavior in a physiologically relevant manner in vitro. This can be used to develop combinatorial therapeutics to promote tissue repair/regeneration, as well as unravel novel mechanisms of disease or injury progression. This talk provides an overview of tissue engineering strategies utilized in my lab to vary extracellular matrix (ECM) presentation in vitro, and how different aspects of cell behavior are modulated. The examples in this talk focus on nerve injury and repair, and can be utilized to study ECM effects on cell metabolism in various healthy and diseased/injured conditions.

Materials and Methods

To create three-dimensional (3D) cell culture platforms, murine and porcine sciatic nerves and spinal cords were decellularized using previously established methods. Afterwards, the acellular tissue matrices were digested in enzymatic-acidic solution to create pre-gel solutions. After pH neutralization and cell encapsulation, ECM composition, fiber thickness, and orientation were varied during gelation. Cell secretome profile, broad metabolic activity (alamarblue assay), phenotypic changes, and ECM deposition were assessed.

Results and Discussions

Preliminary findings from our lab show differential responses by cells to ECM presentations in vitro. Higher concentration of spinal cord ECM to sciatic nerve ECM resulted in higher broad metabolic activity of embedded adipose-derived stem cells (ASCs) in early time points. Thicker ECM fibers generated through temperature-mediated hydrogel formation resulted in significant reactive phenotypic changes of astrocytes. Finally, aligned collagen fiber led to alignment of embedded ASCs and significant modulation of secretome profiles. Further studies are currently ongoing to corroborate these results and identify molecular and cellular mechanisms.

Conclusions

Tissue engineering approaches to modulate ECM presentation provide unique tools to study cell behavior in healthy and diseased/injured microenvironments. These 3D cell culture systems can be used to assess metabolic changes in (patho)physiological contexts as a function of ECM changes.

Acknowledgement

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Existing UA-UAMS Collaboration

Dr. Rob Griffin through Arkansas Integrative Metabolic Research Center

Opportunities for Future Collaboration

Analyses of patient samples, assessing patient-derived cell behavior in controlled, bioengineered 3D culture platforms

Teresita Bellido, PhD, Professor and Chair, Dept. Physiology & Cell Biology, UAMS

Abstract Title

Distinct mechanisms regulate the response of female and male skeletons to sex steroid deficiency and to the bone protective effects of blueberry containing diets.

Other Authors and Affiliations

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Introduction

There is an unmet need for non-sex steroid interventions that prevent bone loss induced by sex steroid deficiency with better compliance and lower side effects than current approaches. Loss of sex steroids causes accumulation of reactive oxygen species (ROS) in bone. ROS, in turn, activate the transcription factor Nrf2 and increase the endogenous antioxidant response (EAR), in an attempt to mitigate damaging oxidative effects.

Materials and Methods

We investigated the ability to prevent bone loss due to sex steroid deficiency of diets containing 10% of 3 types of blueberries rich in antioxidant metabolites fed to 4 mo-old female (F) or male (M) WT and Nrf2 KO littermate mice (N=19-26).

Results and Discussions

In mice fed control diet, ovariectomy (OVX) or orchidectomy (ORX) induced the expected bone loss, which was similar in WT and KO mice, as quantified by % change BMD/month or final BMD. Only the diet with Montgomery berries decreased the rate of bone loss, either fully in F or partially in M. Whereas the berry diet prevented bone loss in both WT and KO F, it only prevented bone loss in WT M. OVX decreased expression of the estrogen response element (ERE)-containing gene C3 and ORX decreased expression of the androgen ARE-containing gene Rhox5 in bone, in WT and KO mice fed control diet. And, C3 or Rhox5 expression remained low in OVX or ORX mice fed the berry diet, indicating that bone protection is not due to estrogenic/androgenic actions of the diet. OVX increased the EAR in bone, but ORX did not. Moreover, the berry diet prevented the increase in EAR induced by OVX in both WT and KO mice, but it did not alter EAR in ORX mice.

