UCONN HEALTH
UNIVERSITY OF CONNECTICUT

The Jackson Laboratory

- ABSTRACT BOOK -

THE FIFTH POSTDOC RESEARCH DAY 2021

Welcome to the fifth annual UConn Health/Jackson Laboratory Postdoc Research Day. While I hope that having this event virtually doesn't become part of the tradition, we felt it was in the best interest of our presenters and the UCH and Jax communities to go online one more year. As always, the main event is our Speak4Science 4-minute rapid talks held on Tuesday, September 21st. This is a great opportunity to hear about the work of many of our postdocs as well as get a quick introduction to the variety of research going on around our campus. That will be followed on Wednesday, September 22nd by our virtual poster session. We are using an innovative Spatial Chat format, which will allow you to control an avatar on your screen to "walk" around to the different posters. As you come near another avatar, such as the poster presenter, you will be able to see and talk to them in real time. On the following pages, you will find a schedule for both the short talks on Tuesday, the posters on Wednesday and their abstracts.

I would like to thank the Health Center Research Advisory Council for their support of this year's event as well as Stephanie Holden, Sarah Wojiski and Catie Sevigny for their great help in putting this event together. We also wish to thank Chris Watson from Venue Audiovisuals for his assistance in managing and producing our online events. Finally, I would like to thank the enthusiastic team of postdocs from our PDRD Planning Committee:

Sherli Koshy-Chenthittayil (UConn Health)
Roberto Vazquez Munoz (UConn Health)
Feyza Yilmaz (The Jackson Laboratory)

Thank you for joining us in this celebration of our postdoctoral fellows and the great work they do to drive the research mission at our two institutions. I hope we can all be together in person again soon.

Be safe and be well,

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

The Fifth

Postdoc Research Day(s)

Tuesday, September 21st, 2021

1:00 – 3:00 **Speak4Science Rapid Talks**

Wednesday, September 22nd, 2021

3:00 – 4:00 **Virtual Poster Session (Groups 1-4)**

4:00 – 5:00 **Virtual Poster Session (Groups 5-8)**

To join events, click here
(Password: postdoc21)

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 the virtual poster sessions on Wednesday. The speaker roster and their corresponding poster group numbers are listed below.

	Name	Title	Affiliation	Group
1	Antwine **McFarland**	*Senescent cells impact bone repair*	UCH	1
2	Wenqiang **Du**	*Scar assists excessive placenta invasion*	UCH	2
3	Mohamadmahdi **Samandan**	In vivo *printing improves would healing*	UCH	4
4	Michael **Arul**	*Injectable nanocarriers for IVD repair and regeneration*	UCH	1
5	Sherli **Koshy-Chenthittayil**	*ABMs and bacterial biofilms*	UCH	3
6	Carissa **Orlando**	*Factors affecting anxious youth outcomes*	UCH	3
7	Shir **Ginzburg**	*COVID lockdown issues*	UCH	3
8	Debolina **Ghosh**	*Migraine therapy may impair bone healing*	UCH	5
9	Binsheng **Wang**	*p21-highly-expressing senescent cells*	UCH	7
10	Lichao **Wang**	*Role of $p21^{high}$ senescent cells in insulin resistance*	UCH	4
11	Maumita **Bhattacharjee**	*Amnion hydrogel for osteoarthritis*	UCH	1
12	Takayoshi **Otsuka**	*Bottom-up approaches for regeneration*	UCH	4
13	Stefan **Sumsky**	*Network drivers of seizure termination*	UCH	5
14	Alice **Burghard**	*How to measure a phantom – testing for tinnitus*	UCH	5
15	Neeraj **Singh**	*Microglial BACE1, a phagocytic regulator*	UCH	6
16	Kevin Child	*Splicing program for heart development*	UCH	6
17	Feyza **Yilmaz**	*Structural variants in the 3q29 region*	JAX	7
18	Mikail **Dogan**	*Antibody assays for COVID19*	JAX	8
19	Duomeng **Yang**	*UBR5 promotes antiviral innate immunity*	UCH	8
20	Tingting **Geng**	*UBXN3B controls B lymphopoiesis via BLNK*	UCH	6
21	Gautam **Srivastava**	*Inhibition of Protein phosphatase-1 by I-3*	UCH	2
22	Yuan **Gui**	*HGF is a key determinant of fibrotic KLM*	UCH	2
23	Roberto **Vazquez-Munoz**	L. johnsonii *inhibits candida biofilms*	UCH	3
24	Linda **Archambault**	*Approaching biofilms from two sides*	UCH	3
25	Eunhee **Yi**	*Extrachromosomal DNA tracking in cancer*	JAX	7
26	Kevin **Anderson**	*Profiling cell state diversity in glioma*	JAX	4
27	Ali **Foroughi pour**	*Integrative deep survival analysis*	JAX	7

Virtual Poster Groups

Group	Name	Time
Group 1	Antwine McFarland Maumita Bhattacharjee Asa Thibodeau Michael Arul	3:00 – 4:00
Group 2	Hasan Baig Gautam Srivastava Yuan Gui Wenqiang Du	3:00 – 4:00
Group 3	Sherli Koshy-Chenthittayil Linda Archambault Roberto Vazquez-Munoz Carissa Orlando Shir Ginzburg	3:00 – 4:00
Group 4	Takayoshi Otsuka Kevin Anderson Mohamadmahdi Samandari Lichao Wang	3:00 – 4:00
Group 5	Debolina Ghosh Meihong Zhu Stefan Sumsky Christopher Lee/Alice Burghard	4:00 – 5:00
Group 6	Neeraj Singh Kevin Child Shyam Sah Tingting Geng	4:00 – 5:00
Group 7	Feyza Yilmaz Binsheng Wang Eunhee Yi Ali Foroughi pour	4:00 – 5:00
Group 8	Mikail Dogan Duomeng Yang Jianan Lin Diana Cadena Castaneda	4:00 – 5:00

Abstracts

1. Antwine McFarland

Bone repair by mediation of senescence cells via specifically time drug release from calcium phosphate discs specifically for elderly women

Antwine W. McFarland Jr.[1], Liisa T. Kuhn[1]

[1]Biomedial Engineering, UConn Health

Surgeons often use the bone morphogenetic protein 2 (BMP-2) product INFUSE to enhance bone healing, but side effects have slowed its use in elderly patients. A critical need to improve elderly bone healing remains. Inactivity is detrimental to their long-term health of elderly patients. Slowed bone healing in the elderly is multi-factorial involving an age associated reduction of osteoprogenitor cells, cell proliferation, and differentiation capabilities. Additionally, chronic inflammation is associated with senescent cell accumulation. Senescent cells have a senescence-associated secretory phenotype (SASP) that stimulates chronic inflammation which inhibits macrophages phenotypical change from pro-inflammatory M1 to an anti-inflammatory M2 phenotypes that resolves inflammation and guides bone formation. Senescent cells chronic inflammatory effects can be downregulated by using anti-cancer drugs identified as senolytics, such as navitoclax (ABT-263), to selectively trigger senescent cell death. An anti-inflammatory drug such as ruxolitinib (Rux), which specifically inhibits JAK pathways, is another way to block the destructive, pro-inflammatory SASP of senescent cells. Preliminary data revealed substantial evidence that the time-window of senolytic or anti-SASP drug delivery is critical for ensuring positive effects and reducing negative effects on bone healing. We hypothesize that the senolytic ABT-263 or the anti-SASP Rux can each positively impact bone healing, but they will require different optimized delivery profiles that align with their effects on senescent cells and the cells involved in bone healing. By utilizing drug loaded calcium phosphate discs that have a timed release, we anticipate that we can provide two new methods for treating slow bone healing in the elderly.

