GSBSE
Graduate School of Biomedical Science and Engineering

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ANNUAL MEETING

SEPTEMBER 20, 2024

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8:00am – 9:00am **Registration & Breakfast**	Friday, September 20, 2024 Wells Conference Center poster set up - students (room 2)	Room 1
9:00am – 9:15am **Welcoming Remarks and event emcee**	**Greg Cox, Ph.D.** GSBSE Program Director *or via zoom by request	Room 1*
9:15am – 9:30am **New Student Introductions**	James Feduccia Lindsey Howland Marisa Johnson Anna Kelly Affan Shaikh Sheikh Fareya Rahman Meena Ramanathan Sam Reynolds Will Sampson Savannah Wakita Michelle Wiese Emma Yvanovich *or via zoom by request	Room 1*
New Faculty Introductions	Emily Spaulding, Ph.D. (MDIBL) Zhao Xuan, Ph.D. (UM) Eva Rose Balog, Ph.D. (UNE) Samantha Barrick (UM) Scott Wood (UNE) *or via zoom by request	Room 1*
9:30am – 10:30am **Oral Presentations**	Selected senior students: (Talks will be judged by faculty) 6 x 10 min Ahmed Almaghasilah Mary Astumian Amanda Ignacz Kodey Silknitter Michael Babcock Michayla Moore	Room 1
10:30am – 11:30am **Poster Presentations**	Session 1 (list students) Abraham Fadahunsi Ahmed Almaghasilah Amanda Ignacz Andrew Ouellette Arad Bustan Courtney Willey Felix Anim Gabie Johnson Hannah Megathlin Hilda Frempong	Room 2

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	Audrie Langlais Becca Peters Brianna Gurdon Carolina Cora Caryl Young Kodey Silknitter John Butts Jordan Miner Josh Hamilton Kehinde Abayomi Mary Astumian	
11:30am – 12:30pm **Lunch**	**Lunch** (set up in the atrium) Potato bar, garden salad w/ various toppings for both options, drinks, dessert	Room 1
12:00pm – 12:30pm	**Steering Committee Working Lunch**	Room 3
12:30pm – 1:30pm **Keynote**	**Aileen Huang-Saad, Ph.D., MBA** Roux Institute	Room 1
1:30pm – 2:30pm **Oral Presentations**	Selected senior students (Talks will be judged by faculty) 6 x 10 min Audrie Langlais Rebecca Peters Madeleine Nowak John Butts Andrew Ouellette Megan Tomasch	Room 1
2:30pm – 3:30pm **Poster Presentations**	Session 2 (list students) Kehinde Adeniran Liza White Logan Douglas Lola Holcomb Madeleine Nowak Madison Mueth Megan Steele Megan Tomasch Cory Diemler Hang Chen Michael Babcock Michayla Moore Olaleye Olajuyin Omodasola Adekeye Rajat Rai Remi Geohegan Shawn David Sophie Craig Tiyasha Banerjee Zach Applebee	Room 2
3:30pm – 3:45pm **Awards for Oral**	**Greg Cox, Ph.D.** GSBSE Program Director	Room 1

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Presentations		
3:45pm – 4:30pm **Meetings**	**Faculty Meeting** *or via zoom by request	Room 3*
	Student Meeting	Room 1
4:30pm – 5:00pm **Networking Social**	**Afternoon Refreshments** Popcorn bar with toppings, coffee, tea	Room 1

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Keynote Presentation

Dr. Aileen Huang-Saad

Associate Professor, Bioengineering
Director of Life Science and Engineering Programs, Roux Institute

Education

PhD, Biomedical Engineering, Johns Hopkins University School of Medicine
MBA, Ross School of Business, University of Michigan
BSE, Bioengineering, University of Pennsylvania

Brief Biography

Aileen Huang-Saad is the Director of Life Sciences, Health, and Engineering Programs at Northeastern's Roux Institute (Portland, Maine) and an Associate Professor of Bioengineering. Leveraging her experience in design, entrepreneurship, education research, and industry, she seeks to close the gap between higher education and professional practice. Specifically, she is designing interdisciplinary experiential education life sciences, health, and engineering programs to attract, retain, and advance talent in the state of Maine. These programs are designed to re-imagine how higher education and communities can work together to impact economic and talent development.

Dr. Huang-Saad most recently came from University of Michigan where she had a fourteen-year history of bringing about organizational change in higher education, leveraging evidence-based practices at the University of Michigan. She created the U-M BME graduate design program, co-founded the U-M College of Engineering Center for Entrepreneurship, launched the U-M National Science Foundation (NSF) I-Corps Node, and developed the U-M BME Instructional Incubator. She is a canonical instructor for both the NSF and National Institute of Health (NIH) I-Corps Programs. Dr. Huang-Saad has received numerous awards for her teaching and student advising, including the 1938E College of Engineering Award, the Thomas M. Sawyer, Jr. Teaching Award, the U-M ASEE Outstanding Professor Award, the International Teaching with Sakai Innovation Award, and the College of Engineering Outstanding Student Advisor Award.

Prior to entering higher education, Dr. Huang-Saad worked in industry gaining experience in new venture biotech, the defense industry, and medical device testing. Dr. Huang-Saad currently serves as Deputy Editor-in-Chief of Springer's *Biomedical Engineering Education* and is a member of the Maine State Workforce Board. She is also an American Institute for Medical and Biological Engineering Fellow.

New students ~ AY24/25

James Feduccia
Biomedical Science (PhD)
Fall 2024
Open Rotation:
Dustin Updike
MDIBL

Education: Bachelor's degree in Biochemistry

Research Interests: Aging, Neuroscience, Biochemistry, and Molecular Biology

Career Goals: My current goals are to become a professor and scientific communicator.

Marisa Johnson
Biomedical Engineering (PhD)
Fall 2024
Open Rotation:
Caitlin Howell
UMaine

Education: Bachelor of Science in Bioengineering

Research Interests: Biomedical Engineering and Cancer Research

Career Goals: To be a professor.

Anna Kelly
Biomedical Engineering (PhD)
Fall 2024
Open Rotation:
Ling Cao
UNE

Education: BS, Worcester Polytechnic Institute

Research Interests: Pain Studies

Career Goals: To be a Professor and continue research on chronic pain

Sheikh Rahman
Biomedical Engineering (PhD)
Fall 2024
Open Rotation:
Karissa Tilbury
UMaine

Education: M.Sc. in Optics & Photonics, Karlsruhe Institute of Technology (KIT), Germany ; B.Sc. in Electrical & Electronic Engineering, University of Dhaka, Bangladesh

Research Interests: Biomedical Optics and Imaging Microscopy

Career Goals: My career goal is to become a researcher and professor in the field of biomedical engineering

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Muthumeena Ramanathan
Biomedical Science (PhD)
Fall 2024
Open Rotation:
Philip West
JAX

Education: B.Sc. (Research) in Biotechnology

Research Interests: I'm interested in inflammation and oncology. I am also keenly interested in developmental biology and wish to study how tissues precisely regulate regeneration and its cessation. The complexities of aging also intrigue me.

Career Goals: I aim to contribute to biomedical science research and improve our understanding of fundamental biological processes and translate these findings to enhance human health.

Samuel Reynolds
Biomedical Science (PhD)
Fall 2024
Direct Admit:
Ian Meng
UNE

Education: B.Sc. Biology, University of Maine

Research Interests: Neuroscience: integrative physiology and behavior

Career Goals: Academic scientist

Will Sampson
Biomedical Science (PhD)
Fall 2024
Open Rotation:
Katherine Motyl
MHIR

Education: B.Sc. Biology, University of Maine

Research Interests:
Neuroscience: integrative physiology and behavior

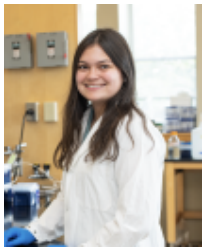
Career Goals: Academic scientist

Affan Mohammed Shaikh
Biomedical Science (PhD)
Fall 2024
JAX Track R1:
James Godwin

Education:B.S in Pharmaceutical Sciences

Research Interests: Developmental biology, Regeneration, Stem cell Biology

Career Goals: Academia, But partly leaning towards industry



Savannah Wakita
Biomedical Science (PhD)
Fall 2024
Direct Admit:
Eva Rose Balog
UNE

Education: B.Sc. Biochemistry

Research Interests: Protein biochemistry, biochemical pathways/cell signaling

Career Goals: Develop novel molecular tools using proteins as a foundation.

Michelle Wiese
Biomedical Engineering (PhD)
Fall 2024
Direct Admit:
Giovanna Guidoboni
UMaine

Education: BS in Biomedical Engineering from Florida International University

Research Interests: Mathematical and computational modeling of organs and organ systems and neural function

Career Goals: To build computational models of organ systems, and eventually teach others how to build computational models.

Emma Yvanovich
Biomedical Science (PhD)
Fall 2024
Open Rotation: Lucas Chang
JAX

Education: BS Biology, BA Psychology

Research Interests: Hematopoiesis, stem cells, translational medicine

Career Goals: Teach as an undergrad biology professor

Poster Abstracts

01

Kehinde Abayomi
Biomedical Science (PhD)
Fall 2023
MDIBL

The role of saeg-2 as a target for transgenerational epigenetic inheritance in lotr-1 mutants in C. elegans

Kehinde Abayomi 1,2; Rhiannon Lewis 1; Noah Lind 1; Catherine Sharp 1; and Dustin Updike 1,2

1 Kathryn W. Davis Center for Regenerative Biology and Medicine; MDI Biological Laboratory, 2 University of Maine, Graduate School of Biomedical Science and Engineering

The Caenorhabditis elegans germline relies on specialized cytoplasmic structures called germ granules to regulate posttranscriptional gene expression and ensure that a memory of germline-licensed expression is maintained across generations. A sub-component of germ granules, called Z granules, is central to the transgenerational epigenetic inheritance (TEI) of small RNA regulated expression. Core Z-granule proteins include the helicase ZNFX-1 and a LOTUS and Tudor domain-containing protein that we named (LOTR-1). LOTR-1 appears to be critical for TEI, and lotr-1 mutants become progressively sterile with each generation. Homologs of LOTR-1 in other species, such as TDRD5 and TDRD7 in mammals, Tejas and Tapas in Drosophila, are required for normal spermatogenesis as well as proper germ granule formation and piRNA silencing of transposons in the germline, respectively. We performed total mRNA and polysome mRNA-Seq, comparing wild-type strains to strains harboring one of three precision-edited alleles of lotr-1. From this, we discovered that lotr-1 mutants consistently and significantly upregulate mRNA encoding SAEG-2, and that this correlates with the loss of 22G small RNAs (secondary piRNAs) targeting saeg-2. SAEG-2 is an ortholog of the mammalian DNTTIP1, a terminal deoxynucleotidyl transferase. SAEG-2 has been implicated in the TEI regulation of small RNA pathways in C. elegans that transmit a memory of egg-laying, foraging and chemotaxis behaviors from parent to offspring. We are currently investigating the role of LOTR-1 and Z granules in the TEI of saeg-2 expression. We are also using an Oxford Nanopore-based Nano3P tail-Seq approach to look for differences in 3'UTR tailing in lotr-1 mutants, that could be attributed to interactions between the LOTUS domain of LOTR-1 and 3'UTR cleavage stimulation factors. These approaches will deepen our understanding of the molecular mechanisms by which LOTR-1 influences saeg-2 regulation, potentially uncovering novel pathways involved in transgenerational epigenetic inheritance in C. elegans.

02

Omodasola Adekeye
Biomedical Science (PhD)
Summer 2021
MDIBL

Protein Tyrosine Phosphatase Receptor Type Q (Ptprq) is involved in maintaining glomerular filtration barrier integrity.

