23rd Annual Biomedical Research Training Program Symposium

KU SCHOOL OF MEDICINE

The University of Kansas

Monday, June 5, 2023

Health Education Building (HEB)

Room B102

Sponsored by:

The Office of the Executive Vice Chancellor, KUMC

TABLE OF CONTENTS

An overview of the

Biomedical Research Training Program

The KUMC Biomedical Research Training Program is in its 24th year. The program represents an outgrowth of the Wesley Foundation/Kansas Health Foundation Training Program in Cancer Research, established two decades ago. This investment led directly to increases in research productivity and external grant support and provided the impetus for our current training program.

This fiscal year, Dr. Robert Simari, the Executive Vice Chancellor, made a substantial commitment of over $200,000 to support this training program. Its purpose is to encourage a strong scientific research environment at KUMC in funded basic science and clinical research laboratories. Major goals of the program are to facilitate the development of externally funded individual pre- and postdoctoral fellowships, institutional training grants, and faculty research grants. The fourteen scholars supported this year (10 Predoctoral and 4 Postdoctoral) are actively involved in biomedical research laboratories affiliated with both basic science and clinical departments.

The continued strength of the KUMC Biomedical Research Training Program will be ensured by the active participation of our faculty and by the productivity of the scholars and their mentors' laboratories. The quality of research presented by the awardees in the Symposium bodes well for the continued success of this program.

Julie A. Christianson, PhD
Program Director

Tina Darrow
Program Administrator

EVENT SCHEDULE

Time	Award Recipient	Mentor	Department
9:00	**Opening Remarks: Julie A. Christianson**		
9:10	Benjamin A Kugler	John Thyfault	Cell Biology & Physiology
9:22	Jordan Trant	Gustavo Blanco	Cell Biology & Physiology
9:34	Paige Minchella	Warren Nothnick	Cell Biology & Physiology
9:46	Tara McQuillan	Julie Christianson	Cell Biology & Physiology
9:58	Alexander P Gabrielli	Russell Swerdlow	Neurology
10:10	Anubhav Chakraborty	Alan Yu	Kidney Institute
10:22	Leena Kader	Erin Young	Anesthesiology
10:34	**Break**		
10:50	Elidh Chowanec	Christy Hagan	Cancer Biology
11:02	Luis M Cortez	Bret Freudenthal	Cancer Biology
11:14	Rosalyn Zimmermann	Michael Washburn	Cancer Biology
11:26	Ayelen Moreno	Michael Soares	Pathology & Laboratory Medicine
11:38	Sagar Rayamajhi	Andrew Godwin	Pathology & Laboratory Medicine
11:50	Kafayat Yusuf	Shahid Umar	Radiology & Cancer Biology
12:02	**Break and Lunch**		
12:30	Keynote Speaker: Ryan Thummel, Ph.D. "Muller Glia in the Retina: when they save the day, when fail to play, and when they go astray"		
1:45	Inaugural Joan Hunt Award		
2:00	Thank you for attending the BRTP Symposium!		

FY 2023
SELECTION COMMITTEE MEMBERS

Program Director:
Julie A. Christianson, PhD
Cell Biology and Physiology

Seth Holwerda, PhD
Anesthesiology

Jeroen Roelofs, PhD
Lejla Zubcevic, PhD
Biochemistry & Molecular Biology

Prasad Dandawate, PhD
Joan Lewis-Wambi, PhD
Cancer Biology

Dale Abrahamson, PhD
Kyle Baumbauer, PhD
Lane Christenson, PhD
Julie Christianson, PhD
Lisa Harlan-Williams, PhD
William Kinsey, PhD
Colin McCoin, PhD
Irfan Saadi, PhD
John Stanford, PhD
Pamela Tran, PhD
Cell Biology & Physiology

Irina Tikhanovich, PhD
AnnWozniak, PhD
Internal Medicine

Maria Kalamvoki, PhD
Mary Markiewicz, PhD
Microbiology,
Molecular Genetics & Immunology

Adam Rouse, PhD
Neurosurgery

Sandy Billinger, PhD
Jill Morris, PhD
Neurology

Courtney Marsh, PhD
Obstetrics & Gynecology

Fariba Behbod, PhD
Pathology & Laboratory Medicine

Udayan Apte, PhD
Qi Chen, PhD
Ken McCarson, PhD
Michele Pritchard, PhD
Pharmacology, Toxicology & Therapeutics

