Food & Science Evening
Saturday, April 22, 2017

The American Society for Clinical Investigation
The 2017 ASCI Council
Young Physician-Scientist Awards

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CV in normal myocardium and modulates activation propagation in peri-infarct ventricular myocardium. These data demonstrate functional control of arrhythmogenic peri-infarct myocardium by sympathetic nerves, and in part explain the temporal nature of arrhythmogenesis.

Presenter: Himisha Beltran, MD

Whole exome sequencing of cell-free DNA in patients with castration-resistant neuroendocrine prostate cancer informs tumor heterogeneity

Himisha Beltran,1,2 Alessandro Romanel,3 Nicola Casiraghi,2 Michael Sigorous,1 David M. Nanus,1 Scott T. Tagawa,1 Mark A. Rubin,2 Matteo Benelli,3 Jenny Xiang,2 and Francesca Demichelis2,3

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We recently identified mechanisms underlying the clonal evolution of castration-resistant adenocarcinoma (CRPC-Adeno) to a neuroendocrine phenotype as a mechanism of resistance to androgen receptor (AR)-targeted therapies in prostate cancer (Beltran et al., Nature Medicine 2016). In the current study, we aimed to develop a noninvasive approach to identify patients that are developing neuroendocrine prostate cancer (NEPC) using circulating tumor DNA (ctDNA).

We performed whole exome sequencing (WES) of matched ctDNA, germline DNA, and metastatic biopsies from patients with CRPC-Adeno and NEPC using a minimum of 50 ng DNA, NimbleGen library prep, Illumina platform (ctDNA >300X). After applying ad hoc partial duplication filtering, we used FACETS segmentation followed by CLONET to calculate the fraction of tumor DNA in the circulation and assess the clonality of genomic lesions including SCNA and SNV.
Primary graft dysfunction (PGD) affects over 50% of lung transplant recipients and remains the predominant cause of both short- and long-term mortality. Neutrophil infiltration into the allograft following ischemia-reperfusion injury (IRI) mediates PGD. While neutrophil depletion can ameliorate PGD, it may not be clinically viable due to the importance of neutrophils in host defense. Accordingly, we investigated the mechanisms responsible for neutrophil trafficking into the lung allograft. We show that a previously unknown subset of pulmonary non-classical monocytes (NCM), retained in donor lungs, are the drivers of neutrophil recruitment following IRI and their depletion abrogates PGD without affecting recipients’ pathogen-directed immunity.

In clinical specimens, fluorescence-activated cell sorting (FACS) and immunofluorescence microscopy were used to characterize human donor lungs after vascular flushing and after reperfusion. For experimental modeling, allogeneic murine single lung transplants between complete MHC-mismatched strain combinations as well as syngeneic transplants were performed, and neutrophil trafficking was analyzed using two-photon imaging and flow cytometry. Histologic evidence of lung injury, PaO2, Evans blue dye leak, and wet-to-dry ratio were used to assess for experimental PGD. FACS was used to quantify myeloid cell populations and sort NCM. RNA-Seq was used for transcriptional profiling of sorted NCM. RT-qPCR and ELISA were used to quantify MIP-2, a key neutrophil chemoattractant, mRNA in NCM and protein levels in pulmonary circulation, respectively.

Perfused murine lungs demonstrated intravascular CD11b+MHCIID64+/–Ly6C+ monocytes (NCM) that constituted ~3% of resident CD45+ cells. These murine NCM shared a functional phenotype with CD14+CD16+ monocytes detected in perfused human lungs. Pre-treatment of donors with clodronate-liposomes (Clo-lip) depleted NCM in donor lungs (<0.3%). Persistence of NCM was associated with rapid neutrophil influx after reperfusion. In mice, donor NCM depletion with Clo-lip; deletion of NR4A1, an orphan nuclear receptor necessary for NCM formation; or CX3CR1, an NCM cell surface receptor/cell adhesion molecule, resulted in suppressed neutrophil influx and protection against PGD. In contrast, treatment with anti-CCR2 antibodies, which selectively deplete the classical Ly6C+ monocytes, but not NCM, worsened neutrophilia (3-fold) and allograft injury. Myd88-trif–/– donor allografts that lack Toll-like receptor (TLR) signaling were also protected. Reconstitution of NCM-depleted donor lungs with wild-type, but not Myd88-trif–/–, NCM restored neutrophil influx. Post-reperfu-

64 CRPC patients were prospectively enrolled. Overall the spectrum of genomic alterations captured by WES ctDNA was consistent with those commonly observed in CRPC, validating the feasibility of the approach. The SCNA similarity between tumor tissue and ctDNA was higher in NEPC compared to CRPC-Adeno \( (P = 0.0001) \), suggesting less intra-patient heterogeneity in NEPC. There was enrichment of \( RB1 \) and \( TP53 \) loss in ctDNA of NEPC and \( AR \) gains in CRPC-Adeno. The overall fraction of missense mutations shared by both ctDNA and tumoral tissue was ~80%. PM161 is a representative patient who developed small cell NEPC after multiple lines of therapy. We compared three different tumor biopsy time points — CRPC-Adeno (lymph node metastasis), CRPC-Adeno (bone metastasis), and NEPC (liver metastasis). Unexpectedly the baseline ctDNA profile (at time of CRPC-Adeno diagnosis) displayed genomic features most similar to the NEPC tissue sample (last time point, liver biopsy). These data suggest that NEPC alterations are detectable in the circulation and potentially prior to the development of NEPC clinical features. We compared the ctDNA exome of another patient, PM0, with his 6 sites of NEPC metastases (obtained 6 days later at autopsy); the relative contribution of tumor alterations in ctDNA was highest for the liver metastasis (similarity 0.59) compared with other sites, suggesting a differential contribution of sites of metastases in the circulation, with implications for the interpretation of single site clinical biopsies.

This is the first study to show that WES of ctDNA is feasible in CRPC and can help elucidate intra-patient heterogeneity and identify the spectrum and frequency of NEPC-specific genomic changes. We have incorporated our WES ctDNA discoveries into a targeted panel for larger-scale validation using prospective cohorts. Our goal is to improve the earlier detection of patients transforming toward NEPC.

Presenter: Ankit Bharat, MBBS, FACS

Donor-derived pulmonary intravascular non-classical monocytes recruit recipient neutrophils and mediate primary lung allograft dysfunction

Ankit Bharat,1,2 Zhihun Zheng,1 Stephen Chiu,1 Mahzad Akbarpour,1 Haiying Sun,1 Paul A. Reyfman,2 Kishore Anekalla,1,2 Hiam Abdala-Valencia,2 Daphne Edgren,1 Wenjun Li,1 Daniel Kreisel,2 Farida V. Korobova,2 Ramiro Fernandez,1 Alexandra McQuattie-Pimentel,2 Zheng J. Zhang,2 Harris Perlman,2 Alexander V. Misharin,2 and G.R. Scott Budinger2

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1,2

- MYD88-trif–/– donor allografts that lack Toll-like receptor (TLR) signaling were also protected. Reconstitution of NCM-depleted donor lungs with wild-type, but not MYD88-trif–/–, NCM restored neutrophil influx. Post-reperfu-
sion donor NCM upregulated TLR2/CD14-MYD88-NFxB pathway genes and MIP-2. Further, MIP-2 was increased in allograft circulation only when functional donor NCM were present and neutralization of MIP-2 abrogated neutrophil influx. Depletion of NCM did not impair the hosts’ ability to mount a neutrophil response to intratracheal or intravenous lipopolysaccharide or pseudomonas inoculation.

Donor-derived NCM, retained in human and murine donor lungs, recruit neutrophils through the production of MIP-2 via a TLR2/CD14-MyD88-NFxB pathway. Depletion of donor NCM represents a novel and clinically relevant therapy, as it may abrogate PGD without affecting recipient host defense.

**Presenter: Michael Chattergoon, MD, PhD**

**YPSA-4 | Poster session 2**

**Recognition and control of HIV by inflammasomes**

Michael A. Chattergoon, Guido Massaccesi, Jeffrey Quinn, Neel Sangal, Rebecca Veenhuis, and Andrea L. Cox

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HIV activates several innate immune pathways including the inflammasome and type I interferon (IFN), and HIV-1-infected individuals have elevated levels of circulating interferon-alpha and IL-18 (inflammasome regulated). The mechanism by which HIV is sensed by the inflammasome is incompletely understood, but Toll-like receptor-8, MyD88, and NALP3 have been shown to be key aspects of the system. Further, inflammasome activity and levels of inflammasome cytokines can be modulated by the state of immune dysfunction and co-infections.

While the effects of interferon on HIV replication and pathogenesis have been described, the significance of the inflammasome in these processes is not understood.

Using a combinatorial approach with mixtures of major cytokine families, we test >400 cytokine combinations to identify cytokines that modulate inflammasome activity, thereby testing the ability of the cytokine milieu to alter inflammasome activity induced by HIV. Having identified key regulatory cytokines, we assessed effect of these cytokines and inflammasome products to suppress HIV replication in human primary CD4+ T-cells.

We find that the cytokines IL-1β, IL-1α, and TNFα augment the inflammasome activity triggered by HIV. The effect of IL-1β was particularly dramatic, increasing transcription of the inflammasome pro-cytokines pro-IL-1β (>200 fold) and pro-IL-18 (>10-fold), and could not be overcome by any cytokine with suppressive activity. The strongest suppressive activity was observed with IFNα and IFNβ; these cytokines completely suppressed inflammasomes except when IL-1β and IL-1α were present. Interestingly, IL-37 also strongly suppressed inflammasome activity. IL-37 is a recently characterized member of the IL-1 family of cytokines that is regulated by inflammasomes. We find that activated T-cells express receptors for all inflammasome cytokines; however, only IL-37 exerts an antiviral effect. In the presence of IL-37 HIV replication was reduced by approximately 70%. In addition to reducing transcription of pro-inflammatory molecules, we also demonstrate that IL-37 induces expression of antiviral factors including IFITM proteins and APOBEC3A in CD4+ T-cells and directly inhibits HIV-1 replication in infected primary human CD4+ T-cells by inhibiting life cycle steps prior to integration of the proviral genome.

The inflammasome response to HIV comprises a complex mixture of cytokines. Some inflammasome products suppress type I interferon activity and augment HIV replication in vitro. IL-37, a novel inflammasome cytokine, has both anti-inflammatory activity and direct antiviral activity. Our data suggest that the balance of activity of inflammasome cytokines affects the innate immune response to HIV and may be important in the earliest response to HIV.

**Presenter: Helen Y. Chu, MD, MPH**

**YPSA-5 | Poster session 1**

**Respiratory syncytial virus transplacental antibody transfer in mother-infant pairs in Nepal**

Helen Y. Chu,1 James Tielsch,2 Joanne Katz,3 Amalia Magaret,1 Subarna Khatri,4 Stephen C. Le Clerq,4 Laxman Shrestha,3 Jane Kuypers,1 Mark C. Steinhoff,6 and Janet A. Englund1,7

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Respiratory syncytial virus (RSV) is the most important viral cause of pneumonia in children worldwide. RSV-specific antibody (ab) protects infants from disease. Maternal vaccines against RSV have the potential to protect the infant through transplacental antibody transfer. The effect of RSV antibody on incidence of RSV infection in infants has not been well described in resource-limited settings.

In a prospective study in rural southern Nepal, wom-
en were enrolled during pregnancy, and maternal and cord blood were collected at birth. Active weekly home-based surveillance for respiratory illness was performed for infants from birth to 180 days, and nasal swabs from symptomatic infants were tested for RSV by PCR. Serum samples from mother-infant pairs were tested using an RSV ab micro-neutralization assay. Ab titers at time of RSV infection in infants were estimated based on a decay rate of 0.026 log₂ titer/day.

From March 2012 to October 2013, serum samples were collected from 299 mother-infant pairs. Cord/maternal RSV ab transfer ratio was 1.03 (SD: 0.07), with a mean RSV ab titer of log₂ 11.4 (1.2) and log₂ 11.8 (1.3) in mothers and infants, respectively. Eight of 25 (32%) preterm infants had ab transfer ratios <1.00, compared to 51 (19%) of 273 full-term infants ($P = 0.11$). Cord blood RSV ab titers did not differ between infants with and without RSV infection (12.3 [1.1] vs. 11.6 [1.3]). Estimated RSV ab titer at time of infection did not differ between infants with and without RSV infection (12.3 [1.2] versus lower respiratory tract infection ($n = 19$; log₂ 9.9 [1.6]; $P = 0.24$). Additionally, cord blood RSV antibody titers did not correlate with age at primary RSV infection ($R = 0.03$; $P = 0.88$).

Transplacental transfer of RSV-specific ab from mother to the fetus was efficient in mother-infant pairs in rural Nepal. Higher cord blood ab titers were not protective against earlier or more severe RSV infection in infants. Potential maternal vaccination strategies will require establishment of a protective cord blood ab titer against RSV infection in infants in maternal vaccine trials.

Presenter: Paul Cohen, MD, PhD

YPSA-6 | Poster session 2

Identification of a beige fat-derived factor regulating hepatic insulin sensitivity

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Obesity is strongly associated with diabetes, cardiovascular disease, and many types of cancer. These obesity-associated conditions account for the leading causes of morbidity and mortality in the United States. New approaches must be developed in order to break the connection between obesity and disease. Adipose tissue is now appreciated to be an endocrine organ with an important role in the pathogenesis of diabetes and other obesity-related diseases. Rodents and humans have white and brown fat. White fat cells store excess energy, and in the obese state, become inflamed and contribute to diabetes. Brown fat cells dissipate energy and protect against obesity and diabetes. Mammals are now known to have two distinct types of brown fat cells. Classical brown fat is developmentally preformed, but more recently, brown-like fat cells have been identified that are embedded within white fat. These cells, termed beige adipocytes, are highly inducible, and when activated, they have similar properties to brown fat, though they come from a distinct developmental lineage. We have shown that the transcriptional coregulatory protein PRDM16 is required for beige fat function. Ablation of beige fat in mice results in significant insulin resistance that most prominently affects the liver. The mechanism by which an alteration in beige fat leads to a dysfunction in hepatic insulin signaling is unknown. We have now found that conditioned media from cultured beige fat cells can directly modulate insulin signaling and glucose production in primary hepatocytes, in a PRDM16-dependent fashion. Using unique murine and cellular models in my lab along with genomic and proteomic techniques, we are now focused on identifying the factor by which beige fat can modulate hepatic insulin signaling. These studies have the potential to provide novel insights into the mechanisms linking obesity to diabetes and could facilitate the development of targeted therapeutic strategies to treat obesity-associated diseases.

Presenter: Matthew Steven Davids, MD, MMSc

YPSA-7 | Poster session 1

Bruton’s tyrosine kinase inhibition increases B cell leukemia/lymphoma-2 dependence and enhances sensitivity to venetoclax in chronic lymphocytic leukemia

Jing Deng, Elif Isik, Stacey M. Fernandes, Jennifer R. Brown, Anthony Letai, and Matthew S. Davids

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The treatment of chronic lymphocytic leukemia (CLL) has recently been transformed by novel oral agents targeting B cell receptor (BCR) pathway kinases that are critical to malignant B cell survival. Ibrutinib is a highly effective and recently FDA-approved agent that inhibits Bruton’s tyrosine kinase (BTK), a key BCR pathway protein. Interestingly, although ibrutinib decreases tumor burden in patients, it typically does not induce a substantial amount of apoptosis...
in vitro, and complete responses in patients are rare. The mechanisms by which ibrutinib interacts with the apoptotic cascade are incompletely understood. A next-generation BTK inhibitor, acalabrutinib, recently entered the clinic and was also found to be highly effective in relapsed/refractory CLL in early-phase clinical trials. As with ibrutinib, the effect of BTK inhibition with acalabrutinib on the mitochondrial pathway of apoptosis has not been fully explored.

Our group previously showed that CLL cells rely heavily on the anti-apoptotic protein BCL-2 for survival. Venetoclax is a highly selective oral BCL-2 antagonist that induces rapid and deep responses in CLL and was recently FDA approved. The mechanism of action and toxicities of venetoclax are distinct from BTK inhibitors, suggesting that the combination of these agents may be feasible in the clinic. BH3 profiling is a functional assay previously developed by our group to interrogate mitochondria to determine their proximity to the threshold of apoptosis, a property called “mitochondrial priming,” as well as their relative dependence on different anti-apoptotic proteins for survival. Dynamic BH3 profiling (DBP) is a new variation of this technique that measures early changes in net pro-apoptotic signaling that are induced in cancer cells treated with anti-cancer agents. We hypothesized that DBP would allow us to assess how BTK inhibitors and venetoclax influence mitochondrial priming and anti-apoptotic dependence in primary CLL cells.

Using DBP, we analyzed alterations in the function of the mitochondrial apoptotic pathway induced by ibrutinib and acalabrutinib. We studied CLL patient samples treated ex vivo with both drugs, as well as in vivo primary samples from CLL patients on clinical trials of both drugs. We found that BTK inhibition enhances mitochondrial BCL-2 dependence without significantly altering overall mitochondrial priming. Enhancement of BCL-2 dependence was accompanied by an increase in the expression of the pro-apoptotic protein BIM. In contrast, treatment with the selective BCL-2 inhibitor venetoclax enhanced overall mitochondrial priming without increasing BCL-2 dependence. Pre-treatment of CLL cells with either BTK inhibitor, whether ex vivo or in vivo in patients, enhanced killing by venetoclax. Our data suggest that BTK inhibition enhances mitochondrial BCL2 dependence, supporting the development of clinical trials combining BTK and BCL-2 inhibition in CLL.

Lungs of healthy individuals, previously considered sterile, are now known to contain diverse communities of bacteria. Though lung microbiota have been associated with alveolar inflammation in disease states, the relationship between lung microbiota and lung immunity in human health is unknown. In murine models, vendor- and litter-specific differences in gut microbiota have been implicated in experimental variation of murine models of systemic inflammation. We hypothesized that murine lung microbiota vary by cage, by litter, and by vendor, and that differences in lung microbiota are correlated with differences in baseline lung immunity.

Forty healthy, genetically identical (C57BL/6) 10-week-old mice were studied. Half were obtained from the Jackson Laboratory, the remainder from Charles River Laboratory. Mice from each vendor represented two distinct litters, and were housed 5 mice per cage. We characterized microbiota from the lungs, tongue, and cecum of each mouse using community sequencing of the 16S rRNA gene (Illumina MiSeq). We measured inflammatory cytokines using a Luminox multiplex assay of homogenized lung tissue.

Lung bacterial communities were highly variable among mice, and differed significantly by cage, by litter, and by vendor ($P < 0.0001$ for all). For instance, the Streptococcus family represented 9.5% of bacterial sequences in lungs of mice from Charles River Laboratory, but only 0.2% of bacterial sequences in lungs of mice from the Jackson Laboratory ($P < 0.001$). The community composition of lung bacteria correlated significantly with alveolar concentrations of IL-1α ($P < 0.01$), IL-4 ($P < 0.0001$), and IL-17 ($P < 0.001$). Decreased diversity of lung bacteria was strongly correlated with increased alveolar concentrations of all three inflammatory cytokines ($P < 0.001$ for all). In contrast, alveolar concentrations of IL-1α and IL-17 had no detectable association with the community composition or diversity of oral or cecal bacteria ($P > 0.05$).

Lung microbiota in healthy mice are highly variable and cluster by cage, litter, and vendor. Inflammatory cytokines
in the lung are strongly correlated with the community composition of lung microbiota, but not with microbiota of the upper or lower gastrointestinal tract. Our results suggest that baseline “immune tone” in the lung reflects local, not remote, host-microbe interactions. Variability in lung microbiota may be an important source of experimental and clinical variability in health and inflammatory lung disease.

Presenter: Jennifer Alzos Downs, MD, MSc, PhD

YPSA-9 | Poster session 1

Effects of schistosomiasis on susceptibility to HIV-1 infection and HIV-1 viral load set-point: a nested case-control study

Jennifer A. Downs,1,2 Kathryn M. Dupnik,1 Govert J. van Dam,3 Mark Urassa,4 Peter Lunotja,4 Dieuwke Kornelis,3 Claudia J. de Dood,3 Pytsje Hoekstra,3 Chifundo Kanjala,4 Raphael Isingo,4 Robert N. Peck, 2 Myung Hee Lee,2,3 Paul L.A.M. Corstjens,2 Jim Todd,4 John M. Changalucha,4 Warren D. Johnson Jr.,1 and Daniel W. Fitzgerald1

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Schistosomiasis is a parasitic worm infection that affects 218 million people worldwide, with most infections in Africa. Prevalence studies suggest that people with chronic schistosomiasis may have higher risk of HIV-1 acquisition and impaired ability to control HIV-1 replication once infected.

Our goal was to determine the impact of schistosome infection on susceptibility to HIV-1 acquisition and viral load set-point in a longitudinal study. We therefore conducted a nested case-control study within a community-based HIV-1 sero-incidence cohort in Tanzania. A population of adults from seven villages was tested for HIV in 2007, 2010, and 2013, and dried blood spots were taken. Approximately 40% of this population has Schistosoma mansoni, and 2% has S. haematobium. We tested for schistosome antigens in the pre- and post-HIV-1 seroconversion blood spots of people who acquired HIV-1. We also tested blood spots of matched controls who did not acquire HIV-1 and calculated the odds that a person with schistosomiasis would become HIV-1-infected compared to these matched controls.

Analysis was stratified by sex. We additionally compared HIV-1 RNA levels in the post-seroconversion blood spots in HIV-1-seroconverters with schistosomiasis versus those without.

In 2010, 3,146 adults who were HIV-uninfected in 2007 were re-tested, and 54 were found to have HIV-1-seroconverted during the three-year interval. In 2013, 2,701 adults who had been HIV-uninfected in 2010 were re-tested, and 40 had newly HIV-1-seroconverted. We obtained dried blood spots for schistosome testing from 37 of the 2010 seroconverters and 36 of the 2013 seroconverters who were randomly selected from among all HIV-seroconverters according to our power calculation.

In total, we compared 73 HIV-1 seroconverters with 265 controls. Women constituted 62% of the HIV-seroconverters. In women, 20 of 45 HIV-1 seroconverters (44%) had schistosome infection at the time of HIV-1 acquisition, compared to 48 of 162 female HIV-uninfected controls (30%). Women with schistosome infections had a higher odds of HIV-1 acquisition than those without (adjusted odds ratio [OR] = 2.5 [1.1-5.8], \( P = 0.035 \)). Schistosome-infected men did not have increased odds of HIV-1 acquisition (adjusted OR = 0.7 [0.3-2.2], \( P = 0.62 \)).

We also quantified HIV-1 viral load RNA level in the 37 HIV-1 seroconverters who were diagnosed with HIV-1 in 2010, before antiretroviral therapy became widely available in the region. The median whole blood HIV-1 RNA level in the 15 HIV-1 seroconverters with schistosome infection was significantly higher than in the 22 without schistosomiasis: 4.4 (3.9-4.6) versus 3.7 (3.2-4.3) log10 copies/ml, \( P = 0.017 \).

We confirm prospectively, in an area with endemic S. mansoni, that schistosome infection increases odds of HIV-1 acquisition in women and raises HIV-1 viral load set-points. Our study suggests that controlling schistosomiasis in African countries may prevent incident HIV-1 infections and decrease HIV-1 viral load set-points, thereby decreasing HIV-1 transmission and delaying disease progression.

Presenter: Maros Ferencik, MD, PhD

YPSA-10 | Poster session 2

Impact of coronary calcium on clinical management in patients with acute chest pain: results from the ACRIN-PA 4005 and ROMICAT II trials

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Coronary artery calcium (CAC) may impair diagnostic assessment of coronary tomography angiography (CTA). It is unclear whether this is associated with inefficient patient management. We determined whether CAC affects efficiency of a coronary CTA strategy in patients with suspected acute coronary syndrome (ACS).

We performed a pooled analysis of the two trials (ACRIN-PA 4005 and ROMICAT II) comparing an initial coronary CTA diagnostic strategy to standard of care in patients who presented to the emergency department with acute chest pain. In the CTA arms of the studies, we investigated differences in downstream testing (beyond CTA), diagnostic yield, and cost across CAC strata (Agatston score 0, 0–10, 10–100, 100–400, >400) and sex. Cost was calculated using hospital cost-accounting systems and physician billing records.

Of 1,234 patients (mean age 51 ± 8.8 years), 80 (6.5%) had obstructive coronary artery disease (CAD; ≥70% stenosis), and 68 (5.5%) had ACS. Prevalence of obstructive CAD (1% to 64%), frequency of downstream testing (4% to 72%), incidence of ACS (1% to 44%), and total (2,337 US$ to 8,484 US$) and diagnostic cost (2,310 US$ to 6,678 US$) increased across increasing CAC strata, translating into overall less efficient management in women (cost per ACS 45,780 US$ in men vs. 101,831 US$ in women; P = 0.011).

Coronary artery disease burden, adverse health outcomes, and cost of care increased with increasing CAC, but did not vary in patients with obstructive CAD. Downstream testing and total as well as diagnostic costs increased with increasing CAC, but were found to be appropriate, as obstructive CAD and adverse outcomes are more prevalent in patients with high CAC. In patients undergoing a strategy with coronary CTA, cost-efficient testing and excellent invasive diagnostic yield can be achieved in patients with high burden of CAC.

Selective retinoids overcome bone marrow (BM) stromal protection of malignant cells

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All-trans retinoic acid (ATRA) causes terminal differentiation and apoptosis of non-APL AML cells in vitro but has not proven clinically effective. BM stroma expressing CYP26 inactivates ATRA and protects leukemia cells from differentiation. ATRA signals through retinoic acid receptors (RARs) α, β and γ induce not only terminal differentiation of AML, but also upregulation of CYP26, potentially forming an even more protective niche for leukemia. We investigated the relative contribution of RARα and RARγ to leukemia differentiation and stromal CYP26 upregulation.

ATRA (pan-RAR agonist), AM80 (strong RARα agonist, weak RARγ), and IRX5183 (RARα-specific agonist) all induced significant differentiation of NB4 APL cells. In contrast, the RARγ-specific agonist CD437 produced no evidence of differentiation. In addition, ATRA induced a 40-fold upregulation of stromal CYP26B1 (P = 0.02), and while CD437 also produced a brisk and sustained upregulation of CYP26B1, the RARα-specific IRX5183 had only modest effects on CYP26B1 levels. Since RARγ activation was dispensable for differentiation, we tested whether the two RARα active agents, AM80 and IRX5183, could bypass stromal-mediated protection against ATRA-induced differentiation. Several AML cell lines were co-cultured with OP9...
BM stroma and treated with ATRA, AM80, or IRX5183 at concentrations of 0.1 to 0.01 mM. We have previously shown that ATRA can induce differentiation of most AML cell lines in stroma-free conditions, but it is inactive in the presence of BM stroma; further, its effect can be restored by inhibition of stromal CYP26 (Su M et al. 2015). Whereas stroma blocked upregulation of CD11b, and inhibition of clonogenicity of NB4 cells by ATRA, both AM80 and IRX5183 showed similar activity in the presence or absence of stroma. Stroma also blocked ATRA’s activity against NPM1-mutated OCI-AML3 cells, but AM80 and IRX5183 were active in both the presence and absence of stroma (e.g., IRX5183 resulted in clonogenic recovery from control of 7.3% ± 2.5% vs 11.2% ± 2.8% on stroma, P = NS). Similar effects were observed using Kasumi-1 core binding factor AML cells.

In conclusion, we found that RARα and RARγ have distinct effects on niche versus AML cells. Stimulation of RARγ does not induce differentiation of AML cells, but leads to upregulation of stromal CYP26B1 and, thus, enhanced resistance to ATRA. In contrast, CYP26-resistant, RARα active synthetic retinoids AM80 and IRX5183 are able to differentiate and eliminate AML cells even in the presence of BM stroma regardless of induction of CYP26 expression. We are currently exploring a phase I/II clinical trial using IRX5183 in non-APL AML (NCT02749708).

Presenter: Matthew Blake Greenblatt, MD, PhD

YPSA-13 | Poster session 1

Identification of a novel periosteal skeletal stem cell

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The periosteum, the lining of mesenchymal tissue on the outer surface of bones, has a unique contribution to bone health, being essential for both fracture healing and the physiologic expansion of bone diameter that maintains the biomechanical strength of bone during aging. Despite this importance, the stem cell progenitor giving rise to bone-forming osteoblasts on the periosteum is unknown. Here we use multi-paramter FACS together with lineage tracing to identify a novel periosteal skeletal stem cell (pSSC) residing on the periosteum that serves as a progenitor of periosteal osteoblasts in both long bones and calvarium in mice. This pSSC displays formal stem cell properties, including clonal multi-
potency and the ability to self-renew after serial transplantation. Furthermore, pSSCs are, after either in vitro culture or transplantation to secondary hosts, able to give rise to their full set of derivatives observed during in vivo lineage tracing, indicating that they sit at the apex of their differentiation hierarchy. Transcriptome analysis of pSSCs demonstrates that they are distinct from either their derivative cell types or from endosteal SSCs residing inside of bone. Furthermore, whereas endosteal SSCs undergo endochondral ossification upon transplantation to secondary hosts, as characterized by formation of a cartilage template and recruitment of hematopoietic elements, pSSCs display distinct functional capacities, engaging in intramembranous bone formation without recruitment of hematopoietic elements or formation of a cartilaginous template, findings consistent with the physiologic restriction of marrow recruitment to the endosteum. Analysis of human periosteal tissue demonstrates the presence of a population with a very similar immunophenotype and similar differentiation capacity, offering evidence for the existence of a human counterpart to murine pSSCs. Taken together, this work identifies a novel periosteal skeletal stem cell, providing an opportunity for future studies to determine how therapeutic and pathologic modulation of the activity of this cell alters bone health.

Presenter: Yogendra Kanthi, MD

YPSA-14 | Poster session 2

Differential biomechanical regulation of endothelial CD39 from vein grafts by pulsatile radial forces

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Human saphenous veins are the most frequently used bypass grafts in coronary and peripheral artery bypass surgery, but are disproportionately prone to failure when compared to arterial grafts due to adverse remodeling. The endothelium senses radial and linear biomechanical forces exerted by blood flow. While thick-walled arteries are subjected to pulsatile strain, thinner-walled venous blood flow in situ is non-pulsatile until transposed as an arterial graft. The impact of cyclic stretching from pulsatile distention on the vein wall is not well elucidated. CD39 is a potent ecto-enzyme at the endothelium: blood interface regulating blood fluidity and suppressing vascular thrombo-inflammation by enzymatically dissipating extracellular ATP and ADP, and facilitating adenosine generation. We recently identified that endothelial CD39 expression can be induced by linear biomechanical forces (laminar shear stress). However, the response of CD39 to cyclic stretch during vein graft arterialization remains unknown. We hypothesized that the vasculoprotective enzyme CD39 is induced by the venous endothelium in response to radial forces associated with pulsatile stretch.