Conclusions

In summary, (1) bone loss induced by estrogen/androgen deficiency is independent of Nrf2; (2) only Montgomery berry diet decreased bone loss rate; (3) berry diet-induced skeletal protection depends on sex (full in F vs partial in M) and is exerted by a mechanism independent of canonical ERE or ARE signaling; and (4) bone EAR in sex steroid deficiency (OVX-increased vs ORX-not altered) or in response to the berry diet (prevention of OVX-induced increases vs not altered-ORX) also depends on sex. We conclude that distinct mechanisms underly the response of the F and M skeletons to (a) sex steroid deficiency, as bone EAR is increased in F but not M, or to (b) bone protection by the berry diet, as Nrf2 is required in M but not in F. Thus, optimal skeletal benefits might be achieved by tailoring antioxidant-rich diets to patients of either sex.

Acknowledgement

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Teresita Bellido, PhD, Professor and Chair, Dept. Physiology & Cell Biology, UAMS

Abstract Title

Sost- and Dkk1-mediated inhibition of LRP5 signaling contributes to diabetes-induced bone disease

Other Authors and Affiliations

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Introduction

Diabetes induces bone fragility by complex and poorly understood mechanisms. Earlier evidence demonstrated increased expression of the antagonists of Wnt signaling SOST/sclerostin and Dkk1 in bones of mice with type 1 diabetes (T1D). Sclerostin and Dkk1 are also detected at higher levels in the serum of T1D patients.

Materials and Methods

To investigate the role of these antagonists, diabetes was induced with streptozotocin (STZ) in mice expressing the LRP5 high bone mass (HBM) mutation pG171V, which is refractory to Sost- and Dkk1-mediated inhibition of Wnt/βcatenin signaling, or control littermate mice (C). Skeletally mature 12-wk old mice randomized by spinal BMD were injected with STZ (45 mg/kg/d, T1D) or vehicle for 5 days. Diabetes was confirmed in both genotypes 10 days after the last STZ injection by measuring blood glucose.

Results and Discussion

LRP5 HBM mice exhibited the expected higher BMD compared to C mice, regardless of the diabetes status. After 4 wks of established T1D, both LRP5 HBM and C diabetic mice exhibited similar reduction in body weight. In addition, diabetic C mice exhibited lower spinal and total BMD compared to C mice receiving vehicle. In contrast, diabetic LRP5 HBM mice exhibited similar BMD compared to vehicle-injected LRP5 HBM mice. Protection from the decrease in BMD induced by T1D was maintained in LRP5 HBM mice even after 8 wks of established diabetes. At this time, both genotypes maintained similar levels of hyperglycemia.

Acknowledgement

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Teresita Bellido, PhD, Professor and Chair, Dept. Physiology & Cell Biology, UAMS

Abstract Title

Pharmacologic inhibition of the proteasome prevents GC-induced loss of both bone and skeletal muscle, but the atrogene MuRF1 is responsible only for muscle atrophy.

Other Authors and Affiliations

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Introduction

Excess of glucocorticoids (GC), either endogenous as in aging or due to glucocorticoid administration as immunosuppressants, leads to loss of bone and muscle mass, i.e. atrophy. Muscle atrophy, in turn, reduces body balance and increases the propensity to fall thus increasing the risk of bone fractures. Atrophy could be due to decreased protein synthesis and/or increased protein degradation. In particular, muscle atrophy induced by GC is associated with increased expression of the E3 ubiquitin ligases like MuRF1 (or Trim63), named atrogene, followed by increased protein ubiquitination and proteasomal degradation, leading to reduced total protein content (1, 2).
We recently showed that GC also increase atrogene expression not only in skeletal muscle but also in bone, in vivo, ex vivo, and in vitro (3). These findings suggest that atrogene upregulation is a common mechanism by which GC induce bone and muscle atrophy.
Objective: To examine whether interference with proteasomal activity or the expression or function of the atrogene MuRF1 prevents GC harmful actions in bone and muscle

Materials and Methods

To assess the role of atrogenes in the musculoskeletal effects of GC, we inhibited the pathway by pharmacologic or genetic means in skeletally mature 4 month-old mice implanted with prednisolone (GC, 2.1 mg/kg/d) or placebo pellets. For pharmacologic means, mice were treated with vehicle or the proteasomal inhibitor carfilzomib (CF) 5mg/kg/d twice a week starting 3 days before pellet implantation. For genetic means, wild type littermate control mice of mice lacking MuRF1-mediated ubiquitination activity due to genetic deletion of the RING domain (ΔR) were also utilized.

