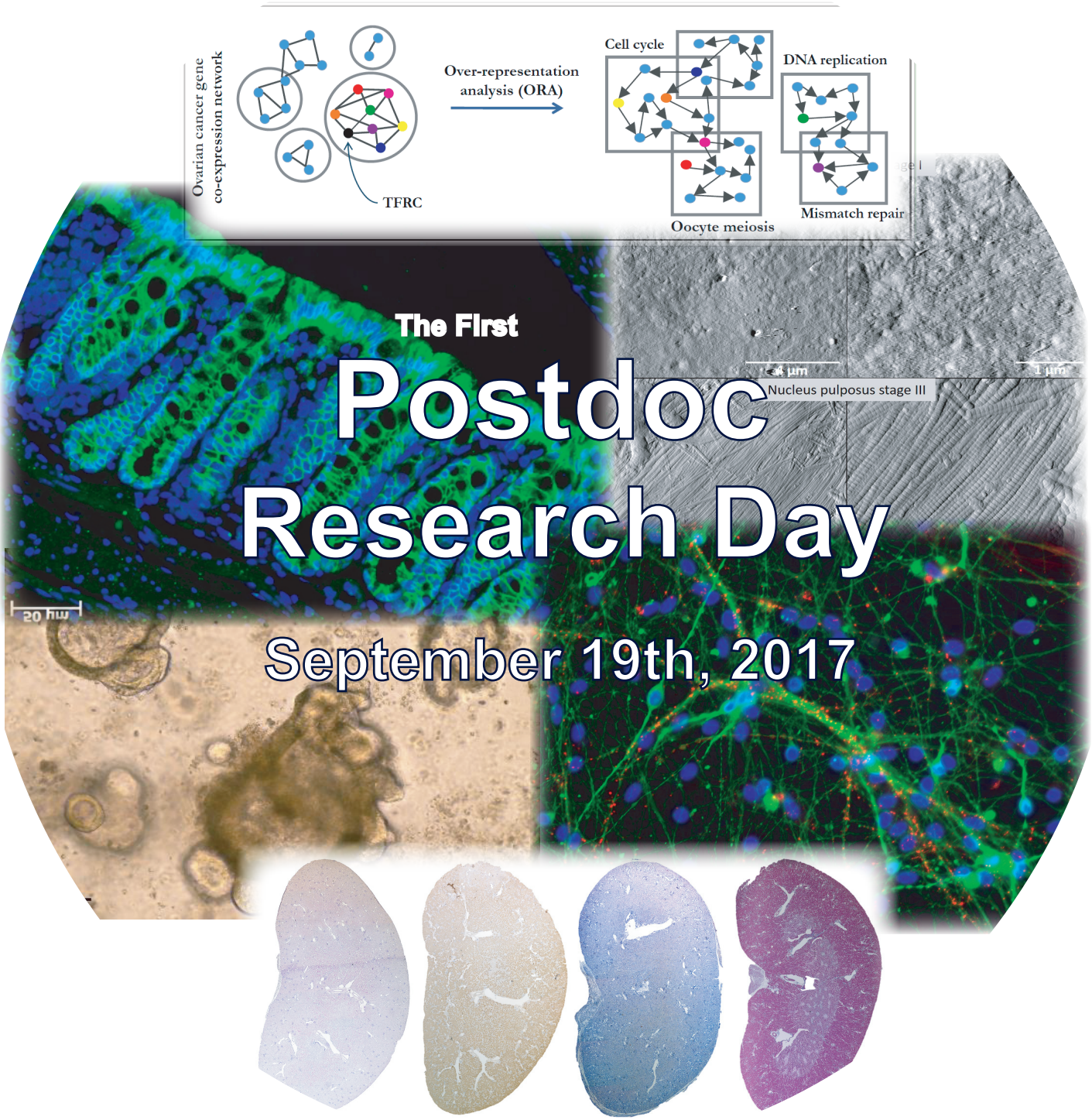


The First

Postdoc Research Day

September 19th, 2017

UCONN HEALTH

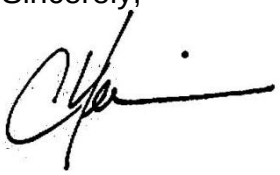
The Jackson Laboratory

Leading the search for tomorrow's cures

Welcome to the first UConn Health/Jackson Laboratory Postdoc Research Day; an event I hope will become an annual tradition. This week we celebrate National Postdoc Appreciation Week and I am delighted you have joined us today to recognize and celebrate the excellent work being done here by our postdoc community. I want to especially thank Dr. Bruce Liang, Dean of the UConn School of Medicine and Dr. Suzanne Rose, Senior Associate Dean for Education for their support for this event as well as our postdocs participating in today's sessions. I am also excited to have postdocs joining us today from The Jackson Laboratory for Genomic Medicine and the UConn Storrs campus. Finally, I am delighted to welcome back Dr. Robert Binder from the University of Pittsburgh, a former UConn Health student and postdoc.

Postdocs often toil in obscurity at universities and research institutes. Today we bring them out from behind their lab benches and computers to share their efforts with all of you. For the postdocs, I hope today they recognize they are part of a community of postdocs who share many of the same struggles and aspirations. I encourage them to become involved in our group activities such as the UConn Health Postdoctoral Association and our monthly postdoctoral seminar series. For the broader UConn Health and Jackson Laboratory communities, I hope you get to know some of the faces of our postdocs and appreciate the excellent research they do here every day.

Sincerely,



Christopher D. Heinen, Ph.D.
Director of Postdoctoral Affairs
UConn Health

We thank the following for their support:

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Cover photo credits: (Clockwise) Martinna Bertolini, Anna Konstorum, Megan Cauble, Noelle Germain, Emily Shearier, Bo Lin

The First

Postdoc Research Day

Tuesday, September 19th, 2017

1:00	**Opening Remarks**	**Academic Rotunda**
1:05	**Speak4Science Part I**	**Academic Rotunda**
2:05	**Coffee Break**	**Rotunda Lobby**
2:20	**Speak4Science Part II**	**Academic Rotunda**
3:15	**Information Session:** Christopher Stoddard – Human Genome Editing Core Ann Cowan – CCAM Microscopy Facility Abbie O'Brien – UConn Foundation Cory Brunson – Postdoctoral Association and UHP	**Academic Rotunda**
3:30	**Keynote Address:** Robert J. Binder, Ph.D. Associate Professor of Immunology Director, Microbiology & Immunology Graduate Program University of Pittsburgh "***A perspective on successful post-doctoral fellowships - the science, the mentor and beyond"***	**Academic Rotunda**
4:30	**Poster Session and Reception**	**Rotunda Lobby**
4:40	**Poster Presentations I (Odd numbers)**	**Rotunda Lobby**
5:20	**Poster Presentations II (Even numbers)**	**Rotunda Lobby**
6:00	**End**	

Our Speak4Science event will feature a series of 4-minute talks by our postdocs. Each speaker will use one slide to broadly introduce their area of research and why it excites them. To learn more about the details of their research, I encourage you to visit their posters later in the day. The speaker roster and their corresponding poster numbers are listed below.

Speakers:

Name	Title	Affiliation	Poster
1. Jitendra Kanaujiya	*Molecular Basis of Rare Bone Disorder CMD*	UConn Health	1
2. Ankita Srivastava	*The Divergent TFIIF of Trypanosomes*	UConn Health	2
3. Sandra Garrett	*CRISPR-Cas Based Immunity in* Pyrococcus	UConn Health	5
4. Varadraj Vernekar	*Growth Factor Delivery for Tendon Repair*	UConn Health	8
5. Maria Romo-Palafox	*Food Marketing and Infant Nutrition*	UConn Storrs	10
6. Jenna Bartley	*Aches, Age and Influenza*	UConn Health	14
7. Charan Devarakonda	*Cellular Stress in Atherosclerosis*	UConn Health	16
8. Alice Burghard	*Is Bigger Better? An Auditory Perspective*	UConn Health	19
9. Milena Milosevic	*Spontaneous Calcium Signals in Development*	UConn Health	21
10. Mason Yeh	*Getting Excited Over a Little Bit of Booze*	UConn Health	23
11. Prem Krishna Shrestha	*Watching Secretion As It Happens*	UConn Health	24
12. Bibbin Paul	*Mitochondria: More Than The Cell Powerhouse*	UConn Health	26
13. Martinna Bertolini	*Novel Oral Mucositis Mouse Model*	UConn Health	27
14. Floris Barthel	*Telomere Length in Cancer*	Jackson Lab	28
15. Liang Gong	*Long-Read Sequencing, Structural Variation*	Jackson Lab	29
16. Anna Konstorum	*Social Network for Ovarian Cancer Genes*	UConn Health	30
17. Kelly Teske	*Synthesis of Targeted Cancer Drugs*	UConn Storrs	32
18. Alyssa Lau	*Tumor-Specific Methylation in cfDNA*	Jackson Lab	33
19. Anil Kesarwani	*Misregulated RNA Splicing in Cancer*	Jackson Lab	31
20. Hakimeh Ebrahimi-Nik	*Neopeptides as Cancer Immunotherapy Targets*	UConn Health	36
21. Jenny Suarez Ramirez	*Environmental Factors Influencing T Cell Fate*	UConn Health	38
22. Ellen Elliott	*DOCK8 Immunodeficiency*	Jackson Lab	39
23. Michael Poe	*Phosphoantigens for Cancer Immunotherapy*	UConn Storrs	37

Keynote Presentation

Dr. Robert J. Binder, Ph.D.