Shawn Frost, PhD
Physical Medicine & Rehabilitation

Hao Zhu, PhD
SHP Clinical Laboratory Sciences

Jacob Sosnoff, PhD
SHP Physical Therapy, Rehabilitation Science

PRE-DOCTORAL SCHOLARS & MENTORS

Anubhav Chakraborty
Alan Yu, PhD
Kidney Institute

Elidh Chowanec
Christy Hagan, PhD
Cancer Biology

Luis M Cortez
Bret Freudenthal, PhD
Cancer Biology

Alexander P Gabrielli
Russell Swerdlow, PhD
Neurology

Leena Kader
Erin Young, PhD
Anesthesiology

Tara McQuillan
Julie Christianson, PhD
Cell Biology and Physiology

***CindyMenjivar**
Jeffrey Bose, PhD
Microbiology, Molecular Genetics & Immunology

Paige Minchella
Warren Nothnick, PhD
Cell Biology and Physiology

Jordan Trant
Gustavo Blanco, PhD
Cell Biology and Physiology

Kafayat Yusaf
Shahid Umar, PhD
Radiology and Cancer Biology

*Scholar not presenting

POST-DOCTORAL SCHOLARS & MENTORS

Benjamin A Kugler, PhD
John Thyfault, PhD
Cell Biology and Physiology

Ayelen Moreno, PhD
Michael J Soares, PhD
Pathology and Laboratory Medicine

Rayamajhi Sagar, PhD
Andrew Godwin
Pathology and Laboratory Medicine

Rosalyn Zimmermann, PhD
Michael Washburn, PhD
Cancer Biology

KEYNOTE SPEAKER

Ryan Thummel, Ph.D.

Associate Professor and Graduate Director
Department of Ophthalmology
Visual and Anatomical Sciences
Wayne State University School of Medicine
Detroit, Michigan

"Muller Glia in the Retina: when they save the day, when fail to play and when they go astray"

Ryan Thummel, PhD completed his undergraduate degree in Pre-Professional Studies and Cognitive Psychology at the University of Notre Dame. After taking a gap year to live in Italy, he returned to his home state of Kansas for his PhD in Developmental Genetics under the mentorship of Dr. Alan Godwin at KU Medical Center. During graduate school he created the first stable zebrafish line to express Cre recombinase and developed an in vivo electroporation technique to inhibit genes of interest in regenerating adult fin tissue. Adapting this technique for use in the retina, as a post-doc in David Hyde's lab at the University of Notre Dame, he definitively showed that Müller glia were the source of retinal progenitors during retinal regeneration in zebrafish. Upon completion of a short post-doc, Dr. Thummel started his own lab at Wayne State University School of Medicine in 2009. His primary research interests are retinal degeneration/regeneration and vision defects associated with hypomyelination disorders. His lab utilizes various cell biology and molecular approaches in zebrafish, mice, and human tissue as model systems. Dr. Thummel is passionate about training the next generation of young scientists, both in the classroom and in the lab. He teaches as part of the first-year medical school curriculum and has mentored 37 trainees – from all levels – in his lab since 2009. He also serves as Graduate Program Director for the MS and PhD programs in his department, module director for a NEI-sponsored P30 Core Grant, and Chair of F03A study section for the NIH.

Scholar Presentations

Benjamin A Kugler, Hepatic Mitochondrial Adaptation to Exercise: The Role of Dynamin-Related Protein 1 (Drp1)

Jordan Trant, Na,K-ATPase affinity for ouabain enhances renal cyst growth in a polycystic kidney disease mouse model.

Paige Minchella, Endometrial RE-1 Silencing transcription factor (rest) is reduced in patients with endometriosis and is associated with changes in neuronal gene expression and inflammation in a knockout mouse

Tara McQuillan, Voluntary wheel running improves hippocampal integrity, cognitive performance, and urogenital hypersensitivity in adult mice exposed to neonatal maternal separation

Alexander P Gabrielli, Primary Mitochondrial Dysfunction Promotes Apolipoprotein E Upregulation in a Neuronal Model

Anubhav Chakraborty, Heterochromatinized proximal promoter of PKD1 limits CRISPR activation of the gene in immortalized cell lines

Leena Kader, The bidirectional impact of arginine-vasopressin receptor 1a (Avpr1a/AVPR1A) and the gut microbiome on visceral hypersensitivity (VH)

Elidh Chowanec, Progesterone Promotes Immunosuppression in the Mammary Gland Through Regulatory T-cell Activity

Luis M Cortez, Structural Effects of Ribonucleotide Insertion into Telomeres

Rosalyn Zimmermann, Multicomplex Integrative Structural Modeling of a Human HDAC 1/2 Interactome

Ayelen Moreno, Invasive Trophoblast Cell-Natural Killer Cell Cooperation in the Establishment of the Hemochorial Placenta

Sagar Rayamajhi, Extracellular vesicles protein biomarkers define the preneoplastic landscape of high-grade serous ovarian cancer

Kafayat Yusuf, Investigating a novel role of DCLK1 in IBD and Colon Cancer

Cindy Menjivar, Identification of the Staphylococcus aureus fatty acid degradation system

Hepatic Mitochondrial Adaptations to Physical Activity: The Role of Dynamin-Related Protein 1 (Drp1)

Benjamin A. Kugler and John Thyfault.