2. Wenqiang Du

Endometrial scar-induced defective decidua is a causal determinant of placenta accreta spectrum

Wenqiang Du[1], Ashkan Novin[1], Junaid Afzal[2], Yasir Suhail[1], Tannin A. Schmidt[1], Kshitiz[1]

[1]Biomedical Engineering Department, UConn Health; [2]Department of Medicine, University of California San Francisco, San Francisco, CA

Placenta accreta spectrum (PAS) disorder describes a life-threatening condition where the placenta invades too deeply into the uterus wall and leads to massive bleeding after delivery. The incidence of PAS has increased rapidly over the last decade from around 1 in 10,000 to 1 in 300 cases of delivery. However, it is yet unclear why and how PAS occurs. The worldwide incidence of PAS is directly linked with the increase in cesarean delivery, which typically leaves a scar on the uterus. We hypothesized that the aberrant decidualization around endometrial scar causes the decidua to change its phenotype from being resistive to placental invasion to being more assistive to invasion. To test this hypothesis, we developed an in vitro placenta accreta model that recapitulated the abnormally deep trophoblast infiltration, a hallmark of PAS. Our model consisted of a scar-mimetic matrix (SMM) and its physiological counterpart inspired by the rigidity, ultrastructure, and ligands of the native tissues. We performed in vitro decidualization of patient derived endometrial stromal cells (ESCs) and found that the SMM-decidualized ESCs (dESCs) were more pro-invasive to trophoblasts. SMM elevated reactive oxygen species in dESCs, which lowered their resistance to trophoblast invasion, as well as elevated matrix

metalloproteinase expression in dESCs, resulting in increased extracellular matrix degradation, thereby paving the way for trophoblast invasion. Our transcriptomic analysis also identified G-CSF as a pro-invasive determinant. Knockout of G-CSF from the dESCs significantly decreased the invasibility of trophoblasts. Our model will advance our mechanistic understanding of PAS, hence innovative therapies.

3. Mohamadmahdi Samandari

***In Vivo* Printing of Growth Factor Eluting Adhesive Scaffolds for Wound Healing**

Mohamadmahdi Samandari[1]

[1]*Biomedical Engineering, UConn Health Center*

Acute and chronic wounds affect millions of people around the world, imposing a growing financial burden on patients and hospitals. Despite the application of current wound management strategies, the physiological healing process is disrupted in many cases, resulting in impaired wound healing. While autografts are considered the “gold standard” approach for the treatment of severe skin injuries, their application is limited by variable tissue integration, incomplete functional recovery, and donor site morbidity. In this study, we demonstrate the benefit of in vivo printed growth factor eluting adhesive scaffolds for the treatment of full-thickness wounds in a porcine model. In vitro and in vivo results show that the in situ GelMA crosslinking induces a strong scaffold adhesion and enables printing on curved surfaces of wet tissues, without the need for any sutures. The scaffold is further shown to offer a sustained release of VEGF, enhancing the migration of endothelial cells in vitro. Histological analyses demonstrate that the administration of the VEGF eluting GelMA scaffolds that remain adherent to the wound bed significantly improve the quality of healing through reduced wound contraction, increased epidermal thickness, and the number of rete ridges, and reduced inflammation. It is demonstrated that improved wound healing is correlated with enhanced vascularization, causing a more regulated physiological response to the injury. The introduced in vivo printing strategy for wound healing applications is translational, easy, and convenient to use in any place, such as an operating room, and does not require expensive bioprinters or imaging modalities.

4. Michael Arul

Injectable nanocarriers for IVD disc regenerations

Michael R. Arul[1]*, Sangamesh G. Kumbar*[1]

[1]*Department of Orthopaedic Surgery, University of Connecticut Health, Farmington, CT, USA.*

Back pain is highly prevalent today and is one of the major causes of disability, and it is related to intervertebral disc (IVD) degeneration. In particular, IVD related injuries in the US affect upwards of 5.7 million people each year. In recent years, most of the repair and regeneration of IVD tissue are carried by injectable hydrogel to promote IVD regeneration. The major drawback of injectable hydrogel is releasing the drug molecule quickly and accumulating hydrogel in the injected site. Also, its degradation is slow on the injected area and causes undesirable side effects such as inflammations and increases the pain or the sensation of swelling at the injection site. Injectable nanocarriers are promising drug delivery systems that directly deliver the drug/proteins to the injured area. It has a more prolonged effect in the active site with reduced risk of infection and easy removal from the injected site. We report the synthesis of polymeric fluorescence dye nanoparticles to load phosphatase Pleckstrin homology domain leucine-rich repeat protein phosphatase 1 (PHLPP1) in the progression of age-related intervertebral disc (IVD) degeneration. Prepared nanoparticles were characterized for particle size, SEM, FTIR, XRD, NMR, drug encapsulation efficiency, in vitro drug release, and in-vitro toxicity. The PHLPP1 was studied for the in-vivo rat model. The NMR, FTIR studies showed the drug had been encapsulated in the

polymeric nanoparticles, and the XRD study revealed the drugs had been converted into crystalline to amorphous form. The results showed that delivery PLHPP1 of the injectable nanocarriers is highly impactful in intervertebral disc (IVD) as nanoparticles showed improved bioavailability and provide controlled release of medication from a single dose to IVD.

5. Sherli Koshy-Chenthittayil

Using agent-based modeling to better understand the interactions between Streptococcus oralis and Lactobacillus paracasei in biofilms

Sherli Koshy-Chenthittayil[1], Linda Archambault[1,2], Anna Dongari-Bagtzoglou[2], Reinhard Laubenbacher[3], Pedro Mendes[1,4]

[1]Center for Quantitative Medicine and Department of Cell Biology, University of Connecticut School of Medicine, Farmington, Connecticut, USA; [2]Department of Oral Health and Diagnostic Sciences, University of Connecticut School of Dental Health, Farmington, Connecticut, USA; [3]Department of Medicine, University of Florida, Gainesville, Florida, USA; [4]Center for Cell Analysis and Modeling, University of Connecticut School of Medicine, Farmington, Connecticut, USA

This talk will provide a description of the mathematical model employed to understand multi-species microbial biofilms. Biofilms are a major source of infections on medical implants and in the oral mucosa. We use agent-based models to investigate these phenomena. In our model, the agents are the bacteria Streptococcus oralis and Lactobacillus paracasei. The agents have characteristics of growth, decay, death, and mechanical interactions. We have explored four different interactions, namely competition for nutrients and space, inhibition of growth of S. oralis by L. paracasei, production of surfactants by L. paracasei which cause both bacteria to detach from the biofilm, and the combination of inhibition and surfactant. By comparing simulations with experimental results, we conclude that simple competition does not explain the experimental observations, and that the interaction could include either inhibition of growth of S. oralis by L. paracasei or the presence of a surfactant.

6. Carissa Orlando

What Affects Child Outcomes? Investigating Additional Factors in a School Nurse-Delivered Intervention for Anxious Youth

Carissa M. Orlando[1], Isaac C. Smith[1], Anneliese DeVito[1], Thomas J. Harrison[1], Kelly Drake[2], Golda S. Ginsburg[1]

[1]Department of Psychiatry, University of Connecticut School of Medicine, Farmington, CT; [2]Anxiety Treatment Center of Maryland, Columbia, MD

As youth experiencing anxiety often present to the school nurse with somatic symptoms of anxiety, nurses can be a valuable gatekeeper for intervention. The current study utilizes data from a pilot trial of the Child Anxiety Learning Modules (CALM), a brief nurse-delivered intervention based on cognitive behavioral principles. Comparisons between CALM and an active control utilizing relaxation strategies (CALM-R) revealed significant reductions in anxiety in both conditions (Ginsburg et al., 2019). The current study investigates additional factors that may contribute to intervention outcomes, independent of the intervention delivered, including nurse characteristics (education, years of experience), intervention delivery factors (competence in delivery, intervention structure, number of sessions), and child engagement. 21 school nurses delivered either CALM or CALM-R to a total of 54 youth with clinically significant anxiety. Binary logistic and multiple regressions were conducted to examine the relationship between predictors and intervention response (measured by the Clinical Global Impression—Improvement scale [CGI-I]) and parent-report of child anxiety at post-intervention (measured by the SCARED). Analyses controlled for child age, gender, race/ethnicity, intervention group, and baseline

anxiety. No predictors were significantly associated with treatment response. Session structure was associated with a significant decrease in parent report of child anxiety post-intervention (p = .043); this suggests that youth who received interventions that incorporated more evidence-based structural elements (i.e., setting an agenda, taught/practicing a skill, assigning homework) had lower anxiety based on parent report at post intervention. These results provide preliminary information on components of successful interventions delivered by non-mental health school-based providers.