Omodasola Adekeye 1,2; Daemon Dikeman 1; Ritu Tomar 3; Will Sampson 1,2; Iain A. Drummond 2

1 The University of Maine, USA; 2 Mount Desert Island Biological Laboratory, Bar Harbor, Maine, USA; 3 Nephrology Division, Department of Medicine, Massachusetts General Hospital, MA.

The podocyte is the key unit of the kidney glomerular filtration barrier comprised of interdigitating foot processes, bridged by slit diaphragms (SD). Dysfunction of the podocyte is a major cause of proteinuria and a leading cause of end-stage kidney disease. Using a transcriptomic approach to discovering novel genes important for podocyte development, we identified protein tyrosine phosphatase receptor type Q (ptprq) to be highly enriched in the developing pronephric glomeruli of zebrafish. Ptprq is a receptor tyrosine phosphatase that dephosphorylates phosphatidylinositol (3,4,5)-triphosphate(PIP3) to phosphatidylinositol (4,5)-biphosphate(PIP2). This process induces and regulates PIP2-dependent signaling, promoting the binding of receptors to the plasma membrane, which is essential for SD formation. However, the relevance of Ptprq in the formation and regulation of podocyte structure and function remains

unknown. We hypothesize that Ptprq regulates the proper interdigitation of the podocyte foot processes and podocyte morphogenesis. To better understand the morphogenesis of podocytes, we studied the function of Ptprq in podocytes. Here we confirmed that zebrafish larvae glomeruli express ptprq. Whole-mount in situ hybridization and immunohistochemistry demonstrated that ptprq is expressed in the zebrafish larval glomerulus and Ptprq protein localizes to the membrane of the podocytes, colocalizing with ZO-1, a podocyte SD marker. Additionally, we used the zebrafish CRISPR G0 screen to test Ptprq function in glomerular development. Our CRISPR efficiency was validated using primary kidney failure. Permeability analysis of the glomerular filtration barrier of this zebrafish crisprant fluorescent polymerase chain reaction (PCR) and fragment analysis. Knockout of ptprq in zebrafish larvae led to whole-body edema, a phenotype associated with showed a disruption of the selective glomerular permeability filter resulting in proteinuria. In conclusion, these data demonstrate that Ptprq promotes the normal function of the podocyte, suggesting that Ptprq may play a crucial role in the development of the podocyte. Our research would identify novel causes of genetic glomerular disease and help inform further analysis of human kidney disease.

03

Kehinde Adeniran
Biomedical Science (PhD)
Fall 2023
JAX

Prioritizing Non-Coding Variants Associated with Type 2 Diabetes using Gene Co-expression Networks

K.R. ADENIRAN 1,2; N. NERURKAR 2,3; M. KOTHARI4; R. BHUIYAN4,5; S. KALES 2; J.C. ULIRSCH 6,7; H. DEWEY 2,3; R. KURSAWE 4; D. BERENZY2; H.K. FINUCANE 6,7,8; M. STITZEL 4,5,9; R. TEWHEY 1,2,3.

1 Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, USA; 2The Jackson Laboratory, Bar Harbor, ME, USA; 3 Graduate School of Biomedical Sciences, Tufts University School of Medicine, Boston, MA, USA; 4The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA; 5 Department of Genetics and Genome Sciences, University of Connecticut, Farmington, CT, USA; 6 Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 7 Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 8Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA; 9 Institute for Systems Genomics, University of Connecticut Health Center, Farmington, CT, USA

Type 2 diabetes (T2D) is a complex disease influenced by both environmental and genetic factors. Genome-wide association studies (GWAS) have identified over 500 loci associated with T2D, with 95% of variants linked to these loci residing in non-coding regions. Variants that overlap cis-regulatory elements (CREs)-which are non-coding regions of the genome that directly regulate transcription of nearby genes-present a significant challenge for functional validation. Due to the indirect effect these variants exert through CREs, it is difficult to infer their biological impact and role in contributing to T2D complexity.

High-throughput techniques, such as massively parallel reporter assays (MPRA), provide insights into the potential of non-coding variants to modulate gene expression. We recently screened 6109 T2D-associated variants with MPRA across 7 different cell types and identified 1756 expression-modulating variants (emVars), which showed a significant difference in gene expression compared to the non-risk allele across all 7 cell types. These emVars were overlapped with T2D-relevant CREs, narrowing the list to 277 high-confidence functional variants. However, while MPRA can narrow the pool of potential causal variants, it does not reveal how these variants modulate genomic pathways relevant to T2D. This limits our ability to determine the mechanism through which variants contribute to T2D pathogenesis.

To address this challenge, we explored Weighted Gene Co-expression Network Analysis (WGCNA) to prioritize variants for in-vitro functional validation. WGCNA groups genes into co-expression modules that may indicate shared biological pathways or functions and can be explored to understand how emVars might disrupt gene regulatory networks in T2D. We assigned emVars to genes on closest genomic proximity and

mapped them to the network, identifying their modules and the extent of their connections within and across modules.

We hypothesize that emVars associated with highly connected genes within the network are likely to have a greater impact on modulating the co-expression structure. This high connectivity suggests functional relationships, co-regulation, and shared pathways among these genes, providing insights into the complexity of the T2D expression network. We plan to use this information to better understand gene regulation and guide the design of in vivo experiments aimed at functionally validating how non-coding variants influence gene regulation.

04

Ahmed Almaghasilah
Biomedical Engineering (PhD)
Summer 2019
UMaine

DPM3 is involved in causing heart failure

Almaghasilah 1; A. Ignacz 1; C. Henry 2.

1 Graduate School of Biomedical Science and Engineering, University of Maine; 2School of Biology and Ecology, University of Maine

A mutation in the dolichyl-phosphate mannosyltransferase subunit 3 (DPM3) gene causes an incurable, life-threatening muscular dystrophy characterized progressive weakness of the skeletal muscle. DPM3 is one of 18 known genes linked to dystroglycanopathies, a group of disorders caused by abnormal glycosylation of dystroglycan protein. Although mutations in DPM3 can lead to either congenital muscular dystrophy or dystroglycanopathies, it is crucial to distinguish between the effects of disrupting dystroglycan glycosylation and disruption of other glycosylated proteins necessary for muscle cell adhesion. Despite this importance, studies on DPM3 are very limited. Aside from the fact that there are few well-established animal models of dystroglycanopathies, those that do exist almost always exhibit a severe muscular dystrophies and/or a shortened lifespan. This limitation hampers further research on dystroglycanopathies. To address this, we generated a dpm3 zebrafish line with 11 base pair deletion using CRISPR-Cas9. Our preliminary results show that dpm3 mutants exhibit mild dystrophy and have a lifespan between 2 to 3 weeks. The confocal phalloidin images of dpm3 mutants revealed that not all mutants exhibited visible dystrophies. Their motility was comparable to their siblings, providing no clear indication of motor dysfunction that might have impaired their ability to fetch the food, potentially leading to death by starvation. While the light-sheet images showed mild dystrophies in the jaw muscle, the mutant displayed normal gastrointestinal motility, indicating that they were digesting food. They also exhibited similar muscle regeneration capacity as their siblings. However, we observed that the heart beats of the mutants decreased steadily over a 9-day period. Additionally, they had smaller somite widths than their siblings. Taken all together, we believe that DPM3 is involved in contributing to heart failures which is rare in patients with dystroglycanopathies. We also hypothesize that involved in contributing to heart failures which is rare in patients with dystroglycanopathies. We also hypothesize that the muscle growth defects are due to impaired hyperplasia and/or hypertrophy.

05

Felix Gershon Anim
Biomedical Science (PhD)
Fall 2022
JAX

Characterizing The Wasted Mouse As A Motor Neuron Disease Model

F.G. ANIM 1,2; J. LI 1,3; R.W. BURGESS 1,2,3.

1 GSBSE, University of Maine; 2 The Jackson Laboratory; 3 Tuft Genetics Program

Translation defects and tRNA modification in motor neurons contribute greatly to neuromuscular disorders due to defective protein synthesis and cell stress response. The translation elongation factor 1A, eEF1A, has two independently encoded isoforms, eEF1A1 and eEF1A2. The well-conserved developmental switch to eEF1A2 expression in muscles and neurons suggests

there may be significant functional differences. In wasted mice (wst/wst), the gene encoding eEF1A2 has a spontaneous 15.8 kb deletion affecting the first exon and all promoter regions. Homozygous mice have neuromuscular abnormalities and survive about 5 weeks. These mice present a valuable opportunity to study early-onset motor neuron disease, given the early onset and aggressive nature of the phenotypic abnormalities. We have confirmed eEF1A2 expression and persistence of eEF1A1 expression in alpha motor neurons beyond post-natal development. The phenotype suggests a critical role of eEF1A2 in motor neurons as the primary defect in the wst/wst mice. We therefore aim to characterize the wst/wst mice in the context of motor neuron disease. Measurement of clinically relevant outcomes employing electromyography, wire hang test, neuromuscular junction innervation, histology of femoral nerves, spinal cord and brain was carried out on 23day old wst/wst and wildtype littermate controls. Gene expression analysis in the spinal cord of wst/wst and wildtype littermate controls was also carried out. Consistent with previous characterizations, the wst/wst mice at about 3 weeks have defects suggestive of neuromuscular abnormalities. Data from electromyography, behavioral tests of motor performance, histopathology of nerves and spinal cord, and neuromuscular junction innervation are currently being quantified. We are also analyzing gene expression data to identify dysregulated pathways in the wst/wst mice. Results gathered will inform the relevance of the wst/wst mouse as a model for studying early onset motor neuron disease and confirm the switch from eEF1A1 to eEF1A2 contributes to the cell-type specificity observed. We will also be able to determine if the integrated stress response resulting from amino acid deficiency and ribosome stalling is contributing to the neuromuscular phenotype.

06

Zach Applebee
Biomedical Engineering (PhD)
Fall 2022
UMaine

Bacterial species respond differently to oil saturation levels in liquid-infused polymers

C. FONG 1,2; E. LEONARD1,2; ***Z. APPLEBEE** 1,2; C. HOWELL1,2.

1 Department of Chemical and Biomedical Engineering, University of Maine; 2 Graduate School of Biomedical Science and Engineering, University of Maine

Liquid-infused polymers are of great interest in biomedical applications due to their unique antifouling properties. Currently, it is believed that the primary mechanism of action is the presence of a free-flowing, continuous liquid surface layer to which contaminants cannot attach. However, recent studies have shown that partially infused polymers which have limited or no liquid layer on the surface can also effectively repel foulants, indicating that we do not fully understand the dynamic mechanisms behind the liquid-infused system. This study investigates infusing silicone polymers with silicone oil to various levels of saturation, incubating them with Escherichia coli, Enterococcus faecalis, and Pseudomonas aeruginosa, and measuring the bacterial colonization and adhesion. Our findings revealed that after 48h of infusion, cells of E. coli showed a low degree of surface colonization overall, with a significant decrease only at 90% oil saturation or above, but E. coli cells adhered better to samples with 10-30% oil than controls with no oil. In contrast, E. faecalis showed higher colonization, which decreased at 70–90% saturation and significantly reduced adhesion at 30–50% saturation. Finally, P. aeruginosa also showed low colonization with a significant decrease at 70–90% but a significantly impacted adhesion at 10-30% oil saturation. Together, the results show that saturation level of silicone oil within the polymer matrix can be a key factor in affecting bacterial colonization and adhesion and should be considered when developing antifouling surfaces based on liquid-infused polymers.