Primary human saphenous vein endothelial cells (HSVEC) were isolated from vein remnants from patients undergoing coronary bypass surgery, and exposed to low and high levels of cyclic stretch in vitro, mimicking arterial and vein graft patterns using flexible membrane-bottomed plates. Cd39 gene and protein expression were quantified by qRT-PCR and immunoblot. Ecto-ATPase and -ADPase activity of CD39 on cultured cells following cyclic stretch was quantified with a malachite green assay. Mice with a haploinsufficiency of Cd39 (Cd39<sup>−−</sup>) underwent carotid bypass surgery with a vein segment as an interposition graft. Subsequent neointimal hyperplasia was compared with similar grafts in wild-type controls.

High (16%, 1 Hz) and low (5%, 1 Hz) levels of cyclic stretch of HSVEC elicited similar morphologic realignment of cells perpendicular to the direction of stretch, but distinct patterns of CD39 expression. Cd39 transcript remained unchanged following 24 and 48 hours of low or high stretch (n = 6, each). Low-level cyclic stretch did not alter CD39 protein expression after 24 or 48 hours compared with static controls (n = 6, each). In stark contrast, cyclic stretch at 16% induced a 130% increase in CD39 protein expression after 48 hours, and a 60% increase after 24 hours compared with static controls (n = 6 each, P < 0.05). We observed corresponding increases of 60% and 100% in CD39-dependent enzymatic hydrolysis of ATP and ADP, respectively, in HSVEC following high levels of stretch (n = 4, P < 0.005).

In vitro, cyclic stretch did not induce transcripts of other exonuclease degrading enzymes. Preliminary studies in a murine model of vein graft disease suggest that CD39 haploinsufficiency exacerbates neointimal hyperplasia in isogenic vein-to-artery grafts compared to wild-type controls.

We have identified CD39 as a novel, stretch-responsive endothelial ectoenzyme in human saphenous veins, which may represent a protective response to venous distention from arterial stretch patterns.
Inflammation has long been appreciated to be a central mechanism driving heart failure pathogenesis. However, the precise mechanistic basis by which inflammation is generated in the diseased heart and contributes to heart failure progression remains poorly defined. We and others have recently demonstrated that under steady-state conditions, the mouse heart contains a diverse and heterogeneous population of tissue-resident macrophages with distinct functions that are derived from a variety of embryonic origins. Following tissue injury, a shift in macrophage ontogeny occurs where resident cardiac macrophages are replaced by damaging inflammatory monocytes and monocyte-derived macrophages derived from definitive hematopoietic progenitors located within the spleen and bone marrow. To date, surprising little is understood regarding the mechanisms that orchestrate recruitment of monocytes to the injured heart and how newly recruited monocytes and monocyte-derived macrophages acquire an inflammatory fate. Using ischemia-reperfusion injury and heart transplantation models in combination with genetic lineage tracing, macrophage depletion, and conditional gene deletion, we tested the hypothesis that macrophage ontogeny shifts are coordinated by a specific subset of resident cardiac macrophages. Consistent with this hypothesis, we demonstrated that CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> resident cardiac macrophages are required for entry of monocytes into the injured myocardium and differentiation into inflammatory CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> macrophages. CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> macrophages are derived from fetal liver hematopoiesis, long-lived, and maintained independent of monocyte input. Mechanistically, CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> macrophages are activated by mitochondrial DNA and IL-1α released from damaged cardiomyocytes through a Myd88- and NF-κb-dependent pathway, resulting in the release of neutrophil and monocyte chemokines (MCP1, MCP3, CXCL2, CXCL5) and inflammatory chemokines (IL-1β, IL-6, TNF-α). CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> macrophage depletion or inactivation of TLR9 and IL-1R signaling in cardiac macrophages was sufficient to dramatically reduce inflammatory monocyte and macrophage recruitment and improve outcomes following ischemic cardiac injury. We further demonstrated that the human heart also contains CCR2<sup>+</sup>MHC-II<sup>high</sup>CD206<sup>-</sup> macrophages with similar functions. Collectively, these findings identify the mechanistic basis by which macrophage ontology shifts are mediated in the injured heart and define a targetable signaling pathway linking cardiomyocyte injury with resultant inflammation.

Continuous genomic evolution has been a major limitation to curative treatment of multiple myeloma (MM). Frequent monitoring of the genetic heterogeneity in MM from blood, rather than serial bone marrow (BM) biopsies, would therefore be desirable. We hypothesized that genomic characterization of circulating MM cells (CMMCs) recapitulates the genetics of MM in BM biopsies, enables MM classification, and is feasible in the majority of MM patients with active disease. To test these hypotheses, we developed a method to enrich, purify, and isolate single CMMCs with a sensitivity of 1:10<sup>6</sup>. We then performed DNA sequencing of single CMMCs and compared them to single BM-derived MM cells. We performed targeted sequencing of the 35 most recurrently mutated loci in 35 currently mutated loci of single cells from 10 MM patients. In addition to DNA sequencing we also performed whole transcriptome single-cell RNA sequencing.

Mutational analysis of 35 recurrently mutated loci in 335 high-quality single MM cells from the blood and BM of 10 patients, including one patient with monoclonal gammopa-
Innate immune activation of platelets drives inflammation and thrombotic complications following trauma

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Trauma is a leading cause of death and disability internationally. Largely considered a disease of excessive innate immune activation, trauma is characterized by activation of Toll-like receptors (TLRs) on various cell types. The cellular dysfunction arising from excessive TLR signaling has been shown to lead to multiple organ dysfunction and death. In parallel to the excessive activation of inflammatory pathways, trauma patients experience both severely impaired hemostasis and excessive late thrombosis, leading to a unique coagulopathy in the severely injured patient. This so-called acute coagulopathy of trauma (ACoT) occurs in as many as 25% of trauma patients and has been a major contributor to morbidity and mortality; however, the pathogenesis is poorly understood. Platelets are key contributors to both hemostasis and thrombosis, and the role of platelet dysfunction in trauma has recently been addressed. Platelets have been extensively studied as hemostatic regulators; however, a rapidly evolving concept is that platelets are also sentinel innate immune responders. Our lab has shown that the critical innate immune receptor Toll-like receptor 4 (TLR4) regulates platelet response to sterile injury. We have demonstrated, using transgenic murine studies, that platelet TLR4 specifically regulates platelet sequestration and organ failure following hemorrhagic shock. Additionally, we have shown that the endogenous danger signal high-mobility group box 1 (HMGB1) is released by activated platelets following injury. Release of HMGB1 results in autocrine and paracrine signaling on platelets through TLR4, providing a unique and novel mechanism of innate immune signaling in platelets. The downstream signaling cascade from HMGB1-TLR4 involves the initiation of novel complex formation involving guanylate cyclase and regulation of cyclic GMP production. This signaling pathway regulates platelet activation, aggregation, adhesion, as well as the formation of thrombus both in vitro and in vivo. We next generated a mouse lacking HMGB1 specifically on platelets (HMGB1\textsuperscript{−/−}), and utilizing models of trauma, hemorrhage, and deep vein thrombosis (DVT), we have discovered a specific role for platelet release of HMGB1 in both micro- and macro-vascular thrombotic complications following injury. The release of HMGB1 from platelets was discovered to occur on platelet-derived exosomes as measured by nanoparticle tracking technology, and adoptive transfusion of platelet exosomes worsened thrombotic complications. Utilizing hydroxychloroquine, an inhibitor of HMGB1 production in other cell types, we were able to markedly decrease thrombosis via specific inhibition of platelet exosome release, resulting in a significant reduction in DVT formation in mice. Taken together, these data suggest that platelet TLR4 and HMGB1 may represent therapeutic targets to attenuate organ injury and late thrombotic complications following trauma.
Presenter: Sahar Nissim, MD, PhD

YPSA-18 | Poster session 2

Mutations in RABL3 alter KRAS prenylation and are associated with hereditary pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers, with limited treatment options despite intensive research efforts. Familial predisposition to PDAC is thought to occur in ~10% of cases, but causative genes have not been identified in most of these families. Uncovering the genetic basis for PDAC susceptibility has immediate prognostic implications for families and can provide precious mechanistic clues to PDAC pathogenesis. Here, we perform whole-genome sequence analysis in a family with high incidence of PDAC and identify a germline nonsense mutation in the member of RAS oncogene family-like 3 (RABL3) gene that has never before been directly associated with hereditary cancer. The truncated mutant allele (RABL3_p.Ser36*) co-segregates with cancer occurrence. To evaluate the contribution of the RABL3 mutant allele in hereditary cancer, we generated rabl3-heterozygous mutant zebrafish and found increased susceptibility to cancer formation in two independent cancer models. Complementary unbiased approaches implicate RABL3 in RAS pathway regulation. RNA-seq and genome-set enrichment analysis of juvenile rabl3 mutants reveals a KRAS upregulation signature. Furthermore, affinity-purification mass spectrometry for proteins associated with RABL3 or RABL3_p.Ser36* identifies Rap1 GTPase-GDP dissociation stimulator 1 (RAP1GDS1, SmgGDS), a chaperone that regulates prenylation of RAS GTPases. Indeed, in vitro studies demonstrate that RABL3_p.Ser36* accelerates KRAS prenylation, and this impact is lost in the absence of H/N/KRAS proteins. Whereas heterozygous rabl3 mutant zebrafish exhibit cancer predisposition, homozygous rabl3 mutant zebrafish develop severe craniofacial, skeletal, and growth defects consistent with human RASopathies, and these defects are partially rescued with the MEK inhibitor trametinib. Our findings support a gain-of-function rather than a null function typically associated with premature protein truncations. The discovered causative RABL3 germline mutation provides new diagnostic opportunities for genetic testing in other cancer families and uncovers an alternative mechanism for dysregulated RAS signaling in development and cancer.

Presenter: Edwin Ostrin, MD, PhD

YPSA-19 | Poster session 1

Myo-inositol reduces pro-tumoral inflammation in a mouse model of lung cancer chemoprevention

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Myo-inositol is a sugar alcohol found in many fruits and grains. It has been studied as a lung cancer chemopreventive agent due to its effect in tobacco-exposed mouse models of lung cancer. However, a recent phase IIb study found that myo-inositol did not significantly reduce the number of pre-malignant lesions found in the airways of smokers. A better understanding of myo-inositol’s exact mechanism of action may permit better selection of subjects who would benefit from chemoprevention.

Transgenic mice carrying activated Kras in the airway epithelium (CCSPCre+; KrasLSL-G12D+/−, or CC-LR) were raised from weaning to 14 weeks of age on a diet containing one of four potential chemopreventive agents or a control diet. CC-LR mice develop histologically apparent premalignant lesions, including bronchial hyperplasia and atypical aden-
Excessive glucagon receptor action in hepatocytes is a major contributing factor to type 2 diabetes (T2D). Accordingly, there has been great interest in developing glucagon receptor antagonists (GRAs) as a treatment for T2D. Although phase II clinical trials have shown that GRAs effectively lower blood glucose in T2D subjects, they increase plasma low density lipoprotein (LDL) cholesterol levels, which has presented a significant block to their development. In this context, recent studies have suggested that cholesterol and proprotein convertase subtilisin/kexin type 9 (PCSK9) levels can be regulated by fasting and perhaps glucagon, but in-depth mechanistic insight is lacking. In order to test the functional importance of hepatic glucagon action on lipid metabolism, we silenced glucagon receptor (GcgR) in obese mice using AAV8–H1-shGcgR to silence the receptor in hepatocytes. Consistent with previous reports, this treatment effectively lowered blood glucose in obese mice without a change in body weight. Moreover, GcgR silencing, like GRAs in humans, significantly increased plasma total cholesterol and LDL cholesterol. In search for the mechanism, we found that inhibition of GcgR significantly lowered hepatic LDL receptor protein levels and increased both hepatic PCSK9 and circulating PCSK9 (295.6 ± 42 in control vs. 597.1 ± 62.1 ng/ml in shGcgR; P < 0.01), without an effect on cholesterol synthesis. To determine causation, we treated GcgR-silenced obese mice with a neutralizing monoclonal antibody against PCSK9 and found that this intervention restored hepatic LDL receptor protein levels and prevented the increase in LDL cholesterol. In order to understand whether the regulation of PCSK9 by glucagon signaling is cell intrinsic to hepatocytes, we assayed PCSK9 in primary hepatocytes treated with scrambled RNA (control) or GcgR siRNA. Consistent with our in vivo data, GcgR silencing significantly increased cellular and secreted PCSK9 protein. In terms of mechanism, GcgR silencing in hepatocytes did not increase Pcsk9 mRNA. Rather, blocking GcgR increased the half-life of PCSK9 protein by suppressing signaling through exchange protein activated by cAMP (Epac). In particular, the ability of GcgR silencing to increase PCSK9 and suppress LDL receptor protein levels was mimicked by hepatocytes lacking Epac. Conversely, treatment with a specific Epac activator lowered PCSK9 and increased LDL receptor protein levels in WT hepatocytes. Thus, GcgR signaling through Epac appears to have critical effects on processes that regulate cholesterol metabolism through PCSK9. These new findings have important implications for the lipid metabolism effects of hepatic glucagon signaling in both normal physiology and metabolic disease and for the development of safer GRA-like drugs to treat T2D.
Inflammation, striatal dopamine receptor binding, and anhedonia in depression

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Growing evidence supports a potential relationship between inflammation and the dopamine (DA) system in major depressive disorder (MDD). Pro-inflammatory cytokines including interferons (IFNs), interleukin-1 (IL1), and tumor necrosis factor-alpha (TNF-α) reduce the availability of monoamines (e.g., DA) by reducing monoamine synthesis and increasing the expression and function of presynaptic reuptake pumps. DA D2 receptor-deficient mice have demonstrated severe activation of astrocytes and microglia and pronounced inflammatory responses. Neuroimaging studies in non-psychiatric samples support inflammation-induced reductions in responsiveness to positive reward and effort-based motivation, core dimensions of the DA-related anhedonia construct. In this study, we hypothesized that MDD patients with increased levels of baseline pro-inflammatory cytokines will show (1) reduced postsynaptic striatal DA D2 receptors and (2) higher anhedonia scores.

We measured striatal DA D2 receptor binding potential (BP) using positron emission tomography (PET) and the selective DA D2 receptor antagonist [¹C]raclopride in 26 unmedicated subjects with MDD. Anhedonia scores were assessed using two different self-reported questionnaires: the Apathy Evaluation Scale (motivational anhedonia) and the Snaith-Hamilton Pleasure Scale (consummatory anhedonia). Baseline pro-inflammatory cytokines (natural log transformations for IFN-γ, IFN-α2, TNF-α, and IL-1α) and D2 binding maps were analyzed according to standard procedures.

Higher levels of baseline pro-inflammatory cytokines were negatively correlated with DA D2/3 receptor binding in the bilateral ventral striatum (VS) (left VS: IL-1α [r = -0.48, P = 0.02]; right VS: IFN-γ [r = 0.45, P = 0.04], TNF-α [r = -0.56, P = 0.008]). We found no significant association between consummatory or motivational anhedonia scores and baseline levels of pro-inflammatory cytokines.

Consistently with previous animal evidence, our results suggest that in patients with MDD, higher levels of pro-inflammatory cytokines are associated with reduced striatal D2 postsynaptic binding. While the clinical implications of the DA-depression-inflammation relationship remain to be further investigated, a better understanding of this relationship might guide future development and testing of novel treatment strategies in patients with DA deficits and increased inflammation.
negative predictive value was 91.9%.

Among 15,075 HRS participants, we identified 1,268 AHRF hospitalizations and 13,117 “at-risk” hospitalizations. AHRF hospitalizations were matched to 1,157 non-hospitalized adults and to 1,017 at-risk hospitalizations. The matched cohorts had indistinguishable demographics, economic status, comorbidity burden, pre-hospitalization functional limitations, and self-rated health. Among patients who survived at least 30 days, AHRF was associated with a 24.4% (95% CI: 19.9%-28.9%, \( P < 0.001 \)) absolute increase in late mortality relative to adults not currently hospitalized and a 6.7% (95% CI: 1.7%-11.7%, \( P = 0.01 \)) absolute increase relative to adults hospitalized with acute inciting event(s) alone. Even among AHRF patients who survived a full year, mortality during the 2nd year remained elevated relative to the matched non-hospitalized cohort, but not relative to matched at-risk hospitalizations. Hospitalization for an acute inciting event(s) accounted for 71.2% (95% CI: 52.7%-89.6%) of the increased odds of late mortality after AHRF.

In this national sample of older Americans, approximately one in four AHRF survivors had a late death not explained by pre-AHRF health status. More than 70% of this increased risk is explained by hospitalization for acute inciting events, while 30% was attributable to respiratory failure. These findings suggest that the inflammation and multi-organ failure associated with ventilator-induced lung injury may resolve faster than the inflammation and immune-suppression that follows sepsis and, clinically, that post-ICU rehabilitation efforts should not be restricted to patients who have undergone mechanical ventilation.

**Presenter: Seth Rakoff-Nahoum, MD, PhD**

**YPSA-23 | Poster session 1**

**Social ecology of sugar utilization by the intestinal microbiota**

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The human intestine is colonized with trillions of microorganisms important to health and disease. There has been an intensive effort to catalog the species and genetic content of this microbial ecosystem. However, little is known of the ecological interactions between these microbes, a prerequisite to understanding the dynamics and stability of this host-associated microbial community. Using a social evolutionary framework to identify public good resources in the intestinal ecosystem, we have uncovered a previously unappreciated rich interaction network based on polysaccharide utilization between members of the order Bacteroidales. Bacteroidales able to grow on polysaccharides use remarkably different strategies for the use of polysaccharides, as some liberate significant amounts of polysaccharide breakdown products (PBP) available for public use, while other members liberate none at all, suggesting that polysaccharide public good liberation is a variable trait under natural selection. These PBP are critical to the survival and dynamics of the Bacteroidales community, as we have identified members that are unable to utilize polysaccharides as energy sources but thrive on the PBP provided by other Bacteroidales. Using genetic and molecular tools, we demonstrate that the enzymes (glycosyl hydrolases) responsible for the generation of PBP are secreted on outer membrane vesicles and can act at significant distances from the producing bacterium. In addition to characterizing the importance of polysaccharide-based public goods in inter-species ecological interactions among the Bacteroidales, we have begun to characterize the social evolutionary ecology of polysaccharide utilization in clonal populations. Surprisingly, we find that in addition to direct and clonal population fitness benefits, some Bacteroidales members have evolved a costly, dedicated cross-feeding system involved in inter-species cooperation that is stabilized by partner choice, a phenomenon in which a partner in a relationship provides weighted reciprocal feedback benefits to the counter-partner from which it receives the greatest fitness benefit. This polysaccharide-based network likely represents foundational relationships creating organized ecological units within the intestinal microbiota, knowledge of which can be applied to impact human health.
Vitreoretinal lymphoma (VRL), the most common lymphoma of the eye, is a rare form of primary CNS lymphoma (PCNSL). Most frequently, a high-grade diffuse large B cell lymphoma, VRL, can cause vision loss, and its prognosis remains dismal: the overall survival time is 3 years after diagnosis. Radiotherapy and chemotherapy are used but remain frequently ineffective, and no standardized treatment regimen exists. Furthermore, no biologically targeted treatments, based on the genetic profile of the tumor, are available, as VRL has hitherto not comprehensively been profiled. To address these unmet needs, we hypothesized that a next-generation sequencing (NGS)-based, National Cancer Institute (NCI) MATCH Trial-modified panel would be able to identify actionable genomic alterations from small-volume, intraocular liquid biopsies.

In this retrospective study, we collected diluted vitreous biopsies from 4 patients with a high suspicion for VRL. Following cytological confirmation of lymphoma (all were diffuse large B cell lymphomas), we subjected genomic DNA from the biopsies to NGS, using a panel containing 126 genes (3,435 amplicons across several hotspots per gene), which was modified from that of the NCI MATCH Trial, a new trial that has matched patients with cancers that have not responded (or never responded) to investigational therapeutics based on their prioritized mutation profile rather than site of tumor origin. Using a validated bioinformatics pipeline, we assessed for the presence of actionable mutations and copy number alterations. In all four small-volume, intraocular liquid biopsies, we obtained sufficient genomic DNA for analysis, even in diluted samples in which the undiluted vitreous was used for cytology and flow cytometry. Using NGS, we found targetable heterozygous gain-of-function mutations in the MYD88 oncogene and confirmed in our cohort the presence of the L265 mutations, previously described using PCR-based assays. For the first time in VRL, we also identified the MYD88 S243N mutation. We also identified two copy number losses in the tumor suppressor CDK2NA in all four cases, and one copy loss of the tumor suppressor PTEN in one sample. In one case, in which vitreous biopsies were originally read as cytologically negative, but which was confirmed as lymphoma when a lesion appeared in the brain two years later, our NGS-based approach detected tumoral DNA in the banked, original liquid biopsy.

We performed the first systematic exploration of the actionable cancer genome in VRL. Our NGS-based approach identified exploitable genomic alterations such as gain-of-function MYD88 oncogene mutations and loss of the tumor suppressor CDK2NA, and thus illuminates new routes to biologically targeted therapies for VRL, a cancer with a dismal prognosis. This precision medicine strategy could be used to nominate novel, targeted therapies in lymphomas and other blinding and deadly ocular, orbital, and ocular adnexal diseases for which few treatments exist.
peribiliary glands (PBGs), the biliary progenitor cell niche. The steps from chronic inflammation to cholangiocarcinoma are not well defined, but are thought to include PBGs hyperplasia. We aimed to elucidate the effect of activated Hh signaling on the biliary progenitor cell niche during homeostasis. We used wild-type (WT) mice, and transgenic murine models of Shh ligand overexpression (pCMV-ShhH14) and Hh pathway inhibition (Gli1−/−). We assessed bileduct (BD) expression of biliary cell markers (CK7; TFF2) with a quantitative reverse transcription polymerase chain reaction and immunohistochemistry; and acidic mucin, typical for cholangiocytes, with alcin blue chemical staining. We had developed the novel 3-dimensional organoid models derived from extrahepatic BDs of WT and pCMV-ShhH14 mice to study the direct effect of Shh overexpression on biliary progenitor cells by enumeration of 5-ethynyl-2′-deoxyuridine (the cell proliferation marker)-positive epithelial cells ex vivo. BD histology was evaluated by a trained pathologist. We demonstrated that Shh expression in pCMV-ShhH14 mice localized to the PBGs. While extrahepatic BDs from WT, pCMV-ShhH14, and Gli1−/− mice showed no macroscopic differences, the area occupied by the PBGs was expanded in the Gli1−/− mice. The pCMV-ShhH14 animals had significantly increased Shh mRNA expression compared to the WT animals. Patched-1 mRNA expression increased in pCMV-ShhH14 and was decreased in the Gli1−/− mice. The pCMV-ShhH14 animals demonstrated a significant increase in CK7 mRNA expression compared to the WT and Gli1−/− animals. pCMV-ShhH14 animals with Shh overexpression in the PBGs had a mild increase in TFF2 mRNA and protein expression compared to the WT animals. The Gli1−/− animals demonstrated a significant decrease in TFF2 mRNA and acidic mucin expression. The biliary organoids derived from pCMV-ShhH14 mice demonstrated increased cell proliferation compared to WT animal-derived organoids ex vivo.

Upregulation of Hh signaling in the biliary progenitor cell niche promotes cholangiocyte differentiation in vivo. Genetic ablation of the Hh pathway in the biliary stroma in vivo and isolation of biliary progenitor cells from their microenvironment in the organoid model promotes biliary progenitor cell proliferation and BT metaplasia. These data indicate the cross-talk between Shh ligand-producing PBGs and receptive stromal cells for normal biliary differentiation in the PBGs at homeostasis.

Bacteremia due to extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) is associated with inadequate empirical therapy and high mortality rates in neutropenic patients. Increased knowledge of ESBL-E colonization rates and risk of subsequent ESBL-E bacteremia in neutropenic populations are needed.

We collected perianal swabs from patients undergoing a hematopoietic stem cell transplant (HSCT) between May 2014 and September 2016 on admission and weekly thereafter until neutrophil engraftment. All patients received prophylactic levofloxacin while neutropenic. Swabs were plated onto selective ESBL agar plates and colonies isolated from these plates were identified to the species level and underwent antimicrobial susceptibility testing using an automated broth microdilution system and Etest (bioMérieux) for new Gram-negative agents. All ceftriaxone-resistant Enterobacteriaceae (CRO-R-E) underwent phenotypic testing for ESBL production. We then determined the prevalence of colonization with ESBL-E prior to HSCT and the incidence of acquiring ESBL-E during the transplant admission in patients not initially colonized. We also assessed the incidence of ESBL-E bacteremia in patients who were and were not colonized on admission. Additionally, we performed multiplex PCR and gene sequencing to characterize the β-lactamase genes harbored by these organisms. Colonizing and bloodstream isolates from patients with CRO-R-E bacteremia underwent further characterization by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) to determine whether the strains were genetically identical.

We analyzed 312 patients, including 212 allogeneic and
A LIN28B-RAN-AURKA signaling network promotes neuroblastoma tumorigenesis


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100 autologous HSCT recipients. Ten percent of patients (31/312) were colonized with an ESBL-E prior to transplant (allogenic: 10%; autologous: 9%) and an additional 3% of patients were colonized with non-ESBL CRO-R-E. Of the 31 pre-transplant colonizing ESBL-E strains, 28 were Escherichia coli and three were Klebsiella pneumoniae, and all but two harbored the ESBL gene blaCTX-M. Anti-microbial susceptibility rates of the colonizing ESBL-E to broad-spectrum β-lactam agents were: cefepime: 23%, piperacillin-tazobactam: 81%, ceftolozane-tazobactam: 84%, and meropenem: 94%. All isolates were susceptible to ceftazidime-avibactam. Of patients not colonized with an ESBL-E prior to transplantation, 5% acquired an ESBL-E on a subsequent swab. Twelve of the 31 patients (39%) colonized with an ESBL-E pre-transplant developed bacteremia due to an ESBL-E during their transplant admission, and all occurred in patients initially colonized with a levofloxacin-resistant organism. In contrast, only one of 281 patients (0.4%) not colonized with an ESBL-E on admission developed ESBL-E bacteremia, and this patient acquired an ESBL-E during the transplant admission (P < 0.001). Of the 12 pairs of colonizing and bloodstream ESBL-E, 11 had identical and PFGE patterns.

HSCT recipients who are colonized with ESBL-E have high rates of bacteremia from their colonizing strain during neutropenia. Assessing for pre-transplant ESBL-E colonization may identify neutropenic patients at high risk for ESBL-E bacteremia who should receive a carbapenem as initial empirical therapy for new onset of fever.

Neuroblastoma, a childhood cancer of the sympathetic nervous system, accounts for approximately 10%-15% of pediatric oncology deaths. Previously we showed that high LIN28B expression is associated with advanced-stage disease and worse patient outcome. Here we focus on defining the oncogenic signaling networks influenced by LIN28B, which binds mRNAs directly and is a master regulator of the let-7 family of tumor suppressor microRNAs.

To discover LIN28B-associated pathways in neuroblastoma, we performed gene set enrichment analysis (GSEA) on mRNA expression datasets and analyzed DNA copy number datasets. We used siRNAs, shRNAs, and microRNA mimetics to perturb transcripts of interest in neuroblastoma cells and measured effects on downstream signaling, protein-protein interactions, and proliferation.

We applied GSEA to mRNA expression profiles from 250 neuroblastoma tumors and found LIN28B expression to be robustly correlated with several biologically relevant gene sets, including “RAN signaling.” We focused on RAN signaling, as RAN is a member of the RAS family of GTPases implicated in the pathogenesis of several malignancies, and we demonstrated a strong positive correlation between LIN28B and RAN expression, most strikingly in the MYCN-amplified context (P = 2.2 × 10^-10). We next analyzed 374 high-risk neuroblastoma tumors and found that 28% of them displayed recurrent somatic copy number gain of chromosome 12q24, the genomic location of RAN, which was associated with increased RAN expression (P = 0.0004) and was inversely related to MYCN amplification (P = 0.0021). Increased RAN expression was associated with stage 4 disease (P = 0.0047) and decreased overall survival (P = 0.0002). To further dissect the LIN28B-RAN relationship, we depleted LIN28B using shRNAs, showing that it reduced RAN RNA and protein levels. LIN28B directly bound RAN mRNA, likely enhancing its translation. As RAN promotes the phosphorylation and activation of Aurora kinase A (AURKA), we then demonstrated that LIN28B leads to AURKA activation via RAN. Moreover, we demonstrated that AURKA is a direct let-7 target, defining a separate mechanism by which LIN28B/let-7 influences AURKA expression. Finally, we showed that RAN depletion resulted in decreased neuroblastoma proliferation, phenocopying LIN28B depletion.

These results demonstrate that enhanced LIN28B expression and chromosome 12q24 gain each independently promote RAN expression and that LIN28B and RAN signaling further converge on AURKA. Collectively, our studies support LIN28B as a master regulator of multiple oncogenes implicated in neuroblastoma pathogenesis. Currently, we are...
investigating the contribution of LIN28 signaling to self-renewal and metastatic dissemination, processes that contribute to the aggressive behavior of high-risk neuroblastoma.

Presenter: Natalie D. Shaw, MD, MMSc

YPSA-28 | Poster session 2

Mutations in SMCHD1 are associated with isolated arhinia, Bosma arhinia microphthalmia syndrome, and facioscapulohumeral muscular dystrophy type 2

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Arhinia (absent nose) is a rare congenital malformation that occurs in isolation or with ocular and neuro-reproductive defects, a triad called Bosma Arhinia Microphthalmia syndrome. The genetic cause is unknown.

We assembled a cohort of 40 patients with arhinia and used next-generation sequencing, rare mutation burden test-
Arhinia was typically accompanied by a high-arched/flat palate, hypoplastic sinuses and maxilla, nasolacrimal duct and choanal atresia, and ocular defects (aniso-micro-ophthalmsia, cataract). The reproductive axis was assessed in 31 subjects, and 97% demonstrated hypogonadotropic hypogonadism. Olfactory structures were absent in all subjects with brain imaging.

We identified rare, heterozygous missense SMCHD1 variants in 86% of cases. All variants were located in the SMCHD1 ATPase domain, which we determined to be under strong evolutionary constraint using regional constraint models. Gene-based burden testing of rare variants confirmed that SMCHD1 was the only gene to achieve genome-wide significance ($P = 2.9 \times 10^{-17}$).