7. Shir Ginzburg

Problems Faced during the Beginning of the COVID-19 Shutdown

Shir Lerman Ginzburg[1], Stephen Schensul[1]

[1]Department of Public Health Sciences, UConn Health, Farmington, CT

At the beginning of the COVID-19 pandemic, people faced multiple issues related to the pandemic, such as loss of employment and decreased access to healthcare. There was widespread uncertainty about the nature of COVID; as such, people turned to various sources of information for updates about the pandemic. However, there was limited data on COVID-19, leading to public misinformation about the disease, which contributed to overburdened healthcare systems and shuttered jobs in the face of physical distancing requirements. In this paper, we inspect the ways in which the problems people faced during the beginning of the pandemic are associated with sources of information about the pandemic and anxiety over the uncertainty surrounding the beginning of the pandemic, as well as key demographic variables, such as gender. We conducted an online survey with Connecticut residents to assess behaviors, knowledge, and attitudes about COVID-19. We found that the pandemic disrupted education and added a significant burden of unpaid labor for women, suggesting that women and people with lower levels of education face more issues with social isolation, loss of income, and abrupt termination of access to childcare and healthcare. Furthermore, following more sources of information is likely to add to issues faced during the pandemic, as the media emphasized the lack of material resources (like toilet paper) during the pandemic, which would add to mental distress.

8. Debolina Ghosh

Inhibition of sensory neuropeptide Calcitonin gene related peptide (CGRP) signaling contributes to delayed bone healing

Debolina Ghosh[1], Natalie Wee[1], Sanja Novak[1], Ivo Kalajzic[1]

[1]Department of Reconstructive sciences, UConn Health, Farmington, CT

Pain transmission during bone injury involves the release of sensory neuropeptides including Calcitonin gene related peptide (CGRP), a 37 amino acid peptide that functions through its major receptor complex CLR (Calcitonin-like receptor), RAMP1 (Receptor Activity Modifying Protein 1) and RCP (Receptor Complex Protein). CGRP inhibition is shown to be beneficial in controlling migraine-associated pain. Since CGRP regulates osteoprogenitor proliferation, differentiation and osteoclastogenesis, our study aims to determine whether inhibiting CGRP signaling can negatively affect bone fracture healing in mice. Using αSMACreERT animals crossed with Ai9 reporter mice, we showed that CGRP expressing nerves are in close proximity to osteoprogenitor cells in the periosteum. In vitro experiments showed that periosteal cell cultures express CLR and RAMP1 and respond to CGRP stimulation by increasing cell proliferation and mineralization. To study the effects of conditional CLR deletion on fracture healing in osteoprogenitor cells, we used αSMACreERT/CLRfl/fl

mice induced by tamoxifen on -2/0/2 days post fracture (dpf) and observed decreased proliferation in fracture callus compared to Cre- mice while periosteal cultures treated with 4-OH-tamoxifen, with CGRP stimulation, led to less mineralization. MicroCT analysis of femurs from SMACreERT/CLRfl/fl mice showed that CLR deletion reduces bone mass by 17.6%. Pharmacological inhibition of CGRP with small molecule inhibitor Olcegepant (BIBN-4096, 10g/day) resulted in significantly decreased callus volume compared to placebo. Hot-plate paw-withdrawal assay at 50°C showed the inhibitor-treated cohort had significantly higher latency toward thermal nociception. Our results indicate that inhibiting CGRP, either by deleting CLR or by using small molecule inhibitor may contribute to delayed bone-healing.

9. Binsheng Wang

p21-Cre mouse model, a novel and powerful model to study p21-highly-expressing senescent cells *in vivo*

Binsheng Wang[1,2], Lichao Wang[1,2], Nathan S. Gasek[1,2], Yueying Zhou[3,4], Taewan Kim[1,2,5], Chun Guo[1], Evan R. Jellison[6], Laura Haynes[1,6], Sumit Yadav[7], Tamar Tchkonia[8], George A. Kuchel[1], James L. Kirkland[8],, Ming Xu[1,2]

[1]UConn Center on Aging, [2]Department of Genetics and Genome Sciences, [3]Xiangya Stomatological Hospital, Central South University, Changsha, China, [4]Center for Regenerative Medicine and Skeletal Development, [5]Biomedical Science Graduate Program, [6]Department of Immunology, [7]Division of Orthodontics, UConn Health, Farmington, CT, [8]Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN

The role of senescent cells has been implicated in various tissue dysfunction associated with aging, obesity, and other pathological conditions. Currently, most transgenic mouse models that investigate senescent cells only target p16Ink4a-highly-expressing (p16high) cells. Here, we generated a novel p21-Cre mouse model, containing a p21 promoter driving inducible Cre, enabling us to examine p21Cip1-highly-expressing (p21high) cells, a previously unexplored senescent cell population. p21high cells are distinct from p16high cells in a number of aged tissues, and exhibit several characteristics typical of senescent cells. By crossing p21-Cre mice with different floxed mice, we managed to monitor, sort, image, eliminate, or modulate p21high cells in vivo. Using our model, we showed that p21high cells can be induced by various conditions, and percentages of p21high cells varied from 1.5 to 10% across a number of tissues in 23-month-old mice, compared to very few (<1%) in 3-month-old mice. Intermittent clearance of p21high cells improved physical function in 23-month-old mice. Our study demonstrates that the p21-Cre mouse model is a valuable and powerful tool for studying p21high cells to further understand the biology of senescent cells.

10. Lichao Wang

Targeting p21Cip1-highly-expressing cells in adipose tissue alleviates insulin resistance in obesity

Lichao Wang[1,2], Binsheng Wang[1,2], Nathan S. Gasek[1,2], Wulin Zuo[3], Rachel Cohn[1,2], Dominique E. Martin[1], Paul Robson[2,3], Ming Xu[1,2].

[1]UConn Center on Aging, 2Department of Genetics and Genome Sciences, UConn Health, Farmington, CT; [3]The Jackson Laboratory for Genomic Medicine, Farmington, CT

Insulin resistance is a common pathological state strongly associated with obesity, representing a major risk factor for type 2 diabetes (T2D) and its complications. Nevertheless, other than exercise and diet, limited mechanism-based strategies exist to alleviate insulin resistance or its complications. Here, using single-cell

transcriptomics (SCT), we identify a small, critically important, but previously unexamined cell population, p21Cip1-highly-expressing (p21high) cells, which accumulate in adipose tissue in obese mice. By leveraging a novel transgene containing a p21 promoter driving Cre, we demonstrate that intermittent clearance of p21high cells can both prevent and alleviate insulin resistance in obese mice. A universal activation of NF-κB pathway in p21high cells is found via SCT analysis, and exclusive inactivation of NF-κB pathway within p21high cells attenuates insulin resistance. Moreover, adipose tissue transplantation experiments establish that p21high cells within visceral adipose tissue are sufficient to cause insulin resistance in vivo. Importantly, a senolytic cocktail, dasatinib plus quercetin, reduces p21high cells in adipose tissue explants isolated from obese human subjects, and mitigates insulin resistance following transplantation into immuno-deficient recipient mice. Our findings lay the foundation for pursuing p21high cells as a new therapeutic target for alleviating insulin resistance, paving the way for future clinical trials of dasatinib plus quercetin in T2D.