1. Department of Cell Biology and Physiology, The University of Kansas Medical Center; Kansas City, KS, 66160,

Hepatic steatosis, excessive fat storage in the liver, has become the most common cause of chronic liver disease worldwide. Exercise can treat and prevent steatosis independent of weight status, which is associated with enhanced mitochondrial function (MitoFX). Further, it has been demonstrated that critical sexual dimorphic differences in hepatic MitoFX in response to diet and exercise affect the risk of developing steatosis. However, it remains unclear how exercise and sexual dimorphism alter hepatic MitoFX or if these enhancements are required to treat hepatic steatosis. Dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission, is reduced in the steatotic livers of humans and rodents, which is associated with reduced MitoFX. Interestingly, female rodents have a higher level of hepatic Drp1, enhanced MitoFX, and protection against steatosis, but with the loss of ovarian function, this protection is lost while hepatic Drp1 activity and MitoFX are reduced. However, following each bout of exercise, Drp1 activity is enhanced, followed by an upsurge in mitophagic flux. In addition, regular exercise increases hepatic Drp1 content, indicating exercise improves mitochondrial quality through the distinct interplay between Drp1-mediated mitochondrial fission and mitophagy. Similarly, the treatment of steatosis after the loss of ovarian function utilizing estradiol replacement and exercise restored Drp1 activity and MitoFX while providing protection against steatosis. Nevertheless, it remains unknown if Drp1 contributes to the enhancement of MitoFX and treatment of hepatic steatosis following exercise. My overall hypothesis is that exercise protects against diet-induced hepatic steatosis, in part, through enhanced Drp1 activity allowing for increased mitophagic flux, which removes low-functioning mitochondria, promoting the enhancement in MitoFX. I will test this overall hypothesis by investigating if hepatic Drp1-mediated mitochondrial fission is essential for chronic exercise adaptations in liver-specific homozygous Drp1 knockout mice fed a high-fat diet or following loss of ovarian function using ovariectomy.

CNa,K-ATPase affinity for ouabain enhances renal cyst growth in a polycystic kidney disease mouse model.

Presenting Author: Jordan Trant, Department of Cell Biology and Physiology
Mentor: Gustavo Blanco, MD, PhD
Contributors: Gladis Sanchez, Jeffrey P. McDermott

Renal cyst progression in autosomal dominant polycystic kidney disease (ADPKD) is dependent on agents circulating in blood. We have previously shown, using different in vitro models, that one of these agents is the hormone ouabain. By binding to Na,K-ATPase (NKA), ouabain triggers a cascade of signal transduction events which enhance ADPKD cyst progression by increasing cell proliferation, fluid secretion, and causing dedifferentiation of the renal tubular epithelial cells. Here, we determined the effects of ouabain in vivo. We show that daily application of exogenous ouabain to Pkd1RC/RC ADPKD mice for 1-5 months, with ouabain levels remaining in the physiological range, augmented kidney cyst area and cyst number compared to saline-injected controls. Also, ouabain favored renal fibrosis, but tissue damage did not significantly alter renal function as determined by blood urea nitrogen (BUN) levels. Ouabain did not have a sex preferential effect, equally affecting male and female mice. By contrast, ouabain had no significant effect on wild type mice. The actions of ouabain on Pkd1RC/RC mice were exacerbated when a second set of mutations engineered in those mice increased the affinity of Na,K-ATPase for ouabain (Pkd1RC/RCNKAα1OS/OS mice). In this case, we found both higher cyst growth and declined kidney function with and without exogenous ouabain. Altogether, these results demonstrate that ouabain stimulates kidney cyst progression in ADPKD not only in vitro, but also in vivo; that NKA, and specifically its ouabain affinity site, is critical for this effect; and that circulating ouabain is an epigenetic factor that worsens the ADPKD phenotype.

No conflicts of interest, financial or otherwise, are declared by the authors.
This research was supported in part by NIH grant DK081431 to Gustavo Blanco, MD, PhD.

CEUTOPIC ENDOMETRIAL RE-1 SILENCING TRANSCRIPTION FACTOR (REST) IS REDUCED IN PATIENTS WITH ENDOMETRIOSIS AND IS ASSOCIATED WITH CHANGES IN NEURONAL GENE EXPRESSION AND INFLAMMATION IN A KNOCKOUT MOUSE MODEL.