07

Mary Astumian
Biomedical Science (PhD)
Summer 2019
UMaine

The Localization of Dystroglycan and Integrin Proteins Within Muscle Cell Membranes

M. Astumian 1; K. Shivanna 2; P.Raut 2; S. Hess 2; C. Henry 1

1 Graduate School of Biomedical Science and Engineering, Department of Biology and Ecology, University of Maine, 2 Department of Physics and Astronomy, University of Maine

Healthy muscle fibers move bone by contracting and transducing force to the tendon, which connects to bone. But in muscular dystrophy and dystroglycanopathies, progressive diseases that affect both muscle and neurological health, muscle fibers detach from the tendon region and fail to function properly. In healthy muscle, muscle membrane proteins integrin and dystroglycan bind laminin proteins in the tendon region, connecting muscle to tendon. In zebrafish dystroglycan mutants and integrin mutants, disrupted laminin deposition and muscle health improved after oxidized nicotinamide adenine dinucleotide (NAD+) treatment. But in one dystroglycanopathy mutant, which lacks dystroglycan glycosylation, muscle health was not improved with NAD+. It is known that glycosylation influences localization and clustering of proteins. We hypothesized that NAD+ alters the localization of dystroglycan in integrin α7 mutants and that the nanoscale localization of laminin receptors is crucial for muscle adhesion. To measure localization, we tested the feasibility of fluorescence photoactivation localization microscopy (FPALM) to image whole, intact zebrafish muscle and determine the subcellular localization of dystroglycan and integrin for the first time. Results were consistent with confocal data. Preliminary data for dystroglycan localization in integrin α7 mutants indicated smaller dystroglycan cluster areas compared to non-mutants. Next, to find the mechanism of NAD+ action, we are testing the impact of NAD+ on dystroglycan localization in integrin α7 mutants. To further modulate membrane protein organization, we generated a tetraspanin CD151 mutant zebrafish, and predict impacts to integrin localization. CD151 mutants had altered neuromuscular junction structure and minor muscle defects. Overexpression of CD151 in dystroglycan mutants resulted in aberrant muscle fiber boundary crossings. This is the first examination of the CD151 in muscle. This research uses FPALM in zebrafish muscle to study nanoscale localization of laminin receptors, the impact of gene mutations and treatments on laminin receptor organization, and to relate localizations to muscle adhesion with the goal being to use the information to find therapies that impact muscle adhesion and function.

08

Michael Babcock
Biomedical Science (PhD)
Fall 2019
UMaine

Improving Rural Cancer Patient Access to Precision Medicines: Maximizing Tissue for Molecular Profiling

M. J. Babcock 1,3; B. King 2; M. Skacel 3.

1 Graduate School of Biomedical Sciences and Engineering (GSBSE), University of Maine, Orono, Maine; 2Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine; 3 Dahl-Chase Pathology Associates, Bangor, Maine.

Introduction: Pre-analytical tumor tissue prioritization is critical for cancer diagnosis, tumor molecular profiling, and tissue archiving for clinical trials and translational research studies. Molecular profiling provides biomarker data for cancer patient access to precision medicines requiring a 30% tumor cell content (in approximately 25 mm2 tissue area). However, histological tissue requirements for a cancer diagnosis often results in an inability to perform genomic profiling. Studies show that up to 30% of advanced lung cancer tumor biopsies are insufficient for molecular subtyping due to a lack of available tissue. There is a critical need for maximizing the use of tissue for cancer diagnosis and molecular profiling for patients to gain access to precision medicines and clinical trial studies. The aim of this study was to determine if pre-analytical molecular profiling workflows improved patient access to precision medicines.

Methods: Unused, discarded tumor tissue associated with level 2-3 histology tissue sections were used as a specimen source for pre-analytical molecular profiling. Retrospective review of pathology and molecular profiling results from de-identified cancer patients (n=2087) from the Northern Light, Eastern Maine Medical Center system between January 1, 2020 and

August 10, 2024. Data review included; tumor %, mutational findings and patient outcomes.

Results: Cohort case review (n=2087) of solid tumor cases (46.6%) with next-generation sequencing results (n=972/2087) demonstrates 70.1% were lung cancers (n=681/972). 28.9% of tested lung cancers (n=197/681) were < 30% tumor content, and 41.6% had at least one actionable finding. 49% of all tumors (n=1038/2087) had at least one actionable finding, and 565 were lung cancers. Of these lung cancers, 182 had an actionable finding.

Conclusion: Our findings demonstrate that pre-analytical molecular profiling tissue stewardship improves rural cancer patient access to precision oncology.

09

Tiyasha Banerjee
Biomedical Science (PhD)
Fall 2023
UNE

Moving Towards the Ellucidation of Catabolic thresholds of Chondrocyte Integrins

Tiyasha Banerjee 1,2,3 and Scott Wood, Ph.D. 1,3

1. Graduate School of Biomedical Science and Engineering; 2. University of Maine; 3.University of New England

Introduction: Osteoarthritis is one of the leading chronic diseases of joints worldwide. As of 2020, 595 million people or 7.6% of the world population were observed affected by osteoarthritis, making it the most common form of arthritis. Chondrocytes are specialized cells which differentiate from clusters of mesenchymal cells. Chondrocytes are the solitary cellular component of hyaline cartilage, and the balance between the anabolic versus catabolic factors is the common pathway that plays a role in cartilage degradation. Chondrocytes play role in synthesis, maintenance, and repair of the cartilaginous matrix by balancing the production of Extracellular Matrix and cartilage-degrading enzymes like metalloproteinases [MMPs] and the production of disintegrin and metalloproteinase with thrombospondin motifs [ADAMTSs]. Chondrocytes are sensitive to various factors like mechanical stress, cytokines (IL-6, TNF-α, IL-1β), chemokines, chondrocytes when exposed to these factors may contribute to the development of osteoarthritis.

Goals: Chondrocyte mechanotransduction (MT) has still not been very clearly investigated due their structure and organization, which is quite different from those of other MT studies. This prevents the translation of any data acquired from previous studies done on integrin-mediated mechanotransduction (IMMT). The biggest challenge faced while culturing chondrocytes is their rapid de-differentiation under standard culture conditions. To tackle these constraints, a micropatterned composite thin-film platform was developed, called the CellWell. We aim to establish a basic catabolic threshold for the tensile strength applied to integrins to understand the level of force applied to specific integrins (α1β1, α5β1) at which we see the switch from anabolic to catabolic conditions by Nuclear factor-κB(NF-κB) activation. When this objective is successfully executed, we expect to establish the catabolic thresholds for α1β1, α5β1, this would allow us to crease a basis for interpretation of results observed in previous studies on forces applied mechanotransduction at the tissue level.

10

Arad Bustan
Biomedical Science (PhD)
Fall 2022
JAX

Investigating the Role of KRAB-Zinc Finger Proteins (KZFPs) in Mitigating Non-syndromic Cleft Lip and Palate (CLP) in A Woolley Snell (A/WySn) Mice

Arad Bustan 1,2; Haley Fortin 1,3; Christopher L Baker 1,2,3

1 The Jackson Laboratory, Bar Harbor, Maine 04609 USA; 2 Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, Maine 04469 USA; 3 School of Graduate Biomedical Sciences, Tufts University, Boston, Massachusetts 02111 USA

Cleft lip and palate (CL/P) is among the most common birth defects of human genetic disorders. The etiology of CL/P is often complex, influenced by both genetic and environmental factors. This complexity makes it difficult to develop potential therapeutics. Humans and mice exhibit many conserved developmental stages during the formation of the lip and palate, making mice a good model system to study congenital defects like CL/P. In particular, the A/WySn mouse shows about 20% prevalence of craniofacial clefting, making this strain an optimal tool for investigating the genetic and epigenetic pathogenesis of CL/P. In this strain, researchers identified two loci, clf1 and clf2, that contribute to palate clefting. The clf1 locus, located on chromosome (Chr) 11, contains an active retroviral Intracisternal A particle (IAP) element. This IAP insertion dysregulates the expression of Wnt9b, a gene critical for proper lip formation. The identity of clf2, located on Chr 13, is unknown; however, we do know that certain genotypes at the clf2 locus are associated with methylation of the IAP and normal lip development. Additionally, the Chr 13 region contains a cluster of KRAB-zinc finger proteins (KZFPs), which are known for their role in repressing transposable elements.

Given the role of clf2 in methylating the IAP near Wnt9b, I hypothesize that clf2 is a KZFP that modifies the CL/P phenotype by repressing IAP activity at the clf1 locus. To test this, I will integrate PacBio long-read sequencing data with a high-throughput, HCR-FlowFISH screen to determine the identity of clf2. As a proof of concept, using PacBio long-read isoform data generated in the Baker Lab, we selected two novel DBA/2J (D2) specific KZFPs to determine if overexpression of each KZFP, in C57BL/6J (B6) and C57BL/6NJ (NJ)—strains from which they are absent—mouse embryonic stem cells (mESCs), results in changes in chromatin accessibility at IAP elements. ATAC-seq analysis shows that overexpression of these novel D2 KZFPs in B6 and NJ results in alterations of chromatin accessibility at repeat regions, including IAP elements.

This suggests that expanding this panel of KZFPs could aid in identifying the gene responsible for repressing the IAP element that leads to the development of CL/P in A/WySn. To do this, I will repress expression of IAP elements by overexpressing KZFPs in A/WySn mESCs and then determine changes in IAP expression using HCR-FlowFISH. Specifically, HCR-FlowFISH utilizes RNA probes and hairpin amplifiers conjugated to a fluorophore that can be used in combination with fluorescence-activated cell sorting to detect expression changes in IAP elements. Ongoing work aims at exploring using this approach to determine the identity of clf2, the gene that represses IAP activity leading to normal lip development.

11

John Butts
Biomedical Science (PhD)
Fall 2019
JAX

Population-scale non-coding variant effect predictions using an in-silico reporter assay

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Over 500 million single nucleotide variants (SNVs) have been identified across diverse human populations. The majority of these SNVs are in non-coding cis-regulatory elements (CREs). CREs dictate the precise spatiotemporal expression of genes and variation within CREs has been linked to evolution, traits, and disease. A powerful approach for characterizing non-coding variants is the use of Massively Parallel Reporter Assays (MPRA), which test the regulatory potential of thousands of sequences in a single experiment. However, it is unfeasible to test all human genetic variation by MPRA, both known or yet to be discovered. Thus, there is a need for a sensitive, scalable, and broadly applicable non-coding variant effect predictor with the experimental accuracy of MPRA.

We recently developed a deep learning model that predicts MPRA activity with accuracy approaching experimental replication in three cell types (K562, HepG2, SKNSH, r = .89 All). We applied this model to variant effect prediction, referring to differences in

predicted activity between alleles as 'skew' and variants with substantial skew as expression-modulating variants or 'emVars.' We find our model accurately predicts emVar skew (r .71-.77), and that predicted emVars correlate with DNase Allele Specific Effects (K562 r = .7) as well as recover causal variants for complex traits in UKBB/BBJ and eQTLs in GTEx comparably to experimentally defined emVars (UKBB precision .79-.91, recall .25-.02, GTEx precision .78-1, recall .25-.02). Given our performance on direct and orthogonal comparisons we applied our in-silico reporter to experimentally intractable repositories, generating variant effect predictions for 575M non-coding SNVs in gnomAD, ClinVar, and COSMIC.