Results and Discussions

GC administration to C57Bl/6 mice for 2 weeks caused the expected reduction in bone mineral density (total BMD -3; femoral BMD -1% change) and muscle weight (quadriceps -22; tibialis anterior -14% change of placebo) in vehicle-treated mice. In contrast, mice treated with the proteasomal inhibitor carfilzomib (CF) 5mg/kg/d twice a week starting 3 days before pellet implantation, were protected from GC-induced loss of bone (total BMD 0%; femoral BMD +3% change) as well as skeletal muscle/body weight (quadriceps -1.7; tibialis anterior -1.8% change of placebo). CF also preserved the strength of the entire posterior musculature compartment (comprising 8 muscles), quantified in vivo by plantarflexion torque testing at all frequency stimulations (10-300Hz). Further, GC administration for 4 weeks induced the expected reduction in muscle weight and function in wild type littermate control mice but not in mice lacking MuRF1-mediated ubiquitination activity due to genetic deletion of the RING domain (ΔR): quadriceps -18 vs +1% and tibialis anterior -8 vs -1% for control and ΔR, respectively (% change of placebo). In contrast, GC administration induced bone loss in both ΔR and control mice to a similar extent (total BMD -2 vs -2.5%; femoral BMD -6.1 vs -6.6% change, for control and ΔR, respectively)

Conclusions

Overall, these results demonstrate that pharmacologic inhibition of the proteasome prevents GC-induced atrophy in both bone and skeletal muscle, and also identify MuRF1 as the sole atrogene responsible for GC-induced atrophy in skeletal muscle, but not in bone.

Acknowledgement

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Leonard Harris, PhD, Assistant Professor, University of Arkansas, Fayetteville, AR

Abstract Title

Drug tolerant BRAF-mutant melanoma populations are characterized by ion channel dysregulation and susceptibility to ferroptosis

Other Authors and Affiliations

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Introduction

We recently reported on a non-genetic, drug-tolerant state of balanced division and death, termed "idling," in BRAF-mutant melanoma cell populations under prolonged BRAF inhibition. The idling state is reversible, i.e., removing drug restores the proliferation rate to that of untreated cells and reapplication of drug reestablishes the idling behavior. Moreover, idling cells are not quiescent but actively divide and die, evidenced by experiments using a genetically encoded cell cycle indicator. By continuing to divide and, hence, replicate DNA, idling cells are believed to have an increased chance of acquiring genetic resistance mutations. It is hypothesized that following drug treatment, a residual tumor mass may be highly enriched in idling cells and that from these acquired drug resistance arises. As such, a window of opportunity might exist between the onset of idling and the acquisition of drug resistance during which a targeted secondary treatment can be deployed to further reduce, or even eliminate, the residual disease.

Results and Discussions

Prior metabolic analyses of untreated and idling melanoma subclones showed that metabolic activity is dramatically reduced in idling cells. Here, we show that RNA sequencing of the same subclones show that expression levels of inositol trisphosphate (IP3) receptors, which regulate metabolic processes via Ca^{2+} flux between the endoplasmic reticulum (ER) and mitochondria, also fall significantly over time under BRAF inhibition. A gene enrichment analysis based on both transcriptomic and epigenomic (chromatin accessibility) data from untreated and idling cell populations, suggests impaired ion channel activity is a characteristic of idling cells, pointing to a role for mitochondrial metabolism in the idling phenotype. This is supported by calcium flux assays that show store-operated calcium entry (SOCE) is significantly altered in idling cells. Finally, idling cell populations are shown to have increased sensitivity to ferroptotic cell death, supporting the idea that residual disease can be targeted by rational secondary treatments.