Paige Minchella, Amanda Graham, Julie A. Christianson, Warren B. Nothnick. Institute for Reproductive and Developmental Sciences, Department of Cell Biology and Physiology, University of Kansas Medical Center, Kansas City, KS.

Endometriosis is a gynecological disease that affects 1 in 10 individuals with a uterus and is commonly associated with pain symptomology, reducing overall quality of life and is highly variable between patients. RE-1 silencing transcription factor (Rest) is a neuronal gene modulator that takes part in regulating neuronal gene activity and inflammation. We analyzed endometrial biopsies from patients with and without endometriosis to assess protein expression of Rest (nuclear and cytoplasmic) and observed a decrease in nuclear Rest compared to cytoplasmic Rest in eutopic and ectopic endometriotic tissue, indicating lower levels of Rest transcriptional activity. To further understand the role of Rest in endometriosis, we generated a Rest conditional knockout (cKO) mouse model, using the progesterone promoter to deplete Rest in the endometrium (Restfl/flPgRCre/+). To assess pelvic sensitivity, we measured the visceromotor response (VMR) to graded vaginal balloon distention in wildtype and cKO mice at 3-, 6-, and 9- months of age. We observed a significant decrease in VMR in the cKO mice, compared to wildtypes, only at the 9-month timepoint. Uterine mRNA levels were quantitated for neuronal and inflammatory components. Neuronal targets (Brain-derived neurotrophic factor, Bdnf; Calcitonin, Calc α/ß; Nerve growth factor, Ngf) had significantly lower mRNA expression in Rest cKO at 3 months as compared to wildtype. In contrast, mRNA expression of inflammatory markers (Chromogranin A, Chga; Tumor necrosis factor, Tnfα) were significantly elevated compared to wildtype. In conclusion, reduced nuclear Rest expression in eutopic/ectopic endometrial tissue correlates with human endometriosis pathology and impacts mRNA neuronal gene expression and inflammation. Further research will be conducted to investigate the impact of Rest deficiency in an endometriosis-induction mouse model. Identification and characterization of Rest as a novel therapeutic target in endometriosis can lead to better management of the disease and improve quality of life in patients.

Voluntary wheel running improves hippocampal integrity, cognitive performance, and urogenital hypersensitivity in adult mice exposed to neonatal maternal separation

Tara E McQuillan, University of Kansas Medical Center Department of Cell Biology and Physiology

Rebecca M Foright, PhD, Jenna M Frick, PhD, Brittni M Levasseur, PhD, Aaron D Brake, MD, Olivia C Eller, PhD, Julie A Christianson, PhD

Early life stress (ELS) exposure has been correlated with pain and cognitive symptom severity in patients with urologic chronic pelvic pain syndrome (UCPPS). Neuroimaging studies in UCPPS patients revealed changes in gray matter volume, neurochemical concentration, and functional connectivity that correlate with pain symptomology. Our lab has established a mouse model of early life stress using a neonatal maternal separation (NMS) paradigm that recapitulates many of the clinical features of UCPPS patients including increased urogenital sensitivity, decreased hippocampal volume, and reduced neuronal integrity. We have previously demonstrated that exposure to acute water avoidance stress (WAS) exacerbates our NMS phenotype, while voluntary wheel running can attenuate NMS-related outcomes. We therefore hypothesize that ELS-induced changes in the hippocampus can be modified by increasing physical activity, thereby attenuating cognitive deficits and urogenital hypersensitivity, particularly after an acute exposure to WAS. Hippocampal integrity is being evaluated using magnetic resonance imaging and spectroscopy (MRI/MRS). We examined cognition with Y-maze performance. Urogenital sensitivity was evaluated as perigenital mechanical withdrawal thresholds in males and visceromotor response to urinary bladder distention in females. We observed that voluntary wheel running attenuated bladder sensitivity in NMS females, even following WAS exposure. Our preliminary data suggest that voluntary exercise increases baseline prostate mechanical withdrawal threshold in NMS male mice but does not prevent WAS-induced sensitivity. Finally, observations of y-maze performance suggest that voluntary wheel running may improve cognitive impairment in NMS males. Completion of this project will provide evidence for the effectiveness of exercise as a long-term treatment intervention for ELS-induced morbidities.