In ClinVar, we call 8,930 emVars, identify enrichments of emVars in pathogenic variants (OR = 2.97, p = 1.87e-226), and accurately discern pathogenic and benign promoter variants (precision .85, recall .42). We nominate 40,460 emVars in COSMIC and identify emVar enrichments in cancer-relevant non-coding features (Recurrence OR = 1.07 p = .015, Cancer Promoter OR = 1.5, p = .011). In gnomAD, we identify 7M emVars across over 500M common, rare, and private SNVs and observe strong evolutionary constraint on loss-of-function (negative skew) variants in promoters (high skew bin, mean phyloP promoter: 2.8, vs. proximal CRE .8 vs. distal CRE .07). These observations demonstrate that our model extends MPRA to the speed and scale required to understand the clinical and evolutionary impacts of human variation genome-wide.

12

Hang Chen
Biomedical Science (PhD)
Fall 2023
JAX

Dissecting the role of chemotherapy-induced host responses in post-therapy metastatic relapse of breast cancer

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Over 90% of breast cancer related deaths are due to the metastatic relapse of tumors in distant vital organs such as lung and liver. While chemotherapy, a major treatment approach for breast cancer, has direct cytotoxicity to epithelial tumor cells leading to tumor remission, it also elicits host responses that may offset the therapeutic efficacy. One of the main host responses is augmentation of the regeneration responses, including inflammation, stromal cell activation, and cellular proliferation, all of which could counteract the tumoricidal effects of chemotherapy. As epithelial tumor cells become drug-resistant, the regenerative responses may instigate local recurrence and metastatic relapse of tumor cells leading to therapeutic failure. Using mouse models of breast cancer, our preliminary study indeed showed a chemotherapy-induced early metastatic relapse of breast tumors in the lung and liver. Furthermore, by single cell transcriptomic profiling, we found that a population of CXCR4+ lung pericytes underwent expansion and upregulation of regeneration-associated genes and signaling pathways. A hypothesis is therefore raised: systemic chemotherapy elicits regenerative host responses of organ-resident stromal cells, which are, in turn, utilized by drug-resistant tumor cells for their metastatic relapse. To test this hypothesis, I am working on 1) determining how chemotherapeutic drugs activate organ-resident stromal cells using spatial transcriptomics and multiplexed imaging technologies, and 2) delineating the molecular mechanisms underlying chemotherapeutic drug-stimulated organ-resident stromal cells to support metastatic tumor growth in the lung and liver using in vivo transgenic mouse models and in vitro assays. The results will establish a foundation for the development of novel strategies to target organ stroma, improve the efficacies of current chemotherapy treatment and reduce metastatic relapse in breast cancer patients.

13

Carolina Cora
Biomedical Science (PhD)
Fall 2021
MHIR

Lipidomics of Cardiac Arrest Patients

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Cardiac arrest (CA) results in over 600,000 deaths annually in the US, with a high mortality rate of approximately 90% due to significant brain trauma. Therapeutic hypothermia (TH) is a common treatment aimed at reducing biological activity and preventing long-term damage by lowering body temperature. However, TH's effectiveness varies, with some patients showing no significant improvement in survival rates compared to normothermic conditions. Our research suggests that the presence of brown adipose tissue (BAT), which regulates body temperature through lipid metabolism, may explain these differences. The presence of 12,13-diHOME in the blood serves as a marker for BAT. We hypothesized that variations in 12,13-diHOME levels correlate with differences in blood lipid profiles in CA patients.

In this study, we analyzed serum samples from 10 CA patients, categorized by low and high 12,13-diHOME levels, measuring 352 lipid species at 24 hours post-resuscitation. Our results revealed distinct lipid profile differences between the two groups. High 12,13-diHOME individuals exhibited an upregulation of ePE(32:3), ePC(32:3), and PE-Cer(16:0) lipids, while low 12,13-diHOME individuals showed increased levels of LPE(18:3) and a higher prevalence of 36 and 34 carbon chain lipids. Conversely, low 12,13-diHOME patients had a reduced presence of 38 carbon chain lipids. Notably, alterations in the phosphatidylglycerol group (PG) metabolism were observed.

These findings suggest that lipid species, particularly those within the PG group, could inform personalized treatment strategies for TH patients. By considering BAT presence and 12,13-diHOME levels, therapeutic approaches can be tailored to enhance patient outcomes. This study highlights the potential for diet personalization and targeted therapies in managing CA recovery, emphasizing the need for further research into BAT's role in TH efficacy.

14

Sophie Craig
Biomedical Science (PhD)
Fall 2021
UMaine

Uncovering mechanisms of JC polyomavirus entry in primary cells

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JC polyomavirus (JCPyV) infects 50-80% of the human population worldwide, establishing a lifelong, asymptomatic infection in the kidney. Under immunosuppressive conditions, the virus can spread to the brain and infect the glial cells. This results in a demyelinating neurodegenerative disease called progressive multifocal leukoencephalopathy (PML). PML is debilitating, with symptoms including motor dysfunction and cognitive impairment, and has no cure or approved treatment. Research to understand the JCPyV infectious cycle is necessary to develop antivirals. Most JCPyV research has been performed in immortalized cell lines, which has led to important advancements. However, immortalized cells are not the most accurate model for infection, and this work has not revealed the cell type-specific JCPyV infectious mechanisms that account for JCPyV pathogenesis in the kidney and brain. Primary cells better represent the phenotype of infected cells in vivo. Differences in signaling pathway activation during infection of immortalized and primary brain cells suggest cell type-specific JCPyV mechanisms of entry and infection. Thus, mechanisms of JCPyV infection must be characterized in primary cells. Using inhibitors, siRNA knockdown, and infection assays, we have begun to

elucidate the mechanisms of viral entry and signaling pathway regulation in primary kidney and brain cells. My research suggests that JCPyV infection occurs by clathrin-mediated endocytosis in primary cells, and my future research will investigate cell type-specific signaling mechanisms during infection. This work illuminates JC polyomavirus infection mechanisms in primary cells, helping to uncover potential targets for antivirals that could reduce the spread of JCPyV and the impact of PML.

15

Shawn David
Biomedical Science (PhD)
Fall 2022
JAX

Hematopoietic stem cell and T cell interactions in Clonal hematopoiesis of indeterminate potential (CHIP)

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Malignant bone marrow disorders such as myelodysplastic syndromes (MDS) characterized by ineffective hematopoiesis and high risk to transformation into acute myeloid leukemia (AML). To better understand progression of such bone marrow disorders to leukemia and devise therapeutic strategies to prevent leukemic states, it is important to study pre-MDS states. One of the most frequently reported pre-MDS states is clonal hematopoiesis of indeterminate potential (CHIP) where expansion of blood cells occurs from a single hematopoietic stem cell (HSC) harboring a pre-leukemic mutation. Although CHIP is an asymptomatic condition found in elderly population, it is known to be associated with increased risk of cardiovascular disease, chronic obstructive pulmonary disease, all-cause mortality and leukemic transformation. The most common mutation in CHIP is known to inactivate DNA methyltransferase 3A or Dnmt3a. Dnmt3a-mutated HSCs are known to have a self-renewal or proliferative advantage over non-mutated HSCs which is enhanced by the inflammatory environment associated with CHIP. However, we have very limited understanding of how Dnmt3a-mutation and the inflammatory environment impacts T cell surveillance to drive progression of CHIP to malignancy. Therefore, the goal of this study is to understand the functional significance of T cell and mutant HSC interactions in Dnmt3a-mutated CHIP.

Based on literature evidence of strong HSPCs and CD4+ T cell bidirectional interactions eliminating aberrant HSPCs and preliminary results from differential gene expression analysis on human and mouse CHIP samples we hypothesize that Dnmt3a mutation in HSPCs downregulate MHC-II expression to evade immune surveillance of CD4+ T cells thus providing Dnmt3a-mutated HSPCs a proliferative advantage over non-mutated HSPC and ultimately leading to a malignant transformation. Here we show that Dnmt3a-mutated HSPCs have lower expression of MHC-II molecules compared to WT HSPCs in vivo. In vitro coculture between Dnmt3a-HSPC presenting foreign antigen (ovalbumin) and CD4+ T cell shows decreased levels of activated CD4+ T cells. Transplanting Dnmt3a-mutant MHC-II +/- stem cells (LSK) into lethally irradiated mice (stem cells ablated) shows higher engraftment of Dnmt3a-mutant MHC-II- stem cells compared to WT MHC-II- stem cells and lower engraftment of Dnmt3a-mutant MHC-II+ stem cells compared to WT MHC-II+ stem cells upon assessing peripheral blood cells in vivo. These data suggest a possible CD4 T cell surveillance evasion by Dnmt3a-mutant cells. New insights gained from future experiments will guide future strategies to abrogate clonal expansion and prolong immune health in pre-MDS states which could potentially decrease the severity and progression of pre-MDS states to malignant transformations.

16

Cory Diemler
Biomedical Science (PhD)
Fall 2021
MHIR

Microglia depletion increases susceptibility for glaucomatous neurodegeneration in ocular hypertensive mice

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Microglia responses occur early in the pathogenesis of glaucoma and other neurodegenerative diseases. In recent years, changes in microglial states have been correlated with later glaucoma severity; however, their specific role(s) are not known. We hypothesize that the depletion of microglia with a dietary CSF1R inhibitor would alter glaucomatous optic nerve damage in an aged ocular hypertensive model.

Dietary PLX5622, a CSF1R inhibitor known to decrease populations of microglia in the retina, was introduced to 9.5mo-old DBA/2J mice (a widely used model relevant to ocular hypertension). Microglial depletion was confirmed with retinal tissue RNA-seq analysis (n=4 per diet per sex). Intraocular pressures (IOPs) were measured, and retinal ganglion cell (RGC) function was assessed by measuring pattern electroretinography (PERG) amplitudes and latency at 9, 10.5, and 12mo of age. (n=10 per diet per sex). At 12mo, optic nerves were evaluated for glaucomatous damage using p-phenylenediamine staining (n=12 per diet per sex). Retinas corresponding to the assessed optic nerves were isolated for confocal microscopy (n=6 per diet).

Pilot studies showed that 75% of retinal microglia are depleted after 3wks exposure to PLX5622. Microglia depletion was further validated by RNA-seq analysis that showed significant downregulation of microglia-specific genes including Tmem119, and P2ry12. 10wks exposure to PLX5622 revealed no significant differences in PERG amplitude and latency, IOP, or RGC soma number between dietary groups. However, analysis of optic nerves showed a significant PLX5622 diet-associated increase in moderate-to-severe optic nerve damage (p= 0.0022).

Our results indicate that reducing the retinal microglial population from 9.5 to 12mo increased susceptibility for glaucomatous neurodegeneration in DBA/2J mice. This suggests a potential beneficial effect of microglia in glaucoma. Experiments are underway to determine whether this overall beneficial effect can be boosted by renewing the microglia pool just prior to IOP onset and optic nerve damage through short term exposure to PLX5622. Future studies will include targeting specific states of microglia through disruption of genes known to control activation including the triggering receptor expressed on myeloid cells (TREM) gene family.