Conclusions

Our long-term goal is to uncover the molecular drivers of the idling phenotype, which we believe is a common phenomenon across cancer types, and to identify novel targets that can kill idling cells and, hence, improve patient outcomes. Toward this end, we have begun building a computational model incorporating Ca^{2+} handling in the ER, Ca^{2+} flux between the ER and mitochondria, mitochondrial metabolism, reactive oxygen species (ROS), and the ferroptosis programmed cell death pathway. The integrated model is based on sub-models previously published in the literature and on new data being generated by experimental collaborators at Vanderbilt University.

Acknowledgement

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Opportunities for Future Collaboration

Our lab is excited about the prospect of forming new collaborations with UA/UAMS researchers. We have extensive experience working side-by-side with experimental collaborators to build computational models that can provide insight into experimental data and suggest new experiments. In addition to this project, we are also currently working on building computational models of intracellular signaling pathways and microenvironmental cell-cell interactions in small cell lung cancer and tumor-induced bone disease.

Ruben Michael Ceballos, PhD, Assistant Professor, Cell and Molecular Biology, UARK

Abstract Title

Differential susceptibility of neuronal neurotransmitter phenotypes to HHV6 infection

Other Authors and Affiliations

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2-Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR

Introduction

Three human β-herpesviruses are described: HHV-6A, HHV-6B, and HHV-7. Initially, HHV-6A/HHV-6B were considered 2 strains of the same virus (i.e., HHV6). However, despite having a high level of overall genomic sequence identity (~90 %), HHV-6A and HHV-6B are now classified as 2 distinct viruses due to limited sequence similarity (e.g., 70%) in critical coding regions and critical physiological/biochemical differences (e.g., preferential use of distinct receptors for viral entry into hosts). HHV-6A/HHV-6B play a role in several neurological disorders, including multiple sclerosis, epilepsy, and chronic fatigue syndrome. Although identified as causal agents in nervous system dysfunction, the contribution/mechanism HHV6-induced epileptogenesis action is unknown. Regarding cell tropism/pathogenicity of HHV-6A vs. HHV-6B would aid understanding of viruses' role in seizure induction. Although HHV-6A/HHV-6B are known to infect glia and cerebellar Purkinje cells, cell tropism of HHV-6A vs. HHV-6B neuronal neurotransmitters phenotypes is unknown.

Materials and Methods

Human embryonic stem cells were plated and grown in CELLStart™ coated vessels as a monolayer. Cells were passaged every 7 days. Different media types were used during re-plating to differentiate cells along specified routes. To prepare virus, HHV-6A strain GS-infected HSB2 cells and HHV-6B strain Z29-infected MOLT-3 cells (courtesy NIH) were thawed and used to infect uninfected HSB2 and MOLT-3 cells. HHV6-infected cells (10^6) were mixed with uninfected cells in a 1:10 ratio. The HHV6-specific U22 gene was targeted for qPCR-based viral titers from cell lines (i.e., HSB2 and MOLT-3 cells) and infected differentiated hNSCs. The existence of intact, fully assembled virions from host cells (e.g., HSB2 cells, MOLT-3 cells, and dHNSCs) was confirmed via transmission electron microscopy (TEM). Morphological changes in infected cultures were examined via light microscopy. Images were obtained from dHNSCs at post-differentiation day (PDD) 7 and PDD 14 at 2 hours post-infection (HPI) and 24 HPI. Fluorescent antibodies and the nuclear dye 4,6-diamidino2-phenylindole dihydrochloride (DAPI) were used to differentiate distinct cell types.