A perspective on successful post-doctoral fellowships - the science, the mentor and beyond

Immunologist Robert J Binder is an associate professor at the University of Pittsburgh, Pennsylvania.

Synopsis Born in London UK and raised in Ghana, Binder was interested in biology at an early age. After studying at University of Ghana in Accra Ghana, he earned his Bachelors degree in Biochemistry and Chemistry with first class honors in 1992. He pursued his doctorate in the US and obtained his Ph.D from the University of Connecticut in 2000 under the tutelage of Professor Pramod Srivastava. Binder continued with a postdoctoral fellowship at the University of Connecticut until he was recruited to the University of Pittsburgh in 2007 to pursue his independent career. In addition to running his lab, Binder serves as the director for the graduate program in Microbiology and Immunology at PITT, the chair of the minority affairs committee of the AAI, chair of the immunology section of ABRCMS, among others.

Career Highlights As part of the Srivastava team during the early 2000's, Binder was involved in examining the cellular and molecular mechanisms of how heat shock proteins elicited immune responses. Binder was eventually able to identify and characterize the HSP receptor, CD91. This work, for the first time, showed how molecules of engoneous/self origin could trigger immune responses. Binders work has been focused on HSPs and CD91 and continues to provide seminal information on the role of these molecules in cancer, infectious diseases and autoimmunity. His current work shows that immunological fitness is, at least in part, dictated by CD91 polymorphism.

In addition to his work at the bench, Binder is passionate about diversity in science, participating in numerous roles and committees to advance and promote diversity in students at all levels in science. He is one of the few African Americans in the field.

Personal life Binder lives in Pittsburgh. Outside of his scientific area of interest, he is deeply interested in astronomy and aeronautics.

Poster Abstracts

1. Jitendra Kanaujiya – UConn Health

Degradation mechanisms of short-lived progressive ankylosis protein (ANKH) in craniometaphyseal dysplasia (CMD)

Jitendra K. Kanaujiya[1], Edward Bastow[1], Zhifang Hao[2], Ernst J. Reichenberger[1], I-Ping Chen[2]

[1]Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, UConn Health, Farmington
[2]Oral Health and Diagnostic Sciences, UConn Health, Farmington

CMD is a rare genetic skeletal disorder characterized by progressive thickening of craniofacial bones and metaphyseal widening of long bones. Autosomal dominant CMD is caused by mutations in the progressive ankylosis protein (ANKH; mouse ortholog ANK). We found that CMD mutations resulted in decreased ANKH/ANK protein expression due to rapid degradation. Here we studied its degradation mechanisms using 1) rat osteosarcoma-derived (ROS) cells overexpressing wt and CMD mutant ANK (Phe377del, S375del); 2) osteoclast from a knock-in (KI) mouse model replicating human CMD; and 3) stem cells from exfoliated deciduous teeth (SHED) of healthy controls and CMD patients.

Our immunoblots showed that inhibitors of proteasomal degradation (MG132, bortezomib, and epoxomicin) significantly rescued exogenous CMD mutant ANK in a dose-dependent manner. ANK is an ubiquitinated protein. Co-transfecting knock-out ubiquitin or mutant ubiquitin which is unable to induce proteasomal degradation (K48R) with ANK leads to increased ANK expression, suggesting the involvement of a ubiquitin-mediated proteasomal pathway. In contrast, endogenous ANKH/ANK was recovered more by bafilomycin A (BFA), an inhibitor of lysosomal degradation, than MG132. In summary, our data suggest that exogenous ANK is mainly degraded via the proteasomal pathway whereas endogenous ANK/ANKH in mouse and human cells is preferentially degraded by lysosomal degradation. We suggest that recovery of functional ANKH by preventing rapid degradation might be a therapeutic strategy to treat CMD.

2. Ankita Srivastava – UConn Health

A heterodimeric, RNA polymerase II-associated factor is required for SL RNA gene transcription, likely representing the missing transcription factor IIF in trypanosomes

Ankita Srivastava, Nitika Badjatia, Ju Huck Lee and Arthur Günzl

Department of Genetics and Genome Sciences, UConn Health, Farmington

Trypanosoma brucei is a lethal human parasite, belonging to the early-diverged, phylogenetic order Kinetoplastida. It transcribes protein-coding genes polycistronically and requires spliced leader (SL) *trans* splicing for the maturation of all mRNAs. SL RNA is a small nuclear RNA and, as *trans* splicing substrate, required in large amounts for the parasite to maintain gene expression. There are ~100 *SLRNA* gene copies on chromosome 9 which are transcribed monocistronically by RNA polymerase II from a concrete transcription initiation site. Previous studies showed that a transcription pre-initiation complex (PIC) is formed at the *SLRNA* promoter, consisting of extremely divergent orthologs of SNAPc, TFIIA, TBP, TFIIB, TFIIH/TFIIE and mediator, leaving TFIIF as the only missing general transcription factor in trypanosomes. Eukaryotic TFIIF is a heterodimeric protein complex that binds directly to RNA polymerase II, facilitating recruitment of the enzyme to the PIC. In addition, TFIIF is crucial for the formation of a stable PIC. We tandem affinity-purified RNA pol II of *T. brucei* and identified co-purified proteins by liquid chromatography-tandem mass spectrometry. We identified all 12 RNA polymerase II subunits, several transcription elongation factors and proteins known to interact with the enzyme. Additionally, we consistently detected

three proteins of unknown function. Two of them associated with the polymerase in seemingly stoichiometric amounts, indicating that they form a complex. Biochemical purification and sedimentation analysis confirmed this notion. Functional analyses *in vivo* and *in vitro* demonstrated that the complex is essential for cell viability, *SLRNA* transcription, and PIC integrity.

3. Noelle Germain – UConn Health

Investigating the efficacy of novel therapeutic approaches for restoring UBE3A expression in human Angelman syndrome neurons

Noelle Germain[1], Jack Hsiao[2], and Stormy Chamberlain[1]

[1]Department of Genetics and Genome Sciences, UConn Health, Farmington
[2]Coriell Cell Respositories, Camden, NJ

Angelman syndrome (AS) is a neurodevelopmental disorder affecting approximately 1 in 15,000 people and is characterized by severe intellectual disability, absent speech, seizures, ataxia, and a happy demeanor. AS is caused by loss of the maternally inherited copy of the UBE3A gene. In non-neuronal tissues, UBE3A is expressed from both parental alleles. However, in neurons, a long non-coding antisense transcript (UBE3A-ATS) originating from the SNURF-SNRPN promoter silences paternally inherited UBE3A. Therefore, loss of maternal UBE3A in AS results in complete absence of the protein in neurons. The intact, but silenced, paternal copy of UBE3A in these cells is an attractive therapeutic target for AS since reactivation of this copy could potentially restore proper levels of UBE3A expression and function. Our preliminary data suggests that UBE3A imprinting is controlled by a delicate balance between expression of the UBE3A-ATS and UBE3A sense transcripts. This balance may be influenced by either decreasing UBE3A-ATS or increasing expression of paternal UBE3A directly. My work aims to 1) further understand the mechanism by which UBE3A-ATS imprints UBE3A and 2) test the efficacy of novel therapeutic approaches for reactivating paternal UBE3A in our human induced pluripotent stem cell (iPSC)-derived neuron model of AS.