17

Logan Douglas
Biomedical Science (PhD)
Fall 2021
MHIR

Developmental Thyrotoxicosis Impacts Neural Systems and Dependent Behaviors Reward

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Thyroid conditions are highly prevalent but up to 60% of cases go undiagnosed. Thyroid conditions are especially prevalent among women and, during pregnancy, altered thyroid hormones (TH) due to maternal thyroid dysregulation may impact the development of the fetal brain, with neurological consequences into adulthood. To elucidate the impact of fetal TH excess on reward-motivated behaviors and the reward-related brain regions, we utilize a mouse model (Dio3KO) with a non-functioning type 3 deiodinase (DIO3). DIO3 breaks down TH into inactive metabolites to ensure hormone availability in developing tissues are maintained at an adequate level for their developmental stage. DIO3 loss of function results in an inability to breakdown maternal TH and TH excess in the fetus, resulting later in life in a range of neurological abnormalities including aggression,

decreased anxiety-like and depression-like behavior, hyperactivity, and hyperphagia. We do not yet understand the effect of developmental thyrotoxicosis on reward-related brain areas such as the amygdala and striatum. Dio3 exhibits transient high expression in these areas during development, suggesting TH excess disrupts the development of reward-related brain regions and associated behaviors. We hypothesized that developmental TH excess results in abnormal programming of reward-related brain regions, with consequences for associated behaviors later in adulthood. We utilized male and female wild-type (WT) and Dio3KO mouse littermates generated via reciprocal crosses of C57BL/6J and 129/SVJ mice heterozygous for the Dio3 mutation. We analyzed reward-motivated behavior using a palatable food reward-conditioned self-administration runway (SAR) and conditioned place preference (CPP) tests. We observed parent-of-origin specific increased motivation for palatable food reward, with Dio3KO mice showing a resistance to extinction in the SAR assay, and Dio3KO mice with a C57BL/6J paternal background showing increased CPP for palatable food reward compared to both wild-type littermates and Dio3KO mice with a 129/SvJ paternal background. The acyl / total serum ratio of ghrelin, a hormone involved in the regulation of reward-motivated neurological circuits, was significantly lower in Dio3KO mice compared to WT controls, and differed based on paternal genetic background but not sex. Gene expression and immunohistochemistry data further indicates alterations to relevant measures of reward such as tyrosine hydroxylase and dopamine decarboxylase within the amygdala and striatum. These experiments suggest that abnormal TH levels during development play a role in the programming of the reward system. As abnormal acyl/total ghrelin ratios have been associated with alcoholism, obesity, and eating disorders, our findings raise the possibility that altered thyroid states during development influence the susceptibility to obesity and addiction via aberrant programming of brain reward circuitries and dependent behaviors.

18

Adeola Abraham Fadahunsi
Biomedical Engineering (PhD)
Fall 2022
UMaine

Fabrication and In-vitro Assessment of Cellulose Nanofiber-Hydroxyapatite Composite For Bone Tissue Regrowth

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Bone is fundamental to the human body, interacting with most physiological systems. Disorders in these systems affect bone anatomy and metabolism, leading to changes in density, porosity, stiffness, strength, and the development of defects. Critical bone defects impair self-healing and require medical intervention. Traditionally, large defects, such as fractures, are treated with grafting, where donor bone is implanted at the trauma site due to its osteo-inductive and conductive properties. However, challenges like donor shortages, tissue rejection, infection risks, and inflammation have driven the search for alternative, biocompatible, cost-effective materials to promote bone regeneration. This remains a key focus in bone tissue engineering research.

Despite its similarity to collagen, a major component of bone, cellulose nanofiber (CNF) has received limited attention in bone repair research. This study aims to investigate a composite material made of hydroxyapatite (HA) and CNF for bone repair. Various CNF-HA ratios were synthesized and analyzed for morphological features using scanning electron microscopy (SEM). Mechanical properties, including compressive, tensile, and flexural strength, were tested according to ASTM standards. Cytotoxicity was assessed by culturing MC3T3 pre-osteoblasts and L929 fibroblasts with the composites. Osteoconductive properties and cellular differentiation were examined via immunofluorescence staining for Osteopontin and Collagen-1, while mineralization was evaluated using Alizarin staining.

19

Remi Geohegan
Biomedical Science (PhD)
Fall 2022
UMaine

Characterizing S. cerevisiae and C. elegans as Models for the Effects of Nuclear Aging on Nuclear Transport

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Aging is the greatest risk factor for many chronic diseases [1]. At present there are 5.6 million people in the United States are over the age of 85; a figure which is projected to rise to 19 million by 2050 [2]. This increase highlights the urgency to develop a better understanding of the underlying mechanisms of aging and their roles in age-associated diseases. Defects in nuclear transport, the process by which molecules move across the nuclear membrane within a cell, are a shared trait that characterize age-associated diseases including Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal Dementia (FTD). This work aims to determine whether the efficacy of nuclear transport is altered with aging using bakers yeast and nematode models.

20

Brianna Gurdon
Biomedical Science (PhD)
Spring 2021
JAX

Linking pathological and cognitive resilience using brain-wide immunohistochemistry in the AD-BXD panel

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The relationship between regional cell composition, brain pathology, and memory impairment in Alzheimer's disease (AD) remains incompletely understood. By integrating brain-wide immunohistochemistry and cognitive outcomes we can achieve unbiased detection of regions of interest and identify cellular correlates of cognitive resilience for causal testing.

Immunohistochemistry was completed to evaluate neurodegeneration, gliosis, amyloid beta (Aβ) pathology, and cell bodies in adult (6 months (m)) and middle-aged (14m) mice of the AD-BXD panel (n=271). Using the QUINT workflow, hemibrain slices were systematically segmented and registered to the Allen Brain Atlas to gain a global perspective of percent cell and pathology coverage. IHC traits were correlated with contextual fear conditioning outcomes to identify regions whose stain composition is associated with memory performance.

We find that Aβ quantified at the presymptomatic time point (6m) is strongly correlated with differences in short- and long-term memory in 14m female 5XFAD carriers. Furthermore, the degree of astrogliosis at 6m in cortical, striatal, and thalamic regions acts as a predictor of cognitive outcomes at 14m. While age-related decreases in NeuN load were not observed among 5XFADs, wide variation in neurodegeneration levels could be attributed to strain differences, and 14m NeuN load was positively correlated with 14m long-term memory among this female 5XFAD population. We categorized strains as resilient or susceptible to pathological and cognitive decline and evaluated the overlap of these traits. These findings highlight the importance of studying the impact of genetic background on AD progression.

21

Josh Hamilton
Biomedical Engineering (PhD)
Fall 2022
UMaine

Quantitative Anisotropy Analysis of the Ductal Connective Tissue Boundary in Breast Cancer Shows Organizational Differences Across Subtypes

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Aim of Study: There is a worldwide shortage of trained pathologists and this shortage is exacerbated in low income countries. There is a need for automated systems to support pathologists and reduce cancer burden in countries with limited access to pathology labs. The most commonly diagnosed cancer worldwide is breast cancer, accounting for over 10% of diagnosed cancers. Our work aims to use the publicly available BReAst Carcinoma Subtyping (BRACS) dataset to develop explainable wavelet based algorithms, automating breast cancer subtyping and determining quantitative changes in the tumor microenvironment between subtypes.

Methods: H&E images larger than 1024 x 1024 pixels with ducts near the center of the image were manually selected from the BRACS dataset for normal tissue (N=448), ductal carcinoma in situ (DCIS) (N=777), and invasive cancer (N=639). The images were color deconvolved to their hematoxylin and eosin components. For every image, four 512 x 512 pixel subsets were obtained from the image corners from both color channels. The 2D Wavelet Transform Modulus Maxima (2D WTMM) Anisotropy Method was used to calculate multiscale anisotropy factors for each corner. The mean image anisotropy factor from the four corners was calculated for each image. The mean and standard deviation across the images for each subtype were then calculated with Wilcoxon tests conducted at each wavelet scale.

Results: Normal and DCIS hematoxylin channel anisotropy factors were statistically significantly different at large size scales ($p < 1.4*10-7$). Their eosin channel anisotropy factors were also significantly different at large size scales ($p < 0.004$). Normal and invasive cancer hematoxylin channel anisotropy factors were significantly different at small scales ($p < 0.006$). Their eosin channel anisotropy factors were significantly different at almost every size scale ($p < 0.009$). DCIS and invasive cancer were significantly different at every size scale for both hematoxylin and eosin channels ($p < 0.002$).

Conclusions: The 2D WTMM Anisotropy method is capable of quantifying organizational changes in the ductal connective tissue interface. Organization of cell nuclei near the ductal boundary seem to be the key for differentiating DCIS from normal ducts. Future work includes segmenting the whole slide images, and classifying subtypes from the anisotropy factors.

22

Lola Holcomb
Biomedical Science (PhD)
Fall 2021
UMaine

Early life exposure to broccoli sprouts confers stronger protection against enterocolitis in an immunological mouse model of inflammatory bowel disease

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Inflammatory Bowel Diseases (IBD) are chronic conditions characterized by inflammation of the gastrointestinal tract that heavily burden daily life, result in surgery or other complications, and disrupt the gut microbiome. How IBD influences gut microbial ecology, especially biogeographic patterns of microbial location, and how the gut microbiota can use diet components and microbial metabolites to mediate disease, are still poorly understood. This study aimed to resolve such questions. Many studies on diet and IBD in mice use a chemically induced ulcerative colitis model, despite the availability of an immune-modulated Crohn's Disease model. Interleukin-10-knockout (IL-10-KO) mice on a C57BL/6 background, beginning at age 4 or 7 weeks, were fed either a control diet or one containing 10% (w/w) raw broccoli sprouts which was high in the sprout-sourced anti-inflammatory sulforaphane. Diets began 7 days prior to inoculation

with Helicobacter hepaticus, which triggers Crohn's-like symptoms in these immune-impaired mice, and ran for two additional weeks. Key findings of this study suggest that the broccoli sprout diet increases sulforaphane concentration in plasma; decreases weight stagnation, fecal blood, and diarrhea associated with enterocolitis; and increases microbiota richness in the gut, especially in younger mice. Sprout diets resulted in some anatomically specific bacterial communities in younger mice, and reduced the prevalence and abundance of potentially pathogenic or otherwise-commensal bacteria which trigger inflammation in the IL-10 deficient mouse, for example, Escherichia coli and Helicobacter. Overall, the IL-10-KO mouse model is responsive to a raw broccoli sprout diet and represents an opportunity for more diet-host-microbiome research.

23

Amanda Ignacz
Biomedical Science (PhD)
Fall 2020
UMaine

A zebrafish model demonstrating that DPM3 functions in both dystroglycan dependent and independent roles in neuromuscular disease progression

Amanda Ignacz 1; Claire Schaffer 1; Mary Astumian 1; Clarissa Henry 1.

1 GSBSE

Skeletal muscle development, growth, and homeostasis relies on post-translational modifications, such as glycosylation. Healthy muscle function also requires the development of neuromuscular junctions (NMJs) and myotendinous junctions (MTJs), all interconnected via cell-matrix adhesion complexes that are replete with glycosylated proteins. Glycosylation is notably disrupted in dystroglycanopathies, which are identified by their role in glycosylation of dystroglycan (DG), a critical transmembrane receptor that anchors the intracellular cytoskeleton to the extracellular matrix. An important question that remains unanswered is if dystroglycanopathy genes contribute to the glycosylation of the myriad of other glycosylated proteins that promote adhesion to the extracellular matrix, and what aspects of neuromusculoskeletal degeneration are specifically due to the disruption of DG glycosylation versus disruption of glycosylation of other proteins. One such dystroglycanopathy gene is dolichyl phosphate mannosyltransferase 3 (dpm3), which contributes a foundational mannose residue as a building block for the glycosylation of DG. We developed a zebrafish model of DPM3-dystroglycanopathy through CRISPR/Cas9 technology that recapitulates the interesting phenotypic variation and disease progression observed in human DPM3 patients. Through use of dpm3 mutants, as well as dpm3;dg double mutants, we have developed a model to elucidate what aspects of DPM3 function are DG-dependent and DG-independent in disease progression. While dg -/- mutants do harbor dystrophy and muscle fiber detachments, the loss of even one copy of wild-type dpm3 dramatically exacerbates NMJ, MTJ, and skeletal muscle degeneration. Interestingly, we have also observed heterogeneity within the dpm3 +/-;dg -/- genotype, ranging from mild posterior muscle degeneration to severe multi-segment degeneration early in development. These findings indicate that DPM3 likely contributes to neuromuscular disease progression via both DG-dependent and DG-independent pathways, and possibly acts in a gene-dose dependent manner. Ultimately, we aim to elucidate DG-independent roles of dystroglycanopathy genes to gain fundamental knowledge for future therapeutic strategies in treating neuromuscular diseases.