11. Maumita Bhattacharjee

Injectable amnion hydrogel as a stem cell delivery system for osteoarthritis treatment

Maumita Bhattacharjee[1,2], Jorge Luis Escobar Ivirico[1,2], Ho-Man Kan[1,2], Lakshmi S. Nair[1,2,3,5,6,7], Cato T. Laurencin[1,2,3,4,5,6,7,8]

[1]Connecticut Convergence Institute for Translation in Regenerative Engineering, University of Connecticut Health, Farmington, CT, USA, [2]Raymond and Beverly Sackler Center for Biomedical, Biological, Physical and Engineering Sciences, University of Connecticut Health, Farmington, CT, USA, [3]Department of Orthopaedic Surgery, University of Connecticut Health, Farmington, CT, USA, [4]Department of Chemical and Biomolecular Engineering, University of Connecticut, Storrs, CT, USA, [5]Department of Biomedical Engineering, University of Connecticut, Storrs, CT, USA, [6]Department of Materials Science and Engineering, University of Connecticut, Storrs, CT, USA, [7]Institute of Materials Science, University of Connecticut, Storrs, CT, USA, [8]Department of Craniofacial Sciences, School of Dental Medicine, University of Connecticut Health, Farmington, CT, USA

"Osteoarthritis (OA) is the most common degenerative joint disease and the leading cause of disability worldwide. Current therapies provide temporary relief but have failed to treat the disease pathogenesis or reversing OA process. There is a growing interest in the use of adipose derived stem cells (ADSCs) for OA treatment and developing biomimetic injectable hydrogels as cell delivery systems. Biomimetic injectable hydrogels can simulate the native tissue microenvironment by providing appropriate biological and chemical cues for tissue regeneration. We have developed a biomimetic injectable hydrogel using amnion membrane, which can self-assemble in situ and retain the stem cells at the target site. The unique advantages of AM such as biocompatibility, anti-inflammatory properties, and ability to accelerate wound healing make it an ideal candidate for fostering tissue regeneration. In the present study, AM was isolated from human placental tissues, decellularized and lyophilized. AM was then solubilized and subsequently modified to form an injectable hydrogel under physiological pH and temperature. The AM hydrogels were characterized to study the swelling, degradation, rheological behavior which showed that the properties of AM hydrogels could be tuned by altering the concentration. We then demonstrated the ability of injectable AM hydrogels to support ADSC viability, proliferation, and stemness. Our studies also showed that ADSCs and AM hydrogel could synergistically exert an anti-inflammatory and chondroprotective effect both in an in vitro and in vivo osteoarthritic environment. This novel ADSC-AM composite with unique chondroprotective and anti-inflammatory properties may lead to the development of a translational strategy to attenuate OA progression.

12. Takayoshi Otsuka

The application of FGF-8b for the cell fate determination

Takayoshi Otsuka[1, 2, 3], Paulos Y. Mengsteab[1, 2, 3, 4], and Cato T. Laurencin[1, 2, 3, 4, 5, 6]

[1]Connecticut Convergence Institute for Translation in Regenerative Engineering, UConn Health; [2]Raymond and Beverly Sackler Center for Biological, Physical and Engineering Sciences, UConn Health; [3]Department of Orthopedic Surgery, UConn Health; [4]Department of Biomedical Engineering, University of Connecticut; [5]Department of Materials Science and Engineering, University of Connecticut; [6]Department of Chemical and Biomolecular Engineering, University of Connecticut

Some of the genetic regulations are conserved between regeneration and embryonic development. Fibroblast growth factor 8 (FGF-8) signaling has an essential role in both limb regeneration and morphogenesis. On the other hand, its expression is rarely seen in adult mammals. Therefore, we aimed to study the effect of FGF-8b on the proliferation and differentiation of mesenchymal stem cells (MSCs) as a model of limb mesenchyme. Rat adipose-derived stem cells (ADSCs) and muscle progenitor cells (MPCs) were isolated and cultured in growth medium and various types of differentiation medium (osteogenic, chondrogenic, adipogenic, tenogenic, and myogenic medium) with or without FGF-8b supplementation. We found that FGF-8b induced robust proliferation regardless of culture medium. FGF-8b enhanced chondrogenic differentiation and suppressed adipogenic and tenogenic differentiation in ADSCs. Osteogenic differentiation was not affected by FGF-8b supplementation. FGF-8b was found to enhance myofiber formation in rat MPCs. Overall, this study provides foundational knowledge on the effect of FGF-8b in the proliferation and fate determination of MSCs. These results propose a potential application of FGF-8b in expansion and fate determination of MSCs towards complex tissue regeneration. Future studies will analyze the effect of FGF-8b under the pathological conditions to enhance complex tissue regeneration.

13. Stefan Sumsky

Network Analysis of Focal Seizure Dynamics Suggests a Possible Mechanism of Seizure Termination

Stefan Sumsky[1], John Greenfield[1]

[1]Neurology, UConn Health

Electrophysiological studies have revealed pathological processes involved in epileptiform activity, but the role of macro-scale networks in seizure initiation, propagation and termination remains unclear. Changes in network structure/function during seizure propagation and termination have not been characterized. Here, we use model-based network estimation to identify network changes during seizures. iEEG data from 10 epilepsy patients from the iEEG.org database were analyzed. Each seizure was divided into 15-second periods and each period was further divided into consecutive 5-second epochs. For each epoch, a multiple input, single output (MISO) state space model was estimated for each channel with all other channels as inputs. The influence of each other channel on the given channel was used to infer a directed network graph between all channels. The resulting networks were analyzed across seizures and patients using degree centrality. Degree was significantly higher than interictal in all regions and periods. By mid-seizure, SOZ degree fell significantly below PSZ and NSZ groups, but rose again during termination. This counterintuitive result may be explained by examining the proportion of incoming vs outgoing connections. During Initiation, the majority of connections in SOZ are outgoing to PSZ channels. This falls to roughly 50/50 incoming vs outgoing during the mid-seizure. Finally, during termination, SOZ connections are mostly from the PSZ group. This suggests a combined SOZ exhaustion and neighboring inhibition mechanism for seizure termination.

14. Christopher M Lee & Alice L Burghard

How to measure a phantom sound. The search for the electrophysiological signature of tinnitus

Christopher M Lee[1], Alice L Burghard[1], Emily M Fabrizio-Stover[1], Douglas L Oliver[1]

[1]Department of Neuroscience, UConn Health

How to measure a phantom sound. The search for the electrophysiological signature of tinnitus. How to measure a phantom – testing for tinnitus. "Tinnitus, a phantom perception of sound with no external auditory stimulus, is experienced by over 15 million Americans. Animal models of tinnitus are crucial for studies on tinnitus; however, they depend on behavioral tests to determine whether tinnitus is present. In our current study, we examined electrophysiological measures of ongoing brain activity and sound-evoked brainstem responses in sound-exposed mice, to validate two behavioral tests of tinnitus: active avoidance (AA), and gap-induced prepulse inhibition of acoustic startle (GPIAS). Tinnitus was induced in awake mice by exposure to high-level sounds (2 kHz wide, 16 kHz centered noise at 113- or 116-dB SPL, 1 hr). In mice that had evidence of tinnitus when tested with AA, neurons tuned to frequencies above the exposure frequency showed higher spontaneous firing than in tinnitus-negative mice. This difference was not evident in mice whose tinnitus status were determined with GPIAS. These results suggest that the GPIAS and AA tests are not equally valid for diagnosing tinnitus in mice, as they do not often show the same frequencies of deficit. Sound-evoked auditory brainstem responses produce characteristic voltage peaks and troughs that represent activity in specific parts of the auditory system. These may be altered following presentation of a continuous one-minute narrowband noise. However, in mice with tinnitus tested with AA, the peak-trough amplitudes of tinnitus-frequency tone responses in the exposed ear were amplified more strongly following the noise than in mice without behavioral evidence of tinnitus. This finding could allow for a quick and non-invasive test for tinnitus.

15. Neeraj Singh

Bace-1 inhibition in microglia enhances amyloid clearance via activating DAM-1 like state with no detrimental effect on synaptic plasticity

Neeraj Singh[1], Mark Benoit[1], Brati Das[1], Xiangyou Hu[1] and Riqiang Yan[1]

[1]Department of Neuroscience, UConn Health, 263 Farmington Avenue, Farmington, USA CT 06030-3401

Abnormal accumulation of β-amyloid peptides (Aβ) causes amyloid deposition and cognitive dysfunction in Alzheimer's disease (AD); thus blocking Aβ generation is being explored for AD treatment. Inhibition of Bace-1, the secretase responsible for Aβ generation, reduces amyloid plaques but fails in clinical trials, attributable to synaptic side effects. Here we demonstrate that targeted inhibition of Bace-1 in microglia has unique advantages. When Bace-1 was deleted in Alzheimer's 5xFAD mice, there were significantly fewer amyloid plaques compared to none-deleted littermates, and this reduction was not due to changes in APP processing, but rather to enhanced Aβ clearance. Using single cell RNAseq, we demonstrated that Bace-1 has unique functions in microglia and that Bace-1 regulates expression of genes important for a disease-associated microglia (DAM) signature. Specifically, conditional deletion of Bace-1 in microglia elevated percentage of microglia expressing genes expression of many genes encoding for ApoE, TREM2 and Rac-1. We also noted unique sets of transcription factors such as Jun, Jund, Btg2, Erg1, Junb and Fos and Fosb to be elevated after Bace-1 deletion in multiple lines of Bace-1 knockout mice. The elevated expression of these genes correlates with higher proportion of microglia in the stage 1 damage associated microglia (DAM-1), which appears to favor phagocytosis .manifested by the shifting of microglia from a disease-associated signature to a homeostatic signature. Conditional deletion of Bace-1 in microglia exhibited signaling gene expression that favor phagocytosis and enhance functions of autophagolysosmes. Remarkably, mice with deletion of Bace-1 in microglia showed no reduction in long-term potentiation, unlike global deletion of Bace-1. Our results suggest that targeted inhibition of Bace-1 in microglia will be a superior strategy for AD treatment.