This discovery was unexpected, as loss-of-function mutations in SMCHD1, in combination with a permissive 4q35 haplotype and truncated D4Z4 repeat cause a rare form of muscular dystrophy (FSHD2). SMCHD1 is an epigenetic repressor that maintains X-inactivation and silences autosomal gene clusters. In FSHD2, loss of SMCHD1 function leads to D4Z4 hypomethylation and abnormal expression of the muscle toxin DUX4. Variants associated with FSHD2 span the entire gene and include missense and truncating mutations; variants associated with arhinia were exclusively missense and within the ATPase domain. However, several FSHD2-specific mutations also localized to the ATPase domain, and at least two FSHD2 variants were detected in our arhinia cohort. The arhinia mutations had the same direction of effect as reported for FSHD2 mutations: 74% of arhinia cases with an SMCHD1 variant had D4Z4 hypomethylation characteristic of FSHD2, while family members without an SMCHD1 variant did not. Further analyses identified two arhinia patients and a father who met all genetic requirements for FSHD2; one patient has asymmetric muscle atrophy, and the father is being treated for muscular dystrophy.

Transient and permanent (CRISPR/Cas9) ablation of the SMCHD1 locus in zebrafish caused abnormal facial cartilage, small eyes, and blunted GnRH-immunopositive terminal nerve projections. Each phenotype was rescuable with wildtype but not with mutant SMCHD1 mRNA, demonstrating assay specificity. RNA-seq and gene-set analyses in human cell lines revealed down-regulation of genes statistically enriched for one phenotype: “depressed nasal tip.”

Rare variants in an evolutionarily constrained region of SMCHD1 are associated with arhinia. Mutations in SMCHD1 also cause an oligogenic form of muscular dystrophy, demonstrating a strikingly diverse phenotypic spectrum from identical alleles and implicating disruption of critical interactions with other loci.
Pancreatic cancer is an almost universally deadly disease with few effective treatments. Surgical resection is the most proven treatment; however, only a handful of patients present with resectable disease. Approximately 30%-40% of pancreatic cancer patients have locally advanced disease, where their tumor is not resectable but has not yet spread beyond the pancreas. These patients often require a combination of powerful chemotherapy agents to destroy microscopic metastatic disease as well as precisely delivered radiation treatments for local control to have any hope for prolonged survival. The chemotherapy and radiation treatments required to treat pancreatic cancer are intense and come with many side effects, particularly gastrointestinal toxicities. For instance, radiation therapy to the pancreatic head requires high doses of radiation to achieve tumor control, but these cannot be safely given to patient because the adjacent duodenum is very sensitive to radiation damage.

The EGLN family of prolyl hydroxylases are key regulators of cell growth, survival, metabolism, and the hypoxic response. We have previously demonstrated that these proteins regulate the radiation sensitivity of the intestinal tract, where EGLN inhibition protects the gut from otherwise lethal doses of radiation. Despite this strong phenotype, it is unknown whether this protective effect can apply to clinically relevant dosing of chemoradiation therapy.

We obtained the EGLN inhibitor FG-4592, which is in the late stages of FDA approval for another indication. We first assessed whether FG-4592 radioprotects wild-type C57BL/6 mice against a dose-escalated radiotherapy regimen to the upper abdomen (75 Gy/15 fractions, BED\textsubscript{10} = 112.5 Gy). Strikingly, 100% of the mice that received the cytoprotectant FG-4592 lived 30 days after treatment, while none of the vehicle mice survived 10 days. Necropsies suggested intestinal bleeding as the chief source of mortality. We then treated genetically engineered KPC mice that develop spontaneous pancreatic tumors with sham RT, dose-escalated RT (75 Gy/15 fractions), or dose-escalated RT with FG-4592 for radioprotection (n = 18-25 per group). Treatment with FG-4592 significantly improved survival compared to sham RT (P < 0.0001) or RT alone (P < 0.03), likely by reducing GI toxicity.

Importantly, we confirmed that FG-4592 does not radioprotect tumors or enhance their growth. Moreover, the overexpression of HIF1 or HIF2 in syngeneic orthotopic pancreatic tumors slows tumor growth, further demonstrating a positive therapeutic ratio of EGLN inhibition.

Thus, these data suggest a new paradigm that uses EGLN inhibitors to reduce normal tissue toxicity to enable curative treatment. This mechanism-based approach could be rapidly translated to the clinic, since FG-4592 could easily be repurposed for cytoprotection of the normal GI tract from chemoradiation damage.

Presenter: Sriram Venneti, MD, PhD

YPSA-31 | Poster session 1

Epigenetic deregulation in genomically silent pediatric posterior fossa ependymomas as a biomarker for patient stratification

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Ependymomas in children occur most commonly in the posterior fossa of the brain and cause significant morbidity and mortality. The pathogenesis of these tumors remains obscure, as recent whole genome and whole exome sequencing efforts have not yielded recurrent genetic mutations. We and others have shown that these tumors exhibit abnormal CpG island methylation, suggesting that epigenetic alterations may be a significant driver. To gain insights into the epigenetics of childhood ependymomas, we performed mass spectroscopy for histone modifications to discover that H3K27 trimethylation was reduced and H2K27 acetylation was increased in these tumors. Despite global reduction in H3K27me3, ChIP sequencing for H3K27me3 revealed genomic enrichment at several loci important for neurodevelopment. In a cohort of 195 childhood posterior fossa ependymoma samples, reduction in H3K27me3 was associated with worse prognosis (P < 0.0001). Tumors with reduced H3K27me3 corresponded to younger children (P < 0.0001), arose mainly from the roof of the forth ventricle and exhibited increased evidence of radiologic and histopathologic invasion. These data have clinical implications for biomarker development and to inform epigenetic approaches to treat PF ependymomas.

Presenter: Philip A. Verhoef, MD, PhD

Type 2 inflammatory responses protect against *Staphylococcus aureus* sepsis

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Sepsis is defined as life-threatening organ dysfunction caused by the body’s response to infection. Sepsis is associated with massive type 1 and type 17 inflammation, but the role of type 2 responses in sepsis remains unknown. To test the role of type 2 immune responses in sepsis, we treated mice with intratracheal IL-33 to induce an innate pulmonary type 2 response, followed by intravenous infection with *S. aureus* to induce sepsis. Surprisingly, type 2 responses were beneficial during acute infection, as IL-33 treatment protected mice from *S. aureus*-induced death. An increase in the ratio of lung eosinophils to neutrophils was revealed in IL-33-treated, *S. aureus*-infected mice, suggesting that type 2 responses suppress lung neutrophilia. Further, no difference was found in splenic granulocytes or lymphocytes, indicating that activation of pulmonary immune responses was sufficient to protect. Infection of PLZF-null mice, which have defective NKT and ILC2 cells normally required for type 2 innate responses after IL-33 treatment, resulted in accelerated mortality compared with wild-type littermate controls. Moreover, IL-33 did not rescue PLZF-null mice from death. However, infection of CD1d-null mice, which lack only NKT cells but have intact ILC2s, demonstrated no difference in mortality. Thus, pulmonary type 2 innate immunity provided by ILC2s is critical for protection against *S. aureus*-mediated death.

Presenter: Taia Wang, MD, PhD

YPSA-33 | Poster session 1

IgG antibodies to dengue enhanced for FcγRIIIA binding determine disease severity

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Dengue virus (DENV) infection in the presence of reactive, non-neutralizing IgG (RNNIg) is the greatest risk factor for dengue hemorrhagic fever (DHF) or shock syndrome (DSS). Progression to DHF/DSS is attributed to antibody-dependent enhancement (ADE); however, since only a fraction of infections occurring in the presence of RNNIg advance to DHF/DSS, the presence of RNNIg alone cannot account for disease severity. We discovered that DHF/DSS patients respond to infection by producing IgGs with enhanced affinity for the activating Fc receptor IIIA due to afucosylated Fc glycans and IgG1 subclass. RNNIg enriched for afucosylated IgG1 triggered platelet reduction in vivo and was a significant risk factor for thrombocytopenia. This indicates that therapeutics and vaccines restricting production of afucosylated, IgG1 RNNIg during infection
may prevent ADE of DENV disease. Thus, we have found that some individuals are inherently more susceptible to severe DENV disease due to production of IgGs with enhanced capacity for mediating ADE. Further studies will determine how patient selectivity in IgG responses that mediate ADE may determine susceptibility to disease caused by other flaviviruses.

Presenter: Michael Emmerson Ward, MD, PhD

YPSA-34 | Poster session 2

**Clinicopathological features of NCL occur in humans with progranulin haploinsufficiency**

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Heterozygous GRN mutations lead to progranulin (PGRN) haploinsufficiency and cause frontotemporal dementia (FTD), a neurodegenerative syndrome of older adults. Homozygous GRN mutations, on the other hand, lead to complete PGRN loss and cause neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease usually seen in children. Since the predominant clinical and pathological features of FTD and NCL are distinct, it is controversial whether the disease mechanisms associated with complete and partial PGRN loss are similar or distinct. Here, we show that PGRN haploinsufficiency leads to NCL-like features in humans, some occurring before dementia onset. Non-invasive retinal imaging reveals pre-clinical retinal lipofuscinosis in heterozygous GRN mutation carriers. Increased lipofuscinosis and intracellular NCL-like storage material also occurs in postmortem cortex of heterozygous GRN mutation carriers. Lymphoblasts from heterozygous GRN mutation carriers accumulate prominent NCL-like storage material, which can be rescued by normalizing PGRN expression. Fibroblasts from heterozygous GRN mutation carriers have impaired lysosomal protease activity. Our findings indicate that progranulin haploinsufficiency causes accumulation of NCL-like storage material and early retinal abnormalities in humans, and implicate lysosomal dysfunction as a central disease process in GRN-associated FTD and GRN-associated NCL. Furthermore, we propose that retinal imaging may be a useful clinical biomarker in monitoring responsiveness to therapeutics in development for GRN-associated FTD.

Presenter: Brendon O. Watson, MD, PhD

YPSA-35 | Poster session 1

**Variability of brain network element activity — a novel metric of brain state**

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The state of brain networks is believed to determine how they carry out their functions — from allowing normal mood to proper cognition. Sleep loss is known to lead to impaired cognition and altered mood; however, we have little evidence regarding how sleep changes or regulates the details of brain activity. Using novel methods for simultaneous recordings of large populations of neurons in frontal cortex of rats, we observed that sleep homogenizes the activity of cortical neurons in any given population of recorded principal neurons. During wakefulness, the population of principal cells in cortex showed a wide distribution of activity rates, but after sleep that distribution became narrowed. Furthermore, sleep sub-states such as REM, non-REM, and microarousals each play distinct roles in this overall narrowing process. Specifically, during non-REM sleep, activity of high-firing-rate neurons decreased, whereas activity of slow-firing neurons increased. The effect of REM was to reduce firing rates across the entire rate spectrum, whereas microarousals, which occur between non-REM epochs, increased firing rates of slow-firing neurons. Thus, the sub-states of sleep differentially affected neurons across the firing rate distribu-
tion, and the net result of sleep was to homogenize the firing rate distribution. These findings are at variance with current homeostatic models and provide a novel view of sleep in adjusting network excitability.

The width of this distribution represents a new measure of network functional state and may relate more broadly to other aspects of brain state.

Relatively, recent pilot work shows that the fast-acting antidepressant ketamine may re-balance cortical networks in a manner commensurate with sleep deprivation — a known antidepressant. However, in contrast to sleep deprivation, the effects of which are reversed after a sleep session, the effect of ketamine network rebalancing lasts for at least 24 hours. This work as well as further characterization of the network changes exerted by ketamine can help the psychiatric community better understand the brain basis of antidepressant action and may lead to novel screening methods for therapeutics.

Presenter: Marc Nathan Wein, MD, PhD

YPSA-36 | Poster session 2

Phosphoproteomic profiling reveals novel salt-inducible kinase targets downstream of parathyroid hormone signaling in osteocytes

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Osteocytes, post-mitotic cells deeply ensconced within bone, orchestrate bone formation and resorption through production of paracrine factors. Parathyroid hormone (PTH) activates receptors on osteocytes to accomplish both of these goals. We recently described that a crucial step in intracellular PTH signaling in osteocytes involves protein kinase A-mediated inhibition of salt-inducible kinase 2 (SIK2). Potent small molecule SIK2 inhibitors, such as YKL-05-099, mimic PTH action in vitro, and, like intermittent PTH treatment, boost bone formation and bone mass in vivo. However, the full range of SIK2 substrates responsible for these effects are currently unknown. Here, an unbiased approach was employed to identify novel osteocyte phosphoproteins whose abundance is decreased by both PTH and YKL-05-099. Ocy454 cells, a conditionally immortalized murine osteocyte-like cell line, were treated with vehicle, PTH (100 nM), or YKL-05-099 (10 μM) for 40 minutes. Samples were digested to peptides, and phosphopeptides were immunoprecipitated using a cocktail of monoclonal antibodies recognizing consensus kinase substrate motifs (RxRxxS*/T*, RRxS*/T*, and LxRxS*/T*) to deeply sample the portion of the phosphoproteome regulated by cAMP signaling and AMPK family kinases. Purified peptides were identified and quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The majority of the 4,920 phosphorylation sites identified were not affected by either PTH or YKL-05-099 treatment. However, 46 unique phosphorylation sites on 17 proteins (UAP1, MEPCE, IRS1, BACH2, SRRM2, LARP1, RALGAPA1, ESY12, INF2, PXN, FOXO3, MEKK3, FRS2, UMPs, STARD13, MED1, ULK1) were down-regulated by both treatments. Studies are now ongoing to characterize the roles of these 17 proteins in PTH signaling in osteocytes. Here we demonstrate that the transcription factor FOXO3 translocates from the cytoplasm to the nucleus in response to either PTH or YKL-05-099 in Ocy454 cells. FOXO3-deficient Ocy454 cells were generated by CRISPR/Cas9-mediated gene deletion. Control and FOXO3-deficient cells were treated with vehicle or PTH (20 nM) for 4 hours, and levels of a panel of 32 PTH/YKL-05-099 regulated transcripts were measured by digital gene expression (NanoString nCounter analysis). The majority of these transcripts showed normal regulation by PTH in FOXO3-deficient cells. However, PTH-induced up-regulation of IGF1 mRNA was defective in the absence of FOXO3. Therefore, FOXO3 represents a novel SIK2-regulated phosphoprotein with a selective role in PTH-mediated gene expression in osteocytes.

Presenter: James Michael Wells, MD

YPSA-37 | Poster session 1

The matrikine AcPGP induces pulmonary hypertension via vascular remodeling through Rho kinase

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Pulmonary hypertension (PH) is a chronic disease associated with poor long-term outcomes. Little is known about the mechanistic role of matrikines in pulmonary vascular smooth muscle remodeling. The collagen fragment acetyl-proline-glycine-proline (AcPGP) is a matrikine that causes neutrophilic inflammation and parenchymal remodeling and serves as a critical regulator of vascular endothelial permeability. Whether the effects of AcPGP are coupled to pulmonary vascular remodeling and PH is unknown. Thus, we tested the hypothesis that AcPGP causes PH through selectively activating Rho kinase.

Female A/J mice were separated into groups and treated with intraperitoneal (IP) phosphate-buffered saline (PBS) control, AcPGP, or AcPGP plus the rho-kinase inhibitor fasudil for 2 weeks. Mice underwent echocardiography, hemodynamic assessment via Millar catheterization, and collection of lung and heart tissue. Right ventricular (RV) hypertrophy was reported by the RV to left ventricle plus septum ratio (RV/LV+S). Vascular morphometry was performed using Image-Pro Premier 9.1 software. Western blot analysis was performed on tissue homogenates using MYPT1 and phospho-MYPT1-Thr696. In a separate experiment, AcPGP was measured by tandem mass spectrometry in C57BL/6 mice that were exposed to hypoxia (10% oxygen) or normoxia (21% oxygen) for three weeks. In a proof-of-concept study, AcPGP was measured on explanted lung tissue from individuals with idiopathic pulmonary arterial hypertension (IPAH) and age-matched controls.

Mice treated with IP AcPGP developed pulmonary hypertension as measured by echocardiography and by direct hemodynamic assessment. We observed a 2.4-fold increase in vascular wall thickness on morphometric evaluation of vessels smaller than 100 μm, as well as the development of RV hypertrophy in AcPGP-treated mice compared to control. AcPGP upregulates MYPT phosphorylation in a RhoA/Rho kinase-dependent manner by Western blot analysis, and these effects were abrogated by fasudil administration. AcPGP levels were increased in animals exposed to chronic hypoxemia. Individuals with IPAH had elevated tissue AcPGP compared to age-matched controls.

AcPGP mediates pulmonary vascular remodeling and pulmonary hypertension through Rho kinase activation. This pathway may identify novel therapeutic targets in IPAH and in PH-related to chronic lung diseases.

Ischemia/reperfusion (I/R) injury accounts for a significant portion of infarct size in acute coronary syndrome (ACS). Autophagy, an evolutionarily conserved process of cell recycling and degradation, plays a critical role in the cellular response to stress. We have reported that re-induction of I/R-suppressed cardiomyocyte autophagy with a histone deacetylase (HDAC) inhibitor, SAHA, affords significant cardioprotection. However, it is not known whether the cardioprotective effect of HDAC inhibitor is exclusively through re-induction of autophagy and its downstream mechanism. Further, newer and more specific therapeutics may eliminate side effects of HDAC inhibitor. Tat-beclin is a novel therapeutic agent whose only biological effect is to induce autophagy, and mitochondrial homeostasis has been implicated to cardiomyocyte survival. Here, we hypothesized that induction of autophagy with Tat-beclin at the time of reperfusion will be cardioprotective and HDAC inhibition protects cardiomyocytes through maintaining mitochondrial integrity and function.

Mice were randomized among 3 treatment groups prior to surgical I/R injury (45-min ligation of left anterior coronary artery followed by 24-hour reperfusion): (a) vehicle control, (b) nonfunctional Tat-scrambled, or (c) Tat-beclin delivered at the time of reperfusion. To define molecular mechanisms, cultured neonatal rat ventricular cardiomyocytes (NRVMs) and immortalized human ventricular cardiomyocytes (AC 16) were subjected to simulated I/R.

Tat-beclin reduced infarct size 31.5% (±10.3%, n = 11, P < 0.013 vs. vehicle). This treatment was associated with improved systolic function (declines in % fractional shortening: 19.8%± 3.7% vehicle; 18.7%± 2.1% inactive peptide; 8.5% ± 1.7% active peptide, P < 0.011 vs. vehicle). In NRVMs subjected to simulated I/R injury, cell death was reduced 41% (±6%, n = 12, P < 0.001 vs. vehicle). Improvements in cell survival correlated with increased cardiomyocyte autophagy measured by the autophagic marker LC3-II.
knockdown (RNAi) of either ATG5 or ATG7, essential proteins required for autophagic flux, eliminated the protective actions of autophagy-inducing therapy in simulated I/R. In AC 16 cells, compared with vehicle control, treatment of HDAC inhibitor (SAHA) increased the intact mitochondrial DNA 80% (±20.1%, n = 6, P < 0.01). Further, SAHA rescued I/R-induced mitochondrial potential loss (20% ± 6%, n = 6, P < 0.05). Tat-beclin treatment had similar effects.

Tat-beclin’s induction of cardiomyocyte autophagy reduces I/R-elicted infarct size and cell death and blunts declines in contractile function. HDAC inhibition and Tat-beclin-induced autophagy seem to protect cardiomyocytes through maintenance of mitochondrial homeostasis. Critically, this cardioprotection occurred when the intervention was imposed at the time of reperfusion, the clinically relevant context.

Presenter: Kelley Yan, MD, PhD

YPSA-39 | Poster session 1

Non-equivalence of extracellular Wnt and R-spondin ligands during Lgr5+ intestinal stem cell self-renewal

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The canonical Wnt/β-catenin signaling pathway governs diverse developmental, homeostatic, and disease processes. The Wnt and R-spondin (Rsps) ligand families synergistically promote β-catenin signaling in vitro, but their interchangeability, functional cooperation, and relative contributions to in vivo Wnt signaling remain unknown. Here, we deconstructed functional roles of Wnt versus Rsps ligands in the intestinal epithelium, an archetypal Wnt pathway-dependent self-renewing tissue supported by Lgr5+ intestinal stem cells (ISCs). We demonstrate that the default fate of Lgr5+ ISCs is lineage commitment, escape from which requires both Rsps and Wnt ligand signals. However, gain-of-function studies using Rsps versus a novel engineered Wnt analog reveal qualitatively distinct, non-interchangeable roles for these ligands in ISCs. Wnts are insufficient to induce Lgr5+ ISC self-renewal, but rather confer a basal competency by maintaining Rsps receptor expression that enables Rsps to actively drive and specify the extent of stem cell expansion. This functional non-equivalence of Wnt and Rsps ligands establishes a molecular precedent for regulation of mammalian stem cells by distinct priming and self-renewal factors, with broad implications for precision control of tissue regeneration.
ic-driven proliferation of exogenously delivered tumor-specific T-cells. We investigated two mechanisms that could be driving this result: (1) depletion of endogenous immune cells including regulatory T-cells (Tregs), and (2) a peripheral and/or local surge of IL-7 and IL-15 gamma chain cytokines. We have measured Treg counts in peripheral blood of lymphopenic mice and confirmed a drastically reduced number of Tregs compared to their lymphoreplete counterparts. To determine whether Treg depletion is required for the intratumoral persistence and efficacy of IC-delivered CAR T-cells, we established a DEREG mouse model that allows for selective depletion of Tregs. Early studies show that temporary depletion of Tregs at the time of CAR infusion has no effect on treatment efficacy. Continued work will investigate whether sustained Treg depletion throughout the duration of tumor-killing activity will alter CAR T-cell efficacy. Ongoing studies include isolating the effects of homeostatic cytokines on CAR T-cell activity using transgenic IL-7 and IL-15 knockout mice. Upcoming studies will involve using our EGFR-specific CAR model to examine how lymphopenia might affect the efficacy and toxicity of targeting a tumor-associated antigen in GBM. Our results will help to clarify the mechanisms by which lymphopenia enhances CAR T-cell efficacy, and have potential to change approaches in the treatment of GBM.

Presenter: Melissa L. Abel

HHMI-1 | Poster session 1

Host lymphodepletion enhances the frequency, persistence, and antitumor efficacy of loco-regionally delivered tumor-specific CAR T-cells against glioblastoma

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Glioblastoma (GBM) is the most common malignant primary brain tumor and is uniformly lethal. Conventional treatment is limited in part by a lack of specificity leading to damage of healthy brain tissue. The adoptive transfer of T-cells genetically engineered to express chimeric antigen receptors (CARs) represents a promising strategy to safely and efficiently target tumor cells within the central nervous system. CARs are artificial transmembrane receptors that couple the antigen-binding regions of an antibody with intracellular signaling components of a T-cell receptor. We have developed human and murine CARs that specifically recognize epidermal growth factor receptor (EGFR), a commonly amplified gene among GBMs, as well as EGFRvIII, a tumor-specific variant expressed in GBMs and other neoplasms. Previous studies from our lab have evaluated loco-regional delivery of EGFRvIII-specific CAR T-cells as a way to bypass variability in T-cell migration and enhance CAR T-cell survival and proliferation. CARs prolonged survival at modest doses, but were still hampered by poor persistence and displayed markers of T-cell exhaustion. In order to improve efficacy, we evaluated the effects of lymphodepletion prior to intra-cranial (IC) EGFRvIII CAR T-cell administration. These studies resulted in 90-100% long-term cures, while immune reconstitution impaired therapeutic efficacy. Additionally, host lymphodepletion enhanced the persistence of CARs in the brain over time and led to a significant decrease in CAR expression of T-cell exhaustion markers. The benefits associated with host lymphodepletion for adoptive T-cell immunotherapy are thought to include the improved functionality and the homeostatic

Presenter: Hena S. Ahmed

HHMI-2 | Poster session 2

Tmprss3 gene expression and gene therapy in a mouse model of human deafness

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This project aims to characterize hearing in a mouse model of deafness resulting from mutations in the transmembrane protease serine 3 gene (Tmprss3), and attempt to recover hearing using viral-mediated gene therapy. Tmprss3 muta-
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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease that is associated with the poorest of prognoses. The dismal outcomes associated with this condition are due in part to the highly active cancer-associated stroma that surrounds and communicates with cancer cells. Cancer-associated fibroblasts constitute a significant proportion of the cancer-associated stroma, and play a major role in the signaling interactions that increase tumor aggressiveness and resistance to therapy. Our project is focused on “reprogramming” cancer-stromal interactions to facilitate increased tumor susceptibility to standard-of-care chemotherapies and to the clinically promising light-based modality photodynamic therapy (PDT). Here, we test the efficiency of FDA-approved differentiation-promoting agents such as vitamin D (calcipotriol), all-trans retinoic acid (ATRA), and 5-fluorouracil (5-FU) to reprogram cancer-stromal interactions in vitro, and probe whether stromal reprogramming improves the cytotoxic efficacy of PDT in three-dimensional (3D) co-culture models comprising PDAC cells and cancer-associated fibroblasts (CAFs). We show that calcipotriol and 5-fluorouracil reduce the expression of α-smooth muscle actin (αSMA), a marker of stromal activation, in basal cell carcinoma-associated fibroblasts (BCAF). In addition, calcipotriol and 5-FU abrogate BCAF and pancreatic cancer-associated fibroblast (PCAF) induced changes in PDAC cell metabolic activity. Together, these results indicate that vitamin D and 5-FU may reprogram CAF-PDAC interactions. We probe whether PDAC resistance to PDT is overcome with this approach, and observe that BCAF and PCAF 3D co-cultures exhibit heightened PDT susceptibility following vitamin D pretreatment, suggesting that stromal reprogramming may be a viable strategy to overcome the profound therapeutic recalcitrance seen in clinical PDAC. In vivo studies are underway to confirm these findings, with the aim of improving clinical PDAC outcomes and augmenting ongoing clinical trials.

**Presenter:** John P. Andrews

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Pancreatic ductal adenocarcinoma resistance to photodynamic therapy

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Pancreatic ductal adenocarcinoma (PDAC) is a devastating disease that is associated with the poorest of prognoses. The dismal outcomes associated with this condition are due in part to the highly active cancer-associated stromal reprogramming with differentiation-promoting agents overcomes pancreatic ductal adenocarcinoma resistance to photodynamic therapy

**Presenter:** Sriram Anbil

HHMI-3 | Poster session 1

Subcortical cholinergic arousal nuclei during seizures: in vivo whole-cell recordings in a rat model

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The mechanism of how seizures impair consciousness is not fully understood, but recent evidence suggests that ictal suppression of subcortical cholinergic arousal circuits, such as...
the nucleus basalis (NB) and pedunculopontine tegmental nucleus (PPTg), may play a role. Functional imaging and extracellular techniques show ictal suppression of activity in the NB and PPTg in a rat model of limbic seizures, but the postsynaptic signaling associated with this depressed firing rate is not known. The purpose of this investigation is to elucidate postsynaptic changes in neurons of the subcortical cholinergic arousal system during seizures.

Whole-cell recording (WCR) from neurons in vivo is a powerful technique for characterizing postsynaptic neuronal signaling from intact physiologic circuits. Whole cell recording is commonly used to access cortical neurons in vivo, but the literature reporting whole-cell recording from deep brain structures in vivo is sparse and in vivo WCR in the PPTg has not been previously reported. In this study, whole cell recordings were obtained in neurons of the PPTg, 6-8 mm deep, measured from the cortex, in the brain of head-fixed, anesthetized rats during limbic seizures.

One aspect limiting the depth of WCR is reproducible creation of long, narrow glass pipettes of sufficiently low resistance. A second obstacle to deep WCR is maintaining a viable pipette tip that can form a seal of >1 GΩ with the target neuronal cell membrane (i.e., gigaseal) after passing through large amounts of tissue. The former problem was addressed using a 2.5 x 4.5 mm filament in a model P-1000 micropipette puller (Sutter Instruments) and creating a multi-line pulling program with a single high-velocity line followed by 3-4 low-velocity lines to produce micropipettes with a 10-mm taper and 4-6 MΩ resistance. To maintain a viable tip during descent through over 6-7 mm of tissue, the first pipette was lowered to the target region and left in place for 1 hour to allow the brain to form a narrow, low-resistance canal through which subsequent pipettes may pass. In addition, intrapipette positive pressure was maintained at 500 mbar throughout the descent, and dropped to <30 mbar at the target region. In this way, brain architecture was minimally perturbed and pipettes could access the PPTg.

Preliminary results of WCR in the PPTg during seizures show hyperpolarization of membrane potential associated with decreased firing in a subset of neurons histologically confirmed to be cholinergic. These data suggest cholinergic arousal systems are inhibited during hippocampal seizures, which may contribute to loss of consciousness. Ultimately these studies are aimed at identifying the synaptic mechanisms of depressed subcortical arousal during seizures, which may lead to new treatments aimed at preventing these changes and improving ictal and postictal cognition.

**Presenter:** Jacob H. Basham

**HHMI-5 | Poster session 1**

**The effect(s) of programmed death ligands 1 and 2 on the treatment of acute myeloid leukemia with anti-CD33 chimeric antigen receptor-engineered T lymphocytes**

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T lymphocytes engineered to express a chimeric antigen receptor (CAR) have shown profound clinical responses in relapsed/refractory B cell malignancies including leukemia, multiple myeloma, and lymphoma. This success of re-targeted T cells has yet to be extended to other hematologic malignancies. To this end, we have developed an immunotherapeutic approach to treat acute myeloid leukemia (AML) using CAR T cells re-directed against the myeloid-specific antigen CD33 (CART-33). CART-33 cells are potent and specific in eliminating AML cells in vitro and in vivo. Despite this, CART-33 cells have shown inadequate in vivo expansion and persistence in NOD-SCID IL2rγ (-/-) (NSG) AML xenograft models. To address this, we assessed the impact of AML-expressed programmed death ligands 1 and 2 (PD-1/L2) on CART-33 activity. PD-L1 inhibits T cell functions upon binding PD-1, an inhibitory receptor expressed on T cells that have been activated. Less is known about PD-L2’s effects. Interferon-gamma (IFN-γ), a primary effector cytokine secreted by CD4\(^+\) and CD8\(^+\) effector T cells, is a known inducer of PD-L1 on AML blasts. Using human AML cell lines U937, Oci-AML3, CMK, MV4-11, and a panel of primary human AML samples, we show that co-culturing AML with CART-33 cells, IFN-γ, TNF-α, or activated CART-33 supernatant can induce upregulation of both PD-L1 and PD-L2 on AML. Antibody-mediated blockade of cytokines in AML cultures mixed with activated CART-33 supernatant revealed that upregulation of PD-L1/L2 is dependent on CART-33 IFN-γ and TNF-α, and independent of other T cell-secreted effector cytokines. Additionally, IFN-γ and TNF-α synergize strongly in upregulating PD-ligands on AML. The kinetics and induction of PD-L2 are distinct from those of PD-L1. Although PD-L1 is well documented to suppress T cell function via ligation of T cell-expressed PD-1, the induction of PD-L1/L2 on AML had no effect on the cytolytic activity of CART-33 cells against AML in short-term (<48 h) cultures. Paradoxically, 24-hour pre-treatment of AML with either IFN-γ or CART-33 supernatant increased AML susceptibility to
Evaluating organic fluorophore phototoxicity: quantification of phototoxic potential and effects

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Visualization of the spatiotemporal relationships underlying biological processes is becoming increasingly vital to our understanding of cellular physiology and pathology. Recent advances in imaging technologies have expanded the utility of live-cell imaging, allowing the visualization of subcellular processes with high spatiotemporal resolution. Phototoxicity, light-mediated damage produced through the absorption of light by endogenous and exogenous chromophores, becomes more limiting to such techniques as excitation wavelength and intensity are increased. Previous reports have identified various phototoxic effects exerted by fluorophores utilized in live-cell microscopy, including the generation of reactive oxygen species, induction of apoptosis, and decrease of mitotic activity; however, a comparison of commonly used fluorophores and excitation wavelengths has yet to be reported due to the lack of a standardized method of evaluation. This study describes the development of an assay designed to compare the phototoxic potential of various small molecule fluorescent dyes starting with the current line of Janelia Fluor dyes. Results obtained through this assay, in combination with those of functional and endpoint analyses, will inform both dye utilization and dye development aimed at mitigating imaging-induced phototoxicity, ensuring that biological systems being studied are not influenced by the tools used to study them.