Results and Discussions

Our results confirm that HHV-6A/HHV-6B can infect glia derived from the differentiation of human neural stem cells (i.e., H-9 cells). Results show that both HHV-6A and HHV-6B can also infect neurons derived from differentiated hNSCs. Immunofluorescence indicates that both viruses infect VGluT1-containing cells (i.e., glutamatergic neurons). Likewise, both viruses infect dopamine-containing cells (i.e., dopaminergic neurons). However, it appears that neither virus establishes productive infection in GAD67-containing cells (i.e., GABAergic cells). The explanation for GABAergic cell resistance to HHV-6A and HHV-6B infection remains to be elucidated. According to image analyses of neurite extension and retraction dynamics, cell death during infection, and time-course of cell clumping events (e.g., syncytia formation), it appears that HHV-6A produces more severe CPEs than HHV-6B at the same endpoint during infection assays and under equivalent conditions (i.e., the same multiplicity of infection, MOI). The data suggest that HHV-6A has higher virulence than HHV-6B on differentiated HNSCs into distinct neuronal neurotransmitter phenotypes. Apparent resistance of GABAergic dHNSCs (i.e., resistant to infection by either virus) is the exception.

Conclusions

Characterizing potential differences in neuronal neurotransmitter phenotype susceptibility to HHV-6A vs. HHV-6B provides critical information to understanding the potential mechanisms for HHV6-induced epileptogenesis. There are several proposed mechanisms for HHV6-induced seizures. Understanding cell tropism, relative virulence, and the genetics/-omics substrates of infection outcomes will allow us to determine the validity of each proposed model.

Acknowledgement

NIH provided HHV6 strains and cell lines for virus propagation. The authors thank the Arkansas Bioscience Institute for 2 one-year grants in 2020 & 2021 to support this work.

Opportunities for future Collaboration

We seek human brain tissue samples for immunofluorescence staining and would like to work with a neurologist specializing in epilepsy who could provide patient blood/CSF samples to test HHV6 (HHV7).

Haven Griffin, UAMS/Little Rock
Giulia Baldini, MD, PhD, Professor, UAMS/Little Rock

Abstract Title

Cholesterol depletion changes localization of MC4R and Gs in Neuro2A cells and primary hypothalamic neurons

Other Authors and Affiliations

Sarah Sullivan and Susan Russell
UAMS, Dept. of Biochemistry and Molecular Biology, Little Rock, AR

Introduction

Control of glucose homeostasis is disrupted in diabetic patients; however, little is known about the mechanism of glucose regulation by neuronal signaling in the brain. In the central nervous system, the melanocortin system is a well-known neuronal pathway that regulates not only energy homeostasis, but also glucose homeostasis. The melanocortin 4 receptor (MC4R) expressed in cholinergic neurons is of particular interest as it is required to maintain normal levels of glucose and insulin in blood. To avoid episodes of hyperglycemia and hypoglycemia, restoration of proper MC4R signaling may be required.

Materials and Methods

Using standard confocal microscopy and airyscan imaging, we measured association and colocalization, respectively, of HA-MC4R-GFP with Gαs and clathrin at the cell surface of Neuro2A cells. To better understand the mechanism of MC4R signaling in vivo, we generated mice that express MC4R-HA and established a protocol for preparing cultures of primary hypothalamic neurons. For measuring functional changes in MC4R in primary neurons, we cultured hypothalamic neurons from mice expressing a Forster Resonance Energy Transfer (FRET)-inducible cAMP sensor under the MC4R promoter. These studies offer tools to determine whether cholesterol reduction, to the same extent as that taking place in diabetes, impairs signaling of MC4R by preventing pre-coupling of MC4R to Gs and/or localization of MC4R to clathrin.

Results and Discussions

Our preliminary data indicate that HA-MC4R-GFP associates with Gαs and clathrin at the cell surface of Neuro2A cells, and that cholesterol depletion leads to the accumulation of receptor at the plasma membrane. To confirm whether MC4R associates with Gαs at the plasma membrane of hypothalamic neurons and whether cholesterol depletion affects the distribution of MC4R, we have established a protocol for culturing primary hypothalamic neurons from MC4R-HA mice and visualizing HA protein using immunofluorescence microscopy. Using FRET microscopy, we have confirmed that MC4R-Cre x CAMPER mice express a functional cAMP sensor, which will allow us to measure changes in cAMP signaling in MC4R-Cre-expressing cells.