4. Giulia Vigone – UConn Health

Synergistic activities of multiple cyclic AMP phosphodiesterases prevent premature meiotic progression and ovulation in mouse ovarian follicles

Giulia Vigone, Leia C. Shuhaibar, Jeremy R. Egbert, Laurinda A. Jaffe

Department of Cell Biology, UConn Health, Farmington

In the mammalian ovary, luteinizing hormone (LH) acts on the granulosa cells that surround the oocyte in preovulatory follicles to cause meiotic resumption and ovulation. Both of these responses are mediated primarily by an increase in cAMP in the granulosa cells. Multiple phosphodiesterases are expressed in the granulosa cells, raising the question of which phosphodiesterases contribute to preventing uncontrolled activation of meiotic resumption and ovulation. Using selective inhibitors of PDE4, PDE7 and PDE8, we showed that each of these inhibitors alone causes no detectable increase in cAMP, whereas a mixture of all 3 elevates cAMP to a level comparable to that seen with LH. Correspondingly, inhibition of PDE4, PDE7 or PDE8 alone has little or no effect on meiotic resumption or ovulation. However, the fraction of oocytes resuming meiosis and undergoing ovulation is increased when PDE4, PDE7, and PDE8 are all inhibited. Thus 3 cAMP phosphodiesterases act synergistically to ensure that meiotic resumption and ovulation do not occur prior to the LH surge. Ovulation requires the synthesis of progesterone and progesterone receptors, and PDE4, PDE7, and PDE8 also function together to suppress their spontaneous synthesis. Our results indicate that multiple cAMP phosphodiesterases act synergistically to suppress premature responses in preovulatory follicles.

5. Sandra Garrett – UConn Health

CRISPR loci in cultured *Pyrococcus furiosus* actively acquire new spacers

Sandra Garrett[2], Masami Shiimori[1], Brenton R. Graveley[2], Michael P. Terns[1,3]

[1] Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA
[2] Department of Genetics and Genome Sciences, UConn Health, Farmington,
[3] Department of Genetics, University of Georgia, Athens, GA

CRISPR-Cas is an adaptive immune system in prokaryotes that provides sequence-specific protection against invaders like bacteriophages. The specificity is achieved by short sequences called “spacers”, which correspond to DNA derived from previously encountered invaders. RNAs are transcribed from CRISPR arrays containing these spacers and they pair with Cas defense proteins to recognize and degrade invader DNA. To develop immunity against novel invaders, the system adopts new spacers in a process termed “adaptation”: a fragment of source DNA (protospacer) is selected, processed, and integrated into the CRISPR array. Here we describe adaptation in the hyperthermophilic archaeaon, *Pyrococcus furiosus*. It incorporated new spacers from both plasmid (foreign) and host genome (self) DNA. The majority of new spacers were derived from DNA immediately downstream from a 5’-CCN-3’ protospacer adjacent motif (PAM) that is critical for invader targeting. Spacers were preferentially acquired from genome or plasmid regions corresponding to transposons, CRISPR loci, ribosomal RNA genes, rolling circle origins of replication, and areas undergoing recombination. A common feature of the highly sampled spacers is that they arise from DNA regions expected to undergo DNA nicking and/or double-strand breaks. Taken together with results from bacterial systems, our findings indicate that free DNA termini and PAMs are conserved features important for CRISPR spacer uptake. Moreover, self-targeting by CRISPR systems may also contribute to host genome stability.

6. Maeva Langouet – UConn Health

Zinc finger protein 274 regulates imprinted expression of transcripts in Prader-Willi syndrome neurons

Maéva Langouët[1,3], Heather Glatt-Deeley[1,3], Michael Chung[1], Clémence Dupont-Thibert[1], Elodie Mathieux[1], Erin Banda[1], Christopher Stoddard[1], Leann Crandall[1], Marc Lalande[1,2]

[1]Department of Genetics and Genome Sciences, UConn Health, Farmington
[2]Institute for Systems Genomics, University of Connecticut, Storrs
[3]These authors contributed equally to this work.

Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia, developmental delay and hyperphagia/obesity and is caused by the absence of paternal contribution to chromosome 15q11-q13. Using induced pluripotent stem cell (iPSC) models of PWS, we previously discovered an epigenetic complex that is comprised of the zinc-finger protein ZNF274 and the SET domain bifurcated 1 (SETDB1) histone H3 lysine 9 (H3K9) methyltransferase and that silences the maternal alleles at the PWS locus. We have knocked out *ZNF274* and rescued the expression of silent maternal alleles in neurons derived from PWS iPSC lines, without affecting DNA methylation at the PWS-Imprinting Center (PWS-IC). This suggests that the ZNF274 complex is a separate imprinting mark that represses maternal PWS gene expression in neurons and is a potential target for future therapeutic applications to rescue the PWS phenotype. Genome-wide *ZNF274* KO may not, however, represent the best strategy since ZNF274 binds across the genome where it is associated with distinct and potentially crucial functions. With this in mind, we characterized the ZNF274 binding sites in order to specifically interfere with ZNF274 binding at the PWS locus to reactivate maternal transcripts, offering a potential therapeutic treatment for PWS patients.

7. Samir Amin – The Jackson Laboratory

Genomic profiling of canine glioma: Comparative analyses with respect to drivers of human glioma
Samirkumar Amin[1], Juan Emmanuel Martinez-Ledesma[2], Beth Boudreau[3], Hoon Kim[1], Kevin Johnson[1], Amy Heimberger[2], Jonathan Levine[3], Roel Verhaak[1]

[1]The Jackson Laboratory for Genomic Medicine, Farmington
[2]The UT MD Anderson Cancer Center, Houston, TX
[3]Texas A&M College of Veterinary Medicine, College Station, TX

Gliomas are the most common (~7 per 100,000 human population) primary malignant brain tumors and typically carry grave prognosis with 5-year survival of 3-5%. Despite advances in molecular and phenotypic characterization of adult gliomas, accurate prognostication and curative treatment modalities are often limited by intratumor heterogeneity and lack of animal translational models that can faithfully recapitulate underlying oncogenic processes and treatment response to newer therapies. Spontaneous gliomas in dogs are common (5-25% in boxers) and follow disease course similar to humans with poor survival outcome. Studying canine glioma at molecular level has several merits: First, it has a distinct advantage over genetically modified animal models to study natural course of glioma in dogs. Second, breed-specific elevated cancer risk, e.g., short-nosed breeds have higher risk for gliomas and smaller effective population size compared to humans potentially allow better characterization driver elements in the development and progression of canine glioma. Finally, such spontaneous occurrence of glioma in immuno-competent dogs can be of value as a translational model system for evaluating mechanism and response to emerging immunotherapies, thereby has potential to improve otherwise poor disease outcome for both, pet dogs and humans. Towards these merits, we have undertaken the first large-scale project to comprehensively characterize both genetic and transcriptomic alterations in 60 tumors and matched normal brain tissues from canine glioma. We show frequently occurring mutations and copy number alterations in canine gliomas and compare these alterations to drivers of human glioma. Using comparative oncology approaches, we aim to locate shared mutational processes across dogs and humans and identify glioma-associated conserved risk loci using haplotype sharing analysis. We will also highlight preliminary findings to deconvolve immune-microenvironment using RNA-seq data on 41 tumors. Our comprehensive study to map molecular underpinnings of spontaneously occurring glioma in immune-competent pet dogs will pave the way not only for better understanding of drivers of glioma but also should optimize potential preclinical immunotherapy trials towards a common objective of better survival outcome in both species.

8. Varadraj Vernekar – UConn Health

Engineered polymeric matrices with controlled protein release for rotator cuff tendon repair
Varadraj N. Vernekar, Anupama Prabhath*, Sangamesh G. Kumbar, Cato T. Laurencin*

Institute for Regenerative Engineering, Department of Orthopedic Surgery, UConn Health, Farmington
*Equal author contribution

Rotator cuff tendon tears result in more than 75,000 surgical procedures annually. Surgical repair yields sub-optimal results, with a 94% failure rate for large tears. This high retear rate is attributed to acellularity, poor vascularization, increased fibrosis, and high stress concentration at the tissue repair site. Surgical repair along with growth factor has shown promising results in animal models. However, growth factor use is limited by short half-life and mode of delivery. In situ growth factor delivery via a polymeric matrix can both protect the growth factor bioactivity as well as release them in a controlled manner at the different stages of tissue repair. However, traditionally used polymeric protein-delivery systems face the challenges of uncontrolled release, acidic degradation products, and incompatible mechanical properties. Therefore, we developed a novel flexible, suturable, and biodegradable PLA-CL polymeric matrix that is able to release proteins in a controlled manner for a period of 4 weeks, with minimal change in pH. The pliability of this

matrix can facilitate surgical handling and manipulation, and withstand tendon mechanics. This matrix will be used to evaluate the augmentation of surgical repair via growth factor delivery in a rat rotator cuff tendon tear model.