No conflicts of interest

R01 DK099611

Primary Mitochondrial Dysfunction Promotes Apolipoprotein E Upregulation in a Neuronal Model

Presenting Author: Alexander Gabrielli, Department of Cell Biology and Physiology
Mentor: Russell Swerdlow, MD
Contributors: Ian Weidling, Lesya Novikova

Alzheimer's Disease (AD) is a debilitating neurodegenerative disorder associated with progressive memory loss and declining cognitive function. Genetic and post-mortem studies implicate mitochondrial dysfunction in Alzheimer's disease (AD)-associated molecular phenomena. Apolipoprotein E (apoE) biology, especially apoE ε4, is also profoundly associated with AD risk. Our understanding of how these processes intersect remains incomplete. We previously reported that SH-SY5Y human neuroblastoma cells completely depleted of mitochondrial DNA (mtDNA) (ρ0 cells) exhibited a 65-fold increase in APOE mRNA, and a robust increase in both secreted and intracellular apoE protein. We investigated this phenomenon in the more acute setting of partial mtDNA-depletion, chemical-induced manipulations of the mitochondrial membrane potential, toxin-induced respiratory chain inhibition, and finally glucose deprivation (starvation conditions). In each instance, we found that a primary mitochondrial dysfunction induced APOE mRNA expression. We identified several transcription factors in the ρ0 cells that appear to contribute to apoE expression, notably C/EBPα and NFE2L2. Similarly, we found a role for two mitogen-activated protein kinases, JNK and ERK, in moderating SH-SY5Y ρ0 cell APOE mRNA levels. These findings have contributed to a mechanistic understanding of how primary mitochondrial dysfunction may induce apoE expression. Recent findings extend this phenomenon in an additional neuronal model – human neural progenitor cells derived from induced pluripotent stem cells. Inhibition of ATP-synthase activity in these cells increased APOE mRNA levels more than 10-fold. Experiments in differentiated human forebrain neurons are pending. Collectively, this work can inform the pursuit of therapeutics for AD that consider the mutual role of both mitochondria and apoE in contributing to pathogenesis, potentially succeeding where amyloid-targeting approaches have failed.

The authors report no conflicts of interest.

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A significant portion of the featured data was published in the Journal of Alzheimer's Disease. A full citation is listed below:
Gabrielli AP, Weidling I, Ranjan A, Wang X, Novikova L, Chowdhury SR, Menta B, Berkowicz A, Wilkins HM, Peterson KR, Swerdlow RH. Mitochondria Profoundly

Heterochromatinized proximal promoter of PKD1 limits CRISPR activation of the gene in immortalized cell lines

Anubhav Chakraborty[1,2], Christopher J. Ward[2], Alan S. Yu[2]
[1]Department of Cell Biology and Physiology, University of Kansas Medical Center, Kansas City, KS, USA
[2]Jared Grantham Kidney Institute, University of Kansas Medical Center, Kansas City, KS, USA

Autosomal dominant polycystic kidney disease is predominantly caused by loss of function mutations in PKD1 and is characterized by progressive enlargement of renal cysts that leads to a decline in renal function. Cysts form when the functional levels of polycystin 1, the protein product of PKD1, decrease below a critical threshold. To explore if increasing the expression of PKD1 using CRISPR activation (CRISPRa) could rescue the disease, we first designed and screened several guide RNAs (gRNA) targeting the proximal promoter of Pkd1/PKD1 in mouse renal cortical collecting duct (M1) and HEK293T cell lines. The maximum increase in Pkd1/PKD1 expression was 2–2.5-fold ($p<0.001$), using pooled gRNAs that directed the dCas9-VPR ribonucleoprotein to the 100 bp region upstream of the transcriptional start site. By contrast, in the positive control genes (Klf1, Nkx2, Oct4, INS, and TTN1) CRISPRa induced much greater increases in expression (5-6000-fold). Both cell lines had a low abundance of PKD1 mRNA (~10 copies/cell). Chromatin accessibility assay showed that PCR of the Pkd1/PKD1 proximal promoter in M1 and HEK293T cells was enriched less than 4-fold after nuclease digestion indicating a heterochromatinized region. Further, H3K27 acetylation and DNase hypersensitivity data from ENCODE showed that heterochromatization of the Pkd1/PKD1 proximal promoter is common to most immortalized cell lines. Our data indicate that heterochromatinized Pkd1/PKD1 proximal promoter can limit the degree to which the gene can be upregulated by CRISPRa.

The bidirectional impact of arginine-vasopressin receptor 1a (Avpr1a/ AVPR1A) and the gut microbiome on visceral hypersensitivity (VH).