Conclusions

Our preliminary data suggest that MC4R and Gs are pre-coupled, and that cholesterol depletion, as occurs in the diabetic brain, leads to the accumulation of inactive MC4R at the cell surface of Neuro2A cells. We have developed methods to test whether these effects of cholesterol depletion also apply in primary hypothalamic neurons. Future studies will examine whether replenishing cholesterol and/or promoting MC4R activity by delivery of mRNA encapsulated into nanoparticles promotes signaling of MC4R through Gs to control glucose metabolism.

Acknowledgement

Provost's Innovator Award
Sturgis Award

Existing UA-UAMS Collaboration

Ryan Tian

Narasimhan Rajaram, PhD, Associate Professor, Biomedical Engineering, UAF

Abstract Title

Optical imaging strategies to determine tumor fate

Introduction

My lab is interested in studying the relationship between tumor oxygenation and metabolism and the role that this relationship plays in promoting cancer progression, metastasis, and treatment resistance. To study this relationship, we develop clinically translational quantitative optical imaging technologies that can not only enable basic science discovery in the lab but also translate these discoveries to measurable optical biomarkers in the clinic to significantly impact cancer treatment. In this talk, I will discuss some of our recent work using different optical imaging strategies to uncover key changes in the tumor microenvironment that are indicative of treatment resistance and the critical partnerships with UAMS that enable these studies.

Existing UA-UAMS Collaboration

Ongoing collaborations -
Ruud Dings and Rob Griffin - UAMS Radiation Biology
Alan J Tackett - UAMS Biochemistry and Molecular Biology
Analiz Rodriguez - UAMS Neurosurgery

Chenguang, Fan, Ph.D., Assistant Professor, ARSC/UAF

Abstract Title

Site-Specific Studies of Lysine Acetylation of Aminoacyl-tRNA Synthetases

Introduction

Aminoacyl-tRNA synthetases (AARSs) charge their cognate tRNAs with corresponding amino acids, playing key roles in ribosomal protein synthesis. A series of proteomic studies have demonstrated that AARSs have much higher levels of lysine acetylation than other proteins in Escherichia coli. But the impact of acetylation on AARSs has not been studied extensively.

Materials and Methods

To study AARS acetylation, 20 site-specifically acetylated variants of three AARSs were generated by the genetic code expansion strategy. Kinetic analyses were performed to biochemically characterize the impact of site-specific acetylation on AARS functions, including amino acid activation, tRNA aminoacylation, and editing activities.

Results and Discussions

The results showed that impacts of acetylation were different between class I and class II AARSs, and also varied among the same class of AARSs.

Conclusions

The results showed that acetylation of threonyl-tRNA synthetase (ThrRS) could affect its editing function.

Acknowledgement

NIH, ABI, UAF

Robert J Griffin, Ph.D., Professor, Dept. of Radiation Oncology, UAMS.

Abstract Title

Biocompatible Delivery of Nanomedicine Using Exosomal Membrane Coatings

Other Authors and Affiliations

Robert J Griffin[1], Hayden Carlton[2], Samir V Jenkins[1], Lauren P. Greenlee[2], David Huitink[2]
1- University of Arkansas for Medical Science
2- University of Arkansas - Fayetteville

Introduction

Nanoparticle platforms have been investigated for local tumor ablation in a variety of modalities, yet targeted particle delivery remains a challenge. A new approach to particle design with magnetic cores encapsulated in a membrane from harvested exosomes will be described based on a new collaboration between our laboratory at UAMS and two UARK laboratories in mechanical and chemical engineering.

Materials and Methods

Our approach diverges from functionalization efforts to instead enable cell-like membranes around each NP that are expected to improve the uptake and trafficking of the nanoparticle inside of cells in vitro and in vivo. This approach could transform the field of tumor oncology, using cell mimicry to enable stability in the circulation and targeted delivery to tumors. It will build on our previous studies in exosomes and cell to cell communication and help us learn more about how exosomes from varying sources and environments can modify recipient cell gene expression and induced effects on proliferation, metabolism and treatment resistance.