24

Gabriela Johnson
Biomedical Science (PhD)
Fall 2022
MDIBL

Development of two mechanistically distinct transgenic axolotl cell-ablation systems

Gabriela Johnson 1,2; Andrew Hart 2; Dr. James Godwin 1,2.

1 GSBSE; 2 MDIBL

Unlike humans, salamanders have a high regenerative capacity that enables them to undergo scar-free wound repair and functionally replace a range of clinically relevant adult structures following injury, including parts of the brain, heart, spinal cord, and limbs. The

axolotl is a type of salamander that is supported by a growing catalog of tissue and cell-specific transgenic lines where the translucent skin facilitates high quality imaging and cell tracking. The ability to assign function to specific cell types in regeneration has been obstructed by the lack of effective cell-specific ablation systems. To solve this problem, we developed two independent transgenic cell-ablation platforms in parallel that genetically sensitize target cells to drug induced cell death in vivo. The metronidazole prodrug-inducible enhanced-nitroreductase enzyme system (NTR 2.0) and the small molecule-inducible Caspase 9 (ihCasp9) ablation systems were compared in transgenic axolotls to evaluate ablation efficiency in different stages of axolotl development. Drug concentrations capable of effectively inducing death in genetically sensitized cells were determined in vitro. In vivo grafting studies were used to optimize drug dose, carrier vehicle, route of administration, and ablation kinetics. These robust genetic systems are the first to be implemented in axolotl and provide researchers with the tools required to conduct detailed studies aimed at understanding cell-specific functions during regenerative or developmental processes in vivo. Investigating the cellular mechanisms required for scar-free wound repair can provide insights for improving the regenerative capabilities of humans.

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Audrie Langlais
Biomedical Science (PhD)
Fall 2020
MHIR

Investigating the impact of morphine on bone via neural-derived extracellular vesicles

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Opioids compromise bone health by reducing bone mineral density and increasing fracture risk, but the causes are not fully understood. Previously, we identified morphine-induced trabecular bone loss in male, but not female, C57BL/6J mice was due to reduced bone formation. Bone loss was also associated with reduced circulating miRNA expression in serum and bone. However, the source of altered miRNA expression and the effects on bone formation have not been investigated. Based on the large-scale changes in miRNA expression, we hypothesized extracellular vesicles may be decreased. To test this, we treated 8-week-old C57BL/6J male mice with morphine (20 mg/kg, s.c.) or vehicle (0.9% saline, N = 6-7/group). After 1 hour, serum extracellular vesicles were isolated for nanoparticle tracking analysis (ZetaView). Within serum, the concentration of extracellular vesicles was significantly reduced ($p < 0.05$), suggesting decreased vesicle secretion contributes to decreased miRNA expression. Based on known opioid effects on the nervous system, we hypothesized that morphine may inhibit neural-derived vesicle secretion in bone. To begin to test this hypothesis, a second cohort of C57BL/6J mice received intratibial injections of Fast Blue (37.5 µg), a retrograde neuronal tracer. After 1 week, the lumbar dorsal root ganglion (DRG) were isolated. As expected, the majority of tibial innervation determined by presence of Fast Blue (FB+), arises from the L1-L2 DRG. Furthermore, a large proportion of the FB+ sensory neurons also express the µ-opioid receptor (Oprm1 RNAScope, Avg. 47.5%), suggesting morphine may impact local vesicle secretion in bone in addition to systemic changes observed in serum. To determine a neural-specific role of morphine on vesicle secretion, we generated a novel reporter mouse, CD63emGFPl/s/l x Baf53bCre/+ which targets neuronal derived CD63-exosomes. In addition to expected GFP expression in the DRG and spinal cord, we also observed overlap with βIII Tubulin+ nerves in bone (vertebrae, femur, tibia), suggesting their release from nerve terminals to recipient cells. Collectively, these studies suggest neural-bone crosstalk is mediated at least in part by extracellular vesicles. Although their composition and function are currently unknown, we hypothesize that changes in neural extracellular vesicle secretion may lead to changes in bone homeostasis, which has implications for opioid and other nervous-system related factors influencing bone.

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Hannah Megathlin
Biomedical Science (PhD)
Fall 2022
JAX

Extending the half-life of human therapeutic antibodies in NSG mice

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Monoclonal antibodies (mAbs) are a powerful therapeutic tool. They are used to treat a diverse set of diseases but are especially useful in cancer treatments. The go to model for preclinical testing of cancer mAbs are humanized NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice. NSG mice are immunodeficient mice that support the engraftment of human immune cells and tissues. However, due to a gain of function mutation in the Fcgr1 gene in the NOD strain background, NSG mice rapidly clear human IgG1, IgG3, and IgG4. Since most therapeutic mAbs are either IgG1 or IgG4 isotypes, this limits their use for preclinical testing of cancer therapeutic mAbs. In order to extend the half-life of mAbs in NSG mice, we created NSG Fcγ Receptor I knock out (NSG-FcγRI KO) mice. To measure human IgG clearance, we administered via tail vein injections three different therapeutic mAbs used to treat cancer, Rituximab (IgG1), Trastuzumab (IgG1), and Prembrolizumab (IgG4), and then measured their levels in the serum over the course of 5 weeks. Pharmacokinetic analysis was performed to determine half-lives of each of the mAbs. We found significant increases in the half lives of each of these antibodies in the NSG-FcγRI KO mice when compared to NSG controls. In the future, we will test whether the increased half life translates into therapeutic efficacy.

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Biomedical Engineering(PhD)
Summer 2021
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Exploring the Role of Integrin α2β1 in Breast Cancer Progression Using 3D Knockdown Spheroid Migration Models

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There is a lack of knowledge in distinguishing low- versus high-risk early-stage breast cancer tumors due to heterogeneity of cancer progression. Therefore, it is essential to develop biomarkers to differentiate invasive cancers. Interestingly, aggressive breast cancer has been shown to have elevated levels of integrin α2β1 which plays a role in cellular migration and metastasis to secondary locations in the body. Therefore, we aim to understand the role of integrin α2β1 on cellular migration using 3D in-vitro spheroid models composed of the MCF10A human breast cancer cell line series (three cell lines that mimic the progression of breast cancer from non-tumorigenic to pre-malignant to metastatic). Using shRNA transduction and adhesion assays, we reduced integrin α2β1 expression (knockdown) to greater than 60% of the original values in all three cell lines which was validated by Western blots and fluorescent staining. Spheroids were generated with a seeding density of 4,000 cells with 2.5% Matrigel and allowed to grow to 350-450 µm in diameter (~48 hours). Spheroids were then embedded in a 2 mg/mL collagen hydrogel for 3D migration experiments where brightfield images were acquired every 12 hours for a total duration of 72 hours with a BioTek Cytation 5. Cellular migration metrics are quantified on a single cell level using a custom FIJI and R analysis pipeline. Preliminary trials demonstrate that integrin α2β1 knockdown has a greater impact on cellular migration in the metastatic cell line compared to the non-tumorigenic and pre-malignant cell lines. In the future, we aim to incorporate human mammary fibroblasts into our model to increase the physiological

relevance of the model as fibroblasts are a top contributor to extracellular matrix remodeling and thus migration potential. Overall, these results demonstrate the potential of integrin α2β1 to be a biomarker for breast cancer progression.

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Michayla Moore
Biomedical Science (PhD)
Fall 2020
MHIR

Molecular Characterization of Human Highly Proliferative Cells (hHiPCs) Isolated from Coronary Artery Bypass Graft Surgery (CABG) patients Identifies IGFBP3, SOST, and ISLR as Downstream Targets of BMP9/ALK1 Pro-angiogenic Signaling

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Stem/progenitor cells are being investigated as a potential cell source to reverse cardiac remodeling and restore cardiac function following injury, but the molecular mechanisms required for improved repair are not well understood. Circulating BMP9 is negatively associated with heart disease risk factors. BMP9/ALK1 signaling is a critical pathway involved in several mechanisms of vascular repair such as angiogenesis and inflammation. We recently identified ALK1 expression in human highly proliferative cells (hHiPCs) with progenitor capabilities isolated from patients undergoing coronary artery bypass graft (CABG) surgery as measured by flow cytometry (delta MFI=2.0) and western blot. We have determined that circulating BMP9 in patients is negatively associated with the number of hHiPC ($p<0.001$) and positively associated with endothelial cell (EC) number ($p=0.008$) suggesting that BMP9 may play a role in hHiPC differentiation into EC. Further, we found upregulation of novel BMP9 regulated proteins, insulin-like growth factor-binding protein 3 (IGFBP3), sclerostin (SOST), and meflin/ISLR, in hHiPC upon BMP9 treatment using LC-MS/MS analysis of the secretome ($p<0.01$, Fold-change (FC)>2.0) and RT-qPCR ($p<0.001$, FC>2.0). Conditioned media collected from BMP9 treated hHiPCs or ECs improved tube formation in the tube formation assay. IGFBP3, SOST, and ISLR upregulation by BMP9 is inhibited upon knockdown and pharmacological inhibition of ALK1 as measured by proteomics ($p<0.01$) and RT-qPCR analysis ($p<0.05$). ISLR is a novel marker of mesenchymal stem cells found to have an important role in maintaining “stemness” and colony formation. hHiPCs treated with novel BMP9 target, ISLR, were analyzed using LC-MS/MS and STRING. We found ISLR treatment enriched for differential regulation of cell adhesion, cell migration, and angiogenesis proteins such as CXCL12, EMILIN1, and ANXA5 ($p<0.05$). Together, these data lay the foundation for investigating BMP9/ALK1 autocrine signaling in potential regulation of progenitor cell maintenance and angiogenesis in heart disease.

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Madison Mueth
Biomedical Engineering (PhD)
Summer 2021
UNE

The RNA-Binding Protein CUGBP Elav-Like Family Member 4 (CELF4) Acts as a Negative Regulator of Sensory Neuron Excitability and Hyperalgesia in Mice

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Chronic pain impacts approximately 25% of the global population and is the #1 reason adults in the US seek medical attention. Despite the availability of various therapeutic strategies, chronic pain management remains challenging due to limitations including side effects and risks associated with pharmacological interventions, variable response to treatment among patients, and limited long-term effectiveness, leaving patients battling untreated and/or recurrent pain. Therefore, there is a significant need for the development of alternative strategies to manage chronic pain. The development and maintenance of persistent pain is dependent on the translation of pronociceptive mRNAs in sensory neurons. Identifying post-transcriptional regulatory mechanisms that

control pronociceptive translation may allow for modulation of sensory neuron sensitivity in persistent pain. Previously, we identified that the RNA-binding protein CUGBP Elav-like family member 4 (CELF4) is expressed in TRPV1+ sensory neurons in the dorsal root ganglia (DRG) and that CELF4 preferentially associates with many transcripts of pronociceptive genes. CELF4 is also a known negative regulator of excitatory neurotransmission in the central nervous system where it regulates the translation of sodium channels. Considering these findings, we generated conditional knockout (KO) mice with Celf4 deleted from adult DRG neurons to investigate its role in pain signaling. This revealed Celf4 KO causes sensory neurons to become extremely hyperexcitable compared to wild-type controls and these mice display robust mechanical and thermal behavioral hypersensitivities. To further investigate this phenotype, we used RNA immunoprecipitation sequencing to confirm CELF4 binding with predicted nociceptive targets and conducted histological analyses to identify changes in expression of these targets within sensory neurons from Celf4 KO mice compared to controls. This revealed that CELF4 exhibits enriched binding with transcripts of neuropeptides critical to pain signaling and that Celf4 KO leads to increased expression of the sodium channel, Nav1.8. These findings support CELF4 as a promising candidate regulatory mechanism within sensory neurons that may be leveraged to reduce sensory neuron sensitivity in persistent pain conditions.