Targeting the bromodomain and extraterminal domain family of proteins in Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a neuroendocrine cutaneous malignancy that is lethal in its metastatic form. The basic helix-loop-helix transcription factor atonal homolog-1 (A TOH1) has been shown to be essential for normal development of Merkel cells and implicated in the pathogenesis of MCC. The bromodomain and extraterminal domain (BET) family of proteins, such as BRD4, modulate transcription of genes by binding to acetylated lysine residues on histones and have promise as therapeutic targets in several cancer types. Here we investigate the therapeutic potential of BET protein in MCC using novel BET inhibitors and degraders.

To investigate the role of BRD4 in MCC, we first screened 17 MCC cell lines for their response to two different classes of BET inhibitors, ZBC-11 and ZBC-246. ZBC-11 is small molecule inhibitor that binds to BRD4 and displaces it from chromatin. On the other hand, ZBC-246 is a fusion of ZBC-11 and thalidomide, which induces proteasomal degradation of BRD4 via the recruitment of ubiquitin complex. Sensitivity to these BET inhibitors was assessed by performing cell viability assays following 5 days of treatment. MCC cell lines were found to be very sensitive to BET inhibition, with most IC50 values at nanomolar concentrations. Since ZBC-246 leads to a complete loss of BRD4 protein in comparison to partial inhibitory activity of ZBC-11, MCC cell lines demonstrated greater sensitivity to ZBC-246 (IC50 60 pM to 73 nM) when compared to ZBC-11 (IC50 15 nM to >6 μM).

In order to identify the gene targets of BRD4 in MCC cells, we performed gene expression analysis on MCC-47 cells treated with both BET inhibitor (ZBC-11) and BET degrader (ZBC-246) at 3 and 24 hours. Microarray analysis demonstrated downregulation of ATOH1 and its downstream target SOX2. To validate this observation, we performed quantitative polymerase chain reaction (qPCR) and Western blotting for ATOH1 following 3- and 24-hour treatment with ZBC-11 and ZBC-246. There was significant downregu-
tion of ATOH1 mRNA and protein at both time points.

Based on this observation, we hypothesized that the suppression of cell growth demonstrated by BRD4 inhibition occurred via suppression of ATOH1. To test our hypothesis, we investigated the role of ATOH1 knockdown on the proliferative capacity of MCC-47 cells. To our surprise, siRNA-mediated knockdown of ATOH1 had no effect on the viability of the MCC-47 cell line, suggesting that the effect of BET inhibitors on MCC cells is independent of ATOH1. As a future direction, we are mining the gene expression data to nominate BRD4-regulated genes that may have a vital role in MCC cell survival.

Taken together, our results demonstrate that MCC cell are very sensitive to compounds targeting the BET family of proteins and provide a compelling rationale for clinical evaluation of BET inhibitors and degraders in MCC.

Presenter: Megan L. Clark

HHMI-8 | Poster session 2

**Leishmania**-specific skin-resident CD4+ T cells are formed from recently activated effector T cells

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Tissue-resident memory T (Trm) cells are critical components of protective immunity against a variety of pathogens. The majority of studies have focused on Trm cells at the site of infection or immunization, where inflammation promotes T cell recruitment. In contrast, few studies have focused on how Trm cells gain access to non-inflamed sites, an important issue for designing vaccines to target these cells. In mice that have resolved a primary infection with **Leishmania**, skin-resident memory CD4+ T cells have recently been shown to provide protection against challenge at sites distant from the initial infection site. This provides a model to determine when and how CD4+ Trm cells enter non-inflamed skin. We found that while **Leishmania**-specific CD4+ T cells enter the site of infection within a few hours, T cells were not found in non-inflamed skin distant from the primary infection site until 2 weeks after infection, and continued to enter the non-inflamed skin for at least 5 weeks. However, using parabiosis of naive mice and immune mice that had resolved infection, we found that **Leishmania**-specific CD4+ T cells present in the immune partner could not enter the non-inflamed skin of the naive partner. In contrast, upon re-challenge of the parabionts, these CD4+ T cells re-gained the ability to enter the non-inflamed skin of the naive parabiont. To understand what allows entry of CD4+ T cells into non-inflamed skin sites, we examined P- and E-selectin ligand (P&ESL) expression, and found that the CD4+ T cells capable of entering non-inflamed skin sites expressed high levels of P&ESL. To further characterize these cells, we examined their proliferation, and found that the cells entering non-inflamed skin sites have recently proliferated, suggesting that only activated effector T cells gain entry to non-inflamed skin. Combined, these data demonstrate that recently activated effector CD4+ T cells, but not memory CD4+ T cells, are capable of entering non-inflamed skin sites, and suggest that P&ESL expression plays a role in this process. Future studies will examine whether P&ESL expression is required for entry of CD4+ T cells into non-inflamed skin sites, identify what other factors are involved, and determine what promotes the retention of Trm cells in the skin. This work will be a critical contribution to the development of vaccines targeting the generation, migration, and retention of pathogen-specific resident memory T cells to relevant tissue sites.

Presenter: Charles Dai

HHMI-9 | Poster session 1

**Application of isotopic tracing analysis reveals heterogeneous patterns of intratumoral dihydrotestosterone biosynthesis from adrenal androgens in localized prostate cancer**

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Androgen deprivation therapy (ADT) by medical or surgical castration is a mainstay of treatment for advanced prostate cancer. ADT disrupts the critical androgen signaling axis required for prostate cancer progression by depleting gonadal testosterone, the canonical precursor to the potent androgen dihydrotestosterone (DHT). While initially effective, ADT is invariably followed by recurrence of castration-resistant prostate cancer (CRPC). A major mechanism of CRPC is the intratumoral biosynthesis of DHT from the adrenal an-
drogen dehydroepiandrosterone (DHEA), via three critical enzymatic steps (DHEA → androstenedione → 5α-androstane
dione → DHT), designated as the “5α-androstane
dione (5α-dione) pathway.” We sought to determine the accessibility of the 5α-dione pathway in ADT-naive, localized prostate cancers to better understand the transition of early-stage disease to CRPC and the potential underlying genotypic drivers of this metabolic phenotype.

The feasibility of an ex vivo culture system was first established using primary tissue from men undergoing prostatectomies for ADT-naive, localized prostate cancer (n = 16). Selective isotopic tracing analysis was employed to measure the efficiency in conversion of isotope-labeled androstenedione (AD) into downstream steroidal metabolites including DHT. Patient primary tissue samples, four different prostate cancer cell lines, and xenograft-derived tumors were incubated in media spiked with isotope-labeled AD over 48 hours, with and without equimolar unlabeled testosterone co-substrate to recapitulate a eugonadal versus androgen-deprived setting. Metabolites in media were captured by liquid chromatography and quantified via a radioactivity detector for tritium-labeled compounds or tandem mass spectrometry for 13C-labeled compounds (LC-MS/MS).

Tracing studies demonstrated that AD is readily converted to 5α-dione and ultimately to DHT in the majority of patient samples, although considerable variation was observed in the degree of metabolic utilization. Accessibility of the 5α-dione pathway was unaffected by the presence of testosterone co-substrate. Distinct metabolic phenotypes of robust versus limited metabolizers of AD were also noted across tested cell lines. To explore potential genotypic drivers of these differential phenotypes, publicly available cancer genomic data were queried for selective enrichment of genomic aberrations in genes expressing known steroidogenic isoenzymes involved in androgen metabolism. Expression of genes of interest was confirmed in cell lines in vitro by real-time PCR and Western blotting and then functionally tested through transient overexpression assays, siRNA-mediated gene silencing, and CRISPR/Cas9-mediated gene editing.

Our studies suggest that adrenal androgens may contribute to the biosynthesis of DHT in both early-stage, ADT-naive prostate cancer and CRPC via the 5α-dione pathway, but metabolic heterogeneity exists in the efficiency of substrate utilization. Active work is focused on the role of copy number alteration among genes in the 17β-hydroxysteroid dehydrogenase enzyme family toward determination of these phenotypes.

In collaboration, the Jacobs and Herold laboratories generated an attenuated herpes simplex virus type 2 (HSV-2) strain deleted in glycoprotein D (HSV-2 ΔgD) and demonstrated that it triggers an FcγRIV-activating response that elicits both antibody-dependent cell-mediated cytotoxicity and phagocytosis (ADCC and ADCP, respectively). The antibodies rapidly clear virus and prevent the establishment of latency in mice and guinea pigs following challenge with several clinical isolates of both HSV-1 and HSV-2. Quantifying ADCC and defining the responsible effector cells is technically challenging. Antibody-dependent cell-mediated cytotoxicity (ADCC) has typically been measured by chromium release assays, which are cumbersome and require the use of a Cr51 radioisotope. Newer methods do away with the radioactive reagent, but can be inflexible, indirect, and expensive. To address these problems, we introduced rfp into HSV-2 (G) ΔgD under control of an EF1α promoter. This reporter virus allows us to quantify the kinetics of ADCC and ADCP by separating infected from uninfected cells. The addition of cell membrane and cytosol markers then allow for direct quantification of the proportion of cells that undergo apoptosis due to effector function. By adding effector cells alongside immune sera or monoclonal antibodies from vaccinated mice, we are able to measure ADCC/ADCP activity as reductions in the viable rfp expressing cell population over time. Viability is measured using the membrane and cytosol markers. Moreover, since gD is required for viral entry, HSV-2 (G) ΔgD::rfp has the additional utility of not spreading from cell to cell. As a result, it does not cause widespread cell death characteristic of HSV infection in wild-type cells. We hypothesize that this system will allow us to very finely evaluate the efficacy of different antibodies in mediating ADCC and ADCP function. Moreover, we propose that this assay can be used as a model to screen effector cell mutants and cells from different species to determine the genes and cytokines essential for ADCC.
function in HSV infection.

Preliminary experiments show that cells infected with 1 multiplicity of infection (MOI) of HSV-2 (G) ΔgD::rfp start expressing the fluorescent marker around 4.5 hours post-infection. Expression increases up to 24 hours, at which point most cells undergo apoptosis due to the high MOI. Prior to infection, we stain non-adherent HEK cells with PKH67, a green fluorescent cell membrane marker, and tag-it violet cell cytosol markers. These target cells are then infected with 1 MOI of HSV-2 (G) ΔgD::rfp. At 1 hour post-infection, we incubate the target cells in pooled serum from HSV-2 ΔgD vaccinated animals and then add these target cells to cultures of activated effector cells (such as splenocytes or bone marrow-derived macrophages [BMDMs], NK cells, or neutrophils). ADCC activity is then quantified with cytometric cell sorting. The proportion of rfp expressing cells that are double positive for the membrane and cytosol markers will decrease over time as infected cells are killed. Preliminary experiments show that serum from HSV-2 ΔgD vaccinated guinea pigs causes BMDMs to kill approximately 20-40% more of infected cells compared to serum from control vaccinated animals (a two to four-fold increase).

Presenter: Joanna Dong

HHMI-11 | Poster session 1

The SRCAP complex subunit YL1 as a novel epigenetic target in melanoma

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Metastatic melanoma remains therapeutically challenging, often resistant or refractory to current targeted and immune therapies. Targeting epigenetic regulators represents an advantageous strategy, as it most centrally affects aberrant transcriptional programs that promote or sustain the malignant phenotype. Such regulators, known as histone variants, contain sequence and structural variants of canonical histones and replace their conventional counterparts in chromatin through deposition by histone chaperone complexes. We previously found that H2A.Z, an H2A variant, is overexpressed in melanoma, with high expression correlating with decreased patient survival. The H2A.Z.2 isoform, in particular, promotes melanoma cell proliferation through increased transcription of E2F gene targets. We additionally showed that H2A.Z-specific chaperone complex SNF2-related CBP activator protein (SRCAP), which functions to replace nucleosomal H2A with H2A.Z, interacts with H2A.Z-containing nucleosomes in melanoma cells. Thus, we hypothesize that SRCAP complex is crucial to the epigenetic machinery controlling transcription in melanoma and its inhibition presents a novel strategy in melanoma treatment.

Using a short hairpin RNA-based loss-of-function mini-screen of the SRCAP complex members in melanoma cell lines, we found that knockdown of the subunit YL1 revealed significant inhibition of melanoma cell proliferation. Further, using gene expression and patient outcome data from The Cancer Genome Atlas (TCGA), YL1 was found to be overexpressed in 22% of cutaneous melanoma patients, and increased expression correlated with decreased patient survival. Mining of available datasets and immunoblot characterization of benign and malignant tissue corroborated increased levels of YL1 protein and miRNA in melanoma compared to melanocytes and benign nevi. Importantly, functional assays of YL1 knockdown in BRAF- and NRAS-mutant melanoma cell lines revealed decreased H2A.Z levels in chromatin, and cell cycle arrest followed by apoptosis.

Our findings suggest YL1 is aberrantly upregulated in melanoma and is crucial to maintain oncogenic proliferation and progression, likely through its role in H2A.Z-associated transcriptional mechanisms. Ongoing RNA and chromatin immunoprecipitation (ChIP) sequencing approaches will further reveal the biological role of YL1 in H2A.Z-mediated gene regulation and H2A.Z genomic occupancy in melanoma cells and its potential as a novel target in future melanoma pharmacotherapeutics.

Presenter: Teresa P. Easwaran

HHMI-12 | Poster session 2

Strategies to characterize the subcellular localization of neuropilin-2 and its secreted ligand semaphorin-3F in the restriction of cortical neuron dendritic spine density and distribution

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Dendritic spines are small protrusions along the dendrite shaft that are the major location for excitatory synaptic input, and
they undergo dynamic regulation following changes in activity and experience. It is critical to maintain excitatory and inhibitory balance for a coordinated and functional nervous system; disruption of this balance is seen in various neurological diseases, such as epilepsy and autism. The semaphorins play critical roles in neural circuit assembly, dendritic spine formation, and neuronal morphogenesis. We have demonstrated that a secreted guidance cue, semaphorin 3F (Sema3F), is a negative regulator of dendritic spine number and synapse formation in the postnatal nervous system. Previous work demonstrates that loss of Sema3F, or its co-receptors neuropilin-2 (Npn-2) or plexin A3 (PlexA3), leads to an increase in spine density in layer V cortical pyramidal neurons and also in dentate gyrus granule cells. Further, Sema3F-/- and Npn-2-/- null mutant mice undergo spontaneous seizures, suggesting an imbalance in excitation and inhibition. It remains unclear what the specific cellular sources are that secrete Sema3F to restrict spine density and synapse formation. Therefore, we have developed a genetic strategy to conditionally knock out Sema3F from selective neuron populations and then assess spine morphology and distribution when Sema3F is removed from these specific cell types. To characterize dendritic morphology and spine distribution in these genotypes, we are labeling layer V pyramidal neurons using a g-deleted rabies virus expressing GFP so that we can achieve sparse labeling restricted to only layer V neurons, allowing for robust spine analysis and quantitation. We plan to determine which cells secrete Sema3F to constrain spine formation and distribution using this approach. Many questions also still remain regarding the expression, subcellular localization, and trafficking of the Npn-2. To address these issues, we have employed CRISPR/Cas9 technology to create an HA epitope-tagged Npn-2 knock-in mouse. This knock-in mouse allows us to robustly assess endogenous Npn-2 cell surface distribution and also how it responds to Sema3F and interacts with the PlexA3 signaling receptor. This will provide understanding of how classical guidance molecules allow for proper cortical circuit assembly, with implications for understanding how dynamic regulation of synaptic morphology and function is maintained to influence synaptic plasticity and scaling in response to changes in neuronal activity.

Axon degeneration is a form of programmed subcellular death resulting in the destruction of axons in injury and disease states. Injury-induced axon degeneration is thought to be independent of classic cell death pathways, as genetic and pharmacologic inhibition of these pathways do not significantly delay or prevent degeneration. Sterile alpha and TIR motif-containing-1 (SARM1) was recently reported to be an essential mediator of an injury-induced axon death pathway, though the mechanism behind its action remains to be solved. Here, we report that SARM1 mediates axon degeneration locally, by a catastrophic depletion of nicotinamide adenine dinucleotide (NAD+). Using a pharmacologically controlled dimerization system, we showed that activation of SARM1 signaling via dimerization of its TIR domain is sufficient to induce axon degeneration. This axonal demise is caused by a catastrophic loss of NAD+ in axons and can be inhibited by supplementation with the NAD+ precursor nicotinamide riboside (NR). The NAD+ consuming enzyme underlying SARM1-dependent axonal death has also remained unknown, although NADases like PARP1 and CD38 have been previously eliminated as candidates. Through a series of experiments, we have now identified the axonal NADase responsible for axon degeneration. This newly identified enzyme provides a novel therapeutic target against the many neurological diseases characterized by axonal degeneration, including peripheral neuropathy, traumatic brain injury, and neurodegenerative diseases.

**Presenter: Chelsea Feldman**

**HHMI-14 | Poster session 2**

**Stromal interaction molecule 1 (STIM1) is required for a normal contractile phenotype in the uterus and is associated with obesity**

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Appropriate regulation of uterine contractility is necessary for successful parturition; inadequate uterine contraction
is associated with prolonged labor, need for induction/augmentation, and cesarean delivery. Maternal obesity (BMI ≥30) increases the risk of obstetric complications secondary to poor uterine contractility. The mechanisms by which obesity affects myometrial contractility are unknown. Stromal interaction molecule 1 (STIM1) is a single-pass transmembrane protein that functions as a calcium (Ca\(^{2+}\)) sensor by activating store-operated Ca\(^{2+}\) (SOC) channels. STIM1 is required for maintaining cellular Ca\(^{2+}\) homeostasis and has been implicated in both excitation-contraction (EC) coupling and metabolic regulation of skeletal muscle, suggesting a possible link between myocyte contraction and obesity. To date, the role of STIM1 in uterine smooth muscle remains unknown. Herein, we characterize the role of STIM1 in the normal myometrium and begin to examine whether STIM1 function is altered in obesity.

STIM1-LacZ reporter mice underwent timed matings; uterine horns were isolated for histological and biochemical analysis. To assess contractility, uterine horns were harvested from STIM1-deficient (STIM1\(^{-/-}\); STIM1\(^{−/−}\)) mice, and myometrial strips were suspended in a tissue organ bath and stimulated with increasing doses of oxytocin. Parallel experiments were performed in both non-pregnant (NP) and term-pregnant (TNL) wild-type (WT) C57Bl/6 mice; muscle strips were pre-treated with a SOC channel inhibitor or vehicle control prior to stimulation with oxytocin. Human myometrial expression was measured in uterine biopsy samples obtained from term-pregnant women undergoing cesarean delivery at Duke University Medical Center. Primary cell lines derived from STIM1-deficient mouse models were used to assess cytosolic and mitochondrial Ca\(^{2+}\) dynamics and cellular metabolism. Finally, STIM1 polymorphisms were genotyped in skeletal muscle samples collected as part of the STRRIDE exercise intervention trial.

STIM1 is expressed in both the murine and human myometrium; STIM1 expression is upregulated in pregnancy and remains elevated throughout gestation and immediately postpartum. In myometrial strips isolated from NP and TNL mouse models, STIM1 is required for synchronous and sustained uterine contraction. Both STIM1 deficiency and SOC channel inhibition result in decreased contractile force, reduced contraction frequency, and diminished basal tone in response to myogenic (spontaneous) and oxytocin-induced stimulation. Preliminary data in both WT and STIM1-deficient mouse models suggests STIM1 expression is altered in obesity and that STIM1 deficiency results in reduced mitochondrial Ca\(^{2+}\) stores, leading to a Ca\(^{2+}\)-related block in oxidative metabolism. Last, initial genotyping experiments suggest STIM1 polymorphisms are associated with elevated BMI in adults.

We have demonstrated that STIM1 is required for a normal contractile phenotype in the myometrium. Our preliminary results suggest STIM1 as a potential link between obesity and myometrial contractility. Further studies are required to investigate this relationship, as understanding the effect of obesity on uterine contractility would lead to improved maternal and perinatal outcomes.

**Presenter:** Gabriel N. Friedman

**HHMI-15 | Poster session 1**

**Real-time decoding of unconstrained upper limb movements using surface electrocorticography signals**

Gabriel N. Friedman, 1,2,3 Mohsen Jamali, 2,3 Keiji Hu, 2,3 Ziev B. Moses, 2,3 and Ziv M. Williams 1,2,3

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Motor deficit is among the most debilitating aspects of injury to the central nervous system. Despite ongoing progress in brain-machine-interface development and in the functional electrical stimulation of muscles and nerves, very little is understood currently about how neural signals in the brain may be used to potentially control movement in one’s own unconstrained paralyzed limb. Here, we examined how neural activity in ventral premotor cortex (vPMC) of a rhesus macaque represents information about both planned and performed reach-return limb movements in free-space. We recorded from high-density electrode arrays and used real-time motion tracking techniques to correlate spatial-temporal changes in neural activity with arm movements made toward objects in space at millisecond precision. We find that many sites in the vPMC encode reaching movement and that neural activity from even a small number of electrodes within the vPMC can be used to accurately predict reach-return movement onset and left-right direction. Moreover, whereas higher gamma frequency field activity was more informative about movement direction during performance, mid-band beta and low gamma activity was more predictive of movement prior to onset. We speculate these dual signals may be used to optimize both planning and execution of movement during natural reaching, with prospective relevance to the future development of neural prosthetics aimed at restoring motor control over one’s own paralyzed limb.

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Reshaping the immune microenvironment in metastatic prostate cancer with a TLR-4 agonist-conjugated anti-CDCP1 antibody and a CD47 antagonist

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Prostate cancer is the second leading cause of cancer death in men in the United States. Severe morbidity and fatal outcome of the disease are directly linked to cancer metastasis. Recent discovery of the role of the immune system in clearance of metastatic prostate cancer cells (mPCs) has spurred interest in therapies that empower immune effectors against cancer cells. Antibodies are a class of drugs that can be designed to specifically target cancer cells and flag them to be killed by the immune system.

By comparing microarray gene expression between normal prostate, localized prostate cancer, metastatic prostate cancer, and a human metastatic prostate cancer cell line (PC-3), we identified a unique surface marker on mPCs, CUB domain-containing protein-1 (CDCP1), and developed a mouse IgG1 anti-human CDCP1 antibody (ACA). Initially, ACA treatment of luciferase-GFP+ PC-3 cells incubated with mouse macrophages did not increase phagocytosis over IgG1 isotype control, suggesting PC-3 cells possess mechanisms to evade antibody-mediated phagocytosis. We found that PC-3 cells highly express CD47, an anti-phagocytic signal and ligand to the SIRP-α molecule on macrophages. Although a CD47 antagonist, GV3, doubled phagocytosis compared to isotype control, addition of ACA did not significantly increase phagocytosis from GV3 alone. Moreover, combination treatment of PC-3 tumors under the renal capsule of NOD.Cg-PrkdcscidIL2rgtm1Wj/J (NSG) mice with ACA and GV3 did not reduce tumor burden compared to isotype treatment.

Characterization of macrophages in the presence of PC-3 cells in vitro and in vivo revealed greater representation of anti-inflammatory M2 macrophages compared with pro-inflammatory M1 macrophages. To promote a pro-inflammatory environment around the tumor and shift the balance of M2 and M1 macrophages, we conjugated our ACA with a Toll-like receptor 4 (TLR-4) agonist (TLR-4 agonist-conjugated anti-CDCP1 antibody, or TACA). Combined treatment of PC-3 cells with TACA and GV3 in vitro resulted in higher phagocytosis than either treatment alone (21.2% vs. 15.0% and 15.5%; P < 0.05). Similarly, TACA and GV3 decreased PC-3 tumor burden in NSG mice compared to isotype treatment, as measured by luciferase-based bioluminescence and percent GFP+ CD45+ cells by FACS (0.65% vs. 3.82%; P < 0.05). Immune profiling of the PC-3 tumors revealed lower overall numbers of macrophages (0.24% vs. 2.36%; P < 0.05), but an increased proportion of M1 (6.03% vs. 0.68%; P < 0.05) and decreased proportion of M2 macrophages (16.5% vs. 66.3; P < 0.05) in TACA+GV3-treated versus isotype control conditions. In conclusion, we have shown that the combination of a TLR agonist-conjugated antibody targeting CDCP1 and the CD47 blocker GV3 allows for a specific and effective mechanism of immune-mediated mPC killing.

Periventricular nodular heterotopia (PVNH) is a rare neuronal migration disorder characterized by uncalcified nodules of neurons ectopically situated along the surface of the lateral ventricles. Loss of function of the human filamin A (FLNA) locus on Xq28 causes the most commonly inherited form of PVNH, predominantly affecting heterozygous females, while exhibiting prenatal or neonatal lethality in most males with the hemizygous variant.

Previous studies have shown that the probability of identifying a pathogenic FLNA variant in an individual with classic bilateral PVNH was 49%, leaving a high incidence of negative molecular genetic testing in individuals, particularly males. The absence of classical loss-of-function FLNA mutations in nearly half of patients with PVNH and the limited number of additional contributory genes suggest that novel mutational mechanisms impacting FLNA may account for many of the apparently FLNA-negative clinical testing results.

Deep sequencing of individuals with PVNH revealed
several candidate mutations impacting conserved amino acids, intronic splice sites, and regulatory regions within FLNA. Most notably, a single base substitution was identified in intron 14, which is known to exhibit tissue-specific retention resulting in the premature termination of the protein. The T>C substitution was confirmed by custom in vitro assays to result in the loss of normal splicing, causing complete retention of the intron and premature protein truncation. These data suggest a novel mechanism where FLNA levels are partially regulated through the inclusion of introns, which in turn dysregulate the normal patterns of retention by intronic splicing mutations in individuals with PVNH, resulting in tissue-specific intronic retention, a reduction of protein and loss of FLNA. Together, these findings suggest that enhanced screening of FLNA through the inclusion of deep intronic and regulatory positions in individuals with typical and atypical PVNH could improve the diagnostic rate of clinical FLNA testing in individuals with PVNH.

Presenter: Daniel R. Hettel

HHMI-18 | Poster session 2

Androgen receptor signaling regulates a feed-forward mechanism of androgen synthesis through transcriptional upregulation of HSD3B1

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Prostate cancer is the second leading cause of cancer related deaths in US men. Androgen deprivation therapy (ADT), by either surgical or pharmacological means, is a mainstay of treatment for metastatic disease; however, ADT invariably fails and results in the formation of castration-resistant prostate cancer (CRPC). CRPC is driven, to some extent, by the synthesis of intratumoral androgens and subsequent stimulation of androgen receptor (AR). 3β hydroxysteroid dehydrogenase/D5→D4 isomerase (3βHSD1) is the rate-limiting step of androgen biosynthesis, making it an ideal target for therapy in CRPC. Currently, little is known regarding the transcriptional regulation of HSD3B1, which is the coding gene for 3βHSD1. The aim of this study was to elucidate the transcriptional machinery responsible for regulation of HSD3B1, uncovering potential drug targets to prolong the life of patients with CRPC. Initial observations utilizing quantitative PCR in LNCaP and LAPC4 prostate cancer cell models demonstrated that transcription of HSD3B1 increased upon androgen stimulation for approximately seven to nine days. This is unique, as canonical androgen response is typically seen within 24-72 hours. Western blotting confirmed that increases in transcription correlated with increases in 3βHSD1 protein. Interestingly, this response also varies between cell models, with CWR22Rv1 and VCaP cell lines demonstrating induction as early as 24 hours and 72 hours, respectively. LAPC4 cells treated with the synthetic androgen R1881 for 10 days were then treated with actinomycin D, an inhibitor of transcription. Actinomycin D treatment did not alter the half-life of HSD3B1 mRNA, which suggests that increases in mRNA seen with prolonged androgen exposure are the result of transcriptional upregulation rather than a change in mRNA stability. AR-stimulated increases in HSD3B1 transcript and 3βHSD1 protein were accompanied by an increase in enzyme activity, as assessed by metabolic flux from [3H]-DHEA to androstenedione and downstream steroids. Taken together, these data demonstrate a role for AR stimulation in HSD3B1 expression. To identify potential AR response elements in the HSD3B1 gene, we queried Cis-rome Research Finder, a public database of ChIP-seq data. We limited the search to those experiments targeting AR in the presence of androgen stimulation, and returned multiple potential binding sites both upstream and downstream of the transcription start site that are currently being explored. Overall, the current data suggest a role for AR signaling in the regulation of HSD3B1 expression, and future studies will work to elucidate whether this is a direct or indirect action of AR and the cofactors required for upregulation of HSD3B1. This will reveal potential therapeutic targets to help extend life for patients with CRPC.

Presenter: Daniel B. Hoffman

HHMI-19 | Poster session 1

Elucidating the role of oxidized macrophage inhibitory factor (oxMIF) in glioblastoma

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Glioblastoma (GBM), a WHO grade IV glioma, is a highly aggressive primary brain cancer, with an average life expectancy of under two years from time of diagnosis in part due to its characteristic invasiveness and resistance to therapeutics. Our lab has identified alterations in secretion of macrophage inhibitory factor (MIF) — a pleiotropic cytokine...
secreted by most cell types with known immunomodulatory and inflammatory properties — as one of the mechanisms of resistance of GBM to bevacizumab, an anti-VEGF monoclonal antibody (Oncogene, in press). MIF has been associated with GBM invasiveness and proliferation in cultured cells, and elevated MIF expression has been associated with worse clinical outcomes. Our studies have demonstrated that MIF drives a pro-inflammatory shift in the GBM microenvironment, and that inhibition of MIF signaling in GBM leads to a more invasive phenotype. Tumor cells are known to produce a build-up of reactive oxygen species (ROS) that may lead to increased production of oxidized MIF (oxMIF), an immunologically distinct redox dependent conformational isoform of MIF. We believe this can explain the discongruent pro-tumoral vs. anti-tumoral effects reported in our study and others. We have found that inhibition of oxMIF via a monoclonal antibody specifically targeting oxMIF decreases GBM cell invasion in Matrigel invasion assays in cell culture. We are analyzing cell culture and in vivo models to fully define the effect of oxMIF inhibition on GBM proliferation, invasion, and modulation of the tumor microenvironment. We believe that elucidating further understanding of the different roles of MIF vs. oxMIF in GBM phenotype may open the doors to new therapeutic options.