Leena Kader, Adam Willits, Julie A. Christianson, Kyle Baumbauer, Jun-Ho La, Abraham Palmer, and Erin E. Young

Presenting Author: Leena Kader

Corresponding Author: Dr. Erin E. Young

Visceral hypersensitivity (VH) is commonly cited as a driver of chronic abdominal pain in disorders of gut-brain interactions (DGBI) where persistent and/or recurrent abdominal pain is a primary symptom regardless of any alterations in bowel habits. Development of VH is influenced by genetic, environmental, and gut microbial colonization factors, yet specific mechanisms that generate VH are incompletely understood. Unfortunately, current treatments primarily focus on symptom management rather than targeting physiological mechanisms responsible for generating VH. We have begun to examine the role of genetic susceptibility and microbiome response dynamics in VH development using a preclinical model of intracolonic zymosan (ZYM) administration. Preliminary data reveal differential susceptibility to ZYM-induced VH between the closely related C57BL/6NTac (Taconic) and C57BL/6J (Jackson Labs) substrains. Comparing whole genome sequencing between strains, we have identified Avpr1a, encoding the arginine-vasopressin receptor 1A (AVPR1A) protein, as our highest priority VH candidate gene based on a differential SNP within the 5' flanking sequence. We have subsequently identified alterations in Avpr1a expression as well as enteric neuron responsiveness that covary with VH. Dynamic strain differences in the location and composition of the gut microbiome correspond to increased enteric neuronal responses to fecal supernatants in VH susceptible mice, implicating these neurons in the interaction between genetic risk and the microbiome. We will present data on the efficacy of targeting Avpr1a overexpression using specific-antisense oligonucleotide (ASO) and on the role of the microbiome in risk for developing VH independently and through regulation of Avpr1a in host tissues.

Progesterone Promotes Immunosuppression in the Mammary Gland Through Regulatory T-cell Activity

Elidh Chowanec

Estrogen and progesterone signaling together drive hormone-receptor positive breast cancer growth and progression. However, studies on the independent role that progesterone and its receptor, the progesterone receptor (PR), play in breast cancer development are limited. Our lab has shown that progesterone signaling promotes mammary tumor growth and changes the immune landscape of the mammary gland towards immunosuppression. Regulatory T-cells (Tregs) are immune cells known to promote immunosuppression in the tumor microenvironment. The data from our lab show that progesterone increases the number of Tregs in the normal mammary gland. To determine if progesterone signaling increases the number of Tregs in the tumor-bearing mammary gland, we used a syngeneic murine tumor model, E0771 cells, engineered to express mouse PR (E0771-PR) or control empty vector (E0771-vec). Mice were treated with progesterone or placebo for 7 days before implantation of the modified E0771 cells. Results from this study show that progesterone promoted tumor growth, as well as changed the immune landscape of the tumor. Flow cytometry analysis showed that PR+ tumors treated with progesterone harbored more Tregs than placebo treated mice. As progesterone treatment promoted tumor growth and Treg recruitment in PR+ tumors, these data imply that progesterone treatment promotes the release of secreted factors impacting the immune cells in the mammary microenvironment. To determine if progesterone treatment results in cytokine changes that may affect Treg recruitment, enzyme-linked immunosorbent assays (ELISAs) were performed using cell culture supernatant from E0771-PR cells treated with progesterone (0-72 hours). E0771-PR cells treated with progesterone showed higher levels of cytokines linked to Treg recruitment and differentiation, such as M-CSF. Our findings demonstrate that progesterone signaling promotes an immunosuppressed microenvironment in the mammary gland through increased Tregs, providing rationale to investigate the use of anti-progestins as a mechanism to overcome the immune-cold tumor microenvironment seen in PR+ breast tumors.

Structural Effects of Ribonucleotide Insertion into Telomeres

Luis M Cortez

Telomeres are protective noncoding DNA caps at the ends of chromosomes that maintain genomic integrity. In most somatic tissues telomeres will shorten with successive rounds of replication and will reach a critically short length, at which point a cell will become senescent or undergo apoptosis. This fate can be avoided if telomeres are maintained through expression of telomerase, a reverse transcriptase that elongates telomeres by adding telomeric repeats. This elongation of telomeres can lead to replicative immortality, one of the hallmarks of cancer. Another hallmark of cancer is genomic instability that arises from DNA damage. The most prevalent form of DNA damage are ribonucleotides (rNTPs) inserted during DNA replication. Left unrepaired these rNTPs will promote genomic instability, changes to the DNA secondary structure, and human diseases. Given the deleterious effects of rNTPs in DNA, cells have evolved the ribonucleotide excision repair (RER) pathway to remove rNTPs. While the impact of rNTPs in the genome is well established to have deleterious effects and promote human disease, it is not known what effect rNTPs will have on telomeric structure and integrity, or how rNTPs at telomeres are repaired to preserve telomeres. One essential DNA secondary structure seen at telomeres is a G-quadruplex (G4). Given the effect that rNTPs have on DNA secondary structure, we examined the effect of rNTP insertion into telomeric sequences on G4 formation and stability. Using circular dichroism spectrophotometry we show that rNTP insertion can alter G4 conformation and can increase or decrease stability depending on the location within the G4. Additionally, we demonstrate that the ability to repair rNTPs depends on the location within the G4.