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Madeleine Nowak
Biomedical Science (PhD)
Fall 2020
MHIR

Plasma Hormones, Mesoderm Specific Transcript, and the Development of Obesity in Mice

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Aim: In the white adipose tissue (WAT) of genetically identical mice, high expression of mesoderm specific transcript (Mest) is predictive for development of diet-induced obesity (DIO). We previously demonstrated that gene expression of endocrine factors from the liver and WAT significantly correlate with WAT Mest in male mice. Our current aims are to determine if (1) differences in circulating endocrine factors are established shortly after weaning and (2) associations between endocrine factors and WAT Mest can be found in females as well as males.

Methods: Previous studies used plasma and tissue samples from a cohort of 40 male C57B6/J mice to identify differences in gene expression and corresponding plasma levels of 3 hepatokines; energy homeostasis associated (Enho, adropin), retinol binding protein 4 (RBP4), and inhibin beta chain E (INHBE). To induce Mest expression associated with changes in adiposity, mice were fed high fat diet (HFD) from 8 to 12 weeks of age. Plasma was collected at 8, 10, and 12 weeks of age, and tissue samples for measures of transcriptional activity were collected at 12 weeks of age.

Our ongoing study seeks to replicate these findings from a cohort of 66 males and 59 females fed HFD from 8 to 12 weeks of age. Phosphorylation of SMAD 2/3 in WAT was measured as a surrogate marker for INHBE signaling. Plasma samples were collected at 4, 8, and 12 weeks of age and will be used to measure plasma adropin, RBP4, and leptin. Tissue samples will be used for measures of transcriptional activity.

Results: In our initial study, mice with high WAT Mest were most susceptible to DIO. Gene expression of WAT leptin and hepatic Enho, Rbp4, and Inhbe strongly correlated with WAT Mest. Plasma leptin, adropin, and RBP4 also correlated with WAT Mest and predicted DIO. Early data from our ongoing study indicates that females show similar differences in the development of DIO as males. Recent data on levels of phosphorylated SMAD 2/3 also clarify the role of INHBE in variable Mest expression in obesity.

Conclusions: Plasma adropin, RBP4, and leptin show early, significant differences that prelude DIO in mice fed HFD and may influence WAT Mest expression. Data on SMAD 2/3 phosphorylation in high and low gainers shed light into a possible mechanism for MEST in obesity.

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Olajuyin Olaleye
Biomedical Science (PhD)
Fall 2022
MDIBL

Inflammatory Signaling Promotes Kidney Regeneration in Adult Zebrafish

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The human kidney is a vital organ needed to maintain body homeostasis and health. It is susceptible to damage, with dialysis and transplantation being the primary treatments. Kidney disease affects over 35 million Americans. The shortage of organ donors and long transplant waiting lists have increased the search for alternative therapies. Kidney regeneration holds immense promise in reducing mortality among kidney disease patients, yet the mechanisms governing this process remain unclear. Unlike mammals, zebrafish regenerate functional kidneys after injury because they maintain a population of quiescent stem/progenitor cells. However, the mechanisms driving the activation and differentiation of these stem cells for regeneration remain unknown.

We employed scRNA-Seq to analyze adult zebrafish kidney stem cell transcriptomes; we identified cytokine receptors, including il-6 signal transducers (gp130 and cntfr), suggesting a role for inflammatory signaling in nephron regeneration. We hypothesized that inflammatory-induced cytokine signaling played a pivotal role in activating kidney stem cells for regeneration. We performed bulk RNA sequencing on the injured kidney, revealing a cluster of inflammatory response-linked cells 7 days post-injury.

This finding prompted us to explore the role of this inflammatory expression in stem cell activation. We injected gentamicin, a nephrotoxic drug, into the zebrafish. 4 days post-injury, qPCR showed significant upregulation of pro-inflammatory markers (il-6, il-1β, tnfα). Additionally, 7 days post-gentamicin injection, we detected increased expression of lhx1a, wnt9b, and fzd9b, essential regulators of kidney stem cell aggregation and regeneration. This upregulation was corroborated with in-situ hybridization, providing direct evidence of stem cell activation in response to inflammation.

To test whether inflammatory responses, in the absence of injury, are sufficient to stimulate stem cell activation and promote new nephron formation, we administered various immune activators, including lipopolysaccharides, zymosan, and poly IC, to induce inflammation in the absence of injury, 7 days post-injection, we also detected upregulation of new nephron markers; in conclusion, our findings highlight the crucial role of inflammatory cytokines in kidney regeneration in adult zebrafish.

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Hilda Opoku Frempong
Biomedical Science (PhD)
Fall 2022
JAX

Assessing the impact of PRDM9 on mammalian genome instability using diverse mouse strains

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The onset of meiosis is characterized by the programed induction of double-stranded breaks (DSBs). DSBs play a key role in homology recognition, pairing, and recombination, but must be properly repaired to ensure stable transmission of an intact genome. In mice and humans, the location of meiotic DSBs is governed by site-specific DNA binding of PR domain containing protein 9 (PRDM9). Once bound to DNA, PRDM9 modifies local histones and recruits the DSB protein machinery. These induced DSBs are repaired via the homology mediated crossover and non-crossover pathways, ensuring genome stability. However, evidence suggests that PRDM9-associated DSBs that arise in repetitive genomic regions can also promote structural mutations via homology-driven non-allelic repair processes. These lead to hypothesis that PRDM9

shapes the genomic landscape of large-scale structural variation (SV) and contributes to karyotypic innovation in mammalian genomes.

To test PRDM9's role as an engine for SV in mammalian genomes, I aim to test for enrichment of SVs at PRDM9 programmed DSB sites across a panel of genetically diverse mice, each harboring a unique PRDM9 allele targeting unique DNA-binding motifs in the genome. Prior work from our lab have already yielded a catalog of SV calls in this strain set. DSB sites will be identified in early meiotic cells using CUT&RUN profiling assays targeting DMC1, a single-stranded DNA binding protein that localizes to PRDM9-directed meiotic DSBs. I will employ bioinformatic approaches to infer DMC1 binding sites from sequenced CUT&RUN libraries, and then test for enrichment of SVs at DSB sites. To assess the feasibility of this approach, I completed a pilot study on 8-week and 12-month-old male mice. I prepared and stained testicular cell suspensions with DyeCycle violet for fluorescence-activated cell sorting (FACS) to specifically isolate early meiotic (leptotene) spermatocytes. I confirmed the purity of the sorted cells using immunostaining for diagnostic cell-stage markers (i.e., synaptonemal complex 3 (SYCP3) and (H2A histone family member X (H2AX)). While cell number yields were lower than expected, I successfully obtained 30,000 and 15,000 cells which prior studies suggest will be sufficient for downstream CUT&RUN library preparation and analysis. I am currently working on optimizing the DMC1 CUT&RUN assay and downstream bioinformatic analysis pipeline. Ultimately, my goal is to correlate sites of DMC1 enrichment with SV breakpoints in genetically diverse mouse strains to elucidate the impact of PRDM9 on mammalian genome instability.

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Andrew Ouellette
Biomedical Science (PhD)
Fall 2019
JAX

Life-long dietary restrictions have negligible or damaging effects on late-life cognitive performance: A key role for genetics in outcomes

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Several studies report that caloric restriction (CR) or intermittent fasting (IF) can improve cognition, while others report limited or no cognitive benefits. Here, we compare the effects of 20% CR, 40% CR, 1-day IF, and 2-day IF feeding paradigms to ad libitum controls (AL) on Y-maze working memory and contextual fear memory (CFM) in a large population of Diversity Outbred mice that model the genetic diversity of humans. While CR and IF interventions improve lifespan, we observed no enhancement of working memory or CFM in mice on these feeding paradigms, and report 40% CR to be damaging to recall of CFM. Using Quantitative Trait Loci mapping, we identified the gene Slc16a7 to be associated with CFM outcomes in aged mice on lifespan promoting feeding paradigms. Limited utility of dieting and fasting on memory in mice that recapitulate genetic diversity in the human population highlights the need for anti-aging therapeutics that promote cognitive function, with a neuronal monocarboxylate transporter encoded by Slc16a7 highlighted as novel target.

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Rebecca Peters
Biomedical Science (PhD)
Fall 2022
MHIR

Atenolol increases circulating P1NP during parathyroid hormone treatment, but does not improve femoral bone outcomes in female C57BL/6J mice

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Atenolol, a β1-adrenergic receptor selective antagonist, is currently being tested in a clinical trial to prevent osteoporosis in postmenopausal women. In a short-term randomized trial in postmenopausal women, atenolol was found to be more protective against bone loss than the non-selective β-blocker propranolol and significantly reduced bone turnover markers, CTX-1 and P1NP. In our previous mouse studies, propranolol promoted bone formation and limited resorption during anabolic PTH treatment. However, whether atenolol may have comparable effects is unknown. The majority of previous preclinical research on β-blocker effects on bone utilized propranolol, therefore we first needed to determine if atenolol dosing would result in exposure levels similar to human serum concentrations (2.26 M). C57BL/6J mice were sacrificed 1 hour after single-dosing (10 mg/kg) via oral gavage, and we found an average exposure of 1.63 µM within the serum and 2.87 µM in the marrow using LC-MS/MS. To determine if atenolol would improve PTH effects on bone, female C57BL/6J mice were treated from 16-20 weeks of age with either vehicle, PTH (80 µg/kg), atenolol (10 mg/kg), or atenolol and PTH. Using µCT, we analyzed trabecular bone within both the primary and secondary spongiosa as well as cortical bone in the femoral midshaft. Overall, PTH increased bone within all regions analyzed as expected. However, atenolol did not alter femoral bone microarchitecture or modulate the response to PTH treatment (N=7-8). Neither serum CTX-1 or P1NP levels were significantly altered by atenolol alone. CTX-1 tended to be increased (p=0.12) and P1NP levels were significantly increased (p=0.0015) with PTH treatment as expected. Interestingly, atenolol and PTH co-treatment increased P1NP levels beyond that of PTH alone (Interaction p=0.0037, pairwise p=0.0173, N=4-7). Future studies will analyze bone microarchitecture within the lumbar vertebrae to investigate if atenolol's effects on bone are site-specific. This work may support the combined use of -blockers with PTH for bone anabolism.

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Rajat Rai
Biomedical Engineering (PhD)
Summer 2023
UMaine

Physiology-guided Classification of Primary Open-Angle Glaucoma Eyes Based on Systolic Velocity Deviation in the CRA

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The multi-factorial nature of primary open angle glaucoma (POAG) adds to challenges related to both diagnosis and treatment. For instance, studies have failed to establish target values of factors such as intraocular pressure (IOP) and blood pressure (BP) that can be considered safe. Here, we leverage the peak systolic velocity (CRAsys) measured using color doppler imaging (CDI) in combination with a mathematical model based on physiological principles to characterize POAG eyes and identify those at a higher risk.