Results and Discussions

The research activities include nanoparticle synthesis, characterizing of the heating efficacy using magnetic of photoactivation of the novel membrane-coated particles, measurement of transport and tumor targeting in a living system, and demonstrating cancer cell killing potential.

Conclusions

We hope to establish a solid preliminary data foundation for pursuing funding from NIH or NSF via demonstration of the innovative effects of using naturally occurring membranes for increased biocompatibility.

Existing UA-UAMS Collaboration

See above.

Shilpa Iyer, Ph.D., Assistant Professor, Fulbright College of Arts and Sciences/ UAF, Fayetteville

Abstract Title

Evaluating the bioenergetics health index ratio in Leigh syndrome fibroblasts to understand disease severity

Other Authors and Affiliations

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6-Dept. of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA

Introduction

Several pediatric mitochondrial disorders, including Leigh syndrome (LS), impact mitochondrial (mt) genetics, development, and metabolism, leading to complex pathologies and energy failure. The extent to which pathogenic mtDNA variants regulate disease severity in LS is currently not well understood.

Materials and Methods

To better understand this relationship, we computed a glycolytic bioenergetics health index (BHI) for measuring mitochondrial dysfunction in LS patient fibroblast cells harboring varying percentages of pathogenic mutant mtDNA (T8993G, T9185C) exhibiting deficiency in complex V or complex I (T10158C, T12706C).

Results and Discussions

A high percentage (> 90%) of pathogenic mtDNA in cells affecting complex V and a low percentage (< 39%) of pathogenic mtDNA in cells affecting complex I was quantified. Levels of defective enzyme activities of the electron transport chain correlated with the percentage of pathogenic mtDNA. Subsequent bioenergetics assays showed cell lines relied on both OXPHOS and glycolysis for meeting energy requirements.

Conclusions

We conclude that whereas the precise mechanism of LS has not been elucidated, a multi-pronged approach taking into consideration the specific pathogenic mtDNA variant, glycolytic BHI, and the composite BHI (average ratio of oxphos to glycolysis) can aid in better understanding the factors influencing disease severity in LS.

Acknowledgement

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Shilpa Iyer, Ph.D., Assistant Professor, Fulbright College of Arts and Sciences/ UAF, Fayetteville

Abstract Title

Pluripotent stem cell models for studying Leigh syndrome

Introduction

Leigh Syndrome (LS) is a classic mitochondrial disease that has no current cure and no adequate cellular model for understanding the rapid fatality associated with the disease (1). Fatality results from excess accumulation of mutant mtDNA leading to failure of mitochondrial-bioenergetics. Other symptoms include developmental, neural, cardiac and muscle impairments. Research in my lab is focused on (a) developing novel metabolic disease biomarkers (2); (b) creating pluripotent stem cell models for studying abnormal development (c) understanding altered mitochondrial dynamics and function towards developing relevant therapies (3). In this talk, I will discuss some of our recent findings in patient fibroblasts and derived human induced pluripotent stem cells for mitochondrial disorders.

Acknowledgement

Thanks to collaborators at UARK (Kyle Quinn, Kartik Balachandran, Raj Rao, Suresh Kumar, Justin Zhan); UGA (Frank West); VA Medical Center (Ed Lesnefsky)

Ruud Dings, Ph.D., Assistant Professor, Radiation Oncology, UAMS

Abstract Title

Metabolic biomarkers of treatment resistance

Other Authors and Affiliations

Samir Jenkins - UAMS
Lisa Rebello -UA
Robert Griffin - UAMS
Narasimhan Rajaram - UA

Introduction

Treatment failure due to intrinsic or acquired resistance remains a significant problem for cancer patients. One of the major challenges is a swift detection of resistance. Namely, currently it can take up to eight (8) weeks before a tumor is deemed resistant in the clinic. So, a rapid and improved detection of treatment resistance facilitates a shift to different treatment modalities to improve clinical outcome and/or optimization of quality of life for the patient.

Materials and Methods

We generated isogenically matched resistant cell lines to optically identify metabolic biomarkers of treatment resistance, using state of the art label-free two-photon excited fluorescence microscopy.