**Presenter: Melody Y. Hu**

**HHMI-20 | Poster session 2**

**Chronic stress impairs reward-directed behavior in a rodent model of depression**

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Depression and other stress-related mood disorders are associated with cognitive dysfunctions, including deficits in reward processing and behavioral flexibility. The prefrontal cortex (PFC), which mediates higher-order cognitive abilities, is particularly vulnerable to stress-induced impairments in both structure and function. However, it is not well understood how chronic stress affects PFC activity at the level of individual neurons and local circuits, or how changes in PFC activity might correlate with behavioral deficits.

To characterize the effects of chronic stress on reward-directed behavior and PFC activity, we performed a longitudinal behavioral and imaging study in mice subjected to chronic social defeat stress (a well-established model of depressive behaviors in rodents). Adult male mice were trained on an operant sucrose preference task involving varying concentrations of sucrose and water rewards to assess adaptability to changing reward values; task performance was measured repeatedly before, during, and after 10 consecutive days of social defeat. In a subset of these mice, two-photon calcium imaging of pyramidal neuron activity in the M2 secondary motor region of medial PFC (mPFC) was recorded while animals were concurrently engaged in the sucrose preference task.

Chronic social defeat stress caused a significant decrease in sucrose preference, nearly abolishing preference for sucrose over water by the end of the stress period. Stressed mice also exhibited a significant increase in total acquisition of both sucrose and water rewards. Notably, chronic stress had a major effect on reward acquisition strategy; while control and pre-stress mice consistently demonstrated reward-directed behavior (in which actions are continually modified in response to changes in outcome value), mice subjected to chronic stress shifted toward a much more repetitive, reward-insensitive pattern of activity over the course of the stress period. Collection and analysis of calcium imaging data in conjunction with the above behavioral data are ongoing.

Our preliminary results suggest that chronic stress impairs reward-directed activity by shifting overall action strategy from a flexible, reward-sensitive approach to a more habitual, reward-insensitive pattern of behavior. Ongoing analysis of imaging data will provide further insight into the contribution of mPFC to reward-directed behaviors and the effect of chronic stress on mPFC neural dynamics.

**Presenter: John P. Huizar**

**HHMI-21 | Poster session 1**

**The orphan nuclear hormone receptor Nur77 regulates the differentiation of natural IgM-secreting plasma cells**

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Innate or “natural” serum IgM provides a critical early layer of immune protection during infection, and also promotes tissue homeostasis by facilitating clearance of debris such as apoptotic cells and oxidized LDL cholesterol. B-1a cells are an innate-like subset of B lymphocytes that has long been associated with natural IgM production, yet body cavity B-1a cells themselves do not secrete substantial quantities of IgM. Instead, they are thought to give rise to IgM-secreting plasma cells that reside in the bone marrow and spleen, but

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the mechanisms controlling this differentiation step remain unknown. Here we present evidence that the orphan nuclear hormone receptor Nur77 is an important negative regulator of this process.

The B-1a repertoire comprises a restricted set of germ-line-encoded B cell receptor (BCR) specificities, many of which are thought to be self-reactive or polyreactive. Using a Nur77-eGFP reporter that we previously showed to be responsive to BCR signaling, we observed higher reporter expression in B-1a compared to B-1b or B-2 cells, suggesting chronic stimulation by endogenous antigens. To determine whether BCR-driven Nur77 expression modulates homoeostatic functions associated with the B-1a compartment, we assessed steady-state serum IgM levels in Nur77-deficient (Nur77hi) mice. Despite unchanged peritoneal cavity B-1a cell numbers, Nur77hi mice exhibit a 4-fold elevation of serum IgM (but not IgG), and a 4- and 10-fold expansion of IgM antibody-secreting cell (ASC) numbers in the spleen and bone marrow, respectively. These ASCs express high levels of intracellular IgM and IRF4, and are largely CD138hi. Chimeric mice with selective Nur77 deficiency in the B-1a compartment recapitulate these phenotypes, establishing that the expanded IgM ASCs arise from B-1a precursors. Interestingly, B-1a cells in Nur77hi mice incorporate bromodeoxyuridine (BrdU) at twice the rate of those in wild-type mice, indicative of increased turnover in this self-renewing population. Consistent with this, half of the IgM ASCs in Nur77hi mice bear an immature, B220lo surface phenotype, suggesting an exaggerated rate of ASC production with accumulation of newly formed plasmablasts. Taken together, these observations support a model in which BCR-driven expression of Nur77 restrains the differentiation of B-1a cells into IgM ASCs. Ongoing work will define the transcriptional program regulated by Nur77 in B-1a cells.

Presenter: Momodou L. Jammeh

HHMI-22 | Poster session 2

ALK4 loss drives epithelial-mesenchymal transition to promote the malignant phenotype in pancreatic ductal adenocarcinoma

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Activin receptor-like kinase 4 (ALK4) is a type 1 transforming growth factor-β (TGF-β) superfamily receptor that mediates signaling from several TGF-β superfamily ligands. ALK4 loss-of-function mutation in PDAC has been associated with an aggressive disease phenotype and poor survival. Here, we explore the mechanisms of ALK4 action in PDAC. Using shRNA-mediated silencing of ALK4 in PDAC cells (Panc-1), we demonstrated that decreased ALK4 expression induces epithelial-mesenchymal transition (EMT) and promotes migration. Specifically, ALK4-silenced cells had decreased expression of epithelial markers E-cadherin and β-catenin, with upregulation of mesenchymal markers N-cadherin and vimentin. In vivo, orthotopic implantation of luciferase expression shALK4-silenced HPNE cells resulted in significantly smaller primary tumor burden relative to shNTC HPNE implanted mice (0.5 g vs 1.5 g, P < 0.01). However, we observed a 3-fold increase in the incidence of mesenteric lymph node and liver metastasis in the shALK4 HPNE mice compared to shNTC. Histologic staining of harvested tissue revealed the upregulation of epithelial marker ZO-1 in shNTC HPNE tumors and higher mesenchymal marker vimentin expression in shALK4 HPNE tumors, suggesting that loss of ALK4 expression promoted EMT in vivo.

Mechanistically, ALK4-silenced Panc-1 cells demonstrated increased expression of integrins αβi and αβj but no increase in receptor tyrosine kinase receptors, including EGFR, VEGF, or FGFR. PDAC-associated desmoplasia provides an extracellular matrix rich in integrin ligands that can activate integrin signaling via focal adhesion kinases and their recruitment of non-receptor tyrosine kinases such as c-Src. Integrins promote a variety of adhesion-dependent effects on tumor progression, including survival, proliferation, invasion, and chemotherapeutic resistance. Currently, CRISPR/Cas9 ALK4 silenced PDAC cell lines are being generated to elucidate the role of integrins as mediators of tumor progression in ALK4 mutant PDAC. Using these cell lines, we will further investigate the activation of MAP kinase and P13/Akt pathways by fibronectin, an integrin αβi and αβj ligand, and any effects it may have on resistance to chemotherapy with gemcitabine.

These data suggest that ALK4 silencing facilitates the development of highly metastatic tumors, albeit with small tumor burden. Further studies directed toward the observed upregulation of integrin receptors will provide deeper insight into the role of ALK4 in PDAC tumor progression.
AP1 is required for Notch1 activation by histone deacetylase inhibitors in neuroendocrine cancers

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Notch signaling is minimally active in neuroendocrine (NE) cancer cells, and the induction of Notch isoforms at the transcriptional level alters the malignant NE phenotype, suppresses cancer cell proliferation, and leads to apoptosis. While histone deacetylase inhibitors (HDACi) induce Notch1 and suppress NE cancer growth, the molecular mechanism underlying this interplay has not been determined.

NE cancer cell lines BON, H727, and MZ-CRC-1 were treated with known HDACi Thailandepsin-A (TDP-A) and valproic acid (VPA), and Notch1 mRNA expression was measured with RT-PCR. Truncated genomic fragments of the Notch1 promotor region fused with a luciferase reporter were used to identify the HDACi-associated transcription factor (TF) binding sites. The key regulatory TF was identified with the electrophoretic mobility shift assay (EMSA). The effect of HDACi on Notch1 level was determined before and after silencing the potential TF by siRNA transfection. In addition, the plasmid of the potential TF was transiently transfected to evaluate its effect on Notch1 induction.

TDP-A and VPA induced Notch1 mRNA in a dose-dependent manner in NE cancer cell lines in nanomolar and millimolar concentrations, respectively. A functional DNA motif at -75 to -60 from the Notch1 start codon responsible for the HDACi-dependent Notch1 induction was identified. The mutation of this core sequence containing the potential TFBSs failed to induce luciferase activity despite HDACi treatment. EMSA showed the greatest gel shift with AP1 in nuclear extracts of NE cancer cells after HDACi treatment. siRNA transfection achieved a greater than 4-fold decrease in AP1 transcript level, which significantly attenuated the effect of TDP-A and VPA on Notch1 induction. Interestingly, AP1 transfection did not alter Notch1 levels, which suggest that the presence of AP1 is necessary but insufficient for HDACi activation of Notch1.

We identified that AP1 is the transcription factor that binds to a specific transcription-binding site within the Notch1 promotor region that triggers Notch1 transcription. Elucidating the HDACi activation mechanism may lead to the development of novel therapeutic options for patients with NE cancers and facilitate the identification of clinical responders and prevent adverse effects.

Modulation of TGFβ in cardiac neural crest-derived cells titrates aneurysm severity in Marfan syndrome mice

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Aneurysm of the proximal aorta is the leading cause of mortality in Marfan syndrome (MFS), a disorder caused by mutations in the gene (FBNI) encoding the extracellular matrix protein fibrillin-1. Fibrillin-1 interacts with latent TGFβ, and tissues (including the aortic wall) from MFS patients and mice show an unambiguous signature for elevated TGFβ activity. Furthermore, antagonism of TGFβ attenuates aneurysm severity in MFS mice. Shprintzen-Goldberg Syndrome (SGS) and Loeys-Dietz Syndrome (LDS) are closely related disorders that show substantial phenotypic overlap with MFS, including aortic root aneurysm. SGS is caused by loss-of-function (LOF) mutations in SKI, encoding a prototypical TGFβ repressor that recruits negative and displaces positive regulators of transcription to TGFβ target genes. Paradoxically, LDS is caused by LOF mutations in positive TGFβ effectors, including ligands (TGFB2 or TGFB3), receptor subunits (TGFBRI or TGFBRII), and intracellular signaling mediators (SMAD3). Contrary to the prediction that mutations in SGS and LDS will drive TGFβ activity in opposite directions, aortic tissue from both disorders shows the same unambiguous signature for high TGFβ signaling as seen in MFS. These findings have generated ambiguity about the precise role of TGFβ in aneurysm progression. We propose a reconciling model drawing upon the observation that aneurysm in all three disorders arise at sites where vascular smooth muscle cells (VSMCs) of differing embryonic origin overlap. We hypothesize that neighboring VSMC lineages respond differentially to a deficiency of TGFβ signaling, and loss of signaling in one lineage causes compensatory secretion of TGFβ ligand that overstimulates its less vulnerable neighbor.

In agreement with this model, we observed that LDS second heart field (SHF)-derived VSMCs in the aortic root show diminished TGFβ signaling and amplified lev-
els of TGFβ expression, while adjacent cardiac neural crest (CNC)-derived VSMCs remain signaling competent. We engineered MFS mice to overexpress a transgenic SKI allele in specific lineages of VSMC during particular time periods, permitting fine control over suppression of TGFβ target gene activity. We also developed a conditional SKI knockout mouse, permitting a targeted increase of TGFβ target gene expression in specific cells. In preliminary studies, MFS mice that overexpress SKI in CNC-derived cells (predicted to decrease TGFβ activity) display decreased aneurysm growth compared to MFS littermates. Additionally, MFS mice that are SKI-null in CNC-derived cells (predicted to increase TGFβ activity) display greater aneurysm growth than MFS mice with functional SKI alleles. As an independent and parallel test of hypothesis, we are developing a transgenic mouse allele that expresses a constitutively active form of TGFBR1 in a spatially and temporally controllable manner. We hypothesize that excessive TGFβ signaling in CNC-derived cells will prove sufficient to cause aneurysm, and that supplementary TGFβ signaling in SHF-derived cells can protect against aneurysm in TGFβ vasculopathies.

**Presenter:** Angad Jolly

**HHMI-25 | Poster session 1**

**Whole exome sequencing analysis of Mayer-Rokitansky-Kuster-Hauser syndrome patients reveals a mixed genetic etiology**


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Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome represents a disorder of Mullerian development with an incidence of 1/5,000 that can include renal aplasia and cervical somite anomalies. While functional models have elucidated numerous candidate genes, only WNT4 (OMIM #603490) variants have been firmly associated with a clinical entity consistent with a diagnosis of MRKH (OMIM #158330).

We conducted whole exome sequencing (WES) analysis of a cohort of 71 MRKH type I and II patients to identify rare, evolutionarily conserved variants that are putatively pathogenic. This analysis resulted in over 20 novel potential candidate disease genes. These genomic findings can be divided into 3 categories: (a) potentially pathogenic variants in Mullerian duct and renal developmental pathway genes (HOXA13, ROBO2, SPRY1, SLIT2, FOXC1 [1 family each], FGFR [3], NOTCH2 [4], RET [7]); (b) potentially pathogenic variants associated with Mullerian duct or renal developmental malformation through functional data or protein-protein interaction (HDAC1, HDAC2, SINEA, DACH2, EYA3 [1 family each], DACH1, EYA2 [2], TBX6 [3], CHD3 [4], PTC1 [5], KIF26B [7], and KIF26A [8]); and (c) potentially pathogenic variants in genes with largely unknown function. For many candidate genes, rare variants were identified at a single locus in more than one family, providing additional evidence for a potential disease association. For candidate genes identified in a single family, efforts are ongoing to identify additional families with rare variation at the same locus, using tools such as GeneMatcher (https://genematcher.org/).

Our findings also provide evidence that some cases of MRKH may represent a blended phenotype resulting from pathogenic variation at more than one locus. In particular, we have identified probands with rare variants at two loci: one associated with a skeletal phenotype, and one associated with urogenital development. For example, one patient harbors a heterozygous variant in PLEKHM1 (p.E942*), implicated in autosomal recessive osteopetrosis (OMIM #611497), and a heterozygous predicted damaging variant in TBX18 (p.F554S), implicated in autosomal dominant congenital anomalies of the kidneys and urinary tract (OMIM #143400). We suggest that each of these variants contributes to development of the blended phenotype, which includes anomalies of urogenital development and thoracocervical osseous malformations.

In summary, we present WES analysis of a MRKH cohort that identifies likely pathogenic variants in genes known or suspected to play key roles in genitourinary development based on known developmental pathways and protein-protein interactions, as well as genes for which limited functional data are available. We additionally provide evidence that, in some individuals, MRKH may result from a blending of two distinct phenotypes in a single individual.
Mycobacterium tuberculosis (Mtb) is now ranked alongside HIV as the leading cause of death among infectious diseases. Mtb is an obligate aerobe that requires the use of flexible energy metabolic pathways to grow and survive. Recently bedaquiline, clofazimine, and Q203 have been used to study energy metabolism in active whole cells of Mtb. However, another form of Mtb exists with minimal metabolic activity, referred to as cell wall-deficient (CWD) Mtb, which was first described in 1883 in infected tissues and granulomas. It has been postulated that CWD Mtb can persist in a dormant state for many years inside the host cell, even though there is no evidence that it is resistant to frontline anti-TB drugs. Ultimately, when the host immune system is suppressed, CWD Mtb reactivates and converts back to pathogenic Mtb. We believe that examining CWD Mtb susceptibility to energy metabolism-targeting compounds is imperative to our understanding of Mtb physiology, and complete sterilization of Mtb infection.

To study the role of the cell wall in oxidative phosphorylation and drug susceptibility, we generated CWD mycobacteria (spheroplasts) in vitro using glycine and lysosome-based approaches. Spheroplasts were isolated from whole cells of Mtb using flow cytometry. We microscopically confirmed spheroplast generation with scanning and transmission electron microscopy. We generated the bioenergetic profiles of spheroplasts using extracellular flux technology (Agilent Seahorse XF96), and measured energy metabolism in the presence of different energy metabolism-targeting compounds and different carbon sources. Using 13C stable isotopes, we did carbon tracing of the metabolites in energy pathways such as glycolysis, tricarboxylic acid cycle, and the pentose phosphate pathway.

Our XF96 results indicated that CWD forms of Mtb show reduced bioenergetic capacity, as is evident by a reduced oxygen consumption rate (OCR). Furthermore, we were able to demonstrate that bedaquiline, which targets ATP synthase, had no effect on cellular respiration of CWD Mtb. However, exposure of CWD Mtb to the uncoupler CCCP rapidly increased OCR, demonstrating a functional electron transport chain. We conclude that understanding Mtb’s ability to regulate bioenergetic pathways in CWD forms and their ability to latently persist in host cells may lead to novel therapeutic interventions that target these processes. By applying cutting-edge bioenergetic technologies to study CWD Mtb, we hope to make innovative contributions toward better understanding Mtb physiology and pathogenicity.
the CD4-binding-site antibodies VRC01 and 3BNC117 and found to be sensitive. At week 20 after infusion, by which point the serum concentration of 10-1074 was well below neutralization level, the unmutated wild-type virus reemerged as the predominant quasispecies, suggesting a potential fitness cost of the mutated virus. Deeper sequencing through a Primer ID-based approach did not reveal preexisting resistant minority variants. Together these data show that HIV-1 resistance emerges rapidly to bNAb 10-1074 through single point mutations; however, these mutations may have a fitness cost given the reemergence of the wild-type virus following clearance of the bNAb. HIV-1 treatment with bNAbs will require a combination of antibodies targeting different epitopes.

Presenter: Pooja Karukonda

HHMI-28 | Poster session 2

ATR kinase inhibition attenuates expression of PD-L1 on murine cancer cells post-ionizing radiation treatment

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Ataxia telangiectasia and Rad3-related (ATR) is a DNA damage-signaling kinase, inhibition of which leads to profound radio sensitization of cancer cells, reported via cell cycle checkpoint blockade. We propose a novel mechanism for ATR inhibitor (ATRi)-induced radio sensitization of cancer cells: blockade of immune checkpoint signaling via decreased cell surface expression of programmed death-ligand 1 (PD-L1), an inducer of T cell exhaustion.

Using flow cytometry, we show an increase in PD-L1 surface protein expression on two immunogenic murine colon cancer cell lines 24 hours after treatment with 6 Gy of ionizing radiation (IR), a sub-lethal dose. Importantly, we show significant attenuation of this increase with addition of ATRi. PD-L1 expression has been shown to increase on apoptotic cells; thus, we also evaluated expression after 2 Gy, a non-lethal dose, which resulted in similar findings. Additionally, we show with annexin-V staining that cell death is not significantly different between IR and IR + ATRi treatment arms after 24 hours. Taken together, these data suggest that signaling mechanisms outside of apoptotic pathways are relevant in our model of ATR-dependent PD-L1 expression.

We proceed to show, via the creation of p53-knockout (KO) cell lines, that the ATR-dependent increase in PD-L1 expression is not dependent on p53, a key downstream effector of ATR kinase. Using real-time polymerase chain reaction (RT-PCR), we have identified interferon regulatory factor-1 (IRF-1), an immune-modulatory transcription factor, as a potential downstream target, as its expression increases after IR, and is subsequently inhibited with ATRi after 2 Gy. We show, however, that ATRi leads to a substantial increase in PD-L1 mRNA levels after 6 Gy, which leads us to believe that there are separate mechanisms in play after different doses of IR, and that after 6 Gy, PD-L1 expression may not be regulated at the transcriptional level.

Future experiments aim to flesh out the mechanistic details behind the immunoregulatory effects of ATRi, a potentially powerful cancer therapeutic when used in combination with IR. We will additionally perform functional studies, including co-culture of cancer cells with T cells to test the overall effects of ATRi on T cell exhaustion, as well as in vivo mouse experiments to look at the direct effects of ATRi on tumor burden and tumor-infiltrating lymphocytes.

Presenter: Ahmed I. Kashkoush

HHMI-29 | Poster session 1

Establishing a multichannel dorsal root ganglion neural interface for restoring sensation and reducing phantom limb pain after amputation

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Recent evidence suggests that electrical stimulation of peripheral nerves can evoke focal sensations that emanate from the missing limb in amputees. Further evidence suggests that restoring physiologic sensations is associated with long-term mitigation of phantom limb pain (PLP). This study aims to characterize the normal patterns of activity in primary afferents (PAs), and to use those patterns in future studies to guide the programming of stimulation parameters for a neuroprosthesis to restore naturalistic sensation and reduce PLP in people with amputation.

We initially characterized PA responses of 125 recorded neurons to standing bidirectional (anterior vs. posterior) platform perturbation tasks using penetrating microelectrode arrays (MEAs) implanted in the lumbar dorsal root ganglia.
A translational rodent model of hepatocellular carcinoma for in vivo studies of cancer dormancy and metabolism

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Despite evidence that novel targeted therapies have led to improved tumor response rates for hepatocellular carcinoma (HCC), the rate of cure remains low owing to recurrent disease, which can result from dormant malignant cells that persist without detection by conventional imaging methods. How dormant cancer cells survive in a growth-restricted environment is poorly understood but may offer insight into improved methods of detection and treatment. HCC cell survival following transarterial embolization (TAE) provides an ideal method through which to study tumor dormancy, as sustained ischemia has been shown to activate the metabolic stress response and induce quiescence. Using an oral diethylnitrosamine (DEN)-induced HCC model in male Wistar rats, we analyzed characteristics to optimize the autochthonous cancer model and described a novel endovascular approach that mimics clinical treatment that is known to induce dormancy. We found that rats with greater body weight (>400 g) at the start of the DEN diet had significantly longer survival than rats less than 300 g (mean 108 vs. 88 days; \( P < 0.0001 \)) without a significant increase in tumor latency (\( P = 0.310 \)). Tranarterial embolization via the right common femoral artery was described and found to be successful for the induction of ischemia. Embolized tumors were fluorescently stained with bromodeoxyuridine (BrdU), pimonidazole, and DAPI to confirm cellular hypoxia and the arrest of proliferation as evidence of dormancy. With this in vivo model of cancer dormancy, quantitative metabolic flux analysis will be performed using mass spectrometry and dynamic nuclear polarization-carbon-13-nuclear magnetic resonance spectroscopy. The detection of altered metabolism may uncover new imaging probes for the improved detection of dormant cancer as well as novel targeted therapies.
The nervous system is important in controlling intraocular pressure (IOP); however, the precise mechanisms of neural control need further evaluation. The mouse enables functional experiments, and so we are characterizing limbal innervation in mice. To understand neuronal functions regulating IOP, we are using modern molecular techniques and fluorescent reporter mice to map limbal neurons and determine pressure-dependent neuronal activity. We used a whole-mount procedure of the anterior mouse eye to study the innervation of the entire limbus in 3D. The Prox1-GFP mouse strain was used to label Schlemm’s canal (SC). The Thy1-YFP strain was used to identify sensory neurons. Antibodies to detect sympathetic neurons (tyrosine hydroxylase), parasympathetic neurons (vesicular acetylcholine transporter, choline transporter), nitrergic neurons (neuronal nitric oxide synthase), and axons (neurofilament) have been used. 3D limbal images generated using confocal microscopy and Imaris were used to map and identify the number and type of neurons and nerve termini in SC and trabecular meshwork (TM). Neuronal activity in response to pressure elevation was assessed using fluorescent neuronal activation sensors (GCaMP). To do this, eyes were cannulated and held at pressures of 13 and 33 mmHg for 30 minutes, followed by fixation, immunolabeling, and confocal microscopy. We have identified sympathetic nerve terminals and possibly non-nociceptive sensory terminals terminating in SC and TM. The TH-labeled termini have varicosities and the YFP-labeled termini appear as bouton-like structures. Using GCaMP calcium sensor mice, we have identified neurons that are activated in the limbus in response to elevated pressure. These neurons are present in the SC and associated TM region. We are constructing a detailed 3-dimensional (3D) map of the neuronal innervation of the AQH drainage structures to facilitate understanding of the neural control of IOP. Both SC and TM are highly innervated with activation of a subset of neurons in response to experimental elevation of IOP.
Polymorphic Alu repetitive elements are a common source of structural variation among humans, accounting for ~17% of structural variants in the human genome. While Alu insertions have been previously implicated in causing rare monogenic diseases, it remains unclear if common Alu polymorphisms affect inherited risk for common diseases. We hypothesize that some Alu variants alter disease risk by modifying mRNA transcript structure, and focus on two mechanisms by which intronic Alu elements affect mRNA splicing. Alu elements may either disrupt existing splice sites and cause exon skipping or introduce new splice sites leading to exonization of Alu sequence.

We have curated a list of 115 intronic polymorphic Alu elements within 100 base pairs of alternatively used exons and are performing minigene splicing assays with subsequent RT-PCR analysis to identify Alu variants that cause exon skipping. To focus on Alu candidates most likely to affect disease risk, we prioritize polymorphic Alu elements in linkage disequilibrium with genome-wide association study (GWAS) signals. Of nine Alu variants assayed in the minigene splicing assay thus far, four cause skipping of an adjacent exon (25-97 base pairs away). In one case the exon is always skipped in the presence of the Alu element, and in three cases the amount of the shorter mRNA transcript increases in the presence of the Alu element but exon skipping is not absolute. Additional polymorphic Alu elements remain to be tested, and follow-up studies will be conducted to characterize the mechanism by which the Alu element causes exon skipping. Alu variants that map near GWAS signals corresponding to diseases of greatest interest will be further evaluated at the endogenous locus using CRISPR/Cas9 genome editing.

In parallel, we are implementing a computational approach to identify Alu exonization events and are developing a bioinformatics pipeline that uses RNA-sequencing data to identify novel Alu-containing transcripts. Because our focus is to detect exonization events from polymorphic Alu elements, many of which are not annotated in the reference genome, accurately mapping the RNA-sequencing reads from these repetitive elements poses a challenge. We are currently optimizing the pipeline to minimize detection of Alu-containing transcripts resulting from read-through transcription and thus identify only true splicing events, and also to minimize detection of non-polymorphic reference genome Alu elements. Alu exonization events detected by the pipeline will be validated by RT-PCR. As with exon skipping events described above, Alu variants of greatest clinical interest will be prioritized for further investigation using genome editing.

Hemodialysis is the most common method of renal replacement in patients with end-stage renal disease. The arteriovenous fistula (AVF) is the preferred method of dialysis access but must adapt, e.g., dilate and thicken, to the higher pressure and flow arterial environment. Although women frequently require dialysis, women have lower rates of AVF maturation compared to men (38% vs. 60%), preventing optimal AVF use. Using a novel mouse model that recapitulates human AVF maturation, we hypothesize that there is a difference in AVF patency and maturation between male and female mice.

A 25-gauge needle was used to create fistulae between the abdominal inferior vena cava (IVC) and aorta in male and female C57BL/6 mice (9-10 wk). Doppler ultrasound was used to monitor aortic and IVC diameters and flow ve-
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Locality at days 0, 3, 7, 14, and 21; shear stress was calculated. AVFs were examined at day 21, and computer morphometry was used to measure AVF wall thickness.

Male mice weighed more than female mice preoperatively and at day 21 (25.4 ± 0.5 g vs. 18.3 ± 0.3 g; \( P < 0.05; n = 15 \)); male mice also had larger suprarenal aortic diameter (1.23 ± 0.05 mm vs 1.06 ± 0.02 mm; \( P = 0.002 \)) but smaller suprarenal IVC diameter (1.53 ± 0.06 mm vs 1.73 ± 0.06 mm; \( P = 0.02 \)) at baseline. After AVF creation, there was increased suprarenal IVC dilation in male mice (53 ± 13% vs 25 ± 5%, \( n = 5-8 \); \( P = 0.04 \)) at day 7; at day 21, there was a trend toward increased IVC dilation in male mice (infrarenal, 86 ± 23% vs. 63 ± 10%, \( P = 0.22 \); suprarenal, 61 ± 17% vs. 42 ± 6%, \( P = 0.23 \)). Velocity in the suprarenal IVC was also increased in male mice, both at baseline (40 ± 6 mm/s vs. 25 ± 2 mm/s; \( P = 0.012; n = 15 \)) and at day 7 (79 ± 16 mm/s vs. 44 ± 6 mm/s; \( P = 0.04; n = 5-8 \)). Similarly, shear stress was increased in male mice at baseline (15 ± 2 vs. 8 ± 1 dynes/cm²; \( P = 0.002; n = 11-15 \)) and day 7 (30 ± 8 vs. 18 ± 5 dynes/cm²; \( P = 0.12; n = 5-8 \)). At day 21, AVF neointimal thickness was similar (\( P = 0.9 \)).

In a mouse AVF model that recapitulates human AVF maturation, preliminary data suggests lower magnitudes of laminar shear stress and increased neointimal thickening in female mice. These findings may suggest mechanisms underlying the diminished rates of AVF maturation in women who need hemodialysis; understanding these mechanisms will be crucial to develop targeted interventions to improve AVF outcomes in female patients. Additional studies are underway to increase sample size, evaluate shear stress frequency, determine expression levels of proteins involved in AVF maturation, and examine vessel elasticity with wire myography.

**Presenter:** Chiraag V. Kulkarni

**HHMI-35 | Poster session 1**

**Unique growth characteristics of E. coli via the NtrB/NtrC two-component system in vitro vs. in vivo — a signal transduction pathway associated with dysbiosis in IBD**

Chiraag V. Kulkarni,1,2 Josephine Ni,2 Eric Barash,2 Manuela Roggiani,3 Mark Goulian,1 and Gary D. Wu2

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Crohn’s disease is associated with bacterial dysbiosis consisting of reduced bacterial diversity and outgrowth of Proteobacteria, notably Enterobacteriaceae. However, a definitive mechanism for the development of dysbiosis is not known. An analysis of the gut microbiome in pediatric Crohn’s disease revealed a genomic signature for nitrogen metabolism associated with dysbiosis. Specifically, a bacterial two-component system responsible for sensing and responding to environmental nitrogen sources, NtrB/NtrC, was associated with dysbiosis, an increase in fecal amino acids, and the abundance of *E. coli*. Herein, we examined the effect of *ntrC* on growth characteristics of *E. coli* under different environmental conditions in vitro as well as its effects on intestinal colonization in vivo.