9. Rebecca Acabchuk – UConn Storrs

Yoga for chronic low back pain: Investigating mechanisms of action and adherence to home practice

Rebecca L. Acabchuk[1,2], Crystal L. Park[3], Divya Ramesh[3], Angela Starkweather[3]

[1]Department of Psychological Sciences, University of Connecticut, Storrs
[2]Institute for Collaboration on Health, Intervention, and Policy (InCHIP), Storrs
[3]Center for Advancement in Managing Pain, School of Nursing, Storrs

Objective: To investigate theory-based mechanisms of action of yoga for chronic low back pain (CLBP) using both quantitative and qualitative assessments, and explore what factors facilitate the development of a regular home practice.
Methods: 12 adults ranging from age 20 to 64 reporting chronic low back pain for 3+ months participated in a 12-week yoga intervention (1x/week for 1 hour) that used Iyengar-style alignment cues and integrated various meditation techniques; material was provided to help establish a daily home yoga practice (i.e., custom video, pose manual, homework log, and written instructions).
Results: All participants who completed the study (*N*=9) adhered to study requirements, attended weekly classes and successfully established a regular home practice despite obstacles. Satisfaction levels were very high; participants reported improved physical movement, reduced stress levels, reduced pain and no adverse reactions. Effect sizes were large for the Brief Pain Inventory (Cohen's *d*= 0.97), medium for self-efficacy (Cohen's *d* = 0.79), small to medium for Quantitative Sensory Testing (Cohen's *d* = 0.28 to 0.69), and small for the Emotion Regulation Questionnaire-Suppression subscale (Cohen's *d* = 0.36). No effects appeared for the Five Facet Mindfulness Questionnaire and additional scales of emotion regulation. Qualitative findings explore mechanisms from a participant perspective, and provide insight into how best to assist participants in developing a daily home practice.

10. Maria Romo-Palafox – UConn Storrs

Parental attitudes about feeding young children

Maria J. Romo-Palafox, Jennifer Harris

Rudd Center for Food Policy and Obesity, University of Connecticut, Storrs

National efforts to improve the diets of young children must recognize the intersectionality between social economic status, culture and ethnicity as well as the role of marketing of baby and toddler food products. Food preferences and eating habits established before age 5 are behaviors that track into adulthood and can determine lifelong health outcomes. Parents of 6-36 month olds (n=1746) surveyed and results highlight the need to focus on helping parents of young children decide which foods to introduce to their toddlers at an early age. Alarming results include toddlers that didn't receive any fruits (5%) or vegetables (20%) in the past month, while half (49.8%) received some kind of sweet. Most toddlers (82%) had 100% fruit juice in the past month, 37% fruit drinks, 11% soda and one-quarter (27%) consumed Pediasure. Most of the parents surveyed agreed with the expert recommendations that toddlers should consume fruits and vegetables daily (92%), that children under 2 should not consume drinks with added sugars (72%), and consume plain whole milk (74%). However, most parents also agreed with marketing claims made by baby and toddler food companies that do not conform to expert recommendations.

11. Megan Cauble – UConn Health

Disc degeneration induced by annular puncture alters the nano- and microstructure of extracellular matrix in murine intervertebral disc

Meagan Cauble[1], Nick Mancini[1], Judy Kalinowski[1], George Lykotrafitis[2], and Isaac Moss[1]

[1]Department of Orthopedic Surgery UConn Health, Farmington
[2]Department of Biomedical Engineering, University of Connecticut, Storrs

Degeneration of the intervertebral disc (IVD) is a common condition closely linked to the development of debilitating low back pain and degenerative disc disease (DDD). The structural mechanisms involved in the development of DDD are not well understood at the micro and nano scale. Characterization of changes to the IVD extracellular matrix (ECM) will lead to better understanding of the degenerative process and will aid in the design of future disease-modifying treatments. Using a murine annular puncture model of IVD degeneration, atomic force microscopy (AFM) imaging revealed progressive changes to ECM architecture. In the annulus fibrosus (AF), progressive degeneration resulted in serpentine bundles of collagen fibrils at the micron scale with no changes to fibril structure at the nanoscale. In the nucleus pulposus (NP), more severe grades of degeneration were associated with a higher density of collagen fibrils with a defined D-spacing. Areas with fibrils aligned in the same direction within the NP were only observed in grade III degeneration. The change in ECM structure induced by annular puncture of the IVD is a previously unrecognized aspect of the impact of disc degeneration and can be used to assess the effectiveness of therapeutics for DDD.

12. Ninna Shuhaibar – UConn Health

Odontoblast processes in developing teeth investigated by serial section electron microscopy

Ninna Shuhaibar[1,2], Arthur Hand[1,2] and Mark Terasaki[2]

[1]Department of Skeletal, Craniofacial and Oral Biology, UConn Health, Farmington
[2]Department of Cell Biology, UConn Health, Farmington

Introduction - The tooth is composed mostly of dentin, the highly mineralized collagenous matrix. In contrast to enamel, dentin undergoes growth throughout life and is essential for limiting damage to and protecting the tooth pulp. The odontoblasts are the cells that form and maintain the dentin. The odontoblasts deposit the predentin and subsequently mineralize it around thin, branching cellular processes. These are called odontoblast processes, and are present within a dentinal tubule. We are investigating the three-dimensional structure of the odontoblast processes, using serial section electron microscopy.

Methods - We used incisor and molars dissected from cardiac perfused 5 week old wildtype mice. After decalcification, we cross-sectioned the hemimandibles, and split them into 5 segments using the 1st, 2nd and 3rd molar as a reference (Smith and Nanci, 1989). We sectioned the tissue with an ultramicrotome (75 nm thick), collected the sections with a tape collector, and imaged them with a SEM.

Results - Our preliminary results indicate that the shape of the odontoblast process in the incisor is a plate, and not a tubule as the textbook figures indicate. We are now testing whether this is true in molars as well.

13. Emily Shearier – UConn Health

Proximal tubule proteins as urinary biomarkers of early renal damage in murine models of obstruction and children with congenital UPJO

Emily Shearier[1], Claire Gerber[1], Miriam Harel[2], Robin Lo[1], Fernando Ferrer[2], Linda Shapiro[1]

[1]Center for Vascular Biology, UConn Health, Farmington
[2]Pediatric Urology Associates

Ureteropelvic junction obstruction (UPJO) is a form of congenital nephropathy that affects 1 in 500 children[1]. This blockage of the ureter results in a wide range of damage to the kidney, resulting in a need for a reliable urine biomarker. The backpressure within the kidney can damage the delicate structures including the proximal tubule brush border. Based on proteomic analysis of brush border lysates, three metallopeptidases (CD10, CD13, and CD26) were chosen to investigate. Each of the metallopeptidases outperformed previously published biomarkers, with the levels significantly higher in the UPJO patient samples compared to the control[2]. Because in human patients it is rare for a biopsy to be collected from the renal tissue, it is difficult to make a direct comparison between urine biomarker levels and the levels of damage. To assess this relationship fully, the mouse model of unilateral ureteral obstruction (UUO) was utilized. Damage was measured using various histological techniques, and was synthesized into a single score. These scores were compared to each ELISA biomarker measurement in the ligated and unligated urine, showing the predictability of each with increasing damage, and further supported the hypothesis CD10, CD13, and CD26 are reliable biomarkers of the associated damage with UPJO.