Multicomplex Integrative Structural Modeling of a Human HDAC 1/2 Interactome

AUTHORS: Rosalyn C. Zimmermann[1*],Kartik Majila[2*], Cassandra Kempf[3*],Charles A.S. Banks[3], Mark K. Adams[3], Sayem Miah[3,5], Janet L. Thornton[1],Mihaela Sardiu[3,5], Laurence Florens[3], Shruthi Viswanath[2], Michael P. Washburn[1]

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ABSTRACT: Histone deacetylases (HDACs) 1 and 2 are Class I HDACs that are members of several transcriptional regulatory complexes including coREST, MIER, NuRD, and SIN3. As such, they are involved in the regulation of many developmental and cellular processes, and both are implicated in most cancers. Although each is members within these complexes, they also are known to interact with a number of proteins outside of the complexes. Our studies utilize crosslinking mass spectrometry (XL-MS) to identify unknown interactors of HDACs 1 and 2. Additionally we seek to further characterize HDAC1 within coREST, NuRD, and SIN3 complexes with integrative structure modeling. Lastly, we leveraged XL-MS to identify a novel MIER complex member (MHAP1) and develop a working model for how HDAC1:MIER1:MHAP1 may interact within the cell.

Invasive Trophoblast Cell-Natural Killer Cell Cooperation in the Establishment of the Hemochorial Placenta

Ayelen Moreno-Irusta[1], Olha Krichevskiy[1,2], Esteban M. Dominguez[1], Khursheed Iqbal[1], Regan L. Scott[1], Marc Parrish[1,2], and Michael J. Soares[1,2,3]

1Institute for Reproductive and Developmental Sciences, Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS 66160,2Department of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS 66160,3Center for Perinatal Research, Children's Mercy Research Institute, Children's Mercy, Kansas City, MO 64108.

Natural killer (NK) cells and invasive trophoblast cells have roles in orchestrating the transformation of the uterus, including uterine spiral artery remodeling during pregnancy. In this research, we explore roles for NK cells and invasive trophoblast cells in transformation of the uterine-placental interface and adaptations that occur in their absence. We used an interleukin 15 (Il15) null rat as a model of NK cell deficiency and a placenta specific 1 (Plac1) mutant rat as a model of invasive trophoblast cell deficiency. Pregnancies with homogenous placental genotypes/phenotypes were analyzed. We used four different breeding combinations: 1) wild type (WT) male x WT female; 2) WT male x Il15-/- female; 3) WT male x Plac1-/- female; 4) WT male x Plac1-/-, Il15-/- female. Pregnancies were examined on gestation day (gd) 7.5 and 18.5. Viability and litter size did not differ among the breeding combinations at gd 7.5. At gd 18.5, double-deficient pregnancies exhibited placentomegaly, significant decrease in fetal survival and spiral artery remodeling failure. Postnatal survival was also negatively affected in offspring. In summary, a "single hit" affecting either NK cells or invasive trophoblast cells affected placental site development; however, a "double hit" affecting both invasive trophoblast cells and NK cells was not limited to the structure of the placentation site but extended to impacts on placental and fetal development and maternal survival. These in vivo findings highlight the plasticity associated with hemochorial placentation. [Supported by KUMC Biomedical Training Program (A.M.-I.), Kansas Idea Network of Biomedical Research Excellence, P20 GM103418 (A.M.-I.), Lalor Foundation postdoctoral fellowships to A.M-I. and EMD, NIH HD020676 and HD099638, HD105734, and the Sosland Foundation.]

Extracellular vesicles protein biomarkers define the preneoplastic landscape of high-grade serous ovarian cancer