16. Kevin Child

Identifying the Role of Alternative Splicing during Human Heart Organogenesis

K. Child[1], T.N. Yankee[1], A. Wilderman[1], J. Cotney[1]

[1]Department of Genetics and Genome Sciences, UConn Health

Congenital Heart Defects (CHD) are one the most frequent congenital abnormalities affecting births worldwide. Many groups investigating CHDs have been focused on heart gene expression during fetal development and later. However, recent evidence has suggested that gene expression dynamics occurs during organogenesis encompassing the first eight weeks of human development which is classified into 23 Carnegie Stages (CS). Our lab has published extensive work detailing the nature of gene expression during these early stages focusing on CS12 to 23. Examination of gene co-expression across this window of development revealed RNA processing and splicing as a major module in this network. To identify alternative splicing events across development we reanalyzed short-read bulk gene expression from embryonic human heart and found several differential splicing events between individual Carnegie stages with enrichment for functions related to heart development, however few overlapped with our previous differential expression results calculated at the gene level. Generation of de novo transcriptome assembly using this data revealed previously unannotated isoforms, even amongst well studied heart related genes. To validate these novel isoforms, we profiled the transcriptome of these same samples using Nanopore long-read sequencing. We validated a number of these novel isoforms including a TBX5 transcript which utilizes previously unannotated 5' exons. These exons could be important for post-transcriptional regulation of TBX5 and be an additional region to screen for in Holt-Oram Syndrome patients. Overall, our analysis identifies previously unknown transcript diversity in the developing human heart which could help explain previously undiagnosed genetic causes of CHD.

17. Feyza Yilmaz

An unprecedented level of complexity in the 3q29 region of the human genome.

Feyza Yilmaz[1], Umamaheswaran Gurusamy[2], Yulia Mostovoy[2], Trenell Mosley[3], Tamim H. Shaikh[4], Mike Zwick[3], Pui-Yan Kwok[2], Charles Lee[1], Jennifer G. Mulle[3]

[1]The Jackson Laboratory for Genomic Medicine; [2]Cardiovascular Research Institute, University of California San Francisco School of Medicine [3]Emory University [4]Department of Pediatrics, Section of Clinical Genetics and Metabolism, University of Colorado School of Medicine

Genomic disorders disrupt dosage-sensitive genes and result in deletion and duplication syndromes. At the 3q29 interval, non-allelic homologous recombination (NAHR) between highly identical copies of segmental duplications (SDs) can lead to deletion or duplication of a 1.6 Mb segment, causing somatic, neurodevelopmental, and psychiatric phenotypes. Risk factors contributing to NAHR at this locus are not understood. We used an optical mapping approach to characterize structural variation at the complex human 3q29 genomic region. Using 16 probands with 3q29 deletion syndrome, two probands with 3q29 duplication syndrome, and 164 phenotypically normal individuals from 26 worldwide populations, we uncovered 19 new haplotypes, increasing the total number of known 3q29 haplotypes by 51%. We show there is significant variation in haplotype prevalence between populations, supported by GRCh38 and recently published telomere-to-telomere haplotypes. Long-read sequencing data offered orthogonal support for 22 haplotypes, including 11 novel haplotypes identified by optical mapping data. In 3q29 deletion/duplication probands, we localized CNV breakpoints to specific paralogous duplicons located within complex SDs and categorized breakpoints into five distinct classes: three deletion and two duplication breakpoint classes. The population-level data presented here highlight the extreme diversity of large and complex SVs within SDs of the complex human 3q29 region. Our

study is the most extensive study revealing the number of haplotypes in the GRCh38 3q29 region. The results from our research will significantly facilitate the investigation of the role of inter-SD structural variation as a driver of chromosomal rearrangements and genomic disorders.

18. Mikail Dogan

SARS-CoV-2 specific antibody and neutralization assays reveal the wide range of the humoral immune response to virus

Mikail Dogan[1], Lina Kozhaya[1], Lindsey Placek[1], Courtney Gunter[1], Mesut Yigit[1], Rachel Hardy[1], Matthew Plassmeyer[3], Paige Coatney[3], Kimberleigh Lillard[3], Zaheer Bukhari[4], Michael Kleinberg[5], Chelsea Hayes[6], Moshe Arditi[7], Ellen Klapper[8], Noah Merin[9], Bruce Tsan-Tang Liang[10], Raavi Gupta[4], Oral Alpan[3] & Derya Unutmaz[1,2]

[1]Jackson Laboratory for Genomic Medicine, Farmington, CT, USA; [2]Department of Immunology, University of Connecticut School of Medicine, Farmington, CT, USA; [3]Amerimmune, Fairfax, VA, USA; [4]SUNY Downstate Medical Center, Department of Pathology, Brooklyn, NY, USA; [5]Calhoun Cardiology Center, University of Connecticut School of Medicine, Farmington, CT, USA; [6]Department of Pathology & Laboratory Medicine and Transfusion Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [7]Department of Pediatric, Division of Pediatric Infectious Diseases and Immunology, Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [8]Department of Pathology & Laboratory Medicine and Transfusion Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA; [9]Department of Internal Medicine, Division of Hematology Cedars-Sinai Medical Center, Los Angeles, CA, USA; [10]Calhoun Cardiology Center, University of Connecticut School of Medicine, Farmington, CT, USA

Development of antibody protection during SARS-CoV-2 infection is a pressing question for public health and for vaccine development. We developed highly sensitive SARS-CoV-2-specific antibody and neutralization assays. SARS-CoV-2 Spike protein or Nucleocapsid protein specific IgG antibodies at titers more than 1:100,000 were detectable in all PCR+ subjects (n = 115) and were absent in the negative controls. Other isotype antibodies (IgA, IgG1-4) were also detected. SARS-CoV-2 neutralization was determined in COVID-19 and convalescent plasma at up to 10,000-fold dilution, using Spike protein pseudotyped lentiviruses, which were also blocked by neutralizing antibodies (NAbs). Hospitalized patients had up to 3000-fold higher antibody and neutralization titers compared to outpatients or convalescent plasma donors. Interestingly, some COVID-19 patients also possessed NAbs against SARS-CoV Spike protein pseudovirus. Together these results demonstrate the high specificity and sensitivity of our assays, which may impact understanding the quality or duration of the antibody response during COVID-19 and in determining the effectiveness of potential vaccines.

19. Duomeng Yang

UBR5 restricts antiviral innate immunity

Duomeng Yang[1], Tingting Geng[1], Andrew G. Harrison[1], Jason Cahoon[1], Penghua Wang[1]

[1]Department of Immunology, UConn Health center, Farmington, CT 06032

When invaded by a virus, a host cell produces a rapid innate immune response initiated by pathogen pattern recognition receptors (PRRs), of which the RLR family (RIG-I and MDA5) are essential for initiation of the innate antiviral immune response to a wide spectrum of RNA viruses. Once engaged by viral RNA, both RIG-I and MDA5 undergo K63-polyubiquitination, oligomerize, and bind to a mitochondrial antiviral signaling protein (MAVS), which ignites a signaling event, leading to transcription of immune genes, in particular type I/III interferons (IFN) that provide an instant protection to the host. Both RIG-I and MDA5 activity is tightly regulated

by posttranslational modifications, among which ubiquitination is one of the best characterized and most important, but still not fully defined. By an unbiased screening of an array of 374 individual ubiquitin E3 ligase knockout cells constructed in our lab, we identified Ubiquitin Protein Ligase E3 Component N-Recognin 5 (UBR5) as an essential activator of RLR signaling. RIG-I and MDA5 signaling was deficient, while the cGAS-STING signaling pathways were normal in the absence of UBR5. K63-ubiquitination of MDA5 was impaired. Recomplement of UBR5 in UBR5-/- cells specifically rescue the RIG-I and MDA-5 protein expression with or without poly I:C. Both RIG-I, MDA5 and UBR5 were essential for controlling Severe Acute Respiratory Syndrome (SARS)-CoV-2 infection in human lung epithelial cells. We herein hypothesize that UBR5 mediates activation of RLR signaling through ubiquitination and is essential for controlling EMCV/SARS-CoV-2 infection.