14. Jenna Bartley – UConn Health

Influenza-induced muscle degradation: A pathway to age-associated disability

Jenna M. Bartley[1,2], Spencer R. Keilich[1,2], George A. Kuchel[1], and Laura Haynes[1,2]

[1]UConn Center on Aging, UConn Health, Farmington
[2]Department of Immunology, UConn Health, Farmington

The elderly have increased susceptibility and severity of infection with influenza (flu) being a top killer. Flu infection is limited to pulmonary epithelial cells; yet myalgia is a common symptom and elderly are at increased risk for disability post-flu. Recent studies from our laboratory were the first to demonstrate a molecular link for this interaction. We demonstrated declines in mobility and altered gait kinetics in both young and aged mice during infection with more prolonged deficits with aging. Gastrocnemius gene expression of inflammatory cytokines was upregulated with flu with more dramatic and prolonged alterations in the aged mice. Similarly, genes involved in muscle degradation and proteolysis were upregulated with infection and remained elevated for longer in the aged mice. This may indicate that flu infection is a previously unrecognized contributor to sarcopenia and frailty in the elderly. Studies just completed in our laboratory have shown that vaccination with recombinant flu nucleoprotein can partially protect mice from functional decrements and muscle gene alterations. Thus, despite decreased vaccine efficacy with aging, vaccination may be a potential strategy to prevent flu-induced disability with aging. Mechanisms are currently being investigated; however, these initial findings provide preliminary highly translational advancements to protect the aging population.

15. Iman Al-Naggar – UConn Health

The HCN channel contributes to adrenergic detrusor relaxation in an age-dependent manner

Al-Naggar I.M.[1], Hardy C.C.[1,2], Taweh O.[3], Kuchel G.A.[1] and Smith P.P.[1,4,5]

[1]UConn Center on Aging, Farmington
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[3]University of Connecticut, Storrs
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The Hyperpolarization- activated Cyclic Nucleotide- gated (HCN) ion channel has recently been described in the bladder, where it may participate in detrusor muscle tension-setting. In other tissues, loss of HCN with aging has been linked to functional declines. We therefore hypothesized that HCN has an age-sensitive expression profile and functional role in adrenergic-induced bladder relaxation. HCN mRNA and protein levels were measured in bladders from young (2-6 m.) and old (18-24 m.) C57Bl/6 female mice, using qRT-PCR and Western blots. Functional testing was conducted by isometric tension studies using urothelium-intact bladder strips from old and young WT and young HCN1KO female C57/Bl6 mice. The impact of 1 µM isoproterenol in the absence and presence of the HCN blocking agents (5mM CsCl and 50µM ZD7288) on tension and spectral content were compared among these groups. HCN1 is dominant in young bladders. Both HCN1 mRNA and protein levels were significantly lower in old bladders. Pharmacological HCN blockade significantly inhibited isoproterenol-induced bladder strip relaxation in WT, but not KO strips. In contrast, blockade of HCN in old bladders enhanced isoproterenol-induced relaxation. We conclude that HCN represents an age-sensitive determinant of bladder responses to sympathetic stimulation. With advancing age, changes in bladder HCN expression could contribute to diminished sensitivity leading to disorders of urine storage and voiding.

16. Charan Devarakonda – UConn Health

CD13 deficiency leads to bigger and vulnerable atherosclerotic plaques

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Atherosclerosis is an inflammatory cardiovascular disorder affecting millions of people worldwide. CD13 is an aminopeptidase that plays a role in trafficking of immune cells, homotypic adhesion as well as in angiogenesis. Based on this knowledge, we wanted to determine the role of CD13 in atherosclerosis. To this end, CD13+/+ LDLR-/- and CD13-/- LDLR-/- (low density lipoprotein receptor) mice were fed regular or high fat diet for 9 or 12 or 15 weeks. At the 15-week time point, CD13-/- LDLR-/- mice had bigger lesions and larger necrotic areas. To understand the cellular mechanisms, CD13+/+ and CD13-/- bone marrow derived macrophages (BMDMs) were incubated with highly oxidized low density lipoprotein (oxLDL). CD13+/+ and CD13-/- BMDMs were found to endocytose similar amounts of oxLDL, but the subsequent generation of reactive oxygen species (ROS) was higher in CD13-/- BMDMs in comparison to CD13+/+ BMDMs. Additionally, CD13-/- BMDMs were found to have reduced autophagy, rendering them more susceptible to oxidative stress. This combination of increased levels of ROS as well as reduced autophagy in CD13-/- BMDMs led to an increase in the number of apoptotic cells. Therefore, lack of CD13 led to an increased susceptibility to ROS and reduced efficiency in handling oxidative stress.

17. Ashley Groshong – UConn Health

Peptide uptake is essential for *B. burgdorferi* growth and is facilitated via a complex and precisely regulated oligopeptide system

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Borrelia burgdorferi (*Bb*), the Lyme disease spirochete is an extreme amino acid auxotroph. The spirochete's genome encodes an expansive oligopeptide transport system: five substrate-binding proteins (OppAs), two heterodimer permeases, and a heterodimer nucleotide binding domain (NBD). Here we undertook a comprehensive analysis to elucidate how it enables *Bb* to meet amino acid requirements throughout the enzootic cycle and assess the importance of the system for *Bb* viability. We determined that the OppAs are differentially and individually regulated. Structural modeling of the OppAs, based on the crystal structure of *Bb* OppA4, demonstrated that the OppAs have unique features that theoretically enable each OppA to bind a different range of peptides. To evaluate the necessity of the Opp system for *Bb* viability, we generated a conditional IPTG-inducible NBD mutant. The NBD mutant confirmed that peptides are required for viability *in vitro* and *in vivo*. Peptide-starved spirochetes exhibited an elongated morphotype lacking division septa, often with flattening of the planar wave form at the cell centers. This is the first demonstration that the Opp system is essential for Bb as well as the first evidence that the Opp system is the primary network and peptides are the primary source for amino acid acquisition.

18. Raj Luxmi – UConn Health

A bioactive peptide amidating monooxygenase, PAM, is released in ciliary ectosomes during sexual reproduction in *Chlamydomonas reinhardtii*

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Cilia/flagella play an important role in mediating intercellular communication by sending and receiving environmental signals in the form of ectosomes, one of several types of extracellular vesicles. Ectosomes have a unique composition and are released in a regulated manner; their function depends on the physiological state of the organism and site of origin. PAM, which produces peptides/proteins with an amidated C-terminus, is localized to ciliary and Golgi membranes in *C. reinhardtii* and in rodents. Knockdown of *PAM* expression in *C. reinhardtii* leads to deficits in ciliogenesis, suggesting a role for PAM activity in cilium generating signaling pathways. In this study, we report that CrPAM is released from cilia as a component of ciliary ectosomes. The release of CrPAM protein and activity in ectosomes is a regulated process. While CrPAM is enriched in ectosomes shed during sexual reproduction, it is depleted from ectosomes shed during the vegetative life cycle; secretion of soluble PAM during mating was not detectable. Based on published RNAseq data, CrPAM expression is upregulated during sexual reproduction. The regulated release of active PAM in mating ectosomes suggests a role for amidation during the process of mating and an evolutionarily conserved role in intercellular communication.

19. Alice Burghard – UConn Health

Hearing with an oversized inferior colliculus – is bigger better?
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The inferior colliculus (IC) is a major hub of auditory processing. After different aspects of an acoustic signal are processed in the auditory brainstem nuclei, this information converges in the central nucleus of the IC (ICC). According to the synaptic domain theory, the inputs from different auditory brainstem nuclei to the ICC cluster in specific sub-regions and, thus, form functional zones superimposed in the ICC. One approach to the detailed study of function and anatomy in the ICC sub-regions is to genetically manipulate the IC during development. In the present study, we used a mouse model with an oversized IC. Developed by Dee et al. (2016), MEK1 (also referred to as mitogen-activated protein 2 kinase 1, MAP2K1) is overexpressed in this mouse in the stem cell zone from which the IC originates. Consequently, the stem cells remain in the proliferation stage longer, thus increasing the number of stem cells, but also delaying neurogenesis. This results in more, but later developed IC neurons. We tested the hearing thresholds of MEK1 mice using click-evoked auditory brainstem response (ABR) and amplitude-modulated frequency following response (AMFR). Our preliminary results show an increased hearing threshold for MEK mice especially in the mid-frequency range. They also show a reduced amplitude growth with increasing stimulus intensity. We also measured the peak synchrony of the AMFR signal and found that synchrony was slightly degraded in most MEK1 mice in comparison to littermates. In summary, our preliminary observations of a mouse with a massively enlarged IC suggest that this structural change may result in more than one type of alteration in the circuitry of the auditory midbrain and more than one hearing phenotype. Bigger may not be better.