Results and Discussions

We identified changes in the optical redox ratio of FAD/(NADH and FAD) following induced radiation resistance. We found a significant ($p = 0.01$) decrease in the optical redox ratio in the radiation-resistant cells after radiation exposure. This change in the redox ratio was indicative of increased catabolism of glucose in the resistant cells after radiation and coincided with the induction of hypoxia-inducible factor 1 alpha (HIF-1α), a key promoter of glycolytic metabolism. Moreover, this was also reflected by increased glucose uptake and reduced glutathione levels, as well as reduced reactive oxygen species (ROS) in the resistant tumor cells. However, as sometimes is the case, these changes could not be explained by changes in mitochondrial function/organization.

Conclusions

Our results demonstrate that the optical redox ratio determination by label-free two-photon fluorescence microscopy holds great clinical promise in the prognosis of treatment resistance.

Acknowledgement

Past and current lab members of the laboratories of Narasimhan Rajaram, Robert J. Griffin and Ruud P.M. Dings. Funding for presented and related research: NCI R01 CA238025, NCI R01 CA245083, Arkansas Biosciences Institute, UAMS Office of the Vice Chancellor of Research & Innovation and the Winthrop P. Rockefeller Cancer Institute. The study was also supported in part by the COBREs Arkansas Integrative Metabolic Research Center (P20GM139768) and Center for Microbial Pathogenesis and Host Inflammatory Responses (P20GM103625).

Existing UA-UAMS Collaboration

Narasimhan Rajaram (UA) – Ruud P.M. Dings (UAMS)

Opportunities for Future Collaboration

We have several validated isogenically-matched treatment resistant cell lines (immortalized normal cells and tumorigenic) against various FDA-approved and experimental therapeutics, as well as matched cell lines with or without adaption to hypoxia.

Shilpa Iyer, Ph.D., Assistant Professor, Fulbright College of Arts and Sciences/ UAF, Fayetteville

Abstract Title

Altered mitochondrial dynamics in Leigh syndrome human induced pluripotent stem cells with mitochondrial DNA mutations

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Introduction

Mitochondria are dynamic organelles undergoing constant fission and fusion dynamics to regulate several vital cellular functions. Alterations in mitochondrial dynamics has been linked to many human disorders from human cancers to mitochondrial disease. The underlying mechanism linking remodeled mitochondrial dynamics and progression of mitochondrial disorders such as Leigh syndrome (LS) is not known. LS is a fatal pediatric neurodegenerative disorder with mutations in mitochondrial and/or nuclear genome, leading to complex and variable clinical symptoms. Elucidating the role of mitochondrial dynamics in regulating abnormal development associated with LS is worthwhile, as this understanding will offer new insights into treatments for incurable disorders.

Materials and Methods

In this study, we performed a comprehensive analysis of mitochondrial morphology and function in five patient-derived human induced pluripotent stem cells (hiPSCs) containing pathogenic mtDNA mutations impacting complex I or V subunit of the electron transport chain. Using MitoTracker Red CMXRos staining and a mitochondrial network morphology analysis (MiNA) Image J tool, we quantitated different mitochondrial shapes in fluorescently labeled hiPSCs. Mitochondrial membrane potential (MMP) was also quantitated in these lines.

Results and Discussions

We observed that LS-hiPSCs exhibited a decrease in MMP and various remodeled mitochondrial shapes when compared with the control BJ-hiPSCs..

Conclusions

To our knowledge, this is the first study that links pathogenic mtDNA mutations to altered mitochondrial dynamics and function in early developmental models for LS.

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Existing UA-UAMS Collaboration

None at this time.

Opportunities for Future Collaboration

1. Jessica Snowden, Division Chief, Pediatric Infectious Disease; Vice Chair for Research, Horace C. Cabe Endowed Chair in Pediatric Infectious Disease, UAMS
2. Andrew Burrow, Clinical and Medical Biochemical Geneticist; Associate Professor of Pediatrics, UAMS
3. Elisabet Børsheim, Professor, Department of Pediatrics, Section of Developmental Nutrition, UAMS