\( \Delta ntrC \) and wild-type MP1, a murine commensal strain of *E. coli*, were cultured under aerobic and anaerobic conditions using lysogeny broth (LB) and M9 minimal media. C57BL/6 mice were pretreated with antibiotics and polyethylene glycol (PEG) per established protocols and subsequently gavaged with a mixture of \( \Delta ntrC \) and wild-type MP1 that have tetracycline-induced fluorescent protein tags. Stool serial dilutions were performed and colony counts obtained weekly for calculation of competitive indices. When cultured aerobically, \( \Delta ntrC \) MP1 exhibited both a growth delay and reduction in growth yield that were more pronounced in LB medium. Anaerobically, \( \Delta ntrC \) showed a growth delay but reached a similar OD in stationary phase compared to wild-type MP1 in both M9 and LB media. Complementation of the *ntrC* deletion in trans restored wild-type growth patterns. Additionally, \( \Delta ntrC \) MP1 showed a modest cell aggregation phenotype during late log and early stationary phase growth, specifically under aerobic conditions. Surprisingly, despite a clear growth defect under all conditions measured in vitro, oral co-inoculation of \( \Delta ntrC \) MP1 with wild-type MP1 in mice resulted in higher engraftment of \( \Delta ntrC \) MP1 compared to wild-type MP1 (~10⁹ colony forming units [cfu] versus 10⁸ cfu) in the gastrointestinal tract. This 2-log competitive advantage for \( \Delta ntrC \) MP1 over wild-type MP1 was sustained for a period of at least 1 month (\( P < 0.01 \)).

Despite the clear growth disadvantage of \( \Delta ntrC \) MP1 under all culture conditions examined in vitro, \( \Delta ntrC \) MP1 led to an engraftment advantage over wild-type MP1 in vivo, demonstrating that the murine gut provides a unique environment for *E. coli* favoring the absence of this nitrogen signaling pathway. This *ntrC*-dependent response in vivo is unique among the 33 two-component signaling systems in the MP1 *E. coli* genome. One possible explanation under current investigation is that the cell aggregation phenotype of \( \Delta ntrC \) MP1 observed in vitro provides a colonization advantage in vivo. This observation may have particular relevance to IBD, where *E. coli* is an important component of the dysbiotic microbiota.
Autologous cell therapy for the treatment of hematological malignancies and primary immunodeficiencies has been limited by the inability to yield functional human hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) in vitro. Although previous approaches have produced immortalized cells with HSC-like morphology and cell surface markers, they have failed to demonstrate murine engraftment in secondary recipients — the gold standard for demonstrating HSC self-renewal and differentiation. Our lab has recently uncovered five transcription factors (TFs) necessary for differentiating iPSCs into hemogenic endothelial cells (HEs), the fetal precursors to HSCs, and confirmed hematopoiesis with multi-lineage secondary engraftment. Building on this discovery and focusing on therapeutic applications, we now set out to characterize and enhance the capacity of HEs to produce more terminally differentiated lymphocytes, for the ultimate aim of B and T cell replacement in immunocompromised hosts.

Following embryoid body formation, human iPSCs derived from bone marrow mesenchymal stem cells were transduced with polyclisotropic lentiviral vectors (RUNX1-ERG, LCOR-HOXA9-HOXA5) on day 3 of endothelial-hematopoietic transition (EHT) induction. A candidate screen of 17 cytokines, epigenetic modifiers, and small molecules aimed at inducing HE proliferation was conducted during EHT induction in a RUNX1c gene reporter assay. Flow cytometry of HEs cocultured with MS5 or OP9-DL1 stromal cells was performed weekly for 6 weeks after initiating lymphoid differentiation.

In the candidate screen, IFN-γ enhanced RUNX1c reporter 2.4-fold during EHT, which was confirmed by flow cytometry showing increased hematopoietic progenitor cells (CD34+CD45+). Several compounds were suppressive to EHT, including IFN-α, which led to loss-of-function EHT enhancement when co-introduced with IFN-γ. After 1 week of lymphoid directed differentiation, the cell population was characterized by the predominance of myeloid progenitors (CD38+CD45+CD45RA-), with roughly 5% of progenitors characterized as common lymphoid progenitors (CD10+CD34+CD45RA-). At 3 weeks, CD19 IgM B cell progenitors as well as mature IgM+ B cells emerged, which were comparable to human cord blood (CB) controls; CD3+CD4+CD8+ T cells were also identified at this time.

We determined that transient exposure to IFN-γ greatly improved the EHT of HEs, which may bolster the typically low engraftment efficiency of iPSC-derived HSCs. In our lymphoid differentiation assay, HEs were comparable to CB in the formation of mature and immature B/T cells. This finding suggests that our approach to creating engraftable HSCs holds significant potential for modeling hematopoietic disease, both in animal models and as a “disease-on-a-dish,” and for developing therapeutic strategies in genetic blood disorders.

Reducing T cell-mediated cardiac injury through TLR9 signaling with CpG oligodeoxynucleotides

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T cell-mediated damage in the heart may occur following various stimuli, including viral infection, autoimmune provocation, and immunostimulatory cancer therapy. The PD-1/PD-L1 pathway prevents or limits T cell-mediated damage to the heart, as shown by mouse models in the Lichtman laboratory, and recently by the increased risk of myocarditis in cancer patients treated with anti-PD-1 antibodies. CpG oligodeoxynucleotides (ODN) are known to induce the expression of type I interferons (IFN) by acting as a ligand for endosomal Toll-like receptor 9 (TLR 9), and type I IFN have been shown to induce the immune checkpoint marker programmed death-ligand 1 (PD-L1) in the heart. However, the therapeutic potential of CpG ODN in T cell-mediated cardiac disease has not been established. We evaluated the immunomodulatory properties of CpG ODN in a variety of murine models of myocarditis. Furthermore, as there is little data regarding the expression of PD-L1 in the human heart, we investigated PD-L1 expression in both diseased and healthy human tissue.

Two models of autoimmunity were used to induce myocarditis in mice. CD4+ T cell-dependent disease was induced with two injections of myosin-heavy chain (MyHC) peptide with complete Freund’s adjuvant into BALB/c mice. CD8+ cytotoxic T lymphocyte (CTL)-mediated disease was induced by adoptively transferring T cell receptor (TCR)
transgenic CTL (OT-I) cells specific for ovalbumin peptide into mice expressing ovalbumin only in the heart under the MyHC promotor (cMy-mOva mice). The expression of PD-L1 as influenced by CpG ODN was evaluated by immunohistochemistry and qRT-PCR in BALB/c mice treated with one intraperitoneal injection of 50 μg CpG ODN or PBS 24 hours prior to sacrifice. Histological sections of normal and mycoiditic human hearts were analyzed for expression of PD-L1 and class II MHC, a marker of interferon activity, by immunohistochemistry.

Relative to PBS-injected mice, CpG ODN-injected mice demonstrated a 3.2-fold increase in PD-L1 mRNA expression in myocardial tissue. Immunohistochemistry performed on CpG ODN-treated mice demonstrated increased PD-L1 protein expression, in an endothelial pattern, compared to PBS-treated mice. We have treated cMy-mOva mice with CpG ODN during OT-I-mediated myocarditis to assess the effects of induced myocardial PD-L1 on disease. Immunohistochemistry of the myocarditic human heart demonstrates focal expression of PD-L1, on both myocytes and endothelial cells, and increased endothelial class II MHC, accompanying T cell infiltration.

CpG ODN markedly induces expression of PD-L1 in murine myocardium, and PD-L1 upregulation in human hearts occurs in the setting of T cell infiltration, as it does in mice. Therapeutic induction of myocardial PD-L1 may represent a therapeutic option for preventing or treating T cell-dependent cardiac disease. Further experimentation to assess the utility of such treatment in preventing disease progression is in progress for various murine models.

**Presenter:** Spencer B. Lewis

**HHMI-38 | Poster session 2**

**Virtual histopathology and immunohistochemistry with stimulated Raman scattering microscopy**

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Differentiating tumor-infiltrated tissue from surrounding normal brain during surgery is extremely challenging. Because the surgeon cannot visualize the tumor margin, peripherial tumor infiltration cannot be accurately assessed, leading to suboptimal resection. This difficulty contributes to high postoperative morbidity and mortality, as well as decreasing median time to local tumor recurrence. Improving intraoperative brain tumor detection is an important step toward improved outcomes in brain tumor surgery.

Stimulated Raman scattering (SRS) microscopy is a label-free optical technique that uses the intrinsic chemical composition of fresh tissue to generate image contrast. We have previously demonstrated exceptional structural correlation between SRS and conventional H&E microscopy, allowing SRS images to yield reliable diagnoses of human glioma tissue. Quantitative SRS has facilitated automatic tumor detection, leveraging the distributions of axons and nuclei to segment tumor infiltration with extremely high accuracy. Until now, however, it has not been possible to execute SRS in a clinical environment. Further, despite the potential for chemically specific SRS imaging, only structural features have been used to make diagnoses with SRS.

Here, we report the first execution of SRS microscopy in a clinical setting, using a portable, fiber-laser-based SRS microscope to image unprocessed specimens from 93 neurosurgical patients. SRS had near-perfect (k > 0.89) concordance with standard methods in a simulation on intraoperative pathologic consultation. We also present a multi-layer perceptron-based on image attribute quantification capable of predicting tumor diagnosis with 86.6% accuracy.

We further investigated how we could leverage the chemical specificity of SRS to image mutational status at the cellular level. Isocitrate dehydrogenase 1 (IDH-1) mutations are the most common mutations in low grade gliomas. These mutations result in a predictable set of metabolomic alterations, including the accumulation of high concentrations of the aberrant oncometabolite 2-hydroxyglutarate (2-HG), which directly mediate tumorigenesis.

Using two isogenic lines of normal human astrocytes differing by the most common oncogenic point mutation in the IDH-1 gene (R132H), we have profiled the Raman spectral changes associated with this mutation, as well as their metabolomic correlates. Further, we have demonstrated that these changes can be induced in wild-type cells by exposing them to cell-permeable 2-HG, and that exposure of IDH-1-mutant cells to a small-molecule inhibitor of the mutant form of IDH-1 reverses these changes.

Because SRS amplifies the Raman signal with no resonant background, we believe this spectral difference can be leveraged to rapidly generate high resolution hyperspectral images of live wild-type and R132H cells in vitro. Importantly, this would represent a unique visualization mutational status without tags or immunohistochemical stains.
Bone fractures are among the most common orthopedic problems that affect individuals of all ages. Acutely after injury, activated macrophages dynamically contribute to and regulate an acute inflammatory response. As a result of this inflammatory milieu, different macrophage phenotypes arise: undifferentiated M0, pro-inflammatory M1, and anti-inflammatory M2. These macrophages work in concert with other cells at the injury site, including mesenchymal stem cells (MSCs), to modulate bone healing. The dynamics of macrophage-MSC interaction and their subsequent effects are not well understood.

In this study, we co-cultured undifferentiated M0 and polarized macrophages (M1 and M2) with primary murine MSCs to determine the cross-talk between polarized macrophages and MSCs and their effects on osteogenesis. At two weeks of culture, MSCs co-cultured with M1 macrophages had reduced alkaline phosphatase (ALP) activity compared to MSCs grown alone and the M0-MSC and M2-MSC groups. However, after four weeks of co-culture, MSCs grown in the presence of macrophages, especially M1 macrophages, had enhanced bone matrix mineralization compared to MSCs grown alone. Despite low ALP activity early in co-culture, the M1-MSC culture produced the most bone.

Bone formation was found to be closely associated with prostaglandin E2 (PGE2) secretion early in osteogenesis (two weeks). To determine the necessity of PGE2 in bone formation, cultures were treated with celecoxib, a cyclooxygenase-2 (COX-2)-selective inhibitor, for one week. This treatment resulted in significantly reduced bone mineralization in all co-cultures but most dramatically in the M1-MSC group. This result confirmed the importance of COX-2 activity and PGE2 in macrophage-mediated enhancement of MSC osteogenesis. The presence of macrophages also reduced the expression of osteoprotegerin (OPG), the decoy RANKL receptor. This finding suggests that macrophages negatively regulate OPG secretion and thus may alter osteoclast activity in addition to enhancing bone formation.

Taken together, our study shows that an initial pro-inflammatory phase modulated by M1 macrophages promotes osteogenesis in MSCs via the COX-2-PGE2 pathway. Gaining a better understanding of the complex interactions between macrophages and MSCs in regulating inflammation provides opportunities to better understand and optimize immune modulation of bone healing and other regenerative processes in the presence of inflammation. As the role of COX-2 and PGE2 extends beyond musculoskeletal diseases into cancer biology, endocrinology, and cardiovascular health, it is imperative to gain a granular understanding of the nuances of COX-2 and PGE2 activity in all systems of the body. Moreover, this study provides a biological explanation for the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) in the setting of inflammation and bone injury.

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of mortality related to cancer in the world. PDAC tumors, characterized by an extensive desmoplastic stromal response and hypovascularity, are likely impacted by significant oxygen scarcity during both initiation and progression. We have demonstrated, using a well-defined autochthonous KrasG12D-driven murine model, that hypoxia and stabilization of hypoxia-inducible factor 1 alpha (HIF1α), a principal mediator of hypoxic adaptation, emerge early during the preinvasive stages of PDAC. Previous data show that pancreas-specific deletion of the Hif1α gene actually accelerated early oncogenesis (PanIN 1-3) through increased recruitment of B-lymphocytes to the mouse pancreatic epithelium, dependent on increased expression of the B cell chemokine CXCL13. In order to further explore the role of HIF in later stages of PDAC, where hypoxia is likely to be more severe, we generated

**Pro-inflammatory M1 macrophages promote osteogenesis by mesenchymal stem cells via the COX-2-prostaglandin E2 pathway**

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**HIF1α, B cells, and pancreatic cancer**

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lentivirus with short hairpin RNAs designed to deplete both HIF1α and related HIF2α in a series of three mouse PDAC cell lines. Using knockdown variants of these PDAC cells, we performed subcutaneous injections into wild-type C57BL/6 mice. Rate of growth, size, and weight of the subcutaneous tumors were followed for three weeks and subsequently harvested for RNA and immunohistochemistry analysis. Gene expression analysis reveals adequate HIF1α and HIF2α ablation. In-vitro proliferation assessment reveals no significant difference in growth in the HIF1α- and HIF2α-knockdown cell lines when compared to controls. However, preliminary subcutaneous tumor analysis reveals subdued growth in HIF1α-knockdown tumors in comparison to control, illustrating the significant effect the tumor’s microenvironment has on PDAC growth.

Further subcutaneous tumor injections will be completed using the remaining PDAC cells, and tumor analysis will be performed with immunohistochemistry (IHC) for differences in cellular proliferation (Ki67), vascular angiogenesis (CD31), and apoptosis (cleaved caspase 3). Leukocyte recruitment will be assessed through both IHC (CD45) and flow cytometry (CD19+, CD43+, IgMhi, CD5-) analysis. Finally, selected PDAC cells will be orthotopically transplanted into wild-type C57BL/6 mouse pancreata to further assess the effects of HIF on late-stage PDAC oncogenesis.

Presenter: Thiagarajan Meyyappan

HHMI-41 | Poster session 1

In vivo induction of insulin-specific regulatory T-cells to prevent the onset of type 1 diabetes

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Type 1 diabetes (T1D) is an autoimmune disease that results in the T-cell-mediated destruction of the patient’s islet β cells, the insulin-producing cells of the pancreas. It is widely theorized that the onset of T1D is due to an imbalance of regulatory T-cells (T-regs) and effector T-cells (T-eff). By restoring the T-reg to T-eff balance, it may be possible to reverse or prevent the onset of T1D. We have demonstrated that the sustained release of TGF-β, IL-2, and rapamycin from poly(lactic-co-glycolic acid) (PLGA) microparticles (known collectively as FactorMP) can induce T-regs in vivo. The FactorMP system has been shown to be efficacious in murine models of dry eye disease, contact dermatitis, and hind-limb transplant.

In order to induce T-regs toward islet β cells prophylactically and prevent their autoimmune destruction, we propose to add a fourth microparticle containing a protein localized to islet β cells to our FactorMP cocktail. Specifically, we will synthesize microparticles that are loaded with the b9-23 insulin peptide, which is an islet-specific autoantigen targeted in T1D. Based on our system’s efficacy in other inflammatory diseases, we hypothesize that the in vivo subcutaneous release of TGF-β, rapamycin, and IL-2 in the presence of b9-23 peptide will result in the induction of islet β cell specific T-regs which subsequently will prevent or reverse the onset of T1D.

To test this hypothesis, we used a model of T1D, in which non-obese diabetic (NOD) female mice spontaneously develop autoimmune diabetes at approximately 12 weeks. We have demonstrated that the subcutaneous injection of the microparticle cocktail increases the percentage of T-regs in the draining lymph node of the NOD mice. To determine the specificity of our induced T-regs, we will employ the b9-23 MHC-tetramer (a complex of MHC II molecules loaded with b9-23 peptide such that it will only bind to T-cell receptors that react to that peptide). We have optimized the staining protocol of the b9-23 tetramer and are currently characterizing the specificity the induced T-regs. Our initial studies in attempting to treat recent-onset diabetes have demonstrated an inability to reverse recent-onset hyperglycemia for longer than a few days. We suspect that this lack of sustained response is due to the rapid progression of disease outpacing the time needed to induce T-regs. Currently, we are exploring the use of a short course of immunosuppression to allow our system to induce significant numbers of T-regs without continued progression of disease. Additionally, we are also determining whether our system can prevent the onset of diabetes when administered in prediabetic 8-week-old NOD mice.
Immune checkpoint therapies, including monoclonal antibodies targeting cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1), benefit a subset of patients with metastatic melanoma, but ability to predict clinical outcomes is limited. This meta-analysis of genomic predictors of outcomes to anti-PD-1 and anti-CTLA-4 therapies in melanoma combines 220 sequenced tumors from three published cohorts and aims to validate existing hypotheses regarding response to immune checkpoint therapies, as well as discover new relationships with greater power.

Mutation annotation files, HLA types, and clinical annotations were obtained from published data. We used standardized pipelines for somatic variant calling, mutational signature deconvolution, and neoantigen prediction. Patients were stratified into clinical benefit (CB) and no clinical benefit (NCB) groups using three previously published response metrics based on objective tumor regression (RECIST) and durations of progression-free survival (PFS) and overall survival after starting immune checkpoint therapy.

Nonsynonymous mutational burden was significantly higher in CB vs. NCB using all three response categorizations. To assess the impact of mutational processes contributing to overall mutational burden, we used a non-negative matrix factorization framework to infer mutational activity in tumors from six signatures previously seen in melanoma: aging (S1), T>C substitutions (S5), ultraviolet light (S7), mismatch repair (S6), alkylating agents (S11), and T>G substitutions (S17). Across all samples, the proportion of mutations in S7 or S11 was positively correlated with mutational burden (Spearman’s rho = 0.66), while S5 and S1 were anti-correlated (rho = -0.62). Additionally, in a multivariate logistic model, S7 activity and S11 activity were each independent predictors of CB adjusting for mutational load ($P < 0.05$), with the sum of S7 and S11 activity being a strong predictor ($P < 0.001$). Of the patients with low mutational burden (less than median) with CB, 79% had a majority of mutations in S7 or S11, compared to only 51% of NCB ($P < 0.01$; Pearson’s chi-squared). Neoantigen burden was strongly correlated with mutational burden, and did not improve the ability to predict CB. In examining mutations in specific genes, more than 500 genes were mutated significantly more frequently in either CB or NCB ($P < 0.05$, Fisher’s exact). Restricting analysis to recurrently mutated genes in cancer and correcting for patient mutational burden, nonsynonymous mutations in ACSL3 and MET and truncating alterations in ARID2 were significantly enriched in CB.

In this meta-analysis of 220 patients, harmonized clinical and whole exome analysis confirmed that mutational burden correlates with clinical response to anti-PD-1 and anti-CTLA-4 therapies in metastatic melanoma, with mutational signatures and alterations in specific genes potentially providing additional predictive power.
Meningiomas are the most frequent primary intracranial tumors. Although the majority are histopathologically classified as “benign,” all can damage precious brain tissue. Despite standard of care, malignant meningiomas have a dismal median overall survival of less than 3 years, necessitating the development of novel therapies.

Inactivating mutations in the NF2 tumor suppressor gene, located on chromosome 22, and/or chromosome 22 loss account for 40-60% of sporadic meningiomas. However, these driver mutations are thought to be associated with tumor initiation rather than malignant progression. Within non-NF2 meningiomas, next-generation sequencing recently revealed driver mutations in TRAF7, KLF4, AKT1, SMO, and POLR2A. Moreover, co-occurring with NF2 mutations and/or chromosome 22 loss, recurrent somatic missense mutations in the SMARCB1 tumor suppressor gene have been identified in sporadic meningiomas, interestingly, with a predilection for malignancy.

SMARCB1, located on chromosome 22, encodes a core subunit of the SWI/SNF chromatin-remodeling complex involved in epigenetic regulation. Functionally, the SMARCB1 protein is implicated in the recruitment of the SWI/SNF complex to specific target genes where chromatin modifications such as histone 3, lysine 27 (H3K27) acetylation, a marker of enhanced transcription, mediate gene expression. However, the precise genomic architecture and molecular mechanisms of SMARCB1-driven meningioma remain unknown, limiting avenues for the development of novel therapies.

In this study, we hypothesized that recurrent somatic missense mutations in SMARCB1 may confer the SWI/SNF complex with a neomorphic function in its repertoire of target genes where aberrant H3K27 acetylation may contribute to meningioma tumorigenesis.

To identify a meningioma cohort enriched in SMARCB1 mutations, targeted screening via molecular inversion probes (MIPs) sequencing of NF2, TRAF7, KLF4, AKT1, SMO, POLR2A, and SMARCB1, in series with chromosome 22 quantitative real-time PCR (q-PCR), was performed. To further characterize the genomic landscape of meningiomas, mutation-unknown samples via targeted screening were analyzed via whole exome sequencing. All candidate variants were confirmed via Sanger sequencing. Additionally, to identify potential genetic and phenotypic relationships, correlation between genetic and clinicopathological variables was performed.

To investigate chromatin status in an unbiased genome-wide manner, chromatin immunoprecipitation (ChIP) against H3K27 acetylation coupled with next-generation sequencing (ChIP-sequencing) was utilized. Moreover, to quantify gene expression in an unbiased transcriptome-wide manner, RNA-sequencing was utilized. Epigenetic enrichment via ChIP-sequencing and gene expression levels via RNA-sequencing were computed for (1) normal adult meningeal tissue, (2) non-SMARCB1-mutant/NF2-mutant/Chr22-loss meningiomas, and (3) SMARCB1-mutant/NF2-mutant/Chr22-loss meningioma.

Our experiments are undergoing analysis. To identify potentially pathogenic target genes, all genes showing differential ChIP binding will be correlated with gene expression values to enrich for genes showing differential regulation, which may be associated with SMARCB1-driven meningioma tumorigenesis in the background of NF2 mutations and/or chromosome 22 loss. Identification of these target genes could open avenues for novel therapies.
Next-generation clinical interpretation algorithms for precision cancer medicine

PHIAL (Precision Heuristics for Interpreting the Alteration Landscape) was originally developed as a method of clinically interpreting cancer genomic data. The first iteration of PHIAL (PHIAL1) interpreted somatic whole exome sequence data using a co-developed database of tumor alterations relevant for genomics-driven therapy (TARGET). While PHIAL1 was successfully able to provide rapid clinical interpretation of genomic data, it was limited to first-order genomic relationships, was unable to rank multiple putatively actionable variants, did not make observations based on somatic-germline interactions, and could not leverage transcriptome data. Additionally, the initial version of the TARGET database (TARGET1) only referenced gene-level alterations to actions, did not record the relative predictive implications or cancer specificities of alteration-action relationships, and had no mechanism for recording relevant literature citations.

Both PHIAL1 and TARGET1 underwent several concurrent revisions to improve their ability to provide accurate clinical annotations. The second version of PHIAL (PHIAL2) was revised to utilize the presence of single nucleotide variations, insertions/deletions, somatic copy number alterations, fusions, and global features (such as mutation burden) together to predict actionability. Updates to the TARGET database (TARGET2) enabled greater specificity in alteration specification (including specification of the alteration and alteration type), added systems for measuring the predictive implication and cancer context of an annotation, and allowed the addition of citations to annotations. Additionally, a web-based system was built to allow other researchers convenient access to TARGET2 and to solicit future additions.

We applied both PHIAL1/TARGET1 and PHIAL2/TARGET2 to a 255-patient cohort with both whole exome and transcriptome sequencing data (146 castration-resistant prostate cancer and 109 metastatic melanoma samples). PHIAL1 identified 1,342 putatively clinically actionable/biologically relevant events across the cohort, with a median of 3 events per patient; 95% of patients had at least one putative event. PHIAL2 identified 2,508 putative events, with a median of 6 events per patient; 98.5% of patients harbored at least one event. Of these events, 8.12% were associated with an FDA-approved therapy and 2.09% with a clinical trial. PHIAL2 identified events in 9 patient samples that PHIAL1 associated with no events.

Current pharmacological management of type 2 diabetes mellitus (T2DM) begins with metformin to improve insulin sensitivity. As the disease progresses, however, several classes of insulinotropic compounds, such as sulfonylureas, are required to achieve glycemic control, with insulin as a final option when glycemic control cannot otherwise be achieved. We previously established fibroblast growth factor 1 (FGF1) as an essential factor in adipose remodeling in response to nutrient status. Through injection of this peptide, we uncovered FGF1 as a potent anti-diabetic compound that normalizes blood glucose levels within hours across a range of diabetic models. Chronic injection of FGF1 achieved sustained glucose lowering and insulin sensitization. These effects were in part mediated through the anti-lipolytic actions of the FGF1-FGFR1 signaling cascade in the adipose tissue. Importantly, suppression of adipose tissue lipolysis and free fatty acid release by FGF1-FGFR1 acted synergistically with insulin. Though FGF1 works with insulin to suppress lipolysis, it enacts transcriptomic changes separate from insulin. This allows FGF1 to lead to persistent glucose lowering in insulin-resistant mice. A single injection of recombinant FGF1 decreased serum free fatty
acids, adipose lipolysis, hepatic acetyl-CoA, and gluconeogenesis, in addition to normalization of blood glucose levels. Continued work on this project will be focused on further understanding the mechanism of FGF1 action, as well as exploring compounds that may synergize with FGF1.

Presenter: Andrej Nedic

HHMI-47 | Poster session 1

In situ signal characterization of dermal sensory interfaces

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Dermal sensory interfaces (DSIs) consist of residual sensory peripheral nerves implanted into de-epithelialized skin grafts placed subcutaneously. DSIs may transduce electrical pulse signals to compound sensory nerve action potentials (CSNAPs) to provide sensation from prostheses. Our purpose is to characterize in situ DSI signal transduction evoked while varying key electrical stimulation parameters of: (1) amplitude, (2) frequency, and (3) pulse width.

Rats were grouped by surgery: innervated skin (IS, n = five), control nerve (CN, n = five), transected nerve (TN, n = five), or DSI (n = ten; historical data). Each DSI was constructed by encasing a transected sural nerve with a de-epithelialized skin graft. Electrical stimulation was applied distally to: the skin at the ankle (IS), the sural nerve (CN), the transected sural nerve (TN), and the skin graft (DSI). Peak-to-peak voltage (Vpp) and percent of stimuli transduced (sensitivity of signal) were recorded at stimulation current amplitudes (threshold [T], T + 50, T + 100, T + 150 μA), frequencies (2, 20, 50, 100 Hz), and pulse widths (30, 50, 100, 500 μsec). Evoked nerve responses were recorded proximally at the sural nerve.

As expected, threshold current required to elicit CSNAPs was significantly higher for both IS (450.0 ± 70.3 μA) and DSI (465 ± 109.9 μA) groups compared to CN (31.0 ± 28.3 μA) and TN (22.0 ± 17.9 μA) (P < 0.05). In the DSI group, increasing stimulation amplitude from threshold to threshold + 150 μA resulted in a linear increase in Vpp (11.4 ± 10.6 to 43.1 ± 24.2 μV; r² = 0.97), importantly, with no significant difference from IS (10.8 ± 2.0 to 17.6 ± 5.9 μV). Both increases were smaller than CN (28.8 ± 5.9 to 146.8 ± 85.6 μV) and TN (63.3 ± 34.5 to 304.7 ± 32.2 μV) (P < 0.05). There were no significant differences in Vpp when varying stimulation frequency for all groups. There were no significant differences in Vpp in CN and TN when varying pulse widths. For IS, CSNAPs were not transduced below 100 μsec pulse width. Electrical stimulation applied to DSIs reliably elicited CSNAPs with 96% or higher signal sensitivity at all tested parameters.

Electrical stimulation applied to DSIs produced CSNAPs similar to normal innervated skin, with better transduction sensitivity. Findings from this study suggest that patterned electrical stimulation is successfully transduced across DSIs in a similar fashion to native skin.

Presenter: Gabriel F. Neves

HHMI-48 | Poster session 2

Investigating context-dependent integration potential of postnatally born interneurons

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Postnatally born neurons hold exciting promise for the treatment of brain injuries. Ideally, replacement neurons should reestablish the connections lost by injured neurons in a way that approximates the brain’s function to pre-injury levels. Thus, understanding the mechanisms that govern integration of immature neurons into functional circuits is of great importance. Neurogenesis in the adult rodent subventricular zone (SVZ) is a robust experimental model for investigating and understanding the integration capacities of newborn neurons. Using this system, we show that SVZ neurons have reduced integration capacities when ectopically transplanted into cortical circuitry versus the olfactory bulb circuitry — their normal target. Interestingly, this behavior was reversed when transplanting undifferentiated neurons into the cortex, suggesting that perhaps SVZ neurons at earlier developmental stages may still retain the necessary cellular plasticity to integrate into different contexts. To provide a source of neurons capable of adult cortical integration, we used Fezf2, a master gene for glutamatergic projection neurons, to alter SVZ neuronal fate. Lentiviral-mediated delivery of Fezf2 to the postnatal neural stem cells and neuronal progenitors led to ectopic Fezf2 expression in differentiated neurons. Presently, we are finalizing characterization of the Fezf2+ neurons, as well as performing live-imaging and electrophysiological experiments on Fezf2+ neurons transplanted into...
G protein-coupled receptors (GPCRs) are cell-surface receptors involved in the signaling and regulation of many physiological processes. They bind an array of ligands, from small molecules to polypeptides, which act to stabilize active receptor conformations. In its active forms, the receptor interacts with G protein, thus initiating signaling cascades that eventually lead to functional outcomes. To prevent overstimulation, G protein-coupled receptor kinases (GRKs) phosphorylate the activated GPCR, which facilitates binding of the adapter protein β-arrestin (βarr) to the receptor. βarr recruitment to the GPCR has two important consequences: (1) desensitization of G protein signaling, since βarr binds to similar region of the G protein, thereby sterically blocking its binding to the GPCR; and (2) internalization of the GPCR-βarr complex, facilitated by βarr interacting with endocytic proteins such as clathrin and AP2. βarrs also serve as signaling molecules, capable of inciting molecular pathways that are distinct from those associated with G protein signaling, which ultimately leads to unique physiological consequences.