20. Mikhail Monakhov – UConn Health

Engineering of near-infrared genetically encoded voltage indicators
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Simultaneous optical modulation and readout of neuronal circuit activities is a promising neurotechnology, which could help us decipher how the brain's electrical signals relate to perceptual, cognitive, emotional and motor functions. During recent years, the use of genetically encoded (optogenetic) actuators such as channelrhodopsin, became overwhelmingly successful. On the other hand, genetically encoded voltage indicators (GEVIs) have not yet been satisfactorily optimized and their combination with optogenetic modulation has been difficult to achieve in practice. One major obstacle is the overlap of the spectral bands of light used to activate opsin-based actuators and at the same time excite and image available GEVIs. We propose to use novel bacteriophytochrome-based fluorescent proteins (FPs) to generate a new class of GEVIs that are excited and emit fluorescence in the near-infrared (NIR) spectrum (e.g. 720 nm). For this, we have engineered and characterized a set of spectrally diverse monomeric NIR FPs, termed miRFPs, and produced an effective FRET pair consisting of miRFP670 and miRFP720 proteins. Using this FRET pair we then designed several ratiometric FRET-based NIR GEVI constructs. We have also engineered several intensiometric NIR GEVI variants based on single NIR FPs, either miRFP703 or miRFP720. Both types of the NIR GEVI constructs demonstrated good plasma membrane localization and exhibited up to 11% ΔF/F in a response to a 100 mV depolarizing step in membrane voltage in patch-clamped HEK293 cells. To

further optimize these NIR GEVI constructs, we apply an all-optical screening platform consisting of imaging hardware and mammalian cells stably expressing optogenetic actuators.

21. Milena Milosevic – UConn Health

Spontaneous calcium signals in human neuronal progenitors

Milena M. Milosevic and Srdjan D. Antic

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Spontaneous calcium signals drive developmental processes, including proliferation, migration, and neurodifferentiation. Therefore, the cellular and molecular mechanisms underlying calcium influx through plasma membrane, calcium release from intracellular stores and all other forms of intracellular calcium signaling have a very important role in defining the response of human neuronal progenitors to the environment. Moreover, the comprehensive knowledge of these mechanisms offer a vast set of possibilities of controlled altering of cellular responses *in vitro*, understanding the driving forces of normal brain development, and possibly offering additional insight into the neurodevelopmental disorders. In this study, spontaneous calcium signals from human fetal neuronal progenitors were detected by multisite optical imaging using a membrane permeable calcium-sensitive dye Oregon Green Bapta-1 AM, LED illumination (470 nm) and two CCD cameras (Neuroplex - RedShirtImaging or Luca S - Andor). The source of ionic calcium underlying these spontaneous transients, as well as the responsible plasma membrane channels/receptors, were revealed by the application of pharmacological treatments that block voltage gated sodium channels, calcium channels, receptors of intracellular calcium stores, transient receptor potential channels, gap junctions, and connexin hemichannels. A specific silencing of individual connexin genes in developing human neurons was attempted by custom made antisense oligonucleotides.

22. Mandakini Singh – UConn Health

Studying the physiology of thin dendrites by voltage-sensitive dye and calcium sensitive dye imaging

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Thin dendrites of pyramidal neurons receive information from thousands of other neurons. The integration of these large number of synaptic inputs onto a single neuron is a complex process, underlying information processing, sensory perception, cognition and motor output. In order to understand synaptic integration in individual cortical neurons, it is necessary to understand dendritic membrane properties and the rules to summate synaptic inputs. The gap in the understanding of these processes is caused by technical limitations as thin dendrites do not tolerate standard microelectrode recordings. To overcome this problem, we utilized optical imaging of physiological signals occurring in distal dendrites. Layer 5 neurons in rodent brain slices were patched (whole-cell) and injected with voltage-sensitive dye JPW-3028, or calcium sensitive dye OGB1. Fluorescent images of dendritic contours were then projected onto the fast CCD camera. Brief pulses of depolarizing current were injected into the cell body to evoke action potentials and synaptic stimulations were used to evoke excitatory synaptic potentials and dendritic NMDA spikes, which were recorded optically in basal dendrites. We characterized both voltage and calcium waveforms underlying local dendritic voltage transients (bAPs, EPSPs and NMDA spikes) and correlated them with the somatic voltage transients recorded via standard patch microelectrode.

23. Mason Yeh – UConn Health

Interactions between ethanol and BDNF modulate presynaptic glutamate release at cortical synapses

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Acute ethanol (EtOH) exposure produces intoxication through its actions on synaptic neurotransmission in the central nervous system. However, the precise pre- and/or postsynaptic targets of EtOH in modulating glutamatergic transmission remain to be fully elucidated. Our group and others have shown that EtOH can modulate the activity of postsynaptic NMDA receptors in CA1 of mouse hippocampus and layers 2/3 of the overlying cortex. In the present studies, we extended this work to investigate the modulation of presynaptic glutamate release by EtOH, and potential interactions with BDNF. In a separate study, we established that BDNF acts at the presynaptic terminal to rapidly potentiate glutamate release in these brain regions, an effect which is dependent on presynaptic NMDA receptors. We adapted this paradigm to probe for the role of EtOH in modulating presynaptic release of glutamate. Interestingly, the effect of BDNF on mEPSC frequency was blocked in the presence of EtOH, suggesting that EtOH may affect presynaptic release properties. Furthermore, our data suggests that presynaptic NMDA receptors are a target of EtOH. These studies may provide valuable insight to the mechanisms underlying the proper coding of information and learning and memory, which may be compromised due to EtOH consumption.

24. Prem Krishna Shrestha – UConn Health

Regulation of secretion by CRH in pituitary cells

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Corticotropes in the anterior pituitary gland are key components in the hypothalamic-pituitary-adrenal (HPA) axis that mediates the endocrine response to stress. Upon stress, the hypothalamic hormones, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) act synergistically to stimulate the release of adrenocorticotropic hormone (ACTH) from corticotropes. Acting via a cAMP-dependent pathway, CRH, the major ACTH secretagogue, depolarizes the cells, increasing cytosolic Ca^{2+} and triggering an exocytotic response. To explore the effects of CRH on regulated secretion, we turned to AtT-20 cells, a well characterized mouse corticotrope tumor cell line which utilizes two well characterized secretory pathways: the constitutive pathway for immediate secretion and the regulated pathway for storage and release in response to stimulation. As expected, these cells elevated the secretion of endogenous prohormone convertase-1 (PC1) and ACTH in response to CRH. Live cell imaging of ectopically expressed neuropeptide Y (NPY)-pHluorin, which localizes to secretory granules, also shows similar induction, but displays highly heterogeneous levels of NPY-pHluorin secretion in different regions of the cell. Furthermore, we found robust induction of regulated secretion with KCl, again differing across regions of the cell. In addition, our calcium sensor data showed CRH increased the frequency of Ca^{2+} transients in cells.

25. Peng Gao – UConn Health

Multicompartmental modeling of basal dendrites in prefrontal pyramidal neurons

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Prefrontal cortex plays a crucial role in advanced cognitive functions. Basal dendrites of prefrontal pyramidal neurons are the major targets for synaptic inputs. Therefore, they are the key for information processing in cortical networks. Previous experimental observation has shown that synaptically-induced dendrite plateau potentials bring the dendrites into a long-lasting depolarized state closer to the threshold for firing action potentials and reducing time constant. In such an "activated" state, the pyramidal cells can respond to synaptic inputs more quickly and easily, facilitating synchronization of firing. In this study, we aim to explore the membrane excitability of basal dendrites with a multicompartmental model of a Neurolucida-reconstructed prefrontal cortex layer 5 pyramidal neuron in NEURON simulator. Specifically, we are going to focus on modeling: 1) the back-propagating action potentials by tuning dendrite passive and active membrane properties to match experimental observations; and 2) the glutamate-mediated dendritic plateau potentials by tuning the AMPA/NMDA receptor properties. The receptors will be modeled both on dendritic shafts and dendritic spines. Finally, this model will be used to develop a large-scale network simulation to potentially provide insights on the impact of dendritic events on the network dynamics.