20. Tingting Geng

A Crucial Role of UBXN3B in B Lymphopoiesis

Tingting Geng[1], Duomeng Yang[1], Andrew G. Harrison[1], Penghua Wang[1]

[1]Department of Immunology, UConn Health, Farmington, CT 06030, USA

Hematopoiesis is finely regulated to enable timely production of the right numbers and types of immune cells to maintain tissue homeostasis. Herein, we report a crucial role of a UBX domain-containing protein UBXN3B in maintenance of hematopoietic homeostasis. Ubxn3b−/− mice have significantly fewer B cells (~10-fold), while more myeloid cells, than Ubxn3b+/+ littermates. Transfer of wild type bone marrow to Ubxn3b−/− mice corrects the B cell deficiency, while reverse transplantation does not. Mechanistically, UBXN3B is essential for precursor B-I (pre-BI) transition to pre-BII by regulating pre-B cell receptor signaling and B cell linker (BLNK) protein stability. This dysregulated immune compartmentalization renders mice highly vulnerable to SARS-CoV-2, typified by reduced production of virus-specific antibodies, increased lung immunopathology and delayed resolution of disease. These results reveal a critical role of UBXN3B in early B cell development and control of immunopathogenesis of respiratory viruses.

21. Gautam Srivastava

Regulation of Protein Phosphatase 1 by Inhibitor-3

Gautam Srivastava[1], Meng Choy[1], Rebecca Page[1], Wolfgang Peti[1]

[1]Department of Molecular Biology and Biophysics, UConn Health, Farmington, CT 06030

Protein phosphatase 1 (PP1) is the most widely expressed and abundant serine/threonine phosphatase that is exceptionally well conserved protein from fungi to humans, in both sequence and function. Dephosphorylation events catalysed by PP1 regulate cell-cycle progression, protein synthesis, transcription, neuronal signalling, among others. PP1 is tightly regulated by its interaction with >200 known regulatory proteins that function to direct phosphatase activity. Biochemical and structural studies have revealed that most of these regulators bind PP1 via ~4-8 residues short linear motifs (SLiMs), and are found within intrinsically disordered regions (IDRs) to mediate protein:protein interactions. Inhibitor-3 (I3) is a known PP1 inhibitor and one of the four most anciently conserved PP1 regulators. The deletion of I3 is lethal in vivo, which highlights its biological importance. However, how I3 binds to PP1 and how it regulates PP1 (it is proposed to be an inhibitor, but also has a function as a chaperone or metal-loader) is currently unknown. Our data have enabled us to understand how I3 interacts with PP1. As predicted, I3 binds PP1 via a canonical PP1 RVxF SLiM (40KVEW43). However, combined biochemical

and molecular data showed that I3 also uses a SLiM referred to as SILK site. Furthermore, residues 60CCC62 have a critical role in the inhibition of PP1. Taken together, our structural data shows how I3 binds to PP1 and allows for further exploration into I3 function.

22. Yuan Gui

The hepatocyte growth factor pathway is a key determinant of the fibrotic kidney local microenvironment

Yuan Gui[1] and Dong Zhou[1]

[1]Division of Nephrology, Department of Medicine, University of Connecticut School of Medicine, Farmington, CT, 06053

The kidney local microenvironment (KLM) plays a critical role in the pathogenesis of kidney fibrosis. However, the composition and regulation of a fibrotic KLM remain unclear. Through a multidisciplinary approach, we investigated the roles of the hepatocyte growth factor /c-met signaling pathway in regulating KLM formation in various chronic kidney disease (CKD) models. We performed a retrospective analysis of single-cell RNA sequencing data and determined that tubular epithelial cells and macrophages are two major cell populations in a fibrotic kidney. We then created a mathematical model that predicted loss of c-met in tubular cells would cause greater responses to injury than loss of c-met in macrophages. By generating c-met conditional knockout mice, we validated that loss of cmet influenced epithelial plasticity, myofibroblast activation, and extracellular matrix synthesis/degradation, which ultimately determined the characteristics of the fibrotic KLM. Our findings open the possibility of designing effective therapeutic strategies to retard CKD.

23. Roberto Vazquez-Munoz

Lactobacillus johnsonii displays antimicrobial activity against oral pathobionts

Roberto Vazquez-Munoz[1], Martinna Bertolini[1], Angela Thompson[1], Jordan Russell[2], Takanori Sobue[1], and Anna Dongari-Bagtzoglou[1]

[1]Oral Health and Diagnosis Sciences, University of Connecticut Health Center; [2]Department of Psychiatry/Medicine, University of Connecticut Health Center

Candida albicans is part of the mucosal microbiota; however, it can become invasive under certain conditions and cause infections (candidiasis). Candidiasis is the leading cause of death in fungal diseases. Moreover, *C. albicans* ability to form biofilms and the rise of drug-resistant strains reduce the efficacy of treatments. Candidiasis may be influenced by the mucosal microbiota. Several bacteria species from the healthy microbiota display anticandidal properties, preventing or reducing candida infections. Among these bacteria, probiotic lactobacilli have been of great interest. *Lactobacillus johnsonii* has been studied due to its properties of clinical relevance; however, its anticandidal activity has not been explored. We isolated an *L. johnsonii* strain, sequenced its genome, and evaluated its anticandidal activity *in vitro*. Our findings show that *L. johnsonii* inhibits the growth of *C. albicans* under planktonic and biofilm stages, in dual-species co-cultures, under different conditions. Cell-Free supernatants also display anticandidal activity, which, supported by the functional genomic analysis, suggest that lactobacilli produce soluble metabolites with anticandidal properties. Lactobacilli preformed biofilm reduced the ability of *C. albicans* to form biofilms. Our results indicate that *L. johnsonii* displays promising properties that reduce the growth of C. albicans.

24. Linda Archambault

Multispecies Interactions in Oral Biofilms: A Collaborative Process of Experimentation and Modeling

Linda S. Archambault[1,2], Sherli Koshy-Chenthittayil[2], Pedro Mendes[2,3], Reinhard Laubenbacher[4], and Anna Dongari-Bagtzoglou[1]

[1]Department of Oral Health and Diagnostic Sciences, University of Connecticut School of Dental Health, Farmington, Connecticut, USA; [2]Center for Quantitative Medicine and Department of Cell Biology, University of Connecticut School of Medicine, Farmington, Connecticut, USA; [3]Center for Cell Analysis and Modeling, University of Connecticut School of Medicine, Farmington, Connecticut, USA; [4]Department of Medicine, University of Florida, Gainesville, Florida, USA

"Humans are colonized by diverse microbial communities consisting of bacteria, viruses, and fungi. Increasingly, disease and modern medical treatments lead to an immunocompromised host environment and disrupted microbial communities (dysbiosis) in which normally harmless commensal microbes can interact to become the agents of disease. Many microbes form biofilms on hard (teeth) and soft (mucosal) surfaces of the oral cavity, on oral appliances and on indwelling medical devices. Biofilm structure protects microbes from immune cells and antimicrobials, making them difficult to eradicate. In this study, we used an iterative collaborative process between experimentation and mathematical modeling to reveal aspects of the mostly unexplored relationship between *Streptococcus oralis* and *Lactobacillus paracasei* in biofilm growth. In vitro imaging of biofilms revealed that when growing together, *S. oralis* growth was curtailed while *L. paracasei* growth remained robust. This difference in growth in the dual-species biofilm was not predicted by a model in which competition for nutrients was the only interaction. Through further modeling and experimentation, we are exploring 2 other possible interactions: production of a biosurfactant that could affect the attachment of both species, and production of an inhibitor that acted only on *S. oralis*. The inhibitory nature of *L. paracasei* on *S. oralis* in biofilms may be exploited as a means of preventing or alleviating mucosal infections.