Extracellular vesicles (EVs) are considered a new class of liquid biopsy biomarkers. EVs are secreted by cells as a form of intercellular communication. These EVs can shuttle nucleic acids, lipids, and proteins from their cell of origin to surrounding cells to regulate the function of the recipient cells. A subtype of EVs, termed exosomes or small EVs (50-200 nm), are of endocytic in origin. sEVs contain a cargo of selectively sorted biomolecules that can mirror the physiological status of the cells of origin. Studies have shown that the most common and fatal form of epithelial ovarian cancer, high-grade serous ovarian cancer (HGSOC), originates from secretory cells in the fimbriated end of the fallopian tube epithelium (FTE). Thus, we hypothesized that FTE-derived sEVs are a new type of biomarkers to monitor early preneoplastic changes. First, we utilized a microfluidic platform to culture human FTE explant to 1) establish the proteome of FTE-sEVs and 2) determine FTE transcripts expressed using digital spatial profiling (DSP). Mass spectrometry-based proteomic profiling reported 295 common FTE sEV proteins. DSP of FTE explants identified 1,480 cancer-associated transcripts. Comparison of FTE transcriptomics and FTE exo-proteomics revealed 61 cancer-associated transcripts encodes for protein sorted in FTE sEVs. We next treated FTE with HGSOC-derived sEVs to study early changes in the preneoplastic landscape. 74 differentially expressed genes (DEGs) were observed following the sEV insult, among which S100A9 (S100 calcium-binding proteinA9), LTF (lactotransferrin), ENO1 (enolase1), ALDOA (aldolase), HSPA2 (heat-shock protein), and TPI1 (triosephosphate isomerase) encode for exo-proteins. As transcript change can result in the change of protein quantity being sorted in sEVs, the exo-protein encoded by these DEGs can be used as potential biomarkers indicative of the disease status. Our studies have established the baseline proteomic profile of FTE-derived sEVs with the identification of candidate exo-proteins in the development of the preneoplastic landscape of HGSOC.

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Investigating a novel role of DCLK1 in IBD and Colon Cancer

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The neoplastic effects of chronic intestinal inflammation in patients with inflammatory bowel disease (IBD) significantly increase their risk of colorectal cancer (CRC). Even with the increased global incidence of IBD, the exact causes of chronic inflammation in patients with IBD and the critical driver of the conversion from IBD to colon cancer remain poorly understood. Tuft cells are a group of unique intestinal epithelial cells that serve a protective role in the gut by sensing and eliminating gut pathogenic infection through various mechanisms. These tuft cells exhibit robust expression of Doublecortin-like kinase 1 (DCLK1), a protein with two major isoforms: DCLK1-L and DCLK1-S. Previous studies have shown that hypermethylation of DCLK-1 promoter encoding DCLK1-L, particularly in colorectal cancer, allows switching to the DCLK1-S isoform that confers an invasive tumor phenotype. Studies from our lab have identified a marked elevation of DCLK1-S in tissue samples of IBD patients. We also recently discovered that DCLK1-S is predominantly expressed in Ly6G+;MHCII- neutrophils, which coincide with elevated levels of inflammation and tissue damage in the colon. In ongoing studies using powerful Cytometry by time of flight, or CyTOF, we have discovered a novel interaction between DCLK1 and MMP13 on the surface and interior of colonic crypt cells. These DCLK1-MMP13 interactions were subsequently confirmed in an unbiased docking study that correlated with significant co-localization of DCLK1-S with MMP13 in the colons of DCLK1-short+19-floxed;Villin-Cre (fl/+) mice when subjected to DSS-induced colitis. We have also confirmed the interaction between DCLK1-S and MMP13 in different colon cancer cell lines. Matrix metalloproteinases (MMPs) perform many functions, including injury and repair, inflammation modulation, and deposition and breakdown of extracellular matrix (ECM). The potential interaction between DCLK1-S and MMP13 suggests a plausible mechanism of colitis progression to colon cancer through ECM remodeling and tissue damage.

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Identification of the Staphylococcus aureus fatty acid degradation system

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Staphylococcus aureus is a ubiquitous pathogen that can infect any anatomical site. Its success can be attributed to its plethora of virulence factors and metabolic diversity. S. aureus can utilize exogenous fatty acids for phospholipid synthesis through the fatty acid kinase complex but other fates for these fatty acids have not been shown. It is well thought that S. aureus does not have a fatty acid degradation (Fad) system, however, during an RNAseq analysis of a fakA mutant, we discovered an operon with the highest fold change to be the fad operon. To test the functionality of the S. aureus fad (safad) genes, we performed complementation assays with E. coli fad mutants using minimal media. We were able to restore growth of E. coli fadA and fadB mutants when providing safadBA genes on a plasmid with a variety of fatty acids. Since the operon is uncharacterized, we analyzed its genetic composition. We identified the translational start site for the first protein, FadX, which differs from the current annotation. We also sought to determine if all genes are co-transcribed as suggested by our RNAseq data. In addition to the upstream promoter, there are two >100 bp intergenic regions between genes that could indicated additional promoters. We generated -galactosidase reporters for the two intergenic regions and one for the upstream of fadX. We only saw activity for the fadX promoter suggesting that there is a fadXDEBA operon. This is supported by reverse transcriptase-PCR with a wild-type, fakA mutant, and a fad strain. These studies strongly support our hypothesis that S. aureus possess a functional fatty acid degradation system.

Dr. Bose serves on the Scientific Advisory Board and as a consultant for Azitra, Inc

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