Methods: Over 900 POAG-diagnosed eyes from the Indianapolis Glaucoma Progression Study (IGPS) with measurements of CRAsyS using CDI were taken. Using a mathematical model based on the physiology of healthy eyes, the individualized IOP, BP. and heart rate (HR) values were used as inputs to estimate the CRAsys that would be expected from a standard healthy eye. The values estimated by the model were then compared with CDI-measured values of CRAsys for each eye in IGPS. The eyes were then classified into three groups based on the difference between the model-estimated and the CDI-measured values [Fig.1]. Finally, clinicaly measured structural and functional markers were compared among these groups using a Kruskal-Wallis test.

Results: Table 1 shows the median values of the markers in all three groups as well as the results of the Kruskal-Wallis test. Group 1, Group 2, and Group 3 include eyes that deviated from the model-predicted standard healthy values by less than 2 cm/s, between 2

and 5 cm/s, and more than 5 cm/s, respectively. Among the three groups, eyes are Group 1 had the highest values for Retinal Nerve Fiber Layer (RNFL) thickness, lowest cup-to-disk (C/D) ratios, and the lowest mean deviation (MD) and patterned standard deviation (PSD). In contrast, Group 3 showed the worst structural and functional markers among the groups

Conclusions: Using the values estimated by a physiology-based mathematical model on standard healthy eyes as a guideline to classify eyes, we were able to identify eyes that were significantly different both in term of structure and visual function. This shows promise in establishing a more discernable characterization of eyes that are at higher risk for glaucoma.

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Kodey Silknitter
Biomedical Science (PhD)
Summer 2020
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Investigating the role of b4gat1 as a facilitator of axon guidance and muscle development

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Understanding the relationship between neural and muscle tissues is critical for the establishment of future therapies for muscular dystrophy. The dystroglycan complex is a glycosylated, transmembrane receptor that binds to extracellular proteins and is critical for muscle, myotendinous junction, and neuromuscular junction development. Dystroglycanopathies are a subset of muscular dystrophy in which one of the 19 proteins responsible for α-dystroglycan glycosylation is non-functional. Patients with a form of dystroglycanopathy arising from mutations in B4GAT1 display brain and eye abnormalities, congenital muscular dystrophy, and a shortened lifespan. Previous studies have found that when B4gat1 is truncated in mice, they display muscular dystrophy and disrupted commissural axon guidance. While many studies regarding dystroglycanopathy focus on either the muscle tissue or neural tissue, there is a gap in understanding the relationship between these tissues and the role dystroglycan glycosylation plays in maintaining this relationship during development. To investigate this, we have generated a novel B4GAT1-associated dystroglycanopathy zebrafish model using CRISPR technology. To characterize this model, we performed immunohistochemistry, longitudinal studies, and muscle resiliency assays. We found that b4gat1-/- primary motor neurons exhibit axon pathfinding defects at 1 day post fertilization. Since muscle development relies on innervation, we expected to notice signs of muscle damage. Interestingly, b4gat1-/- larvae do not display significant signs of dystrophy. However, when treated with electrical stimulation, the b4gat1-/- larvae display a lack of muscle resiliency when compared to their wild-type siblings, indicating that maintenance of the muscle is faulty in this model. Ultimately, this model offers an opportunity to better understand the relationship between muscle and neural tissue within the context of dystroglycanopathy and further our progress of treating this disease.

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Fall 2024
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Plastic nanoparticle toxicity is accentuated in the inflamed intestinal cell model.

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Introduction. Recent advancements in cell-based models of intestinal inflammation aim to assess the impact of nanoparticles on disease progression. These models are crucial for understanding how nanoplastic—characterized by its low density and

tendency to agglomerate with lipids and proteins during in vitro digestion—affects cellular environments. Despite its potential for interaction, predictions indicate that only a small fraction, typically just a few percent, of the nanoplastic material deposits on the cell monolayer, highlighting the challenges in accurately modeling and assessing its impact on intestinal health.

Methods. In this study, we utilized a tri-culture model to investigate the effects of digested polymethacrylate (PMA) nanoparticles on intestinal barrier integrity and macrophage response. The apical compartment of the insert, featuring a fully differentiated monolayer of Caco-2 and HT29-MTX-E12 barrier cells, was exposed to either surface-functionalized PMA nanoparticles—COOH (PMA-) or NH2 (PMA+)—at low (143 µg/cm²) and high (571 µg/cm²) doses for 24 or 48 hours. Beneath this insert, in the basolateral compartment, a monolayer of macrophages, differentiated from THP-1 cells, was maintained. The tri-culture systems were evaluated under two conditions: healthy and inflamed states.

Additionally, In Vitro Sedimentation, Diffusion, and Dosimetry (ISDD) modeling was utilized to predict the deposition of PMA nanoparticles onto the cell monolayers.

Results. ISDD modeling indicated that after 24 hours of exposure, only 8% of the PMA nanoparticles were deposited onto the barrier cell monolayer, with an increase to 12% after 48 hours. For the 24-hour exposure, both low and high doses of PMA did not affect transepithelial resistance, LDH levels, or confocal staining patterns in either the healthy or inflamed models. However, after 48 hours of exposure to the high dose of PMA, the inflamed model exhibited significant disruption in the barrier cell structure, accompanied by a substantial increase in pro-inflammatory cytokine secretion by the macrophages. While the healthy model showed no significant changes, in the inflamed model, both high doses of PMA- and PMA+ led to a notable increase in LDH levels, indicating cellular damage compared to the control.

Conclusion. This study is the first to show an additive effect of nanoplastic and inflammation on the barrier integrity of an immune competent in vitro model. This result was due to both the amount of PMA applied and the duration of exposure.

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Megan Tomasch
Biomedical Science (PhD)
Fall 2019
UNE

Increased excitability of neurons expressing corticotropin releasing factor (CRF) in the central nucleus of the amygdala following neonatal trauma.

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Stressful, traumatic, and/or painful events early in life have been shown to alter both physical and psychological developmental trajectory, and result in a vulnerability to pain- and anxiety disorders later in life. This effect is often observed in adolescents who had previously spent time in the neonatal intensive care unit (NICU) and has been successfully replicated in animal models. Using a rodent model of a typical NICU experience, our lab has observed an altered response to a subsequent stressor (e.g. fear conditioning) in rats that experienced neonatal trauma, with them presenting increased anxiety-like behaviors and increased sensitivity to mechanical stimulation following the second stressor at an age considered “adolescence” for rats, PD 24. Previously we associated these behavioral changes, in males, with changes to cells expressing corticotropin releasing factor (CRF) in the central nucleus of the amygdala (CeA)—with neonatal pain resulting in a reduction of CeA-CRF expression at PD 24. Despite the reduced expression of CeA-CRF at the time of this second, activating stressor, literature suggests that these CeA-CRF+ neurons may be hyperactive. We hypothesize that neonatal trauma alters the response patterns of neurons within the CeA-CRF system, creating a pain-induced neural plasticity that primes for altered responses to future

stressors as well as increased pain sensitivity later in life. Whole-cell current-clamp recordings taken from transgenic rats expressing TdTomato in CRF+ neurons revealed that these cells display distinct firing patterns, the distribution of which was altered by neonatal trauma. Interestingly, neonatal pain also appears to create a resilience to depolarization blockade, allowing CeA-CRF+ neurons to maintain firing at higher current stimulations. Furthermore, our data show increased excitability of CeA-CRF+ cells following neonatal trauma, as evidenced by increased number of action potentials fired in response to current injections, and decreased rheobase. These changes may account for later-life changes in affective, as well as pain-related behaviors.

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Biomedical Engineering (PhD)
Fall 2022
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Maine Paper Technology: A New Way to Create Affordable Fluidic Devices

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Microfluidic devices have many different applications from diagnostic testing to biosensors; however, the production of the devices is complex, expensive, and time-consuming. By leveraging the Maine paper industry technology, we looked to develop cost-effective, mass-manufacturable fluidic devices. Specifically, three different fluidic devices were developed at the microliter/hr, liter/hr, and kiloliter/hr for creating precise emulsions, for continuous water disinfecting using an electrical field and for detecting water contaminates, respectively. The precise emulsion device produced droplets with a volume of 225 pL at a rate of 120 droplets/min. The water disinfecting device was optimized by including a surface texture that focused the electric field. The water contaminants detection device detected methylene blue dye at 5 µg/mL and produced results similar to commercial UV-Vis spectrophotometers. These results demonstrate the potential of Maine paper industry technology to produce cost-effective, mass-manufacturable fluidic devices.

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NBL1 Correlates with Kidney Disease in a Mouse Model for Alport Syndrome, but is not Causal

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Alport syndrome (AS) is a rare genetic condition that often results in progressive loss of kidney function and end-stage kidney disease. Although the causal genes for AS are well characterized, individuals with AS still display a wide range of variation in kidney function and age of onset, suggesting the presence of modifiers. Recently, several studies identified levels of neuroblastoma suppressor of tumorigenicity 1 (NBL1) in the blood to be strongly and independently associated with more severe progression of diabetic nephropathy and IgA nephropathy. We decided to investigate the role of NBL1 in a genetically diverse population of Diversity Outbred (DO) mice with X-linked Alport Syndrome (DO-XLAS) and found a significant correlation between NBL1 and kidney function. However, it is still unclear whether NBL1 is causal or consequential to kidney disease. To test causality, we created an NBL1 knockout (KO) mouse model and confirmed that heterozygous (HET) animals have significantly lower NBL1 levels in their blood compared to wildtype (WT). We then induced AS by breeding a mutated Col4a5 allele into our NBL1 KO mice and investigated differences in kidney function and damage. We did not find a difference in GFR or albuminuria between HET and WT animals, suggesting that NBL1 does not have a causal role in disease progression. We are performing a genetic analysis on 600 DO-XLAS mice to identify the drivers of increased plasma NBL1 levels and better understand the underlying mechanism and relationship with kidney

disease. NBL1 does not appear to have a causal role in disease progression and increased NBL1 levels are more likely to be a consequence of kidney disease. The exact role of NBL1 in kidney disease remains to be elucidated.

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Epithelial Morphogenesis Begins with the Cochlear Progenitors

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The structure of the cochlea is asymmetrically organized, influenced by the crosstalk of several signaling pathways during development. The sensory epithelium, the organ of Corti (OC), is comprised of inner hair cells (IHCs) and outer hair cells (OHCs), which are intercalated by support cells (SCs) across the radial axis. Cells on embryonic day (E)12.5 across the cochlear radial axis continue to proliferate, but on E14.5, only the cells in the future inner sulcus (IS) domain continue to proliferate. The Wnt pathway is essential for stem cell/ progenitor proliferation. On E14.5, Wnt secretion by PORCN is enriched in the IS domain where the “niche of proliferating/progenitor cells” is located. We predict that since the progenitors themselves secrete important morphogens, they influence cochlear epithelial morphogenesis. Through transcriptomic analysis, we identified a gene encoding a transcription factor, Mybl2 that is expressed in the progenitor niche of the cochlea between E12.5 and E14.5.

MYBL2 is known to be important for cell cycle progression in progenitor cells in other tissues but its function has not been investigated in the cochlea. Mybl2 is expressed across the radial axis on E12.5, but expression becomes restricted to the future IS on E14.5. We investigate a novel role for MYBL2 on influencing epithelial morphogenesis in the cochlea. To study the function of MYBL2, we generated Mybl2 cKO embryos. Our data show that Ki67 labeling is decreased in the IS domain of Mybl2 cKO cochleas, suggesting a decrease in proliferation. Our data also show that the size of the sensory domain, specified by JAG1 and SOX2, is increased at the expense of the IS. These data suggest that MYBL2 regulates the size of the sensory domain by repression of Jag1. By E18.5, Mybl2 cKO cochleas showed an expanded sensory epithelium with additional, ectopic IHCs.

These data demonstrate that progenitors play an important role in establishing cochlear epithelial morphology inside and outside of the progenitor niche.