25. Eunhee Yi

Live-cell imaging shows uneven segregation of extrachromosomal DNA elements and transcriptionally active extrachromosomal DNA clusters in cancer

Eunhee Yi[1], Amit D. Gujar[1], Molly Guthrie[1], Hoon Kim[1], Dacheng Zhao[1], Kevin C. Johnson[1], Samirkumar B. Amin[1], Megan L. Costa[1], Qianru Yu[1], Sunit Das[2], Nathaniel Jillette[1], Patricia A. Clow[1], Albert W. Cheng[1], Roel GW Verhaak[1]

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, 06032, USA;2Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

Oncogenic extrachromosomal DNA elements (ecDNAs) promote intratumoral heterogeneity, creating a barrier for successful cancer treatments. The underlying mechanisms are poorly understood and studies are hampered in part by a lack of adequate tools enabling studies of ecDNA behavior. Here, we show that single-cell ecDNA copy numbers greatly vary between tumor cells, both in vitro and in patient glioblastoma specimens, suggesting uneven ecDNA segregation during mitosis. We established a CRISPR-based approach which leverages unique ecDNA breakpoint sequences to tag ecDNA with fluorescent markers in living cells. Applying this method during mitosis revealed disjointed ecDNA inheritance patterns, providing an explanation for rapid ecDNA accumulation in cancer. Post-mitosis, ecDNAs tended to cluster and clustered ecDNAs colocalized with RNA polymerase II, promoting transcription of cargo oncogenes. Our observations provide direct evidence for uneven segregation of ecDNA and sheds new light on mechanisms through which ecDNAs contribute to oncogenesis.

26. Kevin Anderson

Multiomic single nucleus RNA- and ATACseq profiling reveals regulators of glioma cell state diversity

Kevin J. Anderson[1], Kevin C. Johnson[1*], Frederick S. Varn[1], Shannon Bessonett[1], Amit D. Gujar[1], Bill Flynn[1], Elise T. Courtois[1], Ann-Christin Hau[2], Anna Golebiewska[2], Sun Há Paek[3], Simone Niclou[2], Michael L. Samuels[1], Paul Robson[1], Roel G.W. Verhaak[1]*

[1]Computational Biology, The Jackson Laboratory for Genomic Medicine; [2]Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg; [3]Department of Neurosurgery, Seoul National University College of Medicine

**These authors contributed equally*

"The extensive intra- and intertumoral heterogeneity observed in glioma reflects the resistance to therapy and poor prognosis observed clinically. Single-cell sequencing studies have highlighted that glioma heterogeneity reflects the co-existence of cell subpopulations with distinct cell states. Prior studies have also shown that EGFR-amplifying extrachromosomal DNA (ecDNA) elements in IDH-wild-type gliomas can contribute to heterogeneity by driving oncogene amplification through long range chromatin contacts. However, single cell studies have largely focused on analyses of transcriptional profiles, and the epigenetic mechanisms underlying the contribution of ecDNA elements to tumor cell state diversity remain poorly understood. To further our understanding of the regulatory programs that contribute to transcriptional diversity and mediate the distribution of tumor cell states, we profiled primary-recurrent tumor pairs from 23 patient samples with multiomic single-nucleus RNA- and ATACseq, resulting in cells with linked chromatin accessibility and gene expression profiles. Integrative clustering of the tumor cells identified tumor cell states ranging from a stem-like to differentiated-like phenotype that were also associated with differences in chromatin accessibility and inferred transcription factor binding activity. Analyses of chromatin accessibility profiles resulted in the identification of ecDNA, and analyses of ecDNA+ cells highlighted distinct cell states with increased copy number burden, oncogene amplification, and differential chromatin accessibility. These results suggest that a better understanding of extrachromosomal contributions to tumor diversity would aid in development of more efficient therapies.

27. Ali Foroughi pour

Integrative survival analysis of colon adenocarcinoma patients identifies crosstalk between morphology, clinical, and genomic markers

Ali Foroughi pour[1], Jie Zhou[1,2], Rafic Beydoun[3], Fahad Ahmed[3], Jeffrey H. Chuang[1,2]

[1]The Jackson Laboratory for Genomic Medicine, [2]Department of Genetics and Genome Sciences, UCONN Health, [3]Department of Pathology, Wayne State University

Colorectal cancer is one of most common cancers in both men and women. While treatments have improved over the last few decades, effective identification of high risk and potentially relapsing patients has been a challenge. Current standard-of-care relies on boards assessing each patient individually, which is time consuming and susceptible to subjectivity amongst different experts. Machine learning can systematize patient stratification and can serve tumor boards as a valuable tool for clinical decision making. To that end, we have developed a deep learning model using pathologist annotated TCGA-COAD H&E whole slide images, clinical information, and mutation status across a gene panel. While morphological features within images separate cases with strong overall survival (OS) differences (OS<3 years v.s. OS>5 years), they are less informative on patients with 3<OS<5 years. Combining deep learning extracted morphological features with clinical information and genomic markers strongly boosts patient stratification. Additionally, models trained on all data modalities enjoy superior stratification to those that only combine clinical information and genomic markers. In particular, the cross-talk of image features with clinical and mutational information is highly informative of patient risk. We observed that precise tumor detection is crucial for reliably encoding morphological features within images. Our

results show integrative analysis boosts patient risk stratification, and that deep learning is an efficient methodology for such analyses.

28. Hasan Baig

Cloud-COPASI - A cloud-based platform for biochemical simulations

Hasan Baig[1] and Pedro Mendes[1]

[1]Center for Quantitative Medicine, UConn Health

Cloud-COPASI is a web-based tool for running computationally intensive biochemical simulations and analyses on high-throughput and cloud-based computing pools. Cloud-COPASI can connect to existing computing pools, including HTCondor. Alternatively, new 'virtual' computing pools can be launched on the Amazon Elastic Compute Cloud (EC2). A user-friendly interface ensures Cloud-COPASI is accessible to all users and a detailed technical knowledge of cloud or distributed computing is not required.

29. Diana Cadena Castaneda

Human-derived ex vivo systems enabling studies on the modulation of human lung innate and adaptive immunity to respiratory viruses

Diana Cadena[1], Michael Schotsaert[2*], Jan Martinek[1*], Jianan Lin[1], Angela Choi[2], Te-Chia Wu[1], Florentina Marches[1], Lerato Hlaka[1], Sonia Jangra[2], Peter Yu[3], Stefan Kachala[3], Andrew Salner[3], Adam Williams[1], Adolfo Garcia-Sastre[2], and Karolina Palucka[1]*

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT; [2]Icahn School of Medicine at Mount Sinai, New York, NY; [3]Hartford HealthCare Cancer Institute, Hartford, CT

[*]Equal contributors

Our ultimate objective is to define the host mechanisms that shape the immunological status and modulate the immune response to viral infection with respiratory viruses including Influenza and SARS-CoV-2. SARS-CoV-2 infection has multiple effects on the immune system in its acute phase. A proportion of infected individuals develop severe disease, while many other remain asymptomatic. The critical early responses that drive the progression towards severe disease are not understood. The virus first infects respiratory tract, characterized by the presence of not only respiratory epithelial cells, but also tissue resident and recruited immune cells such as myeloid cells. However, little is understood about how the virus regulates the epithelial-myeloid cell crosstalk and how it impacts tissue monocyte/macrophages and dendritic cells and subsequent adaptive immunity. This is highly relevant to human immunology as epithelial barriers orchestrate adaptive immunity.

To this end, we are using organoids to expand lung progenitor cells from primary lung tissues and subsequently derive air-liquid interface (ALI) cultures. ALI cultures are then infected with influenza or SARS-CoV-2 in the presence or absence of leukocyte subsets. Responses to infection of both epithelial cells and immune cells are analyzed by flow cytometry and immunofluorescence. Tissue integrity morphology is assessed using phalloidin staining; also, at various time points, tissue/ALI cultures are harvested to measure cytokine/chemokine production, ciliation and mucus production. In addition, we are using single-cell-RNA-Seq of infected cultures to determine the transcriptional response of the system. This approach will enable us to determine molecular mechanisms underlying alterations at epithelial barriers.