In contrast to this classical understanding of GPCR function, we have recently discovered that some GPCRs in fact are capable of forming mega-complexes, whereby one GPCR actively engages with both a G protein and βarr at the same time. This discovery is supported by our previous negative stain electron microscopy work, which directly observed a conformation of βarr that only engages the phosphorylated receptor tail, leaving the G protein binding interface on the intracellular transmembrane receptor core open. These mega-complexes have been shown to mediate sustained G protein signaling, as the receptor is internalized into intracellular compartments such as endosomes.

In the last few years, our lab has successfully purified a number of GPCR-transducers complexes, including a GPCR-βarr complex stabilized by an antigen binding antibody fragment (Fab) as well as a GPCR–G protein-βarr mega-complex stabilized by both a Fab and a nanobody. Furthermore, we have characterized the overall architecture of these complexes using negative stain electron microscopy. Currently, we are conducting preliminary trials in order to screen for optimal constructs and freezing conditions for high-resolution cryo-electron microscopy (cryo-EM) studies. Structural elucidation of these GPCR-transducer complexes using cryo-EM can provide crucial mechanistic insight into GPCR signaling, as well as pave the way for the creation of pharmaceutical compounds that might modulate pathogenic signaling pathways more specifically.

### Structural analysis of GPCR-transducer complexes using cryo-electron microscopy

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### FOXG1 overexpression and epigenetic landscape in a human iPSC-derived forebrain organoid model of severe, macrocephalic autism spectrum disorder

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Autism spectrum disorder (ASD) is a highly heritable developmental disorder that in some cases is associated with early embryonic cortical overgrowth, macrocephaly, and GABAergic neuronal and synaptic imbalance. FOXG1, a transcription factor involved in early forebrain patterning, has been implicated in contributing to ASD phenotype in a subset of severely affected macrocephalic patients. Previous findings show that FOXG1 is particularly upregulated as well as co-expressed with other ASD-associated genes in these ASD patients’ forebrain organoids. These organoids are a human induced pluripotent stem cell (hiPSC)-derived three-dimensional model system for early telencephalic development. In these patients, increased FOXG1 activity may explain some features of ASD pathophysiology, for example, FOXG1’s known associations with cortical growth and GABAergic predominance. Our previous publication supports this connection, showing that FOXG1 knockdown by RNA interference (RNAi) in these ASD-derived organoids...
normalized several ASD-associated gene network and neurophenotypic changes.

However, to what extent FOXG1 upregulation specifically contributes to ASD, and the details of the FOXG1 regulatory network in health and disease, remain unclear. Additionally, the epigenetic landscape of ASD is not well understood, particularly with regard to FOXG1. Our previous ASD-derived organoids showed increased FOXG1 expression but without mutations in the FOXG1 sequence or upstream promoter regions, suggesting that epigenetic or distal regulatory changes might contribute to these patients’ ASD phenotype.

This project explored these uncertainties surrounding FOXG1 by two avenues. First, by using a doxycycline-inducible FOXG1 overexpression system in organoids derived from ASD patients’ fathers, who serve here as unaffected controls, we used several metrics, including RNA-sequencing (RNA-seq) and immunocytochemistry, to examine the extent to which FOXG1 overexpression alone recapitulates the ASD transcriptomic and neurophenotypic changes seen in organoids derived from their autistic sons. Second, by performing chromatin immunoprecipitation sequencing (ChIP-seq) (markers H3k4me3, H3k27ac, H3k27me3) on organoids derived from ASD patients and their unaffected fathers at time points corresponding with early forebrain development, we probed further into the dynamic developmental transcriptomic and epigenetic landscape of our ASD cohort; for example, we aim to investigate potential epigenetic causes for the previously unexplained overexpression of ASD-associated genes such as FOXG1.

FOXG1 over-expression, RNA-seq, and ChIP-seq experiments are ongoing in several clonal lines derived from multiple families. FOXG1 over-expression experiments, in particular, have already yielded unexpected and interesting results suggestive of the nuanced FOXG1 regulatory environment in pluripotency and neuronal differentiation. This set of experiments using hiPSC-derived forebrain organoids should better illustrate the role FOXG1 overexpression plays in creating the ASD phenotype seen in our patient cohort, as well as illustrate the network of transcriptional and epigenetic changes associated with ASD.

**Presenter: Tolani F. Olonisakin**

**HHMI-51 | Poster session 1**

**Platelet-mediated protection against alveolar injury through the release of thrombospodin-1 during Pseudomonas aeruginosa acute intrapulmonary infection**

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*Pseudomonas aeruginosa* (PA) acute lung infection is a major cause of opportunistic infection, and Pseudomonal sepsis is independently associated with increased mortality. Secretion of exoproteases such as Pseudomonas elastase (LasB), a metallo-endoprotease that degrades tissue elastin and collagen, enhances PA pathogenicity, but host-derived factors that disarm pathogen-encoded proteases are not fully known. Thrombopodin-1 (TSP-1), a multifunctional extracellular matrix glycoprotein involved in cell-cell and cell-matrix interactions, is secreted by activated platelets during inflammation and inhibits neutrophil granule serine proteases neutrophil elastase and cathepsin G. We hypothesized that TSP-1 curtails PA exoprotease activity by potentially inhibiting the major Pseudomonas elastase LasB and that platelets provide protection against pathogen-triggered proteolysis through the release of TSP-1 during acute PA-induced injury. Utilizing natively thrombocytopenic mice deficient in the thrombopoietin receptor Mpl (Mpl-/-), we demonstrate that Mpl-/- mice are prone to severe alveolar injury and succumb to early mortality upon acute *P. aeruginosa* intrapulmonary infection. However, acute *Klebsiella pneumoniae* intrapulmonary infection fails to elicit alveolar injury or enhanced mortality in Mpl-/- mice compared to WT controls, highlighting a specific host-pathogen interaction. Furthermore, PA-secreted bacterial exoprotein(s) mediate the alveolar injury, as cell-free filtered supernatant obtained from *P. aeruginosa* reproduces the phenotype observed with whole live bacteria in Mpl-/- mice. We further show that cell-free filtered supernatant obtained from PA mutant containing a transposon insertion in LasB gene (lasB) lacks exoprotease activity and induces significantly less alveolar injury in Mpl-/- mice compared to parent PA strain. In vitro, Pseudomonas elastase activity is inhibited by rhTSP-1 monomer and purified platelet-derived TSP-1 in a dose-dependent manner. Collectively, our findings suggest that thrombocytopenia...
Cilia are essential to life. Cilia are involved in vision, kidney function, mucin clearance in lungs, and brain development and function. Primary cilia are located on neurons, and are involved in neuronal signaling. This implicates a role of cilia in behavior. Mice without primary cilia in the brain have altered behavior. Bardet-Biedl syndrome (BBS) is a pleiotropic, autosomal recessive ciliopathy. We hypothesize that BBS proteins play a crucial role in anxiety processing and response. To study this, we tested fear conditioning (FC) in a homozygous knockin mouse model (BBS1M390R) of the most common BBS mutation (BBS1M390R) in humans. We discovered that BBS1M390R mice have both impaired cue and context FC (P < 0.01). This FC dysfunction is recapitulated when the Bbsδ gene is knocked out postnatally. To rule out confounding variables on the FC test, we performed control studies. These studies show that BBS1M390R mice have normal motor function, including normal cerebellar function (rotarod performance test) and normal hearing (auditory brainstem response) compared to control mice.

To determine the mechanism for the FC defect, we investigated the electrophysiology of the hippocampus and amygdala. Whole-cell patch recordings of pyramidal neurons of the lateral amygdala show that the frequency and amplitude of mEPSCs (miniature excitatory postsynaptic currents) are smaller in BBS1M390R mice compared to control mice (P < 0.01), whereas field recordings show that long-term potentiation of the hippocampus is not different between BBS1M390R mice and controls. Furthermore, the paired pulse ratio (PPR) is larger in the hippocampus and amygdala of BBS mutant mice compared to the PPR in control mice (P < 0.05). The electrophysiology data point to a vesicle release defect. However, the BBS mouse ultrastructure morphology (synaptic density, vesicle density, vesicle docking density, vesicle size, and mitochondrial density) is normal compared to control mice.

Western blotting of synaptosomes shows that BBS proteins are located in the synapse. In addition, Western blot data show that the GSK/AKT signaling pathway in FC is affected in BBS mutant mice. These findings could explain our results that chronic lithium chloride treatment rescue context-dependent FC in BBS mutant mice. These findings provide a new understanding of the molecular mechanisms underlying anxiety disorders, and uncover a novel role for ciliary proteins in neural signaling.

**Presenter:** Joseph S. Park

**HHMI-54 | Poster session 2**

**A genome-wide CRISPR/Cas9 screen for host factors that mediate cellular invasion of Chlamydia trachomatis**

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Chlamydia trachomatis is a Gram-negative bacterium that is the most common cause of sexually transmitted disease in the US and infectious blindness worldwide. An intracellular obligate parasite, Chlamydia trachomatis invades epithelial cells and replicates within a membrane-bound vacuole known as an inclusion body. To comprehensively identify the host factors that mediate Chlamydia invasion, we undertook a genome-wide CRISPR/Cas9-based screen in cultured human epithelial cells (HT29). We engineered a transgenic C. trachomatis serovar L2 strain expressing the mCherry fluorescent protein and infected a pooled mutant library of HT29 cells at high multiplicity of infection, leading to a very high invasion rate (~90%). Fluorescence-activated cell sorting (FACS) was then used to enrich for cells that did not express an intracellular mCherry signal, which reflects a sub-pool of mutant cells resistant to invasion. The nature of the genetic perturbations in the resistant cells was identified by next-generation sequencing of the chromosomally integrated guide RNAs. Having acquired preliminary hits from the screen, efforts are now underway to validate and characterize the function of these genes during C. trachomatis invasion. We hypothesize that some of the identified pathways are important for bacterial attachment, while others act as functional receptors for the chlamydial type 3 secretion
system. The comprehensive identification of host factors involved in Chlamydia infection will aid in the development of novel therapeutic strategies for treating C. trachomatis infection, which is of global urgency.

**Presenter: Fatemeh P. Parvin-Nejad**

**HHMI-55 | Poster session 1**

**Transcriptomic characterization of ductular and stellate cells in a murine liver injury model at single-cell resolution**

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End-stage liver disease from hepatic fibrosis contributes significantly to US and worldwide morbidity and mortality, yet treatment remains limited. Severe liver disease is accompanied by ductular reaction (DR), comprised of expanded ductular cells (putative liver progenitor cells [LPCs]), hepatic stellate cells (HSCs), and fibrosis. DR correlates with severity of disease and fibrosis; however, its underlying mechanisms are poorly characterized. It is also unclear whether HSCs, the main fibrogenic cells in liver, contribute to regeneration by activating ductular cells, or if ductular cells activate HSCs to enhance fibrogenesis. Characterizing the cellular contributions to fibrogenesis may provide new targets for antifibrotic therapies. The aim of our study was to identify and characterize ductular and HSCs using single-cell transcriptomics. To address this aim, our laboratory has generated a hepatic autophagy-deficient mouse line (Alb-Cre:Atg7flx/flx mice) that develops hepatomegaly, spontaneous fibrosis, and marked expansion of LPCs, allowing us to explore their fibrogenic potential as well as their contribution to hepatic regeneration following injury. To visualize fibrogenic cells in this model, Alb-Cre:Atg7flx/flx mice, a liver-specific knockout of autophagy-related protein 7 (Atg7, a key component of the autophagy pathway), were crossed with Tg(Col1-GFP) mice (expressing GFP driven by the collagen 1a1 promoter) to identify collagen 1-transcribing cells. Livers from these reporter mice were analyzed by immunofluorescence for markers of LPCs (CD133, EpCAM) or HSCs (desmin) and correlated with sites of GFP expression. In addition, LPCs were isolated using in situ perfusion and magnetic cell sorting to enrich for EpCAM-expressing cells, which were analyzed by single cell sequencing using the Fluidigm C1 platform. Single-cell sequencing data were analyzed via t-distributed stochastic neighbor embedding (t-SNE) to define clusters of genetically similar cells. Lists of differentially regulated genes among these clusters were reviewed to further characterize clusters and to identify genes of interest. Double immunofluorescence revealed close proximity between fibrotic HSCs (GFP+, desmin+) and LPCs (CD133+, CK7+), as well as scattered co-localization of GFP and CD133 expression in regions displaying a ductular reaction, suggesting crosstalk of LPCs with HSCs. Single-cell sequencing revealed five clusters of cells; three clusters corresponded to resident hepatic cell populations: hepatocytes (Alb+, CRP+, ApoB+), HSCs (Col1a1+, Lrat+, Vimentin+), and immune cells (IL-1R2+, CD103+, Ccl5+); two clusters displayed upregulated expression of progenitor markers including EpCAM and Sox9; one progenitor-like cluster was specific for upregulation of LPC markers (Sox9, EpCAM), associated with downregulation of the HSC markers Lrat and vimentin, whereas the final cluster expressed a diverse genetic signature, including Sox9, as well as other HSC and endothelial cell markers. These data indicate a diversity of phenotypes within the LPC compartment, highlighting the need to functionally define LPC sub-populations and characterize their contributions to fibrogenesis and repair.

**Presenter: Julia D. Ransohoff**

**HHMI-56 | Poster session 2**

**Characterizing the vicinal RNA-protein interactome across human disease**

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Physical interactions between specific RNAs and proteins play critical roles in diverse cellular functions, and their perturbations contribute broadly to human disease. Here we develop a novel vicinal proteomic approach to study RNA-protein interactions in living cells and characterize its applications in genetic disease, cancer, virology, and skin biology. We use a promiscuous biotin ligase (BirA*) to biotin-tag proteins bound to RNA motifs followed by streptavidin pull-down and Western blot (WB) or mass spectrometry
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Presenter: Mondira Ray

HHMI-57 | Poster session 1

Construction and validation of integrated mRNA and microRNA immune cell signatures to predict survival of patients with breast and ovarian cancer

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Immunotherapy is emerging as a powerful paradigm in cancer treatment. Recent studies have also indicated that the presence of immune cells within the tumor microenvironment plays a critical role in prognosis and survival. MicroRNAs (miRNAs), a class of small non-coding RNA molecules that post-transcriptionally regulate gene expression, have been reported to mediate immune cell recruitment and activation in the tumor microenvironment and directly target cancer-related immune pathways. This study aims to combine mRNA and miRNA signatures in order to determine the fractions and types of immune cells within a specific tumor and use these to predict patient survival in breast invasive carcinoma and ovarian serous cystadenocarcinoma. Preprocessed gene expression array, RNA-seq, miRNA-seq, and survival data from The Cancer Genome Atlas (TCGA) were downloaded from the UCSC Xena Browser. Using regression-based computational methods, we constructed, for the first time, immune cell signatures based on miRNA expression from breast and ovarian cancer datasets. We validated these using statistical analysis and by comparing them to known miRNA signatures for a subset of the cells. Next we applied CIBERSORT, an analytical tool developed by Newman et al., to quantify the relative levels of immune cell subsets in the tumor samples using both CIBERSORT’s own immune cell mRNA expression signature matrix and our constructed immune cell miRNA expression signature matrix. The immune cell subsets most related to survival were identified using univariate Cox analysis. In the breast cancer data, applying both the mRNA expression and miRNA expression signatures yielded the most significantly prognostic immune cell population compared to using either signature matrix alone; M2 macrophages emerged as the most significantly prognostic cell type with an adverse relationship to survival in the breast cancer data ($P = 2.74e^{-7}$). Moreover, using the combination of the mRNA and miRNA expression signatures generated the greatest number of significantly prognostic immune cell types in the ovarian cancer data with

(MS) to identify the proteins, terming our method RaPID, for RNA-protein interaction detection.

We first evaluated iron metabolism regulation by iron responsive element (IRE) RNA motif binding by IRE binding proteins (IREBPs). The IRE L-ferritin (FTL) transcript is mutated in hereditary hyperferritinemia-cataract syndrome (HHCS). RaPID-WB with wild-type FTL IRE motif showed significant IREB2 enrichment, and RaPID-MS identified IREB2 as the top IRE binding partner. HHCS FTL IRE disease-associated point mutation-containing transcripts from patients bearing the London, Paris, and Verona mutations demonstrated decreased IREB2 binding compared to wild-type, offering evidence for the hypothesis that HHCS severity is attributable to impaired IRE-IREBP interactions.

We next used RaPID-MS to identify that RC3H1 binds the SM1v1 motif and that increased RC3H1 leads to decay of RNAs bearing the SM1v1 motif. Given that RC3H1 up-regulation correlates with decreased breast cancer survival, our findings suggest RC3H1 overexpression may perturb post-transcriptional regulation of RNAs bearing SM1v1-like motifs, altering disease course specifically through this mechanism.

Given that RNA viruses co-opt host proteins at their untranslated regions (UTRs), we next applied RaPID-MS to identify the Zika virus (ZIKV) host interactome. We found striking enrichment of cell cycle regulatory proteins, paralleling ZIKV-infected neural progenitor cell (NPC) gene perturbations, and of QKI, an RNA binding protein highly expressed in NPCs. QKI depletion decreased ZIKV viral RNA levels by 90%, suggesting QKI is critical for ZIKV replication. As QKI loss mimics Zika congenital syndrome, this RNA-protein interaction may underlie the overlapping phenotypes.

Finally, we applied RaPID-MS to identify APOL4 as a highly specific protein interactor with wild-type cornedodesmosin (CDSN) transcripts in differentiating keratinocytes, but not with transcripts bearing a mutant allele found in psoriasis. We newly describe the strong induction of APOL4 in terminal keratinocyte differentiation, and propose APOL4 as a novel RBP that regulates CDSN transcripts in differentiating KCS and whose function may be altered in psoriasis. Loss of APOL4 markedly reduced cornedodesmosin RNA and protein levels, suggesting a regulatory relationship that may offer insight into the coordinate regulation of terminal differentiation with lipid barrier formation.

Here we describe the development and broad applications of RaPID, a modular system to identify and validate novel RNA-protein interactions across a range of human disease in living cells. This efficient, cost-effective approach powerfully expands the scope, feasibility, and depth of vidual proteomic discoveries.

The American Society for Clinical Investigation

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Ionizing radiation (IR) is used in many therapeutic settings. Despite its ubiquitous use, our understanding of cellular responses to IR remains rudimentary. Here, we examined alternative splicing, and its regulation in irradiated human cells.

To accomplish this, we exposed cultured B-cells from 10 individuals to 10 Gy of ionizing radiation and performed RNA sequencing before, and two and six hours after radiation treatment. With these data, we first identified alternative splicing events. From about 60 million reads per sample, we found 1,1881 events that were radiation-responsive (ANOVA, FDR <5%). The majority of these events (52%) encode transcript isoforms that have opposing responses to radiation; as such, they would not have been identified as radiation-responsive based on their total gene expression levels alone. These events were found primarily in genes involved in RNA processing, DNA repair, and apoptosis.

To determine how these alternative splicing events are regulated, we measured the expression levels of over 70 splicing factors. We found that the expression levels of 40 splicing factors changed significantly in irradiated cells (ANOVA, FDR <5%). One of these radiation-responsive splicing factors is serine/arginine-rich splicing factor 1 (SRSF1), which promotes exon inclusion. Following radiation exposure, the transcript and protein expression levels of SRSF1 decreased. By sequence enrichment analysis and RNA-immunoprecipitations, we found that nearly one half (48%) of the 645 radiation-responsive cassette exons are targets of SRSF1. These include many genes that promote cell survival. As SRSF1 expression decreases in irradiated cells, it is less effective in promoting inclusion of its target exons; thus, upon irradiation, these cassette exons are spliced out. For example, the proteins resulting from BIRC5, BEAR, and API5 can no longer promote cell survival. Thus, it appears that SRSF1 is reduced in irradiated cells to promote cell death. To follow up this observation, we modulated SRSF1 expression and assessed cell survival in response to IR. Over-expression of SRSF1 rescued the irradiated cells, and resulted in increased cell survival. Conversely, SRSF1-knockout cells did not survive irradiation. Together, our results show that alternative splicing plays a role in ionizing radiation-response by inducing cell death, and that SRSF1 is a key factor in mediating these splicing events. In this presentation, we will describe radiation-induced alternative splicing by discussing the genes that are alternatively spliced, and its regulation by the splicing factor SRSF1.
baseline levels and signal amplitude of phosphorylated zeta-chain-associated protein kinase 70 at the beginning of the TCR cascade. RAGE siRNA decreases the signal amplitude of phosphorylated extracellular signal-regulated kinases 1/2 (Erk 1/2) downstream of the TCR on Western blots. The addition of high-mobility group box 1 protein (HMGB1), a known RAGE ligand, increases the amplitude of phosphorylated Erk 1/2 signal in submaximally stimulated Jurkats transfected with negative control siRNA but not in Jurkats transfected with pooled RAGE siRNAs.

RAGE+ cells may require the receptor for maximal signal through the TCR, and RAGE activation through HMGB1 might further augment the signaling cascade. We postulate that this mechanism permits survival and proliferation of potentially autoreactive T cells in type 1 diabetes.

Presenter: Ashley M. Riley

HHMI-60 | Poster session 2

The role of postsynaptic cell-adhesion molecules in the trafficking of AMPA-type glutamate receptors

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Long-term potentiation (LTP), an activity-dependent, long-lasting change in synaptic strength, is a critical component of synaptic plasticity underlying learning and memory and has been implicated in various neuropsychiatric disorders, including depression and addiction. The mechanism of LTP is dependent on the trafficking of AMPA receptors (AMPARs) to the post-synaptic membrane.

The present project is guided by the unexpected role of which cell adhesion proteins mediate trans-synaptic signaling. Specifically, the project focuses on post-synaptic neuroligin 1, an isoform found in excitatory synapses, binding pre-synaptic neurexin. This heterophilic interaction is present in mature and developing synapses, and its absence has been shown to impair LTP.

Here, we generated various neuroligin constructs where different functional domains of neuroligin 1 are mutated. These constructs were then used to determine which neuroligin domains are necessary for AMPAR exocytosis so that the precise mechanism by which neuroligins are required for LTP can be elucidated. Methodically, dissociated mouse hippocampal cultures where all four neuroligin isoforms have been conditionally knocked out were infected by viruses carrying the different neuroligin 1 constructs. Subsequently, the ability of the neuroligin 1 constructs to mediate LTP was monitored by inducing chemical LTP in these neurons and measuring the exocytosis of AMPARs using immunocytochemistry.

Our data suggest that chemical LTP is impaired upon deletion of neuroligin 1, can be rescued solely by the extracellular domain of neuroligin 1, is impaired when neurexin binding is inhibited, and does not require dimerization. Ongoing work includes investigating novel binding partners to the neuroligin-neurexin complex.

Presenter: Tolulope O. Rosanwo

HHMI-61 | Poster session 1

Modeling fetal hemoglobin reactivation in sickle cell anemia induced pluripotent stem cells

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Human induced pluripotent stem cells (hiPSCs) hold promise for both disease modeling and the development of novel therapeutic treatments for sickle cell anemia (SCA). Such models are practical systems to screen new drug therapies and to examine the effects of gene editing. In our study, we aim to employ hiPSCs to test the therapeutic hypothesis that genetic manipulation of BCL11A, a master regulator of fetal hemoglobin (HbF) expression, will ameliorate sickling. hiPSCs can theoretically produce all cell types, including erythroid cells. However, in vitro modeling of SCA with reprogrammed cells has been limited by their inability to differentiate into β-globin-expressing, enucleated erythroid cells. Here, we propose strategies to produce both in vitro and in vivo models of SCA using these cell types. We derived hiPSCs from sickle cell patients with hemoglobin SS disease seen at our hematology clinic at Boston Children’s Hospital. CRISPR/Cas9-mediated repair of the sickle mutation served as a key control. Using a cocktail of transcription factors promoting self-renewal and multi-potentiality expressed under the control of a doxcycline-regulated promoter (Erg, HoxA9, RORα, Sox, Myb), we began generating conditionally immortalized hematopoietic cell lines that serve as a renewable source of robust erythroid progenitors in vitro. Globin-switching of erythroid progenitors differentiated from these lines was assessed following the trans-
fusion of these cells into immune-radiated mice, with the goal of producing a humanized mouse model of SCA. Concurrently, we further assessed the efficacy of differing in vitro differentiation protocols, laying the groundwork needed to test and evaluate gene knockdown of BCL11A and the CRISPR/Cas9 gene-editing of the erythroid-specific enhancer of BCL11A to reactivate HbF. The generation of hiPSC-SCA models will be critical in broadening the current understanding of the molecular mechanisms of this disease, the development of improved pharmacological treatments, and a future of autologous cell therapy for the cure of SCA.

Presenter: Yohei M. Rosen

HHMI-62 | Poster session 2

The mathematics of variation graphs and progress toward population-backed variant calling, with clinical application to orofacial cleft genomics

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Accurately identifying and describing genetic variation is essential to diagnosis and research. The current approach is to use a single arbitrarily chosen genome as the map or “reference structure” against which genetic variants are described. This method is inadequate in the presence of population-level differences or complex structural genetic variation. Both of these factors are implicated in nonsyndromic orofacial cleft and in many other heritable conditions.

Our project leverages and contributes core technology to an international collaboration led from UC Santa Cruz to change the way in which we interpret human genomes, by using all known human genomes as a reference instead of current single reference genome. This collaboration is developing mathematical structures known as variation graphs in order to replace the single-genome reference. These are designed to incorporate the breadth of genetic diversity from across populations and enhance our ability to classify and study structural genetic variants. We report three significant technical contributions to this field.

We introduce the first probability model of the likelihood that a haplotype was generated from a population cohort represented in a variation graph. We demonstrate computational tractability on a 5,008-haplotype graph of chromosome 22, with time complexity linear in haplotype length. Such a model is fundamental for variation graph genomics. It allows the integration of population data into clinical genomics. It also enables new approaches to genetic epidemiology, since it leverages variation graphs’ ability to encode population frequency and linkage information for all human genetic variation.

Second, we introduce algebraic methods for describing sites of genetic variation on genome graphs. This theoretical work is essential for communicating genetic variants and for efficient variation graph algorithms.

Third, we describe progress toward a next-generation population-aware simultaneous variant calling and haplotype phasing tool. It extends naturally to a technology that simultaneously haplotypes parental trios for the analysis of inherited diseases.

Our first test of our technologies will be identifying causative variants in non-syndromic orofacial cleft. We will analyze whole genome sequences of over 400 parental trios of orofacial cleft patients from multiple populations and compare our graph-based results against a parallel assessment using conventional tools.

While our group’s immediate clinical focus is on orofacial cleft genomics, the technology we are developing has fundamental importance to genomics and will have broad application across many diseases, including other types of structural birth defects, other inherited disorders, and cancer.

Presenter: Andrew M. Sohn

HHMI-63 | Poster session 1

TPOT-MDR: toward automated machine learning pipeline design for GWAS analysis

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Machine learning has been gaining larger traction in efforts toward meeting the demand for the analysis and predicative modeling of big data applications in the biomedical sciences. Effective utilization of machine learning, however, requires domain expertise (i.e., feature engineering, model-selection, hyper-parameter tuning, model validation, etc.), all of which can prove to be a high barrier of entry. Therefore, the existence of off-the-shell tools that make machine learning more accessible can prove to be beneficial. To this end, we have been developing an open-source tree-based approach toward automated machine learning pipeline design, TPOT-MDR, for bioinformatics using evolutionary computation.
Medulloblastoma (MB) is the most common malignant brain tumor in children, accounting for nearly 20% of all pediatric brain cancers. Based on transcriptional profiling studies, MB has been characterized into four molecular subgroups — WNT, SHH, group 3, and group 4 — that have distinct genetics, patient demographics, histology, and clinical outcomes. Group 3 MB carries the worst prognosis, with 5-year survival rates of 50%. Current therapy for MB consists of maximal safe surgical resection, radiation, and chemotherapy, with cisplatin, vincristine, and cyclophosphamide comprising a standard chemotherapy regimen. However, group 3 MB commonly develops resistance and becomes refractory to standard therapy. As these mechanisms of resistance remain poorly understood, we are using the CRISPR/Cas9-based synergistic activation mediator (SAM) system to conduct a genome-wide, gain-of-function, positive selection screen to identify the specific drivers of chemoresistance in group 3 MB. We cloned and sequenced a library consisting of 70,290 single-guide RNAs (sgRNAs) targeting each of the 23,430 coding isoforms from the human RefSeq database. We have transduced this library into a group 3 MB patient-derived model stably expressing the other SAM components (dCas9, VP64, MS2, p65, and HSF1) and then selected with standard-of-care chemotherapy conditions: (1) cisplatin; (2) vincristine; (3) 4-hydroperoxycyclophosphamide; and (4) combination of cisplatin, vincristine, and 4-hydroperoxycyclophosphamide. Modulators of chemotherapy sensitivity will be prioritized by sgRNAs that have activated genes conferring chemoresistance, and we will perform next-generation sequencing to identify those sgRNAs. We will then validate the top hits from our screen and also cross-reference them with recently published whole-genome sequencing data on six pairs of human diagnostic and post-therapy group 3 MBs. These findings will provide insights into the mechanisms of resistance in group 3 MB and inform novel therapeutic targets that may sensitize the tumor to chemotherapy and improve future treatment response.