26. Bibbin Paul – UConn Health

Sideroflexin4: A key player in cellular iron metabolism

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Mitochondria are critical for many cellular functions including energy generation, biosynthesis of amino acids, phospholipids, iron-cofactors and various other metabolic intermediates. Dysfunction of mitochondria are associated with number of inborn errors in metabolism and common diseases. Despite the numerous cellular functions of mitochondria, hundreds of mitochondrial proteins lack functional annotation. Sideroflexin4 (SFXN4) is an inner mitochondrial membrane protein with limited knowledge about its function. Our studies of the mechanism of action of SFXN4 revealed that SFXN4 modulates mitochondrial respiration and iron sulfur cluster biogenesis. SFXN4 knock-out cells exhibited impaired respiratory activity and a phenotype similar to cells with defects in iron-sulfur cluster biogenesis, including increased IRP-IRE binding and a decrease in IRP1/ACO1 aconitase activity. Furthermore, SFXN4 knock-out decreased the activity of iron-sulfur cluster-containing enzymes such as mitochondrial aconitase and succinate dehydrogenase. Based on these observations, we postulate that SFXN4 is important for cellular iron homeostasis through its role in iron-sulfur cluster biogenesis.

27. Martinna Bertolini – UConn Health

Chemotherapy induced oral mucositis in mice without additional noxious stimuli

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Oral mucositis (OM) is a serious side effect of cancer chemotherapy. The pathobiology of oral mucositis remains incompletely understood due to lack of appropriate models. Existing rodent models are intraperitoneal and require radiation, chemical or mechanical injury to the chemotherapy protocol to induce oral lesions. We aimed to develop an OM mouse model that is induced solely by chemotherapy and reproduces macroscopic, histopathologic and inflammatory characteristics of the human condition. Female C57BL/6 mice were given intravenous 5-Fluorouracil (5-FU) injections every 48 hours, for 2 weeks. Epithelial histomorphometric analyses in tongue, esophageal and intestinal tissues were conducted coupled with assessment of apoptosis, cell proliferation, neutrophilic infiltration and the integrity of adherens junctions by immunohistochemistry. Neutropenia was assessed in peripheral blood and bone marrow. Tissues were analyzed for pro-inflammatory cytokines at the protein and mRNA levels. Intravenous administration triggered atrophy of the oral and esophageal epithelium, reduction in cell proliferation and increased apoptosis. Coincidental with these changes were upregulation of NF-κB, TNFα, IL-1β, GM-CSF, IL-6 and KC. Despite neutropenia, increased oral neutrophilic infiltration and reduced E-cadherin was observed in oroesophageal mucosae. We developed a novel experimental tool for future mechanistic studies on the pathogenesis of chemotherapy-induced OM.

28. Floris Barthel – The Jackson Laboratory

Systematic analysis of telomere length and somatic alterations in 31 cancer types

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Cancer cells survive cellular crisis through telomere maintenance mechanisms. We report telomere lengths in 18,430 samples, including tumors and non-neoplastic samples, across 31 cancer types. Telomeres were shorter in tumors than in normal tissues and longer in sarcomas and gliomas than in other cancers. Among 6,835 cancers, 73% expressed telomerase reverse transcriptase (TERT), which was associated with *TERT* point mutations, rearrangements, DNA amplifications and transcript fusions and predictive of telomerase activity. *TERT* promoter methylation provided an additional deregulatory *TERT* expression mechanism. Five percent of cases, characterized by undetectable *TERT* expression and alterations in *ATRX* or *DAXX*, demonstrated elongated telomeres and increased telomeric repeat–containing RNA (TERRA). The remaining 22% of tumors neither expressed *TERT* nor harbored alterations in *ATRX* or *DAXX*. In this group, telomere length positively correlated with *TP53* and *RB1* mutations. Our analysis integrates *TERT* abnormalities, telomerase activity and genomic alterations with telomere length in cancer.

29. Liang Gong – The Jackson Laboratory

Structural variation detection in cancer genome by nanopore long-read sequencing

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The heterogeneity of structural variations (SVs) in cancer genome is associated with tumorigenesis and has key impacts on therapeutic stratification. However, existing short-read based sequencing approaches are limited by specificity and accuracy to reach the robustness and resolution in delineating SV profiles and precise breakpoint junctions, which are critical for the diversity and molecular mechanism of SVs. Recent advance in real-time, nanopore-based DNA sensing has offered promises in speed, cost and read-length in genome scale characterization. The MinION sequencer can generate long reads with no theoretical length limits without relying polymerase kinetics and special library preparation. In this study, we report the establishment of nanopore sequencing platform and associated analytic pipeline Picky to exploit whole-genome SV profiling. Applying this approach in one of the Triple Negative Breast Cancer models, we identified a wide range of tandem duplications (TDs) with different structural complexity. We have validated almost 100% of the randomly selected SVs with more than one nanopore read support. Our result suggests that the MinION system can be adopt to survey SVs from large tumor collections in clinical setting with high specificity and sensitivity, which are valuable for monitoring cancer severity and treatment response.

30. Anna Konstorum – UConn Health

Identifying novel associations for iron-related genes in high-grade ovarian cancer

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Ovarian cancer (OVC) is the deadliest of all gynecological cancers, with a 5-year survival rate of ~45%. The most common subtype, high-grade serous ovarian cancer (HGSOC) is also the most deadliest, with a 5-year survival rate of _35%. It has been found that cancer cells sequester iron by increasing its influx into, and decreasing efflux out of, the cells. Moreover, iron derived from ruptured blood cells may directly contribute to the initiation and progression of OVC. While some pro-oncogenic actions of iron have been elucidated, there still remain many questions regarding how iron can contribute to cancer progression. In this project, we analyzed microarray gene expression data collected from high-grade serous ovarian cancer patients by The Cancer Genome Project (TCGA) to create an undirected, weighted network of genes connected by pairwise correlations. We used network community-detection methods to identify communities of highly correlated genes, and further investigated the composition of the communities that harbored iron-associated genes such as the iron import and export proteins, transferrin receptor (TFRC) and ferroportin (SLC40A1), respectively. Known biological pathways that the genes in these communities were members of were investigated using overrepresentation analysis. Using this method, we identified potentially novel associations between iron-associated genes and biological pathways.

31. Anil Kesarwani – The Jackson Laboratory

Misregulation of RNA splicing in human cancer

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Alterations in splicing factors and pre-mRNA splicing can result in several clonal disorders including cancers. In myelodysplastic syndromes, recurrent mutations in a core splicing factor SF3B1 and aberrant pre-mRNA splicing using cryptic 3′ splice sites (3′SSs) have been reported. Earlier, we presented a mechanistic model of how SF3B1 mutations lead to selection of cryptic 3'SS which are otherwise protected within well-defined RNA secondary structures present in the region between branch point and authentic 3'SS (Kesarwani et al., Oncogene, 2016). We identified anomalies in RNA splicing and expression of several genes including those implicated in cancer. Misregulation of RNA splicing is also widespread in human breast tumors and other cancer subtype. We developed highly scale pipeline on google cloud machine to detect on quantify splicing from TCGA datasets. Here, I will discuss about technical and practical challenges in processing bulk data and show recent findings from our work.

32. Kelly Teske – UConn Storrs

Development of azole antifungal analogues to treat cancers dependent on Hedgehog signaling

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For the treatment of different cancers there remains a need for the design of targeted therapies that, unlike standard chemotherapies, block tumor growth at precise molecular targets without causing cytotoxic effects to healthy tissue. Although known for its role in regulating cell proliferation and differentiation during embryonic development, inappropriate activation of the Hedgehog (Hh) signaling pathway has been implicated in many cancers such as basal cell carcinoma and medulloblastoma. As a result, the Hh signaling pathway has emerged as a promising target for drug intervention. Itraconazole and posaconazole are azole antifungals that have previously been identified as Hh inhibitors with the ability to decrease tumor growth in models of Hh-dependent basal cell carcinoma and medulloblastoma. Using the azole antifungal scaffold, we report specific structural modifications to develop improved analogues with enhanced activity against Hh-dependent cancers.