30. Jianan Lin

Transcriptional profiling of macrophages in situ in metastatic melanoma reveals localization-dependent phenotypes and function

Jianan Lin[1], Jan Martinek[1*], , Kyung In Kim[1*], Victor G. Wang[1,2], Te-Chia Wu[1], Michael Chiorazzi[3], Hannah Boruchov[1], Ananya Gulati[1], Shamreethaa Seeniraj[1], Lili Sun[1], Florentina Marches[1], Paul Robson[1,2], Anthony Rongvaux[4], Richard A. Flavell[3], Joshy George[1], Jeffrey H. Chuang[1], Jacques Banchereau[1], and Karolina Palucka[1,2]*

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT; [2]Department of Genetics and Genome Sciences, UConn Health, Farmington, CT; [3]Department of Immunobiology, Yale University School of Medicine, Howard Hughes Medical Institute, New Haven, CT; [4]Fred Hutchinson Cancer Research Center, Program in Immunology, Seattle, WA; [*]contributed equally

Therapeutic interventions modulating immune function at the tumor site could improve outcomes in cancer. Here we analyzed patient samples of metastatic melanoma, a tumor type highly responsive to T cell-based therapies, and found that tumor-infiltrating T cells are primarily juxtaposed to CD14+ monocytes/macrophages rather than melanoma cells. Using novel immunofluorescence-guided laser capture microdissection technique, we analyzed transcriptomes of CD3+ T cells, CD14+ monocytes/macrophages and melanoma cells in non-dissociated tissue. CD14+ cells localized within tumor nests displayed a specific transcriptional signature distinct from CD14+ cells localized in tumor stroma. When applied to TCGA cohorts, this stromal macrophage gene set distinguished patients with significantly prolonged survival in cutaneous melanoma and as well as other cancers including uveal melanoma, bladder urothelial carcinoma, and low-grade glioma. This signature contains CD2 and Ly75, a gene linked with antigen capture and regulation of tolerance and immunity, and comprises markers of monocyte-derived dendritic cells (DCs). Thus, the stromal CD14+ cell signature represents a novel candidate biomarker and suggests that reprogramming of stromal macrophages to acquire DC function may offer a therapeutic opportunity for numerous metastatic cancers.

31. Shyam Sah

Functional analysis of novel keloid-associated genetic variants

Shyam Kishor Sah[1], I-Ping Chen[2], Ernst J. Reichenberger[1]*

[1]Center for Regenerative Medicine and Skeletal Development, Department of Reconstructive Sciences, University of Connecticut Health, Farmington, Connecticut 06030, USA; [2]Department of Oral Health and Diagnostic Sciences, School of Dental Medicine, University of Connecticut Health, Farmington, CT 06030, USA

Keloid scarring is a chronic inflammatory skin disorder characterized by aberrant activation of fibroblasts, which leads to excessive and prolonged deposition of extracellular matrix and to fibrotic scar tissue that extends beyond the boundary of the original wound. There is increasing evidence for a genetic predisposition for keloid formation. By linkage analysis and whole-exome sequencing, we identified ASAH1L401P and PHLDA3Q108* variants in families with inherited keloid susceptibility. We then introduced the ASAH1L401P and PHLDA3Q108* variants into human induced pluripotent stem cells (hiPSCs) from peripheral blood mononuclear cells using CRISPR/Cas9 editing and differentiated homozygous mutant and wild-type hiPSCs into fibroblasts. ASAH1 is an acid ceramidase that degrades ceramides into sphingosine and fatty acids. PHLDA3 acts as a tumor suppressor. Here we study the effects of the ASAH1L401P and PHLDA3Q108* mutations in hiPSC-derived fibroblasts. We found that ASAH1 activity was diminished in ASAH1L401P fibroblasts compared to wild-type fibroblasts. In contrast, no changes were found in total ceramide content of ASAH1L401P fibroblasts. However, expression levels of ceramide synthase 2 and ceramide synthase 4 were significantly upregulated in ASAH1L401P fibroblast. TGFβ1 levels and collagen production were highly increased in ASAH1L401P and PHLDA3Q108* fibroblasts. TGFβ1 is a central regulator of tissue fibrosis. Both ASAH1L401P and

PHLDA3Q108* fibroblasts were highly proliferative and expressed pro-fibrogenic markers likely via TGFβ1-SMAD2/3-mediated activation of JAK2/STAT3 and Akt pathways. These finding will help to understand keloid pathogenesis and may be useful for development of potential therapeutics.

32. Asa Thibodeau

AMULET: a novel read count-based method for effective multiplet detection from single nucleus ATAC-seq data

Asa Thibodeau[1+], Alper Eroglu[1+], Christopher S. McGinnis[2], Nathan Lawlor[1], Djamel Nehar-Belaid[1], Romy Kursawe[1], Radu Marches[1], Daniel N. Conrad[2], George A. Kuchel[3], Zev J. Gartner[2,4,5], Jacques Banchereau[1], Michael L. Stitzel[1,6,7], A. Ercument Cicek8,9, Duygu Ucar[1,6,7]*

[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, 06032, USA; [2]Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA 94158, USA; [3]University of Connecticut Center on Aging, UConn Health Center, Farmington, CT, 06030, USA; [4]Chan-Zuckerberg Biohub, San Francisco, CA 94158, USA; [5]NSF Center for Cellular Construction, San Francisco, CA 94158, USA; [6]Department of Genetics and Genome Sciences, University of Connecticut Health Center, Farmington, CT, 06030, USA; [7]Institute for Systems Genomics, University of Connecticut Health Center, Farmington, CT, 06030, USA; [8]Computer Engineering Department, Bilkent University, Ankara, 06800, Turkey; [9] Computational Biology Department, Carnegie Mellon University, Pittsburgh, PA, 15213, USA

Similar to other droplet-based single cell assays, single nucleus ATAC-seq (snATAC-seq) data harbor multiplets that confound downstream analyses. Detecting multiplets in snATAC-seq data is particularly challenging due to data sparsity and limited dynamic range (0 reads: closed chromatin, 1: open on one parental chromosome, 2: open on both chromosomes). Yet, these unique data features offer an opportunity to identify multiplets. AMULET (ATAC-seq MULtiplet Estimation Tool, https://ucarlab.github.io/AMULET/) detects multiplets by studying the number of regions with >2 uniquely aligned reads across the genome, an effective alternative to methods based on artificially-generated multiplets (e.g., state-of-the-art ArchR). For benchmarking, we generated data from two primary human tissues: peripheral blood mononuclear cells (PBMCs) and pancreatic islets. When a certain read depth per nucleus is achieved (i.e., >25K), AMULET captured 85% of simulated multiplets (i.e., recall), significantly outperforming ArchR (24%). To estimate precision, we generated a multiplexed PBMC dataset from 3 donors where multiplets between donors can be identified via donor genotypes using Vireo. Despite lower sequencing depth (i.e., 4K median valid reads per nucleus), AMULET detected these multiplets with high precision (0.59) compared to ArchR (0.28). AMULET is a fast and effective multiplet detection tool for improved single cell epigenomic data analyses across diverse biological systems and conditions.

33. Meihong Zhu

Genetically Encoded Calcium and Voltage Indicators – Cortical Population Signals

Mei Hong Zhu[1], Jinyoung Jang[1], Milena M. Milosevic[1], Kumiko Watanabe[1] and Srdjan D. Antic[1]

[1]Institute for Systems Genomics, Department of Neuroscience, UConn Health, Farmington, CT

Genetically-encoded calcium indicators (GECIs) are essential for studying brain function, while voltage indicators (GEVIs) are slowly permeating neuroscience. Fundamentally, GECI and GEVI measure different things, but both are advertised as reporters of ""neuronal activity"". We quantified the similarities and differences between calcium and voltage imaging modalities, in the context of population activity (without single-cell resolution) in brain slices. GECI optical signals showed 8-20 times better SNR than GEVI signals, but GECI signals attenuated more with distance from the stimulation site. We show the exact temporal discrepancy between calcium and

voltage imaging modalities, and discuss the misleading aspects of GECI imaging. For example, population voltage signals already repolarized to the baseline (~ disappeared), while the GECI signals were still near maximum. Temporal summation of GECI signals is highly exaggerated, causing uniform voltage events produced by neuronal populations to appear with highly variable amplitudes in GECI population traces. Relative signal amplitudes in GECI recordings are thus misleading.

Alphabetical Index of Presenters