**Presenter: Matthew A. Stern**

**HHMI-65 | Poster session 1**

**Impact of inhibitory luminopsins in a mouse tetanus toxin model of spontaneous and recurrent epileptiform discharges**

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Identifying small molecule epigenetic modifiers that impact the viability of patient tumor-derived primary glioblastoma cell lines

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Glioblastoma is an aggressive brain tumor with a median survival time of 15 months despite standard-of-care treatment that consists of maximal safe surgical resection, chemotheraphy with DNA alkylating agent temozolomide (TMZ), and radiation therapy. Therapy resistance has been associated with a tumorigenic and self-renewing subpopulation of cells within the bulk tumor termed glioblastoma stem-like cells (GSCs). In addition to genetic and metabolic mechanisms, epigenetic processes are critical for maintenance of the GSC state. Thus, disrupting the epigenetic mechanism that governs the GSC state is a promising therapeutic approach. To this end, our laboratory first conducted a small molecule library screen of 85 epigenetic-modifying compounds to identify novel drugs that reduce the viability of patient tumor-derived, primary glioblastoma stem-like cell lines. To test these drugs in pooled primary cancer lines, a barcoding strategy was utilized by transducing three primary GSC lines with 30 unique base pair sequences paired with expression of either enhanced GFP, mCerulean, or turbo RFP to generate stable lines expressing the fluorescent protein of interest. Preliminary results indicated that 1) qPCR with cell line DNA using primers specific for each barcode and 2) fluorescence intensity of expressed fluorescent proteins correlated well with cell number for each individual line. Using this system, we then screened for epigenetic-modifying drugs that decreased fluorescence intensity in pooled cell lines after administration of the drug library alone and in combination with TMZ for a period of 10 days. This screen yielded 21 drug hits that significantly reduced the viability of at least two of the three cell lines tested compared to the appropriate controls. Using the MTS colorimetric assay as an independent measure of cell number, we then selected eight of these drugs, whose effects on GSCs have not been previously described, for validation of the initial screen results. These drugs represented a number of the major classes of epigenetic modulators, such as DNA methyltransferase inhibitors, histone deacetylase inhibi-
Small cell lung cancer (SCLC) is an aggressive malignancy with significant intratumoral heterogeneity responsible for differential chemoresistance and metastatic capacity among tumor subclones. In particular, mesenchymal subclones in SCLC have been shown to harbor distinct cytokine profiles and activation of pathogenic innate immune signaling networks, including the TBK1 and STAT1 pathways. Recent work has suggested that exposure of these mesenchymal tumor cells to IFN-γ leads to production of double-stranded RNA (dsRNA) through transcription of endogenous retroviral (ERV) elements located in the 3’ UTR of IFN-γ-inducible genes in a sense and anti-sense direction. However, the mechanism of ERV de-repression or the consequences of this dsRNA production for pathologic innate immune signaling in mesenchymal subclones remains unclear. We began by comparing ERV expression patterns against CCLE and TCGA gene expression databases across tumors and found strong correlation of ERV expression with targets of DNA methyltransferases (DNMTs) and the H3K27 histone methyltransferase EZH2. Treatment of the SCLC cell line H69 with the DNMT inhibitor decitabine and the EZH2 methyltransferase inhibitor GSK126 both independently resulted in significant increases in ERV gene expression. In particular, H69 cells treated with EZH2 inhibition adopted a mesenchymal morphology and an increased production of CXCL10, an innate immune cytokine strongly linked to tumorigenesis, pTBK1, a marker of pathogenic TBK1 innate immune signaling, and PD-L1, a T-cell immunosuppressive factor thought to help mediate immune evasion widely across tumors — suggesting EZH2i-induced de-repression of ERVs contributes to tumor propagation and immune evasion. To further characterize the role of dsRNA production in the activation of cytokine circuits in mesenchymal SCLC subclones, we show that treatment of the chemoresistant H69AR cell line with the synthetic dsRNA oligonucleotide poly(I:C) induces CXCL10 production, with this induction shown to be specifically through activation of IFN-β and largely independent of IFN-γ. Importantly, this CXCL10 upregulation is shown to be completely reversed with CRISPR-mediated deletion of the cytoplasmic dsRNA sensor MAVS in the H69AR cell line. In addition, CXCL10 production is partially inhibited by the dual TBK1/IKKe inhibitor MRT67307, and completely inhibited by the JAK1/2 inhibitor ruxolitinib. Taken together, these results support a mechanism in which H3K27 demethylation in an SCLC model leads to epigenetic de-repression of endogenous retroviral elements and production of dsRNA, whose sensing by MAVS fuels a pathologic inflammatory circuit marked by CXCL10, pTBK1, and PD-L1. Hence, these suggest that mesenchymal-associated alterations in histone demethylation lead to de-repression of ERVs and priming of immunogenicity in tumor subclones, identifying unique points of intervention which may have important consequences for immunotherapy in small cell lung cancer.

**Presenter: Anthony Tran**

**HHMI-68 | Poster session 2**

**The impact on transcriptome diversity after oncogenic transformation of human mammary epithelial cells**

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Breast cancer is a leading cause of cancer-related morbidity and mortality worldwide. In the USA, one of eight women will be diagnosed with breast cancer over the course of their lifetime. A major obstacle hindering the treatment of breast cancers is intratumor heterogeneity, since cytotoxic drug sensitivities among different clonal populations may vary. Previously, we have shown that cancer cells display an increased repertoire and abundance of all transcripts within a cell (termed transcriptome diversity) compared to normal cells. The driving mechanism(s) of cellular intratumor heterogeneity has yet to be fully elucidated. In this study, we utilize immortalized human mammary epithelial cells (MC-
F10A) transformed with an array of doxycycline-inducible lentiviral vectors encoding known genetic drivers of breast tumorigenesis (TP53 mutant, BRCA1 mutant, PIK3CA mutant, shPTEN, and c-MYC) alone and in combination to determine their impact on transcriptome diversity based on RNA-seq. Our strategy enables strict temporal control and reversibility of gene expression alterations via the doxycycline-inducible promoter. Transcriptome diversity will also be interrogated using single-cell RNA sequencing to determine how individual cellular transcriptional profiles contribute to the overall population-based transcriptional landscape. These results will be compared to patient tumor samples’ transcriptome diversity and correlated with their respective clinicopathologic characteristics. Advancing our understanding of cellular transcriptome diversity will enable new avenues of treatment for breast cancers resistant to current clinical interventions such as metastatic and triple-negative breast cancers, both of which are characterized by high transcriptome diversity.

Presenter: Wai Lok Tsang

HHMI-69 | Poster session 1

The role of synaptic zinc in tinnitus-related hyperactivity

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Increased neuronal spiking (hyperactivity) in the dorsal cochlear nucleus (DCN), a part of the auditory brainstem, is linked with the induction of tinnitus (Li et al., 2013). This hyperactivity can result from increased excitatory synaptic transmission. Among modulators of excitatory DCN neurotransmission, zinc is a major modulator that inhibits NMDA and AMPA glutamate receptors (Anderson et al., 2015; Kalappa et al., 2015). We hypothesize that noise-induced loss of synaptic zinc in DCN synapses cause an increase in NMDA and AMPA receptors signaling, leading to neuronal hyperactivity and noise-induced tinnitus.

To assess noise-induced tinnitus in mice, we have been using the gap-prepulse inhibition of the acoustic startle reflex (GPIAS) paradigm, which is the most commonly used animal model of tinnitus (Li et al., 2013). This paradigm has been challenged recently due to observations inconsistent with the key assumption that the sound of tinnitus “fills in” the silent gap in background sounds (Campiono et al., 2013; Hickox and Liberman, 2014). Thus, we decided to further develop and employ an additional animal model of tinnitus through a modified version of the conditioned place preference test (“conditioned crossing” test, in our case) (Yang et al., 2011). This model entails the training of mice to associate the perception of a tone to crossing behavior (to cross from one room to the other room), which allows us to infer tinnitus perception from crossing behavior during silent periods.

In the conditioned crossing paradigm, mice are trained to cross from one room to the other room when they hear an external sound, whereas, silence is expected to not produce any crossing behavior. During training, whenever a pure tone is played, the mouse receives a mild footshock if it does not cross to the other room within ten seconds. When the success rate reaches a 70% threshold, mice are noise exposed to induce tinnitus. If the crossing rate during silence is significantly increased after noise exposure, this is considered behavioral evidence of tinnitus.

Over several different iterations of training with varying tone and silence conditions, our current most optimal iteration of training program averages 15.33 days until the mouse is trained. However, with this training program, only one of fifteen mice (6.67%) has yielded behavior consistent with tinnitus, with an increased crossing rate. Refinements to the training programs continue to be tested to better simulate tinnitus tone conditions without compromising efficiency in generating mice with behavioral evidence of tinnitus. Once we establish this behavioral paradigm, we will perform ex vivo electrophysiological and imaging experiments in brain slices to investigate tinnitus-induced changes in zinc signaling. These experiments will determine whether impaired zinc transmission is correlated with tinnitus.

Presenter: Travis Urban

HHMI-70 | Poster session 2

Understanding the mechanisms of a neuron specific proteotoxic stress response

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Abnormal deposition of aggregated protein in neurons is a shared pathologic feature of the major neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, frontotemporal dementia, Huntington’s disease, and ALS. This fact suggests that there is a mismatch between the pro-
duction and clearance of misfolded proteins in neurons. In an effort to understand whether and how neurons recognize and respond to misfolded protein, we found evidence that the heat shock response (HSR), one of the principal coping responses to misfolded protein, is significantly reduced in neurons, both in vitro and in vivo, compared with non-neuronal cells. Further studies by our lab suggested that heat shock factors (HSFs), the principal transcriptional activators of the HSR, may be processed differently by neurons and non-neuronal cells. We believe neuron-specific processing of HSFs may play a role in attenuating a canonical HSR in neurons and are currently studying potential mechanisms of neuron-specific HSF regulation. We also performed RNA-seq to verify the absence of a canonical HSR and to probe for the existence of unique, neuron-specific responses to proteotoxic stress. Our analysis not only confirmed the absence of a canonical HSR in neurons but suggested a novel transcriptional response, which included upregulation of small nucleolar RNAs (snoRNAs). We are currently investigating the role of this transcriptional response, including snoRNA upregulation, as part of a novel, neuron-specific response to proteotoxic stress.

**Presenter: Ashan Veerakumar**

**HHMI-71 | Poster session 1**

Tracking the frequency-power correlation of the subcallosal cingulate local field potential (SCC-LFP) during chronic SCC deep brain stimulation (DBS) for major depressive disorder (MDD)

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SCC-DBS is an emerging intervention for treatment-resistant MDD, with recent innovations to improve targeting by multimodal imaging-guidance. However, subsequent to implantation, no biomarkers exist to guide initial stimulation parameters or adjustments over the course of therapy, leaving such decisions to time-consuming trial-and-error approaches. Utilizing SCC-LFP data collected from the DBS electrode over a course of therapeutic SCC-DBS, we present results of a longitudinal assessment of the SCC as the MDD patient recovers from illness. Of several candidate biometrics, our results highlight fluctuations in the correlation between LFP power and frequency (hereafter defined as the 1/f slope) — previously assessed as a dynamic brain activity biometric relevant for tracking aging and disease state — which suggests DBS increases the randomness of SCC activity in SCC-DBS treatment responders.

MDD patients (n = 6) were implanted with an investigational stimulation/sensing system (Activa PC+S) with institutional informed consent and regulatory approval. SCC-LFP recordings with stimulation off were collected from a 1-month-post-implantation resting phase, weekly during a 6-month treatment phase, and from a 1-week single-blinded discontinuation experiment. 1/f slope was quantified on Welch periodograms of 15-second SCC-LFP epochs using simple linear regression. To validate the 1/f slope measure, we compared recordings between known waking and sleeping hours to attempt a reproduction of previously reported circadian 1/f slope transitions. Comparative statistics include a general linear model (GLM) to compare 1/f slopes across treatment phases, and a paired t test for sleep-wake comparisons. 5 of 6 patients who responded to SCC-DBS treatment by 6 months were included in this analysis.

As reported in previous human and animal studies, we observe a significant increase in the 1/f slope magnitude during sleeping hours (P < 0.05). As supported by the GLM (P < 0.05), the 1/f slope in the left electrode undergoes a decrease in magnitude with stimulation and a mild increase during the 1-week discontinuation experiment, notably not reaching the pre-treatment baseline, but returning to the pre-discontinuation slope levels with resumed stimulation. This study demonstrates through circadian 1/f changes the sensitivity of the SCC-LFP to detect large shifts in SCC neurophysiological activity. 1/f changes across treatment phase, while less pronounced, and observed in a small sample size, suggest that SCC-DBS stably, and potentially therapeutically, increases the randomness of SCC neural activity. The observed time course provides a potential biomarker of clinical response over the course of DBS therapy.

**Presenter: Kathy K. Wang**

**HHMI-72 | Poster session 2**

IL-33 producers modulate interactions between sensory neurons and regulatory T cells in skeletal muscle

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A distinct population of regulatory T cells (Tregs) that accumulates in injured skeletal muscle helps control inflammation and enhances muscle regeneration. These muscle Tregs express high levels of ST2, which encodes the receptor for the alarmin IL-33. The signaling of IL-33 through ST2 on Tregs has been shown to promote Treg proliferation and to promote muscle repair. The primary IL-33 producers in skeletal muscle are a subset of fibro-adipogenic progenitors (FAPs) within the stromal compartment, expressing the markers Sca-1 and PDGFRα. Using immunostaining of muscle whole-mounts, we observed that IL-33-producing FAPs are frequently associated with peripheral nerve bundles as well as small-diameter sensory fibers. In addition, low-input RNA-seq analysis revealed that both components (CALCRL and Ramp1) of the receptor for the pain-related neuropeptide calcitonin gene-related peptide (CGRP) are expressed in FAPs. These observations prompted us to further investigate potential neural-immune system cross-talk, specifically between sensory neurons and muscle Tregs, mediated by IL-33-producing FAPs. We observed that uninjured TRPV1-Cre/DTA mice have fewer muscle FAPs compared to wild-type littermates, while no difference was seen in IL-33 expression. The total percentage of IL-33+ cells was therefore lower in TRPV1-depleted mice due to the decrease in the FAP population. This suggests that TRPV1+ neurons may have some function in supporting the development or maintenance of the muscle FAP population. The fraction of Tregs in the CD4+ compartment was also reduced in TRPV1-depleted muscle. Single-cell RNA-seq analysis further revealed that CALCRL expression was highest in muscle FAP populations expressing the highest levels of IL-33. Intraperitoneal injection of CGRP resulted in a rapid decrease in IL-33 protein within muscle FAPs according to flow cytometric analysis, which was not directly correlated with a decrease in IL-33 mRNA, suggesting that CGRP signaling may trigger the release of IL-33. To determine whether the physiologic effect of CGRP release by nociceptors is mediated by the CGRP receptor on muscle FAPs, we performed intramuscular injections of capsaicin, which activates pain-sensing TRPV1+ neurons and stimulates CGRP release from TRPV1+ sensory afferent fibers. Capsaicin injection resulted in a significant decrease in IL-33 protein within FAPs by flow cytometry, consistent with the effect seen with CGRP injection. When the CGRP receptor antagonist peptide CGRP8-37 was injected prior to capsaicin, no significant difference in IL-33 expression was observed. Furthermore, repeated intraperitoneal injections of CGRP expanded the Treg population in uninjured muscle. Together, these results suggest that activation of nociceptors in skeletal muscle may mediate IL-33 release via CGRP signaling, promoting muscle Treg expansion. Further understanding of the interplay between stromal, immune, and neural components of the muscle ecosystem could reveal new targets for improving regeneration and limiting destructive fibrosis in diseases such as Duchenne muscular dystrophy and age-related sarcopenia.

**Presenter:** Kathy N. Williams

**HHMI-73 | Poster session 1**

**Reciprocal roles for mRNA binding protein IMP1 in exosome secretion and colorectal cancer cell tumor growth**

Kathy N. Williams,1,2 Sarah F. Andres,2 Ranjan Preet,1 Kathryn E. Hamilton,2 Priya Chatterji,2 Rei Mizuno,2 Dan A. Dixon,3 and Anil K. Rustgi2

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Colorectal cancer (CRC) remains the third leading cause of cancer-related death in the United States. Mutations in oncogenes or tumor suppressor genes, such as APC, BRAF, KRAS, SMAD4, and p53, are known mediators of CRC initiation and progression, but less is known about mechanisms of CRC metastasis. The 5-year survival of patients with CRC is <15%, underscoring the importance of studies elucidating new molecular mechanisms contributing to CRC development or progression. Metastasis occurs when one or more cells with tumor-initiating potential dislodge from the primary tumor site and migrate via the blood or lymphatic system to a secondary site, where they proliferate to form additional lesions. In CRC, the liver is often the site of metastasis. Understanding the mechanisms that confer metastatic potential, migration, invasion, and proliferation is paramount to reducing and treating metastatic disease. Recent work shows that mRNA binding proteins play a role in CRC pathogenesis. The insulin-like growth factor 2 (IGF2) mRNA binding protein 1 (IMP1) is highly expressed in CRC patient tumors and correlates with a worse prognosis. IMP1 is critical during development and is overexpressed in cancers, including colorectal cancer. IMP1 overexpression promotes xenograft tumor growth and dissemination into the blood. Exosomes are secreted vesicles that carry intracellular mRNA, miRNA, and/or protein cargo. Exosomes have been implicated in tumor metastasis, namely the preparation of a “pre-metastatic niche,” but the mechanisms governing exosome generation and cargo are not fully characterized. We sought to study roles for IMP1 in CRC metastasis and exosome biology.

We generated SW480 and HT-29 colorectal cancer cell lines that express different IMP1 levels. Cells were cultured...
Hyaluronan (HA) is a large, soluble glycosaminoglycan of the extracellular matrix that has anti-inflammatory effects under physiologic conditions. However, HA is cleaved into low-molecular-weight (LMW) fragments under conditions of cellular or organismal stress, acting as a molecular “switch” that promotes inflammation. In breast cancer, a decrease in HA synthesis has been correlated with decreased tumor cell proliferation and migration. However, the roles of HA fragmentation in the progression of breast cancer are unknown. We predict that HA fragmentation increases during this transition, promoting inflammation through LMW HA-CD44 interactions. To test our hypothesis, the presence/absence of HA fragmentation was determined using gel electrophoresis in breast cancer cell lines. Additionally, qRT-PCR was performed to examine gene expression of the three major hyaluronan synthases (Has1-3) and the three major hyaluronidases (Hyal1, -2 and -PH-20). Our data suggest that as a cancerous lesion progresses, HMW HA production increases (primarily through Has2), but HA fragmentation does not occur until the tumor acquires a more aggressive phenotype (primarily through Hyal1 and Hyal-PH20). We also verified the presence of CD44 protein in normal and cancerous cell lines via flow cytometry and found an increase in CD44 cell surface expression in aggressive tumor cells when compared to normal cells. Following characterization of HA fragmentation and machinery within our system, we found changes in inflammatory cytokines (such as IL8) as downstream effects of CD44 and HA synthesis inhibition. We have currently knocked out CD44 in a breast cancer cell line using the CRISPR/Cas9 method to determine the functional relationship between CD44 and HA using an in vivo mouse model. By targeting CD44 signaling associated with inflammation, new therapeutic approaches can be developed for the treatment of breast cancer.

Presenter: Chung-an M. Wu

HHMI-75 | Poster session 1

Dynamics of human IgE+ B cell differentiation

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Allergic diseases affect over 400 million individuals worldwide and represent a major burden on the quality of life of those afflicted. IgE is a central molecular mediator of the type I allergic hypersensitivity reaction and a critical point that may be targeted therapeutically, but how the IgE antibody response is regulated under physiological conditions is poorly understood. Prior studies in mice showed that IgE+ B cells exhibited a greater propensity for differentiation into antibody-secreting plasma cells and participated minimally in germinatal centers, which are important for the generation of higher-affinity antibody and humoral memory. This suggests a physiological limiter on long-lasting, high-affinity IgE responses; however, whether these properties of IgE+ B cells in mice extend to humans is yet unclear. Here we used a sensitive flow cytometric approach to characterize the differentiation of human IgE+ B cells generated from blood and tonsil lymphocytes in an anti-CD40/IL-4-driven in vitro assay. We observed that IgE+ B cells had a stronger tendency to differentiate into plasma cells when compared with IgG+ B cells, consistent with existing reports in murine models. To better define the molecular signals guiding IgE antibody production, we also examined the effects of IL-21, which had previously been reported to amplify human IgE responses in vitro. We found that increased IgE production...
in the presence of IL-21 was largely due to enhanced B cell proliferation and plasma cell differentiation. In contrast, effects on IgE class switch recombination depended on other signals received by the cell, such as strength of CD40 stimulation or presence of B cell receptor ligation. Our results highlight key similarities and differences in the behavior of human IgE+ B cells relative to more established murine models, and our specific focus on cellular analysis of the IgE response provides new insights that may be relevant to human disease.

**Presenter:** Danwei Wu

**HHMI-76 | Poster session 2**

**Mutation in an autism susceptibility gene, ANK2, leads to loss of L1CAM binding in long axon tracts**

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Multiple genome-wide studies have identified ANK2 as a high-probability gene associated with high functioning autism. ANK2 encodes two major ankyrin-B isoforms, a 220-kD isoform that is widely expressed and a 440-kD isoform that is expressed only in unmyelinated axons in the developing and adult nervous system. De novo frameshift and nonsense human mutations in ANK2 lead to either loss of both 440-kD ankyrin-B and 220-kD ankyrin-B or loss of only 440-kD ankyrin-B, suggesting that 440-kD ankyrin-B is the key autism target. Here, we investigate the role of 440-kD ankyrin-B in the nervous system using two models: (1) mice with loxP sites flanking the giant exon of 440-kD ankyrin-B crossed with mice expressing Nestin-Cre recombinase and (2) mice generated harboring a frameshift mutation (fs2580a) in the giant exon of ANK2. Both mouse lines are viable and fertile. Homozygous mice bearing fs2580a mutation have complete loss of 440-kD ankyrin-B, and heterozygotes exhibit a 50% reduction in expression. In wild-type mice, 440-kD ankyrin-B is found only in neurons and not in supporting glial cells, with highest expression in the neonatal period. 440-kD and 220-kD ankyrin-B are co-expressed in long axon tracts with L1CAM, a cell adhesion molecule linked to a neurodevelopmental disorder in humans characterized by abnormal axonal pathfinding in the corpus callosum and corticospinal tracts. Using the proximity ligation assay to detect association of L1CAM with ankyrin-B, we found a strong proximity signal of L1CAM and ankyrin-B in the long axon tracts of wild-type mice. In contrast, a proximity signal was barely detectable in mice with Y1228H mutation in L1CAM, which eliminates ankyrin binding and causes abnormal long-range axonal targeting. Strikingly, mice lacking only 440-kD ankyrin-B expression but retaining 220-kD ankyrin-B exhibited nearly complete loss of L1CAM proximity signal in long axon tracts, including corpus callosum, internal capsule, and fibrae. We conclude that 440-kD ankyrin-B is the major ankyrin-B isoform interacting with L1CAM in axons. These findings suggest a functional connection between 440-kD ankyrin-B and L1CAM, a protein known to function in axonal pathfinding, which could provide a molecular mechanism for aberrant neural wiring associated with autism.

**Presenter:** Jason Ya

**HHMI-77 | Poster session 1**

**Exploring the role of p63 in cancer**

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p63 is a member of the p53 family (p53, p63, and p73) of tumor suppressor proteins. All three proteins share similar structures, containing a highly conserved set of functional domains and undergoing splicing to generate various isoforms. The most prominent member of the family, p53, is well known for its function in cell cycle control, DNA repair, apoptosis, and other tumor-suppressive roles in a vast number of cancers. However, the contribution of additional p53 family members to tumor suppressor function is not well understood. p63 has a well-established role as a master regulator of epidermal homeostasis, where it is required for progenitor function as well as terminal differentiation. Unlike p53, mutation of p63 is not observed in most cancers, suggesting it may have pleiotropic roles in a cancer setting. This may be in part due to expression of different isoforms in various tissues. Recent studies have shown that the ratio of two major p63 isoforms, one containing the amino-terminal transactivation domain (TAp63) and an alternatively spliced isoform without this domain (ΔNp63), contributes to tumor-suppressive function, with the TAp63 isoform having been shown to possess tumor suppressor activity. Interestingly, the ΔNp63 isoform is frequently expressed and/or amplified in squamous cell carcinomas, suggesting a potential oncogenic role in cancer. To better understand how the function of p63 is altered in the cancer setting, we turned to proteomics to investigate the p63 interactome in cancer cell

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lines. We first inspected the expression level of ΔNp63 at the mRNA and protein level across a variety of epithelial cancer cell lines using qPCR and Western blot analysis. We found high expression of ΔNp63 primarily in squamous cell carcinomas and cervical squamous cell carcinomas. In addition, we assessed the p63 dependency of these cancer cell lines using proliferation assays after siRNA-mediated knockdown of ΔNp63. We identified a number of p63-dependent cancer cell lines originating from epidermal, head and neck, and cervical squamous cell carcinomas. Then, we sought to characterize the p63 protein interactome in these cell lines using a method known as BioID. To achieve this, we introduced a fusion protein of p63 with BirA*, a promiscuous biotin ligase that upon addition of excess biotin to media attaches biotin to proximally interacting proteins. Using streptavidin immunoprecipitation followed by mass spectrometry, we have identified hundreds of ΔNp63-interacting proteins. Candidate interactors will be placed in a CRISPR screen for proliferation to determine the functional role of these proteins in cancer. Ultimately, this study will help us better understand the role of p63 in cancer and identify novel targets for therapy.

Presenter: Hui Yang

HHMI-78 | Poster session 2

A three-component strategy to study neural control of feeding behaviors at the midbrain/hindbrain boundary in mice

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Neural circuits that have evolved to motivate food procurement during scarcity may contribute to excess caloric intake, obesity, and other metabolic diseases. Recently, intensive efforts to understand circuits that control feeding behavior in mice have pointed to an important role for axon projections from the paraventricular hypothalamic nucleus (PVH) to a poorly defined area including the ventrolateral periaqueductal gray (vlPAG), dorsal raphe (DR), or parabrachial nucleus (PBN). We used muscimol nanoinjections to precisely identify the location of these appetite control neurons at this midbrain/hindbrain boundary. With a more precise localization of this appetite control region, we have set out to investigate the neural processes by which these neurons participate in appetite control. For this, we have been developing a three-component strategy to identify novel neuron types involved in feeding behavior. First, we will use single cell RNA sequencing (RNA-seq) to obtain gene expression markers of neurons in this appetite-controlling region, and define neuronal types based on these molecular markers. Second, neuronal activity within this region during the behavior of interest will be monitored by Ca2+ imaging. Third, after in vivo imaging, we will post hoc validate the molecular markers selected from single cell RNA-seq, and correspond Ca2+ dynamics with the molecular identities by multiplexed fluorescence in situ hybridization (fISH). Ultimately, these molecular markers can be used to develop strategies to manipulate the activity of these neurons. This approach may facilitate uncovering the role of neuronal types in a previously poorly understood brain region, and parallelize investigations of in vivo neuronal dynamics of all molecularly defined cell types in a brain region.

Presenter: Rebekah Packer Zahedi

HHMI-52 | Poster session 2

The role of glial unfolded protein response in diet-induced obesity

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When faced with a stressor such as infection, overnutrition, or hypoxia, cellular resources become taxed and proteins can become misfolded, unfolded, and aggregated. The unfolded protein response of the endoplasmic reticulum (UPRER) helps maintain organismal proteostasis through signaling pathways that decrease protein translation and increase protein folding capacity. The UPRER is attenuated as an organism ages, and dysfunction of this system has been associated with numerous neurodegenerative and age-related diseases. One transcription factor from these pathways, X-box binding protein (XBP-1s), exerts significant control over the UPRER. Constitutive neuronal expression of xbp-1s in Caenorhabditis elegans compensates for systemic age-dependent loss of the UPRER through a cell non-autonomous mechanism and significantly increases worm longevity. Furthermore, glial-specific constitutive expression of xbp-1s in C. elegans also results in the activation of the UPRER in distal tissues and extends lifespan beyond the benefit conferred by neuronal-specific xbp-1s expression. The UPRER cell non-autonomous mechanism was recently found to be conserved in mammals, with neuronal xbp-1s expression shown to protect against diet-induced obesity and increase hepatic insulin sensitivity in mice.
We hypothesized that expression of *xbp-1s* in glia of mice would similarly induce the UPRER in distal tissues and prevent diet-induced obesity. To test this, we utilized an inducible Cre/lox site-specific recombination system to constitutively express *xbp-1s* in astrocytes in mice. To evaluate UPRER up-regulation, brains and livers were assessed using immunohistochemistry, quantitative polymerase chain reaction (qPCR), and Western blots. Age-matched cohorts of XBP1-s Cre/lox mice were then placed on a high-fat diet to characterize metabolic function and subsequent development of obesity. The results from this study will hopefully uncover new mechanisms of metabolic regulation and protein homeostasis that could serve as targets of therapy for human metabolic and age-related diseases.

**Presenter:** Xiaojie Zhang

**HHMI-79 | Poster session 1**

**Exercise blunts tumor growth via differential immune system modulation**

Xiaojie Zhang,1,2 Kathleen A. Ashcraft,2 and Mark W. Dewhirst2

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Exercise as a mode of therapy has long been used in managing illnesses from diabetes to coronary artery disease. However, defining the role of exercise in cancer therapy has been limited. In our murine models of orthotopically implanted breast tumor 4T1-luc cells, exercise slowed tumor growth rates, reduced metastasis, and improved survival. We have shown that exercise reduces tumor hypoxia and improves tumor perfusion. These effects have shed light on the mechanisms by which exercise augments tumor killing by chemotherapy and radiation therapy (RT). As an adjunct to RT, exercise delayed tumor growth by 30% compared to irradiated controls. Tumors from exercising mice that reached endpoint (1,500 mm³) exhibited 50% reduction in hypoxic area compared to sedentary mice. Furthermore, exercise led to greater CD31 colocalization to desmin, a marker of vascular maturity, as well as upregulated expression of angiogenic factors like VEGF.

Exercise modulation of tumor hypoxic environment gives rise to the deeper question of the role of the immune system in exercise-impacted tumorigenesis. It has been shown that immune cells are mobilized by exercise, but their activity is inversely related to hypoxic stress. Since exercise improves oxygenation, the present study aims to investigate the novel, in vivo effects of voluntary wheel run-
Variation in short tandem repeats of vasopressin receptor 1a are associated with violent offending

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Vasopressin is an ancient multifunctional neuropeptide whose many effects are mediated by the varied distribution of its three distinct receptors. Short tandem repeat (STR) polymorphisms in the flanking region of the vasopressin receptor 1a (AVPR1a) gene have attracted interest in social neuroscience due to their association with altered AVPR1a expression in the brain and with a number of social behaviors, including pair-bonding, altruism, and aggression. The RS1 STR is a tetranucleotide repeat situated upstream from the transcription start site. Shorter RS1 alleles are associated with autism, lower AVPR1a promoter activity, and increased amygdala activation. We studied the relationship of the RS1 alleles to violent crime in subjects with history of violent crime, and in healthy controls.

The sample consisted of 311 Finns: 127 were incarcerated criminal offenders, and 184 were nonviolent controls. The RS1 STR was genotyped by size with an ABI 3730 Capillary Sequencer and using primers that equivalently amplified the various RS1 alleles. Gene effects on violent crime were tested in logistic regression models and chi-square analyses.

7 RS1 alleles were detected, with lengths ranging from 306 to 330 bp (9 to 15 repeats). In a logistic regression model controlling for gender, the RS1 STR was significantly associated with violent offending ($P = 0.003$). The short (306) allele was associated with an increased prevalence of violent offending in a dose-dependent manner (percentages of violent offenders in non-306 carriers, 306 heterozygotes, and 306 homozygotes were 36%, 50%, and 67% respectively; $P = 0.01$).

Variation in the number of repeats of the RS1 allele, particularly the presence or absence of the short 306 bp allele, may contribute to aggression. This effect may be due to altered expression of the AVPR1a gene in different regions of the brain, a possibility that is now being evaluated.