33. Alyssa Lau – The Jackson Laboratory

Differential methylation profiles in circulating DNA reveal tumor-specific signatures

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DNA methylation plays a critical role in development, gene regulation, and cancer pathogenesis. Unlike cancer mutations profiles, which can vary within a tumor type, DNA methylation is often more consistent, occurring in specific DNA regions. The greater stability of DNA methylation changes in cancer makes the DNA epigenetic modifications 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) promising targets for biomarker development. We focus on the diagnostic and prognostic potential of cell-free 5mC and 5hmC in liquid biopsies. Because the abundance and nucleotide context of circulating cell-free DNA (cfDNA) is highly associated with tissue origins, monitoring cfDNA provides non-invasive access to genetic

and epigenetic information of patients' tumor status. However limited amounts of cfDNA has presented a challenge in identifying and sequencing different epigenetic modification. We adopted and optimized nano-scale oxidative-bisulfite sequencing to achieve single-base resolution of 5mC and 5hmC in cfDNA. Comparing cell-free 5mC and 5hmC patterns between normal and multiple tumor types, we uncovered qualitative and quantitative differences at distinct features of genomic regions. The differential methylation patterns suggest unique gene expression pathways altered in tumor cells. Characterization of 5mC and 5hmC at base-resolution from expanded tumor collections offers potential new avenues to non-invasive diagnosis, monitoring and stratification of cancer progression.

34. Abhijit Rath – UConn Health

Functional interrogation of Lynch syndrome associated MSH2 variants using genome engineering

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Lynch syndrome (LS) is a hereditary cancer predisposition condition caused by mutations in one of the DNA mismatch repair pathway (MMR) genes (*MSH2*, *MSH6*, *MLH1*, and *PMS2*). LS patients suffer a >80% lifetime risk of developing colorectal and other extra colonic cancers. Identification of mutations by directed DNA sequencing, whose effect on gene function is not entirely obvious (variants of uncertain significance; VUS) presents substantial problem to the clinician in terms of proper classification of the disease and thus deciding therapeutic management. Unbiased assessment of impact of VUS mutations is critical to health management of LS patients. To that end, we used human embryonic stem cells to model LS by engineering site-specific VUS mutations and characterizing the impact of individual mutation on MMR function. Briefly, a panel of cell lines were created, each harboring a specific *MSH2* VUS using CRISPR-Cas9 induced DNA cleavage and incorporation of desired mutation using homology mediated repair. To compare efficacy of MMR dependent DNA damage signaling, cell survival was assessed upon challenge with DNA alkylating agent in different cell lines using MTT assay. We aim to better the understanding of functional significance of VUS mutations in order to potentially identify high risk LS patients.

35. Kevin Johnson – The Jackson Laboratory

Characterizing epigenetic intratumoral heterogeneity in glioma using single-cell reduced representation bisulfite sequencing

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Determining the cellular mechanisms that govern glioma heterogeneity can impact the development of novel therapies, protect patients from side effects of unnecessary treatment, and prevent glioma recurrence. Emerging evidence suggests that molecular subtypes in glioma, based on genotype (e.g., *IDH1* mutations) and DNA methylation profiles (glioma CpG Island Methylator Phenotype, G-CIMP), can provide clinically relevant tumor classifications. However, traditional bulk sampling of gliomas to profile molecular features fails to adequately capture the full complement of epigenomic heterogeneity in tumor cells, and may mask deadly features present in less abundant glioma cells. Therefore, single-cell epigenomic resolution is

needed to characterize the epigenetic intratumoral heterogeneity in glioma, detect rare cell populations, and to identify therapeutic vulnerabilities to prevent recurrence. To optimize our investigation of the glioma epigenome we initially performed single-cell Reduced Representation Bisulfite Sequening (scRRBS) on 134 patient-derived glioma sphere-forming cells. Our results highlight that the scRRBS assay is able to cover an average of 150,000 DNA methylation sites and is highly reproducible across biological replicates. Analyses of single-cell DNA methylation profiles from primary IDH-mutant tumors are now underway. Together, our study aims to generate a cellular hierarchy of primary IDH-mutant gliomas shaped by epigenetic programs that drive tumor growth.

36. Hakimeh Ebrahimi-Nik – UConn Health

$CD11c^{+}$ $MHCII^{int/lo}$ bone marrow-derived dendritic cells as adjuvants for neoepitope –based cancer immunotherapy

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Total splenocytes, macrophage and bone-marrow-derived dendritic cells (BMDCs) were compared for adjuvanticity in a tumor rejection assay, where a neoepitope of the BALB/c Meth-fibrosarcoma, was the immunogen. BMDCs showed the highest activity, providing 100% tumor protection. BMDCs were sorted into various sub-populations, each of which was tested individually in parallel in the same assay. The $CD11c^{+}$, $MCHII^{int/lo}$, $CD11b^{hi}$, $CD86^{lo}$, $CD40^{-}$, $CD24^{lo}$ BMDCs provided the highest tumor protection. Interestingly, these cells had higher expression of heat shock protein receptors CD91 and LOX1 as well as mannose receptors and TLRs. The data of this study also showed that dendritic cells exert their adjuvanticity by acting as antigen reservoirs or antigen donor cells as well as antigen presenting cells. These results have obvious implications for neoepitope-based human cancer immunotherapy.

37. Michael Poe – UConn Storrs

Synthesis and biological evaluation of phosphoantigens for gamma-delta T cell stimulation

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Cancer immunotherapy is an attractive option to use one's own immune system to detect and clear cancerous cells. Our approach in this field targets the activation of gamma-delta (γδ) T cell stimulation through the use of phosphoantigens. These specialized Vγ9Vδ2 T cells are known as "early responders" in fighting malignancies and are stimulated by the natural phosphoantigens *E*-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) and isopentenyl pyrophosphate (IPP). The success of synthetic analogs is limited by stability of the bisphosphates *in vivo* and internalization of the ligand to reach the binding site on the butyrophilin protein BTN3A1. Instead, our approach substitutes the bisphosphate structure with a monophosphonate which results in superior stability, in addition to the utilization of prodrug forms of the ligands to allow for rapid permeation of the cell membrane. These protecting groups are then cleaved intracellularly resulting in the active drug form. The combined alterations have led to the pivaloyl-protected phosphonate POM_2-C-HMBP, which has been shown to strongly stimulate the proliferation of γδ T cells. Herein, we present the synthesis of novel phosphoantigens and determine their ability to stimulate γδ T cell proliferation and bind to the target BTN3A1, as we investigate their effectiveness as a potential cancer immunotherapy.

38. Jenny Suarez Ramirez – UConn Health

Environmental factors influence the fate decisions of activated CD8 T cells to support anti-viral immunity

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CD8 T cells are key players for protection against viral infections. Upon antigen recognition, naïve CD8 T cells are activated and they differentiate into effector CD8 T cells (Teff) that are capable of destroying cells containing virus. Because these cells can damage the host tissues, many are eliminated when the virus is cleared while residual populations of quiescent memory CD8 T cells persist. These different subsets of CD8 T cells are identified using surface expression of homing-receptors which guide T cell migration. Some Teff cells express KLRG1 while memory cells express CD103, CD69, and CD62L. The goal of this project is to understand the mechanism that promotes and maintains KLRG1 expression on activated CD8 T cells using a cell culture system. Herein, enriched naïve CD8 T cells are activated with antigen and IL-2. After 48hrs the cells are exposed to bacterial products that induce KLRG1 expression. We aim to identify factors that change the functional properties of activated CD8 T cells which enhance immunity. Potential candidates include DAMPs or ligands of the SMAD4, the signaling pathways that act downstream of the TGFβ receptor.

39. Ellen Elliott – The Jackson Laboratory

DOCK8 is required for efficient metabolic reprogramming in activated CD4+ T cells

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Loss of *DOCK8* in human patients and mouse models results in Hyper IgE Syndrome (HIES), which is characterized by immunodeficiency and elevated levels of IgE. As a result, *DOCK8* patients display severe atoptic dermatitis, allergies, and increased susceptibility to infections. DOCK8 is an atypical guanine nucleotide exchange factor that regulates the actin cytoskeleton via activation of Rho GTPases, but it's role in T cell function is not well defined.

We have employed a *DOCK8*-null mouse model to determine the function of DOCK8 in $CD4^+$ T cells. $CD4^+$ T cell receptor stimulation activates AKT, leading to increased mTOR signaling and MYC expression. MYC induces a metabolic switch in activated T cells, increasing glucose uptake and anaerobic glycolysis. This metabolic switch increases biosynthetic precursors, and is essential for T cell growth and proliferation.

In *DOCK8*-mutant T cells, the metabolic reprogramming of activated $CD4^+$ T cells is altered, and cells fail to grow and proliferate in a normal manner. The upstream pathways that support metabolic reprogramming, including mTOR and MYC activation, are also defective in *DOCK8*-null $CD4^+$ T cells. Our future work will determine the role of DOCK8 in governing coordination of the metabolic program with T cell activation and T helper cell proliferation.

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